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Specific Complex Formation in Aqueous Solutions Involving Mononucleotides and Oligonucleotides

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Data on complex formation between synthetic polynucleotides and complementary oligo(mono)nucleotides are reviewed and analysed. The principal methods for the investigation of complexes are examined and the limits of their applicability are estimated. Attention is concentrated on the complex formation conditions (temperature, ionic strength, pH, concentration, component ratio, etc.). The influence of the nature of the heterocyclic base and the sugar and of the length of the oligonucleotide chain on the stoichiometry and thermal stability of the complexes is discussed.
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I. INTRODUCTION

Numerous studies have been made in recent years on the interaction between complementary mononucleotides, oligonucleotides, and polynucleotides in connection with the expansion of research on the secondary structure of nucleic acids and on the relation between the structures of native macromolecules and their biological functions.

One of the approaches used to elucidate the causes and characteristics of the formation of involved native nucleotide complexes is based on the study of simpler synthetic systems. Recent studies have shown that such systems are convenient models for the investigation of many problems in molecular biology (codon–anticodon interactions[1], the problem of protein biosynthesis[2], DNA replication[3,4], etc.).

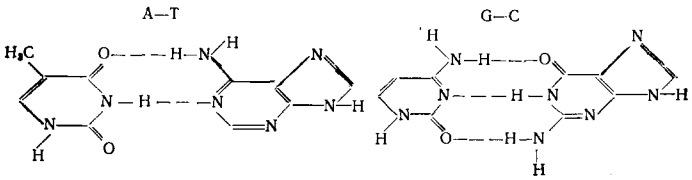
The study of the ability of mononucleotides, oligonucleotides, and polynucleotides to form DNA-like complexes in aqueous solution is also important from the standpoint of bio-organic chemistry in connection with the development of new approaches to non-enzymatic oligonucleotide synthesis on matrices[5-7].

Data published up to 1973 on specific interactions in simplest nucleotide systems are arranged systematically in the present review. Studies on monomer–polymer, oligomer–polymer and oligomer–oligomer complexes are considered, but problems of polynucleotide–polynucleotide interaction, which have been examined in a number of papers and monographs[8-10], as well as certain general problems such as theoretical consideration of the nature of the forces involved in complex formation[11-13] and the modern treatment of the "helix–coil" transition[14-16] are not discussed.

II. FUNDAMENTAL PRINCIPLES OF THE FORMATION OF OLIGOMER(MONOMER)–POLYMER AND OLIGOMER–OLIGOMER COMPLEXES

The capacity for the formation of specific intermolecular complexes in aqueous salt solutions is not characteristic solely of polynucleotides. It has been shown that short oligomers can also form under appropriate conditions ordered intermolecular structures with one another and with polymer molecules[17-25]. The formation of complexes by oligonucleotides and polynucleotides as well as native nucleic acid macromolecules is determined by three fundamental factors: the interaction of the heterocyclic bases (complementary and interplanar), the effect of the solvent, and the electrostatic effects of the phosphate groups.

Complementary (tranverse) interactions result in specific binding to one another of definite heterocyclic bases in adjacent polymeric (oligomeric) chains: adenine interacts with uracil (thymine) and guanine interacts with cytosine[26]:



The theoretical aspects of the problem of the stability of hydrogen-bonded pairs of bases are considered in the monograph of Kochetkov and Budovskii[10].

Interplanar longitudinal "stacking" interactions play an important role in creating a specific conformation of the mononucleotide, oligonucleotide, and polynucleotide chains involved in complex formation and ensure stability of the complex. By virtue of hydrophobic forces, the heterocyclic bases of each chain are arranged in a stack, in an attempt to avoid contact with water molecules; this is caused by the energy gain as a result of the entropy factor associated with the change in the number of structured water molecules around the dissolved substance when its molecules are associated to form a complex. Furthermore, the process is accompanied by a decrease in the surface tension energy due to the change in the surface area of the molecules on passing from the hydrated bases to their association complexes.

Using gel chromatography in conjunction with thermodynamic calculations, Bresler et al.[27] showed that the total

energy of the hydrogen bonds is much higher than the energy of the stacking interaction. On the other hand, it was believed previously that hydrogen bonds do not contribute significantly to the stabilisation of the complex[23].

Apart from the interaction between the bases and the influence of the solvent, the charges of the phosphate groups are an important factor. Whereas the binding of the heterocyclic bases to one another and to water molecules stabilises the complex, the repulsion of the negatively charged phosphate groups destabilises it.

A distinctive feature of the complexes considered in the present review is their marked dependence both on the overall concentration of the nucleotides and on the excess energy of the oligo(mono)nucleotide component in relation to the polymeric matrix[28]. A qualitative explanation of the latter has been given by Pitha and Ts'o[29] and by Uhlenbeck et al.[30] These workers showed that the local concentration of the monomeric units of two polynucleotides of comparable length in the interaction volume is fairly high. Under such conditions, the degree of complex formation is independent of the polymer concentration in solution.

On the other hand, when one of the chains forming the helix consists of mononucleotides or short oligonucleotides, the degree of complex formation is consistent with the law of mass action.

In an oligomer–polymer system the overall concentration of the oligonucleotide affects significantly its local concentration near the polymeric molecules and for this reason the degree of complex formation depends on the overall nucleotide concentration in accordance with the law of mass action.

In monomer–polymer systems the local concentration of the monomeric units is determined directly by the overall concentration of the monomer. Thus the concentration variation of the degree of complex formation in these systems should be most pronounced. The study of various oligonucleotide–polynucleotide complexes revealed a linear relation between $1/T\mathrm{m}$ and $\lg c$ (c is the concentration of the oligomer calculated in terms of the base and $T\mathrm{m}$ is the melting point)[28]. The slope of the experimental straight line depends on the length of the oligonucleotide chain. A general property of complementary complexes is the dependence of their stability on the ionic strength of the solution.

The contribution of electrostatic forces to the free energy of the "helix–coil" transition is specified by the equation[31]

$$\Delta E_{\mathrm{el}} = RT(C_1 - C_2 \lg I),$$

where C_1 and C_2 are empirical constants and I is the ionic strength of the solution.

A linear relation between the melting point and $\lg I$ has been found for polymer–polymer systems[31,32]. It has been shown that the melting point of the complex increases with ionic strength. The same behaviour is characteristic of the oligo A-poly U system[33]. The stability of monomer–polymer complexes depends particularly strongly on the concentration of salts.

The presence of Mg^{2+} ions stabilises appreciably oligomer(monomer)–polymer complexes[34]. For example, an increase of the Mg^{2+} concentration in the $(pA)_4$–poly U system by a factor of 4 (from 10^{-3} M to 4×10^{-3} M) increases the melting point of the complex by 6.5 deg C.[35]

The most important property common to specific intermolecular complexes formed from both short and long nucleotide chains, is the cooperative nature of the decomposition of such structures, which is reflected in a comparatively narrow range of temperatures separating the ordered complex from the disrupted structure. At present the problem of the cooperative nature of the "helix–coil" transition is being solved within the framework of theories in which this process is considered from the standpoint of statistical physics. The essential feature of the cooperative transition is that its different stages do not occur independently but are interrelated and that the disruption of even one section of the helix leads to rapidly progressing breakdown of the entire structure. The cause of cooperative fusion is the breakdown of the interplanar interaction between pairs of bases[36]. The extent of the cooperative transition can be different for different systems and is characterised by the cooperativity factor $\sigma = e^{-E/RT}$, where E is the free energy of the stacking interaction of two isolated pairs of bases linked by hydrogen bonds.

1. Methods for the Investigation of the Complexes

The formation of helical nucleotide complexes in solution is accompanied by a significant change in their properties compared with those of the free components—an increase in molecular weight and rigidity of the structures, an increase in optical activity, the occurrence of hyperchromic and hypochromic effects in the absorption region, etc. This makes it possible to record and investigate the complexes by a number of physicochemical methods: infrared and ultraviolet spectroscopy[5,37], optical rotatory dispersion and circular dichroism[38,39], sedimentation analysis[40], viscometry[34], proton magnetic resonance (^{1}H NMR),[41] calorimetry[42], etc. In studies on oligomer–oligomer and oligomer(mono)–polymer systems optical methods are most widely used and these will be considered in somewhat greater detail.

Ultraviolet spectroscopy. Under complex formation conditions, the ultraviolet absorption intensity changes relative to the overall absorption intensity of the individual components. Depending on the wavelength of the absorbed light, the molar extinction coefficient of the complex can then be smaller (hypochromic effect) or greater (hyperchromic effect) than the sum of the corresponding extinction coefficients of the components or it can be equal to this sum. In the region of the absorption maximum the absorption intensity usually decreases on formation of the complex (Fig. 1). In certain cases where the initial components have an intrinsic secondary structure the formation of the intermolecular complex is accompanied by an increase in absorption[43].

The hypochromic effect is characterised by the percentage of hypochromicity ($\%h$) and reflects the degree of the stacking interaction between the bases in the complex. The temperature variation of the absorption of the solutions at a specified wavelength is usually determined by ultraviolet spectroscopy and the "melting" curves are plotted[9]. A number of thermodynamic characteristics can be calculated from the variation of the thermal stability of the complex ($T\mathrm{m}$) with the chain length of the oligomers and their concentration[33].

In the interaction between homo-oligonucleotides and polynucleotides the wavelength at which the molar extinction coefficient of the complex is equal to the sum of the extinction coefficients of the components serves as a characteristic from which one can infer the approximate

stoichiometry of the complex. The relation between the isochromatic wavelength and the stoichiometry of the complex was established by Stevens and Felsenfeld[44]. Subsequently Blake et al.[45] showed that this characteristic wavelength is 280 nm for 1A.1U double helices and 283.5 nm for the three-chain complex 1A.2U.

