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Thermodynamic studies on interactions between DNA and dye

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Abstract

The interactions in solution between DNA, DNA-I (GC content 26.5%), DNA-II (GC content 42%), and DNA-III (GC content 72%), with various contents of guanine–cytosine base pair (GC content), and 9-amino-acridine (9-AA) or quinacrine with side chains were studied by microcalorimetry and spectrophotometry. The thermodynamic quantities for dye intercalated into adjacent base pairs of DNA were determined.

From the results, the ΔG° values in the DNA-I–, -II–, and -III–9AA systems were estimated to be about -32 kJ, virtually independent of GC content, although the absolute values of ΔH increase with increasing GC content of DNA, suggesting that an interaction between DNA and 9-AA forms a thermodynamically stable complex, depending on the GC content of the DNA.

However, the ΔG° value for the DNA-II–quinacrine system is lower than that for the DNA-I– or DNA-III–quinacrine systems, demonstrating that an interaction between DNA-II and quinacrine forms a thermodynamically stable complex, compared with those in the DNA-I– and DNA-III–quinacrine systems.

An analysis for base specificity of dyes accompanying the intercalation was carried out according to Eq. (7) in the text. The thermodynamic quantities for dye intercalated into GC/GC, AT/AT, and GC/AT base pair sequences were estimated. From these results, the most stable base sequence with respect to the intercalation of 9-AA into adjacent base pairs was the AT/AT base pair rather than the GC/GC and AT/GC base pairs, its thermodynamic quantities being about -31 kJ mol^{-1} for ΔH, 5.0 J K^{-1} mol^{-1} for ΔS, and -33 kJ mol^{-1} for ΔG°. Therefore, 9-AA interacts preferentially with the AT/AT base pair sequence. However, in the quinacrine systems, the most stable base pair for intercalation of quinacrine into adjacent base pairs was the GC/AT base sequence, and its thermodynamic

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quantities being $\Delta H \approx -64\,\mathrm{kJ\,mol^{-1}}$, $\Delta S \approx -12\,\mathrm{J\,K^{-1}\,mol^{-1}}$, and $\Delta G^{\ominus} \approx -68\,\mathrm{kJ\,mol^{-1}}$ suggesting that interaction between DNA and quinacrine is governed by ΔH, and also, that the interaction mode of quinacrine is different from that of 9-AA.

Keywords: Base pair; Cytosine; DNA; Dye; Guanine; Intercalation; Thermodynamics; UVS

1. Introduction

It is well known that DNA interacts with dyes that are characterized by a heterocyclic aromatic chromophore, such as acridine dyes, the interaction exhibiting a binding mode described as the intercalation of a planar aromatic molecule into adjacent base pairs of DNA [1–8].

In our prevoius papers [9, 10], in order to obtain thermodynamic information concerning the intercalation resulting from the interaction between DNA and dye, interactions between DNA containing 42% guanine–cytosine base pairs and 9-aminoacridine (9-AA) dye, and between poly(A) · poly(U) duplex and ethidium bromide dye, were studied by means of microcalorimetry and spectrophotometry, and the thermodynamic quantities of the interaction were determined. From the results, we reported that the mode of interaction produced a thermodynamically stable complex, described as the intercalation of dye inserted into adjacent base pairs of DNA or of the poly(A) · poly(U) duplex. In this paper, in order to obtain thermodynamic information about the base specificity of DNA accompanying interaction of DNA with dye, interactions between DNA with various contents of guanine–cytosine base pair and 9-AA or quinacrine, with a 1-methyl-4-diethyl-aminobutylamino group as a side-chain, were studied by microcalorimetry and spectrophotometry.

We will discuss the dependence of the thermodynamic quantities for the intercalation of dye on the contents of guanine–cytosine base pairs.

