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МОЛЕКУЛЯРНА БІОФІЗИКА

BINDING ENERGY DIFFERENTIATION OF WATER MOLECULES IN DNA HYDRATION SHELL

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An energetics study of water molecule binding to a DNA matrix has been performed. Bound water molecules have been differentiated according to their binding energies, the water molecules being supposed to participate in three types of sorption (hydration): sorption by Langmuir's binding centers, sorption by Henry's ones and multilayer sorption. It has been found that waters of the different sorption types take part in stabilizing a certain DNA conformation to a different degree. Consideration of the obtained values of free binding energies within each sorption type has shown that waters bound to Langmuir's centers mainly advantage A-DNA stabilization while ones bound to Henry's centers facilitate B-DNA stabilization. The water molecules bound in multilayer are more important for stabilization of both A- and B-form in comparison with unordered state, but largely for B-form. This is due to occurring the basic formation of multilayer part of hydration surroundings at high water content in DNA.

KEY WORDS: DNA, conformations, hydration, sorption, binding energy

Structural state of DNA molecule is well known to depend on its water content to a high degree [1-3]. While being wetted or dried a DNA molecule undergoes conformational transitions, i.e., changes of secondary structure. For instance, NaDNA demonstrates an unordered state at relative humidity (r.h.) up to 74-76%, A-form up to 92-94% r.h., then NaDNA exists in B-form [3]. Relationship between DNA structure and its properties makes a problem of study of the DNA-water system undoubtedly of great interest. Determining thermodynamic parameters of the DNA-water systems and their variation in hydration-dehydration process is a part of the problem which is important to come to understanding the DNA organization and functioning. One of such parameters is binding energy of water molecules to the DNA matrix. This energy corresponds to the part of DNA stabilization energy contributed from the hydration surroundings. Integral values of binding energy have already been determined by both direct measurements (microcalorimetry method) [1] and in indirect manner (gravimetry and infrared spectroscopy methods) [1, 4, 5]. However, such data do not tell us about energetic properties of the distinct water molecules in the DNA-water interaction.

In the present paper an attempt to differentiate the energetic characteristics of the distinct water molecules in binding process to NaDNA has been made. The free energies corresponding to the binding processes of water molecules to different hydration center types have been determined by using data obtained by microcalorimetry method and a model of conformation dependent hydration of DNA [6].

THEORY

Generally it is more convenient to consider a binding process of some substance (sorbate) on a matrix (sorbent) in terms of the binding energy excess $\Delta E = E - E_0$ (E is energy of sorbate-sorbent interaction, E_0 is energy of interaction between sorbate molecules in bulk phase) [1]. The greater a value of the binding energy excess ΔE the stronger interaction between the sorbed molecules and the matrix, which characterizes the binding process demonstrably. In case of the hydration within the DNA-water system involved

$$\Delta E(n) = E(n) - E_0, \quad (1)$$

where n is the water content in the system expressed as moles of water per mole of nucleotides ($\text{MH}_2\text{O}/\text{MN}$), $\Delta E(n)$ is the hydration energy excess, $E(n)$ is the hydration (dehydration) energy obtained experimentally, $E_0 = 40.5 \text{ kJ}/\text{MH}_2\text{O}$ – the mean energy of water-water interaction in the bulk phase [1]. So, ΔE represents binding energy of water molecule with a hydration center of DNA.

