Studies on the Heat of Dissociation of Poly(A)·Poly(U) Duplex–Dye Complex Determined by Using the Modified Differential Scanning Calorimeter

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ABSTRACT: The solution properties of poly(riboadenylic acid) (poly(A))·poly(ribouridylic acid) (poly(U)) duplex–dye complexes were studied by a modified differential scanning calorimeter and a spectrophotometer. The heat of dissociation by which dye molecules dissociate from the poly(A)·poly(U) duplex–dye complex were estimated to be 27.0 ± 2.7 kJ per mole of dye for poly(A)·poly(U) duplex–proflavine and 31.4 ± 3.5 kJ for poly(A)·poly(U) duplex–9-aminoacridine systems. Agreement between the heat of dissociation determined from differential scanning calorimetry measurements and the heat of interactions estimated from the heat of mixing in the previous paper is fairly good within experimental error.
    KEY WORDS   Differential Scanning Calorimetry / Ultraviolet and Circular Dichroism Spectra / Poly(riboadenylic acid)·Poly(ribouridylic acid) Duplex / Aminoacridine Dye / Poly(riboadenylic acid)·Poly(ribouridylic acid) Duplex–Dye Complex / Heat of Transition / Heat of Dissociation /

It is well known that an equimolar mixture of poly(riboadenylic acid) (poly(A)) and poly(ribouridylic acid) (poly(U)) forms a duplex of poly(A)·poly(U) having a double-stranded helical structure, and that the stability of this structure depends on such environmental conditions as temperature, ionic strength, and salt concentration.[1–4]

In a previous paper,[5] the heat of mixing of the poly(A)·poly(U) duplex formed from an equimolar mixture of poly(A) and poly(U), and aminoacridine dye was measured and the thermodynamic quantities for poly(A)·poly(U) duplex–dye complexes were estimated. Also, it was reported that the heats of interaction between poly(A)·poly(U) duplex and dye were about -32 kJ per mole of dye for proflavine and -28 kJ for 9-aminoacridine systems, respectively.

In order to supplement the information on the heat of interaction between the poly(A)·poly(U) duplex and the dye determined from the heat of mixing, in this paper we report on the heat of

dissociation of the poly(A)·poly(U) duplex–dye complex obtained by using a modified differential scanning calorimeter.

EXPERIMENTAL

Materials

Poly(riboadenylic acid) (poly(A)) and poly(ribouridylic acid) (poly(U)) were purchased from Yamasa Shoyu Co. Ltd., Japan and used without further purification.

The dyes used in this study were proflavine (PF, Aldrich Chemical Co. Ltd., USA) and 9-aminoacridine (9-AA, Tokyo Chemical Co. Ltd., Japan). All other materials were analytical reagents of commercial products.

The solvent used to adjust the pH was 0.1 mol dm^{-3} Tris–HCl buffer solution (pH 7.60).

Apparatus and Procedure

The differential scanning calorimeter (DSC) (M8056, Rigaku Denki Co. Ltd., Japan) was modified so as to enable measurement of the slight

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heat change in a small amount of sample as reported previously.[4]

The absorption spectra and circular dichroism (CD) spectra measurements were made by a spectrophotometer (Hitachi 124, Hitachi Ltd., Japan) and a spectropolarimeter (J-20, JASCO, Japan), respectively.

RESULTS AND DISCUSSION

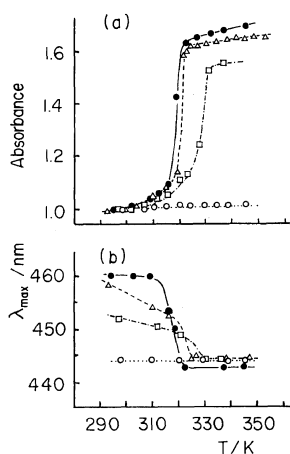*Dependence of Absorbance of Poly(A)·Poly(U) Duplex–Dye Complex on the Temperature*

In a previous paper,[5] the absorption spectra of solutions containing various amounts of the poly(A)·poly(U) duplex in a definite concentration of dyes were measured, and the following information was obtained: the wavelength of the maximum absorbance shifted to red as the concentration of poly(A)·poly(U) duplex increased, and the isosbestic points were about 454 nm for (PF), and about 428 nm for (9-AA). From these facts, it was concluded that the interaction between poly(A)·poly(U) duplex, and dye may correspond to the intercalation process in which the dye molecule is bound to a polynucleotide base.

In order to confirm the thermal stability of the poly(A)·poly(U) duplex, the dependence of absorbance of poly(A)·poly(U) duplex solutions containing various concentrations of dye on the temperature was studied by using the spectrophotometer. The results obtained are shown in Figures 1a and 2a, where the absorbance of the poly(A)·poly(U) duplex–(PF) and –(9-AA) systems at 260 nm are plotted against the temperature. As seen in Figures 1a and 2a, the absorbance at 260 nm for a solution containing only dye shows a definite value which is independent of temperature, but the absorbance at 260 nm for poly(A)·poly(U) duplex–dye complex resembles only that for the poly(A)·poly(U) duplex, thus demonstrating that this increase in absorbance with a rise in temperature for the poly(A)·poly(U) duplex–dye complex corresponds to the helix–coil transition of the poly(A)·poly(U) duplex.

