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Thermal Properties of DNA–Poly(L-lysine) System

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ABSTRACT: In order to obtain information about the interaction between DNA and poly(L-lysine), calorimetric studies of DNA–poly(L-lysine) solutions prepared by direct mixing were carried out using a batch-type microcalorimeter and a differential scanning calorimeter. Furthermore, to complement this calorimetric data, CD spectra of DNA–poly(L-lysine) solutions were also measured. From the results of the calorimetric and the spectrophotometric measurements, the enthalpies of the interaction between DNA and poly(L-lysine) and the conformational change of DNA accompanied by an interaction between DNA and poly(L-lysine) were estimated to be about 4 and 6 kJ, respectively.

KEY WORDS Heat of Mixing / DSC / DNA / Poly(L-lysine) / Enthalpy of Interaction / Enthalpy of Conformational Change /

Many studies on the interaction between DNA and poly(L-lysine) have been carried out primarily by such methods as thermal denaturation[1-3] and circular dichroism.[4-7] This interaction may lead to two types of complexes under the such experimental conditions as salt-gradient (reconstitution)[1-3,5-8] and direct mixing.[2,4,6,7,9] The reconstituted DNA–poly(L-lysine) complex is very different from the complex prepared by direct mixing for both thermal denaturation and circular dichroism. It was reported[4] that in the DNA–poly(L-lysine) complex prepared by the direct mixing, the following two conformations of base pairs of DNA exist: one is the B-form in free DNA regions which does not bind poly(L-lysine) and the other is the intermediate conformation between the B-form and C-one in the bound DNA regions which bind poly(L-lysine). Furthermore, calorimetric studies on the enthalpy change of interaction between DNA and poly(L-lysine) were carried out by a few authors.[10,11] No attempt, however, has been made to obtain the enthalpy change for the conformational change of DNA accompanying the complex formation.

This study provides information on the DNA–poly(L-lysine) interaction and the accompanying conformational change of DNA on the basis of calorimetric measurements of DNA–poly(L-lysine) solutions prepared by direct mixing using a batch-type microcalorimeter and a diferential scanning calorimeter.

EXPERIMENTAL

Materials

Calf thymus DNA and poly(L-lysine) HBr ($\bar{M}_w = 4.0 \times 10^4$) were purchased from Miles Laboratory. The buffer solution used to adjust the pH was 0.01 M SSC (1.5 mM NaCl + 0.15 mM Na Citrate) at pH 7.25.

Apparatus and Procedure

Microcalorimeter. The calorimeter for measuring the heat of mixing of DNA and poly(L-lysine) solutions was similar to the LKB batch-type microcalorimeter and was placed in an air bath kept at 298 ± 0.005 K as reported in previous paper.[12] For each measurement, equal volumes of a DNA solution of known concentration (6×10^{-4} nucleotide unit mol) and a poly(L-lysine) solution were mixed. The measurements were made with varying concentrations of poly(L-lysine).

Differntial Scanning Calorimeter. The differential scanning calorimeter (DSC, Model 8005 Rigaku Denki Co. Ltd.) was modified so as to measure any slight heat change in the sample.[13] A DNA solution of known concentration (0.01 nucleotide unit mol)

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and poly(L-lysine) solutions of various concentrations were mixed at room temperature and about 30 μg of the mixture was used for each DSC measurement. The heating rate was 10 K min^{-1}.

Circular Dichroism. The CD spectra were recorded with a JASCO J-20 spectropolarimeter. The polymer concentrations were about one-fifth of that used for measuring the heat of mixing.

