Russian Chemical Reviews **63** (11) 945–964 (1994)
©1994 Russian Academy of Sciences and Turpion Ltd

UDC 547.963:541.6

The structural-functional organisation of natural peptides

E M Popov

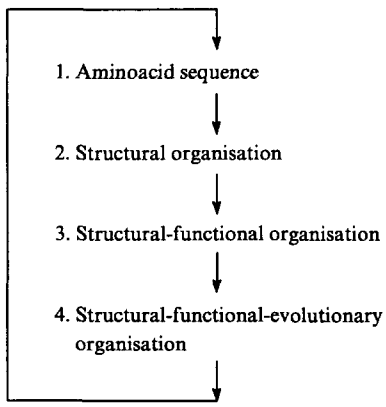
Contents

I.	Introduction	945
II.	Structural organisation of peptides	946
III.	Structural-functional organisation of peptides	949
IV.	The structure and functions of angiotensin II	952
V.	Synthetic analogues of angiotensin	956
VI.	Polyfunctionality of the hormone and heterogeneity of the receptors	958
VII.	Conclusion	963

Abstract. The principles of the structural and structural-functional organisation of the molecules of natural oligopeptides are discussed. The approach to the investigation of the biological activity of peptide hormones directed from structure to function is discussed in general terms and in relation to angiotensin II. It is shown that the polyfunctionality of peptides arises from their ability to exist in a series of conformational states under physiological conditions. A separate function of the peptide is achieved by means of a conformation characteristic of only this function among the conformations most preferred for the free molecule. The method of specific construction of artificial analogues, the steric structure of which corresponds to the set of low-energy physiologically active conformational states of the natural hormone, is examined. The bibliography includes 104 references.

I. Introduction

In the study of natural oligopeptides and proteins, investigators are faced with the need to solve four fundamental problems represented in the following scheme with an indication of the hierarchical relations between them:



E M Popov Department of Organic and Physical Chemistry, Moscow State Correspondence Institute of the Food Industry, Zemlyanoi val 73, 109803 Moscow, Russian Federation. Fax (7-095) 292 65 11. Tel. (7-095) 434 32 41

Received 25 August 1994
Uspekhi Khimii **63** (11) 1004–1025 (1994); translated by A K Grzybowski

The first task involves the establishment of the chemical structure of the compound, namely the determination of the order in which the standard aminoacids are arranged in the peptide chain. The second task involves the identification of the steric form and conformational dynamics of the molecule on the basis of the known aminoacid sequence. The third task involves the study of the dependence of the biological and physiological properties of peptides and proteins on their molecular-structural organisation. Finally, the last task is associated with the consideration of these compounds from the evolutionary standpoint as historical objects. The entire preceding information is now being integrated and the concept of the triunity of the structure, function, and evolution of natural peptides and proteins is being established.

The scheme presented embraces all aspects of the studies on peptides and proteins and classifies them in terms of groups forming a closed cycle in a system of four general coordinates (substance, three-dimensional form, function, and time). The arrows indicate subordinate relations and hence establish a logically justified order in which the fundamental tasks should be considered in the direction from simple to complex or in the given instance from structure to function. This communication describes the fundamentals of this approach and discusses its possibilities in the study of the properties of natural oligopeptides both in general terms and as regards the specific example of angiotensin II.

The theme of the structural and structural-functional organisation of natural oligopeptides has attracted attention for two reasons. Firstly, these compounds are intrinsically extremely interesting in both cognitive and applied respects. They possess unique properties and participate actively in many vital processes. Secondly, the enhanced interest in natural oligopeptides is also due to their genetic relations with proteins. They are close to the latter as regards their evolutionary origin and because they are compounds of the same chemical type. Furthermore, valence and nonvalence interactions between peptides and proteins frequently occur in biological processes, so that the knowledge of the properties of low-molecular-mass peptides is of primary importance in the study of high-molecular-mass compounds of the same nature.

In comparing the properties of natural peptides and proteins, one must bear in mind some uncertainty in their subdivision. The aminoacid sequences encountered in nature form a virtually continuous series of compounds with a gradually increasing number of residues in the chain ranging from several units to many hundreds. It is natural to expect that, on passing from

low-molecular-mass peptides to high-molecular-mass proteins, the conformational properties of the molecules change monotonically and not abruptly. For example, it is evident that the nonvalence interactions between the residues, localised on comparatively small sections of the aminoacid sequence, play the same role in the formation of oligopeptides and polypeptides. The understanding of the steric structure and mechanism of the spontaneous, rapid, and error-free assembly of a protein sequence into the native conformation is therefore impossible without the establishment of the principles of the structural organisation of the low-molecular-mass peptides selected by evolution. Since there are no clearly defined boundaries between natural oligopeptides and polypeptides, the conformational theory of simpler molecules becomes a natural component of the conformational theory of the more complex molecules of the same nature. A definite continuity apparently exists also with respect to the structural-functional organisation of natural oligopeptides and proteins.

II. Structural organisation of peptides

The steric structures of many tens of natural oligopeptides and their synthetic analogues, containing from 5 to more than 30 aminoacid residues, were previously investigated with the aid of the semiempirical method of conformational analysis.[1-3] This resulted in the formulation of the following principles governing the structural organisation of the molecules of these compounds:[2]

1. Under physiological conditions, the steric structure of a natural oligopeptide is determined by a limited set of low-energy compact structures, the stability of which depends on all the nonvalence interactions between the atoms within and between the residues being concerted.

2. The preferred structures of the oligopeptide may differ in the shapes of the peptide skeleton, in the forms of the main chain within the limits of one shape (one structural type), and in the orientations of the side chains, within the limits of one form.

3. The displacement of the equilibrium position between a few conformations of the oligopeptides takes place in accordance with a fully defined low-energy mechanism involving a series of consecutive conformational transitions.

4. The high sensitivity (ready adaptability) of the position of the conformational equilibrium in a natural oligopeptide to the environment (solvent, receptor, enzyme) is due to the rigorous selectivity of the low-energy conformational states as regards specific changes in external conditions.

The question arises what are the common features and what are the differences between the structural organisations of the molecules of natural oligopeptides and proteins. One difference between them is evident—the steric construction of the low-molecular-mass peptides is characterised by a set of structures, i.e. by the position of the conformational equilibrium, while that of the proteins is characterised by a single three-dimensional structure under native conditions. More detailed information about the structural organisation of protein molecules is required for further discussion of this question. We shall use for this purpose the bifurcation thermodynamic model of the spontaneous coiling of the polypeptide chain developed by the author and the physical conformational theory of proteins which makes it possible to predict on the basis of the known aminoacid sequence the geometry of the three-dimensional structure and to estimate quantitatively the capacity of the latter for conformational rearrangements. These data were presented in detail with proof in a monograph.[2] Here we shall deal only briefly with the requirements for the establishment of a genetic relation between the structural organisations of natural oligopeptides and proteins.

The most important feature of the protein chain is the specific conformational nonuniformity of the natural aminoacid sequence, which is responsible for the occurrence of irreversible fluctuations and the appearance of bifurcations (irreversible deviations[4,5]) and hence for the very possibility of the spontaneous formation of a highly organised structure from chaos. One may claim that the essential feature of this phenomenon consists in the existence of a clear-cut interdependence of chemical structure, conformational properties, and irreversible fluctuations. The heterogeneity of the aminoacid sequence is responsible for the difference between the conformational possibilities in its individual sections, which engenders in its turn the thermodynamic nonuniformity of the fluctuations and their differentiation into equilibrium (reversible) and nonequilibrium (irreversible) fluctuation. The combination of the fluctuations and the order in which they follow one another determine the nature and direction of the mechanism of the rapid and error-free self-assembly of the protein chain. This relation is characteristic only of aminoacid sequences selected by evolution.[6] On the other hand, in the case of regular homogeneous or even heterogeneous synthetic polypeptides with a random aminoacid sequence, this random process cannot develop and does not lead to the evolution of the chain from the state of a statistical coil.

In order that the random search mechanism should really lead to the coiling of the chain, the selection of the bifurcation fluctuations need not represent the sorting of all the possible combinations of all the random changes in the entire polypeptide chain, the number of which and the time required for their occurrence being improbably large. Coiling will occur only when structure formation in the protein globule is initiated and developed for a definite time independently in different sections of the sequence. This will occur when fragments differing significantly in their conformational properties alternate in the protein chain. They can be divided approximately into two groups. The first group includes the conformationally rigid fragments the steric structure of which is fully determined by the interactions of their constituent aminoacid residues. The second group includes conformationally labile sections of the chain. They acquire their final steric form after the occurrence of not only short-range but also long-range interactions between the residues. Calculations for a large number of complex natural oligopeptides (25–35 residues in the chain) and for two low-molecular-mass proteins (neurotoxin II and trypsin inhibitor) revealed the following characteristic features in the organisation of their steric structures, which are evidently common to protein aminoacid sequences:

1. The possible conformations of all the fragments of the protein chain are determined to a large extent by the nature and sequence of their constituent aminoacid residues.

2. The conformationally rigid and labile fragments alternate along the polypeptide chain of the protein and in the initial stage of the coiling process are almost independent of one another.

3. The rigid and labile fragments are comparatively small and include not more than 12 residues (the longer rigid fragments have several formation centres).

Subject to the maintenance of the above conditions, the assembly of the proteins begins simultaneously and almost independently on conformationally rigid fragments separated by labile sections of the sequence, i.e. takes place in essence as the assembly of several oligopeptide molecules linked by fairly flexible chains. Random and nonordered selection of all possible fluctuations of virtually autonomous rigid fragments leads sooner or later but inevitably to the appearance in each of them of a bifurcational combination of irreversible conformational changes corresponding to a unique combination of fluctuations of the residues in the fragment. The time necessary for the structural self-organisation of the peptide section from n residues via a random search mechanism is 10^{n-14} s according to Wetlaufer.[7] Consequently, the duration of the assembly of the conformationally rigid fragment with $n < 12$ does not exceed 10^{-2} s, i.e. is entirely realistic.

In proteins, the conformationally labile sections alternating along a chain of approximately the same length also undergo significant changes during this period. The occurrence of internal interresidue interactions in these sections leads to the limitation of conformational freedom. Only a limited set of relatively stable

and, in the absence of long-range interactions, isoenergetic states arises from the enormous set of random states by the same random selection of reversible and irreversible fluctuations during the period $t = 10^{-2}$ s.

It follows from the picture of the coiling of the polypeptide chain described that natural oligopeptide molecules have a structural organisation similar to that of the conformationally rigid fragments of the protein chain. The native conformation of the protein can be represented as an association of oligopeptide molecules in conformations corresponding to their low-energy states and ensuring most effectively the close packing of the protein globule, i.e. forming the maximum number of stabilising contacts between one another and with the sequences connecting them. The isolated conformationally rigid protein fragments, lacking an additional external stabilisation, have a structural organisation which fully corresponds to that of the natural oligopeptide molecules.

The steric structure of the natural polypeptides considered as well as the structure of synthetic oligo- and polyaminoacids depends on the position of the conformational equilibrium rather than being unambiguously determined. A very significant difference consists in the fact that in natural oligopeptides the conformational equilibrium involves a small number of low-energy bifurcational states, which are readily interconverted, and not an enormous number of reversibly fluctuating forms of the statistical coil, as in the artificial peptides.

The knowledge of the principles of the molecular-structural organisation and the ability to predict quantitatively the possible conformations in natural oligopeptides on the basis of the aminoacid sequence constitute the first necessary step towards the understanding of the structural-functional organisation of protein molecules. However, before taking the next step in this direction, it is necessary to consider at least briefly two further questions. The first concerns the reliability of the results of the theoretical conformational analysis and hence of the reliability of the generalisations based on them, while the second concerns the possibilities inherent in the existing experimental methods for the investigation of conformations and the likely success of the empirical approach employed which involves the transition 'from function to structure'.

One's attitude to theoretical calculated data naturally depends on one's certainty that they reflect correctly at least in qualitative terms, if not always strictly quantitatively, the characteristic features of the experimental facts, phenomena, and relations which they describe. An objective idea about the accuracy of the geometrical parameters of the molecules calculated a priori has a special significance in our discussion because the theoretical approach should in fact become the basis for a rigorous solution of the problem of the structural-functional organisation of natural oligopeptides.

The reliability of the calculation of the steric structure of the peptides can be rarely tested by direct comparison of theoretical results with experimental data. Low-molecular-mass peptides exhibit an enhanced, conformational lability, compared with proteins, and their preparation in a crystalline form is therefore a complex and usually an insoluble problem. Consequently the applicability of X-ray diffraction to the study of the steric structures of oligopeptides is extremely limited. Even if it is possible to grow crystals suitable for this purpose and to obtain a diffraction pattern, serious difficulties arise in its interpretation. The method of isomorphous substitution cannot be used in this case to decode the X-ray diffraction pattern, since the incorporation of heavy atoms in the oligomeric molecules constituting the crystal lattice can greatly distort their conformational state; in contrast to proteins, in the study of oligopeptides this method is not really the method of isomorphous substitution. On the other hand, oligopeptides are too complex for their structure to solve with the aid of the direct X-ray diffraction method usually employed in the study of simple organic molecules. Even if it is possible to overcome all the difficulties noted above, nevertheless the problem will still remain unsolved. At best, out of the entire set of low-energy potentially physiologically active conformations of the peptides, only one will become known, namely that tending to form a crystal lattice. There is a possibility that it may have no bearing on the functioning of the compound.

There are at present virtually no crystallographic data on the steric structures of linear low-molecular-mass peptides (with the exception of synthetic dipeptides and tripeptides). X-Ray diffraction analysis has been performed only for relatively rigid macrocyclic natural and artificial oligopeptides and oligodepsipeptides such as gramicidin S, antamanide, enniatin B, valinomycin, and some of their complexes with metal ions.[8] Individual experimental data obtained for these compounds may be compared with the results of theoretical conformational analysis. Such a comparison is particularly instructive for the assessment of the objectivity of the calculated data because the theoretical study was carried out several years before the X-ray diffraction analysis of the same compounds.

The conformational analysis of the cyclic tetradepsipeptides $(Gly-Glyco)_2$, $(MeAla-Lac)_2$, and $(MeVal-Hyiv)_2$, consisting of regularly alternating α-aminoacid and α-hydroxyacid residues with different sets of configurations of the C^{α} atoms (DDDD, DDDL, DDLL, DLLD, LDLD, and LDDD),[9-13] shows that the *cis*, *trans*, *cis*, *trans*-configuration of the main chain is the most stable for these molecules, the peptide and ester groups having the *cis*- and *trans*-configurations respectively. Furthermore, calculation has shown that the introduction of the C^{α} substituents and *N*-methylation only narrow the range of allowed conformations. X-Ray diffraction fully confirmed these conclusions and the geometrical parameters found with its aid for the four diastereoisomers of cyclo-$(MeVal-Hyiv)_2$ with the LDLD, DLLD, DDDL, and LDDD configurations of the C^{α} atoms[8,15-20] agreed quantitatively with the theoretical parameters of the conformations of the corresponding molecules preferred on energy grounds.[10-12] Crystallographic data for enniatin B[21,22] and bovericin[23] also agree satisfactorily with calculated data for the lowest-energy conformation of enniatin B.[24,25] An idea about the similarity of the theoretical and experimental geometrical parameters of the molecules of cyclic tetra- and hexa-depsipeptides can be obtained from Table 1 (see over). Thus, in all cases where it is possible to make a direct comparison between theory and experiment a satisfactory quantitative agreement between the results is observed. However, as already noted, there are few such experimental data and they refer to fairly rigid cyclic oligomeric peptides and depsipeptides.

