Thermodynamic Characterization of Adriamycin Binding to DNA: Dependence of Heat of Interaction on GC Composition of DNA

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ABSTRACT: The interactions between adriamycin (AD) and DNA of various GC content were studied by calorimetry and UV/Vis spectrophotometry. From the results, the thermodynamic parameters accompanying the intercalations were estimated by taking into account the dissociation of the AD dimer to monomer. The stability of the intercalation complex is due to enthalpic and the entropic contributions. The enthalpy change accompanying the intercalations is more exothermic for GC-rich DNA than for AT-rich DNA although the free energy change is independent of GC content of DNA, indicating that the interaction energy of base pair with AD is larger for GC than AT base pairs.

KEY WORDS DNA / Adriamycin / Heat of Mixing / UV/Vis Spectrophotometry / Thermodynamic Parameters / GC Composition of DNA /

One of the most interesting areas in biological research is the interactions between DNA and small molecules. Specifically, the correlation between the intercalations and inhibition of the transcription or replication of DNA is of importance for understanding gene expression. Common intercalators are characterized by heterocyclic aromatic chromophores and include mutagenic aminoacridine dyes¹ and many drugs as antitumor agents.²

Interactions between DNA and intercalators seem due to insertion of planar aromatic molecules between base pairs of DNA, as judged from the results of sedimentation,^{3,4} X-ray diffraction,^{5,6} viscosity,⁷ and the crystal structure of intercalation complexes.^{8,9}

It was previously reported that interactions between the polynucleotide duplex such as poly(A)·poly(U) duplex or poly(I)·poly(C) duplex and mutagenic acridine dye (proflavine and 9-aminoacridine)^{10,11} occur throughout intercalation. However, interactions between

the polynucleotide duplex and adriamycin¹² involve outside, nonintercalation binding. Daunomycin, which has a similar chemical structure to adriamycin, intercalates into DNA^{13–15} and exhibits GC specificity when binding to oligonucleotides as determined from X-ray diffraction studies.¹⁶ From these experimental results, the intercalation of adriamycin into double stranded helical DNA seems due to the GC base pair and/or to the conformation of the double stranded helical structure since the structure of DNA is B-form¹⁷ and that of poly(A)·poly(U) duplex and poly(I)·poly(C) duplex is A-form.¹⁸

In this paper, to obtain thermodynamic information regarding base specificity of interactions between DNA and adriamycin in aqueous solution, we elucidated the thermodynamic parameters of the interaction for adriamycin binding to DNA by measuring the heat of mixing and UV/Vis spectrum, and also explored the effect of GC base composition of

DNA on binding of adriamycin.

EXPERIMENTAL

The DNA samples were *Clostridium perfringens* DNA (GC; 26.5%, DNA(I)), *Escherichia coli* DNA (GC; 50.0%, DNA(II)) and *Micrococcus lyodeikticus* DNA (GC; 72.0%, DNA(III)), which were purchased from Sigma Chemical Co., U.S.A. Adriamycin hydrochloride (AD) was also purchased from Sigma. All measurements were made in 0.1 mol dm⁻³ Tris-HCl buffer solution at pH 7.60.

The calorimeter employed was identical to the LKB batch type microcalorimeter and placed in an air bath kept at 298.15 ± 0.005 K as described previously.¹⁹ For calorimetric measurements, a DNA solution of a known concentration (about 5.0×10^{-4} mol dm⁻³ in nucleotide phosphorus) and an AD solution at various concentrations were mixed. In the calorimetric measurements, the heat of dilution of AD solution was compensated by preparing an AD solution of same concentration in both the sample and reference cells in the calorimeter. The heat of dilution of DNA was negligibly small. The DNA concentration was determined by the phosphorus analysis method.²⁰

The absorption spectra of the mixtures of DNA and AD solutions were measured at room temperature with a Hitachi 220A UV/Vis spectrophotometer. In this case, an AD solution of a known concentration containing various amounts of DNA was employed.

RESULTS AND DISCUSSION

Absorption Spectra of DNA-AD System

The typical adsorption spectra obtained for AD solutions containing various amounts of DNA(III) are shown in Figure 1. As seen in Figure 1, the wavelength of the maximum absorption for the DNA(III)-AD system shifts to red as the concentration of DNA(III) in-

Figure 1. Absorption spectra of adriamycin solution (2.0×10^{-5} mol dm⁻³) containing various amounts of DNA (III) (GC content 72%) in 0.1 mol dm⁻³ Tris-HCl buffer solution at pH 7.60. DNA concentration: —, zero; - - -, 6.0×10^{-5} ; - · - · -, 1.0×10^{-4} ; · · · · ·, 2.6×10^{-4} mol dm⁻³ in phosphorus unit.

creases, and a well-defined isosbestic point is located at about 540 nm. This red shift and isosbestic point are comparable with the results for DNA-aminoacridine dye^{21,22} and polynucleotide duplex-aminoacridine systems^{10,11} which show interactions based on intercalation, suggesting that the interactions between DNA(III) and AD are due to intercalations similar to those between DNA and aminoacridine dye.²³

The absorption spectra for DNA(I) and/or DNA(II) and AD systems are similar to those for DNA(III) and AD system as mentioned above.

