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The Interaction of Metals with Proteins

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Various types of interactions in metal-protein systems are examined, the co-ordinate bonds between metals and proteins and their influence on various levels of organisation in protein molecules are specially considered, and examples of practical applications of metal-protein systems are presented.
The bibliography includes 132 references.

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I. INTRODUCTION

There is now a multiplicity of both novel experimental communications as well as reviews concerning various aspects of the interaction of proteins with metal cations (for example, Refs.1–10). However, most of the published studies deal with specific and comparatively narrow aspects of metal–protein interactions. For example, a number of experimental publications exist in the Soviet literature[11–13] reflecting purely structural aspects of the problem, but a distinct deficiency of review publications is felt.

Reitel[14] believes that a protein can be regarded as a system involving interactions characteristic of linear polypeptides containing between 100 and 1000 aminoacid residues. We think that one cannot limit the treatment merely to interactions of polypeptide chains with one another; one must take into account the entire set of interactions of polypeptide chains with one another, with the solvent, and with high-molecular-weight and low-molecular-weight components and the protein must be regarded as polypeptide chains in a system of interactions. One of the significant types of interaction is that with metal cations.

In the present review an attempt is made to follow a combined approach to the consideration of the interaction of proteins with metals against the background of other types of interactions and to assess the role of metals at various levels of the organisation of the protein. This approach has not so far been realised in published reviews; it can promote both a better understanding of the theoretical aspects of the functioning and properties of protein systems and the solution of practical problems which are important for medicine, biology, and biochemistry as well as a new branch of chemical industry—the technology of the isolation and processing of proteins.

II. BIOLOGICAL FUNCTIONS AND TYPES OF INTERACTIONS IN METAL–PROTEIN SYSTEMS

Historically the interest of investigators in metal–protein interactions has been associated primarily with the biological activity of metal ions[15,16]. In 1973 a collected volume of review articles was published under the title "Metal Ions in Biological Systems", in which biological, chemical, and medical aspects of the role of metals *in vivo* are discussed; they include (as a rule in relation to individual specific examples) also the problems of the interaction of metals with proteins (for example, the role of zinc in the functioning of enzymes[19], the importance of vanadium for the vital activity of plants and animals[20], and the role of copper in haemocyanine[21]).

Proteins incorporating a metal in their composition play an important role in biocatalysis and in the metabolism of all living organisms. These proteins have come to be divided into four groups[22,23]: enzymes, redox proteins, transport proteins, and storage proteins. The activity of many enzymes depends on the presence of cofactors, which frequently consist of metal ions; for example, the activities of arginase and carbonic anhydrase depend on the presence of manganese(II) and zinc(II) ions respectively[24].

Table 1. The role of metal ions in biological processes[17].

Metal	Form of location in organism	Strength of binding to protein	Function
Na, K	mobile	low	charge transfer, osmotic equilibrium, transmission of nerve pulse
Mg, Ca	partly mobile	moderate	formation of structures, "trigger" (initiating) reactions, hydrolysis, transfer of phosphate, regulation of cell functions
Mn, Fe Co, Cu, Mo	static	high	catalysis of oxidation-reduction reactions, formation of active centres in enzymes
Zn	static	high	"superacid" catalysts

The group of redox proteins, which are involved in oxidation-reduction processes whereby electrons are transferred in photosynthesis, in the fixation of nitrogen, in mitochondrial respiration, and in the introduction of an oxygen atom into the molecule or in the elimination of a hydride ion, consists of cytochromes and iron- and sulphur-containing proteins (ferredoxin, rubredoxin).[22] Transport proteins—transferrin, ceruloplasmin, iron–porphyrin oxygen-transferring agents (haemoglobin, myoglobin), non-haemin iron-proteins (haemerythrin), and copper-proteins (haemocyanine), and storage proteins (ferritin) regulate the concentration of metals and substrates in the organism, ensuring their presence at the sites where they are needed[25,26].

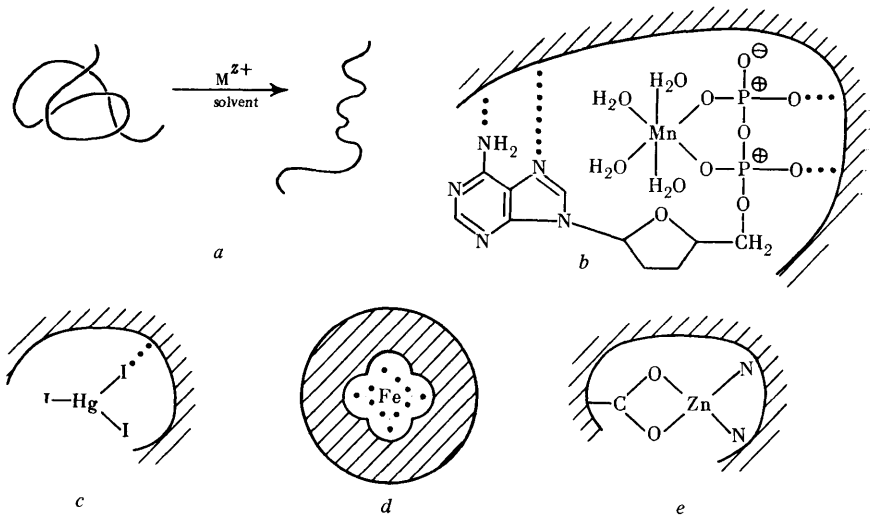


Figure 1. Classification of metal–protein interactions: *a*) indirect interaction via the solvent (lyotropic effect);[27,28] *b*) indirect interaction via an organic ligand[35–37]; *c*) indirect interaction with hydrophobic regions via an inorganic ligand[6,38]; *d*) supermolecular clathrate compounds[39–42]; *e*) coordination interactions with the functional groups of aminoacid residues[19,43].

