Complex formation between spin-labeled polyuridylic acid and pyrimidine nucleosides

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ABSTRACT

Complex formation between poly (U) and pyrimidine nucleosides, uridine and cytidine, was observed using spin labeling technique. The binding of these nucleosides with poly (U) takes place within a narrow range of their concentration and is characterized by a relatively strong cooperativity. It is shown, that both hydrogen bonding and stacking interaction contribute to the complex stability. Some thermodynamic parameters of the process were obtained from the binding isotherms. At 21°C the equilibrium constants for nucleation were found to be 0.23 M^{-1} and 0.42 M^{-1} , and those for chain growth 2.63 M^{-1} and 2.19 M^{-1} for uridine and cytidine respectively. Complex formation of poly (U) with adenosine was also studied by spin labeling method.

INTRODUCTION

The cooperative interaction between homopolynucleotides and complementary monomers results in the formation of complexes which are structural analogues of the corresponding double- and triple-helical polynucleotides. Such complexes are of interest as model systems for investigating the non-enzymic base recognition that, probably, played an important role in synthesis and replication of nucleic acids at early stages of evolution. Besides, studies of polynucleotide - monomer complexes provide valuable information about the nature of the intra- and intermolecular interactions in nucleic acids.

At present, the criteria of polynucleotide - monomer complex formation are well established (1), and the thermodynamic (1-13) and kinetic (14-16) regularities of the process are studied intensively. The stability of such complexes depends on the nature of the polynucleotide template, the concentration of uncomplexed monomer in solution, the temperature (1), as

well as on the salt composition of the medium (17). Upon complex formation, both pyrimidine (1-9) and purine (10,13) polynucleotides can serve as templates, whereas only the purine nucleosides and nucleotides and their derivatives are known as the monomeric components. It is still unclear whether the pyrimidine monomers can form complexes with polynucleotides, although there are no physico-chemical evidences against the formation of such complexes.

In this communication evidences for complex formation between poly (U) and pyrimidine monomers are presented. Spin label method was used to study the polynucleotide - monomer interaction. The complex formation of the spin-labeled poly (U) with uridine, cytidine and adenosine was studied in detail and the thermodynamic characteristics of the process were analysed according to a cooperative model.

MATERIALS AND METHODS

Spin-labeled Polynucleotides

Spin-labeled poly (U) and poly (A) were prepared according to the procedure described in the preceding communication (20). N-(2,2,5,5-tetramethyl-3-carboxypyrroline-1-oxyl)-imidazole was used as a spin label. The extent of spin labeling was 4.2% for poly (U) and 1.5% for poly (A). The nucleosides, uridine, cytidine and adenosine, from "Reanal" firm (Hungary) were chromatographically pure and were used without additional purification. 1,3-dimethyluracil and 4,4'-dimethylcytidine were kindly given by Dr. A.A. Mayevsky.

The concentration of spin-labeled polynucleotides was determined from the absorption measurements on a spectrophotometer VSU-2P (Zeiss, Jena). Since the introduction of small amounts of spin label into the ribose-phosphate backbone of polynucleotides does not affect their UV-spectra (20), the following analytical wavelengths and extinction coefficients were used: spin labeled poly (U) - 260 nm, $9.2 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$; spin labeled poly (A) - 257 nm, $10.5 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

ESR-Measurements

ESR-spectra were measured on a RE 1306 (USSR) spectrometer

operating at 9.5 GHz and equipped with a variable temperature control unit calibrated continuously within 1°C with a copper-constantan thermocouple. The samples were placed in glass capillaries with the inner diameter of about 0.5 mm. The conditions of ESR-spectra recording (microwave power, modulation amplitude, scan time) were selected so as to avoid spectra distortion due to experimental reasons.

