Thermodynamics of the Collapsing Phase Transition in a Single Duplex DNA Molecule

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We observed changes in the conformation of individual single giant DNAs (T4DNA) using fluorescence microscopy at different temperatures, 3∼53 °C, in an aqueous solution containing 1.2 mM spermidine. We found that individual DNAs take either elongated coil or collapsed globule conformation and that the relative population of the globule state increases with an increase in temperature. Based on this temperature dependence, the change in entropy for the coil−globule transition was deduced to be $\Delta S = 32 \pm 8\ k_B$, where k_B is the Boltzmann constant. In other words, at $T_C = 301$ K the change in enthalpy is $\Delta H = 32 \pm 8 k_B T_C (= 19 \pm 5$ kcal/mol chains). The thermodynamics upon the transition in a single T4 molecule from the elongated coil to the collapsed globule is discussed in terms of statistical physics by considering the different contributions to the change in entropy; i.e., elasticity, mixing with the solvent, the translation of counterions, hydration, and charge neutralization. We conclude that the small positive change in entropy for the transition is attributed to the competition between the large positive value originated from the translational entropy on the exchange of counterions and large negative values from the other contributions.

1. Introduction

The transition in the higher order structure of single polymer chains between an elongated coiled state and a collapsed compact state, i.e., the coil−globule transition,[1−5] has recently been the subject of renewed theoretical[6−12] and experimental[12−19] interest. For polymer chains with the contour length much larger than the persistence length, as the quality of the solvent changes from good to poor, the elongated coil shrinks into a collapsed state through the appearance of a so-called θ-state,[10,20] where the size, R, of the chain scales with the number of monomers, N, as in an ideal Gaussian chain, $R \sim N^{1/2}$. Thus, the transition is mild and continuous in flexible chains. On the other hand, for stiffer polymer chains, including double-stranded DNA molecules, most previous experimental studies have indicated that this transition becomes very steep but is still continuous.[21,22] Contrary to such observations, theoretical studies within the framework of a mean field approximation[23] have predicted that this transition becomes discrete when the stiffness of the chain is above a certain threshold. Post and Zimm[4] have provided a theoretical interpretation on the continuous nature of the experimental observations of the "coil−globule" transition in terms of the Boltzmann distribution between the elongated and condensed parts of polymer chains. Although this idea was very interesting, it has been rather difficult to examine in actual experiments. In fact, it has been almost impossible to discriminate between single-chain collapse and multichain aggregation in previous experimental studies on the coil−globule transition of DNA chains. This may be due to the difficulty of measuring individual isolated DNA chains with the currently available experimental tools, such as by measurements of light scattering, sedimentation, viscosity, etc.[18,24,25]

Very recently, based on the single-chain observation of giant DNAs, it has become clear that individual DNAs exhibit an all-or-none transition: according to the symmetry argument of Landau[26] regarding the free energy with respect to order parameters, this discrete transition can be classified as a first-order phase transition.[10,23] Despite the discrete nature of the transition at the level of individual chains, the ensemble average of DNAs always appears to be continuous.[27,28] This discovery of the discrete nature of the transition was achieved by observing single DNA molecules through the use of fluorescence microscopy, FM,[29,30] which makes it possible to clearly distinguish between the collapse of a single molecule and multimolecular aggregation through direct observation of the conformation of individual DNAs.

It has been shown that a coexistence region, with a finite width, exists between the elongated and collapse states. That is to say, the number of segments for a giant DNA chain with the size of several tens of kilo base pairs is enough to induce a large discrete first-order phase transition at the level of individual chains, whereas the number is too small to fulfill the central limit theorem;[27] i.e., in a first-order phase transition for usual macroscopic systems, the width of the coexistence region with a change in an intensive variable should become infinitely small under thermal equilibrium. From the perspective of statistical physics, individual chains constitute a mesoscopic system, where the effect of fluctuation for the ensemble of multiple chains is reflected in the finiteness of the width of the region of coexistence. Thus, since the coexistence region is rather wide, it is possible to measure the relative ratio of the coil and globule states by the single-chain observation of DNAs[29,30] with change in temperature. From such measurements of the Boltzmann distribution between the coil and globule states, the changes in enthalpy and entropy, ΔH and ΔS, with the phase transition of isolated DNA chains can be deduced. In the present article, we describe the thermodynamics of a single DNA chain along these lines. With the current experimental technology, the direct

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measurement of the latent heat for the transition of a single molecular chain seems to be nearly impossible. However, by observing the conformation of individual chains at different temperatures, we have successfully determined ΔH and ΔS for the phase transition in a single DNA chain.