Physicochemical and primarily optical and resonance spectroscopic methods are used widely in the study of the steric structures of peptide derivatives in solution. However, in this case too the structural problem involving these compounds has remained unsolved for a whole series of reasons. The most serious of these is associated with the characteristic features of the formulation of the problem itself, which in the case of natural oligopeptides is more complex than for proteins and synthetic oligo- and poly-aminoacids. The knowledge of only one global conformation, as in the case of proteins, or ideas concerning the statistical mean conformational state, as in the case of synthetic peptides, are insufficient for a rational investigation of the structural-functional organisation of these compounds. A quantitative estimate of the geometrical parameters of a series of structures, the possible conformations of the latter, their interconversions, and the possibility of their occurrence under various conditions is needed here. The derivation of such information is outside the limits of the sensitivity of physicochemical methods and the possibilities for interpretation inherent in them. Furthermore, owing to the complexity of the compounds and the inadequate elucidation of the physical principles of the corresponding phenomena, none of the methods has as yet permitted an unambiguous and sufficiently complete description of even one conformational state of the peptide

Table 1. Theoretical and experimental dihedral angles of the main chains of the molecules of LDLDLD-cyclohexadepsipeptides and LDLD-cyclotetradepsipeptides.

Molecule (method and reference)	Dihedral angle[a]/deg																	
	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	ϕ_3	ψ_3	ω_3	ϕ_4	ψ_4	ω_4	ϕ_5	ψ_5	ω_5	ϕ_6	ψ_6	ω_6
Cyclo-[(MeAla – Lac)$_3$] (calculation [24])	−103	171	180	74	−136	180	−103	171	180	74	−136	180	−103	171	180	74	−136	180
Cyclo-[(MePhe – Hyiv)$_3$] (X-ray diffraction [22])	−103	167	180	74	−133	179	−101	166	−177	73	−164	178	−78	157	178	90	−136	−179
Cyclo-[(MeAla – Lac)$_2$] (X-ray diffraction [12])	−100	125	180	73	47	0	−100	125	180	73	47	0	–	–	–	–	–	–
Cyclo-[(MePhe – Hyiv)$_2$] (X-ray diffraction [20])	−98	136	175	70	44	0	−102	129	177	82	39	2	–	–	–	–	–	–

[a] The reference points for the angles ϕ, ψ and ω correspond to the IUPAC-IUB nomenclature.

Table 6. The relative energies of the low-energy conformations of natural angiotensins (AT) and the synthetic angiotensin II analogues.

Group and number of conformation	Shape	Relative energies of conformations (ΔU/kcal mol^{-1})														
		AT II	[Asn^1]-AT II	AT III	AT I	AT II – (1 – 6)-peptide	[Pro^3]-AT II	[Pro^5]-AT II	[Ala^3]-AT II	[Ala^5]-AT II	[Pro^2]-AT II	[Ala^8]-AT II	[Leu^8]-AT II	[Val^8]-AT II	[Ile^8]-AT II	[Tyr^8]-AT II
A_1	*fe–fef–ef*	0	4.4	7.4	0.8	2.6	0	0	0	0	–	0	0	0	1.3	0
A_2	*ff–fef–ef*	0.9	0	1.2	0	2.8	–	0	0.6	0.9	–	1.3	2.0	2.3	4.0	0
A_3	*ff–fef–ee*	3.5	3.4	5.1	7.9	2.8	–	8.8	2.7	4.6	–	4.7	1.5	4.9	0	3.8
A_4	*fe–fef–ee*	4.0	8.5	7.3	3.0	2.6	4.5	1.8	3.5	4.8	–	3.4	0.3	5.0	0.2	3.6
B_1	*ff–fee–ee*	3.5	5.3	4.1	9.9	3.6	–	6.4	4.3	4.9	–	6.2	4.6	5.4	1.3	3.0
B_2	*ee–fee–ee*	3.7	2.5	1.8	9.6	0	–	6.5	5.8	7.4	4.6	6.5	5.7	7.4	5.1	3.8
B_3	*fe–fee–ee*	4.4	2.6	1.8	–	0.8	–	–	3.8	5.5	–	5.7	2.8	6.2	3.7	5.5
B_4	*ee–fee–ef*	4.6	2.7	0	7.5	0	–	–	5.8	7.4	9.5	6.5	6.3	4.9	7.9	6.2
B_5	*fe–fee–ef*	6.0	3.2	0	8.3	0.8	–	–	5.4	7.0	–	5.8	4.9	2.78	6.0	8.9
B_6	*ef–fee–ee*	7.0	8.0	4.1	–	5.6	–	6.5	7.5	7.4	0	9.3	6.1	5.7	5.4	7.7
C_1	*ff–efe–ee*	7.5	–	9.5	–	5.8	–	–	10.0	7.5	–	7.6	4.1	3.7	4.2	7.1
D_1	*ff–eee–ee*	7.8	9.8	5.3	5.4	2.7	–	–	8.8	9.0	–	8.0	9.1	2.6	–	7.0
E_1	*fe–fff–ef*	7.8	–	9.0	–	5.6	–	7.4	7.3	7.7	–	8.2	4.5	3.8	4.0	7.3
F_1	*ef–eff–ef*	8.0	4.8	4.0	7.6	5.1	–	–	7.9	7.8	0.8	8.0	8.9	5.5	5.0	7.6

Note. A dash indicates that the energy of the conformation exceeds 10 kcal mol^{-1}.

predominating in solution on the basis of the results of experimental measurements.

This conclusion follows evidently from the analysis of the results of virtually all studies on the structure of oligopeptides by physicochemical methods. For example, for the elucidation of the steric structure of the octapeptide hormone angiotensin II, virtually the whole set of the existing methods have been resorted to: EPR and ^1H and ^{13}C NMR spectra, optical rotatory dispersion (ORD) and circular dichroism (CD) curves, IR and Raman spectra, fluorescence analysis, isotope exchange, etc. As a result, different investigators proposed seven completely different models of the steric structure of the octapeptide hormone corresponding to data obtained by one or several methods at once. However, none of these models, reflecting the state of only the main chain of the molecule, satisfies the entire set of experimental facts.[26] Such uncertainty is characteristic also of the treatment of numerous experimental data on the structure of the neurohypophyseal hormone oxytocin,[27] although in this case the study of the structure was facilitated by the presence of a disulfide linkage between the first and sixth aminoacid residues.

In relation to angiotensin and oxytocin, the peptide hormones which have been investigated most comprehensively and in greatest detail during the last two decades, it has been demonstrated convincingly that the existing physicochemical methods have only a limited applicability to the determination of the structural organisation of natural peptides. Without denying the value of experimental data, one can nevertheless state that a solution solely on their basis of the structural problem to the required extent — the determination of the geometry and conformational dynamics of the low-energy structures of the natural peptide — is at present impossible. The purely empirical approach predominating in conformational studies on peptides and incidentally also proteins uses data which are far from reflecting fully the nature of the phenomenon investigated. The existing gap between the required information and the experimental possibilities actually constitutes the reason for the lack of an appreciable progress in this field over a long period (since the 1960s).

At the present time, the possibilities of the existing physicochemical methods are limited to securing data in support of some kind of hypothetical or a priori calculated structure. Furthermore, the experimental test of the theoretical conformational analysis of peptides has its characteristic features. On the one hand, although a satisfactory agreement between the calculated results and experimental data is a positive finding, nevertheless it cannot serve as a reliable criterion of the validity of the theoretical prediction. In addition, strangely enough the lack of agreement does not imply incontrovertible proof that the calculated results do not correspond to reality. The difference between theoretical and experimental data can be induced by causes which do not play a decisive role in a rational study of the structural-functional organisation of the peptide. For example, a clear-cut discrepancy will occur when the entire set of the low-energy conformations is determined correctly and the geometry of each state is estimated satisfactorily but there is incomplete agreement between the dispositions of the conformations on the observed and real energy scales (a difference of 1.5–2.0 kcal mol^{-1} is sufficient).

Two methods have been proposed for the testing of the results of calculations.[2]

The first method for the assessment of calculated results does not require any experimental facts whatever on the steric structure of the peptides. Its essential feature consists in the selection for the theoretical study of objects such that the calculation for the latter entails an internal autonomous check of its results. For example, intramolecular disulfide bonds can serve as natural reference standards.

According to the theory[2,28,29] and Anfinsen's thermodynamic hypothesis,[30] the oxidation of the sulfur atoms of the cysteine molecules forming disulfide bridges in peptides is possible by virtue of the steric predisposition of the corresponding sections of the aminoacid sequence to conformational states in which the Cys residues are close to one another and the side chains have the mutual orientation necessary for the creation of a S–S bond. During the coiling of the polypeptide chain, the cysteine residues approach one another in the lowest-energy conformations of the linear sequence as a result of concerted stabilising nonvalence interactions between all the chain residues. In a multistage conformational analysis of fairly complex peptides, the attainment of the lowest-energy conformations in the concluding state of the calculation may be expected only when all the theoretical postulates underlying the calculations are adequate and the conformational state of the molecule has been described correctly in quantitative terms. The internal check method has been tested in the analysis of a large number of extremely complex cysteine-containing peptides: nonapeptide neurohypophyseal hormones,[27] apamin,[31], tertiapin,[32] and the MSD-peptide[33] containing respectively 18, 21, and 22 aminoacid residues, including cysteine, which forms two disulfide bonds, as well as insectotoxin[34] containing 36 residues, among which there are eight cysteine residues, etc.[1,2]

In the second assessment method, the correctness of the conformational analysis is tested by comparing the results of the calculation of the possible conformations in the oligopeptide fragments with experimental data on the geometry of the crystal structure of the protein. Such a comparison is at present realistic for two low-molecular-mass proteins — bovine pancreatic trypsin inhibitor (58 aminoacid residues in the chain) and neurotoxin II (61 aminoacid residues), for which the first a priori calculation of the three-dimensional structures has been carried out.[35,36] We shall quote data for the trypsin inhibitor, the steric structure of which is described by approximately 300 dihedral angles, which determine the geometry of all the main and side chains (φ, ψ, ω, and χ). It follows from the comparison of the calculated values of these angles and their experimental values obtained by X-ray diffraction with a resolution of 1.5 Å that the conformation of the inhibitor molecule found a priori is fully consistent with the crystallographic structure. The mean square discrepancies between the experimental and theoretical values of the dihedral angles are 13°, 13°, and 9° for the angles φ, ψ, and ω respectively. The discrepancy between the values of the dihedral angles for the side chain χ (18°) is also within the limits of the error of the high-resolution (1.5–2.0 Å) X-ray diffraction analysis of proteins.

Thus the reliability and the fairly high accuracy of the results of the theoretical conformational analysis of oligopeptides have been confirmed by the automatic formation of a regular system of disulfide bonds in the self-checking calculation and by the agreement between the geometries of the theoretical and crystallographic structures in the calculations for protein molecules.

III. Structural-functional organisation of peptides

As happened many years ago, the mechanisms of the physiological action of peptide hormones are studied nowadays both in the absence of the minimum necessary information about coupled receptors and without the knowledge of the structural organisation of the natural peptide cells. Studies are as a rule based on the results of kinetic measurements and biological tests of the hormone and its synthetic analogues, the latter being selected by the method of trial and error, i.e., strictly speaking, blindly. Thus, a purely empirical approach from function to structure predominates in the study of natural oligopeptides.

Neglect of the information about the geometry and possible conformations of molecules implies in essence the search for an a priori nonexistent direct relation between chemical structure and biological activity. Furthermore, in such an approach the existence of a direct and fairly simple relation between individual aminoacid residues and functions of the peptides is postulated. The idea of additivity inevitably implies that the structural organisation of the interacting molecules ceases to be cooperative, that such molecules are no longer complementary, and that biological processes are not induced and multistage. Nevertheless,

it has long been known that this cannot be done because detailed steric correspondences between the interacting molecules, determined mainly by the specific structural organisation of peptides and proteins, underlie biological processes, their interdependence, high sensitivity, rigorous selectivity, effectiveness, and automatic functioning.

When the 'from function to structure' approach is used, the lack of objective information about the steric organisation of peptides, constituting a natural intermediate link between the chemical structure and biological activity of compounds, is made good by phenomenological descriptions and frequently simply by the author's fantasies. A multiplicity of hypothetical ideas of all kinds, sometimes mutually exclusive, concerning the structural organisation of peptides are available in the literature. The proposed treatments of the mechanism of the action of natural peptides are excessively descriptive in character and lack predictive power.[37] This can be readily demonstrated in relation to studies devoted to angiotensin,[26] bradykinin potentiating peptides,[38,39] and neurohyposphyseal hormones.[27]

Thus it must be recognised that the purely empirical route to the establishment of a direct relation between biological activity and chemical structure is relatively ineffective and without prospects as regards the cognitive purely scientific aspect. It is essential to reverse the direction pursued in research, i.e. to employ the 'from structure to function' approach.

In the solution of the problem of the structural-functional organisation of natural oligopeptides, we shall assume that the aminoacid sequence of the hormone and its physiological activity spectrum together with quantitative estimates of each function are known. If the chemical structure of the peptide is known, it is possible to calculate the possible conformations of the molecule and to discover the set of the potentially active low-energy forms. It is required to establish on the basis of these data the relation between the electronic-conformational and physiological properties of the natural peptide. The physiological properties of peptides may be determined by the path of their interaction with the receptors, whereas information about the organisation of the coupled receptors is either completely lacking or is extremely scanty and, in discussing the properties of protein receptors, we shall be forced henceforth to resort to hypothetical ideas about their conformational and functional features.

In the first place, we suggest that the peptide–protein interactions occurring in living organisms have a unique physicochemical nature regardless of the structure of the specific object and the character of the biological function. Consequently, from a general scientific standpoint, all the numerous and extremely diverse functions of peptides and proteins can be subdivided into two groups of phenomena — physical and chemical. The former take place via the nonvalence interactions of peptides and proteins with one another and with molecules of a different kind. They can be static, as in the case of structural proteins, or dynamic, as in the active transport of metabolites. Together with nonvalence interactions, chemical functions include valence interactions which lead to reversible or irreversible changes in the chemical structure of the molecules. Enzymic reactions, in which the entire set of interactions, both nonvalence (physical) and valence (chemical), take place, may serve as an example. The above assumption presupposes that the interactions of a peptide hormone with a protein receptor are in essence analogous to the interactions of a peptide substrate with a proteolytic enzyme. If this is so, then the characteristic features of the structural and structural-functional organisation of the hormone and the substrate are the same, and this can also be said of the receptor and the enzyme. As regards the principles of the structural organisation of the molecules of peptide substrates and peptide hormones, their identity has been reliably established.