The plots of the wavelength of absorption maximum, λ_{max}, in the visible region against the temperature are shown in Figures 1b and 2b, respectively. Figure 1b shows that with an increase in temperature, λ_{max} of the poly(A)·poly(U) duplex–(PF) complex decreases initially and then attains a definite value of 444 nm corresponding to that of a solution containing only (PF). This suggests that the (PF) molecule seems to interact with the poly(A)·poly(U) duplex. This tendency in the poly(A)·poly(U) duplex–(PF) system is the same as that of the poly(A)·poly(U) duplex–(9-AA) system.

From the dependence of absorbance at 260 nm

Figure 1. The dependence of (a) absorbance of wavelength at 260 nm and (b) wavelength of absorption maximum (λ_{max}) on temperature for the poly(A)·poly(U) duplex–proflavine system. [D]/[P]=0.02 (●), 0.06 (△), 0.18 (□), and only proflavin (○). The concentration of nucleotide phosphate, [P], was 1.2×10^{-4} mol dm^{-3}.
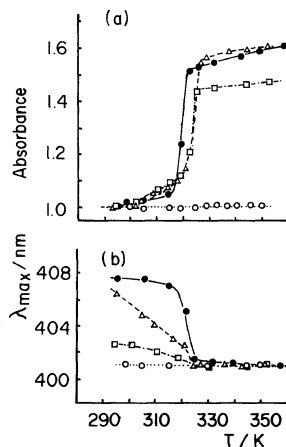
Figure 2. The dependence of (a) absorbance at wavelength of 260 nm and (b) wavelength of absorption maximum (λ_{max}) on temperature for the poly(A)·poly(U) duplex–9-aminoacridine system. [D]/[P]=0.02 (●), 0.09 (△), 0.24 (□), and only 9-aminoacridine (○). The concentration of nucleotide phosphate, [P], was 1.3×10^{-4} mol dm^{-3}.

and λ_{max} on temperature, dye molecules may be considered to dissociate from the poly(A)·poly(U) duplex–dye complex when the double-stranded helical form of poly(A)·poly(U) duplex transforms to the coil form of poly(A) and poly(U) with rise in temperature, according to the following reaction.
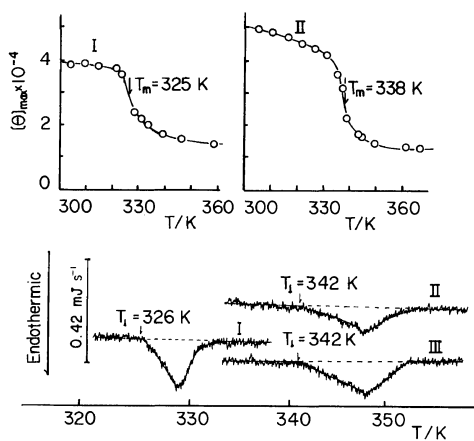
$$\text{Poly(A)}\cdot\text{Poly(U)--Dye} \rightarrow \text{Poly(A)} + \text{Poly(U)} + \text{Dye} \quad (1)$$
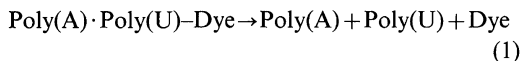
Thermal Properties of the Poly(A)·Poly(U) Duplex–Dye Complex

In order to estimate the heat of dissociation of dye molecules from the poly(A)·poly(U) duplex–dye complex, the DSC measurements of the poly(A)·poly(U) duplex–dye complex solutions of various dye concentrations were made.

The DSC curves obtained show an endothermic peak, having temperature width of 15—20 K, which corresponds to reaction (1) and are shown in Figure 3. As seen in Figure 3, the temperature, T_i, at which an endothermic peak begins to appear, rather than a peak temperature, in the DSC curve is in fair agreement with the transition temperature, T_m, determined from CD spectra measurements.

The apparent heat of transition, ΔH_{app}, per mole of base pair (bpm) for reaction (1) was estimated from the area under the peak of the DSC curve.

The plots of T_i and ΔH_{app} against the ratio, [D]/[P], of the number of moles of dye, [D], to that of nucleotide, [P], are shown in Figure 4.

As seen from Figure 4, T_i and ΔH_{app} for both systems increase at first and then take on definite values as [D]/[P] increases.