2. Experimental Section

Materials and Methods. Bacteriophage T4dC DNA (166 kbp) was purchased from Nippon Gene (Tokyo, Japan). The fluorescent dye 4′6-diamidino-2-phenylindole (DAPI)[31−33] and 2-mercaptoethanol (2-ME) were obtained from Wako Pure Chemical (Osaka, Japan). Spermidine trihydrochloride (SPD) was obtained from Nacalai Tesque (Kyoto, Japan). T4DNA was dissolved in 0.5 × TBE buffer solution (45 mM tris, 45 mM borate, 1 mM EDTA, ethylenediaminetetraacetic acid, pH 7.5). To prevent aggregation, the final concentration of DNA was very dilute: 0.30 μM in nucleotides. As the fluorescent dye, 0.30 μM DAPI was used. It has been confirmed that the persistence length (ca. 50 nm), together with the contour length (ca. 57 μm) of T4DNA remains nearly constant before and after the addition of DAPI.[31−33] To prevent the fluorescence from fading due to illumination, 4% (v/v) 2-ME was added as an antioxidant. The size distribution of DNAs measured by fluorescence microscopy shows a transient change for the initial several tens of minutes after sample preparation. We have confirmed that the size distribution becomes stable within experimental error after 2 h. In the present text, we will address only this latter equilibrium distribution. To minimize the adsorption of DNAs onto the glass surface, special care was taken to clean the glass microscope slides and cover slips thoroughly before the observations. They were cleaned thermally in an electric oven at 500 °C for 1 h and then washed repeatedly with distilled water and ethanol. Finally, they were dried at room temperature (20 °C).

Fluorescence Microscopy. The depth of the sample solution was taken to be relatively large, ca. 200 μm, for measurement by high-resolution microscopy, to avoid the surface effect of the glass plates. The temperature was controlled by using a water-circulating system. The accuracy of the sample temperature was assumed to be ±1 °C. The samples were illuminated with UV light (365 nm). The fluorescence images of DNA molecules were observed using a Carl Zeiss microscope, Axiovert 135TV, equipped with a 100× oil-immersed objective lens. They were recorded on videotape with a high-sensitivity Hamamatsu SIT TV camera. The video images were analyzed with an image processor (Argus 50, Hamamatsu Photonics). Only the moving DNAs with thermal agitation were taken into account, and the adsobed species were discarded in the analysis. To characterize the size of DNA, we measured the long-axis length, L, which was defined as the longest distance in the outline of the DNA image. Due to the technical characteristics of the SIT camera and the resolution limit, there is a blurring effect of about 0.3 μm. The observed L values are given without correction for this blurring effect.

3. Results

Figure 1 shows fluorescent images of T4DNA molecules in the presence of 1.2 mM spermidine at different temperatures, 3∼53 °C, together with corresponding quasi-three-dimensional pictures where the vertical scale represents the intensity of the fluorescence. The fluorescent light-intensity distribution in the figure indicates the spatial density distribution of the DNA segments with a spatial resolution of 0.3 μm. In the example shown here, the experimental condition or the composition of the solution was adjusted so as to observe the coexistence of elongated coiled and collapsed globular chains. Note that with an increase in temperature, the relative proportion of the collapsed DNA chains increases.

Figure 2 shows the changes in the average long-axis length, together with the statistical errors, for individual chains at 25 °C, with a concentration change of spermidine in the DNA solution. At least 100 chains were measured at each spermidine concentration. From this diagram, it is clear that the average size for the coil state and for the globule state remains nearly constant with a change in the spermidine concentration. Thus, the transition is all-or-none for individual DNA molecules. On the other hand, the ensemble average size for all of the DNAs exhibits a continuous change that depends on the concentration of spermidine.[37]

Figure 3 shows the distribution of the long-axis length at different temperatures at a fixed concentration of spermidine (1.2 mM). In this histogram, the morphology, coil or globule, can be easily classified based on the observation of time-dependent changes in the fluorescence images, i.e., intrachain and translational Brownian motion, of individual DNA chains.