The characteristics of the structural-functional organisation of the molecules of enzyme proteins were discovered previously with the aid of a priori (i.e. in the direction 'from structure to function') calculations of the multistage mechanisms of the elementary steps in biocatalysis by α-chymotrypsin and β-trypsin.[40,43] The calculations were based on the general theory of biocatalysis developed by the author.[44] The characteristics of the structural-functional organisation of enzymes can be formulated in a fairly complete form in the following manner:

1. The active centre of a native enzyme is predisposed, firstly, to sorb a specific substrate in a conformation which in the free state of the molecule is one of the most preferred and, secondly, to undergo quite definite intrinsic conformational changes necessary for the subsequent chemical interactions.

2. The nonvalence enzyme–substrate interactions and the formation of the Michaelis complex do not lead to steric strain or enforced deformations in either the substrate or enzyme molecule. The conformational changes occurring in this stage of the catalytic reaction takes place exclusively by virtue of stabilising interactions and not as a result of unfavourable contacts between the enzyme and substrate atoms, which are simply absent.

3. In the Michaelis complex and in the subsequent complexes involved in the multistage catalytic step, polyfunctional closed systems are formed from the atomic groups of the enzyme and the sensitive site of the substrate. These systems are predisposed to undergo the corresponding specific reactions (for example, in the complexes of α-chymotrypsin and β-trypsin these are acylation and deacylation reactions). A chain of interdependent reactions catalysing one another, the initiation of which does not require an activation energy, occur in each of these systems. The role of the Michaelis complex is to create precisely this kind of autocatalytic closed system, and not to convert the substrate into a strained structure (the geometry of which is similar to that of the transition state) and catalytically active groups into unstrained conformations with an enhanced reactivity. The enzymic process is accelerated because termolecular, tetramolecular, and even higher-order reactions are reduced in such a system to a unimolecular interaction.

4. After the formation of the Michaelis complex as well as other intermediate nonvalence complexes, the additional mutual approach necessary for electronic and chemical interactions between the functional groups may be achieved as a result of Brownian collisions and in-phase normal vibrations of the macromolecular formation.

5. The lowest-energy conformational states of the complexes correspond in all the stages of the catalytic step to a productive mutual orientation of the reacting atomic groups of the enzyme and substrate, i.e. to a mutual disposition of the latter such that their maximum predisposition to undergo specific chemical interactions is achieved. The nonproductive conformations of the enzyme–substrate complexes are energetically much less preferred and their occurrence is unlikely. The transitions between all the chemically unstable intermediate states are accompanied by slight conformational changes, which do not give rise to steric strain.

Thus the enzyme performs its function without resorting to an enforced deformation of the sensitive site of the substrate and of the catalytic groups of the active centre. The catalytic step executed by the enzyme is a conformationally unstrained process. The exceptional efficiency and strict specificity of biological catalysts are not achieved as a result of special high-energy electronic-conformational states of the enzyme and substrates, as has been declared in all the existing concepts. On the contrary, the exceptional nature of this spontaneous natural phenomenon, which occurs without error and under very mild conditions, is the extreme simplicity and natural harmony in the interrelations between the enzyme and substrate developed by evolution at all stages. Biological catalysis has few analogies with the usual chemical catalysis and its mechanism cannot be reproduced by the so called congruent model system.

According to the hypothesis put forward above, the principles of the structural-functional organisation of enzymes listed may be applied to protein receptors. The author deliberately refrained from introducing terminological changes and from replacing the

concepts adopted in enzymology by others less specific. Enzyme catalysis, which consists of alternating physical and chemical phenomena, is one of the most complex processes in vital activity. Therefore, the principles described are of a general character and can be readily reduced to a particular special case. If the interactions of a natural oligopeptide with a protein receptor also include both these phenomena, i.e. nonvalence and valence interactions, then in its description it is necessary to replace only certain words. For example, instead of 'enzyme' one should put 'protein receptor' and the word 'substrate' should be replaced by 'peptide hormone', or both should be changed. On the other hand, if the peptide–receptor interactions are purely physical phenomena, then in the description one must omit everything concerning the chemical aspect of enzyme catalysis.

We now have all the necessary criteria which must be satisfied by the structural-functional organisation of natural oligopeptides. Firstly, it must correspond to the structural organisation of these molecules already known, i.e. must be a consequence of the latter. Secondly, it must complement the structural-functional organisation of protein receptors put forward above. Thirdly, it must not conflict with the known experimental facts concerning the physiological activity of many natural peptides and their synthetic analogues.

All the conditions mentioned above are satisfied for the following principles of the structural-functional organisation of natural oligopeptides.[1,27,39]

1. The spectrum of the functional activity of a low-molecular-mass peptide is determined by the existence under physiological conditions of equilibrium between several structures. In other words, the steric structure of a natural peptide molecule is predisposed to perform not one but several definite functions ('conformational equilibrium — polyfunctionality').

2. An individual function of the peptide is performed via a conformation, valid only for itself, of the most preferred structures forming part of the free molecule, i.e. the uniqueness of the geometry and of the dynamic properties of the corresponding conformational state of the given aminoacid sequence determine the selectivity and the specificity of each of its specific physiological activities ('one conformation — one function').

3. The effectiveness of the activity of the natural oligopeptide when all its functions operate can be accounted for by the alternation of the intramolecular interactions stabilising the preferred conformations and by the relative ease of the conformational transformations. In other words, the rapid response of the conformational equilibrium to a change in the medium ensures a sharp increase in the concentration of the required structure at the required place ('specific external conditions — actual conformation').

Having considered the principles of the structural and structural-functional organisation of natural oligopeptides and proteins, we shall now return to the consideration of the fundamental problem, posed above, concerning the search for a relation between structure and function and an attempt will be made to formulate this problem more rigorously and to outline a general approach to its solution. We recall that the aminoacid sequence of the peptide and its physiological functions, together with quantitative estimates of the activity of each, are known.

As already mentioned, the first step consists in the solution of the direct structural problem for the oligopeptide, i.e. in the determination for the latter of a set of the most probable low-energy conformational states. We begin with the hypothesis that only energy-preferred conformations or some of them can operate as physiologically active structures of the oligopeptides through which its functions are effected.

The next step in the determination of the relation between structure and biological activity is based, on the one hand, on the knowledge of the geometry and conformational properties of all the structures of the test peptides relevant to physiological activity and, on the other, on data concerning the entire spectrum of its activity. Thus the problem arises of the correlation between the set of known conformations and the set of known functions, i.e. of the elucidation of the relevant conformation for each physiological activity of the peptide. In the absence of concrete information about the geometry of the protein receptor, this problem does not have an unambiguous solution. The situation is appreciably simpler in the similarly formulated problem of enzyme–substrate interactions, where not only the organisational principles governing the molecules but also their possible conformations and three-dimensional structures are known.

Evidently the unique high-energy conformational state of the free substrate, which complements the geometry of the active centre of the enzyme, is in this case relevant to the performance of its function. In our case, the active centre of the receptor is unknown, so that its selection is impossible without additional information.

In the absence of data concerning the steric structures of the receptors interacting with various conformations of the peptide considered, the necessary information can be secured with the aid of a set of synthetic analogues of the natural sequence corresponding to special requirements. Each compound in this set must be monofunctional and hence should assume only one of the series of actual conformations of the natural peptide complementary to the active centre of the corresponding receptor. Thus synthetic analogues possessing prespecified electronic-conformational properties are essential for the solution of the problem of the structure and function of natural oligopeptides. Compared with the direct structural problem (a priori calculation of the conformations from the known aminoacid sequence), the problem arising here has the opposite formulation and we shall therefore call it the converse structural problem.[1]

The converse structural problem consists in the theoretical determination of the nature of the chemical modifications necessary for the differentiation of the conformation relevant to the corresponding function of the natural oligopeptide. A more general aim involves the specific formation of the chemical structure of the molecule known beforehand to assume the steric structure necessary for the performance of a fully defined function. The solution of this problem within the framework of the 'from structure to function' approach gives rise to extensive prospects not only for the quantitative study of the functioning of natural oligopeptides but also for the modelling of the active centres of enzymes and the search for medicinal preparations, substrates, inhibitors, antibodies, etc., such a search acquiring a specific aim. As already mentioned, hitherto artificial analogues have been synthesised purely intuitively by the method of trial and error.

The method which the present author has proposed for the solution of the converse structural problem is based on the quantitative conformational theory of natural peptides and proteins,[2,28,29] in particular on the estimation of the special role of the short-range interactions and of the spatial organisation of the aminoacid sequences selected by evolution and on the use of the natural classification of peptide structures.[29] This method has been described in detail elsewhere.[1] Before synthesis and biological tests, it permits the construction of specific artificial analogues the steric structure of which corresponds to the set of low-energy physiologically active conformational states of the natural oligopeptides.

The steps in studies on these lines may correspond to the following scheme:

1. Theoretical conformational analysis of the natural oligopeptide and the determination of all the most energy-preferred and hence potentially physiologically active conformational states (solution of the direct structural problem).

2. The creation of a series of artificial analogues, the set of steric structures of which corresponds to the set of low-energy physiologically active conformations (solution of the converse structural problem).

3. The synthesis and biochemical study of artificial model analogues, the determination of the spectrum of the physiological

activity of the natural oligopeptide, the quantitative estimation of its functional activity, the study of the specific interactions with receptors, etc.

4. Analysis of the results of the calculation and the experiment and the determination at the atomic-molecular level of the relation between chemical structure, the possible conformations, and the effectiveness of the physiological activity of the oligopeptide.

The essential feature of this scheme is that the experimental study of the structure, the biological activity, and the mechanism of the functioning is preceded by theoretical modelling resorting to conformational analysis and to quantum-chemical methods. In this respect, the order in which the studies are performed in the direction 'from structure to function' corresponds to an engineering approach.

In the treatment below, two interrelated problems, solved simultaneously, are considered. One of them is more specific and reduces to the establishment of the structural-functional organisation of the hormone angiotensin II with the aid of the approach described above. The second, more general problem involves the testing of the approach in a specific analysis and the estimation of its real possibilities. The choice of angiotensin II as the object of study was dictated by the fact that this natural oligopeptide has now been most thoroughly investigated and is therefore most suitable for testing the new approach.

IV. The structure and functions of angiotensin II

Angiotensin II, an octapeptide tissue hormone, forms part of the renin–angiotensin system in which its biogenesis and decomposition take place. Angiotensin II is the most powerful of the known pressor agents in the blood circulation system. It stimulates the contraction of the peripheral arterioles throughout the organism and hence increases the arterial pressure. In addition, angiotensin II activates the secretion of a series of hormones (mainly aldosterone), influences the operation of the heart, liver, and the central and peripheral components of the nervous system, and also induces a series of other responses in the mammalian organism. Table 2 lists the known functions of the hormone with an indication of the sites where they are manifested in organisms.

The decapeptide angiotensin I, which is a direct precursor of angiotensin II, is formed, according to the scheme presented below, from angiotensinogen, a globular blood protein, when the latter is acted upon by the proteolytic enzyme renin.

Table 2. The principal functions of angiotensin II and the sites where they are performed in the mammalian organism.

Function	Organs and tissues	Ref.
Stimulation of the contraction of smooth muscles	Smooth muscles of arterioles	45
Regulation of the secretion of aldosterone	Adrenal cortex	46
Regulation of the arterial pressure, secretion of vasopressin, intensification of thirst and appetite for salt	The brain (stem, hypothalamohypophyseal system, establishment of the neuronal thirst cycle, etc.)	47–49
Regulation of kidney haemodynamics, of the secretion of renin, and of the permeability of kidney channel walls	Kidneys (kidney arterioles, kidney channel walls, cells of the juxtaglomerular apparatus)	50–52
Increase in the strength of cardiac contractions	Heart muscle	53
Stimulation of the secretion of catecholamines	Adrenal medulla	54

Almost all tissues possess peptide activity which leads to the rapid decomposition of angiotensin II. Two varieties of angiotensin II have been isolated from mammalian blood. One of them is presented in the scheme, while the other differs from it by the Ile residue in the 5-position.

$Asp^1-Arg^2-Val^3-Tyr^4-Val^5-His^6-Pro^7-Phe^8-$
$-His^9-Leu^{10}-Leu^{11}$

↑ renin

Angiotensinogen

$Asp^1-Arg^2-Val^3-Tyr^4-Val^5-His^6-Pro^7-Phe^8-His^9-Leu^{10}$

Angiotensin I

↑ kinase II

$Asp^1-Arg^2-Val^3-Tyr^4-Val^5-His^6-Pro^7-Phe^8$

↑ aminopeptidase

Angiotensin II

$Arg^2-Val^3-Tyr^4-Val^5-His^6-Pro^7-Phe^8$

Angiotensin III

The chemical synthesis of angiotensin II in 1961 [55] initiated the study of its physiological functions. With the aid of numerous synthetic analogues of the hormone, it was possible to show that the natural peptide interacts with a series of receptors located in various tissues of the mammalian organism and possesses a fairly broad spectrum of biological activity.

Numerous studies on the steric structure of angiotensin II in solution have been published. Virtually the entire set of the existing physicochemical methods have been used in them: thin-film dialysis,[56] titration of ionogenic groups,[57] hydrogen exchange,[58] all types of optical and resonance molecular spectroscopy,[59–62] as well as a number of others. The results of the investigations and their interpretation are extremely contradictory. Different workers have proposed numerous significantly different structures for angiotensin II.[26] Each of them concerns exclusively the main chain of the molecule and satisfies only a limited range of experimental data (see, for example, Refs 26, 45, 58, 63, and 64). The positions of the side chains of the aminoacid residues, which constitute a large proportion of the molecule and determine its conformational state, were completely disregarded in the discussion of the structure of the hormone.

The first studies on angiotensin II by dialysis through thin films proved to be contradictory. In some investigations,[65, 66] it was concluded that the hormone molecule has a single compact form in solution, whilst in another investigation[56] the existence of conformational equilibrium between two forms was postulated.

The conclusions reached by different investigators from the analysis of kinetic data on the isotopic substitution of a proton in the hydrogen bonds of angiotensin II were no less contradictory. Scheraga and coworkers[67] noted that the rates of exchange of all the amide protons were the same. Lenkinski et al.[68] speak of an anomalously low rate of exchange of the His^6 amide proton, while Bleich et al.[69] differentiate the Val^3 and Val^5 residues for the same reason. Printz et al.[63] divided the amide protons into three groups in terms of the rate of exchange, the Asp^1 and Arg^2 protons being assigned to the group with the highest rates. In the classification proposed by Marshall,[70] all the exchanging protons were divided into four groups, the amide protons of all the angiotensin II residues, with the exception of Asp^1 and Phe^8, being assigned to a single group with the same rate of exchange.

Table 3. The number of low-energy conformations, having the same form of the main chain, calculated in the literature.[26,80–83]

Literature source	De Coen and Ralson (1977)[80]	Galaktionov, Nikiforovich, et al. (1979)[26]	Nikiforovich, Galaktionov, and Chipens (1983)[81]	Marchionini, Maigzet, and Premilat (1983)[82]	Nikiforovich, Vesterman, et al. (1987)[83]
80	10	1	1	2	0
26	1	6	4	0	2
81	1	4	5	0	2
82	2	0	0	5	0
83	0	2	0	0	6

The dissociation constants of the ionogenic groups of the hormone, obtained by potentiometric titration[57] and with the aid of NMR and CD spectra,[71] led to the conclusion that the N- and C-terminal groups of the peptide chain are close to one another, which permits their interaction. The distance between the groups is much smaller than the corresponding distance for angiotensin in the statistical coil conformation. In addition, it has been postulated[71] that all the ionogenic groups are accessible to the solvent and that the imidazole ring of the His^6 residue interacts with the benzene ring of the Tyr^4 residue and is remote from the Phe^8 residue.