The rotational correlation time, τ_c , of the spin label was calculated from the expression involving the quadratic term of the nitrogen quantum number m (21,22). The following tensor elements are used: $A_{XX} = A_{YY} = 14$ MHz, $A_{ZZ} = 87$ MHz, $g_{YY} = 2.0062$, $g_{XX} = 2.0087$, $g_{ZZ} = 2.0027$ (23), which give the following expression for τ_c :

$$\tau_c = 6.5 \times 10^{-10} \times \Delta H_0 \times (\sqrt{h_0/h_+} + \sqrt{h_0/h_-} - 2) \text{ sec.}$$

where ΔH_0 is peak-to-peak separation of the central hyperfine component of the ESR-spectrum in gauss; h_0 , h_+ and h_- are the amplitudes of the peaks corresponding to the nitrogen quantum number 0, +1 and -1, respectively.

In some cases the ratio between the amplitudes of the central and low field hyperfine components, h_0/h_+ , was used to characterize the label motion.

All ESR-spectra were measured three times with the scan time of 8 minutes, and the mean values from three measurements were used in the calculations. Samples were allowed to equilibrate for 10 minutes before measurements.

Thermodynamics of Polynucleotide-Monomer Binding Equilibria

At present, a few models for binding of small ligands to biopolymers are suggested (24-28). In this study Schwarz model (26) was used, which describes cooperative binding of ligands to linear biopolymers with one type of equivalent binding sites. According to the model, the interaction between binding sites is limited to the nearest neighbors. Two equilibrium constants, K' and K , are sufficient to describe the cooperative polynucleotide - monomer binding equilibria. K' is the equilibrium constant for binding of a monomer molecule with an isolated binding site and K is the equilibrium constant for binding of the monomer with the site in the immediate neighborhood of an already bound one. In the case of polynucleotide - monomer com-

plexes, K' is related directly to the free energy of hydrogen bonding, whereas K is the product of both hydrogen bonding and stacking interaction. These constants are related by a simple expression:

$$K = q \cdot K' \quad /1/$$

where q is the stacking coefficient, determining the free energy of the cooperative interaction of the nearest neighbor monomers on the polynucleotide template:

$$\Delta G_{st} = -RT \ln q \quad /2/$$

The fraction of binding sites occupied, θ , is related to the concentration of free monomer, C , through following expression:

$$\theta = \frac{1}{2} \left\{ 1 - \frac{(1 - KC)}{[(1 - KC)^2 + 4KC/q]^{1/2}} \right\} \quad /3/$$

This equation describes well the isotherms of monomer binding with the complementary polynucleotides and can be easily transformed into

$$\left. \frac{\partial \theta}{\partial \ln C} \right|_{\theta=0.5} = \frac{1}{4} \sqrt{q} = \frac{1}{4} \cdot \exp \left(- \frac{\Delta G_{st}}{2RT} \right) \quad /4/$$

similar to Hill's equation (24).

In this study, the equations /1-4/ were used to determine the thermodynamic parameters ΔG_{st} , K' , K and q from the isotherms of binding of the nucleosides with the spin-labeled poly (U). The methods of constructing the binding isotherms from the ESR data are presented in section RESULTS.

RESULTS

Complex Formation between Spin-Labeled Poly (U) and Uridine

The ESR-spectrum of the spin-labeled poly (U) and those of mixtures of poly (U) with uridine and cytidine in 10^{-2} M NaCl, 10^{-2} M tris-HCl, pH 7.5 at 21°C are presented in Figure 1. The spin label rotational correlation times, τ_c , calculated from the ESR-spectra are equal to 1.24 nsec, 2.00 nsec and 2.24 nsec, respectively. The increase in τ_c values observed for the mixtures of poly (U) with uridine and cytidine is indicative of the changes in poly (U) conformation induced by these nucleosides. The conformation of poly (U) becomes more rigid in the presence

Figure 1. ESR-spectra of: a - 3.87 mM spin-labeled poly (U), b - 3.87 mM spin-labeled poly (U) + 0.75 M of uridine, c - 3.87 mM spin-labeled poly (U) + 0.95 M of cytidine. Conditions: 10 mM NaCl, 10 mM tris-HCl, pH 7.5, 21°C.

of uridine and cytidine, and the ESR-spectra approximate that of the spin-labeled poly (U) at 2°C where this polynucleotide has an ordered double-helical structure (19).