Figure 4 shows a van't Hoff plot of the relative populations of the globule and coil states, P_g/P_c, based on the measurements of individual DNAs for at least 100 molecules at each fixed temperature. The plot fluctuates rather largely owing to the fact that the measurements were carried out on the individual molecules with the number of $\sim 10^2$. Thus, we can only estimate the thermodynamic parameters roughly from the graph. From the x-intercept, the transition temperature T_C is found to be 301 K where $P_g/P_c = 1$. The change in entropy, ΔS, for the discrete transition between the coil and globule states is deduced to be $\Delta S = 32 \pm 8\ k_B$ per DNA molecule, where k_B is the Boltzmann constant ($k_B = 1.38 \times 10^{-23}$ J/K). Thus, the change in enthalpy at $T_C = 301$ K is $\Delta H = 32 \pm 8\ k_B\ T_C$ (= 19 ± 5 kcal/mol chains). In Figure 4 the plot looks somewhat curved, suggesting that more delicate treatment of the thermodynamic parameters may be necessary. However, as the number of measured molecules at each point was only on the order of one hundred, we have not tried to evaluate the detailed thermodynamics properties, such as the temperature dependence of specific heat, $C_p(T)$.

4. Discussion

As shown in Figure 2, the transition between the elongated and collapsed states induced by the addition of a trivalent amine is markedly discrete at the level of individual DNA chains, which corresponds well to previous experimental findings on the discrete character of the transition induced by the addition of various kinds of condensation agents.[34−42] The present results indicate that the transition induced by a change in temperature is also largely discrete for individual DNAs.

As for the condensation of long DNAs with cation, Bloomfield[43−45] has reported a comprehensive report on its physicochemical mechanism. We would like to indicate that their report is very important and useful as the milestone by which to obtain the precise sketch on the past studies on this problem. As was suggested by them, in the past experiments on "DNA condensation" discrimination of the process of a single-chain event from that of multichain interaction has not been definite. In other words, the term of "DNA condensation" has usually implied the meaning of the assembling phenomenon from plural number of DNAs.

Thus, the past experimental studies on the "DNA condensation" have shown that the condensation is highly cooperative