It is also possible to differentiate a fifth group of metal-proteins—kidney proteins responsible for the binding and elimination of heavy metals from the organism, although so far in the literature they are not combined in a separate class and there is no complete information about their physiological role.

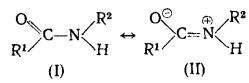
In Table 1 [17] an attempt is made to treat systematically the role of metals in biological processes. Evidently the biological activity of metals is not always determined directly by the strength of their interaction with the protein.

Various types of interactions occurring in metal–protein systems are presented below (Fig. 1). The suggested five types of interactions are fairly conventional and do not exhaust all theoretically possible cases. Nevertheless this scheme, which is not free from internal contradictions, permits a systematic treatment of most of the experimental data available at present.

The first type of interaction—mediated by the solvent—involves the interaction of metal ions and proteins (the lyotropic effect, Fig. 1*a*). Since an aqueous medium is most characteristic of natural systems, we shall consider the influence of metal ions on the protein in such media. The stability of the structure of such protein molecules in solution is usually determined by the hydrogen bonds formed by peptide groups, dipole–dipole interaction of polar groups, and hydrophobic interaction[27,28]. The lyotropic effect of metal ions is of a combined type and includes the following versions: interaction with charged groups, interaction with dipoles (including the peptide bond), and weakening of the hydrogen bond[29].

The interaction of metal salts with charged groups of the protein has a significant influence on the solubility of the latter, since association of the cationic and anionic groups on the surface of the protein with counterions leads to the neutralisation of these groups and weakens the electrostatic attraction between protein molecules. As a result, when salts in low concentrations are added, the solubility of the protein increases.

Proteins contain various readily polarisable functional groups capable of interacting with ions and consisting of dipoles. Most of these dipoles are formed by peptide bonds, which can exist in two *meso*-forms (I) and (II):



(I) (II)

By virtue of the double bond between the carbonyl carbon and the nitrogen in form (II), the rigidity of the structure, necessary for the arrangement of the polypeptide chains in native proteins, is ensured. The addition of metal ions can influence the contribution of mesomeric forms (I) and (II), can weaken the double bond character, and can promote free rotation[30].

Under the influence of metal ions, hydrogen bonds can weaken and even fully rupture if the affinity of the polar group for the ion is comparable to the affinity for its analogue in the hydrogen bond. The rupture of the hydrogen bond, which is an element of the native conformation of the protein, leads to the uncoiling of the peptide chain.

Hydrophobic interactions differ from all other lyophobic interactions by the fact that their energy relations are determined by two factors: firstly, the dispersion forces between the non-polar molecules in contact and, secondly, by the change in the structure of the solvent (water) on interaction with non-polar molecules. Contacts between non-polar molecules and water diminish the entropy as a result of the increase of the fraction of ordered elements in water. The minimisation of such contacts (the transfer of molecules to a non-polar environment or their association) is therefore accompanied by an increase of entropy and a shift of the system to a more favourable energy state[31].

The arrangement of the polypeptide chain in space is such that the hydrophobic aminoacids form the nucleus of the globules, while the hydrophilic aminoacids are distributed on its surface. As a result of electrostatic interaction, the metal ions exert a considerable influence on the structure of

water[32,33], altering the difference between the energies of the water aggregates on the hydrophobic surface of the protein molecule and disturbing the thermodynamic equilibrium in such a way that the aminoacid residue, previously oriented inwards, can become oriented outwards, i.e. the protein molecule with a compact arrangement can uncoil (become denatured). The uncoiling of the molecules results in their aggregation and the reduction of the solubility of the protein. Such increase of the energy of the interaction of protein molecules with one another and the so-called salting out usually occur at high metal salt concentrations in solution[34].

Interactions of the second type are also indirect (via the organic ligand) and occur in bridged complexes (Fig. 1*b*). Such binding is characteristic of many enzymes and transport proteins, including porphyrin-containing proteins[35-37]. In Fig. 1*b* this type of interaction is illustrated schematically for the binding of manganese(II) to creatine kinase via the nucleotide substrate and the formation of a ternary complex: manganese–adenosine diphosphate–creatine kinase[35].

Interactions of the third type are characterised by the addition of heavy metal ions to the protein molecule in hydrophobic pockets (Fig. 1*c*). The addition product is stabilised by van der Waals forces acting between the inorganic readily polarisable ligand and the hydrophobic region of the protein molecule[6,38]. Such addition is characteristic of mercury and gold iodides. In addition, there is a possibility of a direct interaction of certain metal cations with these regions, which is accounted for by the structure of the electron shell of these cations, responsible for their hydrophobic character and the extremely low affinity for water molecules. A high affinity for hydrophobic regions explains the accumulation of Hg^{2+}, Ag^{+}, and Cu^{+} ions in fatty tissues[6].