According to Fermandjian and coworkers,[72,73] the ORD and CD spectra indicate the presence of an antiparallel structure in the angiotensin II molecule. The above workers interpreted similarly the vibrational spectra of the hormone.[60]

A series of studies have been devoted to the NMR spectra of angiotensin II, which yield most information about the steric structure of the molecule. However, here too there is no unanimity in the interpretation of the experimental data. Some workers believe that the spectra indicate the existence of angiotensin II in solution in a single conformation which has a predominantly extended form. Others[75] concluded that the molecule has a coiled form, in which the aromatic side chains of the Tyr^4 and His^6 residues are arranged parallel to one another and interact effectively, whilst the carbonyl group of the Asp^1 residue forms a hydrogen bond with the amino-group of the Tyr^4 residue. The study of the ^{13}C spin–lattice relaxation time led Sekatsis et al.[76] to the conclusion that the conformational mobility of the first two residues is high and that there is no bend in this part of the molecule. Using the same method, Deslauriers et al.,[77] reached a different conclusion. They believe that all the residues of angiotensin II, with the exception of Tyr^4, have approximately the same conformational freedom; the mobility of the side chain of the Tyr^4 residue, which interacts with many other residues of the molecule, is restricted to the greatest extent. The vicinal constants for all the aminoacid residues of angiotensin II[70,78,79] have values consistent with all those corresponding to each residue in the low-energy regions *R* and *B* and hence to 2^7 combinations of the conformational states of the residues in the peptide chain of the molecule.

The reasons for the low effectiveness of the physicochemical methods for the investigation of the steric structures of oligopeptides have already been described in detail. We shall only recall that, for the investigation of the structural-functional organisation of the molecules of these compounds, it is insufficient to know only the global conformation, i.e. the most preferred conformation as regards energy. Here a quantitative estimate of the geometrical parameters of a whole set of structures, their possible conformations, and the probability of their occurrence under various conditions is needed. Such information is so far beyond the limits of sensitivity of physicochemical methods and the possibilities for interpretation inherent in them, so that the semiempirical method of conformational analysis has been resorted to in order to solve the problems listed above. However, here too the results are fairly modest and contradictory. Table 3 presents the results of the calculations of the steric structure of angiotensin II carried out by different workers at different times.[29,80–83] Comparison shows that the sets of low-energy conformations found for the same hormone are not the same. Furthermore, none of them conflict with data obtained in a very wide range of studies carried out on this compound. The reasons for such significant discrepancies have been discussed in detail in a monograph.[2] Therefore we shall briefly note below only certain features of the approach of Galaktionov and coworkers[26,81] to the conformational analysis of natural oligopeptides. From our viewpoint, it is more logical for the solution of the direct structural problem than the approaches used by other investigators.[80,82]

In order to reduce the number of conformations considered with the simultaneous expansion of the critical range of energies, Galaktionov and coworkers[26,81] considered in a preliminary step models in which all the aminoacid residues, other than Arg and Pro, had been replaced by alanine residues. Next, all the possible optimum conformations were determined for the model peptide and the best of these then served as the initial structural variants of the main chain of the real peptide. This procedure is based on what is in our view a fundamentally incorrect hypothesis that the main chain plays a dominant role in determining the possible conformations of the peptides.

If this were the case and the one-third of all the atoms constituting the peptide skeleton did indeed determine the positions of the remaining two-thirds of atoms belonging to the side chains, then all the natural oligopeptides and proteins should have either identical forms of the main chain or a very limited number of such forms. As regards synthetic oligopeptides and polypeptides consisting of glycine and alanine residues and actually lacking side chains, their steric structures should have been described by single rigid structures and not by the state of a statistical coil, as happens in reality. Thus the hypothesis of Galaktionov and coworkers[26,81] is far from representing the true picture. The variations in the steric forms of proteins are almost as numerous as the variations in the order of disposition of the 20 standard aminoacid residues in this chain.

In both the first and second stages of the calculation, where the optimum mutual orientation of the side chains of the test peptides is sought for the selected steric forms of the main chain, mainly the electrostatic interactions of the ionogenic groups were emphasised (it was assumed that they are the main factor in the structural stabilisation). It is difficult to agree with this too because the arrangement of the peptide chain in the native conformation or (in the presence of a series of low-energy structures, as in the case of oligopeptides) the attainment of the conformational equilibrium takes place in aqueous solution under physiological conditions, which prevent an effective electrostatic interaction, abolishing it almost completely ($\varepsilon = 81$).

A characteristic feature of all the theoretical studies of the steric structure of angiotensin II[26,80–83] is the absence of any kind of classification of the conformational states of the molecule let alone one which would be justified from the physical standpoint and would embrace all possible structural variants arranged systematically in accordance with subordinate interrelations in terms of taxonomic categories. All the estimates of the optimum conformations in the studies under discussion were based on the relative values of the overall energy without a quantitative analysis of the contributions by individual intraresidue and interresidue

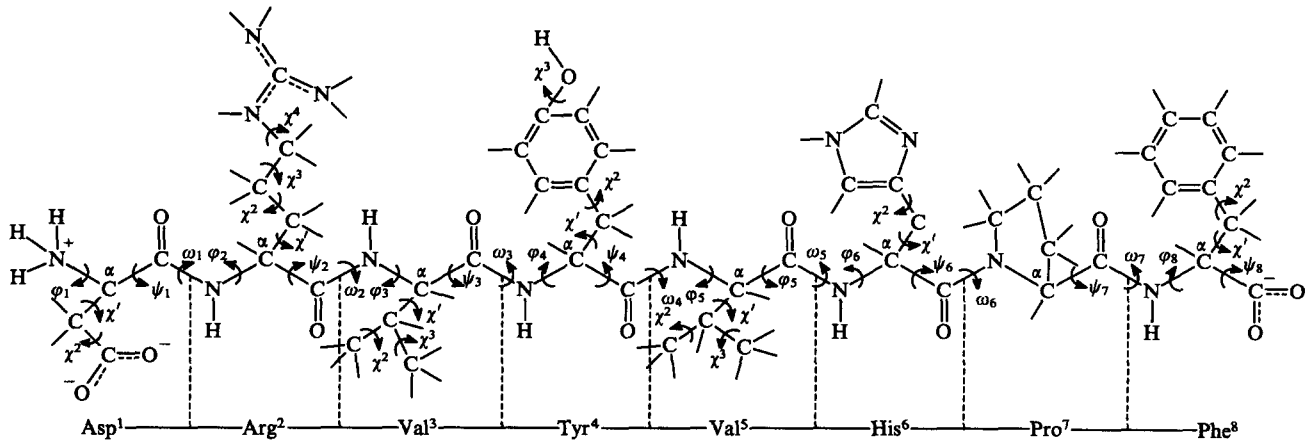


Figure 1. The calculated model of the angiotensin II molecule.

interactions in the structural variants of all the possible forms of different types of the main chain. There is therefore no guarantee that the results of the calculations[26,80–83] are free from either random omissions or incorrect assessments of the data obtained. Table 3 serves to confirm this conclusion.

Thus the available conformational data for angiotensin II could not be used to test the 'from structure to function' approach. This factor led the author of the review and Gogitidze to undertake their own analysis of the conformational states of this molecule.[84] The method of calculation, the notation, and the results are considered briefly below.

The structural organisation of the angiotensin II molecule was investigated on the basis of the conformational theory and the corresponding computational method developed in previous studies[2,85] on the assumption of the rigid valence scheme presented in Fig. 1. The possible conformations of the octapeptide hormone are determined by 41 values of the dihedral angles of rotation about the axes of the main chain (φ, ψ, ω) and the side chains (χ^1, χ^2, ...). The potential functions and the semiempirical parameters used in the calculation to estimate the nonvalence, electrostatic, torsional, and hydrogen bonding interactions were indicated previously.[86,87] The natural classification of the peptide structures in terms of three levels of steric organisation, namely the conformation, form, and shape of the main chain, was used in the calculation and in the description of the results. The classification was put forward in previous communications.[2,85,87]

A system of identifiers was used to designate the conformations. According to this system, the conformational state of each residue is defined by X^n_{ij}, where X characterises the form of the main chain of the residue (R, B, L, and P), n is the number of the residue in the sequence, and the symbols ij = 11, 12, 13, 21 etc. correspond to the position of the side chain (χ^1, χ^2, ...); the subscript 1 corresponds to values of the angles in the range from 0° to 120°, 2 corresponds to angles from 120° to −120°, and 3 corresponds to angles from −120° to 0°. Apart from its form, the main chain is characterised also by its shape or structural type described by the combination of the symbols *f* and *e*, which denote respectively the coiled and uncoiled configurations of the virtual $C^\alpha - C^\alpha_i - C^\alpha_{i+1} - C^\alpha_{i+2}$ bonds. The configuration is defined quantitatively by the virtual angle of rotation $\theta = \psi_i + \phi_{i+1}$ around the $C^\alpha_i - C^\alpha_{i+1}$ bond. The designations and the reference points for the angles of rotation correspond to the generally accepted nomenclature.[88]

The conformational analysis of the octapeptide hormone was carried out in three stages. The first included the independent calculation of five conformations overlapping as regards three aminoacid residues of the tetrapeptide fragments, the second stage involved the calculation for the hexapeptide fragment Asp¹ – His⁶, and the third involved the calculation for the entire angiotensin molecule.

The conformations of the hormone with relative energies from 0 to 8.0 kcal mol^{-1} are presented in Table 4. The first four

Table 4. The relative energies (ΔU/kcal mol^{-1}) and energy contributions (in kcal mol^{-1}) by the nonvalence (U_{nv}), electrostatic (U_{el}), and torsional (U_{tors}) interactions in the preferred optimum conformations of the angiotensin II molecule.

Group and number of conformation	Shape	Conformation	ΔU	U_{nv} [a]	U_{el}	U_{tors}
A_1	*fe–fef–ef*	$R_{23}\,B_{1212}\,R_{222}\,B_{212}\,R_{122}\,B_{31}\,R\,R_{11}$	0	−38.4	−3.5	4.2
A_2	*ff–fef–ef*	$R_{23}\,R_{3222}\,R_{222}\,B_{212}\,R_{122}\,B_{31}\,R\,R_{11}$	0.9	−41.4	−0.9	5.5
A_3	*ff–fef–ee*	$R_{21}\,R_{3222}\,R_{222}\,B_{212}\,R_{122}\,B_{31}\,R\,R_{11}$	3.5	−38.0	−1.4	5.2
A_4	*fe–fef–ee*	$R_{23}\,B_{1212}\,R_{222}\,B_{212}\,R_{122}\,B_{31}\,R\,R_{11}$	4.0	−36.7	−0.3	3.1
B_1	*ff–fee–ee*	$R_{23}\,R_{3222}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,B\,B_{31}$	3.5	−38.4	0.9	3.2
B_2	*ee–fee–ee*	$B_{21}\,B_{1222}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,B\,B_{31}$	3.7	−36.7	0.3	3.1
B_3	*fe–fee–ee*	$R_{21}\,B_{1222}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,B\,R_{31}$	4.4	−36.8	0.5	3.0
B_4	*ee–fee–ef*	$B_{21}\,B_{1222}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,R\,R_{11}$	4.6	−36.3	−1.1	4.3
B_5	*fe–fee–ef*	$R_{23}\,B_{1222}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,R\,R_{11}$	6.0	−36.0	0.1	4.1
B_6	*ef–fee–ee*	$B_{13}\,R_{2122}\,R_{222}\,B_{212}\,B_{222}\,B_{11}\,B\,R_{31}$	7.0	−34.1	0.8	2.6
C_1	*ff–efe–ee*	$R_{23}\,R_{3222}\,B_{122}\,R_{312}\,B_{222}\,B_{11}\,B\,B_{31}$	7.5	−33.2	0.2	2.8
D_1	*ff–eee–ee*	$R_{23}\,R_{3222}\,B_{222}\,B_{112}\,B_{222}\,B_{11}\,B\,B_{11}$	7.8	−33.7	0.7	3.2
E_1	*fe–fff–ef*	$R_{23}\,B_{1222}\,R_{222}\,R_{312}\,R_{222}\,B_{31}\,R\,R_{11}$	7.8	−31.0	−0.3	2.3
F_1	*ef–eff–ef*	$R_{23}\,B_{2122}\,R_{122}\,R_{312}\,R_{222}\,B_{31}\,R\,R_{11}$	8.0	−35.9	2.4	3.8

[a] The energy of hydrogen bonds has also been included in U_{nv}.

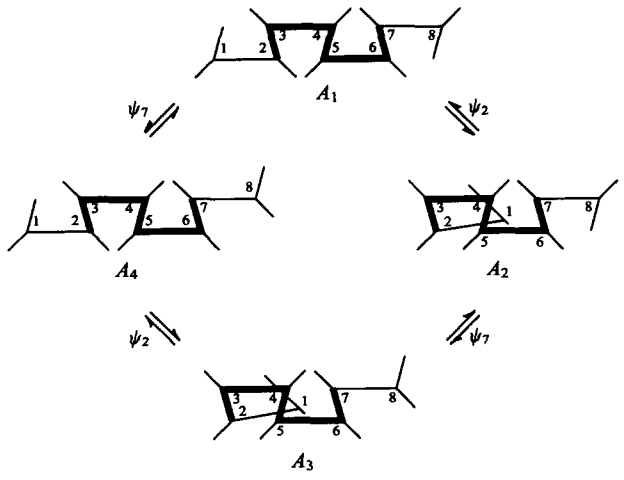


Figure 2. The shapes of the peptide skeleton and the pathways followed in the interconversions of the low-energy conformations of group *A* (Table 4).

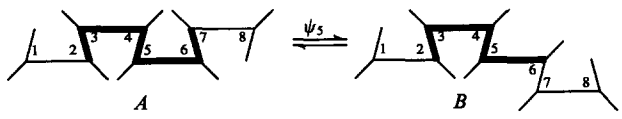


Figure 3. The interconversion of group *A* and *B* conformations (Table 4).

