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Fluctuations in Order–Disorder Transitions in the DNA–Ligand Complexes with Various Binding Mechanisms

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Abstract—The melting of the DNA–ligand complex is considered theoretically for the ligands binding with the DNA by two mechanisms. The obtained results describe the experimentally observed behavior of such quantities as the denaturation degree and the correlation length depending on the concentration of ligands. It is shown that the heat and cold denaturations of the DNA–ligand complexes exhibit the same cooperativity, as the heat denaturation of the pure DNA. At the same time, the temperature range of the cold denaturation is essentially narrower than the interval for the heat denaturation of the pure DNA and the DNA–ligand complexes.

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1. INTRODUCTION

In biopolymers, the phenomenon of the helix–coil transition has been known since the 1960s [1–3] and continues to be actively investigated [4–9]. Such unflagging interest is caused by two factors. From the biological point of view, the unwinding of the double helix of the DNA is related to such genetically important processes as transcription and translation. From the physical point of view, the double helix of the DNA is an example of a one-dimensional cooperative system with the large-scale correlations [1]. The theory of order–disorder transitions in the DNA was considered in [2, 10, 11]. When investigating the structure and function of the DNA, the interactions with water, ions and low-molecular compounds dissolved in water (ligands) are particularly important. The effect of such interactions was studied in [12, 13]. The interaction of ligands with the double helix of the DNA is one of the fundamental factors for many intracellular processes. Thus, for example, the ligands with the reduced specificity to the sequence often have an ability of regulation of gene expression, translation and transcription [14]. Because of the complex structure of the DNA double helix, the various ways of non-covalent binding of ligands to the DNA are possible: intercalation [15], bis-intercalation [16], the narrow-groove binding [17], the wide-groove binding [18], their combination [19], as well as the binding by means of nonclassical mechanisms [20].

The present work is devoted to the influence of fluctuations on the helix–coil transition of the double helix of the DNA, which binds with the ligands in two ways, with the native and the denaturated DNA. The influence of ligands which bind with the DNA by means of two mechanisms on the behavior of the correlation length and the degree of the DNA denaturation, has been investigated.

2. THEORY OF MELTING OF THE DNA–LIGAND COMPLEXES

The standard model of melting of the DNA–ligand complexes was used [1, 21]. It was assumed that the DNA molecule consists of N base pairs. In the area of the helix–coil transition, each base pair can be in one of two states: helix or coil. It is supposed that the molecule is divided into n sections containing N_1 coil base pairs and N_2 helix base pairs. The total number of base pairs is constant in the process of melting, so

$$N = N_1 + N_2 . \tag{1}$$

The solution also contains two types of ligands with different binding parameters. Suppose that the binding constants of ligands with the denaturanted DNA have the values K'_1 and K''_2, and the binding constants with the native DNA are K'_2 and K''_2. The total number of ligands is k, the number of ligand molecules associated with the single-stranded sections by the first mechanism is equal to k'_1 and by the second one is k''_2, and the number of molecules associated with the double-stranded sections by the first and second mechanisms is equal to k'_2 and k''_2, respectively. Let F_1 and F_2 be the free energies of the coil and helix states per the base pair, respectively, F_0 is the free energy of the initialization of the helix, Ψ_i^α the free energy of binding of the ligand ($i = 1,2$ and $\alpha = ',''$), and W the number of microstates of the macromolecule corresponding to a given energy. Thus, the free energy of the system can be estimated as [21, 22]

$$F = F_1 N_1 + F_2 N_2 + nF_0 + \sum_{i=1,2}\sum_{\alpha=',''} k_i^\alpha \Psi_i^\alpha - T\ln W . \tag{2}$$

Using the method of the most probable distribution, the following system of equations for the concentrations $c_i^\alpha = k_i^\alpha / N_i$ was obtained:

$$\begin{aligned}
\frac{c'_1 r'_1/2}{1 - c'_1 r'_1/2} &= \frac{PK'_1}{2}\left[\frac{2D}{P} - 2(1-\vartheta)c'_1 - 2(1-\vartheta)c''_1 - \vartheta c'_2 - \vartheta c''_2\right],\\
\frac{c''_1 r''_1/2}{1 - c''_1 r''_{1_2}/2} &= \frac{PK''_1}{2}\left[\frac{2D}{P} - 2(1-\vartheta)c'_1 - 2(1-\vartheta)c''_1 - \vartheta c'_2 - \vartheta c''_2\right],\\
\frac{c'_2 r'_2}{1 - c'_2 r'_2} &= \frac{PK'_2}{2}\left[\frac{2D}{P} - 2(1-\vartheta)c'_1 - 2(1-\vartheta)c''_1 - \vartheta c'_2 - \vartheta c''_2\right],\\
\frac{c''_2 r''_2}{1 - c''_2 r''_2} &= \frac{PK''_2}{2}\left[\frac{2D}{P} - 2(1-\vartheta)c'_1 - 2(1-\vartheta)c''_1 - \vartheta c'_2 - \vartheta c''_2\right],
\end{aligned} \tag{3}$$