We studied thoroughly the dependence of τ_c of the spin-labeled poly (U) on the concentration of the added uridine. This dependence is shown in Figure 2. Noteworthy, that the τ_c of the spin-labeled poly (U) practically does not change with the addition of uridine up to 0.15 M (it means that uridine does not exert any influence on poly (U) conformation until a certain threshold of its concentration is reached), then increases abruptly and finally reaches the plateau at uridine concentrations of over 0.65 M. Such changes in τ_c can not result from the effect of bivalent metal ions on the conformation of poly (U). Figure 2 shows also the dependence of τ_c for spin-labeled poly (U) on uridine concentration in the presence of 0.1 M EDTA. It can be seen, that the addition of EDTA produces practically no effect on dependence of τ_c upon concentration of uridine. However, if instead of uridine we use 1,3-dimethyl-

Figure 2. Dependence of the rotational correlation time, τ_c , of spin-labeled poly (U) on the concentration of: (●) uridine, (+) uridine in the presence 0.1 M EDTA, (○) 1,3-dimethyluracil. Conditions are the same as in Figure 1.

uracil with the chemically blocked centers responsible for hydrogen bonding, the τ_c of the spin-labeled poly (U) does not change in a wide concentration range of the monomer (see Figure 2). These data indicate, evidently, the direct interaction of uridine with poly (U) template, inducing the changes of polynucleotide conformation. It is clear, that hydrogen bonding is essential for this interaction.

As in the case of polynucleotide - purine monomer complexes, the interaction of uridine with poly (U) is cooperative, and to determine the thermodynamic parameters of complex formation it is necessary to know the binding isotherm. The fraction of poly (U) sites occupied, θ , at different uridine concentrations can be determined as follows:

$$\theta = \frac{\tau_c - \tau_c^{\min}}{\tau_c^{\max} - \tau_c^{\min}} \quad 15/$$

where $\tau_c^{\min}$ is the rotational correlation time of spin-labeled poly (U) in the absence of uridine, $\tau_c^{\max}$ is the rotational

correlation time of the spin-labeled poly (U) at uridine concentrations corresponding to the saturation of the polynucleotide template (Figure 2), and τ_c is the rotational correlation time of spin-labeled poly (U) at intermediate concentrations of uridine. The isotherm of binding of uridine on poly (U), obtained in this way, is presented in Figure 3.

It should be noted that in this case the concentration of the monomer is more than two orders higher than that of the polynucleotide, thus, there will be practically no difference between the total and free concentration of uridine in the solution, i.e. the binding isotherm would not change if the logarithm of the total of free monomer concentration is plotted as abscissa.

The thermodynamic parameters of the interaction of uridine with the spin-labeled poly (U) calculated from the binding isotherm in accordance to the equations /1-4/ are as follows :

$\partial\theta/\partial\ln C = 0.85$ at $\theta = 0.5$; $\Delta G_{st} = -1.42$ kcal/mole; $K=2.63 \text{ M}^{-1}$, $K' = 0.23 \text{ M}^{-1}$ and $q = 11.4$.

Complex Formation between Spin-Labeled Poly (U) and Cytidine

As it follows from Figure 1, the addition of cytidine to the spin-labeled poly (U) results in an essential change of

Figure 3. Isotherm of binding of uridine with the spin-labeled poly (U) at 21°C.

the spin label correlation time, τ_c , indicating an increase in rigidity of poly (U) structure. The dependence of τ_c for the spin-labeled poly (U) on the total concentration of cytidine in the solution is given in Figure 4. The character of τ_c changes with cytidine concentration is similar to that for interaction of poly (U) with uridine. The correlation time does not depend on the concentration of the methylated cytidine derivative, 4,4'-dimethylcytidine, in which the centers responsible for hydrogen bonding are blocked chemically. These results show clearly the specific binding of cytidine to poly (U) with formation of a stoichiometric complex (saturation of polynucleotide template at high cytidine concentrations). It is clear, that hydrogen bonding is an essential factor of this interaction. Using the expression /5/ we constructed the isotherm of binding of cytidine with poly (U) (see Figure 5). The binding of cytidine to poly (U) is cooperative and takes place only in the case when a certain concentration threshold is reached.