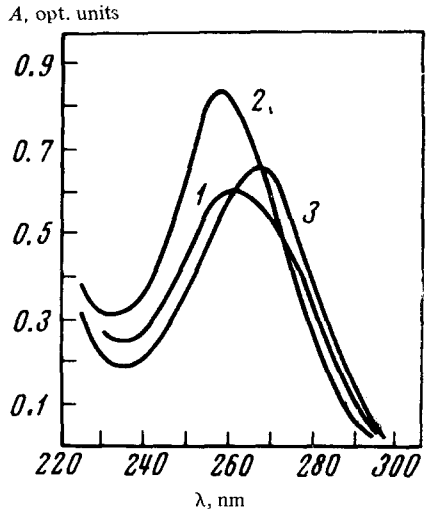


Figure 1. Ultraviolet spectra: 1) of the complex $A(pA)_7 \cdot d(pT)_8$; 2) of the component $A(pA)_7$; 3) of the component $d(pT)_8$ (9.4×10^{-6} M nucleotides, 1 M NaCl, pH 7.0, −5°C).[36]

Thus ultraviolet spectroscopy can yield all the necessary information about the interaction in the given system and its only disadvantage is that it is inconvenient for the investigation of solutions with a high concentration of nucleotides.

Optical rotatory dispersion (ORD) and circular dichroism (CD). In the formation of complexes of synthetic oligonucleotides and polynucleotides appreciable changes are observed in ORD and CD spectra: the shift of the 0–0 transition point to the short-wavelength region and the change in the amplitude of the Cotton effect[46–48]. The ORD or CD curve for the complex is usually compared with the overall curve obtained by adding together the curves for the individual components (taking into account their mole fractions in the complex) or with the ORD or CD curve for the mixture of the components at a temperature greater than the melting point of the complex. Useful information about complex formation is also provided by the "melting" curve reflecting the temperature variation of the molar ellipticity of the molar rotation measured at a specified wavelength. It is noteworthy that a hypsochromic shift of the ORD and CD spectra, a change in optical activity, and hypochromic effects in ultraviolet spectroscopy can be caused not only by the formation of specific complexes but also by the formation of various homoassociation.complexes. As a result, the experimental data for systems where a specific interaction is accompanied by the homoassociation of the components must be corrected[39].

Infrared spectroscopy. The stretching vibration wavenumbers of the NH_2, HN, and O=C< groups of the pyrimidine and purine bases change when these groups are involved in the formation of hydrogen bonds[49,50]. This is the basis of the method for the investigation of complex formation by infrared spectroscopy, whereby one can detect the occurrence of complementary interactions in the system.

The structure of the complex can be determined in two ways: (a) by comparing its infrared spectrum with that of a complex of known stoichiometry (the infrared spectra of polymer–polymer complexes with an appropriate nucleotide composition are usually employed as standards)[51]; (b) by measuring the variation of the intensity of the characteristic absorption band of the complex with mixture composition for a constant overall concentration of both components[10].

Infrared spectroscopy makes it possible to estimate quantitatively the degree of complex formation from the intensities of the bands appearing as a result of the interaction and the plot of this intensity against temperature constitutes the melting profile of the complex[52,53].

Infrared spectroscopy has many advantages over the methods discussed above. Since the infrared spectra of the individual components, their complexes, and self-aggregates as a rule differ from one another, this makes it possible to distinguish the interaction products and to infer the occurrence of non-specific interactions in this system[52]. An important advantage of the method is that it permits the employment of high nucleotide concentrations and complex formation can be studied under conditions which rule out the application of other optical methods. This is particularly valuable in the study of monomer–polymer systems where the concentration of the components plays a decisive role in the complex formation process[53]. A disadvantage of the method is the low intensity of the absorption bands arising as a result of the formation of hydrogen bonds.

2. The Stoichiometry of the Complexes

The most important characteristic of the complex is its stoichiometry. For synthetic homo-oligonucleotides and polynucleotides, there is a possibility of the formation, apart from the usual double helix, of a triple helix, the second polynucleotide (pyrimidine) chain being incorporated into the complex evidently via the formation of hydrogen bonds with the imidazole rings of the purine bases. Such structures are known for the A-U(T) pair in the crystalline state[54,55].

Studies in recent years showed that the stoichiometry of oligomer–polymer complexes is not determined unambiguously by the component ratio on mixing, but depends on a number of factors (temperature, ionic strength of the solution, bivalent metal ions, oligomer chain length, etc.).

The most common method for the determination of the stoichiometry of complexes involves the plotting of mixing curves (the so called Job method of continuous variations)[56]. For example, the ultraviolet absorption of mixtures containing different proportions of the reactants at a fixed overall concentration is measured and the optical density of the mixture is plotted against the mole fraction of one of the components. The extremum on the mixing curve indicates the stoichiometry of the complex. ORD,[57] CD,[48] and infrared spectroscopy[34] are also used to plot mixing curves.

The stoichiometric ratio of the bases interacting in the complex can be determined directly from the mole fraction of one of the components at which the extremum is observed:

$$r = \frac{(1-x)}{x},$$

where x is the mole fraction of one of the components and $1-x$ is the mole fraction of the other component.

When the extremum is broad, the stoichiometry of the complex is found by determining the point of intersection of the straight lines obtained by producing the linear sections of the mixing curve. However, when the curve is asymmetric (for example on formation of a three-chain complex) the extrapolation can lead to a gross error [58,59]. If self-aggregation of the components takes place in the system together with complex formation, additional terms taking into account the change in absorption and concentration due to self-aggregation, are introduced into Job's formula [47]. The calculation of the stoichiometric ratio is extremely difficult. In some cases it is possible to select the wavelength at which the self-aggregation process does not significantly influence the ultraviolet absorption of the system, whereupon the extremum on the mixing curve coincides with the maximum concentration of the complex, as in the absence of aggregation. For example, the stoichiometry of the complexes involving guanylic acid oligonucleotides is usually studied at 275 or 290 nm. [60]

The method based on mixing curves is used in the study of oligomer–oligomer and oligomer–polymer complexes. In the monomer–polymer interaction the complex formation depends very markedly on the concentration of the monomeric component, although in most cases there is no correlation between the component ratio in solution and the stoichiometry of the complexes [39]. The stoichiometry of the interaction is then determined directly by the ratio of the amount of monomer bound in the complex to the total amount of polymer in the solution (when the polymer molecules are completely bound in the complex). This ratio can be found by plotting the Ts'o isotherm [34] or the Scatchard diagram [61]. The interaction energy between nearest neighbours and the binding constant of the bases can be calculated from such graphs [62]. The stoichiometry of the complexes is also determined by viscometry, gel filtration [28], and equilibrium dialysis [63].

3. The Stability of the Complexes. Thermodynamic Parameters

The thermal stability of the complex is one of the main characteristics derivable directly from the experiment. Fig. 2 illustrates the thermal melting curve for the complex obtained by ultraviolet spectroscopy. If it is assumed that the optical density at a low temperature (below the plateau) corresponds to the completely helical structure and the optical density at the end of melting corresponds to the free components, then the proportion θ of the bases interacting in the complex can be calculated at any point in the transition range from the formula

$$\theta(t) = \frac{A_\infty - A_t}{A_\infty - A_0},$$

where A_∞ is the optical density of the solution of the fully denatured complex and A_0 is the optical density of the solution under optimal complex formation conditions.

The thermal stability of the complex is characterised by two parameters—the melting point (T_m) and the width of the transition range (ΔT_m) [64]. The melting point is the midpoint on the transition curve defined as the temperature at which the fractions of the helical and non-helical sections are equal, i.e. $\theta = 1/2$. For a given complex under constant external conditions, the melting point is constant. The width of the transition range is determined by the difference between the temperatures at which the tangent to the $(1-\theta)$–$t°$ fusion curve at the melting point intersects the lines corresponding to $1-\theta = 0$ and $1-\theta = 1$. ΔT_m reflects the cooperative nature of the transition, i.e. the degree of synchronisation of the breakdown of all the units of the helical structure with increase of temperature. For synthetic homopolynucleotides, the width of the transition range is related to the cooperativity factor σ as follows:

$$\Delta T_m = 12.4\, R \frac{T_m^2}{\Delta H} \sigma^{1/2},$$

where ΔH is the enthalpy of the transition.

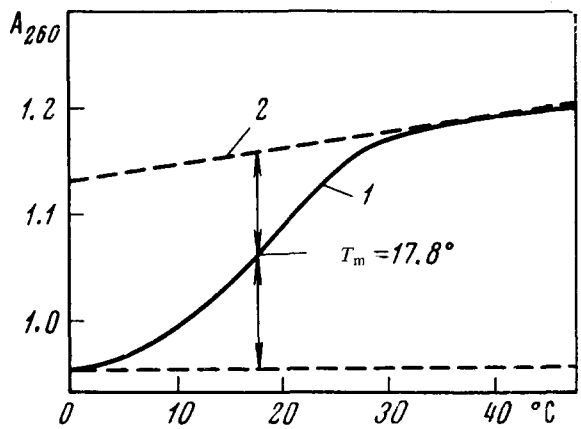


Figure 2. Temperature variation of the optical density A_{260} of the intermolecular complex $(Ap)_5U(pU)_4$ (1 M NaCl, pH 7.4): 1) experimental curve; 2) calculated absorption of the solution in the absence of interaction [63].

The theory of the cooperative "helix–coil" transition, developed in terms of modern statistical physics for polynucleotide models [65–67], is also applicable in the study of oligomer–oligomer and oligomer(monomer)–polymer interactions [68–72]. According to this theory, the first base pair formed in the oligomer–polymer (oligomer) interaction is much less stable than the subsequent pairs. The formation of each successive base pairs is associated with a specific stability constant, which rapidly approaches a limit in the transition range. It has been established that in most cases the differences between the stability constants of all the base pairs, other than the first, are insignificant and the cooperative transition can be described in terms of a model with two parameters.

Accordingly, the formation of a double oligomer–oligomer helix is represented schematically as follows:

$$A + \overset{\beta S}{B} \underset{k_{10}}{\overset{k_{01}}{\rightleftarrows}} D_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} D_2 \underset{k_{22}}{\overset{k_{22}}{\rightleftarrows}} \cdots \underset{k_{N(N-1)}}{\overset{k_{(N-1)N}}{\rightleftarrows}} D_N,$$

where S is the stability constant of the base pair, βS is the stability constant of the first base pair, and k_{ij} are the rate constants of the corresponding reactions.

where A and B are the interacting oligomers (oligomer and polymer), N is the length of the oligomer chain, D_i the partly helical structure with i pairs of bases, βS the stability constant of the first pair of bases, and S the stability constant of any of the succeeding pairs.