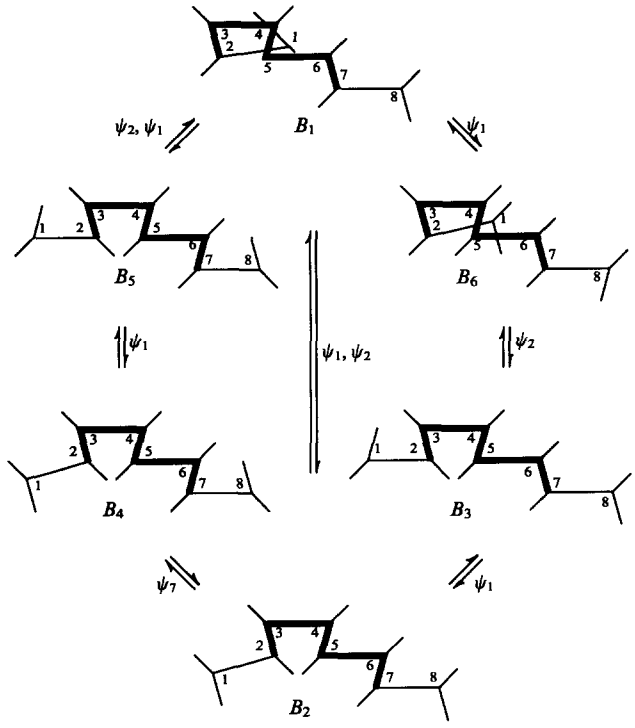


Figure 4. The shapes of the peptide skeleton and the pathways followed in the interconversions of the low-energy group *B* conformations (Table 4).

structures with $\Delta U = 0-4$ kcal mol^{-1} constitute group *A*. A rigid type *fef* nucleation of the central section of the molecule for relatively mobile N- and C-terminal dipeptide fragments is characteristic of these conformations. Fig. 2 illustrates the shapes of the peptide skeletons of these structures and the routes followed in their interconversions. All the changes in the conformational states of the main angiotensin chain within the limits of group *A*, corresponding to the low-energy $R \rightarrow B$ transitions, are affected through rotations about the $C^{\alpha}-C'$ bond of the Arg^2 or Pro^7 residue via a change in the corresponding angle ψ by 180°.

The next six conformations with a relatively low energy ($\Delta U = 3.5-7.0$ kcal mol^{-1}), combined in group *B*, also have the same structure of the central Val^3-Pro^7 section. It belongs to the *fee* shape and differs from the structure of this section in the preceding conformations by only one residue (Val^5). The transition between representatives of groups *A* and *B* is of the low-energy type and reduces to a change in the dihedral angle ψ_5 (Fig. 3). The side chain of the His residue is then moved away from the phenolic ring of the side chain of the Tyr residue, which leads to a slight (~1.0 kcal mol^{-1}) destabilisation of the conformations of group *B* relative to *A*. The shapes of the six low-energy conformations of group *B* and the routes followed in their interconversions are illustrated in Fig. 4.

The best conformations of groups *C*, *D*, *E*, and *F* (Table 4) are appreciably inferior to conformations of groups *A* and *B* as regards energy. They are less compact and are inferior primarily as regards the energy of the nonvalence interactions. The next representatives of these groups have energies exceeding 10 kcal mol^{-1}.

Table 5. The dihedral angles of rotation (in degrees) in the A_1 and B_1 (in brackets) conformations of the angiotensin II molecule.

Residue	Main chain			Side chain			
	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4
Asp^1	−1	−60	−178	80	−92	–	–
	(−50)	(−60)	(180)	(178)	(−93)	(−)	(−)
Arg^2	−151	160	179	61	−179	64	−179
	(−86)	(−35)	(−176)	(−60)	(−178)	(180)	(180)
Val^3	−97	−60	179	178	−179	−179	–
	(−97)	(−61)	(−177)	(179)	(−180)	(−180)	(−)
Tyr^4	−90	146	−169	170	61	180	–
	(−91)	(44)	(−476)	(−175)	(90)	(180)	(−)
Val^5	61	−38	175	70	179	−175	–
	(−99)	(105)	(177)	(180)	(−179)	(180)	(−)
His^6	−85	126	174	−60	102	–	–
	(−122)	(153)	(174)	(59)	(92)	(−)	(−)
Pro^7	−60	−31	179	180	180	180	–
	(−60)	(124)	(−178)	(180)	(180)	(180)	(−)
Phe^8	−95	−38	–	60	86	–	–
	(−96)	(121)	(−)	(−60)	(92)	(−)	(−)

Thus the spectrum of the energy distribution of the optimum conformations of angiotensin II consists of several principal levels (*A*, *B*, *C*, ...), determined by the conformational states of the central fragment Val^3-His^6, and of a comparatively small number of sublevels differing in the conformational states of the labile N- and C-terminal dipeptides; the first can assume steric forms of all possible types (*ee*, *ef*, *fe*, *ff*) and the second can assume forms of only two types (*ee*, *ef*). The energy distribution of the conformations in each family depends to the greatest extent on the number and effectiveness of the stabilising contacts between the residues in the labile and rigid fragments.

It follows from the comparison of the energy contributions (presented in Table 4) by the nonvalence, electrostatic, and torsional interactions to the low-energy conformations of the angiotensin molecule that the nonvalence, mainly dispersion interactions (or, in other words, the packing density of the aminoacid sequence) are of decisive importance in the stabilisation of the steric structure. There is no clear-cut correlation between the quantities U and U_{nv}, although the trends in their variation are similar. The group *A* conformations are on average more compact than the group *B* conformations ($U_{nv}A = -38.6$ kcal mol^{-1} and $U_{nv}B = -36.4$ kcal mol^{-1}) let alone the group *C* and *D* conformations. The geometry and energy of the Val^3-His^6 fragment are virtually constant in the conformations of each group.

Thus the overall result of the solution of the direct structural problem for angiotensin II may be reduced to the claim that the structural organisation of the molecule is described by a comparatively small set of easily interconverted low-energy conformational states, which can be subdivided into several families. The states of the *A* and *B* families are most preferable in terms of energy and entropy. The geometrical parameters of their best representatives (A_1, B_1; Table 4) are presented in Table 5.

V. Synthetic analogues of angiotensin

The study of the possible conformations of angiotensin II [84] made it possible to discover the structural organisation of the hormone and to determine thereby the set of low-energy steric forms which are potentially biologically active. The solution of the next problem, concerning the structural-functional organisation of angiotensin II, consists in discovering within the discovered set of structures those relevant to the occurrence of physiological activities and in the establishment of specific relations between these structures and functions. The development of research on these lines requires the use of experimental data on the functional features of angiotensin II. The effect engendered by angiotensin II in the mammalian organism is the sum of diverse responses, the nature of which depends on the organs and tissues subjected to the action of the hormone (Table 2). The available experimental data show that, like the majority of other peptide hormones, angiotensin II is polyfunctional.

A large number of experimental data obtained in recent years constitute evidence in support of the heterogeneity of the angiotensin II receptor. This process will be examined in detail later and for the moment we shall only note that the polyfunctionality of angiotensin II and the heterogeneity of its receptors can be related naturally to the molecular structural organisation of the hormone, which has been investigated theoretically. Its predisposition to perform a series of functions is manifested by the existence under physiological conditions of several steric forms having similar energies and readily interconverted. The high effectiveness and rigorous selectivity of the interactions of angiotensin II with various receptors are associated with the fact that each of its functions is performed only via the conformation relevant to the given receptor, among the set of preferred structures of the free molecule.

Thus the search for the structural-functional organisation of angiotensin II reduces to the elucidation of the relevant conformation for each biological activity of the peptide. In the absence of necessary data on the potential surfaces for binding sites, the solution of this problem requires additional information. Data on the biological activity of synthetic analogues of natural peptides are normally employed as information of this kind. However, when one sets up a series of analogues in a random fashion without a preliminary study of the possible conformations of both the natural peptide and its artificial analogues, one seeks in essence a simple relation between individual residues of the aminoacid sequence of the hormone and its functions during the investigation. Since the hormone-stimulated allosteric effects arise as a result of multiple and not point contacts between the potential surfaces of the ligand and the receptor complementary to one another (otherwise there would have been no selectivity of the hormonal activity), the disturbance of the function on replacement of even one residue may be a consequence of a whole series of causes: the disappearance of the required functional group, the loss of the necessary dynamic properties of the relevant conformation, the prohibition of the latter as a consequence of the steric strain arising on replacement of the residue, the shift of the conformational equilibrium owing to changing conditions in the interaction with the environment, etc.

Consequently, the random replacement of individual residues does not permit a solution of the problem of the structural-functional organisation of hormones. This is indicated by the lack, during the course of several decades, of appreciable progress in studies on the relation between the structures and functions of angiotensin II, the encephalins and endorphins, neurohypophyseal hormones, bradykinin potentiating peptides, as well as a number of others, which have been prosecuted resorting to a multiplicity of artificial analogues. All this indicates the need to employ a special approach in the study of structural-functional relations for peptide hormones which would make it possible to abandon the method of trial and error and would permit a deliberate choice in the search for synthetic analogues before their synthesis and biological tests.

In this connection, we shall consider the study of Nikiforovich et al.[81] in which an attempt was made to identify the biologically active conformational states of angiotensin II using the results of theoretical analysis together with experimental data. It is assumed that the conformations of the molecules of the hormone and of two of its analogues (conformations A_1, A_2, ..., A_n), which exhibit a high affinity for a definite receptor, and the conformations B_1, B_2, ..., B_n, which are inactive, are known. The authors postulate that the conformations present in the sets of low-energy states of the hormones and the analogues A_1, A_2, ..., A_n and those absent from the sets B_1, B_2, ..., B_n are biologically active structures of angiotensin II.

In this approach, use was made of three synthetic analogues of angiotensin II with a high affinity for the receptor of the smooth muscle tissue of the rat and one without such affinity. However, calculation showed[81] that, among the conformations found for angiotensin II and three of its active analogues, there are none having identical fragments. The absence of such agreement is most probably evidence that the analysis of angiotensin II and of its analogues had been incorrect. However, the authors[81] took what is from our point of view a fundamentally incorrect step without questioning their results. They abandoned the principle that the potential surfaces of the hormone and the receptor are complementary and assumed that a point correspondence is sufficient for the formation of a productive complex. In order to select conformations, they took into account only two distances (and even then with variations within very wide limits) between the C^α atoms of the Val^3 residue and the C^α atoms of the His^6 (4.2–6.2 Å) and Pro^7 (6.7–8.2 Å) residues.

The chosen criterion is extremely ineffective, since generally speaking tens of completely different structures of angiotensin II, differing both in the form of the main chain and in the orientations of the side chains, may satisfy it. In particular, the above ranges of interatomic distances embrace one of the most favoured

conformations of [Pro^2]-angiotensin II for which we carried out calculations[89] and which is discussed below. It is an analogue the affinity of which for the receptors of the smooth muscle tissue is only 0.7% of the affinity of the natural peptide.

However, the choice of a more reliable criterion for the selection of conformations is not the only point at issue. The problem would have remained unsolved even if it had been found that angiotensin II and its active analogues have sets of low-energy conformations in common, which must have been observed for a correct solution of the direct structural problem. The employment of theoretical conformational analysis in the absence of information about the receptors and with a random choice of analogues does not solve the problem of the structural-functional organisation of the natural peptide.

Under the conditions of the polyfunctionality of angiotensin II and the heterogeneity of its receptors, the relations between the low-energy conformations and functions of the hormone may be established with the aid of an extremely small series of synthetic analogues, provided that each compound of the series assumes only one state out of the set of the preferred conformations of the natural peptide. Then all the analogues are able to perform only one hormonal function each or, more precisely, are able to reproduce the interaction of the hormone with only one receptor. When the analogues are monofunctional, their assembly can reproduce the entire spectrum of biological activity of the natural peptide. The problem arising in this case consists in the theoretical determination of the nature of the chemical modifications of the aminoacid sequence of the hormone which are necessary to differentiate a particular conformation relevant to the corresponding function of the natural peptide and the prohibition of all others (converse structural problem). In other words, one is dealing with the deliberate chemical construction of artificial analogues each of which is known beforehand to assume the steric structure necessary for the performance of a fully defined function.

Previously the author proposed a method for the solution of the converse structural problem.[1] It is based on the quantitative conformational theory of natural peptides and proteins,[2] in particular on the estimation of the special role of the nonvalence short-range interactions (intraresidue interactions) of the atoms in the spatial organisation of natural aminoacid sequences and on the use of the natural classification of peptide structures into shapes, forms, and conformations.[2] The short-range interactions have a property extremely important for the solution of the converse structural problem — they are able to determine fully the initial sets of allowed conformational states of the residues. When the peptide chain coils up, the conformational state of each residue is selected only from these sets. Therefore, by influencing in a controlled manner the changes in the short-range interactions, one can deliberately form sets of conformational states of the residues and hence act on the spatial organisation of the peptide effectively and purposefully. It has been shown[1] that this can be achieved by comparatively simple means which have been developed by nature itself in the course of the evolution of the organic world: it involves the replacement in the sequence of one standard residue by another differing significantly in its possible conformations (for example, the replacement of Gly by Ala or Pro), the chemical modification of the standard residue (*N*-methylation, replacement of a L-aminoacid by the D-isomer, etc.), and the replacement of an aminoacid by a hydroxyacid. It is also possible to exchange the Cys residues for Ala and exclude thereby the formation of disulfide bonds without introducing steric complications or, conversely, the residues which are spatially in close proximity may be replaced by Cys, promoting thereby the formation of macrocycles strengthened by S–S bridges. Thus there are many possibilities for the deliberate construction of a set of artificial analogues of the hormone.

We shall now consider the results of the solution of the converse structural problem for angiotensin II.[89] The relative energies of the interactions of atoms not linked by valence bonds in the preferred conformations of the hormone itself and in the related low-energy conformations of a series of its natural and synthetic analogues are compared in Table 6 (p. 948). We recall that the main aim of the investigation[89] was to test the concept of the heterogeneity of angiotensin receptors and to identify the conformations of the hormone responsible for definite functions on the basis of the approach proposed.[1] The starting points were, on the one hand, the aminoacid sequence of the hormone and hence its possible conformations and, on the other, the spectrum of the biological activity of the hormone and quantitative assessments of the activity of each function. After the determination of the minimum necessary set of synthetic analogues for each of them, equally exhaustive biological tests of the analogues are required. Therefore, it was necessary to base the treatment on the available experimental data. Although such data are most extensive for angiotensin II, nevertheless an acute deficiency of experimental facts concerning the biological tests of synthetic analogues is felt here also. The synthetic analogues were as a rule tested solely in terms of one function, so that the total spectra of their biological activities, which are very important in the given instance, remain unknown. The availability of such information would make it possible to extend greatly the results of our study.

The solution of the converse structural problem for angiotensin II was based on the known[84] set of the energy-preferred conformations of the natural molecule. Calculation showed that the structural organisation of the octapeptide hormone can be represented by a limited number of low-energy conformations which can be divided into two groups — *A* and *B* — depending on the geometry of the central tetrapeptide Val^3–His^6 fragment (Table 6). In each group, the conformations differ from one another by the states of the N- and C-terminal dipeptides and can be in their turn subdivided into structures with a free side chain of the Asp or Arg residue.