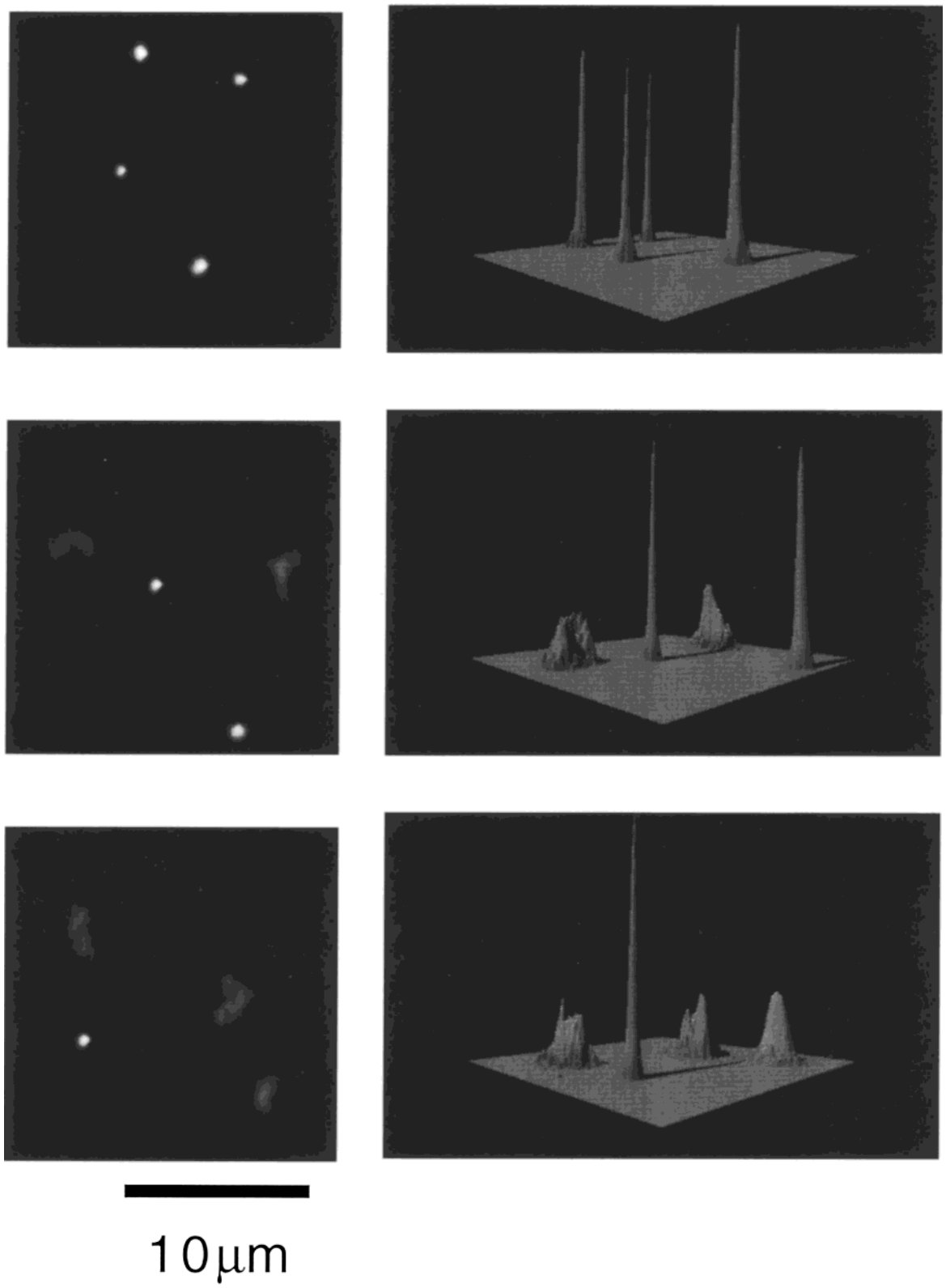

Figure 1. Left: Fluorescence images of T4DNA molecules at 53 °C (top), 25 °C (middle), and 10 °C (bottom). Right: Corresponding quasi-three-dimensional representations of the fluorescence images, where the vertical scale indicates the fluorescence intensity.

but continuous. On the contrary, in the present study we have obtained data on the temperature dependence of the transition of individual DNA chains, indicating that the transition is markedly discrete on the level of individual chains. In the Discussion section of this article, we have tried to perform the analysis with the framework of statistical physics on the single-chain event. Our discussion on the thermodynamics concerning single chain would be, therefore, rather different from the current physicochemical analysis on "DNA condensation"; i.e., the former is discussed based on the fact that the transition is markedly discrete on individual chains, whereas current studies have been interpreted based on the observations with continuous nature in the transition. Although many studies have been carried out on the calculation of the partition function on various kinds