RESULTS AND DISCUSSION

Heat of Mixing and Circular Dichroism

The heats of mixing of the DNA–poly(L-lysine) solutions, in which the molar ratio, r, of the poly(L-lysine) to the nucleotide of DNA was less than 0.4, were measured at 298 $\pm$ 0.005 K. When r was more than 0.4, calorimetric measurement was impossible because DNA precipitated from the solution. The heats of mixing for this system were positive and this accords with the result obtained by Giancotti *et al.*,[11] but is opposite to the sign obtained by Ross and Shapiro.[10] This discrepancy may be due to the difference of the ionic strength of the solutions, as pointed out by Giancotti *et al.*[11]

The plots of the heats of mixing, ΔH_{mix}, per mol of base pair(bpm) of DNA against r are shown in Figure 1. As seen in Figure 1, though the experimental data are somewhat scattered, the ΔH_{mix} shows a definite value of about 10 kJ which is nearly independent of r, even allowing for such uncertainty. In order to complement the calorimetric data, we also measured the CD spectrum under the same experimental conditions.

The CD spectrum obtained for $r=0.02$ is similar to that for $r=0$ as shown in Figure 2, suggesting that the DNA molecule adopts a B-form. On the contrary, when r is more than 0.02, the positive maximum of the CD spectra shifts to red and the intensity of this maximum decreases with an increase in r. This red shift and the decrease in intensity at the positive maximum resembling the CD change of DNA in the high NaCl concentration[4,14] may be considered to correspond to the existence of the intermadiate conformation between B-form and C-one. Accordingly, when r is more than 0.02, the ΔH_{mix} of DNA–poly(L-lysine) solutions is comprised of the following two composites: the enthalpy of interaction between DNA and poly(L-lysine) and the other is the heat of conformational change of DNA. Those enthalpy changes will be presented latter.

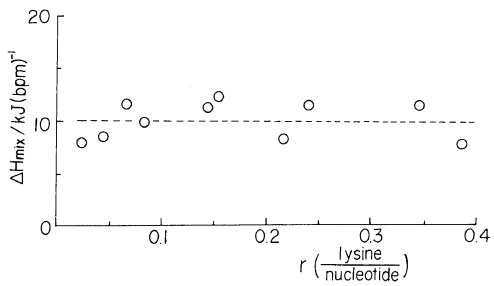

Figure 1. Plots of heat of mixing, ΔH_{mix}, per mol of base pair (bpm) of DNA against the molar ratio, r, of residue of ply(L-lysine) to nucleotide of DNA for DNA–poly(L-lysine) system at 298 K.

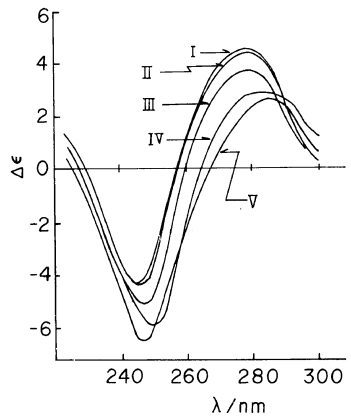

Figure 2. CD spectra of DNA–poly(L-lysine) solutions at various r: I, 0; II, 0.019; III, 0.18; IV, 0.48; V, 0.80.

DSC Measurement

The DSC curves of DNA–poly(L-lysine) system show two endothermic peaks differing from the peak of the DNA solution which is free from poly(L-lysine), as shown in Figure 3. These peaks cor-

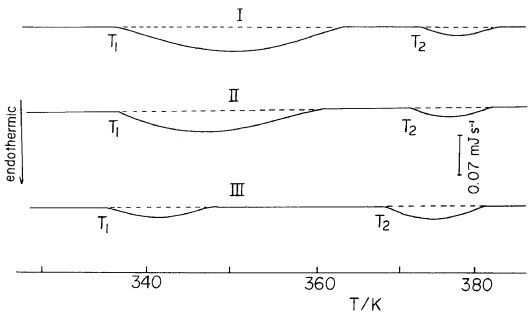

Figure 3. DSC curves of DNA–poly(L-lysine) solutions at various r: I, 0.01; II, 0.03; III, 0.10.

respond to the biphasic melt of the DNA–poly(L-lysine) complex as reported by other authors.[2,9] Thus, the peak obtained at a lower temperature, T_1, corresponds to the helix–coil transition of free DNA which does not bind to poly(L-lysine) and the peak at higher temperature, T_2, corresponds to the helix–coil transition of bound DNA which binds to poly(L-lysine).

