PROPERTIES OF DOUBLE-STRANDED DNA AS A POLYELECTROLYTE

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ABSTRACT The stability of the structure of double-stranded DNA in the salt-free solution is discussed on the basis of the polyelectrolyte theory. Assuming that DNA is an infinitely long rod, and the formation of double strands is divided into combining process and folding process, the free energy changes required in these processes are calculated by the use of the exact solutions of two-dimensional Poisson-Boltzmann equation for the one rod and the two rod systems.

By strong depression of electrostatic interaction due to counter-ion condensation phenomena, the free energy change is remarkably decreased so that the double-stranded structure of DNA can be stabilized by energy of hydrogen bonds between base pairs. The increase of the activity coefficient of a counter-ion upon heat denaturation of DNA is also explained.

INTRODUCTION

It has been observed that the conformation of DNA greatly changes upon its heat denaturation (1). Sharp changes of optical rotation, viscosity and ultraviolet absorption, and the reduction of molecular weight in a very narrow range of temperature suggested a transition of a helical (double-stranded) structure of DNA to a random coil (single-stranded) structure.

This separation and unfolding of double-stranded DNA has been usually treated as a problem of statistical dynamics of chain conformation. Ozaki *et al.* (2) have succeeded in theoretically explaining the linear relationship between the melting temperature of DNA and its content of guanine-cytosine base pair observed by Marmur and Doty (3).

On the other hand, only a few investigations upon these phenomena have been made on the basis of the electrolyte theory. Since a DNA molecule is a highly charged anionic polyelectrolyte, the effect of electrostatic interaction can not be neglected in discussing these phenomena. In this paper, some characteristic polyelectrolyte properties of DNA in a salt-free medium are studied based upon the exact solutions of two-dimensional Poisson-Boltzmann equation for a rod (4–6) and for two parallel rods (7, 8). It will be shown that counter-ions are strongly con-

densed and the coulomb repulsion between two single-stranded DNA molecules is greatly suppressed by this counter-ion condensation.

MODEL OF DOUBLE-STRANDED DNA

At low salt concentration both double-stranded DNA and unfolded single-stranded DNA must be stretched by large coulomb repulsion between ionized groups. Therefore, we adopt a model of infinitely long rods with uniform surface charges for DNA molecules. According to the Watson-Crick model of double-stranded DNA, the approximate values are: pitch of the double helix, 34 A; diameter of the double helix, 20 A; distance between two neighboring phosphate groups along the helical chain, 6.8 A; and spacing of phosphate groups projected on the axis, 3.4 A.

Let us divide the process of formation of double-stranded DNA from two single-stranded DNA's into the following two processes (Fig. 1); (I) combining process

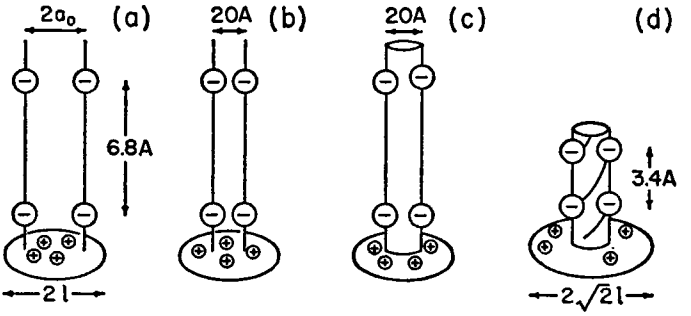

FIGURE 1 Model of double-stranded DNA formation in salt-free solution. (a)→(b): combining process. (c)→(d): folding process.

and (II) folding process. In the combining process, two parallel (infinitely thin) rods, each bearing an electronic charge of a phosphate group per 6.8 A, approach from the large distance $2a_0$ (a) to the distance of 20 A (b). In the folding process, a rod with radius 20 A and uniform surface charge density (two electronic charges per 6.8 A) (c) is compressed to half of its original length forming the double helical structure (four electronic charges per 6.8 A) (d).

A circular free volume model is applied to these rods. The diameter of the free volume for the double-stranded helix (d) is larger than that for the stretched double-stranded structure (c) by a factor of $\sqrt{2}$, because the free volume of a DNA molecule should be kept constant. Even for two parallel rods at large distance (a) a cylindrical free volume model is applicable as suggested in a previous paper (8).