where K_i^α are the binding constants, D the total concentration of ligands, r_i^α the number of binding centers of one ligand, P the total concentration of phosphate groups, and ϑ the degree of helicity. The equation for the effective parameter of helix growth has the form

$$S^* = S\frac{\left(1 - c'_1 r'_1/2\right)^{2/r'_1}\left(1 - c''_1 r''_1/2\right)^{2/r''_1}}{\left(1 - c'_2 r'_2\right)^{2/r'_2}\left(1 - c''_2 r''_2\right)^{2/r''_2}}, \tag{4}$$

where $S = \exp\left(\frac{F_1 - F_2}{T}\right) = \exp\left(-\frac{\Delta H}{RT} + \Delta S\right)$ is the parameter of the helix growth of the pure DNA, ΔH the change of enthalpy, ΔS the change of entropy at the formation of one base pair. In fact, the model with the free energy (2) reduces to the model of the pure DNA with the overdetermined parameter of the

helix growth (4), which depends on the concentration of ligands and parameters of ligands binding to the DNA.

The degree of helicity ϑ is the parameter of order describing the helix–coil transition, which is defined as

$$\vartheta = \frac{1}{N}\langle N_2 \rangle, \tag{5}$$

where $\langle ... \rangle$ is the thermodynamic average. Thus, the degree of helicity is the average fraction of base pairs, which are in the helix phase. At the same time, the fluctuation effects are also essential, especially for describing the cooperativity of the helix–coil transition, the average length of the helical section, and so on.

To take into account the fluctuation effects, we considered the behavior of the correlation length of the double-stranded DNA defined as the scale of the exponential decay of fluctuations along the chain. For this to be done, a model equivalent to (2) and developed to describe the helix–coil transition in the polypeptides and the DNA [23–25], however, more suitable than (2) to describe the fluctuation effects. The energy of the model can be represented as

$$H\{\gamma\} = U\sum_{i=1}^{N}\delta_i^{(\Delta)}, \tag{6}$$

where N is the number of repeating units, U the stabilization energy of base pair, $\delta_j^{(\Delta)} = \prod_{k=0}^{\Delta-1}\delta(\gamma_{j+k},1)$, $\delta(x,1)$ the Kronecker symbol, and $\gamma_i = 1,...,Q$. The variable γ_i describes the state of the i-th repeating unit. It is supposed that the total number of conformations is equal to Q, $\gamma_i = 1$ corresponds to the helix state, and the remaining $(Q-1)$ – to conformations in the coil-like state. Besides, the parameters of the models (2) and (6) are related to each other as

$$U(\{c\},\{r\}) = -RT\ln S^* + RT\ln(Q-1), \tag{7}$$

$$\exp\left(-\frac{F_0}{RT}\right) = Q^{1-\Delta}. \tag{8}$$

Here S^* is the parameter of the helix growth (4). Thus, the stabilization energy of the base pair becomes concentration-dependent. Parameter Δ has the meaning of the persistent length of single-stranded DNA and is estimated as $\Delta \approx 7$.

The fluctuation effects can be described with the use of the pair correlation function

$$g_2(r) = \langle \delta_j^{(\Delta)}\delta_{j+r}^{(\Delta)} \rangle - \langle \delta_j^{(\Delta)} \rangle \langle \delta_{j+r}^{(\Delta)} \rangle, \tag{9}$$

which in this case describes the statistical interdependence of the formation of two base pairs, spaced apart from each other by r base pairs along the chain. As is well known, for a one-dimensional system the pair correlation function decays as [24]

$$g_2(r) \sim \exp\left(-r/\xi\right), \tag{10}$$

where ξ is the correlation length. As it was shown in [24], the correlation length of the one-dimensional system satisfies the relation

$$\coth\left(\frac{1}{2\xi}\right)=\frac{1}{\vartheta(1-\vartheta)}\frac{\partial\vartheta}{\partial\ln S}. \tag{11}$$

The maximum value of the correlation length is at the melting point (at the half-transition point $\vartheta = 1/2$) and is estimated as [24]

$$\xi_0 \approx \frac{1}{2}Q^{\frac{\Delta-1}{2}}. \tag{12}$$

3. RESULTS AND DISCUSSION

To account for the influence of the ligands described above, the degree of helicity and the correlation length were calculated on the basis of equations (3)–(6). In the present work, we have concentrated on the influence of ligands that have a greater affinity to the native DNA than for the molten one. The solution of the system of equations (3) had been obtained numerically.