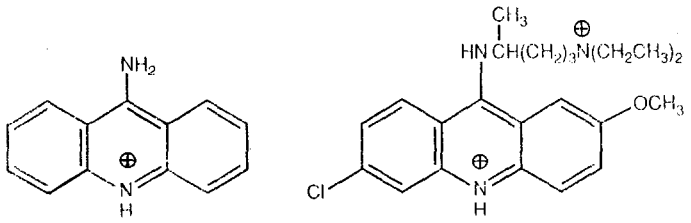
2. Materials and methods

2.1. Materials

The deoxyribonucleic acids, DNA, used in this study were *Clostridium perflingens* DNA, containing 26.5% [11] of guanine–cytosine base pairs (GC content) (DNA-I), calf thymus containing 42% [11] of GC content (DNA-II), and *Micrococcus lysodeikticus* containing 72% [11] of GC content (DNA-III), which were purchased from Sigma Chemical Co., USA.

The 9-aminoacridine hydrochloride (9-AA) and quinacrine dihydrochloride hydrate dyes were purchased from Nakarai Chemicals Ltd., Japan, and Funakoshi Medical Co. Ltd., Japan, respectively; their chemical structures are shown in Fig. 1.

The DNA samples were first purified several times according to the usual methods such as phenol–chloroform extraction to remove contaminants, e.g. protein-containing DNA.



9-Aminoacridine Quinacrine

Fig. 1. Chemical structures of 9-aminoacridine and quinacrine.

All other materials were of analytical reagent grade.

The solvent used in this study was 0.01 mol dm^{-3} phosphate buffer solution at pH 7.00.

Water used to prepare the buffer solution was passed through an osmotic membrane, deionized by ion exchange resin, distilled, and finally purified using a Milli-Q SP reagent water system (Millipore Co. Ltd., Japan).

2.2. Measurements

Absorption spectra of mixtures of DNA solution, of various GC contents, and dye solution were measured at room temperature with an ultraviolet (UV) absorption spectrophotometer (Hitachi 220A, Japan). UV spectra of DNA solutions of various concentrations at a given concentration of dye (9.0×10^{-5} mol dm^{-3}) were measured.

The calorimeter used for measurement of the heats of mixing of DNA and dye solutions was an LKB batch-type microcalorimeter, as reported previously [12], except that the microcalorimeter was placed in an air bath controlled by circulating water kept at 298.15 ± 0.002 K.

For calorimetric measurements, equal volumes (1.0 cm^3) of DNA solution of known concentration, 5.0×10^{-4} mol dm^{-3}, and dye solutions of various concentrations were mixed in the sample cell. Equal volumes (1.0 cm^3) of buffer solution and dye solution, with a concentration the same as that in the sample cell were mixed in the reference cell, thus eliminating the heat of dilution of the dye.

The concentrations of DNA solutions were determined spectrophotometrically using the extinction coefficients, $\varepsilon_{260} = 6900$ for DNA-I, $\varepsilon_{260} = 6700$ for DNA-II, and $\varepsilon_{260} = 6000$ for DNA-III from an analysis of the phosphorus in DNA [13].

3. Results and discussion

3.1. UV spectral measurement

In order to obtain information concerning the GC content dependence of DNA interactions with dye, UV absorption spectra for 9-AA and quinacrine solutions

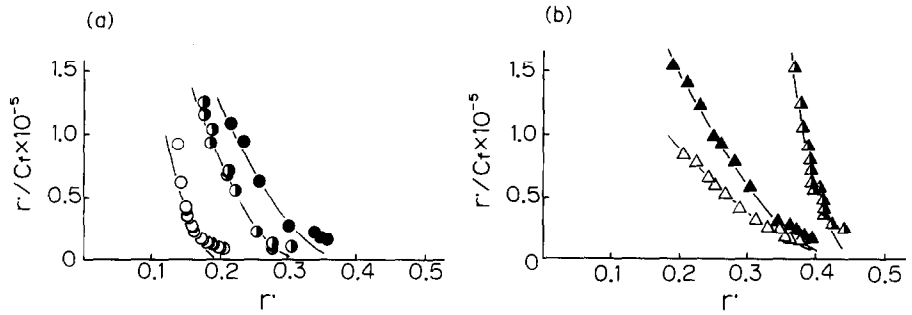


Fig. 2. Scatchard plots for DNA of various GC content. (a) 9-AA and (b) quinacrine system: ○, △, DNA-I; ◑, ◭, DNA-II; ●, ▲, DNA-III.

with various amounts of DNA in 0.01 mol dm^{-3} phosphate buffer solution were measured at room temperature using a spectrophotometer.

