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Thermodynamic characterization of the 9-aminoacridine intercalated into DNA

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Abstract

The interaction of DNA with 9-aminoacridine was studied by means of calorimetric and spectral measurements, and the thermodynamic quantities for 9-aminoacridine intercalated into DNA were determined. Changes in free energy ΔG, enthalpy ΔH, and entropy ΔS accompanying the intercalation process were estimated to be about -36 kJ mol^{-1}, -22 kJ mol^{-1} and 47 J K^{-1} mol^{-1} respectively, suggesting that the interaction between DNA and 9-aminoacridine appears to form a stable complex from the viewpoint of free energy and that this complex formation is governed by both enthalpy and entropy.

INTRODUCTION

It is well known that heterocyclic aromatic dyes interact with DNA having a double-stranded helical structure by hydrogen bonding between complementary base pairs in aqueous solution, and also that the DNA–dye complexes have an interaction mode which is called intercalation, based on the insertion of acridine dyes between adjacent base pairs of DNA [1].

In our previous papers [2–4] it has been suggested that the location of the intercalated dye in the DNA–dye complex depends on the presence of an amino group in the dye and the position of substitution of the amino group of the dye from the thermodynamic point of view [2]. Furthermore, the enthalpy change accompanying the intercalation of 9-aminoacridine (9-AA) into the poly(A) · poly(U) duplex is appreciably less than that for the poly(I) · poly(C) duplex, although the free energy change of intercalation of 9-AA is slightly less for the former duplex than for the latter [3,4]. These results may indicate that the interaction of 9-AA into adjacent base pairs of nucleic acid depends on the base sequence and/or the conformation of the polynucleotide duplex, since the conformation of the poly(A) ·

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poly(U) duplex is A-form and that of the poly(I) · poly(C) duplex is A′-form [5].

In this paper, in order to obtain pertinent information about the conformation of the nucleic acid and/or the base sequence in relation to the intercalation process of 9-AA, the interaction of DNA having the B-form with 9-AA was studied by means of calorimetric and spectral methods.

The thermodynamic quantities of 9-AA intercalated into DNA were evaluated by combining the calorimetric and spectral results. We discuss the influence of the conformation of DNA and the base pair sequence on the intercalation process of 9-AA.

EXPERIMENTAL

Materials

Calf thymus DNA containing 42% of guanine–cytosine base pairs was purchased from Sigma Chemical Co. Ltd., USA. 9-Aminoacridine hydrochloride was purchased from Nakarai Kagaku Co. Ltd., Japan. Both were used without further purification. All other materials were of analytical reagent grade. All measurements were made in 0.01 mol dm^{-3} phosphate buffer solution at pH 7.00.

Water was passed through a reversible osmotic membrane, distilled and, finally, deionized by an ion exchange resin.

Apparatus and procedure

The calorimeter used for measurements of the heats of mixing of DNA and 9-AA solutions was the same LKB batch type microcalorimeter as reported previously [6]. In the calorimetric measurements, DNA solution, at a known concentration of 5.0×10^{-4} mol dm^{-3} in DNA nucleotide, and 9-AA solutions of various concentrations were mixed in equal volumes of about 1.0 cm^3 in a sample cell. In the reference cell, the buffer solution and 9-AA solution at the same concentration as in the sample cell (to compensate for the heat of dilution of 9-AA) were mixed in equal volumes of about 1.0 cm^3. The DNA concentration was determined spectrophotometrically using $\epsilon_{260} = 6700$, which was the value estimated from phosphorus analysis of the DNA [7].

To obtain the binding parameters between DNA and 9-AA, the absorption spectra of DNA–9-AA solutions were recorded with a Hitachi 220A spectrophotometer. DNA solution at a concentration of 1.4×10^{-3} mol dm^{-3} in DNA nucleotide and 9-AA solution at a concentration of 9.0×10^{-5} mol dm^{-3} were mixed in equal volumes and successive portions of 9-AA solution at 4.5×10^{-5} mol dm^{-3} were then added. Thus the concentration of 9-AA was fixed and that of DNA was varied.