Interactions of the fourth type involve the formation of supermolecular clathrate compounds formed by storage proteins, a characteristic representative of which is ferritin (Fig. 1*d*). A ferritin species contains 24 identical protein subunits, within which is incorporated an inorganic nucleus approximately 73 Å in diameter containing up to 4500 iron(III) atoms in the form of a basic hydrogen phosphate gel[39-42]: $(FeOOH)_8(FeO{-}OPO_3H_2)$.

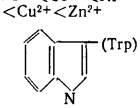
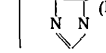
Finally, interactions of the last type, which have been most thoroughly investigated, involve the formation of coordinate bonds between the metal and the functional groups of the aminoacid residues in the polypeptide chain. In Fig. 1*e* these are illustrated schematically for the interaction of zinc with a glutamic acid residue and two histidine residues in the enzyme carboxypeptidase A.[19] Interactions of this type play the most significant role in the structure and functioning of metal–protein systems and must be considered in greatest detail.

III. COORDINATE BONDS BETWEEN METALS AND PROTEINS

Metal complexes with aminoacids and peptides have served for many years as model systems for the investigation of the reactions between metals and proteins. An exhaustive review of the literature up to 1968 was published in 1969;[44] in recent years a multiplicity of novel investigations of new metal complexes with aminoacids and of their practical applications have been published[45,46].

However, despite the extensive literature data, on the basis of results of the binding of metals to aminoacids, it is difficult to draw inferences about metal–protein compounds, since the binding of the metal to the functional groups of the protein differs significantly from its binding to the same groups in small peptides and aminoacids, where the cooperative nature of the interaction of the functional groups, which may have approached one another in the tertiary structure of the protein, is very important. On the other hand, the functional groups in the polypeptide chain are separated by tens of aminoacid residues and act comparatively independently of one another, obeying the geometrical requirements imposed by the structure of the protein. This steric structure can change on coordination of the metal ion, which complicates the study of this process still further. The active centres of enzymes are located as a rule in non-polar pockets within the protein structure where the metal ion is coordinated in fact under the conditions of a non-aqueous medium. Furthermore, the number of coordinating groups in the protein is greater than required to bind the metal, so that the metal ion has a "choice". The protein contains groups with unlike charges which can facilitate or hinder the approach of the cation to the potential ligand without being directly involved in complex formation.

Table 2. Classification of metals and ligands in terms of the HSAB theory[6,47-49].*

Type of compound	Class A (hard)	Intermediate	Class B (soft)
Acids	Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{3+}	$Fe^{2+}<Co^{2+}<Ni^{2+}<Cu^{2+}<Zn^{2+}$	Cu^{+}, Ag^{+}, Hg^{2+}, Pd^{2+}, Pt^{2+}
Bases	H_2O, $RCOO^{-}$ (Asp, Glu),	 (Trp),	R_2S (Met), RS^{-} (Cys),
	ROH (Ser, Thr), RNH_2 (Lys, Arg)	$-C_6H_4OH$ (Tyr)	 (His)

*Preferred coordination series: $RCOO^{-} > RNH_2 > RS^{-} > H_2O$ in class A and $RS^{-} > RNH_2 > ROH \gg H_2O$ in class B.

When one comes to consider the probable centres of the interaction of metals with the functional groups of the proteins, one usually begins with the concept of hard and soft acids and bases (HSAB, Table 2).[47-49]. In terms of this concept, all metal ions have acid properties and are divided into hard (class A) and soft (class B). Hard metal ions have a low polarisability as a consequence of their small radii and a high positive charge. They form the most stable complexes with donor atoms which have a high electronegativity, a low polarisability, and small radii and are difficult to oxidise, i.e. with hard bases (for example, N, O, or F). Soft metal ions have a low positive charge and are readily polarised and form the most stable complexes with readily oxidised and polarised ligands—soft bases (for example, S, P, or I). There exist also intermediate acids and bases exhibiting both hard and soft properties depending on the conditions.

In relation to protein systems, hard metal ions endeavour to combine with hydroxy- and carboxy-groups and water molecules. Coordination to sulphur-containing groups and the imidazole residue of histidine is preferred for soft metal ions. Since certain metal ions occupy an intermediate position in this classification, their softness or hardness depends on the bases surrounding them. The arrangement of the metal cations and ligands in the hardness–softness series makes it possible to predict qualitatively the relative strengths of the possible metal–protein coordinate bonds.

Table 3 presents the stability constants of the complexes of certain cations with aminoacids in model experiments and with aminoacid residues in peptides and proteins. Comparison of

the stability constants of metal complexes with aminoacids in Table 3 and the hardness series in Table 2 shows that the majority of cations behave as intermediate species even in the simplest model systems, while the experimental constants for peptide and even more so protein complexes are inconsistent either with the HSAB theory or with the stability constants of the aminoacid complexes. The quantities obtained experimentally in the study of metal–protein equilibria can be referred to as constants only as a matter of convention—the vast majority of the constants published in the literature are stoichiometric quantities calculated for individual complexes within a narrow range of concentrations of both the protein and the cation. The data presented in Tables 4 and 5 show that, in different concentration ranges and at different ionic strengths, different experimental constants can be obtained[57–59].