Statistical analysis of this scheme, taking into account the possibility of the existence of partly bound structures D_i, showed that β is extremely small ($\beta \ll 1$) and the constant S is very large ($S \gg 1$).[68-70] For relatively short oligomers, this makes it possible to neglect the concentrations of the partially bound structures $[D_i]$ and to treat complex formation in real systems in accordance with the "all or nothing" model:

$$A + B \overset{\beta S_N}{\rightleftarrows} D_N .$$

According to Applequist and Damle[68,69], this assumption does not introduce a significant error into the calculation of the thermodynamic parameters and ensures a simple quantitative relation between the thermodynamic parameters and the melting point, the slope of the fusion curve, length of the oligonucleotide, and its concentration in solution.

Table 1. Thermodynamic parameters of the "helix-coil" transition for a number of oligomer(monomer)–polymer systems[66].

Complex	Complex formation conditions	ΔH_1, kcal mole^{-1}	ΔS, kcal mole^{-1} deg^{-1}	β, mole^{-1}	Refs.
Poly U-adenosine (1A.2U)	D_2O; 0.15M NaCl, pD=7.0	−22.0	—	—	52
Poly U.2-amino-adenosine (1A.2U)	ditto	−22.2	—	—	52
Poly U-adenine (1A.2U)	»	−24.2	—	—	52
Poly U-2,6-diamino-purine (1A.2U)	»	−22.9	—	—	52
Poly C-5′-GMP (1G.2C)	D_2O; 0.2M NaCl, pD=7.0	−16.2	—	—	50
Poly U-oligo A (5′-phosphate)(1A.2U)	1M NaCl; 0.001M $MgCl_2$ pH 7.4	−14.4±3	−41.1±8	$0.1 \cdot 10^{1\pm1}$	35
Poly U-oligo A (3′-phosphate)(1A.2U)	ditto	−15.2±2.5	−43.7±7	$0.5 \cdot 10^{1\pm1}$	35
Poly U-oligo A (1A.2U)	0.1M NaCl; 0.01M $MgCl_2$, pH 7.0	−12.4±2.5	−38±7	$5 \cdot 10^{1\pm1}$	73
Poly U-oligo A (2′ → 5′)(1A.2U)	ditto	−11.6±3	−39±10	$1 \cdot 10^{2\pm1}$	73
Poly C-oligo G (1G.1C)	0.2M NaCl, pH 6.2	−7.0±3	−17.3±7	$1 \cdot 10^{1\pm0.5}$	74
Poly C-oligo I (1C.1I)	0.01M $MgCl_2$; pH 7.0	−6.2±0.3	−17.4		75

To simplify the calculations, it is assumed that ΔH_1 and ΔS_1 (the molar enthalpy and entropy of formation of a pair of bases), are the same for all the pairs and that β is independent of temperature. Then the enthalpy and entropy of the transition are proportional to the oligomer chain length:

$$\Delta H = N\Delta H_1, \quad \Delta S = N\Delta S_1 .$$

Damle[70] calculated a number of thermodynamic parameters for several oligomer(monomer)–polymer systems from the experimental data of other investigators. The results of these calculations are presented in Table 1.

A monomer–polymer system, for which thermodynamic calculations can be made even when the monomers are aggregated in solution, constitutes a real system most closely corresponding to the "all or nothing" model[71].

Later investigations of a number of oligomer–oligomer complexes showed that in real systems β is a function of temperature and the enthalpy of formation of the first base pair is significantly different from the molar enthalpy of formation of the subsequent pairs[36,71,76-78]. Thus, although the simplified theory of Applequist and Damle does in fact make it possible to estimate to a first approximation the thermodynamic characteristics of complex formation in the system as a whole, it does not reflect quite correctly the thermodynamics of the individual stages of this process.

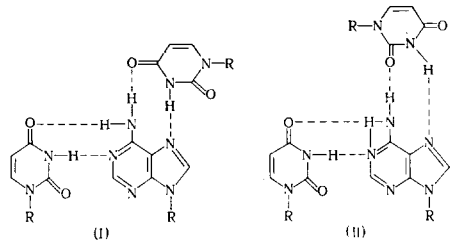
III. COMPLEXES OF MONOMERS WITH POLYNUCLEOTIDES

The formation of complexes of monomers with complementary polynucleotide with rigorous structural specificity has been observed for only one combination of components: a purine monomer and a pyrimidine polymer[34,39,41,50,53]. It has not so far been possible to obtain the corresponding complexes involving pyrimidine monomers[50]. This is probably because the smaller surface area of the pyrimidine base compared with that of the purine base leads to a smaller degree of their overlapping in the formation of a pseudopolymeric structure in the complex and therefore to a lower stabilisation of the complex via the stacking interactions of the covalently non-bonded monomers. For this reason, such complexes should be significantly less stable, if they are formed at all, than the complexes of purine monomers.

A characteristic feature of the monomer–polymer complexes is their marked dependence on the concentration of the monomeric component. The polymer concentration does not actually influence the stability of the complexes—a tenfold increase of the amount of poly U did not affect the stability of the poly U-2-aminoadenine system[63].

Stoichiometry of monomer–polymer complexes. Among the monomer–polymer systems, the interactions of adenosine or its derivatives with 2 poly U have been most widely investigated[34,50,78-80]. Whereas oligomer–polymer or polymer–polymer systems lead to the formation of double and triple helical structures[28], the formation of triple-stranded complexes consisting of two polynucleotide chains and one pseudopolymeric chain formed by the monomers is prefered in the monomer–polymer interaction. The stoichiometry of these complexes (1Pu-2Py) can probably be accounted for by the thermodynamics of the complex formation process. The formation of an ordered secondary structure is as a rule associated with a decrease of entropy which is unfavourable for the process. In this case the heat of reaction should provide the driving force for the process. According to the results of Ross and Scruggs[81], the formation of a triple-stranded complex involving the monomer is accompanied by a large entropy change (about −42 cal mole^{-1} deg^{-1}) compared with the entropy of formation of the triple polymer–polymer helix (about −32 cal mole^{-1} deg^{-1}). Evidently the heat of formation of the double-stranded complex is insufficient to compensate such a high entropy change and in a monomer–polymer system the addition of another chain, which ensures a further enthalpy gain, is necessary. The authors attribute the differences between the entropies of formation of monomer–polymer and polymer–polymer complexes to a greater degree of disorder in the monomer in solution compared with the rigid molecules of polynucleotides. Felsenfeld and Miles[53]

proposed two possible structures for the monomer–polymer complexes A.2U, differing in the disposition of the poly U chains: the chains are antiparallel and parallel in the first and second structures respectively (see below).



(I) (II)

Both structures satisfy the requirements of stoichiometry. However, the study of the infrared spectra of the complex A.2poly U showed that the poly U chains are linked to the adenine residues by different carbonyl oxygen atoms[37]: one of the chains forms pairs of hydrogen bonds via $-C_{(4)}=O$ and $N_{(3)}-H$ and the other via $-C_{(2)}=O$ and $N_{(3)}-H$. This finding supports structure (II), in which the second polynucleotide chain is linked to the adenine bases in accordance with the scheme of Haschemeyer and Sobel[55].

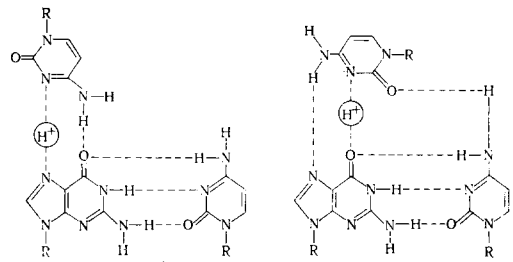
The structure with the antiparallel disposition of chains is also entirely likely for the monomer–polymer complex. In this complex the polynucleotide chains can belong to the same poly U molecule folded in two and are linked to one another via the adenine monomers.

On the basis of the results of sedimentation analysis, Huang and Ts'o[34] suggested the formation of a double-stranded complex of adenosine with poly U at 20°C. However, this conclusion was not confirmed by CD [79] and equilibrium dialysis[63] studies on the system. Damle[80] believes that the triple-stranded complexes 1A.2poly U constitute the only form of interaction between adenosine and poly U regardless of the concentrations of the components and temperature. It should also be noted that the infrared spectra and the melting curves obtained by Howard et al.[50] indicate the complete dissociation of the triple-stranded complex, $A.U_2 = A + 2U$, without the intermediate formation of the complex 1A.1U, The formation of the complex 1A.1U instead of 1A.2U should be reflected in a change in the infrared spectra—the appearance of an intense absorption band at 1672 cm^{-1}, while the 1657 cm^{-1} band is characteristic of the 1A.2U structure[53].

As a result of the presence of substituents in the purine base in the positions responsible for the binding of the second polynucleotide chain, the complex either does not form at all (for example in the case of tebericidin) or a very high concentration of the reactants is required. Thus Huang and Ts'o[34] failed to detect the formation of the complex of $N_{(6)}$-methyladenosine and poly U. On the other hand, Howard et al.[50] discovered the formation of a 1 : 1 complex of 9-methyl-6-methylaminopurine with poly U after increasing the concentration of the reactants.

The interaction of guanine monomers with poly C depends to a large extent on the pH of the solution. Thus at pH 7.8, where the protonation of poly C is impossible, guanosine 5'-phosphate forms the complex 1G.1C with poly C.[52] In the range pH ≤ 7, the complex has the structure 1G.2C, although in this case the formation of a triple-stranded helix requires the presence of the protonated form of poly C^+ at a fairly high pH. However, potentiometric titration has shown that the protonation of polycytidylic acid is facilitated in the present of guanosine, the pK of poly C being displaced towards the neutral region[39].

Among all the schemes proposed at present for the binding of 1G.2C,[74,79] most workers give preference to structure (A) (see below).



(A) (B)

One should note that melting of the triple-stranded complexes 1G.2C takes place in different ways in neutral and acid solutions[39]. At pH 6.4 the complex decomposes to the initial components as the temperature increases, while at pH 4.5, the guanine monomers are "melted out" and the double-stranded structure of poly C and poly C^+ remains. Such denaturation of the complex is more likely for structure (B), in which the parallel poly C and poly C^+ chains are linked. Evidently one can not rule out the possibility that different structures exist at different pH.