INTERACTION BETWEEN TWO SINGLE-STRANDED DNA'S

Suppose that two infinitely long rods with uniform surface charge density (Ne_0 per unit length) and radius b are arranged parallel at distance $2a$ in their free volume of

radius l (Fig. 2). When we take a polar coordinate system (r, θ), the Poisson-Boltzmann equation for this system is expressed by a non-linear partial differential equation in the following way:

$$\frac{\partial^2\phi}{\partial x^2} + \frac{1}{x}\frac{\partial\phi}{\partial x} + \frac{1}{x^2}\frac{\partial^2\phi}{\partial\Theta^2} = \kappa^2 a^2 e^{\phi(x,\theta)} \tag{1}$$

where

$$\kappa^2 = (4\pi Ne_0^2/DkT)\left\{\int_0^{l/a}\int_0^{2\pi} e^{\phi} x\, dx\, d\theta\right\}^{-1} \tag{2}$$

$$\phi = e_0\psi/kT,\ r/a = x, \tag{3}$$

and e_0, ψ, k, D, and T are an electronic charge, electrostatic potential, Boltzmann constant, dielectric constant of water, and absolute temperature, respectively. This

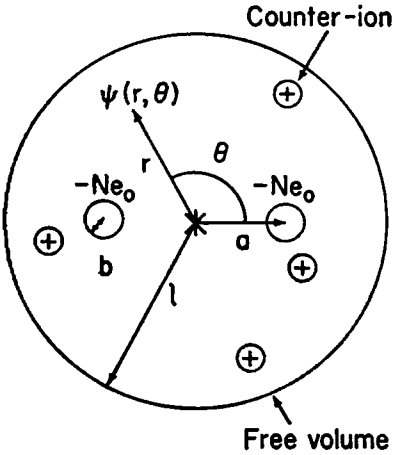

FIGURE 2 System of two infinitely long parallel rods.

Poisson-Boltzmann equation has been exactly solved (7, 8), and the solution is written in the forms:[1]

$$\phi = \begin{cases} -2\ln[C_1\tau^{(1-\epsilon)/2}F_1F_1^* - C_2\tau^{(1+\epsilon)/2}F_2F_2^*], & |1-t| \leq 1 \quad (4) \\ -2\ln[C_3\tau^{(1-\delta)/4}F_3F_3^* - C_4\tau^{(1+\delta)/4}F_4F_4^*], & |1-t| \geq 1 \quad (5) \end{cases}$$

where

$$\tau = (1-t)(1-t^*), \qquad t = x^2e^{2i\theta}, \qquad t^* = x^2e^{-2i\theta} \tag{6}$$

and F_i, F_i^* signify hypergeometric functions and C_i constants. Parameters δ and ϵ are determined by two transcendental equations including l/a, b/a, and N.

When the rods are infinitely thin, the force between two rods can be written as follows (8):

$$\partial f/\partial a = -(1/a)\{4\nu - (1-\delta^2) - 2\nu^2\}, \qquad (\nu \leq 1), \tag{7}$$

[1] In reference 8 the power of τ was partly misprinted.

$$\left.\begin{aligned} f = Fe_0^2/D(kT)^2,\ \nu = Ne_0^2/DkT \\ (1 - \delta^2) = \kappa^2 l^2/2 \end{aligned}\right\} \tag{8}$$

where F is the free energy of the system and the parameter ν is proportional to the charge density along the rods. For example, ν can be considered as 1 in the stretched state of single-stranded DNA molecule in water at 300°K. When a tends to 0, δ becomes $1 - 2\nu$ for $(1/2 \geq \nu \geq 0)$ and 0 for $(1 \geq \nu \geq 1/2)$; reference 8. Consequently, in such cases, the force between two rods becomes

$$\partial f/\partial a \begin{cases} = -(1/a)(2\nu^2), & (1/2 \geq \nu \geq 0) \\ = -(1/a)[1 - 2(1 - \nu)^2], & (1 \geq \nu \geq 1/2) \end{cases} . \tag{8a}$$

The relations between ν and the force for various distances between rods are shown in Fig. 3, where the dashed curve shows the pure coulomb repulsion between rods, *i.e.*,

$$\partial f/\partial a = -2\nu^2/a \tag{9}$$

In this combining process, the following facts should be emphasized: (I) The coulomb repulsion between two rods is remarkably decreased by the depression of the electrostatic potential by counter-ions condensed around two rods. (II) The force between rods is no more increased when ν becomes larger than unity. This is due to the fact that counter-ions begin to condense on each rod when ν exceeds unity and excess charges $\nu - 1$ of rod are all compensated by counter-ions. (III) The force between rods is just half of coulomb repulsion in the limit of $a \to 0$ when $\nu = 1$. As a/l increases, the force decreases and finally for $a/l = 0.5$ it becomes zero, independently of the charge. This suggests that the equidistant arrangement of rods is most stable.