RESULTS AND DISCUSSION

Heat of mixing

The heats of mixing for the DNA–9-AA system were measured over the concentration range 5.0×10^{-6}–1.0×10^{-4} mol dm^{-3} of 9-AA by using a microcalorimeter at 298.15 ± 0.005 K. The results obtained proved to be exothermic, demonstrating that an interaction takes place between DNA and 9-AA. The observed heat of mixing $\Delta_{mix}H$ per nucleotide of DNA is shown in Fig. 1, curve a, where $\Delta_{mix}H$ is plotted against the molar ratio r of 9-AA to DNA nucleotide. As seen in Fig. 1, curve a, the absolute value of $\Delta_{mix}H$ increases at first and then levels off at $r > 0.12$ with an increase in r. The definite value at $r > 0.12$ seems to signify the termination of the interaction between DNA and 9-AA.

Spectral measurements

In order to confirm whether or not the exothermic nature of $\Delta_{mix}H$ is attributable to the interaction of DNA with 9-AA, the absorption spectra obtained for 9-AA solutions with various amounts of DNA were measured at room temperature, and the results obtained are shown in Fig. 2. As seen in Fig. 2, the wavelength of the maximum absorbance undergoes a red shift as the DNA concentration increases, and an isobestic point for the DNA–9-AA system exists at 427 nm, in line with that reported previously [3,4],

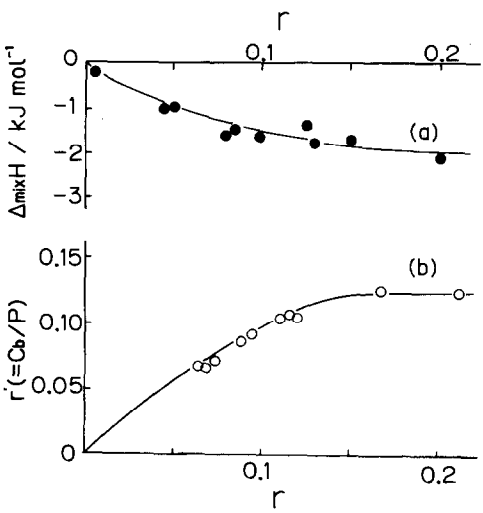

Fig. 1. Dependence of (curve a) the heat of mixing per mole of DNA nucleotide $\Delta_{mix}H$ and (curve b) the molar ratio r' ($= C_b/P$) of bound 9-AA (C_b) to DNA nucleotide (P) on the molar ratio r of 9-AA to DNA nucleotide.

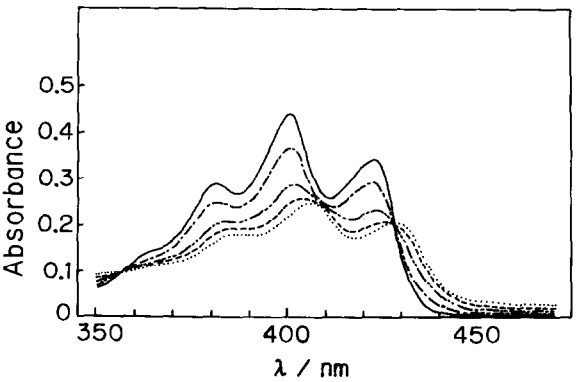

Fig. 2. Typical absorption spectra of 9-AA solutions (4.5×10^{-5} mol dm^{-3}) containing various concentrations of DNA: ———, zero; — · — · —, 1.6×10^{-4}; — · · — · · —, 3.7×10^{-4}; - - - - - -, 4.7×10^{-4}; · · · · · ·, 7.0×10^{-4} mol dm^{-3}.

suggesting that the interaction between DNA and 9-AA takes the form of intercalation due to insertion of 9-AA between the base pairs of DNA in a similar fashion to the poly(A) · poly(U) and poly(I) · poly(C) duplex–dye systems. Thus the exothermic nature of $\Delta_{mix}H$ corresponds to the enthalpy change involved in the intercalation process.

The percentage of 9-AA bound to DNA was calculated by the method of Peacocke and Skerrett [8] and the results obtained are shown in Fig. 1, curve b, where the molar ratio r' ($= C_b/P$) of bound 9-AA (C_b) per DNA nucleotide (P) is plotted against the molar ratio r of total 9-AA to DNA nucleotide. As seen in Fig. 1, curve b, the r' value increases at first and then levels off at $r > 0.12$ with an increase in r. This tendency is in good agreement with that of $\Delta_{mix}H$ as shown in Fig. 1, curve a. It is therefore suggested from these results that the interaction between DNA and 9-AA in the intercalation process terminates at $r = 0.12$.