The thermodynamic parameters of complex formation between the spin-labeled poly (U) and cytidine were found to be: $\partial\theta/\partial\ln C = 0.57$ at $\theta = 0.5$, $\Delta G_{st} = -0.97$ kcal/mole, $K = 2.19 \text{ M}^{-1}$, $K' = 0.42 \text{ M}^{-1}$ and $q = 5.2$.

Figure 4. Dependence of the rotational correlation time of spin-labeled poly (U) on the concentration of: (●) cytidine, (○) 4,4'-dimethylcytidine. Conditions are the same as in Figure 1.

Figure 5. Isotherm of binding of cytidine with the spin-labeled poly (U) at 21°C.

Complex Formation between Spin-Labeled Poly (U) and Adenosine

Complex formation between adenosine and poly (U) has been studied in detail in a number of laboratories, and at present the conditions and thermodynamic characteristics of this interaction are well known (1,2,5,30). We studied the cooperative binding of adenosine to the spin-labeled poly (U), firstly, in order to show, that the spin label method makes it possible to get information on the thermodynamic parameters of polynucleotide - monomer binding equilibria and, secondly, in order to compare the data of ESR spectroscopy with the results obtained by other physico-chemical methods.

The ESR-spectrum of the spin-labeled poly (U) and that of the mixture of poly (U) with adenosine in 10^{-2} M NaCl, 10^{-2} M tris-HCl, pH 7.5 at 2°C are presented in Figure 6. The addition of adenosine to the spin-labeled poly (U) results in an essential transformation of the ESR-spectrum, thus, indicating an abrupt decrease in rotational mobility of the spin label. This is, evidently, related to the formation of the triple-helical complex 2 poly (U). I adenosine whose ESR-spectrum is a superposition of at least two types of ESR-spectra arising from the spin labels with different degree of immobilization. It should

Figure 6. ESR-spectra of: a - 3.87 mM spin-labeled poly (U),
b - 3.87 mM spin-labeled poly (U) + 14 mM adenosine.
Conditions: 10 mM NaCl, 10 mM tris-HCl, pH 7.5, 2°C.

be noted that the ESR-spectrum of the triple-helical 2 poly (U). 1 adenosine complex practically coincides with the ESR-spectrum of triple-helical 2 poly (U). 1 poly (A) complex formed from the spin-labeled poly (U) and non-labeled poly (A) (Figure 7a). However, the ESR-spectrum of such complex, formed from the non-labeled poly (U) and spin-labeled poly (A) (Figure 7b) differs essentially from those of complexes of the spin-labeled poly (U) with adenosine and non-labeled poly (A). This indicates clearly that the structure of the complex of adenosine with poly (U) is completely similar to the structure of 2 poly (U). 1 poly (A) complex.

To characterize the spin label mobility at different concentrations of adenosine we used the ratio between the central and low field hyperfine components of the ESR spectrum, h_0/h_+ . The dependence of this parameter for poly (U) on total concentration of adenosine in the solution is shown in Figure 8.

Proceeding from these data it is possible to construct the binding isotherm, θ being determined in the following way:

$$\theta = \frac{(h_0/h_+) - (h_0/h_+)_{\min}}{(h_0/h_+)_{\max} - (h_0/h_+)_{\min}}$$

Figure 7. ESR-spectra of 2 poly (U) . 1 poly (A) complex at 21°C. a - 3.87 mM of spin-labeled poly (U) + 1.93 mM of non-labeled poly (A), b - 3.87 mM of non-labeled poly (U) + 1.93 mM of spin-labeled poly (A) at pH 7.5.

The meaning of $(h_o/h_+)_{\min}$ and $(h_o/h_+)_{\max}$ is clear from Figure 8. Figure 9 illustrates the isotherm of binding of adenosine to the spin-labeled poly (U) template, constructed in this way.

Figure 8. Dependence of h_o/h_+ for spin-labeled poly (U) on adenosine concentration. Conditions are the same as in Figure 6.

Figure 9. Isotherm of binding of adenosine with the spin-labeled poly (U) at 2°C, pH 7.5.

