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SURFACE DIFFUSION OF BOUND WATER IN HYDRATE SURROUNDINGS OF DNA

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Diffusion of water bound to a DNA molecule along its surface has been studied. A lattice model has been proposed to describe this process. The DNA sorbing surface has been considered as a fractal one. The values of the water diffusion coefficient, which depend on relative humidity of environment, have been estimated in the framework of the model within each interval of the ambient relative humidity where the DNA restores its conformation. They increase monotonously within each interval involved to about $5 \cdot 10^{-11}$, $1.7 \cdot 10^{-10}$ and $3.5 \cdot 10^{-10}$ m²/s for partly disordered form of DNA, A- and B-DNA, respectively. Also, we estimated values of longitudinal diffusion coefficient, i.e. the diffusion directed along the helix axis, which for conformational states considered run into about $9 \cdot 10^{-11}$, $3.8 \cdot 10^{-10}$ and $7 \cdot 10^{-10}$ m²/s. At the highest relative humidities the diffusion coefficient values tend to the values of the water self-diffusion coefficient for DNA solutions. We consider this fact as a result of increasing the number of the water molecules indirectly bound to the DNA binding sites.

KEY WORDS: DNA molecule, water sorption, bound water, surface diffusion, fractal dimension

Ion-hydrate surroundings play a fundamental role in determining a structure and physical properties of a DNA molecule [1-3 and references therein]. Conformational state of a DNA molecule depends strongly on its water content as well as on concentration and type of the counterions [1-5]. Therefore, the problem of studying the properties of the DNA surrounding elements is of great interest. One of the base physical characteristics of interactions between the ion-hydrate shell and the DNA molecule is a water diffusion coefficient near the DNA molecule surface.

Beginning with the work of Wang [6], researchers studied the diffusion process systematically. *In vivo* most often a DNA molecule is in a closely packed and ordered form. This fact caused an interest to investigate the diffusion process in ordered DNA samples both experimentally and theoretically. There have been obtained the diffusion coefficients of counterions and water for macroscopic, i.e. intermolecular, diffusion process (for review see [7] and references therein). As for a water diffusion, data on the diffusion coefficient of bulk water and water in dilute solutions of DNA, in which characteristics of the hydration water are spread, are available [6,8]. Besides, in the work [9] the diffusion coefficients of water molecules in oriented wetted DNA films have been experimentally determined. However, theory of the bound water diffusion in a DNA-water system with a small water content has not been developed enough yet.

This paper is an attempt to stop a gap in this field to some degree. We have carried out estimation of the water diffusion coefficients in the hydrate surroundings of an individual NaDNA molecule for various water contents (in the whole relative humidity (r.h.) range).

THEORY

Molecules adsorbed on a solid surface are in the thermal motion and in the presence of a concentration gradient of an adsorbate phase a diffuse stream against the gradient direction appears. In the present paper a simple model of adsorbed water translational diffusion along a DNA matrix is proposed. As a basis we took the approach described by Frenkel [10] in the kinetic theory of real crystals. As known the theory of such a type is used to describe self-diffusion in liquids and solids. Classic Frenkel-Eyring theory supposes a molecular motion in liquids and real crystals to be a one-particle one, a particle travelling in a potential field spatially periodic and stationary [10]. The motion consists of oscillations near an equilibrium position in the vicinity of the bottom of a potential well and periodic jumps over the barrier to a new equilibrium position. In case of a biopolymer-water system we assume that an application of this theory is possible as a certain approximation due to existence of the distinct water binding sites on the biopolymer surface and a low water content in the system. That is to say "condensed" state of the DNA-water system is studied here.

In order to describe the diffusion process let us consider the polymer matrix as a lattice structure with a period equal to a . In this case migration of water molecules adsorbed is a thermal-activated process due to

energy barriers on the adsorbent surface. The energetic heterogeneity in such a structure arises because of spacing of the matrix elements. Let us assume also that each water molecule adsorbed is disposed in a lattice knot (a binding site) which, in its turn, is situated within a nucleotide. The diffusion process is considered as a process of a water molecule release from one binding site with subsequent occupation of the nearest one by a water molecule. One should note that in the DNA-water system involved there is no bulk water, therefore, a process of exchange with bulk water is not considered.