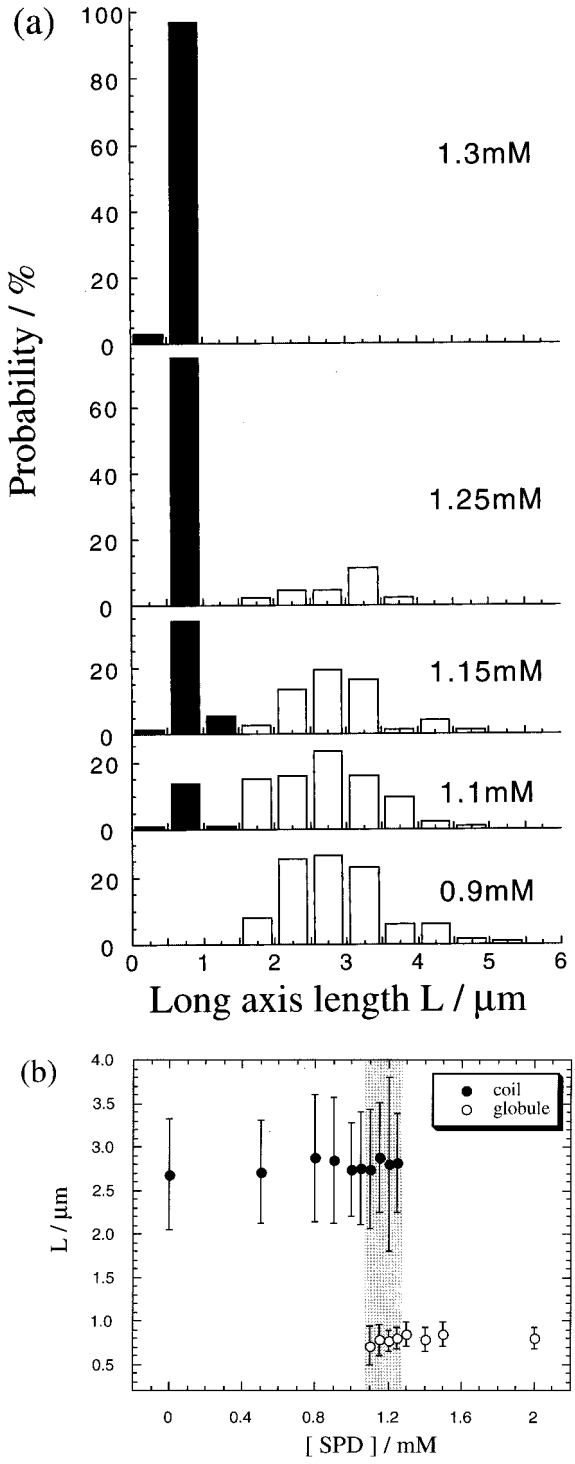

Figure 2. (a) Histogram of the long-axis length, L, for fluorescence images of T4DNAs with a change in the spermidine concentration, [SPD^{3+}]. (b) The mean long-axis length and standard deviation of the coil and globule chains at different spermidine concentrations.

of macromolecular systems from the side of physicists,[9,10,20] there has been almost no report on the direct comparison between theory and experiment on the thermodynamic variables in single chain level, at least on the temperature effect. Although our discussion on the following would be still immature, we believe that theoretical studies with minimum phenomenological parameters, or with nonempirical evaluation on the parameters deduced from the condensation of partition function, are useful for future development on the field of macromoleculer science, especially on polyelectrolytes and also on polyampholites.

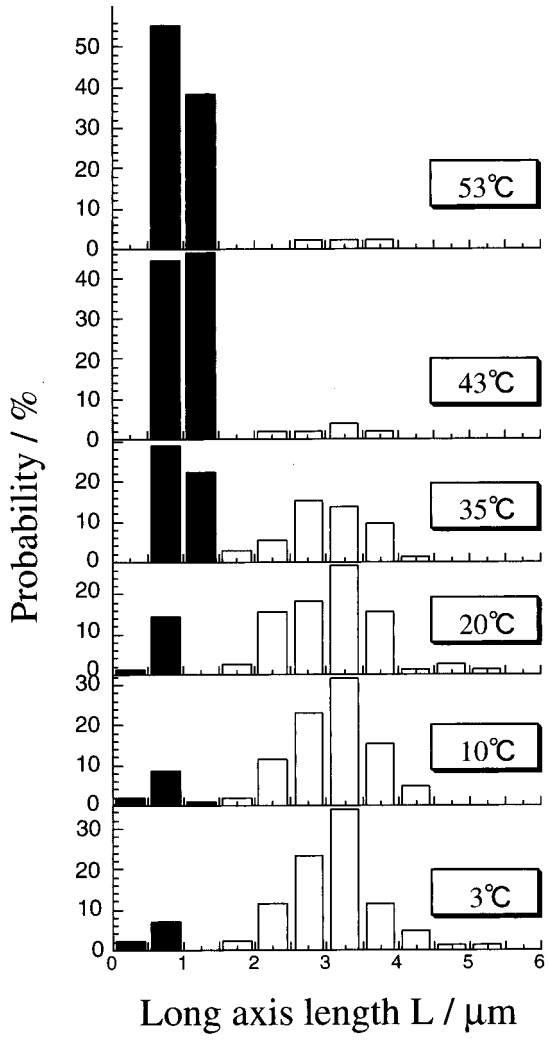

Figure 3. Histogram of the long-axis length, L, at different temperatures. The filled and open bars indicate globule and coil DNAs, which were discriminated on time-successive video images.