In mammalian organisms, angiotensin II is encountered in two varieties differing in the nature of the residue in the first position (Asp or Asn), which has only a weak effect on the functional spectra and physiological activities of both hormones. It is seen from Table 6 that the possible conformations of the natural [Asn^1]-AT II analogues are very similar to those of AT II with the Asp^1 residue in the first position. Only the change in the global conformation in group *A* merits attention. In the AT II molecule, the conformation A_1 has the lowest energy. The Arg^2 residue makes a significant contribution to it stabilisation. The hydrophobic part of the side chain of this residue enters into effective dispersion interactions with almost all the residues of the Val^3–Pro^7 fragment (-11.5 kcal mol^{-1}), while the guanidine group, carrying an integral positive charge, interacts also with the C-terminal Phe^8 residue, which is negatively charged (-2.9 kcal mol^{-1}). The aspartic acid residue hardly interacts with the remainder of the molecule and is oriented towards the surrounding medium. In the A_2 conformation, the energy of which (0.9 kcal mol^{-1}) exceeds only slightly the energy of A_1, the situation changes: the side chain of the Arg^2 residue is free, while that of Asp^1 is bound.

In contrast to AT II, in the [Asn^1]-AT II molecule there is no negative charge on the first residue and the stabilising energy of its electrostatic interaction with the Arg^2 residue is therefore sharply weakened. This is in fact the primary cause of the smaller preference for the A_1 conformation compared with A_2, which becomes the most favourable.

Calculation for angiotensin III, differing from angiotensin II by the absence of the first residue in the sequence, led to an interesting result, which was satisfactorily correlated (this will be demon-strated below) with the physiological activity. The removal of Asp^1 alters significantly the position of the $A \rightleftarrows B$ conformational equilibrium, displacing it to the right. The B_4 or B_5 conformation becomes the most favourable, which amounts to absolutely the same thing, and the A_1 conformation, which is best for angiotensin II, acquires a high relative energy 7.4 kcal mol^{-1} (Table 6, p. 948) and its occurrence becomes unlikely.

One of the causes of the increase in the energy of the A_1 conformation relative to B_4 is associated with the greater destabilising electrostatic energy of the interactions of the positive charges on the main and side chains of the Arg^2 residue; in the A_1 conformation, the energy of the nonvalence interactions of the arginine residue is $+14.0$ kcal mol^{-1}, while in B_4 it is $+9.0$ kcal mol^{-1}. The second cause of the shift of the conformational equilibrium in the absence of the N-terminal Asp^1 residue from angiotensin consists in more compact packing and hence a lower energy of the interresidue dispersion interactions of the valence nonbonded atoms of the Arg^2 side chain in the B_4 conformation (-22.1 kcal mol^{-1}) compared with the A_1 conformation (-17.6 kcal mol^{-1}).

Angiotensin I (Table 6) is a decapeptide containing the His and Leu residues in the C-terminal part of the sequence. The increase in chain length affects appreciably the energy distribution of the conformations. It leads to a stronger determination of the conformational states of the peptide, i.e. to a restriction of the conformational freedom. Of the structural set of angiotensin II, three group A conformations (A_1, A_2, and A_4) are actually allowed, A_1 and A_2 having the lowest energies, as before. The A_2 conformation is more favourable in terms of energy than A_1, since its potential surface is more complementary to the surface of the additional His^9-Leu^{10} dipeptide fragment. The appreciable increase in the energy of group B conformations in angiotensin I, which renders them virtually prohibited, can be explained, firstly, by the significantly weaker interactions of the His^9-Leu^{10} section with the remainder of the molecule (for example, their energy is -6.2 kcal mol^{-1} for B_1, whereas those for A_2 and A_1 are respectively -11.3 and -9.4 kcal mol^{-1}) and, secondly, by the loss, for steric reasons, of a series of stabilising interactions of the Phe^8 residue, which occupies terminal and intermediate positions in angiotensin II and angiotensin I respectively.

Whereas the lengthening of the peptide chain by two residues (AT I) lowers the relative energy of the group A conformations, its shortening by two residues compared with angiotensin II [At II$-$(1$-$6)-peptide] has the opposite effect: two of the four group B conformations acquire the lowest energy, which does not exceed 1.0 kcal mol^{-1}. However, the changes are not quite so marked as in the case of AT I and the possible conformations of the hexapeptide analogue of angiotensin, lacking a series of stabilising interactions following the loss of two residues, are naturally more numerous. Not only group A conformations but even group C_1-F_1 and especially D_1 conformations become realistic under certain external conditions.

The replacement of the valine residues in the third and fifth positions in the angiotensin II molecule by proline {[Pro^3]-AT II and [Pro^5]-AT II (Table 6)} and alanine {[Ala^3]-AT II and [Ala^5]-AT II} residues had as its aim the introduction of a definite and previously known steric hindrance to prevent the occurrence of a large number of conformations (the first two peptides). On the other hand, the aim was to render the aminoacid sequence of angiotensin II more labile and to estimate the restricting effect of fairly bulky valine residues branches at the C^α atom on the formation of the steric structure of the molecule (the second pair of peptides).

Being in the R form, the residue preceding proline has a higher energy owing to the overlap of the van der Waals radii of the hydrogen atoms of the methylene group of the proline ring. The B and then the L forms are most preferred for this residue. For this reason, the Arg^2-Pro^3 section of the [Pro^3]-angiotensin II analogue can only have the expanded e shape. Consequently, the occurrence of the six out of the fourteen possible low-energy conformations of angiotensin II with the R form of Arg^2 and the f shape of the dipeptide Arg^2-Pro^3 becomes unlikely for the synthetic analogue. Only the A_1 and A_4 structures with relative energies of 0 and 4.5 kcal mol^{-1} remain preferred. The energies of the remaining structures exceed 10.0 kcal mol^{-1}.

When Val^5 is replaced by Pro^5, neither group A nor group B conformations are prohibited, since the Tyr^4-Val^5 section has the uncoiled form in both cases. Nevertheless, calculation showed that here the situation is in principle similar to that just examined above, i.e. a decrease in the relative energy of the A conformations and an increase in that of the B conformations is observed.

Calculation showed that the replacement of Val^3 and Val^5 by alanine, which seemingly should have provided considerable conformational freedom to the main chain of the hormone within the limits of the same minima on the multidimensional potential surface, hardly affects the position of the conformational equilibrium. As before, the group A structures in a polar medium are preferable to the group B structures, while the C_1-F_1 structures have equally high energies. Therefore one may expect that the physiological activity spectra of the alanine analogues reproduce fairly completely the spectrum of the natural angiotensin II, in contrast to the proline analogues.

In [Pro^3]-AT II, the replacement involves the residue which not only dictates the purely steric conditions for the establishment of the conformational states of the molecule, as in the four synthetic analogues of the natural hormone examined above, but undoubtedly also plays an extremely important role in specific recognition or in the consequences of the hormone$-$receptor interactions. Such duality complicates somewhat the treatment of the results of the theoretical conformational analysis of the analogue (Table 6). It is remarkable that the B_6 and F_1 conformations, having a very high energy in angiotensin II, are the most preferred in [Pro^2]-AT II.

We shall consider a further five analogues of angiotensin II. The Phe residue of angiotensin II was replaced in succession by the Ala, Leu, Val, Ile, and finally Tyr residues, i.e. by hydrophobic residues with a gradually increasing bulk. The most significant changes in the set of energy-preferred conformations compared with the natural octapeptide were then observed. Group A structures predominate in all cases. The energy distribution of the [Tyr^8]-AT II conformations is virtually the same, as might have been expected, as that in angiotensin II.

The choice for calculation of all the angiotensin II analogues presented in Table 6, especially the last six, is dictated by the availability of the corresponding experimental data, which will be analysed in the next section in the light of the results of the conformational analysis described.

VI. Polyfunctionality of the hormone and heterogeneity of the receptors

The knowledge of the quantitative characteristics of the structural organisation of angiotensin II and it analogues is a necessary condition for the rational investigation of the structural-functional organisation of the hormone. However, this is still far from sufficient. The attempt to discover the physiological characteristics of angiotensin(II), like those of any other natural oligopeptides, encounters fundamental difficulties arising owing to the lack of knowledge of the causes of the polyfunctionality of peptide hormones. The lack of clarity in this problem is due to the extremely scanty information about the hormonal receptors. In other words, the problem of the structural-functional organisation of the hormone has to be solved in essence in the absence of information about the properties of its partners in the interactions.

There are two points of view on the cause of the polyfunctionality of angiotensin and other peptide hormones. According to one of them,[90] the ability of a particular hormone to stimulate completely different processes in different parts of the organism can be accounted for by the presence of several receptor proteins specific to the given hormone, i.e. by the heterogeneity of the receptors. This point of view does not conflict with the known structural organisation of the hormone molecules. Natural oligopeptides in the native state are not rigorously determined three-dimensional structures, as in the case of high-molecular-mass proteins, but consist of sets of comparatively few steric forms with a position of conformational equilibrium which is readily

altered by external forces.[2] The results of earlier investigations of the possible conformations of bradykinin-potentiating peptides[39] and neurohypophyseal hormones—oxytocin, vasopressin, vasotocin, and mesotocin—agree with this assumption.[27,91]

A different treatment of this phenomenon arises on the basis that each hormone is capable of forming a productive complex only with a single receptor and hence of inducing always the same allosteric change in its conformation. If the receptors are the same, then the polyfunctionality of the hormone is determined no longer by the specificity of the hormone–receptor interactions themselves but by the characteristic features of the systems receiving the signal which has been sent. In this case, the diversity of the physiological activities of the hormone is caused not by the unique structure of its molecule and the characteristic features of the interactions with the receptor protein but by the specificity of the structural organisation of the systems, which respond differently to the same signal arising from the same hormone–receptor complexes interacting with them.

The hypothesis of the heterogeneity of the hormonal receptors, in particular the angiotensin II receptors, has been confirmed by the results of studies carried out in recent years. Thus this is indicated also by the different nature of the interaction of angiotensin II analogues with the receptors in different tissues (for example, by the presence of several analogues with selective activity),[92–95] by the different effects of cations on the binding of the hormone itself to the receptors in the tissues of different organs,[95] and by the observation of hormone receptors, differing in molecular mass, in the tissues of rabbit liver,[96] the human placenta,[97] and the adrenal cortex of the rat and the dog.[98]

However, the most significant evidence in support of the heterogeneity of the receptors as a cause of the polyfunctionality of angiotensin II was obtained recently when nonpeptide antagonists of the hormone were employed.[90,99] The use of a new class of nonpeptide antagonists in the study of angiotensin II receptors made it possible to discover the presence of different types of specific angiotensin II receptors in the mammalian organism. Thus two complex organic compounds, which the authors designated as DUP-89 and WL-19, were employed in one of these studies[99] for the identification of the receptors in the tissue of the rat adrenal cortex. It was found that each of them may bind only to a receptor of a fully defined type, whereas angiotensin II exhibits a high affinity for both receptors. As a result, it was shown that even in a single tissue there are two receptors for the hormone, which assumes a different form in the cells of this tissue. Later the receptor having an enhanced affinity for the WL-19 antagonist was detected in the rat and human uterus and in the rat brain.[10] The use of other nonpeptide antagonists permitted also the differentiation of different types of angiotensin II receptors in tissues of the rat liver and rat brain vessels.[100,101]

Thus a whole series of experimental data indicate convincingly the existence of a set of angiotensin II receptors which impose different conformational requirements on the hormone. One of the tasks in the 'from structure to function' approach which we have been developing in relation to a specific object actually involves the establishment of a quantitative relation between the existing experimental data and the structural organisation of the angiotensin II molecule and thus achieves a more rigorous basis for the treatment of the polyfunctionality of peptide hormones in terms of the heterogeneity of the receptors.

If our hypothesis is valid and a fully defined conformation from the set of low-energy readily interconverted steric forms discovered for angiotensin[84] does indeed interact with only one receptor and hence is responsible for the performance of only one function, then the next task consists in the determination for each physiological activity of the hormone of a conformational state relevant to only this activity, i.e. complementary to the corresponding receptor. Thus the energy-preferred conformations known from theoretical analysis have to be correlated with the experimental functions. Since the structures of the receptors let alone of their active centres are unknown, this problem cannot be solved, as already mentioned, without resorting to additional information. Such information may be obtained by studying synthetic analogues of the hormone.

Synthetic analogues have already been used in endocrinology for many decades. However, despite the expenditure of enormous efforts and financial resources, the results obtained with their aid indicate a low effectiveness of this approach, particularly in the scientific, cognitive respect. We suggest that the problem is not with the approach itself (at the present time there is no alternative to it) but with the satisfactory employment of its potential.

Virtually all the studies on these lines suffer from three serious defects. The first, most significant, is due to the random selection of the artificial analogues. The second weak feature, which also precludes the rise of investigations of this kind above an empirical level, involves the neglect of the possibilities of the theoretical analysis of the conformational states of both the hormone itself and of the selected synthetic analogues. Finally the third deficiency, characteristic of studies in this field, consists in the absence of not only a theoretical basis but also of a single systematic approach to the experimental investigation of hormones. Despite the multiplicity of studies in this field, it is still not possible to compare even in only one case the activity of the hormone with the activities of synthetic analogues throughout the functional spectrum. As a rule, in the study of biological activities of a natural peptide, different sources, different tissues, and, what is most significant, different sets of analogues are employed in each case.

When the peptide hormones are polyfunctional and the receptors are heterogeneous but there is no information about the structure of the latter, the most important, key problem, the solution of which can only be attempted with the aid of the corresponding theoretical developments, consists in passing from the random, purely intuitive search for synthetic analogues by the method of trial and error to their purposeful selection corresponding to special requirements. In order to be useful in the study of the relation between the structure and functions of a hormone, each of its synthetic analogues must be as far as possible monofunctional, i.e. must perform one of the functions of the natural peptide and hence must be able to assume, out of the set of the relevant low-energy conformations, only one steric form complementary to the active centre of the corresponding receptor. A more general aim is the deliberate formation of the chemical structure of the molecule known beforehand to assume the steric structure needed for the performance of the planned fully defined function.

We shall now return to the object of our study—angiotensin II, for which the direct and converse structural problems have already been solved (Sections IV and V).[84,89] However, in a rational study of the hormone[102] it was necessary to deviate somewhat from the procedures dictated by strict logic. This enforced deviation, although not fundamental, nevertheless does not lead to the complete elucidation of the possibilities of the 'from structure to function' approach. This is due to the lack of the necessary experimental literature data, although angiotensin II is in fact one of the most fully investigated peptides. The known experimental facts are very numerous and concern both the hormone itself and many hundreds of its synthetic analogues. However, the weak features of the purely empirical approach, noted above, are characteristic of all these studies as well as the analogous studies on other hormones: the random selection of the analogues, the lack of knowledge of the principles governing the molecular structural organisation of the hormone and its derivatives, and also the lack of a systematic approach to the investigation of functions.

We have been unable to find experimental data on any of the enormous number of the synthetic analogues of angiotensin II investigated which would characterise its activity over the entire spectrum of the biological activity of the hormone. Only a few analogues have been discovered for which quantitative data concerning three functions of angiotensin II have been obtained: the stimulation of the contractions of the smooth muscles and the

Table 7. The pressor activities and quantitative characteristics of certain varieties of the biological activity of natural angiotensins and a number of their synthetic analogues.