Table 3. The stability constants of metal complexes of aminoacids ($\lg K$) and aminoacid residues in peptides and proteins ($\lg K'$).*

Aminoacid	Fe^{3+} [51]	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
	lg K					
Glycine	—	7.8	8.9	11.0	15.4	9.3
Aspartic acid	11.4	8.5	10.7	12.7	15.4 [52]	10.4
Glutamic acid	12.1	—	8.1	10.3	14.4 [53]	3.5
Arginine	8.7	—	6.9	9.1	13.8	8.1
Cysteine	—	—	—	—	—	9.9 [55]
Methionine	9.1	—	—	—	—	—
Histidine	4.7	9.3	12.7	15.9	—	12.0
			lgK′			
Cysteine	—	—	—	—	—	7.0 [56]
Histidine	—	—	—	—	3.1—4.4 [54]	2.5 [55]

*Unless otherwise stated, the results were taken from Albert[59].

Note. The numbers in square brackets denote references (Ed.of Translation).

Table 4. The coordination binding of zinc(II) ions to bovine serum albumin.

pH	Medium	t, °C	lg K	References
5—9	0.15 *M* KCl	25	2.9	[57]
6.5	0.2 *M* $NaCH_3COO$	30	3.9	[58]
6.8	0.5 *M* $NaClO_4$	25	9.6	[59]

The scatter of the experimental quantities can be accounted for by many factors. The constant for the binding of a cation by a protein molecule depends on the change of the hardness of the metal in the course of its desolvation and coordination, the changes in the ionic strength of the system, the pH, and the size of the solvation shell, and the conformational changes in the protein. Apart from these factors, account must be taken of the fact that the change in the concentration of the metal ion in a protein solution leads to the binding of the metal at new sites and gives rise to the possibility of oligomerisation or even polymerisation of the protein species, which alters the stability constants. It is at present impossible to take into account all the factors enumerated above in the experimental determination of the binding constant. Thus the determination of the true constant for the binding of the cation to the protein or a specific fragment of the polypeptide chain is very difficult both experimentally and mathematically.

Formally the interaction with the protein can be represented by the equation[6]

$$pM^{z+}+qH^{+}+rA^{y-}=M_pH_qA_r^{(zp+q-ry)},$$

where p, q, and r are the numbers of metal ions (M^{2+}), protons, and protein molecules (A^{y-}), which form the complex. Here A^{y-} is the base of the polynuclear protein molecule in which all the dissociable protons have been split off.

Despite all the difficulties, significant advances have been made in the quantitative estimates for coordination interactions. As an example, one may quote a study[60] in which the coordination interaction of copper with ribonuclease was considered (Table 5); the sites where copper(II) is coordinated at the functional groups of the aminoacid residues of ribonuclease have been determined exactly.

Table 5. Coordination interaction of copper(II) with ribonuclease[60].

Ligands and their positions in primary structure	Degree of saturation of coordination centre, %		
	pH 5.5; 10^{-5} M $CuCl_2$	pH 5.5; 5 × 10^{-5} M $CuCl_2$	pH 7.0; 3.5 × 10^{-5} M $CuCl_2$
α-NH-R, Lys^{-1}; carboxyl, Lys^{-1}; γ-carboxyl, Glu^{49}; hydroxyl, Ser^{80}	37 ± 4	98 ± 8	24 ± 5
Imidazole, His^{119}; γ-carboxyl, Glu^{111}; H_2O bound to γ-carboxyl, Glu^{7}	28 ± 4	86 ± 7	33 ± 7
Hydroxyl, Ser^{21}, hydroxyl, Ser^{22}; hydroxyl, Ser^{23}; α-NH-R, Ser^{24}; guanidine, Arg^{39}	27 ± 4	72 ± 11	42 ± 5
Imidazole, His^{105}	14 ± 3	67 ± 8	29 ± 5
γ-Carboxyl, Glu^{86}	13 ± 4	44 ± 8	—
Imidazole, His^{119}, H_2O bound to γ-carboxyl, Asp^{121}	—	—	47 ± 6
Guanidine, Arg^{10}; hydroxyl, Ser^{50}; β-carboxyl, Asp^{53}	6 ± 3	22 ± 7	—

In the study of the interaction of zinc(II) ions with serum albumin (L) various workers observed and demonstrated the existence of several forms of the complex having the general formula Zn_pL_r: ZnL, Zn_2L, Zn_6L_2, $Zn_{16}L_4$ and $Zn_{26}L_6$.[57–59] The formation of $Zn_{16}L_4$ and $Zn_{26}L_6$ is accompanied by the formation of two new bridges comprising zinc(II) ions with a zinc–imidazole binding constant of approximately $10^{3.3}$.

