Thermodynamics in Folding Transition of DNA

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SUMMARY: Recently, it has been found that individual giant DNA molecules exhibit a discrete transition, or first order phase-transition, between the compact folded state and the elongated coiled state, i.e., the folding transition. In order to clarify the thermodynamics in the folding transition of single DNA molecules, we have studied the temperature effect on the bimodal distribution of conformation for the ensemble of T4DNA chains (166 kbps) in both poly(ethylene glycol) (PEG) and spermidine (SPD), using single-chain observation with fluorescence microscopy. From the van't Hoff relationship, the entropy change in the transition from the compact state to the unfolded state is deduced as, ΔS = +11, +38 *k*/molecule in the aqueous solution of PEG with sodium chloride and potassium chloride, respectively, where k is Boltzmann's constant, whereas, ΔS with SPD is estimated to be –32 *k*/molecule. The values of ΔS with the transition are discussed in term of the translational entropy of counterions together with the hydration effect.

Introduction

In order to clarify the mechanism of DNA compaction in cell, it is very important to understand the coil-globule transition. It has been considered that the coil-globule transition is steep but continuous, or a kind of second-order phase transition[1),2)]. Contrary to current studies, it has been found that individual giant DNA molecules exhibit an all-or-none-type transition between the elongated coil state and compact folded state, using single-chain observation with fluorescence microscopy[3)-5)]. It has also been clarified that the transition in the ensemble of DNA chains becomes continuous, indicating that there is a rather wide region for the coexistence of unfolded and folded chains with respect to the change in the concentration of the condensation agent, such as poly(ethylene glycol) or polyvalent cations[6),7)]. The purpose of the present study is to examine the effect of temperature on the switching of the higher order structure of giant DNAs. By adding a temperature-controlling unit to the system for single-chain observation by fluorescence microscopy, we have measured the change

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in the size distribution (long-axis length) of DNA as a function of temperature. On the basis of these experimental data, in this article we briefly discuss the thermodynamics in the folding transition on individual DNA molecules relative to the enhanced binding of counterions accompanied with the folding transition.

Experimental section

Sample preparation: To examine the effect of temperature on the all-or-none-type transition of individual DNA chains we have deduced suitable experimental conditions for folding transition, i.e., the coexistence region of the folded and unfolded chains, as follows[8),9)].

(a)PEG+NaCl: The PEG solution was adjusted so as to contain the following chemicals: PEG 100 mg/ml, 2-ME 2 %(v/v), DAPI 0.6 μM, and DNA in nucleotides 0.6 μM, NaCl 0.4 M. The prepared samples were allowed to stand at ambient temperature 23 $\pm$ 2 ℃ for 30 min before the observation. The detailed procedure of sample preparation is shown in Ref. 8.

(b)PEG+KCl: Potassium chloride (KCl) was obtained from Wako Pure Chemicals. In the PEG + KCl solution, the procedure of sample preparation is the same as in the PEG + NaCl solution except the final concentration: PEG 100 mg/ml, 2-ME 2 %(v/v), DAPI 0.6 μM, and DNA in nucleotides 0.6 μM, KCl 0.52 M.

(c)SPD: The SPD solution was adjusted as follows: SPD 1.2 mM, DAPI 0.3 μM, 2-ME 4 %(v/v), T4DNA 0.3 μM in nucleotides. The prepared samples were stand at ambient temperature $\pm$2 ℃ for 2 hours before the observation. The detailed procedure of sample preparation is shown in Ref. 9.

Fluorescence microscopy: The procedure of single molecule observation using fluorescence microscopy is shown elsewhere[8),9)].