Compound	Smooth muscle [45]								Adrenal medulla [47, 103]		Adrenal cortex [46, 103]			Pressor activity (%) [45]
	rabbit aorta				rat stomach									
	α_E	pD_2	RAf(%)	pA_2	α_E	pD_2	RAf(%)	pA_2	RA(%)	pA_2	RA(%)	RSIB(%)	pA_2	
AT II	1.0	8.85	100	–	1.0	7.75	100	–	100	–	100	100	–	100
[Pro^2]-AT II					1.0	5.64	0.7							1.3
[Pro^3]-AT II			40 [a]											40
[Pro^6]-AT II														10
AT III	1.0	7.62	5.6	–	1.0	7.5	56	–	15	–	130	100	–	38
[Asn^1]-AT II	1.0	8.11	18	–	1.0	7.8	105		100	–		100		100
[Ala^8]-AT II	0			7.7	0			7.22	21	–				–
[Leu^8]-AT II	0			8.78	0			8.6		8.53			7.0	–
[Ile^8]-AT II	0			9.21	0			8.36		9.31			8.53	–
AT I	1.0	7.53	5.1		1.0	7.1	22		95		2	5		50

Note. α_E = internal activity (the ratio of the maximum response induced by the agonist to the maximum response induced by AT II); pD_2 = negative logarithm of the agonist concentration inducing an effect equal to half the maximum effect (affinity); pA = negative logarithm of the antagonist concentration reducing the effect of the agonist by a factor of two; RAf(%) = relative affinity (the affinity of the agonist relative to the affinity of angiotensin II); RA = relative activity (the ratio of the activity of the agonist to the activity of angiotensin II for doses with average effectiveness); RSIB = relative strength of the inhibition of binding (ratio of the concentrations of the analogue and angiotensin II inducing the displacement of 50% of labelled AT II in binding to a specific receptor); a gap in the Table denotes the absence of data on the given type of activity and a dash denotes the absence of the given type of activity.

[a] In relation to rat uterus receptors.

regulation of the secretion of aldosterene and catecholamines. The corresponding activities of these analogues and the natural peptides are compared in Table 7.

Among the quantitative estimates of the effectiveness of the interaction of the hormone and its analogues with receptors, the internal activities (α_E) and affinities for the receptor (pD_2) characterise most directly the characteristic features of the structural organisation of the molecules (see the footnotes to Table 7). For analogues with a full internal activity, the effective concentration of the antagonist required for the performance of the biological function and hence the probability of a steric structure of the given analogue complementary to the active centre of the receptor and corresponding to the given conformational state of the hormone can be inferred from the values of pD_2. Indeed, the greater the displacement of the position of the conformational equilibrium for the antagonist from that relevant to the given function of the steric structure, the higher should be its concentration for the attainment of a definite effect and hence the lower its affinity for the receptor.

On the other hand, the probability of the occurrence of the relevant conformations is determined by the relative internal energies, which we have found for the hormone and its analogues in the solution of the direct and converse problems. By comparing the data in Tables 6 and 7, we shall attempt to obtain an idea about the significance of a particular conformational state for the performance of the corresponding function, i.e. about the structural-functional organisation of angiotensin II. We may note that Table 6 might be supplemented by any number of examples however large. For example, it is not difficult to calculate the possible conformations of all these hundreds of synthetic analogues of angiotensin II which have been synthesised and subjected to biological tests but which, alas, have been non-systematic and incomplete. However, for the existing level of experimental studies, the expansion of Table 6 would contribute little to the solution of the problems postulated and presented in Table 7.

The solution of the direct problem[84] showed that the possible conformations of angiotensin II may be described by a set of a few low-energy forms subdivided into two representative groups *A* and *B* and the single forms $C_1 - F_1$ (Table 6). Within the limits of one group, the conformations have the same steric structure of the central $Val^3-Tyr^4-Val^5-His^6$ tetrapeptide section of the molecule and differ from one another only in the orientations of the N- and C-terminal dipeptide fragments.

The results obtained in the solution of the converse problem[89] show that replacements of aminoacids in the angiotensin II sequence can lead to significant changes in the possible conformations of the molecule. The replacements not involving the functional residues do not entail the appearance of new low-energy steric forms, giving rise to a displacement of the conformational equilibrium within the limits of the preferred forms of the natural peptide.

As can be seen from Table 7, the modifications of the chemical structure of the hormone are reflected selectively in the effectiveness of the activities characteristic of angiotensin II. Since the experimental data refer to the interactions of the hormone and its analogues with the acceptors of the smooth muscle and adrenal cortex tissues, we shall attempt initially to identify the optimum conformational states of angiotensin II complementary to precisely these receptors.

The proline-containing analogues may be of special interest, since the replacement of any residue in the aminoacid sequence by Pro has a significant effect on the possible conformations of the entire molecule. We shall compare two such analogues with each other and with angiotensin II as regards steric structure and biological activity— [Pro^2]-AT II and [Pro^3]-AT II.

The replacement of the second residue in the aminoacid sequence of the hormone by proline leads to the almost complete loss of the affinity for the smooth muscle receptors by the [Pro^2]-AT II analogue (the relative affinity does not exceed 0.7% in this case), while the replacement of the third residue leaves the affinity of [Pro^3]-AT II for the receptors of the same tissue fairly significant (40%) compared with the hormone itself. On the other hand, highly concentrated doses of the second compound have no effect whatsoever on the secretion of aldosterone.[92]

The conformational states of the two proline-containing analogues also differ sharply. Among all the low-energy conformations of the [Pro^3]-AT II molecule, the group *A* representatives are the most realistic (Table 6). On the other hand, these conformations hardly occur for [Pro^2]-AT II; the equilibrium is markedly displaced towards the B_6 and F_1 conformations, which have the highest energies in the set of angiotensin II.

Thus comparison of the conformational and biological properties of the hormone and of two of its proline-containing analogues lead to the hypothesis that the group *A* steric structures are relevant to the interactions with the receptors of the smooth muscle tissue. However, this assumption may not prove to be entirely rigorous owing to the nonequivalence of the replacements. Whereas the replacement in the case of [Pro^3]-AT II is homologous (Val^3 is replaced by Pro^3) and therefore has an exclusively steric effect on the possible conformations of the molecule, in the case of [Pro^2]-AT II it is nonhomologous (Arg^2 is substituted by Pro^2) and, apart from the changes in the system of intramolecular nonvalence interactions of the atoms, involves the loss of a guanidine group, which may play an important functional role in the mutual relations between the hormone and the receptors.

Furthermore, the hypothesis that type *A* conformations are relevant to the contractile function of angiotensin II in the smooth muscles of arterioles is seemingly inconsistent with the available data for another proline analogue—[Pro^5]-AT II. Although there is virtually no quantitative information about its functions in the literature, nevertheless it is known that the pressor activity of this analogue is an order of magnitude lower than that of the natural peptide (Table 7), which indicates its low affinity for the smooth muscle tissue receptors. However, the group *A* conformations responsible, according to our hypothesis, for the contractile function are most preferred in [Pro^5]-AT II. At first sight, the contradiction is extremely serious, since in this case the replacement of Val^5 by Pro^5 is homologous. Nevertheless the discrepancy may be only apparent, since the loss of the pressor activity by the given analogue is susceptible to a different interpretation.

Calculation of the conformations of the [Pro^5]-AT II molecule showed that the inclusion of the Pro residue between Tyr^4 and His^6 leads to a significant limitation of the conformational freedom of the peptide chain and to the deformation of the group *A* steric structure of the molecule without causing, however, the displacement of the dihedral angles φ and ψ outside the limits of the *fef* shape of the central tetrapeptide section. Thus, whereas the distances between the C^α atoms of the tyrosine and histidine residues in this group of conformers are about 5.5 Å for angiotensin II, for the [Pro^5]-AT II analogue they are 7.4 Å. Furthermore, the activity of the compound may be influenced by the fact that the Pro^5 residue limits significantly the mobility of the side chains of adjacent residues, which undoubtedly interact effectively with the receptors.

Although our hypothesis that the group *A* conformations must participate in the operation of the contractile function of angiotensin II is fairly controversial nevertheless it will remain a working hypothesis.

Important evidence in support of this hypothesis follows from a comparison of the possible conformations and biological activities of angiotensin II and angiotensin III—a natural heptapeptide which differs from the octapeptide hormone considered by the absence of the N-terminal Asp^1 residue. Both compounds have the same spectrum of biological activities, but their effectiveness is significantly different except when they function in the tissues of the adrenal cortex. Numerous investigations have shown that, although the effectiveness of angiotensin III in its action on the remaining tissues is reduced compared with angiotensin II,

nevertheless the heptapeptide hormone induces in the adrenal cortex tissue the same aldosterone-stimulating effect as the octapeptide hormone[103] and, according to certain data,[46] is actually superior to the latter.

If our hypothesis concerning the cause of the polyfunctionality of the hormones is correct, then the angiotensin III molecule should have the corresponding possible conformations adequately reflecting the observed similarity and difference in its biological activity compared with angiotensin II. Indeed, the elimination of the N-terminal residue displaces sharply the conformational equilibrium towards group *B* structures: the $B_4(B_5)$ conformation becomes most preferred and is then followed in this respect by $B_2(B_3)$, B_1, and B_6, which are inferior to the most favourable conformation by 1.8 and 4.1 kcal mol^{-1} respectively (Table 6). In group *A*, there is only one low-energy conformation — A_2 (1.2 kcal mol^{-1}). Thus, among the steric structures related to the low-energy conformations of angiotensin II, in the case of angiotensin III the most probable conformations are $B_4(B_5)$, A_2, $B_2(B_3)$, as well as B_1 and B_6, i.e. there is a sharp displacement of the conformational equilibrium towards representatives of group *B*.

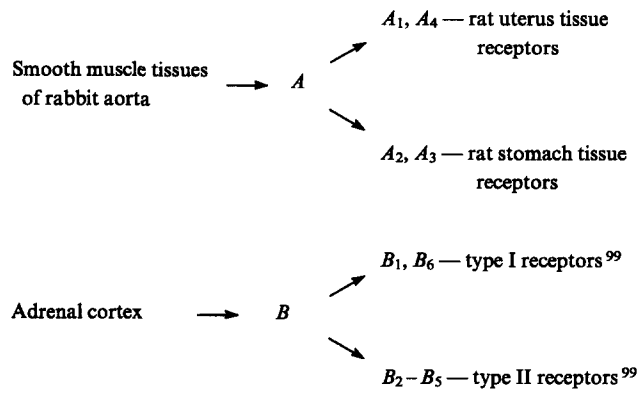
It has been reported[99] that, of the two types of receptors (I and II) isolated from the adrenal cortex tissue with the aid of the DUP-89 and WL-19 nonpeptide antagonists already mentioned, which bind the angiotensin II molecules equally effectively, only type II receptors[99] exhibit a high affinity for angiotensin III. On the other hand, the affinity of the heptapeptide hormone for type I receptors is reduced compared with the type II receptors by a factor of 12.

The effectiveness of the interaction of angiotensin III with the smooth muscle tissue receptors depends on the source and the organ. The affinity of the hormone for the receptors of the rat stomach tissue is lower by a factor of two than that of angiotensin II, while that for the receptors of the rabbit aorta tissue is reduced by a factor of almost 20.[45] The decrease in the affinity of angiotensin III for the receptors observed in these cases can be accounted for by the higher energy of the group *A* conformations responsible, according to our hypothesis, for the interaction of the hormone with the smooth muscle tissue receptors. The latter apparently have different requirements for the conformational state of the agonist. In this case, the A_1 and A_4 states can be assigned to the class of relevant states for the interaction of the hormone with the receptors of the rabbit aorta tissue, while the A_2 and A_3 states may be regarded as relevant to the interaction of the hormone with the rat stomach tissue receptors.

The high affinity of angiotensin III for the adrenal cortex receptors is consistent with the maximum probability of the occurrence of group *B* conformations, which permits the conclusion that these are relevant to the aldosterone-stimulating activity of the hormone. One can go further and postulate that the $B_4(B_5)$ and $B_2(B_3)$ conformations are complementary to the active centres of type II receptors,[99] while the B_1 and B_6 conformations are complementary to the active centres of type I receptors.[99]

[Asn^1]-AT II, another analogue of angiotensin II, is almost fully provided with all the functions of the [Asp^1]-AT II hormone under consideration. The interactions with rabbit aorta tissue receptors are an exception. The possible conformations of the [Asn^1]-AT II and [Asp^1]-AT II molecules are also very similar (Table 6), if one disregards the differences between the energies of the A_1 and A_4 conformations and hence between the probabilities of their occurrence, the probability being significantly smaller in the case of [Asn^1]-AT II. The affinity of this molecule for the rabbit aorta receptors is also appreciably reduced (Table 7). Thus the results of the conformational analysis and of the biological tests on this analogue agree with our conclusion that the A_1 and A_4 conformations are complementary to the rabbit aorta receptors.

Thus comparative analysis of the conformational properties and biological activities of the hormone and its analogues has shown that there is a relation between the biological activities and conformational states of angiotensin II, according to which the structural variants of group *A* interact with the receptors predominating in the smooth muscle tissues and those of group *B* interact with the receptors predominating in the adrenal cortex tissues, the representatives of each group being in their turn subdivided into subgroups according to the following scheme:



The classification of the conformations in terms of subgroups is consistent with a fully defined system of intramolecular contacts between atoms not linked by valence bonds.

In solving the direct problem for angiotensin II, it was established that the Arg^2 residue makes the greatest contribution to the stabilisation of the A_1, A_4, and B_2-B_5 conformations. The Asp^1 side chain is oriented towards the medium and interacts electrostatically only with the positively charged guanidine group of Arg^2; the energy of the interaction of Arg^2 with the rest of the molecule does not exceed 1 kcal mol^{-1} and is in addition destabilising in character.

Another system of intramolecular contacts obtains in the A_2, A_3, and B_1, B_6 conformations; the Arg^2 side chain is oriented in the direction away from the molecule and interacts only weakly with the other residues, while Asp^1 has numerous stabilising contacts.

The conformations of different subgroups differ also as regards the nature of the stabilising interactions. Whereas the A_2, A_3, and B_1, B_6 conformations are preferred as regards the energy of the nonvalence interactions between the atoms, the A_1, A_4 and B_2-B_5 conformations are preferred as regards electrostatic interactions and hydrogen bonds. The alternating responses of the conformations of both subgroups to the polarity of the medium combined with the ease of their interconversions makes the position of the conformational equilibrium in angiotensin II extremely sensitive to external conditions, primarily to specific interactions with receptors.

Some of the conformations of angiotensin II presented in Table 6 differ in the position of the C-terminal Phe^8 residue, which is of exceptional importance for the performance of the functions of the hormone. The elimination of this residue deprives angiotensin of virtually all its physiological activities. Thus [des-Phe^8]-AT II was found to be active only in relation to the rat brain tissues. The octapeptide analogues in which Phe^8 has been replaced by other aminoacid residues as a rule bind effectively to the hormone receptors but do not stimulate the corresponding signals.[45] The results of calculations for the [Ala^8]-, [Leu^8]-, and [Ile^8]-AT II analogues presented in Table 6 demonstrate relatively small changes in the possible conformations of these molecules compared with the natural peptide. On the other hand, it follows from Table 7 that none of them possesses the full spectrum of the biological activities of angiotensin II.