It is of particular interest to note that, although fairly numerous cations can be bound by any protein molecule, there exist certain protein–cation pairs, exhibiting a specific affinity for one another, whose interaction constants, corresponding to certain physicochemical parameters of the medium, are higher by several orders of magnitude than the binding constants for other pairs. This applies primarily to the so-called "metal-dependent" enzymes and the cations participating in the formation of their active centres, for example, zinc(II) in alcohol dehydrogenase[61] and manganese(II) in pyruvate carboxylase[62]. Furthermore, there exist in nature proteins without enzymic activity and having a hitherto unelucidated biological function, which bind selectively certain cations: the Ag-binding protein in rat

liver[63], the Au-binding protein in rat kidneys[64], the Zn-binding factors in rat liver and muscles[65] and in cow's milk[66], and the Cd-binding proteins in the lymph of earthworms[67].

The most probable metal coordination centres in the polypeptide chain are presented in Table 6. The terminal amino-groups (No.1 in Table 6) are the most usual sites for the binding of metals despite their high pK, since the nitrogen atoms of the amino-groups are strongly electron-donating, while the oxygen atoms of the carboxy-group are separated from them by not more than four atoms and can form a chelate ring[50,68]. Coordination to the terminal amino-groups without chelate formation is a much rarer phenomenon and is characteristic of silver and platinum[69,70]. Carboxy-groups (No.2 in Table 6) either participate in chelate formation together with the terminal groups or interact with metals capable of monodentate coordination[19,71,72].

Table 6. Preferred centres for the coordination of metal ions in proteins.

No.	Functional group	Metal	Example of coordination	Refs.
1	Terminal amino-groups	Zn(II), Ag(I), Pt(II)	Pt with α-chymotrypsin	68
2	β,γ-Carboxy-groups (Asp, Glu)	Fe(II), Ag(I), Cu(II), Zn(II), Ca(II), Mn(II)	Zn(II) with Glu residue in carboxypeptidase A	19
	ditto	ditto	Mn with Asp and Glu residues in concanavalin A	69
3	Phenolic OH (Tyr)	—	$(UO_2)^{2+}$ with Tyr residue in tosyl elastase	73
	ditto	—	Fe with Tyr residue in haemerythrin	74
4	Imidazole (His)	Cu(II), Zn(II), Co(II), Fe(II), Ni(II), Cd(II)	Cu(II) with imidazole residue of haemocyanine	75,76
	ditto	ditto	Zn(II) with imidazole residue of insulin	91,23
5	Sulphydryl (Cys)	Ag(I), Hg(II), Mn(II) Fe(II), Co(II), Ni(II), Zn(II), Pb(II), Cu(II)	Cu(II) with Cys residue in "blue" copper-containing proteins	77
	ditto	ditto	Fe with Cys residue in rubredoxin	23
6	Thioester (Met)	Pd(II), Pt(II), Ag(I), Cu(I), Hg(II)	Pt(II) with Met residue in ferricytochrome *c*	80
	ditto	ditto	Cu(II) with Met residue in azurin	23
7	Peptide	Na(I), Ca(II), Co(II), Hg(II), Cu(II), Zn(II), Ni(II), Co(III), Pd(II), Pt(II)	Co(II) and Hg(II) with amide oxygen in biuret	82
	ditto	ditto	Zn(II) with glycylglycine	83

Coordination to metal ions is not very typical for the hydroxy-group of tyrosine (No.3 in Table 6) and the examples in Table 6 [73,74] are perhaps some of the few instances known at the present time. Serine and threonine are more often involved in coordination via the nitrogen atom of the amino-group and the carboxyl oxygen[50,58].

The nitrogen atoms of the imidazole group of histidine are the most important centres for the binding of metals (No.4 in Table 6). The coordination activity of this group can be explained by the fact that the nitrogen of the imidazole groups is an effective electron donor capable of forming stable covalent bonds at the physiological pH.[75,76] The sulphur atoms of methionine, cystine, and cysteine interact preferentially with soft cations (Nos.5 and 6 in Table 6). The sulphur atom is highly polarised in the field of metal ions and is a powerful electron donor. Either monodentate coordination only to the sulphur atom or bidentate complex formation with participation of a nitrogen or oxygen atom in addition to sulphur are usually encountered[77,78,80]. Coordination with participation of a bivalent sulphur atom may be complicated by the oxidation of sulphur to the quadrivalent state and the corresponding reduction of the metal ion[79].

The oxygen of the peptide group (No.7 in Table 6) has weakly basic properties and interacts with metals when chelate formation with participation of a neighbouring amino-group is possible. The oxygen of the peptide group interacts with alkali or alkaline earth metals and this interaction is apparently ionic[81]. The peptide nitrogen binds metals only when the process is accompanied by its deprotonation[84].

IV. THE EFFECT OF METALS ON VARIOUS LEVELS OF THE ORGANISATION OF THE PROTEIN

It may be regarded as firmly established that any local microchemical changes in the protein macromolecule (addition of a low-molecular-weight compound, addition or elimination of the metal, change in the valence of the added metal) [19,29,59,85–96] can lead to significant conformational and configurational rearrangements in the biopolymer. Up to the present time, there is no information about the inclusion in the polypeptide chain of the protein molecule of any fragments other than aminoacids. The question of whether one can assume that the coordination of the functional side groups of the aminoacid and of α-peptide groups to metal cations entails a change in the primary structure is more in the nature of a philosophical-methodological kind and its answer is outside the framework of this review.