In addition, Scatchard plots prepared for the DNA–9-AA system to obtain binding parameters for the intercalation process are shown in Fig. 3. The plots give a straight line in the range up to $r' = 0.12$. The binding constant K and the number of binding sites per DNA nucleotide n are estimated from the slope and the intercept on the vertical axis of the straight line respectively; the values obtained are listed in Table 1, together with those for the polynucleotide duplex–9-AA system reported previously [3,4].

Thermodynamic quantities for 9-AA intercalated into DNA

In order to obtain thermodynamic parameters for the intercalation process of the DNA–9-AA system at $r' < 0.1$, the following analysis was

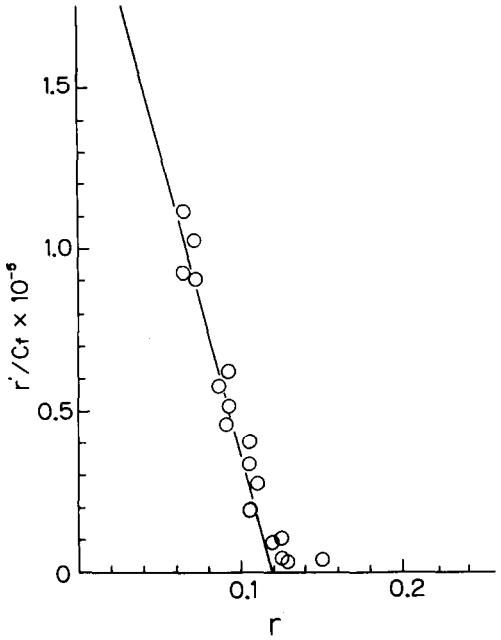

Fig. 3. Scatchard plots of the DNA–9-AA system; C_f and r' here refer to the concentration of free dye and the molar ratio of bound 9-AA to DNA nucleotide respectively.

tested. Assuming that the DNA–9-AA complex is formed by the following reaction between DNA and 9-AA

$$\text{DNA} + \text{9-AA} \rightleftharpoons \text{(DNA–9-AA) complex} \tag{1}$$

the binding constant K can be expressed as follows:

$$K = \frac{C_b}{(nP - C_b)(C - C_b)} \tag{2}$$

TABLE 1

Binding constant K and number of binding sites per DNA nucleotide n for intercalation of 9-AA into DNA and/or polynucleotide duplexes estimated from Scatchard plots

System	K ($dm^3\ mol^{-1}$)	n
DNA	1.9×10^6	0.12
Poly(A)·poly(U) duplex [a]	1.2×10^5	0.21
Poly(I)·poly(C) duplex [b]	9.0×10^4	0.05

[a] Ref. 3.
[b] Ref. 4.

TABLE 2

Enthalpy change ΔH per mole of 9-AA accompanying the intercalation process

$C \times 10^5$ (mol dm^{-3})	r [a]	r' [b]	$-\Delta H$ (kJ mol^{-1}) [c]
0.5	0.010	0.0099	21.5
2.35	0.047	0.0464	23.6
2.5	0.050	0.0492	21.4
3.75	0.075	0.0734	23.8
3.95	0.079	0.0772	21.2
4.90	0.098	0.0942	18.1 ave. 22

[a] r denotes the molar ratio of 9-AA to DNA nucleotide.
[b] r' denotes the molar ratio of bound 9-AA to DNA nucleotide.
[c] Here, mol means mole of 9-AA.

where P is the molar concentration of DNA, C_b is the molar concentration of 9-AA bound to DNA and C is the total molar concentration of 9-AA respectively. From eqn. (2), C_b can be expressed as follows:

$$C_b = \frac{1}{2}\left(nP + C + \frac{1}{K} - \sqrt{\left(nP + C + \frac{1}{K}\right)^2 - 4nPC}\right) \tag{3}$$

C_b can be obtained relative to C using K and n values as listed in Table 1 for a given concentration P of DNA.