As it should be expected, the binding of adenosine with poly (U) is cooperative and takes place within a narrow range of its concentration. The isotherm of binding of adenosine to poly (U) is practically of the same shape as it is in the case of binding of uridine with this polynucleotide (Figure 3), however, they differ essentially in the range of monomer concentrations at which the binding takes place.

The thermodynamic parameters of interaction of adenosine with the spin-labeled poly (U) obtained from the binding isotherm are the following: $\delta\theta/\delta\ln C = 0.79$ at $\theta = 0.5$, $\Delta G_{st} = -1.27$ kcal/mole, $K' = 25 M^{-1}$, $K = 252 M^{-1}$ and $q = 10.1$.

DISCUSSION

Despite the numerous investigations of the polynucleotide - monomer binding equilibria carried out during the past 15 years, no complexes between pyrimidine monomers and polynucleotides were discovered so far. Since the stability of polynucleotide - monomer complexes is contributed mainly by the stacking of the monomers on the template (8), it can be assumed, that because of the weak stacking tendency of pyrimidine heterocycles (18), the stability of their complexes with polynucleotides is essen-

tially lower than that for complexes formed from purine monomers.

Proceeding from the well-known fact that the melting temperature of polynucleotide - monomer complexes increases strongly with the concentration of free monomer in the solution (1, 30), as well as from the mass action law, it could be expected, that for polynucleotide - pyrimidine monomer complex formation a sufficiently higher [monomer]/[polymer] ratio is required. It is obvious, however, that at a very high [monomer]/[polymer] ratio it is difficult to observe the complex formation by the spectral methods and equilibrium dialysis used commonly for these purposes.

In this study we made use of the fact that the complex formation is always associated with structural rearrangement of the polynucleotide, that can be easily detected by the well developed spin labeling technique (29). The spin label bound covalently with 2' OH ribose groups is most suitable for this purpose because, firstly, it is sensitive to the changes in polynucleotide conformation (20) and, secondly, the acylation of 2' OH ribose groups in the polynucleotide does not involve the centers responsible for hydrogen bonding and stacking interaction of bases.

The data obtained are undoubtedly indicative of the cooperative interaction between uridine, cytidine and poly (U). The plateaus on the curves T_c versus concentration of the nucleosides (Figures 2 and 4) clearly indicate the stoichiometric interaction. Unfortunately, we cannot obtain information about the stoichiometry of these complexes from ESR spectroscopy. It seems, however, most probable that the stoichiometry of complexes between uridine, cytidine and poly (U) is 1 : 1, since it is difficult to give a reasonable scheme of hydrogen bonding in coplanar triplets U·U·U and C·C·U. Besides, it is known (19) that poly (U) forms the ordered double-helical structures at low temperatures, whereas triple-helical structures for poly(U) have not been observed.

All thermodynamic characteristics of the complex formation obtained from the isotherms of binding of uridine and cytidine with poly (U) are given in Table 1. For comparison, the thermo-

Table 1. Thermodynamic parameters of polynucleotide - monomer complex formation. a) - calculated from the binding isotherms presented in the references. b) - the error of ΔG_{st} determination is about ± 0.1 kcal/mole

Complex	ΔG_{st} , kcal/mole	K' , M^{-1}	K , M^{-1}	$q = \frac{K}{K'}$	Ref.
2 poly (U) . 1 deoxyadenosine	-1.9	18 ^{a)}	550 ^{a)}	30.5 ^{a)}	(1)
2 poly (C) . 1 deoxyguanosine	-2.3	9.8 ^{a)}	625 ^{a)}	63.5 ^{a)}	(1)
2 poly (U) . 1 adenosine	-1.2	54.7 ^{a)}	476 ^{a)}	8.7 ^{a)}	(5)
poly (U) . 6,9-di- methylaminopurine	-2.51	5	500	100	(8)
2 poly (C) . 1 guanosine	-2.8	14.4 ^{a)}	1852 ^{a)}	129 ^{a)}	(6)
2 poly (C) . 1 guanosine-5'- phosphate	-2.6	12.6 ^{a)}	1429 ^{a)}	113 ^{a)}	(6)
2 poly (U) . 1 adenosine	-1.27 ^{b)}	25.0	252	10.1	this study
poly (U) . uridine	-1.42 ^{b)}	0.23	2.63	11.4	this study
poly (U) . cytidine	-0.97 ^{b)}	0.42	2.19	5.2	this study

dynamic parameters of a number of the well-known polynucleotide-purine monomer binding equilibria are presented there. It can be easily seen that the complexes of poly (U) with pyrimidine nucleosides are characterized by extremely low values of the equilibrium constants for nucleation K' , and for chain growth K , which are about hundredfold lower than those for complexes formed by purine monomers. Thus, it becomes clear why high concentration of uridine and cytidine are required to form complexes with poly (U).