The effects of metals on the secondary structure has been most fully studied in relation to collagen[29]. The structural unit of collagen is the cylindrical tropocollagen molecule which consists of three helically intertwined polypeptide chains. The stepwise (with a displacement corresponding to 1/4 of the length) parallel arrangement of the triple helices of tropocollagen ensures the construction of the collagen fibrils. The addition of metal ions to the system can lead either to the stabilisation of the native helical structure of the polypeptide chains of collagen or to their uncoiling and the denaturation of the protein. A series of cations are given below in order of their increasing denaturing effects on collagen[29]:

$$\underset{\text{helix}}{\longleftarrow} \qquad \underset{\text{coil}}{} \qquad WO_4^{2+} < Al^{3+} < NH_4^+ < Rb^+,\ K^+,\ Na^+,\ Cs^+ < Li^+ < Mg^{2+} < Ca^{2+} < Ba^{2+}.$$

The uncoiling of the polypeptide chains of collagen is associated with the increase in the number of groups bearing like charges in the protein under the influence of the metal cations. The repulsion of like charges leads to swelling, an increase in the volume of the molecule, and a rearrangement of the helical chain into a more ordered configuration (coil).

The above series of "dehelicising" activities of cations is valid only in relation to collagen; for other proteins, series of this kind have not yet been set up and may not correspond at all to that illustrated above, particularly since in the organisms of animals (*in vivo*) the coordination of collagen to calcium actually promotes the formation of helices and an increase of the rigidity of the supermolecular formations[85].

The dependence of the catalytic properties of enzymes on the presence of metal cations in solution is a sensitive indicator of the changes in the tertiary structure of the protein. In the case of "metal-independent" enzymes the change in catalytic activity may be caused by the effect of cations,

mediated by the solvent, which induces changes in the physicochemical parameters of the medium. This type of interaction was examined above in Section I. In the case of "metal-dependent" enzymes the change in catalytic activity is associated with configurational changes occurring as a result of the direct coordination of the metal to the protein. The change in configuration and the loss of catalytic activity occur either as a result of the removal of the metal from the active centre, of the competitive replacement of one cation by another, or as a result of the addition of a further metal ion even to one side of the active centre[19].

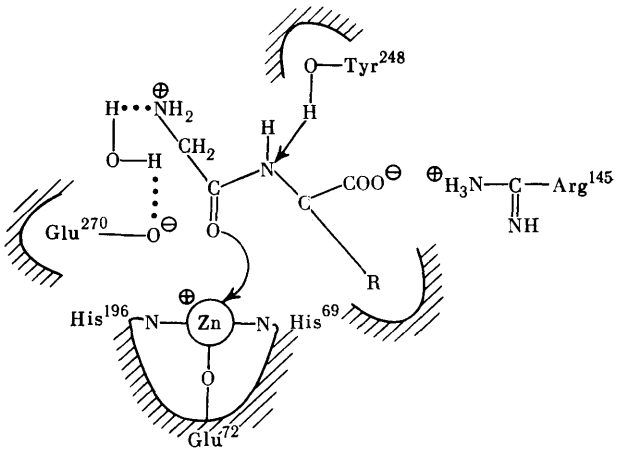


Figure 2. Orientation of the substrate on the surface of carboxypeptidase A.[87]

The optimum catalytic activity, i.e. the existence of the enzyme in the conformational state most favourable for the reaction, is ensured over a comparatively narrow range of concentrations of the added cations. For example, in carboxypeptidase A the Zn^{2+} ion is involved in the orientation of the substrate on the surface of the enzyme molecule (Fig. 2). The removal of the metal leads to a shift of the glutamine (Glu^{270}) and tyrosine residues, subsequent changes in the molecular configuration, and loss of activity[19,86]. An increase of the concentration of the zinc ions in solution is accompanied by the addition of further cations to the enzyme, uncoiling, and polymerisation of the protein molecules via hydrophobic interactions and the formation of salt bridges[59].

In the light of the foregoing considerations, "metal-independent" enzymes strictly speaking do not exist—all enzymes respond to the presence of cations in the system. The only difference is in the concentration of cations giving rise to this effect: Na, K, and Li act indirectly via the solvent and their activity is manifested at high concentrations; Zn, Cd, and Mn, which are coordinated directly to the protein molecule, are active in microamounts.

It is frequently impossible to draw a clear boundary between the changes in the secondary, tertiary, and higher-order structures, since the changes in one of the levels of the organisation of the protein molecule entail the rearrangement of the protein in all its structural levels. This can be well illustrated in relation to the oxygenation of haemoglobin[88,89]. Oxygenation begins with the addition of the O_2 molecule to one of the four haemoglobin sub-units with formation of the O–Fe bond, the iron atom of certain aminoacid residues being displaced from the initial position (Fig. 3). These changes are responsible for further transformations of the tertiary and quaternary structures. After the oxygenation of two sub-units, the molecule as a whole assumes the oxy-configuration, while the two remaining sub-units retain the previous tertiary structure and the latter is now strained, which subsequently facilitates their oxygenation.