Results and discussion

In the PEG solution with NaCl, we chose a concentration of [NaCl] = 0.4 M to investigate the effect of temperature[8)]. Figure 1 shows the distribution of the long-axis length L in the coexistence region of the folded and the unfolded chains at different temperatures[8)]. It clearly exhibits a bimodal profile. The portion corresponding to the unfolded coil state tends to increase with increasing temperature. By evaluating the relative populations of the folded and unfolded states, we can estimate the free energy difference ΔG between the folded and unfolded states: $\Delta G/kT = -\ln(P_f/P_u)$, where P_f

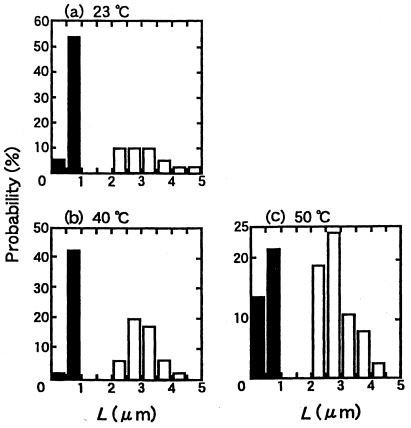

Figure 1 Distribution of the lon-axis length L at different temperatures. The temperature is (a)23℃, (b)40℃, and (c)50℃, respectively. Permitted from ref. 8.

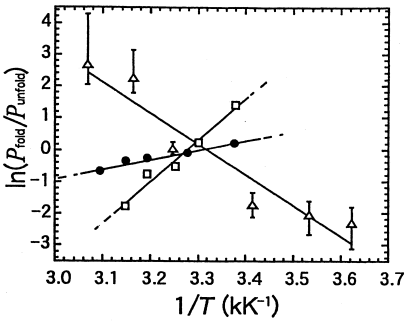

Figure 2 Temperature dependence of the folding/unfolding transition in a $\ln(P_{fold}/P_{unfold})$ vs. 1/T plot at different solutions. The solutions are PEG+NaCl (●), PEG+KCl (□), and SPD (△), respectively. Permitted from ref. 8 and 9.

and P_u are the probabilities of the folded and unfolded states, respectively. Figure 2 shows that $\ln(P_f/P_u)$ changes linearly with $1/T$[8]. Here, P_f and P_u are obtained from the bimodal profile in the distribution of the long-axis length L as shown in Fig. 1. From the slope, we tried to evaluate the entropy change in the folding transition ΔS., Here we omitted the temperature dependence of enthalpy $H(T)$ because within the narrow temperature range (~30K) the evaluation of $H(T)$ is speculative. T_c, the temperature at $P_f = P_u$, is estimated to be 306 K. From the slope, ΔS is determined to be +11.3 k per DNA molecule, where k is Boltzmann's constant. The heat content of the transition at T_c is $\Delta H = +4.77 \times 10^{-20}$ J/molecule (28.7 kJ/mol).

Here, we briefly discuss the mechanism in the folding transition of single DNA molecule. It should be noted that many previous studies have considered, at least implicitly, that the coil-globule transition is steep but continuous, or a kind of second-order phase transition[1),2)]. Recently, we have studies a theoretical model to interpret the intrinsic property of the folding transition of single DNA chains, including the effect of counterions and coexisting low-molecular-weight salt ions[10),11)]. Using this theoretical model, it has become clear that the change in translational entropy before and after the folding transition plays a significant role in determining the free energy[8),9)]. The translational entropy of small ions S_{tra} is described as follows[8)-11)],

$$S_{tra} = \left[\sum_i N_i \ln(\frac{V}{N_i})\right]_{in} + \left[\sum_i N_i \ln(\frac{\Omega}{N_i})\right]_{out} \quad (1)$$

where N_i is the number of ions (charge i), V the effective volume of DNA and Ω the