The equilibrium constant for hydrogen bonding of cytidine with poly (U) is higher than that for uridine. This result is consistent with a theoretical calculation of the hydrogen bonding energy in the base-pairs U·U and C·U (32). The stacking

coefficient found in our experiments is about two-fold higher for uridine as compared to that for cytidine. However, both experimental studies (18) and theoretical calculations (31) show a sufficiently higher stacking tendency of cytidine in aqueous solutions. This discrepancy between our results and literature data can be explained by different mode of overlapping of base planes in the polynucleotide - monomer complexes and upon self-association of free monomers in the solution.

It is striking that the free energy of uridine and cytidine stacking on poly (U) is much higher as compared to that for self-association of these nucleosides in the solution (33). Most probably, that one of the reasons of obtaining such abnormally high values for the free energies of uridine and cytidine stacking on poly (U) is related to the model of cooperative binding used in this study. This model does not take into account the immediate binding of nucleosides to poly (U) in form of associates (oligostacks). However, the thermodynamic parameters of binding of oligostacks to poly (U) can sufficiently differ from that for interaction of monomeric form with template.

We calculated the dependence of uridine monomer, dimer, trimer and tetramer concentrations on the total concentration of uridine from the isodesmic model of association taking the association constant for uridine to be equal to 0.64 M^{-1} (33). Since at high concentration of the monomer the binding of its small part with the polynucleotide will not practically affect the distribution of uridine oligostacks in the solution, we constructed the isotherms of binding of such oligostacks with poly (U) and calculated the thermodynamic parameters of complex formation (Table 2).

It can be, probably, concluded from the data in the Table that the binding of uridine oligostacks with the polynucleotide is more preferable as compared to the binding of its monomeric form. Indeed, an increase in the size of the uridine oligostack results in an increase of the equilibrium constants for both nucleation K' and chain growth K , while the free energy of the interaction between oligostacks and the template is reduced (Table 2). The experimental confirmation of this can be found

Table 2. Thermodynamic parameters of binding of uridine oligostacks with poly (U) template. All values were calculated per mole of uridine in monomeric form

Uridine oligostack	$\Delta G_{st}, \text{kcal/mole}$	K, M^{-1}	K', M^{-1}	$q = \frac{K}{K'}$
monomer	-1.86	4.17	0.17	24.4
dimer	-0.99	13.5	2.5	5.4
trimer	-0.55	57.9	22.4	2.6
tetramer	-0.34	268.0	149.4	1.79

in the study (34) in which the interaction of different uridine oligomers with poly (A) was investigated. Although no complexes of poly (A) with uridine and dimer UpU were found, an abrupt increase in the binding constant with the increase in oligomer size was shown: 4 M^{-1} for $(\text{Up})_2\text{U}$, 49 M^{-1} for $(\text{Up})_3\text{U}$ and 353 M^{-1} for $(\text{Up})_4\text{U}$.

Thus, the results described in this paper are indicative of the existence of complexes between pyrimidine monomers and polynucleotide. However, all thermodynamic parameters of the interaction of uridine and cytidine with poly (U) calculated from the binding isotherms are, probably, some effective values. It follows from this study that precise thermodynamic description of complex formation calls for a more exact model, taking into account the differences in the parameters of interaction of different oligostacks with polynucleotide template.

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REFERENCES

1. Davies, R.J.H., Davidson, N. (1971) *Biopolymers*, **10**, 1455-1479.
2. Huang, W.M., Ts'o, P.O.P. (1966) *J.Mol. Biol.*, **16**, 523-533.
3. Howard, F.B., Frazier, J., Singer, M.F., Miles, H.T. (1966) *J.Mol. Biol.* **16**, 415-439.