From the results, the percentage of dye bound to DNA with various GC contents was calculated according to the method of Peacocke and Skerrett [14]. Scatchard's plots were determined for DNA solutions with various GC contents mixed with dye solutions. The results obtained are shown in Fig. 2, where r'/C_f values are plotted against r', together with those for the DNA-II–9-AA system reported previously [9]; r' is the molar ratio of the molar concentration of bound dye C_b to the base pairs of DNA [bp], and C_f is the molar concentration of free dye.

The binding constant K and number of base pairs occluded by dye molecule n can be determined according to McGhee and Hippel's neighbor-exclusion binding model [15], as follows

$$\frac{r'}{C_f} = K(1 - nr')\left(\frac{1 - nr'}{1 - (n-1)r}\right)^{n-1} \quad (1)$$

From Eq. (1), the K and n values can be estimated by fitting the experimental data. The results obtained are summarized in Table 1, together with those for DNA-II–9-AA solutions, as reported previously [9]. As seen in Table 1, the K values for 9-AA systems decrease with increasing GC content of DNA, whereas the

Table 1
Binding constant K and number of base pairs occluded by dye molecule n, according to Scatchard plots

System	DNA	$K \times 10^{-6}$ in $dm^3\ mol^{-1}$ [a]	n
9-Aminoacridine	DNA-I	0.50	4.6
	DNA-II	0.43	3.0
	DNA-III	0.35	2.4
Quinacrine	DNA-I	0.23	2.2
	DNA-II	2.70	2.2
	DNA-III	0.40	2.3

[a] Here mol refers to mole of dye.

K values for quinacrine systems depend on the GC contents of DNA. Thus, the K value for DNA-II–quinacrine solutions is larger than those of quinacrine systems with DNA-I and DNA-III solutions, demonstrating that quinacrine with a side chain interacts extensively with DNA-II.

3.2. Heat of mixing

From the results of the UV spectra, it was determined that DNA–dye complexes, described as intercalations are formed by interaction between DNA and 9-AA, and DNA and quinacrine.

In order to obtain thermodynamic information about an interaction between DNA and dye, the heats of mixing for the DNA-I, -II, and -III–9-AA, and –quinacrine systems were measured at 298.15 ± 0.002 K using an LKB batch-type microcalorimeter.

The heats of mixing for all systems were exothermic, demonstrating that there is an interaction between DNA and dye, as expected from the UV spectra, assuming that the change in enthalpy based on dimerization of dye is negligibly small, the dye used in this study being very dilute.

The observed heat of mixing, $\Delta_{mix}H$ converted to mole of base pair (bpm) of DNA, is shown in Figs. 3(a) for 9-AA and 3(b) for quinacrine systems, together with that of DNA-II–9-AA solution as reported previously [9]; $\Delta_{mix}H$ is plotted against molar ratio r of dye to base pair of DNA.

As seen in Fig. 3, the absolute value of $\Delta_{mix}H$ for both systems first increases and then levels off at $r > 0.2$ with an increase of r, suggesting that the values for both systems at $r > 0.2$ correspond to termination of the interaction between DNA and dye. The absolute values of $\Delta_{mix}H$ for the DNA-II–quinacrine and for the DNA-I–9AA systems are the highest for their respective systems, demonstrating that quinacrine interacts strongly with DNA-II, and that 9-AA interacts strongly with DNA-I, compared with DNA of other GC contents.