The study of the role of substituents in the heterocyclic ring of the purine component involves in most cases attempts to a quantitative estimation of the individual contributions of hydrogen bonds to the stabilisation of the complex. Evidently the contributions to the interaction energy due to other forces involved in the formation of the structure also vary on formation of a new hydrogen bond. For this reason, it is sometimes difficult to determine the main cause of stabilisation[42,50].

It has been observed that the introduction of substituents in the 1-, 6-, and 7-positions prevents the interaction of the monomer with the corresponding polynucleotide[34,39,82–84] Thus $N_{(1)}$-methyladenosine, where the introduction of the methyl group effects simultaneously the two sections responsible for the binding, is incapable of interacting with poly U.[42,50] Sarocchi et al.[39] also note that poly C does not form a complex when guanosine is replaced by 7-methylguanosine, 6-chlororibosylpurine, 6-thioguanosine and 2,6-diaminoribosylpurine.

Howard et al.[50] observed that complexes of 2-aminoadenosine and 2-aminoadenosine 5'-phosphate with poly U are more stable than the corresponding complexes of adenosine and 5'-AMP. The 2-amino-group participates in the formation of an additional hydrogen bond with one of the poly U chains, which results in the formation of a system resembling 1G.2C. The stoichiometric formula of the complexes of 2-aminoadenosine with poly U is 1A.2U.[63]

Substituents which do not prevent the formation of hydrogen bonds have hardly any influence on the capacity of the monomer for complex formation and on the stability of the complex. For example, the stability of complexes of poly U with various 3- and 9-alkyl-substituted adenines is close to that of complexes with unmodified adenine[34]. The formation of a complex of poly U with 8-aza-2,6-diaminopurine instead of adenine has been observed[50].

The sugar residue in the monomer molecule is not involved in the formation of the sugar phosphate skeleton which strengthens the chain, as happens in polynucleotides. For this reason, the replacement of the purine base by the appropriate nucleoside does not as a rule have a significant

influence on the stability of the complex, although Ts'o noted in a number of cases some stabilisation by the carbohydrate group. The nature of the sugar residue (ribose or deoxyribose) and its configuration (D- or L-ribose) is apparently unimportant at the level of monomer–polymer interactions[34]. A comparative study has been made of a number of systems of homopolymers and copolymers (poly AC, poly UC, poly UG) with monomers by equilibrium dialysis in combination with ultraviolet spectroscopy[63]. The authors established that the copolymer systems obey the same relations as the homopolymer systems. The employment of a mixture of monomers made it possible to compare the strength of the complementary interaction of individual monomers. For example, a study of the formation of complexes by poly UC with a mixture of dG and dA or dG and 2-aminoadenosine revealed a greater degree of binding in the second case.

Effect of the terminal phosphate group. Complexes of mononucleotides with polynucleotides have been least thoroughly investigated among the monomer–polymer systems, although they are of considerable interest from the biological point of view.

The first study in which mononucleotides were detected and investigated was carried out by Howard et al.[52] At high nucleotide concentrations (0.10–0.25 M) the presence of double- and triple-stranded structures, formed by guanylic acid mononucleotides (5'-GMP and 5'-dGMP) with polycytidylic acid, was established by infrared spectroscopy. It was noted that at pD 7.8 the infrared spectrum of the complexes is similar to that of the complex GpGpGp. .poly C, the stoichiometric formula of which is 1G.1C according to Lipsett[74]. At pD ≤ 7 new absorption bands appeared in the spectra of the mononucleotide–polynucleotide complexes, indicating the formation of triple-stranded helices containing the protonated form poly C^+.

Guanylic acid monomers, which are known to show a tendency towards self-aggregation and the formation of gels, sometimes precipitate from a nucleotide concentration of 0.01 M even in solutions at low ionic strength[39]. It is extremely likely that at this high nucleotide concentration the investigators observed the interaction between poly C and complex guanylic acid gels. Sarocchi et al.[39] investigated mixtures of guanylic acids with poly C in weakly acid dilute (overall nucleotide concentration 0.002 M) solutions. The formation of the complex 1G.2C noted under these conditions depends on the pH of the solution: complex formation was observed only at pH ≤ 6. In all probability this upper pH limit to the stability of the complexes is caused not only by the necessity for the protonation of poly C to form a triple-stranded complex but also by the need to suppress the dissociation of the secondary phosphate groups of the mononucleotide. The difference in the behaviour of the complexes of 5'-GMP and 3'(2')-GMP with poly C merits special attention: the latter complex is stable over a wider pH range. Guanylic acids with a phosphate group in the 5'- or 3'(2')-position are known to exhibit different tendencies towards self-aggregation and form gels of different structures[85–87]. However, the differences between their complexes with poly C have not so far been explained.

The study of Ts'o and Huang[40] occupies a special place among those dealing with the interaction of nucleoside mono-, di-, and tri-phosphates with polynucleotides. In solutions with a moderate concentration of the components in the presence of $MgCl_2$ the authors observed that the formation of complexes by purine nucleotides with the corresponding polynucleotides entails the precipitation of the complex. It was not possible to detect complexes in the supernatant. On the basis of these observations, the authors suggested that complex formation of mononucleotide–polynucleotide systems requires an additional driving force, which may be a phase transition. Complexes of monomer triphosphates proved to be less stable than complexes of monophosphates. Studies on the formation of complexes by cyclophosphates[88] showed that they give rise to more stable complexes with polymers than nucleotides. This can probably be explained by the decrease of the charge of the phosphate, which is particularly important for mononucleotides. The conditions governing the complex formation and the characteristics of a number of monomer–polymer complexes are given in Tables 2 and 3.

Table 2. The interaction of purine bases and nucleosides with polynucleotides.

Monomer	Polynucleotide	Conc., M: of monomer	Conc., M: of polymer	Complex formation conditions	Stoichiometry of complex	M.p. of complex, °C	References
Adenosine	Poly U	0.015	0.030	0.15*M* NaCl	1A·2U	18.5	50
ditto	ditto	0.030	0.015	0.6*M* NaCl, pH 6.9	1A·2U	—	42
»	»	0.015	$0.925\cdot10^{-2}$	0.1*M* NaCl	1A·2U (5°)	11	34
»	»	0.015	$0.925\cdot10^{-2}$	0.4*M* NaCl	1A·1U (20°)	18	34
»	»	0.015	$0.925\cdot10^{-2}$	0.02*M* $MgCl_2$		31	34
»	»	$0.5\cdot10^{-3}$	$3.6\cdot10^{-3}$	1*M* NaCl + 0.01*M* $MgCl_2$ + 0.01 M Na cacodylate; pH 6.97		—	80
»	»	$12\cdot10^{-3}$	$3.6\cdot10^{-3}$	0.1*M* NaCl + 0.01 M Na cacodylate; pH 6.97	1A·2U	—	80
D-Adenosine	»	0.015	0.030	0.15*M* NaCl	1A·2U	18.5	50
L-Adenosine	»	0.015	$0.925\cdot10^{-2}$	0.02*M* NaCl	1A·2U		34
2-Amino-adenosine	»	0.015	0.030	0.15*M* NaCl pH 6.9	1A·2U	31	42
ditto	»	0.034	0.030	0.125*M* NaCl, pH 6.9	1A·2U	36	42
»	»	0.030	0.015	0.6*M* NaCl, pH 6.9	1A·2U	47.7	42
8-Aza-2,6-diamino-purine	»	0.015	0.030	0.14*M* NaCl	1Pu·2U	—	50
Adenine	»	0.067	0.100	0.15*M* NaCl	1A·2U	24	50
2,6-Diamino purine	»	0.045	0.090	0.15*M* NaCl	1A·2U	44	50
Guanosine	Poly C	0.034	0.0688	0.125 M Na cacodylate; pH 6.9	1G·2C	36	50
ditto	ditto	$1.38\cdot10^{-3}$	$0.75\cdot10^{-3}$	0.2 M Na cacodylate; pH 6.7	1G·2C	—	39

Analysis of studies of the dependence of monomer–polymer systems on the monomer concentration leads to the conclusion that the interaction requires a definite minimum monomer concentration (the "lower threshold"), which depends on the nature of the monomer, the pH of the solution, ionic strength, and the presence of Mg^{2+} ions. On further increase of the monomer concentration, the thermal stability of the complexes rises, but, after the attainment of a concentration characteristic of the given conditions, saturation takes place, the degree of complex formation reaching a limit and not changing further. Graphically the process can be represented by the Ts'o isotherm[39]. According to Huang and Ts'o, the existence of the "lower concentration threshold" can be accounted for by the fact that the interaction of monomers with polymers is preceded by the self-association of the monomeric molecules in solution:

$$nM \rightleftarrows M_n,$$

where M is the monomer molecule and n the number of monomeric units in the aggregate ($n = 1, 2, 3, \ldots$).

The aggregates formed (stacks) then interact with the polymer to form the corresponding complexes. The formation of the stacks initiates the complex formation process and determines the minimum monomer concentration in solution.

Table 3. The interaction of nucleoside phosphates with polynucleotides.

