Polymer Journal, Vol. 15, No. 9, pp 657—661 (1983)

The Thermodynamic Characterization of Poly(I)·Poly(C) Duplex–Aminoacridine Dye System

Kazuo KANO, Yoshihiro BABA, Akihiro KAGEMOTO,† and Charles L. BEATTY*

Department of Chemistry, Osaka Institute of Technology, Omiya Asahi-ku, Osaka 535, Japan
**Materials Science and Engineering, University of Florida, Gainesville, Florida 32611, U.S.A.*

(Received March 29, 1983)

ABSTRACT: Thermodynamic quantities of dyes (proflavine, PF and 9-aminoacridine, 9AA) bound to the poly(I)·poly(C) duplex in the intercalation process have been evaluated from measurement of the heat of mixing. The free energy change for the duplex–PF system is smaller than that for the duplex–9AA system, indicating that the intercalation of PF into the poly(I)·poly(C) duplex is more stable than the intercalation of 9AA, as in the case of that in the poly(A)·poly(U) duplex–dye system reported previously. This conclusion was drawn from the heat of interaction between PF and poly(I)·poly(C) duplex being larger than that between 9AA and poly(I)·poly(C) duplex. In addition, the enthalpy change of the poly(I)·poly(C) duplex–dye system is smaller than that of poly(A)·poly(U) duplex–dye one, suggesting that the conformational change of the poly(I)·poly(C) duplex in the intercalation process is considerably larger than that of the poly(A)·poly(U) duplex.

KEY WORDS Poly(I)·Poly(C) Duplex / Proflavine / 9-Aminoacridine / UV Spectrum / Heat of Mixing / Intercalation Process / Thermodynamics of Interaction /

Aminoacridine dyes are bound to DNA in two different ways, depending on the concentration of dye: The first type corresponds to strong binding[1] and/or the intercalation process[2] in which dye molecules are bound to the DNA base at dilute concentration of dye. The second type corresponds to the weak binding[1] and/or the stacking process[2] in which dye molecules are bound to the side of DNA molecules without base specificity as the concentration of dye is increased.

The interaction in the intercalation process seems due to an interaction of the flat aromatic dye molecules between the base pairs of DNA as reported by many investigators.[3-9] However, the exact steric location of the intercalated dye has not yet been established.

In the previous papers,[10,11] it was shown that the heat of interaction between the DNA and dye with amino group differs from that between DNA and dye without amino group, however, the intercalated dyes in DNA keep a similar stable condition. These facts sugggest that the location of the intercalated dye in DNA depends on the presence of amino group of dye.[10] The intercalated dye also depends on the position of substitution of the amino groups. The intercalated dye with an amino group on either the 3- or 6-position is more stable than that with an amino group on the 9-position, as inferred from the fact that the heat of interaction between proflavine and the poly(A)·poly(U) duplex is larger than that between 9-aminoacridine and the poly(A)·poly(U) duplex.[11]

In this paper, in order to obtain pertinent information on the base specificity of nucleic acid in the intercalation process of the dye, the heat of mixing of the poly(I)·poly(C) duplex with aminoacridine dye was measured and thermodynamic quantities of the intercalated dye in the poly(I)·poly(C) duplex

† To whom correspondence should be addressed.

were evaluated.

EXPERIMENTAL

Poly(riboinosinic acid), poly(I) and poly(ribocytidylic acid), poly(C) were perchased from Yamasa Shoyu Co., Ltd., Japan. The dyes used in this study were proflavine (PF, from Aldrich Chemical Co., Inc., U.S.A.) and 9-aminoacridine (9AA, from Tokyo Chemical Co., Ltd., Japan). All other materials were of analytical reagent grade. All measurements were made in 0.1 mol·dm^{-3} Tris-HCl buffer solutions at pH 7.60.