The percentage of bound AD to DNA was calculated according to the method by Peacocke *et al.*,²⁴ and the results obtained for each system are shown in Figure 2, where the amount of bound AD per nucleotide phosphorus, r is plotted against the molar ratio, AD/P of AD to nucleotide phosphorus, P. It is seen in Figure 2 that r increases linearly at first, and then in the AD/P region of about 0.15 to 0.20, levels off with increasing AD/P. These results mean the termination of interaction between DNA and AD for intercalation in the AD/P region of about 0.15 to 0.20.

In addition, Scatchard's plots for each system are shown in Figure 3 to obtain binding parameters. From the plots, the binding parameters were estimated and are given in Table I. It is apparent that the binding parameters for

Figure 2. Dependence of the amount of bound adriamycin per mole of DNA phosphorus, r and the heat of mixing per mole of DNA phosphorus, ΔH^M on the molar ratio, AD/P of adriamycin to DNA phosphorus. (a) and (a'), DNA (I) (GC content 26.5%); (b) and (b'), DNA (II) (GC content 50%); (c) and (c'), DNA (III) (GC content 72%).

Figure 3. Scatchard plots for adriamycin solution of various GC content of DNA: DNA (I), 26.5%; DNA (II), 50%; DNA (III), 72%.

Table I. The binding parameters for intercalation of DNA–adriamycin complex as estimated from spectrophotometry

GC content %	K_b^a	n^b
26.5 (DNA (I))	4.4×10^6	0.17
50 (DNA (II))	4.3×10^6	0.17
72 (DNA (III))	4.3×10^6	0.16

^a Binding constant.

^b Number of binding sites per DNA phosphorus.

each system have similar values.

Heat of Mixing for DNA and AD Solutions

In order to estimate the enthalpy change of complex formation accompanying intercalation, the heats of mixing for each DNA–AD system were measured over the AD concentration range of 5×10^{-5} to 1×10^{-4} mol dm⁻³ at 298.15 ± 0.005 K. In this case, the DNA concentration was kept constant at 5.0×10^{-4} mol dm⁻³ in nucleotide phos-

phorus.

All systems proved to be exothermic, indicating that the complex between DNA and AD was formed. The observed heats of mixing, ΔQ at different AD concentration, C and molar ratio, AD/P of AD to DNA phosphorus, P are listed in Table III. The results obtained are presented graphically in Figure 2, where the heat of mixing per mole of phosphorus of DNA, ΔH^M is plotted against AD/P. As seen in Figure 2, the absolute value of ΔH^M for each system increases at first, and then reaches a definite value at a high value of AD/P. This behavior of ΔH^M against AD/P is comparable with that of r against AD/P obtained from the spectral measurements in Figure 2. We suggest that ΔH^M obtained is based on intercalation. In addition, the limiting value of ΔH^M becomes more exothermic with increasing GC content, suggesting that the evaluated heat by mixing may reflect GC content dependence of the heat of interaction of AD with a base of DNA.

Furthermore, the observed heat of mixing ΔQ may contain the heat based on the dissociation of AD dimer to monomer because aromatic compounds such as dye (ethidium bromide, proflavine) or antibiotics (daunomycin, adriamycin) form the dimerization in water. By taking into account the heat of dissociation of AD dimer to monomer, we estimated the heat of interaction between DNA and AD monomer in the next section.

Thermodynamic Parameters for Binding of AD to DNA

Assuming that the DNA-AD complex for mixture of DNA and AD is formed between DNA and monomeric AD and that the dimeric form of AD does not bind DNA, the reaction in the mixing solution of DNA and AD can be expressed as follows,

with the dissociation constant K_d for AD dimer to monomer for reaction (1) and the binding constant K_b of DNA-AD complex formation for reaction (2). Before the mixing of DNA and AD solutions, we have

$$K_d = C_{fi}^2 / C_{2i} \quad \text{and} \quad C = C_{fi} + 2C_{2i} \quad (3)$$

where C_{2i} and C_{fi} are the initial dimer and monomer concentration of AD without the DNA solution, and C is the total concentration of AD. From eq 3, we have

$$C_{2i} = \frac{1}{2} \left(\frac{K_d}{4} + C - \sqrt{\left(\frac{K_d}{4} + C \right)^2 - 4C^2} \right) \quad (4)$$

