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THERMODYNAMIC STUDIES ON THE INTERACTION BETWEEN NUCLEIC ACID AND DRUG BY CALORIMETRY

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ABSTRACT

The heats of mixing of the DNA-AD, Duplex I-AD and Duplex II-one systems were measured at 298.15 ± 0.005 K by using an LKB batch type twin microcalorimeter with a help of spectral measurements. From the results of DNA-AD system obtained, the interaction between DNA and AD is mainly caused by intercalation manner by referring to the result of spectral measurement. And, we estimated the thermodynamic quantities based on the intercalation process. While, from the results of Duplex I-AD and Duplex II-one systems, we concluded that the interactions of these systems may be corresponded to an electrostatic force as binding process rather than an intercalation process.

INTRODUCTION

In the previous papers (refs.1,2,3,4), we evaluated the thermodynamic quantities of interactions between DNA and/or poly(A) poly(U)duplex or poly(I)·poly(C) duplex with double-stranded helical structures and aminoacridine dyes by means of calorimetry with a help of spectrum, and reported that the difference of thermodynamic quantities on the basis of intercalation process obtained depended on whether dye molecule has the amino substituents or not, and also depended on base-pair species of the polynucleotide duplexes on the basis of intercalation process.

In this paper, in order to obtain information about the interactions between adriamycin and DNA, poly(A)·poly(U)duplex, and poly(I)·poly(C)duplex, we studied from the heat of mixing of the DNA-Adriamycin, poly(A)·poly(U)duplex-Adriamycin, and poly(I)·poly(C)duplex-Adriamycin systems at 298 K with a help of spectral measurements, and will discuss the interaction between Adriamycin and DNA and/or poly(A)·poly(U)duplex or poly(I)·poly(C)duplex with double-stranded helical structures from thermodynamic aspect.

Methods

Materials

Calf thymus DNA of which composition contains 42% of guanine-cytosine base-pair was purchased from Sigma, Type I, U.S.A. and poly(riboadenylic acid);poly(A),

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poly(ribouridylic acid); poly(U), poly(riboinosinic acid);poly(I), and poly(ribocytidylic acid); poly(C) were purchased from Yamasa Shoyu Co. Ltd., Japan. Adriamycin(AD) employed in this study was Sigma, U.S.A. All other materials were analytical reagents of commercial products. The solvent used was 0.1 mol dm^{-3} Tris-HCl buffer solution at pH 7.60.

Apparatus and procedure

The calorimeter employed was an LKB batch type twin microcalorimeter at 298.15 $\pm$ 0.005 K. For calorimetry, DNA and polynucleotide concentrations were kept at a definite value of about 5.0 x 10^{-4} mol dm^{-3} in phosphorus of nucleotide. While, AD concentration was varied. Poly(A)·poly(U)duplex(Duplex I) and poly(I)·poly(C)duplex(Duplex II) with double-stranded helical structures formed from an equimolar mixture of poly(A) and poly(U), or poly(I) and poly(C), respectively were mixed in AD solution. The concentrations of DNA and polynucleotide were determined by analysis of phosphorus, respectively (ref.5).

In order to obtain information about interaction between AD and DNA and/or Duplex I, Duplex II, the absorption spectra of solutions containing a known concentration of AD and various amounts of DNA and/or Duplex I and Duplex II were measured by using a spectrophotometer (Hitachi 220A, Japan).

RESULTS AND DISCUSSION

Spectral measurements

Typical absorption spectra of AD solutions containing various amounts of DNA and/or Duplex I or Duplex II are shown in Figs.(1-a), (1-b), and (1-c), respectively, together with Figs. (1-d), (1-e), and (1-f) reported previously. As seen in Fig. (1-a), for DNA-AD system, the wavelength of the maximum absorption of AD shifts to red and the hypochromic effect is observed with an increase of concentration of DNA, and there gives an isosbestic point, demonstrating that interaction between DNA and AD is based on an intercalation manner as reported previously (refs.1,2,6,7,8). While, as seen in Figs. (1-b) and (1-c), both absorption spectra of Duplex I-AD and Duplex II-one systems are different from those for DNA-AD system; there shows few red shift of wavelength of maximum absorption of AD and also few hypochromic effect with an increase of the concentrations of Duplex I or Duplex II , and furthermore, there exists no isosbestic point for both systems. From these facts, we suggest that the reaction mechanism of the interactions between Duplex I and AD,and/or Duplex II and one may be different from that between DNA and AD comparing with the results of previous papers (refs.1,2,3,4) and from the results reported by other investigators (refs.9,10).

The percentage of AD bound to DNA calculated according to Peacocke et al.