Let $w=n/N$ be the probability of finding a binding site being occupied by a water molecule, where N is a total number of binding sites in the DNA sample, n is number of the binding sites occupied. Within time interval t a binding site is occupied for time $t_{oc}=wt$ and the number of occupation events M is equal to wt/τ , where τ is the lifetime of a water molecule in the bound state or the duration of an occupation event. Then, $\tau_{tr}=t/M = \tau/w$ is the mean time between release of one binding site and occupation of another one or the time for which a water molecule displacement between the two binding sites equal to a occurs. In other words τ_{tr} is the time between two "collisions" of the water molecule with knots, i.e. the translational time.

The water molecules are assumed to diffuse according to Fick's law. In the case of one-dimensional diffusion one can write down some modified solution of the Fick's diffusion equation, which spatially corresponds to a Gaussian's distribution of squared standard deviation $\sigma^2=2\mathcal{D}_1t$ [8]:

$$P(x_0|x, t) = (4\pi\mathcal{D}_1t)^{-1/2} \exp\left(-\frac{(x-x_0)^2}{4\mathcal{D}_1t}\right), \quad (1)$$

where parameter \mathcal{D}_1 is a diffusion coefficient (for the case of one-dimensional diffusion); $P(x_0|x, t)$ is a probability of finding a molecule at the moment t in the point with coordinate x provided it was in the point x_0 at the zero time. The squared standard deviation σ^2 is equal to the mean square displacement along a single direction of a molecule from its starting point. An analogy with the problem of random walks of a particle can be traced here. Taking into account the fact that the value of the mean square displacement from starting point for time τ_{tr} is fixed (equal to a^2) and assuming displacements along different directions are statistically independent, we have $\langle x^2 \rangle = a^2 = 2\mathcal{D}_1\tau_{tr}$, i.e.:

$$\mathcal{D}_1 = \frac{a^2}{2\tau_{tr}} = \frac{a^2}{2\tau} w \quad (2)$$

By analogy with the case of one-dimensional diffusion the diffusion coefficients of two- and three-dimensional diffusion can be obtained. They are expressed as $\mathcal{D}_2 = a^2/4\tau_{tr}$ and $\mathcal{D}_3 = a^2/6\tau_{tr}$, respectively. The diffusion coefficients for the different dimension problems are seen to differ only with a numerical factor. It is obvious this factor is equal to $1/2d$, where d is a dimension of the diffuse problem. Thus, in a general case we have:

$$\mathcal{D} = \frac{a^2}{2d\tau} w. \quad (3)$$

Therefore, in consideration of the diffusion process of bound water along the DNA surface a question of DNA molecule dimension arises. To assume the diffusion involved as a two-dimensional one is incorrect since the DNA molecule is known to have a very complicated structure of its surface. In connection with this fact it is necessary to note that surfaces of the most sorbents are very uneven. Such surfaces are characterized with a fractal dimension, D . The values of D lie within interval $2 \leq D \leq 3$ although some sorbents have a fractal dimension even a little greater than 3 [11]. Also, it is interesting that the fractal dimension values of water clusters bound to DNA molecules are within the same interval of the D values [12]. The problem of the fractal dimension of DNA surface has been considered in work [13]. In the work the fractal dimensions of the A- and B-DNA surfaces, \bar{D} , have been obtained by using a method of determination of DNA accessible surface [11, 14]. They are equal to about 3.0 and 2.5 for A- and B-form of DNA, respectively.

Thus, calculating the water diffusion coefficients in the hydrate surroundings of the NaDNA by using the equation (3), we have to substitute in this equation the fractal dimension \bar{D} for a topological one d for each form of the DNA molecule:

$$\mathcal{D} = \frac{a^2}{2\bar{D}\tau} w. \quad (4)$$

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The evaluative calculations were realized in the following way. From the whole region of r.h. from 0 to 100% (that corresponds to the number of water molecules per nucleotide (n) from 0 to about 22 [2]) three intervals were chosen by using a criterion of the existence of certain DNA forms having their definite structural characteristics in these intervals. Three distinct DNA structures exist in the r.h. region involved: a B-like form [1] or in P-form [15] (r.h. $\leq 72\%$, $n \leq 7$), A-form (r.h. = 78-90%, $n = 8-15$) [2], and B-form (r.h. $> 92\%$, $n > 16$) [2]. Therefore, the water diffusion coefficients were estimated for the following intervals of n :

- (1) $n \leq 7$ (B-like form);
- (2) $n = 8-15$ (A-form);
- (3) $n = 16-22$ (B-form).