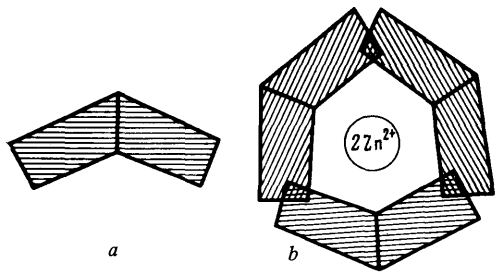


Figure 4. The interaction of insulin with zinc: *a*) dimer; *b*) hexamer.

The change in the quaternary structure has been studied most thoroughly for the interaction of proteins with zinc[90–92]. For example, in the case of insulin, which consists of a dimer comprising two polypeptide chains in solution, the addition of zinc ions results in the formation of a hexamer, in which three dimers are linked to two zinc ions. The coordination

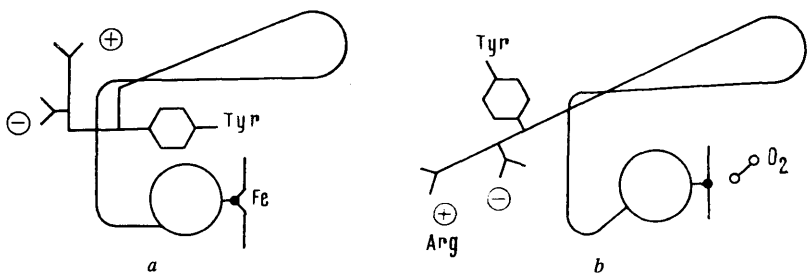


Figure 3. Change in the structure of haemoglobin in the oxygenation process[88]: *a*) deoxy-form; *b*) oxy-form.

of zinc to insulin alters the packing density of the polypeptide chains in the hexamer—they are virtually intertwined with one another (Fig. 4). An increase of the concentrations of the protein and zinc ions in solution increases the size of the aggregates.

In the general case the phenomenon of the aggregation of the protein under the influence of cations is characteristic of solutions of all proteins. The nature of the aggregation depends on the properties and concentration of the protein and the cations. It has been observed[93] that X-ray diffraction by metmyoglobin crystals begins to change when the Zn^{2+}:protein ratio becomes $\geq 3:1$. A further increase of the concentration of zinc ions leads to the disruption of the crystal and the appearance of a brown deposit of the insoluble zinc proteinate. Polymerisation and subsequent precipitation with increase of the concentration of zinc ions have been observed for bovine serum albumin[99]. The interaction of copper(II) ions with insulin also results in the formation of a polymeric protein complex[94].

Table 7. The types of possible steric structures in the aggregation of protein under the influence of cations: $pM^{z+} + rA^{y-} + qH^{+} \rightarrow M_pH_qA_r^{(pz+q-yr)}$.

Type of coordination	Metal	Protein	p	r	Structure
1	Zn^{2+}	Insulin	2	3	soluble species
2	Ca^{2+}, Mn^{2+}, Ni^{2+}	Actin	2	2	fibril formation
3	Ca^{2+}, Mg^{2+}	Casein	3	2	three-dimensional network (gel)
4	Fe^{3+}	Casein	3	1	coagulate
	Zn^{2+}	Metmyoglobin	4	1	coagulate

When actin reacts with calcium, manganese, strontium, and nickel ions, a linear polymer is formed; the interaction with zinc[95] and lanthanum[96] ions leads to non-linear polymerisation. The type of three-dimensional structure produced by the protein as a result of the formation of salt bridges between the protein species is determined by the number of cations which can be bound by the protein species and by the packing density of the species linked by salt bridges (Table 7 and Fig. 5).

When calcium ions are coordinated to actin, each protein species binds not more than two cations and can become aggregated via two salt bridges with not more than two other protein species—aggregates with a linear structure and soluble in water are formed (Table 7, type 2). When calcium ions are coordinated to casein, each casein micelle can bind several ions, which interact with the carboxyl and phosphate groups located on the surface of the caseine micelle. At a protein concentration of 12–15% and a calcuim ion concentration of 0.02–0.1 *N*, a continuous three-dimensional network of protein molecules is formed throughout the volume of the system (Table 7, type 3), which leads to gel formation[97,98]. A decrease of the protein concentration and an increase of the calcium ion concentration lead to closer packing of the protein species, the flocculation of the protein, and the formation of a deposit. Magnesium acts similarly[99]. Iron cations are coordinated to a much greater set of functional groups of the protein molecule than calcium and magnesium, so that the addition of iron to the protein solution gives rise to a closer packing of the protein species owing to a disordered aggregation (Table 7, type 4), which results in precipitation over a wide range of protein and metal concentrations[99].

V. PRACTICAL ASPECTS OF THE APPLICATIONS OF METAL–PROTEIN SYSTEMS

Historically, the practical applications of metal–protein interactions began long before the principal concepts of the chemistry of proteins were formulated. The traditional employment of metal–protein interactions for the conservation of food products and in the leather industry constitute a negligible proportion of the modern applications. Their use is developing most successfully in fields such as biotechnology, the food industry, medicine, and others.