In order to obtain the change in enthalpy based on the intercalation of dye inserted into adjacent base pairs of DNA, the net heat of interaction ΔH, converted to mole of dye, can be determined from $\Delta_{mix}H$

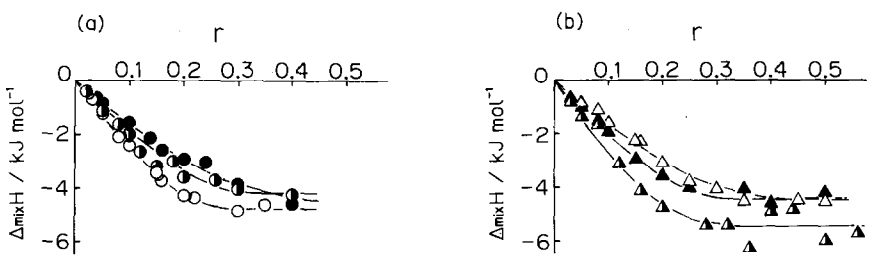


Fig. 3. Dependence of heat of mixing per mole of base pair of DNA, $\Delta_{mix}H$, on the molar ratio r of 9-AA (a) and quinacrine (b) to base pair of DNA. Symbols as in Fig. 2.

$$\Delta H = \frac{\Delta_{mix} H}{C_b/[bp]} \tag{2}$$

where [bp] is the molar concentration of the base pair of DNA, and C_b the molar concentration of bound dye to DNA as described in the UV spectral measurements.

According to Eq. (2), ΔH can be calculated using $\Delta_{mix} H$ and the molar ratio $C_b/[bp]$ of bound dye to base pair of DNA.

Under the assumption that a DNA–dye complex is formed according to the equilibrium reaction

$$\text{DNA} + \text{dye} \rightleftharpoons (\text{DNA–dye}) \text{ complex} \tag{3}$$

the binding contant K can be expressed

$$K = \frac{C_b}{([bp] - nC_b)(C - C_b)} \tag{4}$$

where C is the total molar concentration of dye.

From Eq. (4), $C_b/[bp]$ can also be expressed as

$$\frac{C_b}{[bp]} = \frac{1}{2}\left[\frac{1}{n} + \frac{1}{nk[bp]} + r + \sqrt{\left(r + \frac{1}{nK[bp]} + \frac{1}{n}\right)^2 - \frac{4r}{n}}\right] \tag{5}$$

Using the K and n values estimated from the UV spectral measurements, and according to Eq. (5), $C_b/[bp]$ can be calculated for each r.

Using the $\Delta_{mix} H$ and $C_b/[bp]$ values for each r, ΔH in Eq. (2) can be estimated for each r. The results are summarized in Table 2, and shown in Fig. 4, where ΔH is plotted against r, together with that for DNA-II–9-AA solution as reported previously [9].

As seen in Fig. 4, the absolute value of ΔH for both systems at $r < 0.2$ exhibits a definite value, while, that at $0.2 < r < 0.3$ decreases and then reaches a definite value with an increase in r, suggesting that the interaction between DNA and 9-AA, and between DNA and quinacrine is divided into two different modes depending on r. At $r < 0.2$, the interaction between DNA and 9-AA and quinacrine, corresponds to the change in enthalpy based on the intercalation of dye inserted into adjacent base pairs of DNA. At $r > 0.2$, the interaction modes between DNA and 9-AA, and quinacrine, may correspond to the change in enthalpy based on intercalation and side-binding resulting from an electrostatic interaction between the negatively charged PO_4^- group in the main chain of DNA and the positively charged $\rangle NH^+$ group of 9-AA, and also between the negatively charged PO_4^- group of DNA and the positively charged $/NH^+$ or $-N^+(CH_2CH_3)_2$ groups of quinacrine.

However, it is very difficult to solve this problem due to a lack of information concerning the interaction modes between DNA and dyes. Further study will be needed to solve these problems.