The intervals $n = 7-8$ and $n = 15-16$ correspond to conformational transitions from the B-like form to A-form and from A-form to B-form of DNA, respectively. Therefore, it is problematical to say about the DNA structure in these intervals. In connection with this the calculations for these values of n cannot be performed by using procedure described.

The τ values necessary for the diffusion coefficient estimations have been calculated by using an equation linking the lifetime of a water molecule in the bound state with the hydration energy excess $\Delta E(n)$:

$$\tau(n) = \tau_0 \exp(\Delta E(n)/RT) \quad (5)$$

where τ_0 (equal to about $2.5 \cdot 10^{-10}$ s [16]) is the lifetime of a water molecule in the bound state in bulk water at room temperature; R is the universal gas constant; T is temperature; n is water content expressed as number of moles of water per mole of nucleotide ($\text{MH}_2\text{O}/\text{MN}$), which corresponds to the number of the binding sites occupied. Besides, for calculation of $w = n/N$ figuring in the equation (4) the N value is taken as the total number of water molecules per a nucleotide necessary to form the DNA hydration shell and equal to about 22 (in other words $N = n$ at 100% r.h.).

In energy terms a water molecule can be considered "bound" if its energy of interactions E with an hydrate-active center exceeds the mean energy of water-water interaction in liquid phase ($E_0 = 40.5 \text{ kJ}/\text{MH}_2\text{O}$). Then the hydration energy excess ΔE is equal to $E - E_0$. We made use of data on dependence of the hydration energy excess on n obtained by direct microcalorimetry measurements for NaDNA [2].

In the first interval of n ($n \leq 7$) mainly phosphates are hydrated [17] although hydration of sugars and bases is also observed [2]. Within this interval DNA molecule exists in partly disordered state, structural parameters of the sugar-phosphate backbone resembling ones for B-form of DNA [1]. Since NaDNA is supposed to be in the B-like form, which is structurally similar to the B-form, the surface dimension \bar{D} for the B-like form is accepted the same one as for the B-form of DNA.

In the second interval of n ($n = 8-15$) where NaDNA is in the A-form there are the following features of hydration: the clear primary shell of sugars and bases are absent while the most polar groups, phosphates, are hydrated and their hydration shells are high-ordered (in contrast with B-form); in the grooves there are no water spines, however, functional groups exposed to the grooves are hydrated; the water bridges binding adjoining phosphates are formed along each strand of DNA; there also exist the water bridges binding the phosphate groups of different strands across the major groove, which tighten its edges [3, 18].

In the last interval of n ($n = 16-22$) where the B-form exists, the hydration reaches its high level: phosphates are hydrated significantly; there are hydration shells of bases and sugars; the binding sites in the grooves are taken by water molecules (thus, the water spines in the grooves are formed); the water bridges bind bases and phosphates of the opposite strands [3, 18, 19].

Estimates of the values of the lattice parameter a were found from consideration of water molecule transitions between various hydration centers. These transitions are supposed to occur both within the same DNA strand (e.g. between phosphate groups) and between strands across and inside the grooves. Besides, in the two latter cases (A- and B-form) the formation of water bridges was allowed for [3, 18, 19]. Taking into account the possible hydration centers, their implication to the hydration process with water content growth, and distances between them, after averaging over the distances for possible transitions we obtained the a values for B-like form, A- and B-form equal to about 8.2 Å, 6.9 Å and 7.7 Å, respectively.

RESULTS AND DISCUSSION

Dependence of the lifetime of a water molecule in the bound state, τ , on r.h. (the water content in the system) is presented in fig. 1.

As one can see from this figure the τ values decrease about by factor of 50 when the water content in the system changes from low to high values. This fact clearly demonstrates decreasing the energy of interaction of water molecules with the hydrate-active centers. In the work [9] the experimental results of pulsed proton NMR