The coordination interaction of polyvalent cations with collagen constitutes the basis of the processes involved in the tanning of leather and furs by aluminium and chromium salts[100–105]. The tanning of collagen-containing material by salts makes it possible to regulate its practically important properties such as resistance to moisture, elasticity, and mechanical strength. Treatment of leather and wool by fluorozirconates[106,107] increases the stability of the material without diminishing its elasticity.

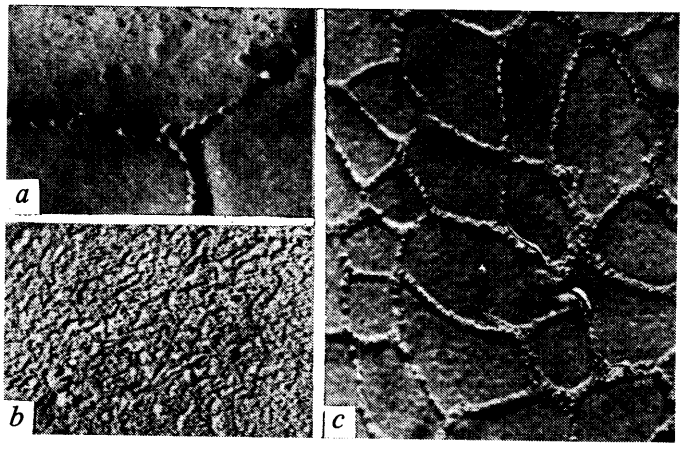


Figure 5. The aggregation of proteins under the influence of metal ions: *a*) fibril-formation; *b*) three-dimensional network (gel); *c*) coagulated material.

Regulation of the solubility of proteins as a result of interaction with salts has found extensive applications in the food industry, in the conversion of isolated protein mixtures into food products. The addition of magnesium and calcium salts to solutions of milk and plant proteins makes it possible to obtain structured systems imitating the fibrous structure of meat products[108,109] and gel-like systems[97,98]. Calcium salts are used to precipitate the lipoprotein complex (the cheese clot) in cheese making[110]. Aluminium salts are used to prepare artificial protein skins for sausages[111] and iron(III) salts serve to increase the mechanical strength of the skins on artificial protein caviare[112,113].

Bivalent and tervalent iron salts are used to precipitate food proteins from very dilute solutions[114–117], which is the most economical method of concentrating and isolating proteins. The converse process is also known—the elimination of heavy metal ions (mercury, copper, lead, tin, etc.) from water by coprecipitation with proteins[118].

Isomorphous complexes of proteins with heavy metals are widely used in electron microscopy[119] and in the X-ray diffraction analysis of proteins[120]. Selective complex formation with certain cations is employed in affinity chromatography for the fractionation and preparative isolation of pure proteins[121,122]. It may be hoped that communications concerning the application of rigorously selective complex formation to the converse problem—the isolation of certain cations from mixtures and dilute solutions—will appear in the immediate future.

Coloured protein complexes of metal cations are used not only for analytical purposes (the biuret reaction with copper salts) [123] but also for the preparation of food dyes (the red colour of the bivalent iron complexes of proteins in the presence of ascorbic acid).[124]

Sodium chloride, which is used traditionally to conserve food products, suppresses the activity of the majority of enzymes at high concentrations. At a certain concentration specific to each enzyme system sodium chloride can activate enzymes. This property has been used in biotechnology for the autolytic processing of the biomass of micro-organisms[125]. Calcium, magnesium, and zinc cations are used to induce the autolysis of micro-organisms[126]. The use of zinc is of greatest interest. The addition of its salts at low concentrations makes it possible to activate selectively enzymes in different groups—for example, one can activate nucleases, leaving proteases in an inactive state[127].

The application of metal–protein interactions in medicine is only just beginning. Quite recently reports have appeared of the detection of antibacterial activity in heavy metal complexes (silver, mercury, and gold) of plant proteins[128]. These studies are extremely promising for the creation of new medicinal preparations with a narrowly specific activity. Peroral administration of the coordination compounds of metal cations and proteins has been suggested for the treatment of disorders caused by the deficiency in the organism of microelements such as zinc, copper, iron, chromium, and manganese[129]. The most promising results have been obtained in the treatment of deficiency of iron[130] and zinc[65].

Supermolecular, colloidal metal–protein compounds, similar to natural clathrate compounds, are also used in medicine. Thus a microdisperse insoluble complex of Fe_3O_4 with serum albumin has been administered intravenously as a special contrasting substance for X-ray studies[131]. Analogous colloidal compounds of CaO and MgO with plant proteins are used to regulate the activity of gastric juice[132].

* * *

Many problems of the structure and functioning of metal–protein systems still await solution. Nevertheless practical necessity requires the employment of metal–protein interactions in various branches of industry, medicine, and science. Each year new applications are being discovered for these systems, while the theoretical problems are still not fully investigated and are hardly likely to be solved in the immediate future.

Most attractive are the prospects for the employment of metal–protein interactions in medicine—for the regulation of enzyme activity and the creation of new drugs with a narrowly specific activity, in metallurgy—for the concentration, isolation, and purification of rare and non-ferrous metals in complex mixtures and dilute solutions (sea water), and in the food industry.

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