Monomer	Polynucleotide	Concn., M: of monomer	Concn., M: of polymer	Complex formation conditions	Stoichiometry of complex	M.p. of complex, °C	Refs.
5′-AMP	Poly U	0.026	0.050	0.075*M* $MgCl_2$,	1A·2U	14	51
ditto	ditto	0.010	0.010	0.03*M* $MgCl_2$, pH 5.8	1A·2U complex in ppt		40
2-NH_2-5′-AMP	»	0.030	0.060	0.18*M* NaCl	1A·2U	11	50
5′-ATP	»	0.020	0.010	0.08*M* $MgCl_2$	1A·2U	11	51
5′-dATP (or 5′-ATP)	»	0.020	0.010	0.04*M* $MgCl_2$, pH 7.0	1A·2U complex in ppt		40
5′-GMP	Poly C	0.054	0.109	*pD* 7.0	1G·2C	~23	52
ditto	ditto	0.115	0.115	*pD* 7.8	1G·1C	~15	52
»	»	0.010	0.010	0.04*M* $MgCl_2$ pH 7.0	1G·1C complex in ppt		40
»	»	$0.16 \cdot 10^{-2}$	$0.1 \cdot 10^{-2}$	1*M* NaCl, pH 5.3–5.6	1G·2C		39
2′(3′)-GMP	»	$0.2 \cdot 10^{-2}$	$0.1 \cdot 10^{-2}$	1*M* NaCl, pH 3.0–6.0	1G·2C		39
5′-GTP	»	0.02	0.04	0.04*M* $MgCl_2$, *pD* 6.1	1G·2C	25	51
ditto	»	0.02	0.01	0.08*M* $MgCl_2$, pH 7.0	1G·1C complex in ppt		40
»	»	0.02	0.04	0.08*M* $MgCl_2$, pH 7.0	1G·2C complex in ppt		40

There exists also another point of view. Felsenfeld and Miles [53] believe that the self-aggregation of monomers must be regarded as a process competing with specific complex formation. The mutual orientations of the purines in the stacks and in an ordered complex are probably different, since in the former case the orientation is determined mainly by the hydrophobic interaction of the aromatic rings and in the second by the action of stereospecific forces. Since there is no rigorous mutual orientation of the monomers in the stacks (this is indicated by the ORD and CD spectra of the monomer solutions), the interaction of the stacks with the polynucleotide should involve their decomposition and the rearrangement of the monomers in accordance with the stereochemical requirements of the helix.

There is at present no reliable information about the involvement of the monomer–polymer complexes in natural biological processes. However, the inhibition of the peptide synthesis directed by the polynucleotide matrix in a cell-free system in the presence of complementary monomers, observed by Maxwell et al. [2], suggests that the monomer–polymer interactions can control the transfer of genetic information in the cell. Such control can occur at different levels of information transfer—in both transcription and translation stages.

IV. COMPLEXES OF OLIGONUCLEOTIDES WITH POLYNUCLEOTIDES

Oligomer–polymer complexes are more stable than the monomer–polymer complexes, which makes it possible to use them as convenient models for the investigation of complex formation. Oligomers containing not only purine bases (as happens in monomer–polymer interactions) but also pyrimidine bases [3,34,81,89] take part in the complex formation process. The structures and physical properties of the oligomer–polymer complexes are close to those of the polymer–polymer association complexes.

The stoichiometry of the A—U(T) complexes is not determined unambiguously by the component ratio in solution, but does depend on a number of factors, one of which is the nature of the oligonucleotide. Thus, according to Cassani and Bollum [28], at a moderate ionic strength the oligomers of thymidylic acid forms a complex 1T.1A with poly dA even in the presence of Mg^{2+}, while the structure 1A.2T is produced in the system of short dA oligomers and poly dT. The reason for the different stoichiometries is probably that in the 1Pu.2Py triple helix, formed with participation of oligo dA, there are breaks in the adenylic acid chain alone. In the triple-stranded complex oligo dT–poly dA, two chains of the helix consist of oligomers. Other conditions being equal, the stability of the latter complex should be much lower than that of the former [81].

The effect of the nature of the sugar residue on the structure of the polynucleotide chains and helices has been discussed in the literature for several years [90–95]. Marked differences have been observed between the CD spectra of the complexes poly-(A-A-U).poly-(A-U-U) and their deoxy-analogues, which may be due to the difference between their carbohydrate components [91].

It has been shown for polymer–polymer systems that the stability of the complexes of deoxypolynucleotides and mixed complexes is lower than that of the corresponding ribopolynucleotide systems [90]. The same behaviour is observed for oligomer–polymer complexes, the nature of the sugar residue of the polynucleotide component playing a particularly important role. The two-chain structure characteristic of the complex $d(pT)_{10}$.poly dA gives way to a triple-stranded structure when poly dA is replaced by polyrA (Fig. 3). [28]

Possibly the role of the 2′-OH group in the stabilisation of the triple-stranded helix is associated with the formation of additional hydrogen bonds in which the hydroxy-group can be involved as a proton acceptor [92] or donor [93]. Ts'o et al. [94] suggest the formation of intramolecular hydrogen bonds between 2′-OH and the $N_{(3)}$ atom of purine, the $O_{(2)}$ atom of pyrimidine, or the phosphate group. However it is still not clear how this can promote the introduction of a second polynucleotide chain into the complex.

A number of other investigators [90,92] suggested that the effect of the 2′-OH group on the structure and stabilisation of the helices is due to a change in the conformation of the sugar ring and the interaction of the ribose 2′-hydroxyl with solvent molecules. There is as yet no sufficiently convincing evidence in favour of a particular point of view.

By varying the chain length of the oligonucleotide in the system $d(pA)_m$–poly dT, it has been shown that there is a critical value of m (characteristic of the given ionic strength of the solution) at which the interaction of an equimolar mixture of the oligomer and a polymer begins to form a double-stranded complex [28]. The formation of

a triple-stranded helix is characteristic of all oligo dA whose length is smaller than this value, regardless of the component ratio in solution. The authors noted that, near the critical value of m-, an equilibrium is established in the equimolar mixture of the components between the double- and triple-stranded complexes: there is a two-stage melting curve, the optical density at 260 nm in the first fusion stage and the decrease of the optical density at 284 nm being due to the complex 1A.1T, while the optical density at 260 nm in the second fusion stage indicates the decomposition of the complex 1A.2T (Fig. 4). A true equilibrium is probably observed in the system: the ratio of the double- and triple-stranded complexes did not change even on incubating the solution (5°C) for three months.

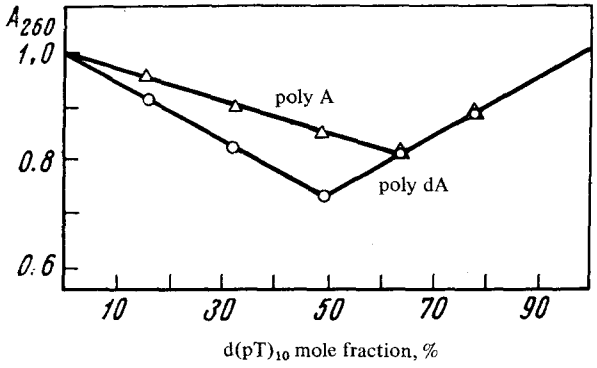


Figure 3. Mixing curves for complexes of $d(pT)_{10}$ with poly A and of $d(pT)_{10}$ with poly dA (1 M NaCl, pH 6.8, 23°C).[28]

As in the interaction of polynucleotides[96,97], an increase in ionic strength promotes the formation of triple-stranded oligomer–polymer helices. Thus Rich[89] observed a change in the stoichiometry of the two-helix oligo dT. .poly A complex and the formation of a triple-stranded structure with increase of the concentration of LiCl. Certain bivalent metal ions play an even more effective role in the stabilisation of triple-stranded complexes. For example, in order to obtain the complex 1A.2U in the system $(pA)_4$–poly U, the 1 M NaCl solution can be replaced by 0.001 M magnesium chloride solution[35]. The main function of the salts introduced into the solution is to shield the charged phosphate groups of oligo- and polynucleotides. The greater effectiveness of bivalent cations compared with univalent cations is a consequence, according to Michelson[9], of the differences between the dissociation constants of the corresponding metal phosphate salts. One cannot rule out the possibility that polyvalent cations can bind simultaneously the phosphate groups in different chains, which should also promote the formation of triple-stranded complexes.

The influence of ionic strength and bivalent metal ions on the interaction of polyanions such as oligo- and polynucleotides is very great. This factor sometimes plays a decisive role in the formation of the complex and with the given stoichiometry. Thus the interaction of oligo A with poly U in the presence of Mg^{2+} leads to the formation of the triple-stranded complex 1A.2U regardless of the component ratio in solution[98].

Some data on the stoichiometry of oligomer–polymer complexes formed with participation of the oligonucleotides of adenylic, deoxyadenylic, and thymidylic acids are presented in Table 4.

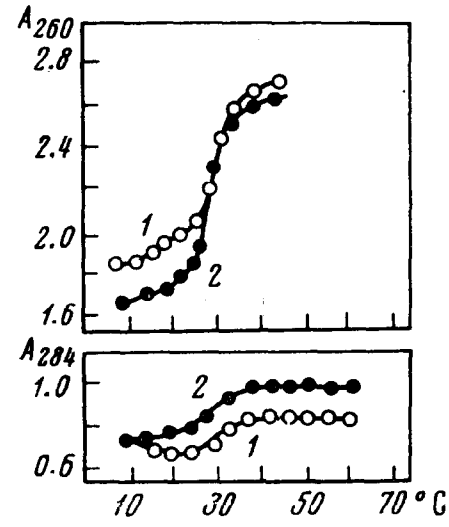


Figure 4. The melting of the structures formed by $d(pA)_8$ with poly dT for different component ratios in solution: 1) 1A–1T; 2) 1A–2T (0.15 M NaCl, pH 7.0).[28]

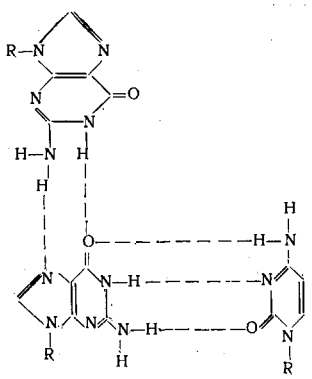
Table 4. Effect of various factors on the stoichiometry of A–U(T) complexes.