The calorimeter used was the same as that described previously.[10] The poly(I)·poly(C) duplex with a double stranded helical structure was formed from an equimolar mixture of poly(I) and poly(C) and mixed with a dye solution. In the calorimetric studies, the poly(I)·poly(C) duplex concentrations were about 5.0×10^{-4} mol dm^{-3} with respect to the phosphate unit of polynucleotide and the concentration of dye in solution was varied. The polynucleotide concentration was determined by analysis of phosphorus as described previously.[10]

The absorption spectra of the mixtures of poly(I)·poly(C) duplex and dye were measured with a Hitachi 124 spectrophotometer. In these measurements, the dye concentration was fixed and the amount of poly(I)·poly(C) duplex was changed.

RESULTS AND DISCUSSION

Spectral Measurement

The absorption spectra taken for dye solutions containing various amounts of poly(I)·poly(C) duplex are shown in Figures 1(a) and (b). It is seen that the wavelength of the maximum absorbance shifts to the red as the poly(I)·poly(C) duplex concentration increases, and the well defined isosbestic points for poly(I)·poly(C) duplex–PF and –9AA systems are about 454 and 428 nm, respectively, quite comparable to the results of DNA–PF,[5] poly(A)·poly(U) duplex–PF,[11] and DNA–9AA,[12] poly(A)·poly(U) duplex–9AA systems.[11] Specificically, the interaction between the poly(I)·poly(C) duplex and PF or 9AA appears to lead to intercalation similar to the DNA–dye and poly(A)·poly(U) duplex–dye systems.

The percentage of dye bound to the poly(I)·poly(C) duplex was calculated according to Peacocke and Skerrett,[13] and the results are shown in Figures 2(a) and 3(a), where the amount of bound dye per polynucleotide phosphate, r, is plotted against the mole ratio, D/P, of dye, D, to polynucleotide phosphate, P. It is seen that for both

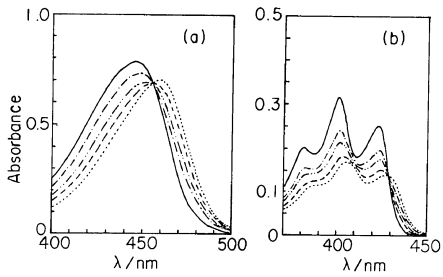

Figure 1. Typical absorption spectra of dye solutions containing various concentrations of poly(I)·poly(C) duplex in 0.1 mol dm^{-3} Tris-HCl buffer solution at pH 7.60. (a): Poly(I)·poly(C) duplex–proflavine system; proflavine concentration, 5.0×10^{-5} mol dm^{-3}. Concentration of nucleotide phosphate: ——, zero; —·—, 3.0×10^{-4}; —··—, 6.0×10^{-4}; – – –, 1.0×10^{-3}; ·····, 2.5×10^{-3} mol·dm^{-3}. (b): Poly(I)·poly(C) duplex–9-aminoacridine system; 9-aminoacridine concentration, 3.2×10^{-5} mol dm^{-3}. Concentration of nucleotide phosphate: ——, zero; —·—, 1.2×10^{-4}; —··—, 2.0×10^{-4}; – – –, 4.8×10^{-4}; ·····, 8×10^{-4} mol dm^{-3}.

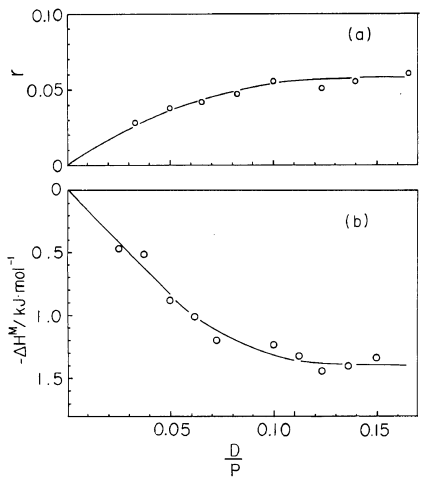

Figure 2. The dependence of (a) the amount of bound dye per mole of polynucleotide phosphate, r, and (b) the heat of mixing per mole of poly(I)·poly(C) duplex phosphate, ΔH^M, on the mole ratio, D/P, of proflavine, D, to polynucleotide phosphate, P, for poly(I)·poly(C) duplex–proflavine system in a 0.1 mol·dm^{-3} Tris-HCl buffer solution at pH 7.60.