The net heat of interaction $\Delta H'$ accompanying the intercalcation of dye inserted into DNA for both systems was estimated by extrapolating the experimental data to the limit of $r \to 0$. The results are listed in the fourth column of Table 3, together with those of the DNA-II–9-AA solution reported previously [9].

Table 2
Bound concentration of dye C_b and enthalpy change ΔH per mole of dye accompanying the intercalation process with various concentrations of dye C at a given concentration of DNA [bp]

System	DNA	$10^5 \times C$ in mol dm^{-3}	r [a] C/[bp] [c]	r' [b] C_b/[bp] [c]	$-\Delta H$ in kJ mol^{-1} [d]
9-AA	DNA-I	1.5	0.03	0.0299	25.4
		2.5	0.05	0.0497	24.9
		4.0	0.08	0.0795	26.2
		5.0	0.10	0.0993	24.4
		7.5	0.15	0.1482	23.2
		8.0	0.16	0.1577	23.9
		10.0	0.20	0.1933	22.2
		11.0	0.22	0.2057	21.1
		15.0	0.30	0.2161	22.4
		17.5	0.35	0.2169	21.2
	DNA-II	1.0	0.02	0.0198	21.3
		2.5	0.05	0.0497	23.1
		4.0	0.08	0.0795	20.8
		5.0	0.10	0.0993	20.0
		6.0	0.12	0.1191	22.0
		7.5	0.15	0.1488	21.5
		9.0	0.18	0.1765	17.0
		10.0	0.20	0.1977	18.5
		11.9_6	0.26	0.2483	15.0
		15.0	0.40	0.2897	14.1
		20.0	0.40	0.3265	14.0
	DNA-III	2.0	0.04	0.0397	16.5
		2.5	0.05	0.0497	17.2
		5.0	0.10	0.0993	16.0
		7.0	0.14	0.1388	15.6
		8.0	0.16	0.1585	16.4
		10.0	0.20	0.1978	14.8
		12.0	0.24	0.2369	12.9
		15.0	0.30	0.2943	13.4
		20.0	0.40	0.3772	12.1
Quinacrine	DNA-I	2.5	0.05	0.0495	16.8
		4.0	0.08	0.0792	14.2
		5.0	0.10	0.0989	15.4
		7.5	0.15	0.1481	14.9
		8.0	0.16	0.1579	14.3
		10.0	0.20	0.1970	15.6
		12.5	0.25	0.2454	15.2
		15.0	0.30	0.2928	13.8
		17.5	0.35	0.3385	13.3
		22.5	0.45	0.4119	10.8
		25.0	0.50	0.4302	10.4
	DNA-II	1.5	0.03	0.0300	25.4
		2.5	0.05	0.0500	27.5
		4.0	0.08	0.0799	26.4

Table 2 (continued)

System	DNA	$10^5 \times C$ in mol dm^{-3}	r [a] C/[bp] [c]	r' [b] C_b/[bp] [c]	$-\Delta H$ in kJ mol^{-1} [d]
		6.0	0.12	0.1199	25.8
		8.0	0.16	0.1598	25.7
		10.0	0.20	0.1997	23.5
		14.0	0.28	0.2795	19.3
		16.0	0.32	0.3192	16.9
		18.0	0.36	0.3587	17.3
		20.0	0.40	0.3976	12.1
		22.0	0.44	0.4332	11.0
		25.0	0.50	0.4514	13.1
		28.0	0.56	0.4531	12.5
	DNA-III	1.5	0.03	0.0298	21.2
		2.5	0.05	0.0497	20.1
		4.0	0.08	0.0795	19.1
		5.0	0.10	0.0994	19.4
		7.5	0.15	0.1489	19.6
		10.0	0.20	0.1982	17.7
		12.5	0.25	0.2472	16.1
		17.5	0.35	0.3425	11.7
		20.0	0.40	0.3855	11.7
		25.0	0.50	0.4306	9.6

[a] r, molar ratio of dye to base pair of DNA. [b] r', molar ratio of bound dye to base pair of DNA. [c] [bp], mole of base pair of DNA. [d] mol, mole of dye.