System	Component ratio in solution	Complex formation on conditions	Stoichiometry of complex	Refs.
$(pA)_m$—poly U	1A-2U	1*M* NaCl, 0.001*M* $MgCl_2$, 0.002*M*		
$m \geqslant 3$		phosph. buffer, pH 7.4	1A·2U	35
	1A-1U	ditto	1A·2U	35
	1A-2U	0.001*M* MgCl, phosph. buffer, pH 7.2	1A·2U	35
	1A-2U	0.1*M* NaCl, 0.01*M* $MgCl_2$, 0.05*M* cacodylate buffer, pH 7.0	1A·2U	99
	1A-1U	0.1*M* NaCl, 0.002*M* phosph. buffer, pH 7.4	1A·2U	35
$A(pA)_m$—poly U				
$m \geqslant 2$	1A-2U	0.5*M* NaCl, 0.04*M* phosph. buffer, pH 7.3	1A·2U	81
	1A-1U	0.1*M* NaCl, 0.002*M* phosph. buffer, pH 7.4	1A·1U	35
$m=1$	1A-1U	0.001*M* $MgCl_2$, 1*M* NaCl, 0.002*M* phosph. buffer, pH 7.4	1A·1U	35
$d(pA)_m$—poly dT	1A-2T	0.04*M* phosph. buffer, pH 7.0	1A·2T	28
$m \leqslant 12$	1A-1T	ditto	1A·2T	28
$m > 12$	1A-1T	»	1A·1T	28
$m = 4$–25	1A-1T	0.008*M* $MgCl_2$, 0.04*M* phosph. buffer, pH 7.0	1A·2T	28
$m \leqslant 16$	1A-1T	0.15*M* NaCl, 0.015*M* citrate buffer, pH 7.0	1A·2T	28
$m > 16$	1A-1T	ditto	1A·1T	28
$d(pT)_m$—poly dA	1A-1T	0.04*M* phosph. buffer, pH 7.0	1A·1T	28
	1A-2T	0.008*M* $MgCl_2$, 0.04*M* phosph. buffer, pH 7.0	1A·1T	28
$d(pT)_m$—poly A	1A-2T	1.0*M* LiCl, 0.01*M* cacodylate buffer, pH 6.8	1A·2T	28

Stoichiometry of G–C complexes. The study of the formation of complexes involving G–C pairs is made extremely difficult by the tendency of guanylyl derivatives to undergo self-association. Numerous studies[99–102] have shown that, under conditions favourable for complex

formation (low temperature, high ionic strength), polyguanylic acid exists in the form of stable aggregates. For this reason, it has not so far been possible to obtain complexes of poly G with cytidylyl oligonucleotides. With decrease of the degree of polymerisation, the tendency towards self-aggregation falls appreciably (but does not disappear altogether), which makes it possible to detect the G–C interaction in the systems oligo G.poly C.

The first data on the stoichiometry of such complexes were obtained by Lipsett[60,74], who investigated the formation of complexes by short guanylic acid oligomers (GpG, GpGp, and GpGpGp) with poly C. On the basis of mixing curves, she concluded that, depending on the temperature and the incubation time, these oligonucleotides can form two types of complexes in a neutral medium: 1G1C (see p. 56) and 2G.1C, in which the guanine bases are linked:



The author believes that the two-stage melting of the complex confirms this structure (Fig. 5).

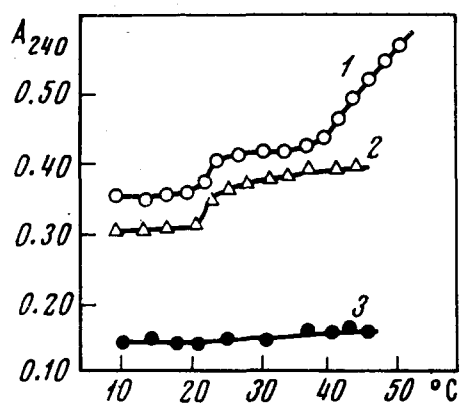


Figure 5. Melting curves: 1) for the complex GpGpGp. .poly C (2G.1C); 2) of the self-association complex GpGpGp; 3) of poly C (0.2 M NaCl, pH 6.2).[74]

However, the mixing curves, which do not have a distinct extremum, show that complex formation in the system is complicated by other, secondary processes, possibly the formation of the self-aggregate GpGpGp. One cannot rule out the possibility that the first stage in the melting profile (Fig. 5) is due to the melting of the self-aggregate GpGpGp, while the second reflects the decomposition of the double-stranded complex 1G.1C.

In the region pH < 6 the oligo G-poly C mixing curve has a distinct minimum at a cytidine mole fraction of 0.66, which indicates the presence of a triple-stranded complex 1G.2C.[74] The formation of a structure of this type was discussed earlier in connection with complexes of guanosine monomers with poly C and is associated with the protonation of polycytidylic acid. Possible schemes for the binding of the bases in the 1G.2C structure were presented above (p. 61).

Thus the results concerning the stoichiometry of oligomer–polymer complexes lead to the following conclusions. Complementary oligo- and poly-nucleotides with a monotonic sequence can form in aqueous solutions double- and triple-stranded helices. The stabilities of the complexes are not the same and are determined in many respects by the structure of the oligomer (the nature of the heterocyclic bases, chain length, etc.). However, with an appropriate choice of conditions (component ratio, pH, ionic strength), it is possible to obtain complexes having the required stoichiometry. In this connection we may recall that the formation of triple-stranded structures is promoted by the presence of purine groups in the oligomer, the presence of ribose residues in the polynucleotide chain, a high ionic strength, and the presence of Mg^{2+} ions. The formation of double-stranded complexes is as a rule observed at a low concentration of salts in the absence of bivalent metal ions. The pH of the solution plays an important role in the stoichiometry of the complex formed in the G–C interaction.

The stability of oligonucleotide–polynucleotide complexes. Thermodynamic parameters of the systems. Triple-stranded polynucleotide complexes can give rise to single-stage or two-stage melting depending on the ionic strength of the solution[90]. The effect of the concentration of the salts on the fusion curves for oligomer–polymer complexes was not specially studied. However, in most of the oligonucleotide–polynucleotide systems the thermal melting profiles are single-stage regardless of ionic strength[35,81,98]. This implies that triple-stranded complexes of oligomers with polymers dissociate, like double-stranded complexes, directly to the initial components without passing through a double-stranded intermediate.

The presence of G–C pairs stabilises the native DNA helices[103]. This is also characteristic of oligomer–polymer systems and is reflected by an increase in the melting points of the complexes containing the G–C pairs. Thus the melting point of the complex of GpG with poly C is 26°C, while that of the complex of ApA with poly U is only 6°C under analogous conditions[35,60].

The thermal stability of the complexes depends on which type of oligomer (purine or pyrimidine) is involved in complex formation. It has been observed that the complexes oligo Pu–poly Py are more stable than oligo Py–poly Pu. For example, the melting point of the complex $(pA)_8$.poly U is 52.8°C, while that of the complex $(pU)_8$. .poly A is 20°C.[104] The reason for the difference between the thermal stabilities of such structures is probably the greater strength in the case of the purine derivatives of the interplanar interactions stabilising the oligomers–polymer helices.

Lipsett et al.[35] showed that, under optimal complex formation conditions, the percentage of hypochromicity in the oligo A-poly U system is 35 for all oligomers, starting

with the trinucleotide, i.e. corresponds to the hypochromic effect of the formation of a triple-stranded helix poly A. .2poly U. Consequently the chain formed by adenylyl oligomers in the complex can be regarded as a continuous pseudopolymeric chain, in which the individual units (oligomers) are linked by vertical interactions.

The situation is different for pyrimidine oligonucleotides. The hypochromic effect observed in the formation of a complex of oligo dT with poly A is smaller than the hypochromic effect for the corresponding polymer–polymer complex. Rich and Tinoco[105] suggested that thymidylic acid oligomers interacting with a polynucleotide are not linked to one another and form independent short helices.

The values of the hypochromic effect for the complexes formed by oligo dT with poly A were calculated for oligomers of different lengths with the aid of the quantitative theory of the hypochromic effect developed by Rich and Tinoco[106].

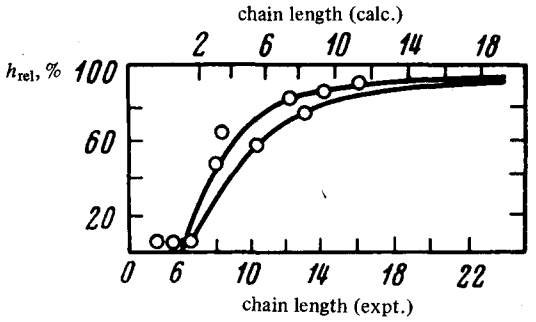


Figure 6. Variation of the hypochromic effect of the complex oligo dT.poly A as a function of the oligomer chain length at 262.5 nm ($h$rel, % is the percentage of hypochromicity calculated in relation to the hypochromicity of the complex poly A.poly U at pH 6.9).[105]

Fig. 6 shows calculated plots (continuous curve) of the relative hypochromic effect as a function of the length of the oligomer chain, the circles representing the experimental hypochromic effects. The experimental hypochromic effect for the heptamer corresponds to the theoretical value calculated for the dimer. This difference does not change with increase in the oligomer chain length up to the eicosanucleotide and makes it necessary to conclude that for any oligo dT 4–6 bases (probably 2–3 at each end) are not included in the stacking interaction, which probably results in a less ideal and stable structure than for the complex oligo A.2poly U.

With increase in the oligomer chain length, the stability of oligonucleotide–polynucleotide complexes rises[60,74,88,103]. A linear relation between the melting point and lg m (or $1/m$), where m is the oligomer chain length, has been established both theoretically[28] and experimentally[85]. The relation shows that the melting points of complexes of short oligomers differ very appreciably from one another, but for longer oligomers the differences between the melting points of the complexes become insignificant. The observed relation is consistent with the statistical-mechanical treatment of cooperative oligomer–polymer interactions described by Magee et al.[67] and Damle[70]. Michelson and Monny[99,104] studied the thermal stabilities of complexes as a function of the oligomer chain length (m) and established that, for a given series of oligomers, the plots of the reciprocals of the melting points against lg m are parallel, i.e. their slope is independent of the nature of the polynucleotide involved in the formation of the complex. It is determined by the structure of the oligomer (the nature of the heterocyclic bases, the presence of terminal phosphate groups, the type of internucleotide bond, etc.). There is a possibility of a break in the $1/T$m–lg m relation, indicating a change in the stoichiometry of the interaction. Indeed, since double- and triple-stranded complexes have different thermal stabilities, the slopes of the plots for these complexes should be different.

The system oligo I–poly C is convenient for the investigation of oligomer–polymer interactions[107]. The stoichiometry of the resulting complexes is always 1I.1C and their optical properties are similar to those of the poly I. .poly C systems[8,59]. However, the stability of the oligo I. .poly C complexes is much lower than that of oligo A.2poly U. Thus the complex ApA.2poly U is not difficult to obtain, while the complex IpI.poly C could not be detected and the minimum length of the inosine oligomers forming complexes with poly C was 5–6 monomeric units depending on the conditions[104].