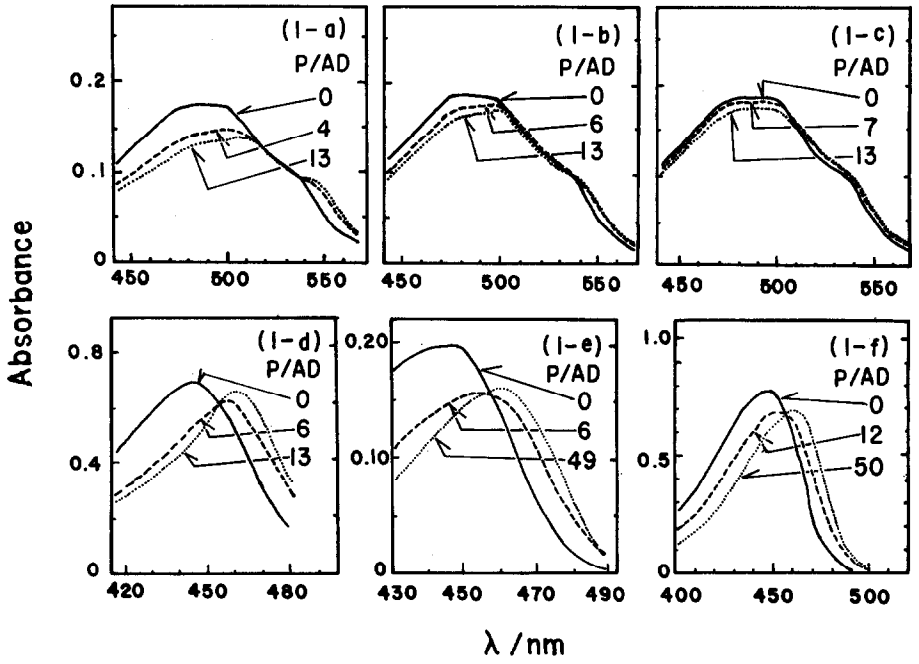

Fig. 1. Typical absorption spectra of AD solutions containing various concentrations of DNA(1-a), Duplex I(1-b), Duplex II(1-c) and of PF* solutions containing various concentrations of DNA(1-d), Duplex I(1-e), Duplex II(1-f).

* See refs. 1,3,4.

(ref.11) is shown in Fig.(2-a), where the amount of AD bound per nucleotide phosphorus, r is plotted versus the molar ratio, AD/P, of adriamycin, AD to DNA phosphorus of nucleotide, P. As seen in Fig.(2-a), r increases rather steeply at first and then, at AD/P values ranging from 0.18 to 0.20, levels off upon further increase. This may be suggested the termination of interaction between DNA and AD in the intercalation process.

Heats of mixing

The heats of mixing of DNA-AD, Duplex I-AD, and Duplex II-AD systems were measured over the concentrations of AD ranging from 2.5×10^{-5} to 1.3×10^{-4} mol dm^{-3} by an LKB batch twin microcalorimeter at 298.15 $\pm$ 0.005 K. All systems proved to be exothermic, indicating that the complex formations between DNA and/or Duplex I, Duplex II and AD were made, under the assumption that the heats of dilution of DNA, Duplex I, and Duplex II are negligibly small, and those of AD are compensated in each other vessel.

The observed heats of mixing per mole of phosphorus of nucleotide unit, ΔH^M against various molar ratios, AD/P of adriamycin, AD to phosphorus of nucleotide,

P are plotted as shown in Fig.(2-b). As seen in Fig.(2-b), the absolute value of ΔH^M increases monotonously at first and then levels off over a AD/P value of about 0.20 for DNA-AD system. These may seem to be reasonably comparable to the results as shown in Fig.(2-a), suggesting that ΔH^M is mainly caused by the interaction between DNA and AD based on the intercalation process by referring to the result of spectral measurement as mentioned above. On the other hand, for the Duplex I-AD and the Duplex II-AD systems, ΔH^M are too small compared with the results of DNA-AD system. From the results of spectral measurements and heats of mixing, the interaction between Duplex I, or Duplex II and AD may be different from the interaction based on the intercalation process between DNA and AD.

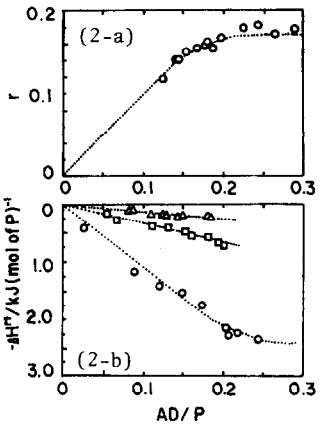

Fig. 2. The dependence of the amount of bound AD per nucleotide phosphorus, r and of the observed heat of mixing, ΔH^M on the molar ratio, AD/P of AD to phosphorus of nucleotide, P.
(2-a):DNA-AD system
(2-b): ○ ;DNA-AD system
□ ;Duplex I-AD system
△ ;Duplex II-AD system

Thermodynamic quantities

In the preceding papers (refs.1,4), we studied on thermodynamics of DNA and/or polynucleotide duplexes with double-stranded helical structures and aminoacridine dyes systems and derived the following expression:

$$\frac{\Delta Q}{V} = \frac{\Delta H}{2}\left\{\frac{1}{K} + n\cdot P + C - \left[\left(\frac{1}{K} + n\cdot P + C\right)^2 - 4\cdot n\cdot P\cdot C\right]^{1/2}\right\} \qquad (1)$$

where $\Delta Q/V$ is the observed heat of mixing per volume of the mixture, ΔH the heat of interaction, K the binding constant, n the number of binding site per nucleotide phosphorus, and C the total concentration of dye, respectively.