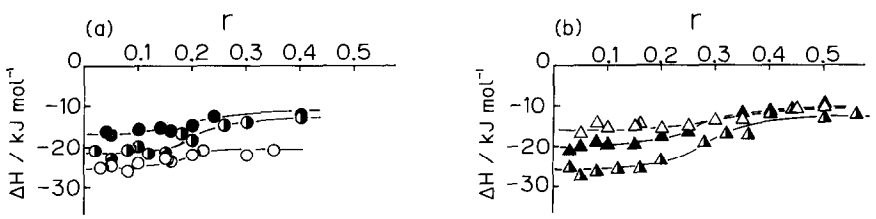


Fig. 4. Plots of the change in enthalpy ΔH accompanying interaction between DNA and 9-AA (a) and quinacrine (b) against the molar ratio r of dye to base pair of DNA; mol here refers to mole of dye. Symbols as in Fig. 2.

As seen in Table 3, in 9-AA systems the absolute value of $\Delta H'$ decreases with increasing GC content. However, in quinacrine systems, $\Delta H'$ for DNA-II is larger than that for DNA-I– and DNA-III– quinacrine systems, suggesting that the interactions between DNA-I and 9-AA and between DNA-II and quinacrine form complexes that are thermodynamically more stable than those of other members of the same series of DNA–dye systems.

Table 3
Thermodynamic quantities for DNA–dye systems in which the DNA has various contents of guanine–cytosine base pair

System	DNA	ΔG in kJ mol^{-1} [a]	ΔH in kJ mol^{-1} [a]	ΔS in J K^{-1} mol^{-1} [a]
9-AA	DNA-I	−33	−26	24
	DNA-II	−32	−21	38
	DNA-III	−32	−17	50
Quinacrine	DNA-I	−31	−16	48
	DNA-II	−37	−25	40
	DNA-III	−32	−20	39

[a] mol refers to mole of dye.

3.3. Thermodynamic quantities

Thermodynamic quantities for the intercalation of the dyes can be estimated using the K and $\Delta H'$ values. The changes in free energy $\Delta G^{\ominus}$ and entropy ΔS can be calculated from $\Delta G^{\ominus} = -RT \ln K$ and $\Delta S = (\Delta H - \Delta G^{\ominus})/T$, respectively.

The results obtained are summarized in Table 3, together with those of DNA-II–9-AA solution as reported previously [9].

As seen in Table 3, $\Delta G^{\ominus}$ for the 9-AA systems is about −32 kJ, virtually independent of GC content although the absolute value of ΔH depends on base sequence of the DNA.

However, the absolute value of $\Delta G^{\ominus}$ for the DNA-II–quinacrine system is larger than those for DNA-I and DNA-III, although the $\Delta G^{\ominus}$ for DNA-I– and DNA-III–quinacrine are the same, demonstrating that the interaction between DNA-II and quinacrine forms a thermodynamically more stable complex than those for the DNA-I– and DNA-III–quinacrine systems.

However, ΔS values for both systems are positive, although ΔS values for complex formation are usually negative.

It is difficult to explain why ΔS accompanying this complex formation should be positive, due to a lack of information. However, one possible explanation can be suggested. The value of $\Delta_{mix}H$ (exothermic) estimated from calorimetric measurements may be low because changes in the enthalpy (endothermic) for the dissociation of stacking between DNA bases and changes in the conformation (endothermic) for the elongation of DNA accompanying intercalation were not taken into consideration.

3.4. Possibility of base specificity for intercalation

It was pointed out above that 9-AA can interact with DNA-I as suggested from the $\Delta H'$ value, although $\Delta G^{\ominus}$ is independent of GC content of DNA for 9-AA systems, and that quinacrine can intersect with DNA-II, as suggested from the $\Delta G^{\ominus}$ and ΔH results, respectively.