If the total concentration of ligands in the solution is comparable to the concentration of phosphate groups, i. e. $2D/P \sim 1$ and $PK'_1 \gg 1, PK''_1 \gg 1, PK'_2 \gg 1, PK''_2 \gg 1$, then the DNA–ligand complexes exhibit high- and low-temperature denaturation combined with a wide 'window' of the stability of the DNA double helix coinciding with the region of the helix–coil transition of the pure DNA (Fig. 1, curve *1*). In this case, the key point is the higher affinity of the ligands to the native DNA than for the denaturated one. In this case, the high-temperature denaturation of the DNA–ligand complexes shifts essentially to the high temperature region as compared to the pure DNA. At the same time, the DNA–ligand complexes are in the native state at temperatures where the pure DNA remains denaturated. When the temperature is lowered, the DNA–ligand complex undergoes the 'cold' denaturation, while the pure DNA becomes helical (Fig. 1, curve *2*).

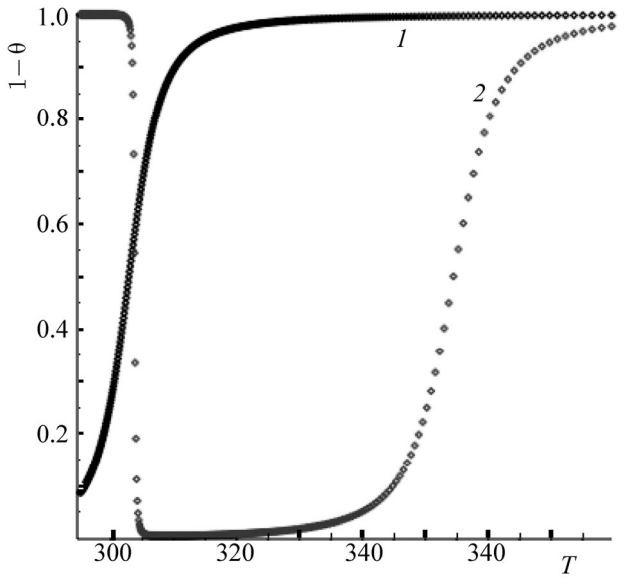

Fig. 1. Dependence of the degree of denaturation 1 – θ on temperature: *1* – for the pure DNA, *2* – for the DNA–ligand complex with the binding parameters $r'_1 = 2$, $r''_1 = 2$, $r'_2 = 4$, $r''_2 = 4$, $K'_1 = 2.9 \times 10^4$ M^{-1}, $K''_1 = 10^2$ M^{-1}, $K'_2 = 9.6 \times 10^5$ M^{-1}, and $K''_2 = 10^7$ M^{-1}.

For proteins and nucleic acids, the phenomenon of 'cold' denaturation was observed experimentally [26–29]. The mechanism of this phenomenon was considered in a large number of publications [25, 26, 28–30]. The 'cold' denaturation of the DNA–ligand complexes is accompanied by the redistribution of ligands related to the molten regions of the DNA (Fig. 2, curve *3*). While the concentration c_2^{α} of ligands associated with the native regions remains constant during heat and cold denaturation, the concentration of ligands associated with the molten sections is sharply decreasing in the region of cold denaturation. Thus, the stabilization of the molten phase is caused by the ligands that bound to the denaturated DNA.