The apparent heats of the helix–coil transition per mol of base pair for free DNA and bound DNA, ΔH_1 and ΔH_2, are obtained from the peak area at the transition temperature, T_1 and T_2, respectively. The plots of ΔH_1, ΔH_2, T_1, and T_2 against r are shown in Figure 4. As seen in Figure 4, T_1 shows a definite value which is independent of r when r is less than 0.05 and increases slightly when r is more than 0.05, whereas T_2 shows a defnite value nearly independent of r. On the other hand, ΔH_1 decreases and ΔH_2 increases as r increases.

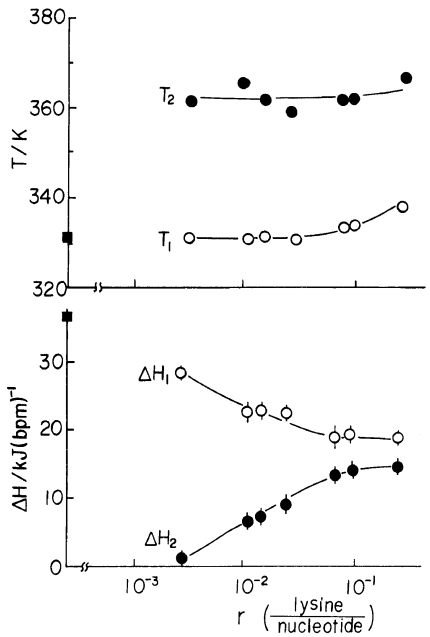

Figure 4. Plots of transition temperature, T, and transition enthalpy, ΔH, of DNA against r. T_1 (○) and T_2 (●) are the transition temperature at low and high temperature regions, ΔH_1 (○) and ΔH_2 (●) are the heat of helix–coil transition at low and high temperature regions, respectively.

Enthalpies of Interaction of DNA–Poly(L-lysine) and Conformational Change of DNA

The results obtained from the heats of mixing and DSC measurements for the DNA–poly(L-lysine) system may be explained by the following two step: One is the helix–coli transition of free DNA at lower temperature, as follows.

DNA(helix)–DNA′ (helix)–poly(L-lysine)

$$\xrightarrow{\Delta H_1} \text{DNA(coil)–DNA′(helix)–poly(L-lysine)} \quad (1)$$

where DNA(helix) and DNA′(helix) represent DNA which adopts the B-form and the intermediate conformation between B-form and C-one, respectively. The apparent heat of the helix–coil transition, ΔH_1, of B-form is related to the net transition heat, ΔH_m, by

$$\Delta H_1 = \frac{m_1}{m}\Delta H_m \quad (2)$$

where m and m_1 are the total number of moles of DNA and number of moles of DNA with the B-form, respectively. ΔH_m corresponds to the transition heat at $r=0$ and this value is 35 kJ per mol of base pair.

The other step is the helix–coil transition of the bound DNA at a higher temperature.

DNA(coil)–DNA′(helix)–poly(L-lysine)

$$\xrightarrow{\Delta H_2} \text{DNA(coil)–poly(L-lysine)} \quad (3)$$

The apparent transition heat, ΔH_2, of the intermediate conformation between B-form and C-one of DNA is related to the net transition heat, $\Delta H_m'$, and number of moles, m_2, of the DNA′(helix) by

$$\Delta H_2 = \frac{m_2}{m}\Delta H_m' = \frac{m-m_1}{m}\Delta H_m' \quad (4)$$

The apparent heat, ψ, is expressed as the sum of ΔH_1 and ΔH_2 from eq 2 and 4 as follows.

$$\psi = \Delta H_1 + \Delta H_2 = \Delta H_m' + (\Delta H_m - \Delta H_m')\frac{\Delta H_1}{\Delta H_m} \quad (5)$$

Accroding to eq 5, the plots of ψ against $\Delta H_1/\Delta H_m$ must be linear, and $\Delta H_m'$ is estimated from the intercept of the straight line. $\Delta H_m'$ obtained from the straight line as shown in Figure 5 is about 29 kJ per mol of base pair.