For DNA one can distinguish several types of hydration centers different in their character and strength of binding [7]. Dividing sorption (hydration) into three types is shown to be adequate to describe the DNA hydration almost completely [8]. There are the following types of sorption: a) by Langmuir's binding centers supposedly lying on nitrogenous bases (hydration with saturation); b) by Henry's binding centers disposed apparently on cation-phosphate groups (hydration without saturation); c) multilayer sorption (hydration of previously occupied binding sites). It can be shown that such a separation is possible in the framework of

Gascoyne-Pethig theory provided one chooses appropriate partial distribution functions [6, 9]. We neglect heterogeneity of binding centers within one hydration type not to complicate the problem. Thus, a binding energy of water molecules with hydrate-active centers ΔE is considered as a sum of three summands representing the contributions to the value of the energy from water molecules sorbed after three mentioned types:

$$X_L(n)\Delta E_L + X_H(n)\Delta E_H + X_{ML}(n)\Delta E_{ML} = \Delta E(n) \quad (2)$$

where $X_L(n)$, $X_H(n)$, $X_{ML}(n)$ are the fractions of water molecules bound to Langmuir's, Henry's binding centers and in multilayer with energies ΔE_L , ΔE_H , and ΔE_{ML} , respectively. It is necessary to note that the partial energies are considered not to depend on the water content but to be constant for a certain type of sorption.

It is more general to describe sorption in free energy terms, therefore, proceeding to the free energies of sorption we obtain:

$$X_L(n)\Delta G_L + X_H(n)\Delta G_H + X_{ML}(n)\Delta G_{ML} = \Delta G(n) \quad (3)$$

where ΔG_L , ΔG_H , and ΔG_{ML} are the free binding energies contributed to the total free binding energy ΔG from water molecules bound after different types.

In the framework of the equilibrium thermodynamics: $\Delta G = \Delta H - T\Delta S$ or in the units of RT $\Delta G = \Delta H - \Delta S$. (Herefrom and then throughout all thermodynamic parameters are expressed in the units of RT per mole of water.) Thus, considering the total changes of binding entropy ΔS and enthalpy ΔH corresponding to the total change of sorbed water molecule number, we have:

$$X_L(n)\Delta G_L + X_H(n)\Delta G_H + X_{ML}(n)\Delta G_{ML} = \Delta H(n) - \Delta S(n) \quad (4)$$

The fact that DNA hydration depends on conformational state of the biopolymer [2, 3] allows us to assume that the energy contribution of each type of bound water is conformational-dependent. As NaDNA exists in three possible conformations over the whole r.h. range (unordered state, A- and B-form), we take into account the energy contributions from water molecules sorbed after types concerned to the stabilization of these conformational states:

$$X_L(n)A(n)\Delta G_L^A + X_L(n)B(n)\Delta G_L^B + X_L(n)U(n)\Delta G_L^U + X_H(n)A(n)\Delta G_H^A + X_H(n)B(n)\Delta G_H^B + \\ + X_H(n)U(n)\Delta G_H^U + X_{ML}(n)A(n)\Delta G_{ML}^A + X_{ML}(n)B(n)\Delta G_{ML}^B + X_{ML}(n)U(n)\Delta G_{ML}^U = \Delta H(n) - \Delta S(n) \quad (5)$$

where $A(n)$, $B(n)$, $U(n)$ are the fractions of A-, B-conformation and unordered form in DNA secondary structure, respectively; ΔG_k^m is a desirable value of energy contribution to the stabilization of m -th conformation from water molecules participating in hydration of k -th type ($k=L, H, ML$; $m=A, B, U$).

The values of the total change of binding enthalpy $\Delta H(n)$ have been determined in work [1] by using microcalorimetry technique. This technique allows one to obtain values of $\Delta H(n)$ in a certain narrow interval of r.h. (from ϕ_1 to ϕ_2) or water content (from n_1 to n_2). $\Delta H(n)$ corresponds to the evaporation enthalpy of Δn water molecules from DNA matrix, where $\Delta n = n_2 - n_1$ and $n = (n_1 + n_2)/2$. Dependence of entropy ΔS on water content n was obtained by Bolbukh and coworkers [10] by using a hydration isotherm analysis. Since there are nine unknowns ΔG_k^m in equation (5), the values of ΔH and ΔS were taken at nine different values of water content n . Thus, writing down equation (5) for nine n values, one can obtain a closed set of equations:

$$\left\{ \begin{array}{l} X_L^{(1)}(n)A^{(1)}(n)\Delta G_L^A + X_L^{(1)}(n)B^{(1)}(n)\Delta G_L^B + X_L^{(1)}(n)U^{(1)}(n)\Delta G_L^U + X_H^{(1)}(n)A^{(1)}(n)\Delta G_H^A + \\ \quad + X_H^{(1)}(n)B^{(1)}(n)\Delta G_H^B + X_H^{(1)}(n)U^{(1)}(n)\Delta G_H^U + X_{ML}^{(1)}(n)A^{(1)}(n)\Delta G_{ML}^A + \\ \quad + X_{ML}^{(1)}(n)B^{(1)}(n)\Delta G_{ML}^B + X_{ML}^{(1)}(n)U^{(1)}(n)\Delta G_{ML}^U = \Delta H^{(1)}(n) - \Delta S^{(1)}(n), \\ \quad \dots \quad \dots \quad \dots \\ X_L^{(9)}(n)A^{(9)}(n)\Delta G_L^A + X_L^{(9)}(n)B^{(9)}(n)\Delta G_L^B + X_L^{(9)}(n)U^{(9)}(n)\Delta G_L^U + X_H^{(9)}(n)A^{(9)}(n)\Delta G_H^A + \\ \quad + X_H^{(9)}(n)B^{(9)}(n)\Delta G_H^B + X_H^{(9)}(n)U^{(9)}(n)\Delta G_H^U + X_{ML}^{(9)}(n)A^{(9)}(n)\Delta G_{ML}^A + \\ \quad + X_{ML}^{(9)}(n)B^{(9)}(n)\Delta G_{ML}^B + X_{ML}^{(9)}(n)U^{(9)}(n)\Delta G_{ML}^U = \Delta H^{(9)}(n) - \Delta S^{(9)}(n). \end{array} \right. \quad (6)$$

Binding energy differentiation of water molecules in DNA hydration shell

The values of parameters $A(n)$, $B(n)$, $U(n)$, $X_L(n)$, $X_H(n)$, $X_{ML}(n)$ can be determined from a model of conformation dependent hydration of DNA [6] where they are variables and ΔG_k^m included as parameters. This model takes into account mutual influence of the DNA hydration and conformational state of DNA, allowing one to simulate hysteresis phenomenon during hydration-dehydration cycle. Considering jointly the set of equations (6) and one featuring in the model of conformation dependent hydration of DNA, we have closed problem. The problem can be solved by using successive approximation method in which the found values of variables of one set are substituted into another set as parameters and then the procedure repeats.

RESULTS AND DISCUSSION

The most reasonable and rational values of ΔG_k^m resulted from solving the problem considered are presented in tab. 1.

Table 1. Values of the free binding energy changes for water molecules bound after different sorption types and participating in stabilizing the possible conformational states of NaDNA

| ΔG_k^m | Type of conformational state stabilized (m) | | |
|--------------------------|---|--------|-----------------|
| | A-form | B-form | Unordered state |
| Type of sorption (k) | | | |
| L | 3.99 | 3.69 | 3.57 |
| H | 1.97 | 2.16 | 1.86 |
| ML | 0.18 | 0.19 | 0.01 |

Analysis of ΔG_k^m values within each sorption type shows that waters bound to Langmuir's centers mainly advantage A-form stabilization while ones bound to Henry's centers facilitate B-form stabilization. This agrees