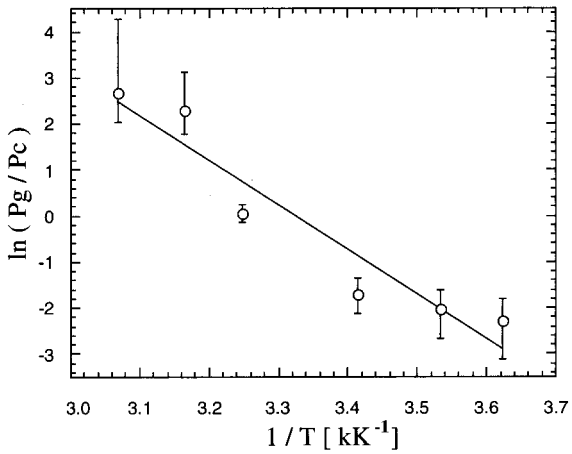

Figure 4. Temperature dependence of the ratio of the globule and coil populations. From the slope and y-intercept, the thermodynamic parameters are estimated to be $\Delta S = 32 \pm 8\ k_B$ and $\Delta H = 32 \pm 8\ k_B T_C$ at $T_C = 301$ K per DNA molecule.

The transition caused by a change in the concentration of a condensation agent, such as spermidine,[37] is believed to be due to a change in the chemical potential of the environment. Both the chemical potential and temperature are intensive variables of the system. On the other hand, the degree of swelling/

shrinking, or the effective volume, is an extensive variable. The discrete nature of an extensive variable, such as volume or the degree of swelling/shrinking, with respect to a change in an intensive variable, such as temperature or chemical potential, indicates that the collapsing transition of individual long DNA chains is a first-order phase transition. Thus, the free-energy profile of the degree of swelling/shrinking is bimodal, with two minima separated by a barrier of more than several k_BT.

Generally, in a first-order phase transition caused by an increase in temperature, a condensed state changes into a disperse state. Contrary to this general trend, our present results show that a DNA chain undergoes a transition from a disperse coil state into a condensed collapse state with an increase in temperature. As has been well established in polymer science, the elastic modulus of a polymer chain is determined by the change in configurational entropy. Thus, the change in elastic entropy, ΔS_{ela}, of a chain should become negative with a collapsing transition; the elastic free energy, $\Delta F_{ela} = -T\Delta S_{ela}$, increases with the transition from a coil to a globule. In contrast, the experimental result in Figure 4 indicates that the net change in entropy, ΔS, is positive in a coil−globule transition. As will be discussed below, this opposite tendency for the temperature dependence of density in DNAs is attributable to the increase in the translational entropy, ΔS_{tra}, of the counterions accompanied by the collapsing transition.

Considering the above point concerning the change in entropy, we would like to perform a semiquantitative estimation of the net change in entropy with a phase transition in a single DNA chain. For simplicity, the free energy of a single DNA chain will be written as the sum of several component

$$F = F_{ela} + F_{mix} + F_{tra} + F_{hyd} + F_{neu} + F_{ele} \qquad (1)$$

where F_{ela} ($= -T\Delta S_{ela}$) is the elastic energy with a change in chain conformation, F_{mix} reflects the mixing entropy between DNA segments and solvent molecules, F_{tra} corresponds to the translational entropic contribution of small ions, F_{sol} is the effect of the solvation of ions, F_{neu} is the effect of charge neutralization on the collapsed DNA, and F_{ele} represents electrostatic interactions. The first three terms are almost purely entropic, whereas the last three terms contain both enthalpic and entropic contributions. (In our system, enthalpy H is almost the same as the internal energy U, since the volume of the solution is nearly constant.) The coil and globule states are present in a 1:1 ratio at around 28 °C (Figure 3). Thus, at this temperature, the free energies of the elongated coil and collapsed globule states should be the same. Since the proportion of the collapsed globule state increases with an increase in temperature (Figure 4), the change in entropy ΔS of the globule with respect to the coil is positive and the corresponding change in enthalpy ΔH is positive. In accordance with our observation, it has been reported[40,48,49] that the condensation or aggregation of DNAs with multivalent cation is enhanced by the increase of temperature. Unfortunately, even on the level of individual chains, it is very difficult to estimate ΔH based on pure theoretical considerations. The physicochemical evaluation of polyelectrolytes is a long-standing problem that remains fraught with theoretical ambiguity, since it is difficult to interpret the spatial correlations between ions interacting with each other through Coulombic interaction in a precise manner. Instead, we would like to try to evaluate the entropic contributions of the first five terms in eq 1. This discussion is expected to be useful also for future investigations on the precise evaluation of the enthalpic contributions in eq 1.

