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DNA–LIGAND COMPLEXES MELTING: THE EFFECT OF MULTIPLE BINDING MECHANISMS

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Helix–coil transition of complexes DNA with two ligands of different binding mechanisms to native and melted regions of biopolymer is considered using the most probable distribution method. It was shown that obtained biphasic behavior of helix–coil transition curves depends on both binding affinity and concentration of ligands in solution. Thermodynamic behavior of the ligands having higher and lower affinity to the native DNA is compared.

***Keywords*:** Helix–coil transition; biphasic denaturation; multiple binding.

Introduction. The basic model of the helix–coil transition in biopolymers for theoretical treatment has been known since the 1960s [1, 2] and considerable attention has been paid to it quite recently [3–8]. The theory of helix–coil transition in biopolymers, particularly for DNA has been already proposed [10–13]. Alongside with it, the theories were developed to understand the interaction peculiarities of small molecules with DNAs, which have different conformations. From this point of view, the thermodynamic description of simultaneous interaction of different ligands with DNA plays a vital role. Current paper presents a theoretical model of the helix–coil transition of DNA–ligand complexes, when two types of ligands bind to the double-stranded (ds-) or to single-stranded (ss-) DNA contemporaneously. The suggested model is an effective way of analyzing the experimental results, obtained for the simultaneous binding of two different ligands (which have different binding parameters) with ss- and ds-DNA [12]. Theory.The macromolecule is divided into "n" regions consisting of N_1 coil base pairs and N_2 helical base pairs. During the melting process the total number of base pairs remains constant

$$N = N_1 + N_2.$$

Let us suppose that the ligands that are bound with ss-regions of DNA are characterized with K_1' and K_1'' binding constants and in case of ds-regions binding,

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constants are K_2' and K_2''. The total number of ligands is equal to k, the number of molecules which are bound with ss-regions by the first mechanism is equal to k_1', by the second mechanism is equal to k_1, and the number of molecules which are bound with ds-regions by the first mechanism is equal to k_2', by the second mechanism is equal to k_1''. Let F_1 and F_2 are the free energies per base pair at the coil and helical states, correspondingly, F_0 is the free energy of helix initialization, i is the free energy of binding ($i = 1,2$ and $\alpha = ',''$) and W is the number of microstates in macromolecule, corresponding to the given energy. The total free energy can be expressed [14]:

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

The helicity degree parameter of the helix–coil transition can be defined as:

$$\vartheta = \frac{1}{N} \langle N_2 \rangle, \tag{2}$$

where $\langle \dots \rangle$ is the thermodynamic average. Applying the most probable distribution method the value of $c_i^{\alpha} = \dfrac{k_i^{\alpha}}{N_i}$ has been obtained.

$$\begin{aligned}
\frac{c_1' r_1'/2}{1 - c_1' r_1'/2} &= \frac{PK_1'}{2} \left[\frac{2D}{P} - 2(1-\vartheta)c_1' - 2(1-\vartheta)c_1'' - \vartheta c_2' - \vartheta c_2'' \right], \\
\frac{c_1'' r_1''/2}{1 - c_1'' r_1''/2} &= \frac{PK_1''}{2} \left[\frac{2D}{P} - 2(1-\vartheta)c_1' - 2(1-\vartheta)c_1'' - \vartheta c_2' - \vartheta c_2'' \right], \\
\frac{c_2' r_2'/2}{1 - c_2' r_2'/2} &= \frac{PK_2'}{2} \left[\frac{2D}{P} - 2(1-\vartheta)c_1' - 2(1-\vartheta)c_1'' - \vartheta c_2' - \vartheta c_2'' \right], \\
\frac{c_2'' r_2''/2}{1 - c_2'' r_2''/2} &= \frac{PK_2''}{2} \left[\frac{2D}{P} - 2(1-\vartheta)c_1' - 2(1-\vartheta)c_1'' - \vartheta c_2' - \vartheta c_2'' \right],
\end{aligned} \tag{3}$$

where D is the total concentration of ligands, r_i^{α} is the number of binding sites per ligand, P is the total concentration of phosphate groups.