An analytical expression for free energy F of the system has not been derived yet because of mathematical difficulties. Therefore, the free energy change F_I accompanying the change of the distance between rods from a_0 (initial distance) to 10 A was obtained by numerical calculation of the integration of the force, namely,

$$F_I = \int_{a_0}^{10A} -(\partial F/\partial a)_l \, da \tag{10}$$

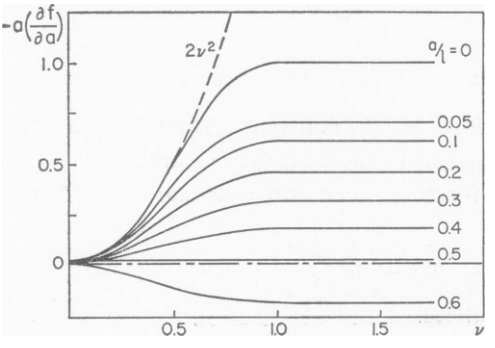

FIGURE 3 Relation between $-a(\partial f/\partial a)$ and ν.

In general, the electrostatic potential in the two-dimensional system is a function of ν and the ratio a/l. Therefore, the free energy change F_I in the combining process should be expressed by ν and the ratio $a_0/10$ A. The relation between F_I (kcal/mole of base pairs) and $a_0/10$ A at 300°K is shown in Fig. 4, where l is chosen as $l = 2a_0$ (equidistant arrangement of rods).

At 300°K, F_I is 1.45 kcal/mole of base pairs for $a_0 = 500$ A (if we assume molecular weight of DNA is 10^6, this value of a_0 roughly corresponds to 10^{-7} mole/litre of DNA solution), and 0.37 kcal/mole of base pairs for $a_0 = 50$ A (10^{-5} mole/litre). If counter-ions were not present in the system, the internal energy change resulting from coulomb repulsion between rods would become 4.7 for $a_0 = 500$ A and 1.95 kcal/mole of base pairs for $a_0 = 50$ A.

With the increase of temperature, ν is decreased from unity, however, $(\partial f/\partial a)$ is slightly decreased in the region of $0.9 \leq \nu \leq 1$ (see Fig. 3). Therefore, from equation (8), the free energy change in the combining process is approximately proportional to T^2. At 333°K ($\nu = 0.9$), F_I becomes 1.73 and 0.44 kcal/mole of base pairs for $a_0 = 500$ A and 50 A, respectively.

FREE ENERGY CHANGE IN FOLDING PROCESS

For the system of a single infinitely long rod of radius b in the free volume of radius l, the free energy F is derived as follows (9):

$$
\begin{aligned}
F &= F_e + F_0 \\
F_e &= -U + NkT \ln \frac{\{(l/b)^2 - 1\}\{(1-\nu)^2 - \delta^2\}}{2\nu} \\
U &= NKT\left\{\frac{(1+\delta^2)}{\nu} \ln (l/b) + \frac{1}{\nu} \ln \frac{(1-\nu)^2 - \delta^2}{(1-\delta^2)} + 1\right\} \qquad (11) \\
F_0 &= NkT \ln C
\end{aligned}
$$

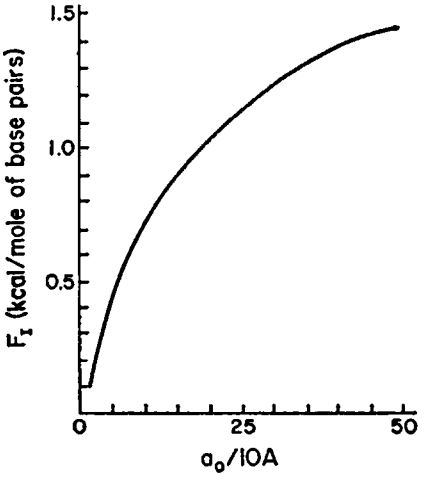

FIGURE 4 Relation between free energy change in the combining process F_I and $a_0/10$A at 300°K.

where F_e, F_0, U, and C are electrostatic free energy, standard free energy, internal energy, mean concentration of the counter-ion in the free volume, respectively. Constant δ is determined by the equation

$$\nu = \frac{1 - \delta^2}{1 + \delta \coth \{\delta \ln (l/b)\}}. \tag{12}$$

The free energy change F_{II} in the folding process can be calculated from these equations (Table I). As shown in the table, the free energy change in the folding process is approximately proportional to T.