After equilibration of the mixture of DNA and AD solution, we have

$$K_d = \frac{C_f^2}{C_2} \quad (5)$$

$$K_b = \frac{C_b}{C_f(nP - C_b)} \quad (6)$$

$$C = C_f + C_b + 2C_2 \quad (7)$$

where C_f , C_2 , and C_b are the concentrations of monomeric AD, the dimeric AD, and the bound AD in DNA-AD complex, respectively, and C is the total concentration of AD, n the number of binding site per nucleotide phosphorus of DNA, and P the concentration in nucleotide phosphorus of DNA, respectively. From eq 5 to 7, we have

$$\begin{aligned} C_f^3 + \left(\frac{K_d}{2} + \frac{1}{K_b} \right) C_f^2 + \left(\frac{K_d}{2} nP + \frac{K_d}{2K_b} - \frac{K_d}{2} C \right) \\ \times C_f - \frac{K_d}{2K_b} C = 0 \end{aligned} \quad (8)$$

The observed heat $\Delta Q/V$ per volume of mixture obtained from the mixing of DNA and AD solutions is given by

$$\frac{\Delta Q}{V} = C_b \Delta H_b + (C_{2i} - C_2) \Delta H_d \quad (9)$$

where ΔH_b is the heat of binding of AD to DNA, ΔH_d the heat of dissociation of AD dimer to monomer, respectively.

Table II. Observed heat of mixing per volume of solution, $\Delta Q/V$, the heat of mixing, ΔH^M , and the heat of binding of adriamycin to DNA, ΔH_b , with concentration of adriamycin, C , and with molar ratio, AD/P

$C \times 10^5$ mol dm ⁻³	AD/P	$-\Delta Q/V$ J dm ⁻³	$-\Delta H^M$ ^a kJ mol ⁻¹	$-\Delta H_b$ ^b kJ mol ⁻¹
DNA (I) (GC content 26.5%) system				
2.51	0.050	0.308	0.62	13.8
3.04	0.062	0.528	1.06	18.9
3.95	0.078	0.668	1.32	18.9
4.97	0.099	0.861	1.71	19.6
5.86	0.114	0.777	1.52	15.7
7.44	0.149	0.834	1.67	14.0
8.49	0.170	0.933	1.87	14.5
9.92	0.197	0.956	1.90	14.8
11.20	0.224	0.957	1.91	15.0
DNA (II) (GC content 50%) system				
1.00	0.020	0.230	0.46	23.9
1.94	0.038	0.527	1.04	28.5
3.22	0.063	0.736	1.44	24.7
3.96	0.079	0.785	1.57	21.9
4.02	0.081	0.825	1.66	22.6
4.85	0.097	1.101	2.19	25.0
6.52	0.130	1.317	2.63	22.9
9.04	0.183	1.237	2.48	17.9
DNA (III) (GC content 72%) system				
0.99	0.020	0.236	0.47	24.6
1.50	0.029	0.488	0.96	33.7
2.52	0.051	0.750	1.52	31.4
3.74	0.075	0.887	1.77	25.7
5.58	0.111	1.349	2.69	26.7
6.00	0.120	1.444	2.89	26.7
7.14	0.143	1.714	3.44	27.1
8.56	0.172	1.613	3.25	23.4

^a Heat per mole of DNA phosphorus.

^b Heat per mole of adriamycin.

The solution of eq 8 was obtained by an iterative procedure using a computer. According to eq 9, we obtained ΔH_b for a given C using values of K_d ($=1100$ at 298 K) and ΔH_d ($=40$ kJ mol⁻¹) reported by Crescenzi and Quadrifoglio²⁵ and K_b and n -values (Table II) obtained from Scatchard's plots. ΔH_b obtained as a function of AD concentration for each system is summarized in the last column of Table II and shown in Figure 4. It is clear that ΔH_b is independent of

Figure 4. Enthalpy change of binding, ΔH_b , of adriamycin to various GC content of DNA corrected for dimerization of the adriamycin as function of adriamycin concentration. GC content of DNA: ○, 26.5%; ●, 50%; △, 72%.

Table III. Thermodynamic parameters for adriamycin binding to DNA at 298 K^a

GC content		ΔG_b	ΔH_b	ΔS_b
%		kJ mol ⁻¹	kJ mol ⁻¹	JK ⁻¹ mol ⁻¹
DNA (I)	26.5	-38	-16	74
DNA (II)	50	-38	-23	50
DNA (III)	72	-38	-27	37

^a Mol here refers to moles of adriamycin.