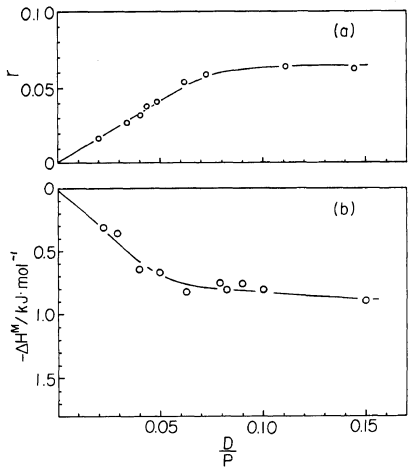

Figure 3. The dependence of (a) the amount of bound dye per mole of polynucleotide phosphate, r, and (b) the heat of mixing per mole of poly(I)·poly(C) duplex phosphate, ΔH^M, on the mole ratio, D/P, of 9-aminoacridine, D, to polynucleotide phosphate, P, for poly(I)·poly(C) duplex–9-aminoacridine system in a 0.1 mol·dm^{-3} Tris-HCl buffer solution at pH 7.60.

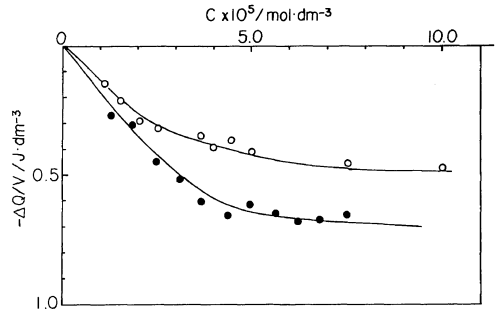

Figure 4. The dependence of observed heat of mixing per total volume of solution, $\Delta Q/V$, on the concentration of dye, C, in a 0.1 mol dm^{-3} Tris-HCl buffer solution (pH 7.60) containing poly(I)·poly(C) duplex of 5.0×10^{-4} mol dm^{-3} with respect to phosphate units at 298 K; ●, poly(I)·poly(C) duplex–proflavine system; ○, poly(I)·poly(C) duplex–9-aminoacridine system. Solid line, $\Delta Q/V$ calculated from eq 5 and the optimized ΔH, K, and n values presented in Table I.

poly(I)·poly(C) duplex–PF and –9AA systems, r increases rather sharply at first and then, at D/P values ranging from 0.07 to 0.09, levels off with a further increase in D/P. These results suggest the termination of interaction between poly(I)·poly(C) duplex and dye in the intercalation process at about 0.07 to 0.09 of D/P.

Heats of Mixing

The heats of mixing for the poly(I)·poly(C) duplex–PF and –9AA systems were measured over the dye concentration from about 1.0×10^{-5} to 10×10^{-5} mol dm^{-3} with a twin microcalorimeter at 298.15 ± 0.005 K. All these systems proved to be exothermic, indicating a considerable interaction between poly(I)·poly(C) duplex and dye. Thus, it may be assumed that the heat of dilution of the poly(I)·poly(C) duplex is negligibly small.

The results obtained are shown in Figures 2(b) and 3(b), where the heat of mixing, ΔH^M, per mole of duplex phosphate against mole ratio, D/P, of the dye, D, to poly(I)·poly(C) duplex phosphate, P, is plotted. The absolute value of ΔH^M increases monotonously at first and then at D/P values of about 0.08 for the PF system and about 0.06 for the 9AA system, levels off with a further increase in D/P. These dependences of ΔH^M on D/P are comparable to those of r as seen in Figures 2(a) and 3(a), respectively, suggesting that ΔH^M is mainly associated with the intercalation process.