Assuming that the Eq.(1) can be applied to the present study, we are able to calculate the value of $\Delta Q/V$ for a given value of the total concentration of AD, C. According to Eq.(1), in order to obtain reasonable theoretical $\Delta Q/V$ curve, which reproduces the experimental data in the concentration region employed, K, ΔH, and n are treated as adjustable parameters and adjusted. Consequently, the best fit curve between calculated value and experimental one is obtained.

In this case, the concentration of phosphorus of nucleotide, P was fixed to be about 5.0×10^{-4} mol dm^{-3}. However, in the Duplex I and Duplex II, assuming here that the interaction between Duplex I, or Duplex II and AD may be mainly caused by electrostatic force and the n-value in these systems is about n=1, which corresponds to one mole of AD bound to one mole of phosphorus of nucleotide, K and ΔH of Duplex I, or Duplex II and AD systems can be evaluated.

The values of DNA-AD and Duplex I-AD and Duplex II-one systems obtained respectively are listed in Table 1, and the theoretical $\Delta Q/V$ curves are in good agreement with the experimental data.

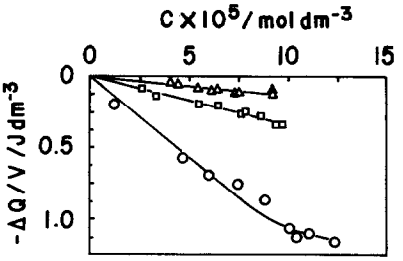

Fig. 3. The dependence of the observed heat of mixing per volume of the mixture, $\Delta Q/V$ on the concentration of AD, C

○ :DNA-AD system
□ :Duplex I-AD system
△ :Duplex II-AD system

Solid line is theoretical curve calculated according to Eq.(1).

Free energy change, $\Delta G(= RT \ln K)$ and the entropy change, $\Delta S(=(\Delta H - \Delta G)/T)$ are also estimated and the results obtained are also summarized in Table 1, together with the values obtained previously.

As seen in Table 1, ΔG-value of the intercalation process between DNA and AD

TABLE 1
Thermodynamic quantities of AD bound to DNA, Duplex I, and Duplex II at 298.15 K.

	DNA / Duplex	K / $dm^3 mol^{-1}$*	ΔG / kJ mol^{-1}*	ΔH / kJ mol^{-1}*	ΔS / J $K^{-1} mol^{-1}$*	n
AD	DNA(GC 42%)	1.4×10^6	-35	-11	80	0.21
	poly(A)·poly(U)	1.3×10^5	-29	-3.4	86	1.0
	poly(I)·poly(C)	4.9×10^4	-27	-1.4	86	1.0
PF	DNA(GC 42%)**	8.0×10^5	-34	-30	12	0.17
	poly(A)·poly(U)***	8.0×10^5	-34	-32	6.7	0.25
	poly(I)·poly(C)***	2.0×10^5	-31	-25	20	0.06

* mol refers to mole of adriamycin bound.
** See ref. 2.
*** See ref. 4.

is in good agreement with that of DNA-PF system estimated previously. For each process, ΔH in the DNA-AD system is considerably lower than that of DNA-PF system, indicating that the stability of the binding in the latter process may be governed by ΔH compared with the former process, but ΔS of the former process may make a large contribution to the stability of binding as expected.

It is very difficult to explain these facts due to a lack of some information in this study. Further study will be needed.

While, ΔH of the Duplex I-AD and Duplex II-one systems is considerably lower than that of DNA-AD system, indicating that the interaction for the former both systems may be different from that for the latter system: that is, from results of the absorption spectral measurements as mentioned above and as pointed out by a few investigators (refs.9,10), we would suggest that the interaction process of the former both systems may be the interaction mainly caused by an electrostatic interaction between the negative charge of phosphorus of nucleotide backbone and the positive charge of aminosugar moiety of AD molecule rather than the intercalation process such as DNA-AD system.

However, the interactions of Duplex I-AD and Duplex II-AD systems as mentioned above may be taken by an electrostatic interaction although that of Duplex I-PF and Duplex II-PF systems is caused by the interaction based on the intercalation process as pointed out previously such as DNA similarly. The reason why the reaction mechanisms for Duplex-AD and for Duplex-PF systems, respectively are different is difficult to make clear from present study. Further study will be needed.

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