Change in Elastic Entropy, ΔS_{el}. With the Flory approximation modified by Birstein,[46] elastic entropy can be approximated as

$$S_{ela}/k_B = -3/2\,[(V_o/V)^{2/3} + (V/V_o)^{2/3}] \qquad (2)$$

where V is the effective volume of one DNA coil, and V_o is the DNA volume in the Gaussian state. Based on the results of our research on the hydrodynamic radius,[47] the ratio of the volumes of the coil and globule is on the order of 10^5, and the coil is only slightly larger than the corresponding Gaussian chain. Thus, by taking $V_g/V_o \sim 10^5$ and $V_c/V_o \sim 1$, $\Delta S_{ela}/k_B$ is roughly estimated to be $\sim -2 \times 10^3$.

Change in Mixing Entropy, ΔS_{mix}. The mixing entropy is given as

$$S_{mix}/k_B = -V/d^3 \times (1-\phi)\ln(1-\phi) \qquad (3)$$

where d is the size of an elementary lattice cell in the Flory−Huggins theory and is assumed to be the same as the diameter, 2 nm, of duplex DNA. ϕ is the volume fraction of DNA inside the effective volume V: $\phi = Nbd^2/V$, where N is the number of Kuhn segments in one DNA molecule, and b is the length of a Kuhn segment. For T4DNA, N is ca. 550 and b is ca. 100 nm. By placing these values into eq 3, $\Delta S_{mix}/k = -7 \times 10^3$.

Change in Translational Entropy, ΔS_{tra}. The translational entropy of freely moving small ions in a solution of DNA can be written as[12]

$$S_{tra}/k = -\sum_i (P_i^{in} \ln(P_i^{in}/V)) - \sum_i (P_i^{out} \ln(P_i^{out}/\Omega)) \qquad (4)$$

where Ω is the effective volume outside the DNA. P_i is the number of ith ions; in our system the ions are monovalent ions (sodium and tris ions), SPD^{3+} (spermidine with a triple positive charge), and small negative ions. In the coil−globule transition of DNA, the contribution of small negative ions to ΔS_{tra} would be negligible. Thus, we assume that the change in translational entropy is due to ion exchange between monovalent ion and trivalent SPD^{3+}.

According to the hypothesis of counterion condensation, about 20% of the counterions do not condense on the polyelectrolyte chain in aqueous solution.[6,25] This suggests that the number of effective negative charges Q in a single T4DNA is $\sim 6 \times 10^4$, by taking the number of phosphorus moieties as 3×10^5 in T4DNA. As monovalent ions exist two orders more than the trivalent ions in our experimental conditions. A certain amount of monovalent cation is expected to remain as the "condensed state". The number of monovalent ions that can be exchanged with SPD^{3+} is, thus, considered to have a maximum limit Q. The change in S_{tra} would be below the maximum value deduced from the following equation:

$$(\Delta S_{tra})^{max}/k_B = -P_{3+} \ln(P_{3+}/V_g) - P_+ \ln(P_+/V_c^*) \qquad (5)$$

where P_{3+} and P_+ are the number of spermidine and monovalent ions that can be exchanged with the transition. V_g ($=1 \times 10^{-22}$) is the volume of globule state. V_c^* is the effective volume for the cations fluctuating under the atmosphere of the negatively charged phosphate group in the coil DNA, respectively. By taking the Debye length on the depth of the ionic atmosphere, we assume that $V_c^* = 3 \times 10^{-22}$ [m^3], where it is noted that V_c^* is much smaller than the volume obtained from the radius of gyration: $V_c = (4/3)\pi R_g^3$. As the density of the segment in elongated coil DNA is very small,[47] we adapt V_c^* instead of V_c; V_c^* is the volume for the long cylinder with the radius of the Debye length and with the long axis of the contour length