In order to confirm information about the base specificity of dyes based on the interaction between DNA and dye with respect to the $\Delta H'$ value, the changes in enthalpy $\Delta H_{GC/GC}$, $\Delta H_{AT/AT}$, and $\Delta H_{GC/AT}$ accompanying the interactions between the base pair sequences guanine (G)–cytosine (C) (GC/GC), adenine(A)–thymine(T) (AT/AT) and (guanine–cytosine)–(adenine–thymine) (GC/AT), and 9-AA or quinacrine dyes were estimated.

Assuming that the three types of intereactions, between GC/GC and dye, between AT/AT and dye, and between GC/AT and dye, occur in numbers given by the probability of the existence of such pairs when the DNA molecule corresponds to a random base sequence, $\Delta H'$ can be represented

$$\Delta H' = \Delta H_{GC/GC} n_{GC}^2 + \Delta H_{AT/AT} n_{AT}^2 + 2\Delta H_{GC/AT} n_{GC} n_{AT} \quad (6)$$

where n_{GC} and n_{AT} are the fractions of GC and AT base pairs in DNA molecules, respectively.

Because n_{AT} is equal to $1 - n_{GC}$, $\Delta H'$ of Eq. (6) can be modified as a function of n_{GC}

$$\Delta H' = (\Delta H_{GC/GC} + \Delta H_{AT/AT} - 2\Delta H_{GC/AT}) n_{GC}^2 + 2(\Delta H_{GC/AT} - \Delta H_{AT/AT}) n_{GC} + \Delta H_{AT/AT} \quad (7)$$

According to Eq. (7) the $\Delta H_{GC/GC}$, $\Delta H_{AT/AT}$, and $\Delta H_{GC/AT}$ values can be determined from the best fit of the experimental ΔH data for each system; these are listed in the third column of Table 4. The changes in entropy for each base pair sequence were estimated in the same way and, using these values, the changes in free energy were determined at 298.15 K. These results obtained are summarized in the fourth and fifth columns of Table 4, respectively. For 9-AA systems, the absolute value of $\Delta H_{AT/AT}$ is very large compared with $\Delta H_{GC/GC}$ and $\Delta H_{GC/AT}$, suggesting that 9-AA interacts predominantly with AT/AT base pairs, and forms a thermodynamically stable complex.

Table 4
Thermodynamic quantities for the intercalation process of 9-AA and quinacrine into base-pair sequences of DNA

System	Base-pair sequence	ΔH in kJ mol^{-1} [a]	ΔS in J K^{-1} mol^{-1} [a]	ΔG in kJ mol^{-1} [a]
9-AA	GC/GC	−11	69	−31
	GC/AT	−13	58	−30
	AT/AT	−31	5	−33
Quinacrine	GC/GC	8	55	−8
	GC/AT	−64	12	−68
	AT/AT	15	73	−7

[a] mol refers to mole of dye.

However, in quinacrine system, the absolute value of $\Delta H_{GC/AT}$ is considerably larger than $\Delta H_{GC/GC}$ and $\Delta H_{AT/AT}$, demonstrating that quinacrine interacts strongly with the GC/AT base-pair sequence rather than with the GC/GC or AT/AT base-pair sequences, and forms a thermodynamically stable complex.

Thus, the intercalations of the 9-AA and quinacrine molecules depend specifically on the base sequence of DNA.

However, the changes in entropy accompanying the intercalation of 9-AA and quinacrine into GC/GC, AT/AT, and GC/AT base-pair sequences are positive. The reason why the change in entropy accompanying complex formation is positive is very difficult to explain due to a lack of information, such as the effect of dehydration around DNA, the conformational change of DNA accompanying the intercalation, etc.

Further study is in progress to elucidate these problems by investigating interactions between base-pair sequences such as poly[d(AT)] · poly[d(AT)], poly[d(AC)] · poly[d(GT)], poly[d(AG)] · poly[d(CT)], and poly[d(GC)] · poly[d(GC)] and dyes.

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