AD concentration although the calculated values are scattered. The average value (solid line in Figure 4) of ΔH_b obtained for each system is listed in Table III, together with values of the free energy change ΔG_b and the entropy change ΔS_b based on the intercalation process calculated from the equations, $\Delta G_b = -RT \ln K_b$ and $\Delta S_b = (\Delta H_b - \Delta G_b)/T$.

The thermodynamic parameters estimated for DNA-AD interaction are comparable with those for DNA-daunomycin interaction using calorimetry,^{26,27} but the DNA-daunomycin was characterized by negative entropy change from van't Hoff analysis.²⁸ This discrepancy is based on differences in enthalpy change: The enthalpy change estimated from calorimetry is significantly less than that from van't Hoff analysis although the magnitude of free energy

change has the same value. However, it is very difficult to assess this discrepancy between calorimetric and van't Hoff methods for enthalpy change based on intercalation process.

As seen in Table III, AD intercalated into DNA exists as a stable complex from the negative value of ΔG_b for each DNA-AD system. This stability of the complex is due to negative enthalpic and positive entropic contributions, although the formation of the complex is generally characterized by negative values for both enthalpy and entropy changes.

The entropic gain accompanying the interaction process seems to result from increase in the degree of disorder of water molecules in hydration shells surrounding the DNA-AD complex as in the reverse process of solubilization of hydrocarbons in water.^{29,30}

Furthermore, it is of interest that the enthalpy change decreases with increasing GC content although the free energy change is independent of GC content, demonstrating that the mechanism of binding of AD into DNA depends on the base sequence of DNA molecules.

Base Specificity for Intercalation

Assuming that the three types of DNA-AD interaction (*i.e.*, between GC pairs, AT pairs, and GC-AT pairs) occur in the numbers given by the probability of existence of such pairs when the DNA molecule corresponds to a random base sequence, the enthalpy change of DNA-AD interaction is represented as,

$$\Delta H_b = \Delta H_1 n_{GC}^2 + \Delta H_2 n_{AT}^2 + 2\Delta H_3 n_{GC} n_{AT} \quad (10)$$

where ΔH_1 , ΔH_2 , and ΔH_3 are the enthalpy changes between GC/GC base pairs and AD, AT/AT base pairs and AD, and GC/AT base pairs and AD, respectively, and n_{GC} and n_{AT} are the fractions of GC and AT base pairs in DNA molecules, respectively.

Since n_{AT} is equal to $1 - n_{GC}$, ΔH_b may be rewritten as a function of n_{GC} ,

Figure 5. Dependence of the enthalpy change, ΔH_b (○) and entropy change, ΔS_b (△) accompanying intercalation of adriamycin on GC content of DNA.

Table IV. Thermodynamic parameters for the intercalation process of adriamycin into base composition of DNA^a

Base pair sequence	ΔH_i	ΔS_i	ΔG_i
	kJ mol ⁻¹	J K ⁻¹ mol ⁻¹	kJ mol ⁻¹
GC/GC	-29	29	-38
GC/AT	-29	28	-37
AT/AT	-5	111	-38

^a *i* is number (1, 2, and 3) in text
Mol refers to moles of adriamycin.

$$\Delta H_b = (\Delta H_1 + \Delta H_2 - 2\Delta H_3)n_{GC}^2 + 2(\Delta H_3 - \Delta H_2)n_{GC} + \Delta H_2 \quad (11)$$

The plots of ΔH_b estimated from calorimetry versus GC fraction of DNA are shown in Figure 5, and the solid line in Figure 5 shows the best fit curve of eq 11 by least squares treatment. Each heat of interaction, ΔH_i (*i* = 1, 2, and 3) as estimated from this curve is shown in Table IV.

The entropy change ΔS_i for the intercalation process was estimated in essentially the same way as that of ΔH_i as mentioned above. The results obtained are also summarized in Table IV, together with values of the free energy change, ΔG_i calculated by $\Delta G_i = \Delta H_i - T\Delta S_i$ at 298 K.

As seen in Table IV, the thermodynamic parameters for AD intercalated between GC/GC base pairs and between GC/AT base pairs have similar values, but the enthalpy change for AD intercalated between AT/AT base pairs is considerably larger than that between GC/GC base pairs although the free energy change has similar value. From these facts, we suggest that the interaction mode between GC base pair and AD is different from that between AT base pair and AD.

CONCLUSIONS

The thermodynamic parameters of the intercalation process for DNA-adriamycin interaction were estimated from calorimetry, taking into account the self association of the AD molecule. The intercalated AD into DNA was due to both enthalpic and entropic contributions. The entropic gain accompanying intercalation seems to result from increase in the degree of disorder of the hydration shell surrounding the DNA-AD complex compared with the hydration of DNA and AD. In addition, the interaction energy of base with AD is larger for GC than AT base pairs although the free energy change for the intercalated AD between base pairs is independent of the base composition of DNA.

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