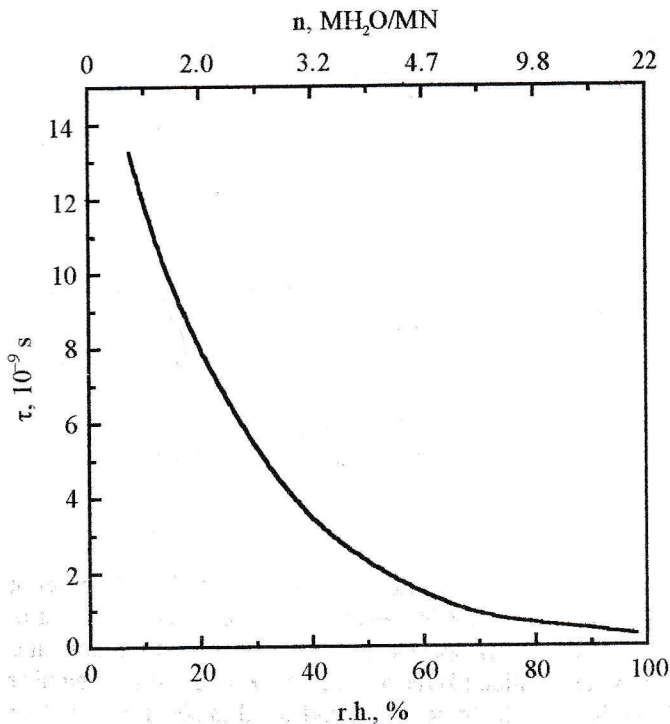


Fig. 1. Dependence of the lifetime of a water molecule in the bound state on r.h. (water content in the NaDNA-water system).

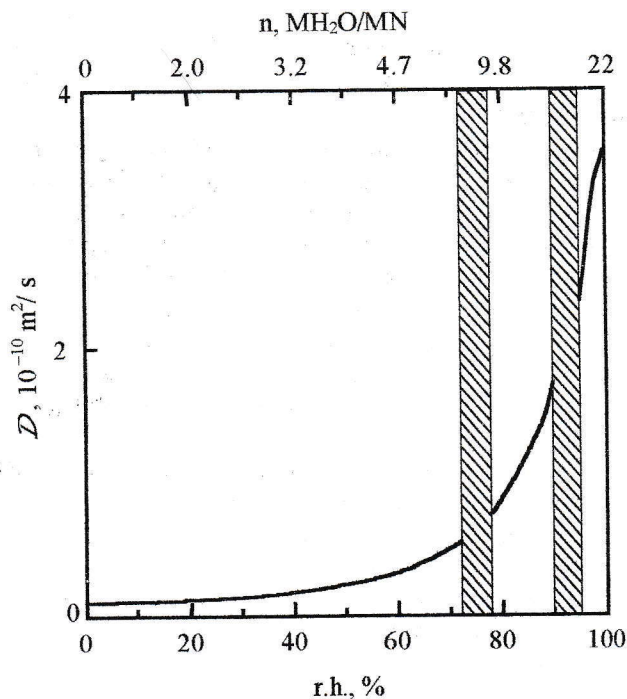


Fig. 2. Dependence of the diffusion coefficient of the bound water molecules along the NaDNA surface on r.h. (water content). Hatched areas correspond to r.h. intervals of conformational transitions, which have been eliminated from consideration in the present study.

relaxation and diffusion study of water sorbed in solid oriented NaDNA samples at moderate r.h. (48.8% - 86.6% r.h.) are reported. Our τ values are in good agreement with the estimate of τ (about 10^{-9} s) obtained in this work in the appropriate water content region.

Real DNA naturally is not "static" object and its structure fluctuates. However, times of these motions are on the time scale of microseconds. These times are much greater than ones considered in our case. Therefore, we did not take these motions into account in the present model.

Dependence of the diffusion coefficient \mathcal{D} calculated from equation (4) with appropriate values of the parameters on r.h. (water content) is plotted in fig. 2. Three sections of the dependence correspond to the hydration intervals where the diffusion coefficient estimation has been performed: in the first section r.h. $\leq 72\%$ ($n \leq 7$), in the second section r.h. = 78-90% ($n=8-15$), and in the last one r.h. $> 92\%$ ($n > 16$).

The dependence of the diffusion coefficient \mathcal{D} increases monotonically in each section of graph (fig. 2). Such a behavior of \mathcal{D} values can be explained by reducing the part of the water molecule "strongly" bound to the DNA due to multilayer sorption with small binding energies.

Steps of the \mathcal{D} values in case of a passage from one section of the graph to another one are probably connected to rearrangement of secondary structure of the DNA molecule and hydration shell, i.e., of geometry of the system considered, during conformational transitions.