The equilibrium mean value of N_2 is obtained from the minima of free energy (1) over the variables N_2 and n such as

$$\frac{\partial F}{\partial N_2} = \frac{\partial F}{\partial n} = 0. \tag{4}$$

In the absence of ligands the helicity degree $\vartheta = \vartheta(S, \sigma)$ is governed by the helix growth parameter for the pure DNA:

$$s = \exp\left(\frac{F_1 - F_2}{T} \right) = \exp\left(\frac{\Delta H}{RT} + \Delta S \right), \tag{5}$$

where ΔH is the enthalpy and ΔS is the entropy of one base pair formation. The helicity degree is also governed by cooperativity parameter:

$$\sigma = \exp\left(-\frac{F_s}{RT} \right), \tag{6}$$

where F_s is the free energy of junction between the helical and coil regions. In presence of ligands the parameter of helix growth is changed as

$$s^* = s\frac{\left(1-c_1'r_1'/2\right)^{2/r_1'}\left(1-c_1''r_1''/2\right)^{2/r_1''}}{\left(1-c_2'r_2'\right)^{2/r_2'}\left(1-c_2''r_2''\right)^{1/r_2''}}, \tag{7}$$

where s is expressed by (5).

Results and Discussion. The helicity degree has been calculated using the equations (2),(3) and (7). In current work the ligands that have higher and lower binding affinity to native DNA has been compared. Solution of the equations (4) has been obtained numerically.

Let us consider the two most interesting cases:

Total Concentration of Ligands in Solution is Very Low $\frac{2D}{P} \ll 1$.

Calculations show that in case of higher affinity of ligands to the helical regions of DNA, the denaturation curve is shifted to the low values of the parameter S, i.e. to the high temperatures. Thus, the low ligand concentrations lead to the stabilization of ds-DNA helical structure. In case of higher affinity to the denatured state, even the small concentration of ligand in solution $\frac{2D}{P} \ll 1$ leads to the destabilization of the native state and gives hot and cold denaturation transitions (Fig. 1).

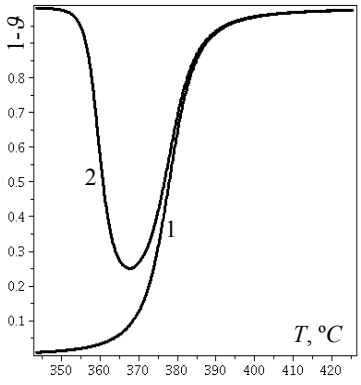

Fig. 1. Dependence of the degree of denaturation $1-\vartheta$ vs temperature: 1) for the pure DNA; 2) for $2D/P = 2.8\cdot 10^{-2}$, $r_1' = 2$, $r_1'' = 2$, $r_2' = 4$, $r_2'' = 4$, $K_1' = 9.6\cdot 10^5$, $K_1'' = 10^7$, $K_2' = 2.9\cdot 10^4$ and $K_2'' = 10^2 M^{-1}$. The enthalpy and entropy of one base pair formation are taken as $\Delta H = -33.3\ kJ/mol$ and $\Delta S = -88.1\ J/mol\cdot K$.

For the illustrative purposes we used the enthalpy $\Delta H = \frac{\Delta H_{GC} + \Delta H_{AT}}{2}$ and the entropy $\Delta S = \frac{\Delta S_{GC} + \Delta S_{AT}}{2}$ of one base pair formation. The values of parameters ΔH_{GC}, ΔH_{AT}, ΔS_{GC}, ΔS_{AT} are taken from the statement [15]. While the temperature of hot denaturation is only slightly disturbed by small concentration of ligands in solution (D), the cold denaturation temperature is substantially lower than for

the hot one. The increases of the ligands concentration suppresses the native state formation and at the certain concentrations degree of denaturation is always higher than $1/2$ (Fig. 2).