It was found that each ΔH_{app} has a definite value, when [D]/[P] is in the region of about 0.05 to 0.15 for (9-AA) and about 0.12 to 0.22 for (PF) systems, suggesting that these regions for both systems correspond to the intercalation process pointed out in the previous paper.[5]

The apparent heat, ΔH_{app}, corresponds to the sum of the net heat of helix–coil transition, ΔH_t, of poly(A)·poly(U) duplex and the heat of dissociation, ΔH_D, of dye from poly(A)·poly(U) duplex–dye complex according to the reaction (1). Thus ΔH_D is given by the difference between ΔH_{app} and ΔH_t which corresponds to the heat of the helix–coil transition at [D]/[P]=0. However, in order to convert this ΔH_D into that per mole of dye, the percentage of bound dye to poly(A)·poly(U) duplex must be estimated exactly.

In the treatment of Peacocke and Skerrett,[6] the amount of bound dye per mole of nucleotide phosphate, r, was plotted against the [D]/[P] and the results obtained are shown in Figure 5a.

Figure 3. Typical DSC curves and heating curves of CD spectra for various concentrations of proflavine. [D]/[P]=0 (I), 0.12 (II), and 0.21 (III). The concentrations of nucleotide phosphate, [P], used in DSC and CD spectra measurements were 1.0×10^{-2} and 4.0×10^{-4} mol dm^{-3}, respectively.
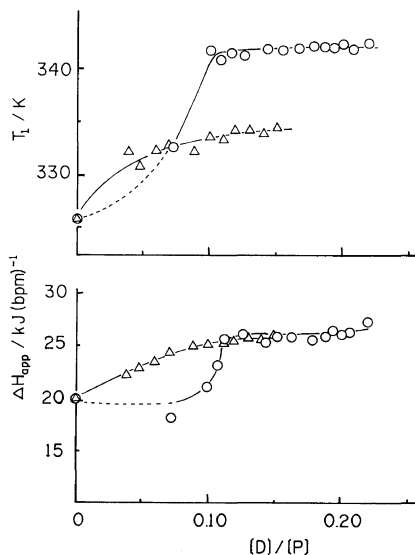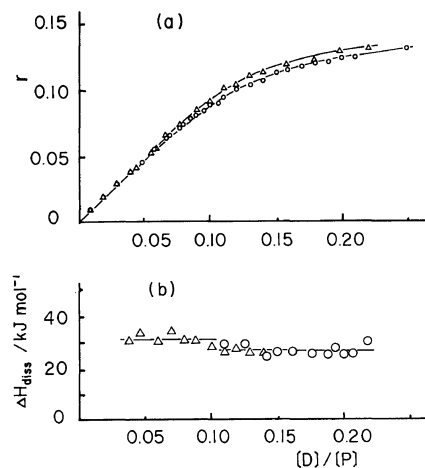

Figure 4. The dependence of the transition temperature, T_i, and the apparent heat of helix–coil transition, ΔH_{app}, of poly(A)·poly(U) duplex on the concentration of dye ([D]/[P]): $\bigcirc$, proflavine; $\triangle$, 9-aminoacridine. The concentration of nucleotide phosphate, [P], was 1.0×10^{-2} mol dm^{-3}.

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Figure 5. The dependence of (a) bound dye per nucleotide phosphate, r, and (b) heat of dissociation, ΔH_{diss}, per mole of dye on the concentration of dye ([D]/[P]): $\bigcirc$, proflavine; $\triangle$, 9-aminoacridine systems.

In Figure 5a, for both poly(A)·poly(U) duplex–(PF) and –(9-AA) systems, r increases at first and then reaches a definite value as [D]/[P] increases.

A definite value to which r approaches corresponds to the termination of 1:1 interaction between the poly(A)·poly(U) duplex and the dye for the intercalation process as was suggested in the previous paper.[5]

By using the value of r corresponding to the intercalation process, the heat of dissociation per mole of dye, ΔH_{diss}, for each [D]/[P] are calculated and plots of ΔH_{diss} for poly(A)·poly(U) duplex–(PF) and –(9-AA) systems against [D]/[P] are also shown in Figure 5b.

Figure 5b shows that the values of ΔH_{diss} are

Table I. Heats of dissociation, ΔH_{diss}, per mole of dye of intercalation process for poly(A)·poly(U) duplex–dye complexes

Dye	ΔH_{diss} kJ mol^{-1}	ΔH^{a} kJ mol^{-1}
Proflavine	27.0 ± 2.7	-32
9-Aminoacridine	31.4 ± 3.5	-28

[a] Heat of interaction per mole of dye estimated from heat of mixing.

27.0 ± 2.7 kJ per mole of dye for poly(A)·poly(U) duplex–(PF) and 31.4 ± 3.5 kJ for poly(A)·poly(U) duplex–(9-AA) systems and that they are nearly independent of [D]/[P].

The results obtained are listed in Table I, together with the results of the previous paper.[5] As seen in Table I, ΔH_{diss}-values for both systems are in good agreement with those calculated by the theoretical treatment in previous paper[5] within experimental error.

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