One of particular cases of the bound water diffusion is a longitudinal one \mathcal{D}_1 , i.e. the diffusion directed along the DNA helix axis. Such a type of diffusion is supposed to be a one-dimensional diffusion. Thus, in the model one should consider the biopolymer matrix as a one-dimensional lattice (in the equation (4) \bar{D} is equal to 1) and, therefore, use the equation (2) to estimate values of the longitudinal diffusion coefficient \mathcal{D}_1 . The values of the lattice parameter a , which corresponded to the mean displacement of a water molecule in the direction of the helix axis, were taken as the averaged values of the axis projections of the water molecule transitions considered in the

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previous case of diffusion along the fractal surface. For the three n intervals involved the a values are equal to $\sim 7\text{\AA}$ for $n \leq 7$, $\sim 6.1\text{\AA}$ for $n=8-15$ and $\sim 6.7\text{\AA}$ for $n=16-22$. In this case the w values were the same ones as for the diffusion along the fractal surface.

Dependence of the longitudinal diffusion coefficient \mathcal{D}_1 calculated from equation (2) with appropriate values of the parameters on r.h. (water content) is presented in fig.3. It is seen from this figure that the dependence obtained demonstrates similar features as one in the previous case of diffusion. However,

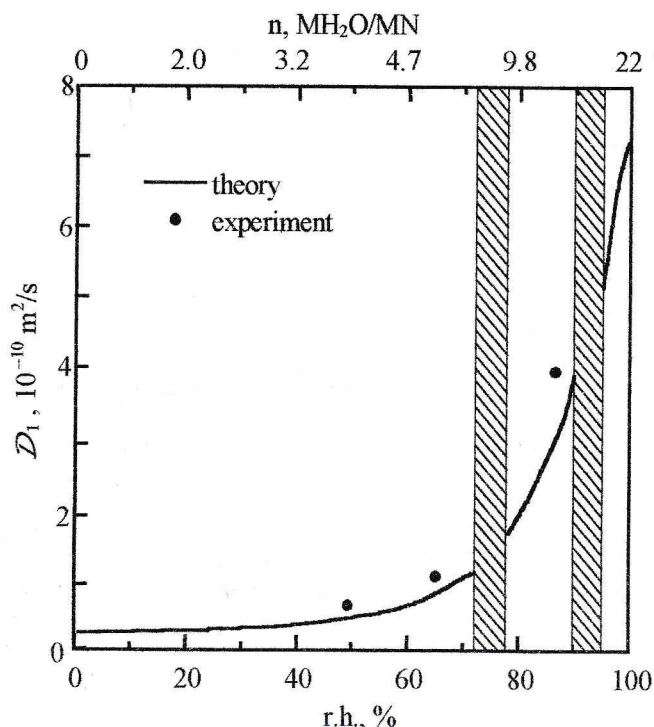


Fig. 3. Dependence of the longitudinal diffusion coefficient of the bound water molecules on r.h. (—). For comparison the experimental data are presented (•) [9]. Hatched areas correspond to r.h. intervals of conformational transitions, which have been eliminated from consideration in the present study.

equations (2) or (4) it is seen while the water content in the sample grows the τ values decrease and the w values increase, and this will cause the gradual increasing of the diffusion coefficient values. They will finally tend to a limiting value.

Our calculations have been made for hydrate surrounding of NaDNA, however, we believe that the procedure described in the present paper is suitable for a case of DNA with other counterions.

The diffusion of the bound water molecules along the DNA surface studied in the present paper could contribute to long-range effects of conformational reorganization [20]. In any case such a water diffusion should be taken into account when local conformational changes of a DNA molecule are considered.

REFERENCES

- Lindsay S.M., Lee S.A., Powell J.M., Weidlich T., DeMarko C., Lewen G.D., Tao N. J., Rupprecht A. // *Biopolymers*. 1988. V.27. P.1015-1043.
- Maleev V. Ya., Semenov M. A., Gasan A. I. and Kashpur V. A. // *Biophysics*. 1993. V. 38. P.789-811.
- Saenger W. // *Ann.Rev.Biophys.Biophys.Chem.* 1987. V.16. P.93-114.
- Lavalle N., Lee S.A., Rupprecht A. // *Biopolymers*. 1990. V.30. P.877-887.
- Weidlich T., Lindsay S.M., Rupprecht A. // *Phys. Rev. Lett.* 1988. V.61. P.1674-1677.
- Wang J.H. // *J. Am. Chem. Soc.* 1955. V.77. P.258-260.
- Andreasson B., Nordenskiöld L., Eriksson D.-O., Rupprecht A. // *Biopolymers*. 1994. V.34. P.1605-1614.
- Stejskal E.O., Tanner J.E. // *J. Chem. Phys.* 1965. V.42. P.288-292.
- Lahajnar G., Zupancic I., Rupprecht A. // *Biophysics of Water*. New York: Wiley. 1982. P.231-234.
- Frenkel Ya. I. *Kinetic theory of liquids*. L. Nauka, 1975. 592p.