volume outside DNA. Figure 4 shows a schematic representation of the distribution of small ions around a single DNA chain in the environment containing PEG chains. It has been confirmed that the change in the effective volume in the folding/unfolding transition is very large; $V_u/V_f \approx 10^4 \sim 10^5$, where V_u and V_f are the effective volume in the unfolded and folded states, respectively[12]. Thus, one can expect a significant increase in S_{tra} from the compact folded state to the elongated unfolded state. Using a known value of V_u/V_f, Eq. (1) gives the increase in entropy in the folding transition of single DNA molecule; $\Delta S_{tra} = +10^4 \sim 10^5\ k$. This estimated entropy difference S_{tra} is much larger than the experimental value of $\Delta S \sim +11.3\ k$, although both values are positive[8]. This difference is attributed to the so-called hydration effect[13),14)]. It has been established that small ions interact rather strongly with water molecules. Thus, the increase in the translational entropy of small ions upon dissociation is almost compensated by the decrease in entropy accompanied by their "complexation" with water molecules[13)], and residual small entropy change corresponds to the observed change in entropy in the experiment. Figure 3 shows a schematic representation of the distribution of PEG and monovalent cations around a single DNA molecule.

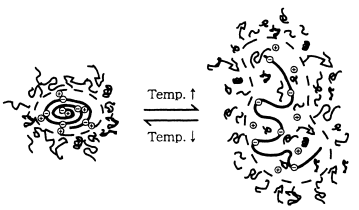

Figure 3 Schematic representation of the distribution of PEG and monovalent cations around a single DNA molecule.

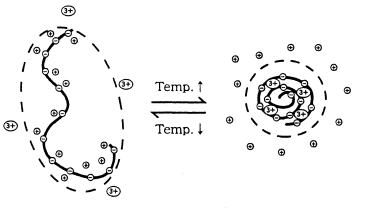

Figure 4 Schematic representation of the distribution of mono- and trivalent cations around a single DNA molecule.

To confirm the contribution of the hydration effect in the folding/unfolding transition, the experiment in the presence of potassium chloride KCl in place of NaCl was performed. From the van't Hoff relationship, it has been clear that ΔS and T_c are estimated to be + 38 *k*/molecule and 305 K as shown in Fig. 2, respectively. The value of ΔS is larger than three times of that in the PEG + NaCl solution though the condition in the sample preparation is very similar to that in the PEG + NaCl solution. This result strongly supports that the observed entropy change is increased with decreasing the hydration effect.

Furthermore, the interesting result in related to the translational entropy of counterions

has been found[9]. The temperature effect in the folding/unfolding transition of DNAs by spermidine (SPD), a trivalent amine, is opposite to that by PEG; with increasing temperature, DNAs exhibit a discrete transition from the unfolded state to the folded state. According to the van't Hoff relationship, the entropy change from the folded state to the unfolded state and T_c are estimated to be –32 *k*/molecule and 301 K as also shown in Fig. 2, respectively[9]. Such temperature dependence is attributed to the increase in the total translational entropy due to the ion-exchange between the mono- and trivalent cations; the counterions are changed from the monovalent cations to the trivalent cations with increasing temperature. Figure 4 shows a schematic representation of the distribution of mono- and trivalent cations around a single DNA chain. Here, we emphasize that the folding transition from the unfolded state to the folded state with increasing temperature can not be explained by the previous theories[1),2)]. Thus, it has become clear that the translational entropy of counterions and the hydration effect around them play a significant role in the folding/unfolding transition of single DNA molecules. Further experiments on the change of conformation of individual DNAs at different concentrations of PEG, NaCl, KCl and SPD, and also with different cations should be performed to obtain useful information for estimating the contribution of the translational entropy of counterions and the hydration effect.

Conclusion

We observed the unfolding/folding transition of single DNA molecules in PEG and SPD solutions. On the basis of the temperature-dependence of the relative probability of the unfolded and folded states, the change in entropy ΔS from the folded state to the unfolded state is evaluated to be +11.3, +38 *k*/molecule in the PEG + NaCl and PEG + KCl solutions, respectively. While in the presence of SPD, ΔS is evaluated to be –32 *k*/molecule; the temperature effect is opposite to that in the PEG solutions. The values of observed ΔS are attributed to the translational entropy of counterions together with the hydration effect.

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