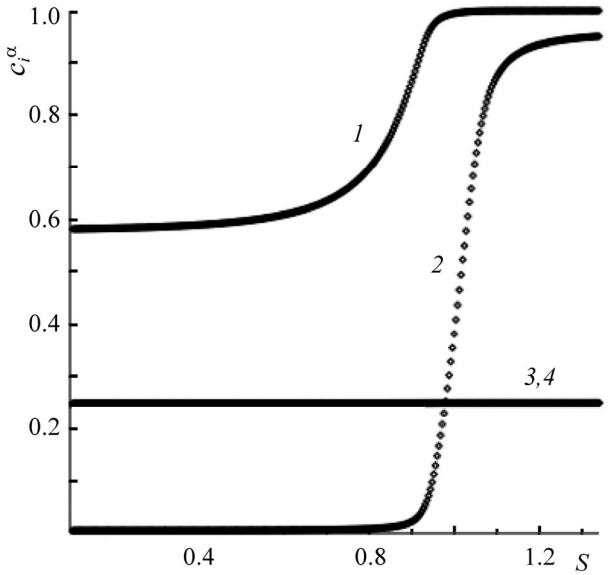

Fig. 2. Dependence of the concentration of ligands c_i^{α} on the parameter of growth of the helix S. Curves *1*, *2*, *3*, and *4* describe the behavior of the concentrations c'_1, c''_1, c'_2 and c'', respectively. The results are obtained for the values of binding parameters: $r'_1 = 2$, $r''_1 = 2$, $r'_2 = 4$, $r''_2 = 4$, $K'_1 = 2.9 \times 10^4$ M^{-1}, $K''_1 = 10^2$ M^{-1}, $K'_2 = 9.6 \times 10^5$ M^{-1}, and $K''_2 = 10^7$ M^{-1}.

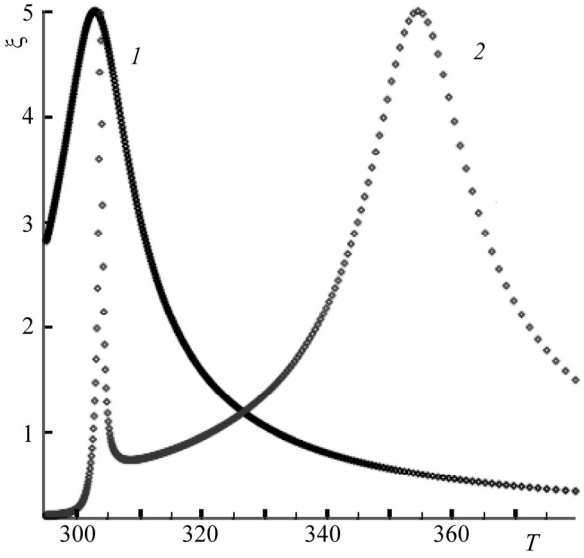

Fig. 3. Dependence of the correlation length ξ on temperature: *1* – for the pure DNA, *2* – for the DNA–ligand complex with the binding parameters $r'_1 = 2$, $r''_1 = 2$, $r'_2 = 4$, $r''_2 = 4$, $K'_1 = 2.9 \times 10^4$ M^{-1}, $K''_1 = 10^2$ M^{-1}, $K'_2 = 9.6 \times 10^5$ M^{-1}, and $K''_2 = 10^7$ M^{-1}.

The correlation length was computed on the basis of equations (3)–(6) and (11). As can be seen from Fig. 3, the temperature dependence of the correlation length of the DNA–ligand complex is characterized by two peaks corresponding to cold and heat denaturation. The high-temperature peak is shifted towards higher temperatures as compared to the case of the pure DNA. As follows from the relations (8) and (12), the cooperativity of the transition is determined by the maximum value of the correlation length and is practically the same for both the pure DNA and the DNA–ligand complexes in the regions of cold and heat denaturation. The temperature range of heat denaturation does not differ from that for the heat denaturation of the pure DNA and the DNA–ligand complexes. At the same time, the interval of transition for cold denaturation of the DNA–ligand complexes is essentially narrower. Thus, it can be argued that the interval of transition is not a single-valued index of the cooperativity of the order–disorder transition.

4. CONCLUSION

The fluctuation effects arising from the melting of the DNA–ligand complexes are considered. It is suggested that ligands are bound to the DNA by means of two mechanisms. The temperature dependences of the degree of denaturation and the correlation length characterizing the order–disorder transition are computed. In particular, it was shown that the maximum value of the correlation length at the transition point, which characterizes the cooperativity of the heat and cold denaturation that occurs for the DNA–ligand complexes does not differ from that for the heat denaturation of the pure DNA. In this case, the temperature interval of cold denaturation is essentially narrower than the interval for the heat denaturation of both the pure DNA and the DNA–ligand complexes, which in turn means that the narrowness of the transition interval is equivocal characteristic of the cooperativity of the order–disorder transition.

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