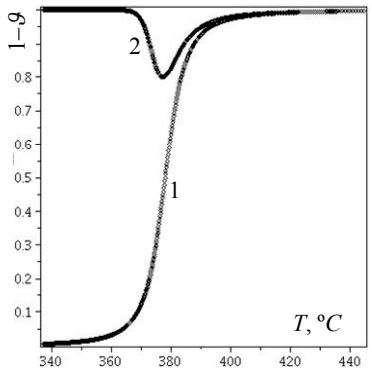

Fig. 2. Dependence of the degree of denaturation $1-\vartheta$ vs temperature: 1) for the pure DNA; 2) for $2D/P = 1.4\cdot 10^{-1}$, $r_1' = 2$, $r_1'' = 2, r_2' = 4$, $r_2'' = 4$, $K_1' = 9.6\cdot 10^5$, $K_1'' = 10^7$, $K_2' = 2.9\cdot 10^4$ and $K_2'' = 10^2 M^{-1}$. The enthalpy and entropy of one base pair formation are taken as $\Delta H = -33.3\ kJ/mol$ and $\Delta S = -88.1\ J/mol\cdot K$.

Total Concentration of Ligands in Solution is Comparable with Those for the Phosphate Groups $\dfrac{2D}{P} \sim 1$ *and* $PK_1' \gg 1$, $PK_2' \gg 1$, $PK_2'' \gg 1$.

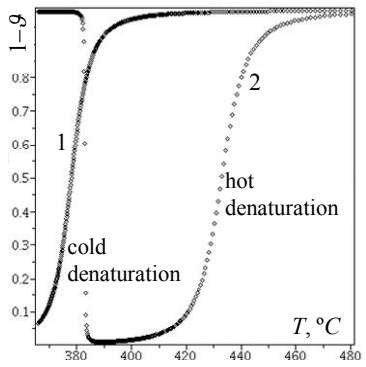

Fig. 3. Dependence of the degree of denaturation $1-\vartheta$ vs temperature: 1) for the pure DNA; 2) for $2D/P = 1.67\cdot 10^{-1}$, $r_1' = 2$, $r_1'' = 2$, $r_2' = 4$, $r_2'' = 4$, $K_1' = 2.9\cdot 10^4$, $K_1'' = 10^2$, $K_2' = 9.6\cdot 10^5$ and $K_2'' = 10^7 M^{-1}$. The enthalpy and entropy of one base pair formation are taken as $\Delta H = -33.3\ kJ/mol$ and $\Delta S = -88.1\ J/mol\cdot K$.

DNA–ligand complexes with higher affinity of ligands to the native state exhibit both high-temperature and low-temperature denaturation with the wide "window" of stable double-helix in the area of helix–coil transition of the pure DNA (Fig. 3). The high-temperature denaturation of DNA–ligand complex is substantially

shifted to the high-temperatures region in contrast with the pure DNA. At the same time, at high temperatures, when the pure DNA still remain denatured, DNA–ligand complex exhibits native state. With the temperature decrease DNA–ligand complex undergoes cold denaturation, while the pure DNA forms helical structure.

Thus, the appropriate choice of the binding constants allows to control the order parameter of the double-stranded DNA in quite wide temperature range. This mechanism should be especially important for the control of polymer nano-composites crystallization [14], where it is needed to cool the sample, but at the same time to keep the DNA molecules at the ordered state.

Thus, we can conclude from the obtained results, that:

Conclusion.

- DNA complexes with two types of ligands, that have different binding parameters with ss- and ds-DNA exhibit both hot and cold denaturation independent from the affinity to the native or denatured states.
- In case of higher affinity to the native state, there is a range of high temperature, at which DNA–ligand complex is in native state.
- In case of higher affinity to the native state, the temperature of hot denaturation of DNA–ligand complex is significantly lower, than it is in case of pure DNA.
- In case of higher affinity to the denatured state the temperature of cold denaturation of DNA–ligand complex is substantially less than it is in case of pure DNA.

The helicity degree is suppressed by the high affinity of the ligands to the ss-DNA.

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