The variation of the stability of the oligomer–polymer complexes with oligomer concentration, which decreases with increase in the oligomer chain length, has been determined. Thus the melting point of the complex A(pA)$_6$.2poly U decreases by 13 deg on 1000-fold dilution of the solution, while the melting point of the complex A(pA)$_4$.2poly U, containing a shorter oligoadenylate, decreases by 14 deg on 100-fold dilution[29]. Blake[33] confirmed this behaviour in a study of the stability of the complexes (Ap)$_4$.2poly U as a function of the tetramer concentration. Cassani and Bollum[28] noted an appreciable influence of the oligomer concentration on the width of the thermal transition ΔTm.

It was noted in a number of cases that the stability of the oligomer–polymer complexes depends on the nature of the sugar ring. The complexes of the ribo-series apparently have a higher thermal stability than those of the deoxyribo-series, which is largely consistent with the characteristics of the polymer–polymer interaction[95,108]. Knorre and coworkers[109] showed that, when the sugar residue of the terminal nucleoside in the oligomeric component is modified, the capacity of the latter for complex formation persists. Thus 2′,3′-*O*-benzylidene derivatives of oligoadenylates give rise to complexes whose properties are identical to those of the complexes of unmodified oligomers, the benzylidene group being displaced from the helix.

The available data about the influence of the terminal phosphate group on the stability of the complex are fairly contradictory. Uhlenbeck et al.[30], who investigated the thermal stabilities of the complexes formed by (Ap)mA and (Ap)m$_{+1}$ with poly U, noted the destabilising effect of the 3′-phosphomonoester group. Thus the introduction of a terminal 3′-phosphate into the tetramers and pentamers reduces the melting points of the complexes of these oligomers with poly U by 8 deg, which the authors believe can be explained by the increased electrostatic repulsion between the oligomers and the polymer chains in the presence of a terminal phosphomonoester group. A similar effect, decreasing appreciably with increase in chain length, has been observed for the system oligo G–poly C.[74]

However, according to Lipsett's data[35], only the terminal 5'-phosphate group has a destabilising effect, while the 3'-phosphomonoester group increases the thermal stability of the complex (Table 5). The author does not explain this fact satisfactorily. Presumably the presence of the phosphomonoester groups should destabilise the complex regardless of their positions in the oligomer chain and the degree of destabilisation can be different for the 3'- and 5'-phosphates, since these groups are known to have different effects on the secondary structures of the oligonucleotides themselves[110].

Table 5. Effect of the terminal phosphate group on the melting points of the complexes oligo A.2poly U (0.18×10^{-3} M nucleotide, 1 M NaCl, 0.001 M $MgCl_2$, pH 7.4).[35]

Position of terminal phosphate	Oligomer chain length			
	2	2	4	5
5'-Phosphomonoester group	7	17	29.5	37
3'-Phosphomonoester group	5	23	33.2	40.5
Without phosphomonoester group	6.2	17.4	31.3	

The formation of complexes by poly U with 5'-oligodeoxyadenylate amides, differing in the structure of the amide fragment and the length of the nucleotide chain, has been investigated by ultraviolet spectroscopy[111]. It was observed that the amide group does not alter the symmetry of the complex (1A.2U), but reduces somewhat its melting point. The destabilising influence of the amide group depends on its structure and decreases with increase in the oligomer chain length.

The nature of the internucleotide linkages in the oligomer molecule has a definite influence on the thermal stabilities of the complexes. It has been noted that the fusion of complexes of isomeric oligonucleotides with the 2'-5'-phosphodiester linkage in the systems oligo A–poly U and oligo G–poly C gives rise to a transition of the same width as complexes of the oligomers with the natural internucleotide linkage, but their melting points are somewhat lower[35,74].

The influence of the defects in the nucleotide sequence of the complementary oligomer on the stability of the oligonucleotide–polynucleotide complexes is of great interest. It has not been established unambiguously whether the non-complementary bases are incorporated into the helix or whether they form loops with breakdown of the stacking interaction between adjacent heterocyclic bases. A study of polynucleotide complexes revealed that the steric disposition of the non-complementary bases in the helix depends on their nature and number[30,112–115].

When the content of the non-complementary base is above a critical value, which depends on the type of system, a complex is not formed[114,115]. Only the frequency and length of the non-complementary sections of the chain are important[59], while the requirements which must be met by their positions in the chain as well as the steric requirements for the formation of complementary pairs at the ends of the helical section are less rigorous than within the section[82].

Uhlenbeck et al.[30] investigated the influence of terminal non-complementary bases in the oligomer on complex formation in the systems X(pA)m–poly U and (Ap)mX–poly U (X = C, U, or G) and reached the following conclusions.

1. The presence of a non-complementary base at one of the ends of the oligonucleotide lowers the stability of the complex.

2. The non-complementary base is displaced from the helix and the polymer is filled without gaps, for example in the interaction between poly U and the oligomers $C(Ap)_4$ and $(Ap)_4C$ the minimum on the mixing curve should occur at a uridine mole fraction of 0.667 if all the oligomer bases interact with the uracil residues, while the experimental curve has a minimum at a uridine mole fraction of 0.612. This means that the cytosine residues do not occupy any sites on the matrix.

3. The introduction of a cytosine residue in the middle of the oligomer has a weaker distabilising effect. The authors believe that this is largely due to the greater degree of solvation of the non-complementary cytosine ring, which has a higher dipole moment than the guanine and uracil rings.

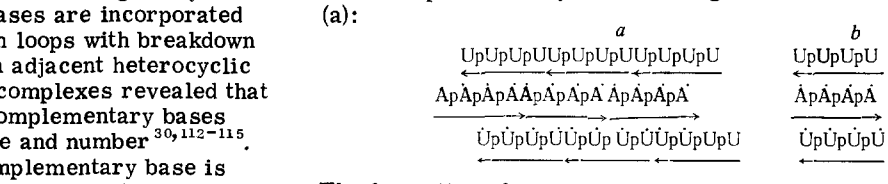
4. A non-complementary base at the 3'-end of the oligomer molecule destabilises the complex to a greater extent than the same base at the 5'-end.

V. INTERACTION OF COMPLEMENTARY OLIGONUCLEOTIDES

The study of complexes of complementary oligonucleotides is particularly important, since it yields information about the stability of short helices, which appear to be important structural elements in ribonucleic acids[53]. However, heterogeneous oligomers with a complex nucleotide sequence are so far almost inaccessible to study owing to the difficulty of their synthesis. Oligomeric complexes formed by oligonucleotides with a monotonic sequence have been most thoroughly studied. The kinetics of complex formation involving flow and relaxation (temperature jump) methods have been studied recently in systems of this kind[36,57,116–118].

Complexes of oligonucleotides with a monotonic nucleotide sequence are apparently not truly oligomeric. These are the so called "concatomers" having a structure similar to that of polymer–polymer complexes and differing from the latter only by the presence of breaks in the chains of the helix.

For example, the triple-stranded complex 1A.2U, formed by the tetranucleotides ApApApA and UpUpUpU, can be represented by the following concatomeric scheme (a):



The formation of such structures rather than dimeric or trimeric complexes is confirmed by the higher molecular weight of the complexes, greatly exceeding three times the molecular weight of the oligomer[48,81]. The formation of concatomeric structures in those cases where it is possible is probably thermodynamically more favourable than the formation of individual short helices of type *b*.

The stoichiometry of concatomeric complexes has been inadequately studied. It has been shown for the interaction of short oligo A oligomers with oligo U by CD, infrared, and ultraviolet spectroscopic methods[53,81] that the stoichiometric formula of these complexes is 1A.2U regardless of the component ratio and the ionic strength of the solution.

Pörschke[23] determined the critical oligomer chain length ($n = 7$) above which the formation of double complexes is possible. The author believes that for shorter oligomers ($n \leq 7$) both double and triple helices are formed.

On the basis of the ultraviolet spectrum of the complex oligo A.oligo dT, Hoggett and Maass[36] suggest the stoichiometric formula 1A.1T. Complexes of oligo G with oligo C have not so far been studied.

The thermal stabilities of the concatomeric complexes formed by adenylic acid oligomers with oligo U or oligo dT have been studied in fair detail[5,36,81]. The stability of such complexes greatly depends on the length of the oligomers and their concentration in solution. Thus the complex $A(pA)_3.3U(pU)_3$ (nucleotide concentration 0.12 M; 0.04 M $MgCl_2$; pH 7.0) melts at 17°C, while $A(pA)_5.2U(pU)_5$ has a melting point of 27°C under these conditions[53]. The shorter the oligomers the higher should be their concentration for complex formation to be appreciable. Thus the complex formation reaction between $A(pA)_7$ and $d(pT)_8$ takes place even at a nucleotide concentration of 8.5×10^{-6}, while the trinucleoside diphosphates ApApA and UpUpU interact only at a concentration of 0.12 M.[53]

The problem of the minimum oligomer size necessary for complex formation has been discussed in the literature for a long time[5,48,103]. Studies by Brahms et al.[119] showed that, at an overall nucleotide concentration of 10^{-4} M, the smallest oligomers are the heptamers. Felsenfeld and Miles[53], who increased the nucleotide concentration by a factor of 1000, demonstrated the existence of complexes of tri- and tetra-mers. The interaction between the complementary mono- and di-nucleotides in aqueous solutions has not so far been observed, although it does apparently occur in organic solvents (chloroform, dimethyl sulphoxide)[120,121].

Complex formation depends on the nature of the oligomers. Thus the formation of a complex in the system oligo dT–oligo dA at a nucleotide concentration of 0.001 M is observed starting with the pentamer of thymidylic acid and the tetramer of adenylic acid[5]. Maurizot et al.[122] observed a relation between complex formation and the structure of the sugar. Thus a comparative study by the CD method of the oligomer mixtures dA_n–dT_n and rA_r–rT_n ($n = 3$–8) in 4.7 M KF solutions at −20°C and a nucleotide concentration of 0.001 M established that deoxyribo-oligomers begin to form a complex starting with trimers, while their ribose analogues begin to form complexes starting with hexamers.