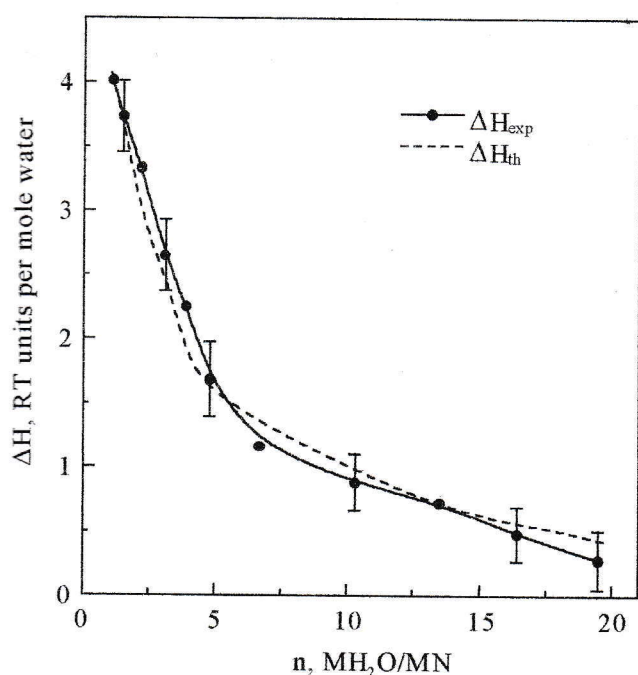


Fig. 1. Dependencies of enthalpy change obtained experimentally [1] and calculated in this work on the water content.

with an idea that hydration of nitrogenous bases and one of phosphates play the leading role in stabilizing the A- and B-form of DNA, respectively [2, 3]. Water molecules bound in multilayer are more important for stabilization of both A- and B-form in comparison with unordered state although they stabilize largely B-form than A-form. This is due to the fact that the basic formation of multilayer part of hydration surroundings occurs at high water content in DNA [2, 3].

Comparison of the results obtained with experimental ones [1] is presented in fig 1. In this figure the experimental values of enthalpy change ΔH_{exp} [1] and the ΔH_{th} values calculated from set (6) by using ΔG_k^m obtained here and $\Delta S(n)$ [10] are plotted as dependencies on the water content n . It is seen that the curves coincide well enough over the interval of the water content considered. Deviation of the theoretical results from the experimental ones does not exceed the values of experimental error, which attests adequate approximation in the calculation of ΔG_k^m achieved during solving the problem. Decrease of the enthalpy values with the water content growth in the system indicates that the fraction of water molecules

strongly bound to primary hydration centers (Langmuir's and Henry's ones) on the DNA matrix reduces due to increase of weak secondary sorption of water, i.e. multilayer sorption.

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РАЗДЕЛЕНИЕ МОЛЕКУЛ ВОДЫ В ГИДРАТНОЙ ОБОЛОЧКЕ ДНК ПО ЭНЕРГИЯМ СВЯЗЫВАНИЯ

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В данной работе проводилось изучение процесса связывания молекул воды с матрицей ДНК. Произведено разделение молекул воды гидратной оболочки ДНК по их энергиям связывания. При этом предполагалось, что молекулы воды могут участвовать в процессах сорбции (гидратации) трех типов: сорбции на Лэнгмюровские центры связывания, сорбции на Генриевские центры связывания и мультислойной сорбции, т.е. сорбции на уже занятые места связывания. Обнаружено, что молекулы воды, сорбированные по различному типу, принимают участие в стабилизации определенной конформации ДНК в различной степени. Анализ полученных значений свободных энергий связывания молекул воды в пределах каждого типа сорбции показал, что вода, связанная с Лэнгмюровскими центрами, главным образом способствует стабилизации А-ДНК. В то же время основная роль молекул воды, связанных с Генриевскими центрами заключается в стабилизации В-ДНК. Молекулы воды, образующие мультислой, наиболее важны для стабилизации как А-, так и В-формы ДНК по сравнению с неупорядоченным состоянием, хотя в большей мере это касается В-формы. Такое поведение молекул мультислоя может объясняться тем фактом, что основное формирование мультислоя происходит при высоком содержании воды, т.е. когда идет образование В-формы ДНК.

КЛЮЧЕВЫЕ СЛОВА: ДНК, конформации, гидратация, сорбция, энергия связывания