Hitherto we have considered the activities of the hormone and its analogues on the smooth muscle and adrenal cortex tissues. There is very little information about the interactions of angiotensin II with the receptors in other tissues. The known facts, concerning, for example, the receptors of the medulla of the

adrenal glands,[104] indicate that their structural requirements as regards the nature and steric structure of the C-terminal section of the aminoacid sequence are less rigorous. Thus [His^9-, Leu^{10}]-AT II, which is a precursor of the octapeptide hormone having a low affinity for the receptors in the smooth muscle and in the adrenal cortex, manifests the same activity as angiotensin II in the adrenal medulla. The synthetic analogue [Ala^8]-AT II, which behaves, as already mentioned, as an antagonist of angiotensin II in most tissues, nevertheless does not block the activity of the hormone in the medulla and actually exhibits some agonistic activity. Thus the lengthening of the peptide chain from the C- end and the replacement of Phe^8 by Ala^8 do not prevent in the given instance either the binding to the receptor or its stimulation.

VII. Conclusion

In conclusion we may note an important feature of the functioning of angiotensin II, which consists in the interdependence of all its numerous physiological activities in the mammalian organism. A number of factors, such as a decrease of the concentration of Na^+ ions in the blood, a decrease in the volume of circulating blood, and a fall in the arterial pressure lead to an increase in the secretion of renin by the kidneys and hence to an increase in the concentration of angiotensin II in mammalian blood.

The interaction of the hormone with the receptors located in different tissues induces responses designed to return the corresponding parameters to normal, the hormone acting at the tissue, systemic, and central levels. Thus the angiotensin II-induced increase in the tonus of the smooth musculature of the arterioles and the intensification of the secretion of catecholamines by the adrenal medulla increase the arterial pressure.

The retention of water and Na^+ ions in the organisms is effected by the hormone mainly via stimulating the secretion of aldosterone by the adrenal cortex, by the stimulation of the secretion of vasopressin by the hypothalamohypophyseal system, and also by the direct action of the hormone on the kidneys. The above activities of angiotensin II are supplemented by its effect on the central and peripheral divisions of the nervous system, which mediates the pressor effects and also intensifies thirst and the appetite for salt. This leads in its turn to an increase in the volume of the circulating blood and the normalisation of the pressure.

Thus the change in external conditions induces in the organism a complicated set of responses stimulated by a unique factor, namely angiotensin II, and directed towards a single aim — the maintenance of a constant internal medium within the organism.

The ability to effect productive interactions with a series of receptors at once and hence to perform simultaneously the corresponding number of coordinated functions is ensured by the structural-functional organisation of angiotensin II which the present author has investigated.

References

1. E M Popov *Mol. Biol.* **19** 1107 (1985)
2. E M Popov *Strukturnaya Organizatsiya Belkov* (Structural Organisation of Proteins) (Moscow: Nauka, 1989)
3. E M Popov *Bioorg. Khim.* **18** 1229 (1992)
4. P Glansdorf, I Prigogine *Thermodynamic Theory of Structure, Stability and Fluctuations* (Translated into Russian; Moscow: Mir, 1973)
5. I Prigogine *From Being to Becoming — Time and Complexity in Physical Sciences* (Translated into Russian; Moscow: Mir, 1985)
6. E M Popov *Priroda* (6) 59 (1993)
7. D B Wetlaufer *Proc. Natl. Acad. Sci. USA* **70** 697 (1973)
8. G N Tishchenko *Kristallokhimiya* (Crystal Chemistry) Vol. 13 (Moscow: Nauka, 1979)
9. E M Popov, G M Lipkind, S F Arkhipova, in *Konformatsionnye Raschety Slozhnykh Molekul* (Conformational Calculations for Complex Molecules) (Ed. A I Perel'man) (Minsk: Narodnaya Asveta, 1970) p. 120
10. E M Popov, V Z Pletnev, in *Konformatsionnye Raschety Slozhnykh Molekul* (Conformational Calculations for Complex Molecules) (Ed. A I Perel'man) (Minsk: Narodnaya Asveta, 1970) p. 129
11. E M Popov, V Z Pletnev *Biofizika* **16** 407 (1971)
12. V Z Pletnev, E M Popov *Izv. Akad. Nauk SSSR, Ser. Khim.* 991 (1970)
13. V Z Pletnev, E M Popov *Bioorg. Khim.* **1** 160 (1975)
14. V Z Pletnev, E M Popov *Khim. Prir. Soedin.* 220 (1973)
15. G N Tishchenko, Z Karimov, N V Nazimova *Bioorg. Khim.* **1** 375 (1975)
16. G N Tishchenko, Z Karimov, V V Borisov *Bioorg. Khim.* **1** 378 (1975)
17. G N Tishchenko, N V Nazimova, V I Andrianov, Z Karimov *Bioorg. Khim.* **1** 386 (1975)
18. G N Tishchenko, V I Smirnova, L N Zeibot, N V Nazimova, V I Andrianov *Bioorg. Khim.* **2** 885 (1976)
19. J Konnert, I L Karll *J. Am. Chem. Soc.* **91** 4888 (1969)
20. A I Geddes, D Akrigg *Acta Cristallogr., Sect. B* **32** 3164 (1976)
21. G N Tischenko, Z Karimov, B K Vainstein, A V Evstratov, V T Ivanov, Yu A Ovchinnikov *FEBS Lett.* **65** 615 (1976)
22. V Z Pletnev, N M Galitskii *Bioorg. Khim.* **3** 1611 (1977)
23. J A Hamilton, L K Stainrauf, E Braden *Biochem. Biophys. Res. Commun.* **64** 151 (1975)
24. E M Popov, V Z Pletnev, A V Evstratov, V T Ivanov, Yu A Ovchinnikov *Khim. Prir. Soedin.* 616 (1970)
25. Yu A Ovchinnikov, V T Ivanov, A V Evstratov, V F Bystrov, N D Abdullaev, E M Popov, G M Lipkind, S F Arkhipova, E S Efremov, M M Shemyakin *Biochem. Biophys. Res. Commun.* **37** 668 (1969)
26. S Z Galaktionov, G V Nikiforovich, G I Chipens, M D Shendorovich *Angiotenzin. Molekulyarnye Mekhanizmy Deistviya* (Angiotensin. Molecular Mechanisms of Action) (Riga: Zinatne, 1979)
27. V S Spasov, E M Popov *Bioorg. Khim.* **7** 25; 502 (1981)
28. E M Popov *Mol. Biol.* **9** 578 (1975)
29. E M Popov *Int. J. Quantum Chem.* (16) 707 (1979)
30. C B Anfinsen *Science* **181** 223 (1973)
31. E M Popov, P N Mel'nikov *Bioorg. Khim.* **5** 1471 (1979)
32. A L Lomize, E M Popov *Bioorg. Khim.* **8** 1474 (1982)
33. A L Lomize, E M Popov *Mol. Biol.* **17** 1212 (1983)
34. E M Popov, V N Shvyrkov, V Z Spasov *Bioorg. Khim.* **8** 61 (1982)
35. E M Popov, L I Izmailova, Sh M Musaev, R E Aliev, N A Akhmedov, I S Maksumov *Bioorg. Khim.* **8** 776 (1982)
36. A A Zaval'nyi, E M Popov *Mol. Biol.* **16** 129 (1982)
37. G I Chipens, L K Polevaya, N I Veretennikova, A Yu Krikis *Struktura i Funktsiya Nizkomolekulyarnykh Peptidov* (Structure and Function of Low-Molecular-Mass Peptides) (Riga: Zinatne, 1980)
38. M Monnier, L Dubler, R Cochter, P F Maier, H Tabler, G Schoenenberger *Experientia* **33** 548 (1977)
39. E M Popov, N N Sevast'yanova *Bioorg. Khim.* **7** 1478 (1981)
40. E M Popov *Mol. Biol.* **11** 5 (1977)
41. G M Lipkind, I S Maksumov, E M Popov *Bioorg. Khim.* **1** 966 (1975)
42. I S Maksumov, G M Lipkind, E M Popov *Bioorg. Khim.* **2** 632; 737 (1975)
43. E M Popov, N M Godzhaev, R E Aliev *Mol. Biol.* **20** 367 (1986)
44. E M Popov *Strukturno-Funktsional'naya Organizatsiya Belkov* (Structural-Functional Organisation of Proteins) (Moscow: Nauka, 1992)
45. D Regoli, W K Park, F Rioux *Pharm. Rev.* **26** 68 (1974)
46. M J Peach, J A Ackery *Fed. Proc.* **35** 2503 (1976)
47. D J Ramsay *Front. Neuroendocrinol.* **7** 263 (1982)
48. M T Schiavone, R A S Santos, K B Broshihan, M C Khosla *Proc. Natl. Acad. Sci. USA* **85** 4095 (1988)
49. A J Prange, C B Nemeroff, M A Lipton, in *Psychopharmacology: Generation of Progress* (New York: Wiley, 1978) p. 637
50. P J Harris, L G Navar *Am. J. Physiol.* **248** 621 (1985)
51. G P Brown, J G Douglas, J Krontiris-Litowitz *Endocrinology* **106** 1923 (1980)

52. F E Freedlander, T L Goodfriend *Fed. Proc.* **36** 481 (1977)
53. T B Rogees *J. Biol. Chem.* **259** 8108 (1984)
54. M J Peach, F M Bumpus, P A Khairalah *J. Pharmacol. Exp. Ther.* **176** 366 (1971)
55. K Arakawa, F M Bumpus *J. Am. Chem. Soc.* **83** 728 (1961)
56. A Y Ferreira, O G Hampe, A C M Paiva *Biochemistry* **8** 3483 (1969)
57. L Juliano, A C M Paiva *Biochemistry* **13** 2445 (1974)
58. J D Glickson, W D Cunningham, U G R Marshall *Biochemistry* **12** 3684 (1973)
59. R R Smeby, K Acha, F M Bumpus, M M Harsh *Biochim. Biophys. Acta* **58** 550 (1962)
60. S Fermandjian, P Fromageot, A M Tishenko, J P Leicham, M Lutz *Eur. J. Biochem.* **28** 174 (1972)
61. J M Matsoukas, G T Moore *Spectrosc. Lett.* **21** 477 (1988)
62. J M Matsoukas, G T Moore *Arch. Biochem. Biophys.* **248** 419 (1986)
63. M P Printz, H P Williams, L C Craig *Proc. Natl. Acad. Sci. USA* **69** 378 (1972)
64. E Nabedrick, C Thiery, P Calvest, S Fermandjian *FEBS Lett.* **58** 273 (1975)
65. L C Craig, E J Harfenist, A C Palladini *Biochemistry* **3** 764 (1964)
66. M T Franze de Fernandes, A E Delius, A C Paladini *Biochim. Biophys. Acta* **154** 223 (1968)
67. T B Paiva, A C Paiva, H A Scheraga *Biochemistry* **2** 1327 (1963)
68. R E Lenkinski, R L Stephens, N R Krishna *Biochim. Biophys. Acta* **667** 157 (1981)
69. H E Bleich, R E Galardy, M P Printz *J. Am. Chem. Soc.* **95** 2041 (1973)
70. G R Marshall *Biochemistry* **12** 19 (1973)
71. K Lintner, S Fermandjian, P Fromageot, M S Khosla, R R Smeby, F M Bumpus *FEBS Lett.* **56** 366 (1975)
72. S Fermandjian, J L Morgat, P Fromageot *Eur. J. Biochem.* **24** 252 (1971)
73. D Grefts, S Fermandjian, P Fromageot, M S Khosla, R R Smeby, F M Bumpus *Eur. J. Biochem.* **61** 297 (1976)
74. I P Sekatsis, Yu A Antsans, D A Bisenietse, G I Chipens *Izv. Akad. Nauk SSSR, Ser. Khim.* 478 (1988)
75. F M Bumpus *Fed. Proc.* **36** 2128 (1977)
76. I P Sekatsis, E E Liepin'sh, Yu A Antsans, A A Berga, G I Chipens *Bioorg. Khim.* **7** 971 (1981)
77. R Deslauriers, A C Paiva, K Schaumburg, J C P Smith *Biochemistry* **14** 878 (1975)
78. J D Glickson, J Dadok, G R Marshall *Biochemistry* **13** 11 (1974)
79. G R Marshall, H E Bosshard, W H Vine, J D Glickson *Nature (London), New Biol.* **245** 125 (1973)
80. J L De Coen, E Ralson *Biopolymers* **16** 1929 (1977)
81. G V Nikiforovich, S G Galaktionov, G I Chipens *Konformatsii Peptidnykh Bioregulyatorov* (Conformations of Peptide Bioregulators) (Moscow: Meditsina, 1983)
82. C Marchionini, B Maigzet, S Premilat *Biochem. Biophys. Res. Commun.* **112** 339 (1983)
83. G V Nikiforovich, B Vesterman, J Betins, L Podins *J. Biomol. Struct. Dynam.* **4** 1119 (1987)
84. T V Gogitidze, E M Popov *Bioorg. Khim.* **19** 517 (1993)
85. E M Popov *Int. J. Quantum Chem.* **16** 707 (1979)
86. N A Akhmedov, G A Akhverdieva, N M Godjaev, E M Popov *Int. J. Pept. Protein Res.* **27** 95 (1986)
87. E M Popov *Fiz. Mol. (Kiev)* **8** 69 (1980)
88. IUPAC-IUB Commission on Biochemical Nomenclature *Biochim. Biophys. Acta* **262** 1 (1971)
89. T V Gogitidze, E M Popov *Bioorg. Khim.* **19** 693 (1993)
90. B P Rowe, K L Grove, D L Saylor, R S Speth *Eur. J. Pharmacol.* **186** 339 (1990)
91. V Z Spasov, E M Popov *Bioorg. Khim.* **7** 502 (1981)
92. H Micami, T Ogihara, K Katahira, N Imai, J Kubahara, F M Bumpus, M C Khosla *J. Cardiovasc. Pharm.* **9** 242 (1987)
93. D A Biseniyatse, Yu E Antsas, M V Myshlyakova, G G Kubrik, E A Porushkevich, M I Ratkevich, G I Chipens *Bioorg. Khim.* **13** 149 (1987)
94. G J Trache, J A Ackery, M J Peach *J. Cardiovasc. Pharm.* **3** 838 (1980)
95. R B Howard, R R Smeby, in *Handbook of Hypertension* Vol. 8 (Eds W H Birkenhäger, J L Reid) (Amsterdam: Elsevier, 1986) p. 389
96. M A Ravi Kiron, R L Soffer *J. Biol. Chem.* **264** 4138 (1989)
97. M Tence, A Petip *Mol. Cell Endocrinol.* **63** 111 (1989)
98. A M Capponi, K J Catt *J. Biol. Chem.* **255** 12081 (1980)
99. R S L Chang, V J Lotti *Mol. Pharmacol.* **37** 347 (1990)
100. R S L Chang, V J Lotti, T B Chen, K A Faust *Biochem. Biophys. Res. Commun.* **171** 813 (1990)
101. R C Speth, K H Kim *Biochem. Biophys. Res. Commun.* **169** 997 (1990)
102. T V Gogitidze, E M Popov *Bioorg. Khim.* **19** 773 (1993)
103. S Salman, A Baucal, S Water, F M Bumpus, K J Catt *Endocrinology* **97** 275 (1975)
104. M J Peach *Circ. Res.* **28** 107 (1971)