The effect of the ionic strength of the solution on complex formation in the majority of oligomer–oligomer systems has been studied as a rule at high ionic strengths (0.5–1.0 M NaCl) and in the presence of $MgCl_2$.[5,36,48,81] It was noted that, when oligo dT reacts with oligo A, the relation between the melting point and $\lg I$ (I is the ionic strength of the solution) deviates somewhat from linearity[36], in agreement with the behaviour of the system poly dT–poly A.

The formation of concatomers[108] is probably favourable also in this case, when the oligomers have an alternating nucleotide sequence. For example, oligo (CA) likewise forms overlapping structures with oligo (TC).[123]

However, when the oligomer has a self-complementary alternating sequence, intermolecular complex formation is not observed. Studies by Scheffler and coworkers[124,125] showed that the structure formed on folding the oligomer chain in two is more favourable for the oligonucleotide $d(TA)_m$. The melting point of the complex then also increases with the oligomer chain length, but is independent of the oligomer concentration.

Complexes of oligonucleotides with a non-monotonic nucleotide sequence were begun to be studied only in the last few years when considerable advances were made in chemical methods for the synthesis of oligomers with a specified sequence[126]. It was established that the interaction of non-monotonic oligonucleotides, uncomplicated by self-aggregation, involves the binding of two (or three) molecules of complementary oligomers with formation of short oligomer–oligomer helices[48,63,127,128]. In this case the general rules are approximately the same as for the formation of concatomeric structures.

The thermal stabilities of a number of complexes of nucleotides with a fairly wide variety of compositions were studied by Khorana and coworkers[127,128], who established that in the presence of Mg^{2+} there is a possibility of complex formation involving relatively short oligonucleotide blocks. Thus complexes of tetranucleotides with deca- and eicosa-nucleotides were obtained at a low temperature (0–5°C).[123] It was noted that, with increase of the content of G–C pairs, the oligomer–oligomer helices are stabilised. A similar behaviour was discovered also in a study by Martin et al.[129]

The attempt to obtain shorter oligomer–oligomer helices was unsuccessful. Jaskunas et al.[47], who investigated by the ORD method a large number of systems consisting of complementary trinucleoside diphosphates, found complexes only for the GpGpG–GpCpC pair (at a nucleotide concentration of 0.01 M in the presence of 0.001 M $MgCl_2$ at pH 7 and 1°C). However, the complex formation process is complicated so much by the self-aggregation of the trimers GpGpC that it is impossible to infer the stoichiometry of the interaction.

If the oligomer chain length is equivalent to 8–10 nucleotides, it is possible to obtain fairly stable complexes also from oligomers without guanylic and cytidylic acids[116]. Martin et al.[129] studied the capacity for complex formation of the self-complementary oligonucleotides $(Ap)_mU(pU)_{n-1}$, in which m and n were varied from 3 to 7. The marked concentration variation of the degree of complex formation for such oligomers suggests the formation of intermolecular complexes and rules out the possibility of intramolecular interactions. It was established that the structure of the complex formed is determined by the ratio A/U in the oligomer chain. A double helix is formed for $m \geq n$ but, when $m < n$, the results of sedimentation analysis and the ultraviolet spectra indicate the formation of larger aggregates, probably containing triple-stranded sections. For identical lengths of the A and U blocks in the oligomer ($m = n$), the melting point of the complex increases appreciably with chain length $N = m + n$ (Table 6).

The enthalpies ΔH_1 quoted in Table 6 refer to the formation of 1 mole of A–U pairs and were calculated from the slopes of the concentration variation of the melting point of the complex. The absolute value of ΔH_1 increases with increase in chain length. This deviation from Applequist's ideal model[68], for which ΔH_1 is independent of the oligomer chain length and the overall heat of reaction is defined by the expression $\Delta H_N = N\Delta h_1$, is not surprising, since it is clear that the contributions to the stabilisation of the helix are by no means the same for all the base pairs. Hoggett and Maass[36] showed that, in the interaction of oligo T with oligo A, the enthalpy of formation of the first base pair $\Delta H_\beta S$ is positive although low. The enthalpies of formation of terminal base pairs can also differ significantly from the enthalpies of formation of the base pairs within the oligomer chain.

The stabilities of the oligomer–oligomer complexes having the same length and nucleotide composition but different nucleotide sequences are evidently not the same. Indeed, the oligonucleotides $(Ap)_6C$ and $G(pU)_6$ form a more stable complex (m.p. 25°C) than $C(pA)_6$ and $(Up)_6G$ (m.p. 19°C),[48] which may be due to a difference between the stacking interactions of the bases in the isomeric structures[130]. Differences have also been observed in the stabilities of the series of complexes $AnCG.CGU_n$ (n = 6 or 8), $AnG_2.C_2U_n$ (n = 4 or 5), and $AnG_3.C_3U_n$ (n = 4 or 5) differing in the positions of the G–C pairs, which the authors attribute to differences in the entropy changes[131].

Table 6. Certain characteristics of the complexes formed by the oligonucleotides $(Ap)mU(pU)_{n-1}$ (10^{-4} M nucleotide, 1 M NaCl, pH 7.0).[129]

Oligonucleotide	$N=(m+n)$	$-N\Delta H_1$, kcal mole^{-1}	$-\Delta H_1$, kcal mole^{-1}	M.p., °C
$(Ap)_3U(pU)_2$	6	—	—	—13
$(Ap)_4U(pU)_3$	8	51	6.4	12
$(Ap)_5U(pU)_4$	10	72	7.2	23
$(Ap)_6U(pU)_5$	12	85	7.1	32
$(Ap)_6G(pU)_4\cdot(Ap)_4C(pU)_5$	14	107	7.6	40
	10	—	—	29*
				33**
$(Ap)_4CpG(pU)_4$	10	76	—	42.6

*The melting point of the complex calculated for a nucleotide concentration of 0.5×10^{-4} M.
**ditto for a nucleotide concentration of 2×10^{-4} M.

The introduction of a non-complementary nucleotide into the oligomer molecule hinders the formation of a complex and lowers its stability. Thus a study has been made of the capacity for interaction of the two oligomers $(Ap)mC(pU)n$ and $(Ap)mG(pU)n$, but in separate solutions and not in a mixture as in the previous study[132]. The cytidine and guanosine monomers disturb the self-complementarity of these oligomers and the destabilisations of the complexes due to them are not the same. The oligomer containing cytidylic acid forms a less stable intermolecular complex than the oligomer with guanylic acid (Fig. 7).

For comparison, the same figure presents the melting profile for the complex formed by $(Ap)_5U(pU)_4$ under analogous conditions. Analysis of the melting points of these complexes for different m and n showed that the introduction of the cytosine residue into the oligomer molecule lowers the stability just as much as the removal of two A–U pairs. The cytidylyl nucleotide probably forms a loop in the structure and displaces from the helix at least a pair of nucleotides adjoining it in the chain. The higher stability of the complex formed by the oligomer $(Ap)mG(pU)n$ compared with that of the oligomer $(Ap)mC(pU)n$ can be explained on the assumption that the guanylyl residue is not displaced from the helix, like the cytidine residue, but is incorporated in it, forming a G–U pair. The oligomeric molecules should then be displaced relative to one another:

ApApApApGpUpUpUpU
UpUpUpUpGpApApApA .

This structure is confirmed by the fact that its stability is close to that of the complex

ApApApApApUpUpUpU
UpUpUpUpApApApApA .

If 4–6 cytidylyl residues are inserted in the oligomer between homogeneous blocks, the molecule folds in two with formation of a single-stranded cytidylyl loop and a structure simulating the tRNA fragments[133]. Complexes of oligomers, in which not all the bases are paired have also been studied, in particular those formed by deca- and nona-nucleotides or deca- and hepta-nucleotides[127]. It has been noted that the thermal stabilities of these complexes decrease with the increase of the non-complementary backbone. For example, the complex of two eicosanucleotides, forming a helix from ten nucleotide pairs.

GTACCCTGTCAGAGGCCAAC
GCTCCCTTAGCATGGGACAG ,

has no cooperative melting profile at all[128], while the complex of a deca- and nona-nucleotide (GAACCGGAGA and TCTCCGGTT) melts over a fairly narrow temperature range (m.p. 40°C).[227] The destabilisation of the eicosanucleotide complex is due to the absence of an ordered steric structure from the single-stranded section of the helix. The inclusion of the single-stranded section in the complex with another oligomer leads to a sharp increase of the stability of the structure[127, 128].

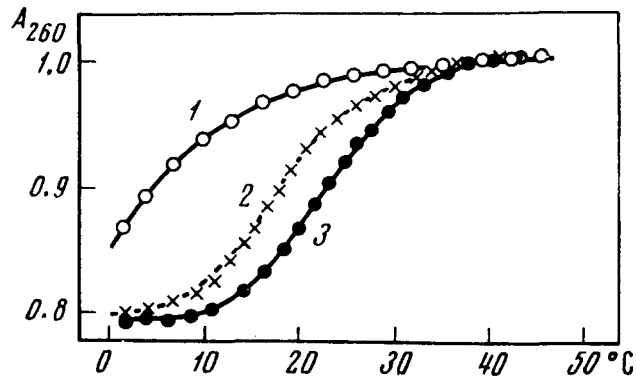


Figure 7. Melting curves for intermolecular oligomer complexes: 1) $(Ap)_5C(pU)_5$; 2) $(Ap)_5G(pU)_5$; 3) $(Ap)_4ApU(pU)_4$ (2.5×10^5 nucleotide, 1 M NaCl).[132]

Analysis of the fundamental relations established in the study of the complementary properties of mono- and oligonucleotides will undoubtedly help in predicting, in a broad outline, the behaviour of systems which have not so far been investigated and in assessing the scope of the complex formation, the stoichiometry of the interaction, etc. However, these characteristics of comparatively simple synthetic systems cannot be applied mechanically to oligomer–oligomer interactions involving native macromolecules with a complex steric structure. Complexes of synthetic oligonucleotides with native polymeric molecules or their fragments, studies on which began recently[134–139], are probably more useful models.

A number of new publications appeared recently. Studies on monomer–polymer [140–143] and oligomer–polymer complexes [144–146] are being continued. Oligomer–oligomer systems which both monotonic [147] and non-monotonic sequences of bases [148–150] are being investigated vigorously.

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