Meanwhile, when the B-form of DNA converts to coil, the following two processes may be considered:

1. The helix of the B-form of DNA directly converts to the coil form

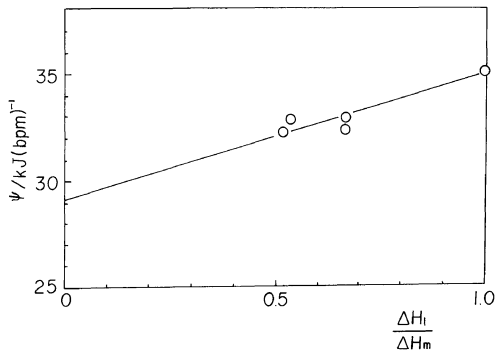

Figure 5. Plots of ψ against $\Delta H_1/\Delta H_m$ according to eq 5.

$$\text{DNA(helix)} \xrightarrow{\Delta H_m} \text{DNA(coil)} \qquad (6)$$

2. The helix of the B-form of DNA transforms to the intermediate conformation and then to the coil form,

$$\text{DNA(helix)} \xrightarrow{\Delta H_c} \text{DNA}'\text{(helix)} \xrightarrow{\Delta H_m'} \text{DNA(coil)} \qquad (7)$$

The heat of the helix–coil transition, ΔH_m, of the B-form is the sum of the heats of the conformational change, ΔH_c, from the B-form to the intermediate conformation and the heat of the helix–coil transition, $\Delta H_m'$, of the DNA with the intermediate conformation,

$$\Delta H_m = \Delta H_c + \Delta H_m' \qquad (8)$$

From eq 8, ΔH_c may be estimated to be about 6 kJ, since $\Delta H_m'$ and ΔH_m are 29 kJ and 35 kJ, respectively.

On the other hand, the heat of mixing, ΔH_{mix}, of DNA and the poly(L-lysine) solutions consists of ΔH_c and the heat of interaction, ΔH_i, between DNA and poly(L-lysine),

$$\Delta H_{mix} = \Delta H_c + \Delta H_i \qquad (9)$$

Since ΔH_c is 6 kJ and ΔH_{mix} is 10 kJ, ΔH_i will be estimated as about 4 kJ per mol of base pair.

The values of the enthalpy of interaction, ΔH_i, between DNA and poly(L-lysine) and the enthalpy of the conformational change, ΔH_c, estimated are listed in Table I, together with the values reported by other authors.[10,11]

As seen in Table I, ΔH_i reverses its sign from a negative to a positive value since the molecular weight of poly(L-lysine) increases. Furthermore, to obtain the net value of ΔH_i, we must consider other factors such as the conformational change of poly(L-lysine) exsisting an extended coil at a neutral pH,[14] the effect of dehydration of DNA accompanied by the interaction between DNA and poly(L-lysine),[4] the electrostatic interaction between the phosphate group of DNA with negative charges and the lysine residue with positive charges,[2] and the effect of ionic strength under the experimental conditions.[11]. To elucidate these effects, further studies will be needed.

Table I. The values of the enthalpy of interaction, ΔH_i between DNA and poly(L-lysine) and enthalpy of conformational change, ΔH_c, from B-form to intermadiate conformation between the B-form and C-one

p[a]	ΔH_i/kJ(bpm)$^{-1}$	ΔH_c/kJ(bpm)$^{-1}$
192	4.0 ± 2.8	6
50	-2.5[10]	
100	6.3[11b]	
1000	22.1[11b]	

[a] Degree of polymerization of poly(L-lysine).
[b] Converted to the value per mol of base pair(bpm) of DNA from one per mol of the lysine residue given by Giancotti *et al.*[11]

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