Further, the heat of interaction ΔH per mole of 9-AA between DNA and 9-AA can be related to the heat of mixing $\Delta_{mix}H$ per mole of DNA nucleotide as follows:

$$\Delta H = \frac{\Delta_{mix}H}{C_b/P} \tag{4}$$

From eqns. (3) and (4), ΔH can be obtained for each concentration of 9-AA. The ΔH values obtained are listed in Table 2 and the plots of ΔH

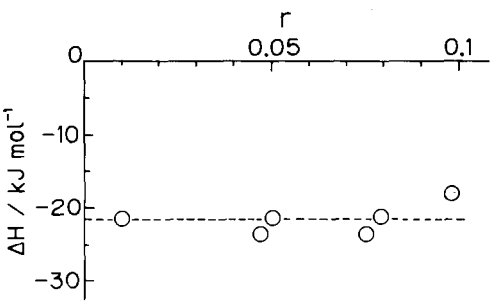

Fig. 4. Plots of the enthalpy change ΔH per mole of 9-AA against r.

TABLE 3

Thermodynamic quantities for 9-AA intercalated in DNA and/or polynucleotide duplexes

System	K ($dm^3\ mol^{-1}$)	ΔG ($kJ\ mol^{-1}$)	ΔH ($kJ\ mol^{-1}$)	ΔS ($J\ K^{-1}\ mol^{-1}$)
DNA (B-form)	1.9×10^6	−36	−22	47
A–U [a] (A-form)	1.2×10^5	−29	−28	3.3
I–C [b] (A'-form)	9.0×10^4	−28	−21	23

[a] Ref. 3.
[b] Ref. 4.

against r are shown in Fig. 4. As seen in Fig. 4, ΔH obtained at $r < 0.1$ shows a definite value which is nearly independent of r, and this definite value is about −22 kJ per mole of 9-AA on average, although the experimental values show some scatter. We suggest that a ΔH value of $-22\ kJ\ mol^{-1}$ corresponds to the net enthalpy change accompanying the intercalation between DNA and 9-AA. In addition, the binding mode of the DNA–9-AA system at $r > 0.1$ corresponds to intercalation and outside binding.

The free energy change ΔG and the entropy change ΔS in the intercalation process of the DNA–9-AA system can also be calculated from $\Delta G = -RT \ln K$ by using the K value listed in Table 1 and $\Delta S = (\Delta H - \Delta G)/T$ respectively. The thermodynamic quantities estimated in such a way are summarized in Table 3, together with those of poly(A) · poly(U) and poly(I) · poly(C) duplexes as reported previously [3,4]. As seen in Table 3, the absolute value of ΔG for the DNA–9-AA system is larger than that of the poly(A) · poly(U) and poly(I) · poly(C) duplex–9-AA systems, demonstrating that the DNA–9-AA complex is more stable than the polynucleotide duplex–9-AA systems. It is also seen from Table 3 that the absolute magnitude of ΔH takes the order poly(A) · poly(U) duplex > DNA > poly(I) · poly(C) duplex, suggesting that 9-AA may bind more strongly to the adenine–thymine base pair than to the guanine–cytosine base pair of DNA. On the other hand, it is worth noting that ΔS accompanying the intercalation process of 9-AA is a positive value, although ΔH is a negative value similar to the value for other intercalated dyes and drugs [9].

The DNA–9-AA complex formation may be attributed to the cooperative action between the behaviour of ΔH and ΔS. It is also an interesting problem that DNA–9-AA complex formation is governed by both ΔH and ΔS, whereas, poly(A) · poly(U) duplex–9-AA complex formation is dominated by ΔH. These differences in binding modes seem to depend on the conformations, such as the B-form of DNA and the A-form of the poly(A) · poly(U) duplex and also seem to depend on the effects of hydration and/or dehydration surrounding DNA accompanying the intercalation. However,

it is very difficult to analyse the phenomenon exactly owing to a lack of certain information such as the effects of hydration and/or dehydration surrounding DNA and the change of stacking energy between base pairs of DNA accompanying the intercalation of 9-AA. It is also very difficult to establish the reason why ΔS accompanying complex formation takes a positive value. However, the possible explanation is as follows. The positive entropy change in aqueous solution may reflect a solvent effect such as the structure disruption of both DNA-bound water [10] and hydrophobically restricted water surrounding the dye [11]. The values of ΔS listed in Table 3 provide the water structure effect by promoting an interaction of the stacking type between 9-AA and the DNA base. Thus the water structure effect in binding 9-AA depends on the conformation of the double-stranded helical structure of the nucleic acid. The water structure effect for the B-form is greater than that for A-form, because high relative humidity favours the B-form rather than the A-form [12,13]. Further study is needed.

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