4. Ts'o, P.O.P., Schweizer, M. (1968) *Biochemistry*, 7, 2963-2971.
5. Pitha, P.M., Huang, W.M., Ts'o, P.O.P. (1968) *Proc. Nat. Acad. Sci., USA*, 61, 332-339.
6. Sarocchi, M.T., Courtois, Y., Guschlbauer, W. (1970) *Eur. J. Biochem.*, 14, 411-421.
7. Damle, V.N. (1972) *Biopolymers*, 12, 1789-1816.
8. Hoffman, G.W., Porschke, D. (1973) *Biopolymers*, 12, 1611-1623.
9. Bangerter, B.W., Modestas, Z. (1974) *Biopolymers*, 13, 567-575.
10. Davies, R.J.H. (1976) *Eur. J. Biochem.*, 61, 225-236.
11. Klump, H. (1975) *Biochim. Biophys. Acta*, 383, 117-122.
12. Scruggs, R.L., Ross, P.D. (1970) *J. Mol. Biol.*, 47, 29-40.
13. Davies, R.J.H., Diamond, S.P. (1978) *Biochim. Biophys. Acta* 518, 1-6.
14. Davies, R.J.H. (1972) *J. Mol. Biol.*, 63, 117-137.
15. Hoffman, G.W., Porschke, D. (1973) *Biopolymers*, 12, 1625-1638.
16. Porschke, D. (1977) in *Chemical Relaxation in Molecular Biology* (Pecht, I., Rigler, E., eds) Springer-Verlag, Berlin, pp.191-218.
17. Schleich, T., Gould, G.K. (1974) *Biopolymers*, 13, 327-337.
18. Ts'o, P.O.P. (1974) in *Basic Principles in Nucleic Acid Chemistry* (Ts'o, P.O.P., ed) Acad. Press, New York, vol. 1, pp. 517-584.
19. Thrier, J.C., Dourlent, M., Leng, M. (1971) *J. Mol. Biol.*, 58, 815-830.
20. Petrov, A.I., Sukhorukov, B.I. (1980) *Nucl. Acids Res.*, vol. 8, N 18.
21. Kivelson, D. (1960) *J. Chem. Phys.*, 33, 1094-1106.
22. Stone, T., Buckman, T., Nordio, P.L., McConnell, H.M. (1965) *Proc. Nat. Acad. Sci. USA*, 54, 1010-1017.
23. Libertini, L.J., Griffith, O.H. (1970) *J. Chem. Phys.*, 53, 1359-1367.
24. Hill, T.L. (1960) *An Introduction to Statistical Thermodynamics*, Addison-Wesley, Reading, Mass., pp.235-241.
25. Schmitz, K.S., Schurr, J.M. (1970) *Biopolymers*, 9, 697-715.
26. Schwarz, G. (1970) *Eur. J. Biochem.*, 12, 442-453.
27. Zasedatelev, A.S., Gursky, G.V., Volkenstein, M.V. (1971) *Molekulyarnaya Biologiya*, 5, 245-251.
28. Burr, J.G., McDowell, T.L., Christian, S.D. (1972) *Biochem. Biophys. Res. Commun.*, 56, 21-28.
29. Berliner, J. (1976) *Spin Labeling. Theory and Application*, Acad. Press, New York.
30. Gukovskaya, A.S., Gukovsky, I.Ya., Chikh, V.P., Sukhorukov, B.I. (1980) *Biopolymers*, 19, 453-468.
31. Poltev, V.I., Shulga, S.M. (1978) *Studia Biophysica*, 70, 51-61.
32. Poltev, V.I., Bruskov, V.I. (1978) *J. Theor. Biol.*, 70, 69-83.
33. Plesiewicz, E., Stepień, E., Bolewska, K., Wierzychowski, K.L. (1976) *Biophys. Chem.*, 4, 131-141.
34. Lewis, J.B., Brass, L.F., Doty, P. (1975) *Biochemistry*, 14, 3164-3171.