Thermodynamic Quantities

Assuming that the poly(I)·poly(C) duplex–dye complex formation in the intercalation process obeys the following reaction

$$\text{poly(I)}\cdot\text{poly(C) duplex}+\text{dye} \rightleftharpoons (\text{poly(I)}\cdot\text{poly(C) duplex–dye}) \quad (1)$$

we express the binding constant, K, as

$$K=\frac{r}{(n-r)\cdot C_f} \quad (2)$$

where n is the number of binding sites per poly(I)·poly(C) duplex phosphate, r, the amount of bound dye per poly(I)·poly(C) duplex phosphate, and C_f, the concentration of free dye. The heat of interaction, ΔH, of poly(I)·poly(C) duplex–dye complex per mole of dye can be expressed by

$$\Delta H=\frac{\Delta Q}{C_b\cdot V} \quad (3)$$

where ΔQ is the observed heat, C_b, the concentration of bound dye related to r and the molar concentration, P, in phosphate of the poly(I)·poly(C) duplex by $C_b=r\cdot P$, and V is the total

Table I. Thermodynamic quantities for the intercalated dye in a polynucleotide duplex estimated from the heat of mixing at 298 K

Duplex	Dye	K[a]	ΔG	ΔH	ΔS	n[b]
		$dm^3\,mol^{-1}$	$kJ\,mol^{-1}$	$kJ\,mol^{-1}$	$J\,K^{-1}\,mol^{-1}$	
Poly(I)·poly(C)	PF	2.0×10^5	−31	−25	20	0.06
	9AA	9.0×10^4	−28	−21	23	0.05_5
Poly(A)·poly(U)[c]	PF	8.0×10^5	−34	−32	6.7	0.25
	9AA	1.2×10^5	−29	−28	3.3	0.21

Mol refers to moles of dye.

[a] Binding constant between polynucleotide duplex and dye.

[b] Number of binding sites per nucleotide phosphate.

[c] See ref 11.

volume of the mixture of poly(I)·poly(C) duplex and dye solutions.

The total concentration of dye, C, is the sum of C_b and C_f

$$C = C_b + C_f \tag{4}$$

From eq 2 to 4, ΔQ is represented by the following function of C, in which the negative sign of the right side must be chosen because the $\Delta Q/V$ vanishes at zero concentration of the dye.

$$\frac{\Delta Q}{V} = \frac{\Delta H}{2}\left\{\frac{1}{K} + n\cdot P + C - \sqrt{\left(\frac{1}{K} + n\cdot P + C\right)^2 - 4\cdot nPC}\right\} \tag{5}$$

The values for ΔH, K, and n which give the best fit of the calculated values by eq 5 to the observed ones can be determined by optimization of eq 5. The solid lines in Figure 4 show the best fit curves calculated with the trial-and-error estimated values for ΔH, K, and n summarized in Table I.

The free energy change, ΔG, and the entropy change, ΔS, in the intercalation process of the poly(I)·poly(C) duplex–dye system can be calculated from the following equations

$$\Delta G = -RT\ln k$$
$$\Delta S = (\Delta H - \Delta G)/T \tag{6}$$

The numerical results are listed in the forth and sixth columns of Table I, which includes the thermodynamic quantities obtained for the poly(A)·poly(U) duplex–dye system in the previous paper.[11]

As seen from Table I, the free energy change for the poly(I)·poly(C) duplex–PF system in the intercalation process is smaller than that for the poly(I)·poly(C) duplex–9AA system, suggesting that the intercalated PF is more stable than the intercalated 9AA. This result is similar to those for poly(A)·poly(U) duplex–PF and –9AA systems.

However, the intercalated dye in the poly(I)·poly(C) duplex is less stable than that in the poly(A)·poly(U) duplex, because the free energy change for the poly(I)·poly(C) duplex–dye system is considerably larger than that for the poly(A)·poly(U) duplex–dye system. This seems due to the enthalpy change in the intercalation process as described below.

As seen from Table I, the enthalpy change, ΔH, for the poly(I)·poly(C) duplex–dye complex formation in the intercalation process depends on the position of amino substituent on the dye molecule, as in the poly(A)·poly(U) duplex–dye system; $-\Delta H$ for PF which has amino groups at the 3- and 6-positions is larger than that for 9AA which has an amino group at the 9-position.