TABLE I

FREE ENERGY CHANGE (KCAL/MOLE OF BASE PAIRS) IN THE FOLDING PROCESS

l(A)	300°K	333°K
100	1.06	1.15
1000	1.99	2.25

(*c*) $\nu = 2$, $b = 10$ *A*, radius of the free volume = l. (*d*) $\nu = 4$, $b = 10$ A, radius of the free volume = $\sqrt{2}l$.

The depression of energy by counter-ions is also remarkable in the folding process. For example, in the system without counter-ions, the internal energy change of this process becomes 12.8 kcal/mole of base pairs for $l = 1000$ A at 300°K.

STABILITY OF DOUBLE-STRANDED DNA

We assume that a total free energy change ΔF required to bring two single-stranded DNA's into a double-stranded DNA can be approximated by the sum of the free energy changes due to the combining process, (*a*) to (*b*), and the folding process, (*c*) to (*d*). The free energy difference between (*b*) and (*c*) may be small as compared with those in combining process and holding process, since the activity coefficient difference between (*b*) and (*c*) is small as compared with those in both processes (see Table II). Moreover, this free energy difference can be cancelled by

TABLE II

ACTIVITY COEFFICIENT OF THE COUNTER-ION

	Combining process		Folding process	
a_0 (A)	(*a*)	(*b*)	(*c*)	(*d*)
50	0.49	0.35	0.41	0.21
500	0.49	0.28	0.31	0.16

the free energy difference between the real double-stranded DNA and (d), because both these differences of energy originate in a similar uniforming procedure of discrete electronic charges. (See Discussion for more detailed descriptions). Then, ΔF is given by

$$\Delta F = F_{\mathrm{I}} + F_{\mathrm{II}} \tag{13}$$

Fig. 5 shows the relation between ΔF and the temperature.

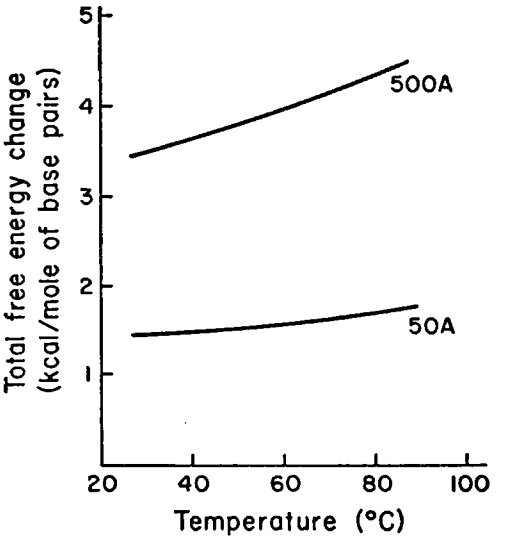

FIGURE 5 Relation between total free energy change and the temperature for $a_0 = 50$A and 500A.

A conformational free energy change ΔF_c due to the change of chain conformation was estimated by Longuet-Higgins and Zimm (10) as follows: there are five valence bonds per base in each chain that are held fixed in the helix but can twist freely in the randomly coiling form. If we assume that each chemical bond may have three rotational positions of minimum potential energy, then, a conformational entropy change per base pair is given by

$$\Delta S = k \ln 3^{2\times 5}. \tag{14}$$

Therefore, ΔF_c from freely coiling single-stranded DNA to double-stranded DNA becomes $\Delta F_c \simeq 11 \times kT \simeq 6.6$ kcal/mole of base pairs at 300°K. This gives a maximum value for ΔF_c of DNA molecules, since in the stretched state of single-stranded DNA in the salt-free solvent the freedom of chain conformation may be greatly reduced.

It follows from these results that 10 kcal/mole of base pairs or somewhat less of free energy is required to stabilize the double-stranded DNA at room temperature. If we suppose that the mean number of hydrogen bonds per base pair is 2.5 (*i.e.*

content of guanine-cytosine base pair is 50 per cent), the energy of a hydrogen bond should be about 4 kcal/mole or less, which is a quite reasonable value.

Marmur and Doty (3) showed that the melting temperature of natural and synthetic DNA is linearly increased with the content of the guanine-cytosine base pair. This fact can be qualitatively understood from the above results, since ΔF_c is proportional to the temperature, and ΔF increases approximately linearly with the temperature.