quantitative differences are observed. The values of the longitudinal diffusion coefficient are greater than the values of the fractal surface one. This is due to the fact that the general case of the surface diffusion comprises the diffusion processes in all directions over the surface (including slow lateral diffusion) and, therefore, total process goes slower than the longitudinal diffusion. Our \mathcal{D}_1 values agree with the experimental ones presented in fig.3 [9] in the appropriate intervals. The experimental data considered correspond to the case of diffusion parallel to the DNA helix axis. The good agreement between the theoretical and experimental results attests adequacy of the longitudinal diffusion process description by using the equation (2). At high r.h. the \mathcal{D} values calculated tend to the water self-diffusion coefficient for DNA solutions obtained in the work [6].

It is obvious if water content in the DNA-water system grows further the values of the diffusion coefficient will gradually increase, approaching the value of the diffusion coefficient for bulk water ($2.5 \cdot 10^{-9} \text{ m}^2/\text{s}$). Qualitatively such a behavior of the \mathcal{D}_1 (and \mathcal{D}) values can be demonstrated in the framework of the model. From the third section shape of the \mathcal{D}_1 (and \mathcal{D}) dependence as well as from the

11. Feder E. Fractals. M. Mir, 1991. 262p.
12. Bul'enkov N. A. // Biofizika. 1991. V.36. P.181-243.
13. Virnik K.M., Tolstorukov M.Ye. // Vest. problem v biol. i meditsine. 1998. No.2. P.5-16.
14. Nauchitel V. V. and Bayramov Sh. K. // Molekulyarnaya biologiya. 1991. V.25. P.162-171.
15. Zehfus U.H., Johnson W.C. // Biopolymers. 1984. V.23. P.1269-1281.
16. Gordeev G.P., Haydarov T. // Water in Biological Systems and Their Components. L. Lenigrad. Univ., 1983. P.3-9.
17. Falk M., Hartman K.A., Lord R.C. // J. Am. Chem. Soc. 1963. V.85. P.387-391.
18. Saenger W., Hunter W.N., Kennard O. // Nature. 1986. V.324. P.385-388.
19. Drew H.R., Dickerson R.E. // J. Mol. Biol. 1981. V.151. P.535-556.
20. Kim U.-S., Fujimoto B.S., Furlong C.E., Sundstrom J.A., Humbert R., Teller D.C., Schurr J.M. // Biopolymers. 1993. V.33. P.1725-1745.

ПОВЕРХНОСТНАЯ ДИФФУЗИЯ СВЯЗАННОЙ ВОДЫ В ГИДРАТНОМ ОКРУЖЕНИИ ДНК

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Рассматривается поверхностная диффузия молекул воды, связанных на матрице ДНК. Для описания этого процесса предлагается решеточная модель. Сорбирующая поверхность ДНК рассматривается в качестве фрактальной. В рамках предложенной модели произведены оценки коэффициента диффузии воды в интервалах относительной влажности окружающей среды, в которых ДНК сохраняет свое конформационное состояние. Рассчитанные величины оценок коэффициента поверхностной диффузии связанных молекул воды увеличиваются с ростом содержания воды в ДНК и достигают значений $5 \cdot 10^{-11}$, $1,7 \cdot 10^{-10}$ и $3,5 \cdot 10^{-10}$ м²/с для частично разупорядоченного состояния, А- и В-формы соответственно. Кроме того, были получены оценки значений коэффициента продольной диффузии, т.е. диффузии, направленной вдоль оси спирали. Максимальные значения этих оценок приблизительно равны $9 \cdot 10^{-11}$, $3,8 \cdot 10^{-10}$ и $7 \cdot 10^{-10}$ м²/с для рассматриваемых конформационных состояний. При высоких относительных влажностях значения коэффициентов диффузии стремятся к величинам коэффициента самодиффузии воды в растворах ДНК. Этот факт рассматривается как результат увеличения числа молекул воды, слабо связанных с сорбирующими центрами ДНК.

КЛЮЧЕВЫЕ СЛОВА: молекула ДНК, сорбция воды, связанная вода, поверхностная диффузия, фрактальная размерность