This fact may be attributed to hydrogen bonding between the phosphate group with negative charge in the nucleotide backbone and the amino group on the 3- and/or 6-position as reported by Peacocke *et al.*[14] The energy of this hydrogen bond may correspond to the difference in ΔH between the poly(I)·poly(C) duplex–PF and –9AA systems and/or the poly(A)·poly(U) duplex–PF and –9AA systems, which is −4 kJ for poly(I)·poly(C) duplex–

dye systems, just as for the poly(A)·poly(U) duplex–dye systems. Thus, the hydrogen bond energy between the phosphate group with a negative charge in the backbone of the polynucleotide duplex and the amino group at either the 3- and/or 6-position is about −4 kJ.

The intercalation process of a dye molecule in the nucleic acid of a double stranded helical structure may be caused by interaction between base pairs of the nucleic acid and dye molecule. For this to occur, extension and unwinding of the nucleotide backbone in the immediate vicinity of the intercalated dye is required, leading to a decrease in the sedimentation coefficient and an increase in viscosity, as found by Lerman.[2] Unwinding of the polynucleotide duplex is generally considered necessary for the intercalation process. Thus, the enthalpy change, ΔH, accompanying this process is partitioned into the energy of conformational change, ΔH_1, which is required for unwinding of the nucleotide backbone corresponding to opening up the base paris of the duplex, and the net interaction energy, ΔH_2, which is that of the interaction of the duplex base pairs with the dye molecule.

Table I indicates that the smaller absolute value of ΔH for the poly(I)·poly(C) duplex–dye complex than that for the poly(A)·poly(U) duplex–dye complex is due to the difference in the conformational change of these polynucleotide duplexes because ΔH_1 depends strongly on the base pair sequence, whereas ΔH_2 does not, as reported by Miller *et al.*[15] Thus, the conformational change of the poly(I)·poly(C) duplex in the intercalation process may be larger than that of poly(A)·poly(U) duplex since the translation distance of 3 Å between base pairs in the poly(I)·poly(C) duplex is smaller than 3.1 Å in the poly(A)·poly(U) duplex.[16]

The number of dye binding sites per duplex phosphate, n, of the poly(A)·poly(U) duplex–dye system is larger than that of the poly(I)·poly(C) duplex–dye system in the intercalation process. This suggests that the dye molecule is more easily intercalated into the poly(A)·poly(U) duplex than into the poly(I)·poly(C) duplex.

REFERENCES

1. A. Blake and A. R. Peacocke, *Biopolymers*, **6**, 1225 (1968).
2. L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961).
3. E. Fredericq and C. Houssier, *Biopolymers*, **11**, 2281 (1972).
4. J. B. Lepecq and C. Paoletti, *J. Mol. Biol.*, **27**, 87 (1967).
5. C. H. Lee, C. T. Chang, and J. G. Wetmur, *Biopolymers*, **12**, 1099 (1973).
6. A. Blake and A. R. Peacocke, *Biopolymers*, **5**, 383 (1967).
7. A. Blake and A. R. Peacocke, *Biopolymers*, **5**, 871 (1967).
8. B. J. Gardner and S. F. Mason, *Biopolymers*, **5**, 79 (1967).
9. G. Lober, H. Shutz, and V. Kleinwachter, *Biopolymers*, **11**, 2439 (1972).
10. S. Tanaka, Y. Baba, and A. Kagemoto, *Makromol. Chem.*, **182**, 1275 (1981).
11. S. Tanaka, Y. Baba, A. Kagemoto, and R. Fujishiro, *Makromol. Chem.*, **182**, 2837 (1981).
12. D. O. Jordan and L. N. Sansen, *Biopolymers*, **10**, 399 (1971).
13. A. R. Peacocke and J. N. H. Skerrett, *Trans. Faraday Soc.*, **52**, 261 (1956).
14. N. J. Pritchard, A. Blake, and A. R. Peacocke, *Nature*, **212**, 1361 (1966).
15. K. J. Miller, R. Brodzinsky, and S. Hall, *Biopolymers*, **19**, 2091 (1980).
16. V. Bloomfield, D. M. Crothers, and I. Tinoco, Jr., "Physical Chemistry of Nucleic Acid," Harper & Row, Publishers, New York, N. Y., 1974.