CHANGE OF THE ACTIVITY COEFFICIENT OF COUNTER-ION

In the present free volume model the activity coefficient γ is given by the ratio of the counter-ion density at the circumference of the free volume to the total charge of rods (8, 11, 12), *i.e.*,

$$\gamma = \begin{cases} \dfrac{1 - \delta^2}{2\nu} & \text{for single-stranded DNA} \\ \dfrac{1 - \delta^2}{4\nu} & \text{for double-stranded DNA} \end{cases} \tag{15}$$

These relations show that γ is proportional to the ratio of the counter-ion density at the circumference of the free volume to the total charge of rods. The numerical results at 300°K are shown in Table II. The difference of the activity coefficient between (*a*) and (*d*) becomes 0.28 and 0.33, for $a = 50$ A and 500 A, respectively.

Ascoli *et al.* (13) observed that the activity coefficient of sodium counter-ion in a sodium-DNA solution sharply increased upon heat denaturation. The change of the activity coefficient was about 0.37 in their experiment. They ascribed this increase of the activity coefficient to the decrease of counter-ion binding at the stretching of a DNA molecule. In the present model, however, the change of the activity coefficient at the stretching of double-stranded DNA, *i.e.* difference between (*d*) and (*c*), is rather small as compared with the experimental value. In order to explain the result of their experiments, the total change of the activity coefficient accompanying the stretching and the double-strand separation should be taken into consideration.

DISCUSSION

In our present model, rod-like DNA molecules are assumed to be in parallel and equidistant arrangement. These assumptions are based upon the facts that parallel arrangement of charged rods minimizes the free energy of the system (14) and that the equidistant arrangement is most stable (8). The characteristic feature of the system of rod-like polyelectrolytes is no doubt the strong depression of electrostatic interaction between rods by counter-ion condensation. If there were no

such depression, the total energy change required in forming a double-stranded DNA would become very high and the double-stranded structure could not be stabilized by the energy of hydrogen bonds.

Doty *et al.* (1) showed that, if the temperature is slowly decreased after heat denaturation of DNA, the separated single-stranded DNA recombines and renatures. In these processes, the phenomena of counter-ion condensation decrease the coulomb repulsion and may facilitate the process of the renaturation.

It is interesting that a three- or four-stranded structure of polynucleotide has been proposed. For such systems, the counter-ion condensation around rods occurs more strongly and may stabilize these structures.

Uniform distribution of electronic charge on single- and double-stranded DNA's has been assumed for the calculation of free energy. To estimate the effects of this assumption, we can refer to the results of Table II, which shows the difference of activity coefficient of counter-ions between states (*b*) and (*c*). The activity coefficient in (*b*), two rods at distance 20 A, is a little lower than that in (*c*), one uniform rod of diameter 20 A. This means that the interaction free energy of the counter-ions and two rods is a little lower than that of the counter-ions and one uniform rod. Such an activity coefficient difference is also expected between state (*d*) and real double-stranded DNA having discrete charges. The interaction free energy of the latter is a little lower than that of the former. Therefore, the free energy difference due to the uniforming process of charge, *i.e.* the difference between (*b*) and (*c*), and the free energy difference due to a reverse process, *i.e.* the difference between (*d*) and real double-stranded DNA, have a tendency to be compensated by each other.

Although, as mentioned above, the total change of electrostatic free energy in double-strand formation is not so much influenced by the uniforming process, the binding of counter-ion is much affected by this process. For example, in the case of the discrete charge distribution of real DNA's, the binding of counter-ions is more localized than in the uniform charge distribution. This localization may have a great effect on the stability of specific conformation of DNA, especially for the system of polyvalent counter-ion.

One of the problems concerning the present results is that the energy change or the change of the activity coefficient is a function of *l*, *i.e.*, a function of the concentration of DNA in the solution. It should be determined whether or not this concentration dependence is really observed in DNA solutions. Another problem is that the present calculation is carried out for the salt-free system. Due to mathematical difficulties, the Poisson-Boltzmann equation cannot be exactly solved for the system with salt. However, the present results may be valid at a low salt concentration, since Oosawa has theoretically proved (12) that the interaction between rods is not affected by the presence of salts as long as the rod is infinitely long and thin. In a real system, DNA is not an ideally infinitely long and

thin rod, and the electrostatic interaction is dependent on the presence of salt, especially in high concentration.

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