TABLE 1: Changes in the Contributions to Entropy in the Collapsing Transition of a Single T4DNA Molecule

	theoretical value	experimental value
$\Delta S_{ela}/k_B$	-2×10^3	
$\Delta S_{mix}/k_B$	-7×10^3	
$\Delta S_{tra}/k_B$	$+2 \times 10^6 \sim +2 \times 10^5$	
$\Delta S_{hyd}/k_B$	$\pm(10^5 \sim 10^4)$	
$\Delta S_{neu}/k_B$	$-10^6 \sim -10^4$	
$\Delta S_{obs}/k_B$		$+32$ (± 8)

(57 μm). Thus, with $P_{3+} = Q/3 = 2 \times 10^4$ and $P_+ = Q$, the maximum ΔS_{tra} is calculated to be 2×10^6 k_B.

$$\Delta S_{tra}/k_B = 2 \times 10^6 \sim 2 \times 10^5 \tag{6}$$

Change in Hydration Entropy, ΔS_{hyd}. Due to the ion exchange of monovalent and trivalent cations that accompanies the transition, the degree of hydration would be greatly changed.[48,49]

In relation to the change in the hydration, recently we have found that effective negative charge in collapsed DNA decreases on the order of 1/20 as that in elongated state, from the measurement of electrophoresis (unpublished result). This clearly suggests that the collapsed globule is a kind of neutral ionic crystal, except for the its surface. Through the charge neutralization, the charged groups, both on phosphates and counterions, would be dehydrated significantly.

However, it is very difficult to evaluate the change in entropy due to such ion exchange. Referring the comprehensive data on the ion solvation,[48] the change in the entropy of a single sodium ion, accompanied with the ion exchange, is expected to be on the order of -1 $k_B \sim -10$ k_B. By multiplying such magnitude in the entropy change by the number of exchangeable monovalent ions $P_+ = Q$, the increase in the hydration entropy, with the release of monovalent ions induced by the collapsing transition, is estimated to be on the order of -10^5 $k_B \sim -10^4$ k_B.

It is also necessary to consider the effect of dehydration in the polar and hydrophobic groups in DNA with the collapsing transition. As dehydration means the enhancement of the mobility of water molecules, positive change in the entropy is expected. Based on the past literature,[50] ΔS on hydration would be on the order of $+10^5$ k_B. Thus, the net change in the hydration entropy would be

$$\Delta S_{hyd}/k_B = \pm(10^5 \sim 10^4) \tag{7}$$

At the present stage, it is difficult to proceed the discussion on the contribution of the hydration/dehydration to the entropy. As it has been well established that the alkaline metal ions, Li^+, Na^+, K^+, and Rb^+, have markedly different nature on the hydration, further experiments on the collapsing transition with different monovalent ions would afford useful information on the hydration entropy. This is because chemical differences in the cationic species with the same monovalency could have negligible effects on the other entropic contributions in Table 1.

Change in Entropy due to Charge Neutralization, ΔS_{neu}. As was mentioned above, we have found that the globule is about 1/20 as mobile as the coil. Since the hydrodynamic radius of the coil is 2 orders of magnitude greater than that of the globule, the effective charge of the globule is more than 3 orders of magnitude smaller than that of the coil. Thus, Q ($\sim 6 \times 10^4$) monovalent ions in aqueous solution of volume Ω ($\sim 10^{-15}$ [m^3]) are absorbed into a collapsed globule of volume V_g. Thus, the maximum change in translational entropy due to charge neutralization effect is

$$\begin{aligned}(\Delta S_{neu})^{max}/k_B &\sim -Q \times \ln(\Omega/V_g) \\ &\sim -6 \times 10^4 \ln(10^{-15}/10^{-22}) \\ &= -1 \times 10^6\end{aligned}$$

The actual ΔS_{neu} would be

$$\Delta S_{neu}/k_B = -1 \times 10^6 \sim -1 \times 10^4 \tag{8}$$

5. Conclusion

In Table 1 we have summarized the estimated changes in entropy on the individual components. The observed positive change in ΔS_{obs} with the transition from the coil to globule in a single T4DNA molecule is the result of the summation of these values. It has become clear that the elastic and mixing terms are minor in the collapsing transition, in contrast to the standard descriptions of "coil−globule" transition in polymer textbooks.[10,20] It is expected that the thermodynamics of the phase transition of giant DNAs is sensitively dependent on changes in the chemical environment. This suggests that opposite temperature dependence of the transition would be found for the collapse of giant DNA with other kinds of condensation agents.[35,51]

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