Equilibrium Dialysis Studies of the DNA-Acridine Complexes

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The strong binding of acridine dyes with DNA was studied by means of equilibrium dialysis. Ten acridine derivatives were used to elucidate the effect of the dye structure on the nature of strong binding. In the strong binding, up to one dye molecule per 4—6 nucleotides was bound with the change of free energy in 5—9 kcal mol^{-1}. The introduction of bulky groups to the 10 or 3,6-positions of the acridine ring led to a marked decrease in both the association constant and the maximum number of binding sites per DNA phosphate. These findings suggest that, when a dye molecule is intercalated, the dye is located in such a way that the bulky groups attached to the acridine ring produce some steric hindrance to the binding.

The binding of acridine dyes to DNA has been extensively studied because of their biological activity and the general interest for the binding interaction of a macromolecule with small molecules.[1,2] It is generally believed that at least two types of binding processes are involved: weak and strong binding processes.[3] The weakly bound dye molecules are thought to be outside the double helix and stacked along the phosphate backbone.[4,5] Lerman[6,7] proposed the intercalation model for the strong binding, in which the dye molecules are inserted in a sandwich-like way between adjacent base pairs of DNA by the extension and the local unwinding of the helix. Though the general features of the model are widely accepted,[1,2] the exact location of the strongly bound dye molecules is not yet known. One of approaches to elucidate the possible location of the intercalating dye is a systematic investigation of the effects of the dye structure on the binding nature.

In the present study, the nature of strong binding was examined by means of equilibrium dialysis, using five 3,6-diaminoacridines and five 10-alkylderivatives of acridine orange. The aims of this study are: (1) to determine the thermodynamic parameters for the strong binding, (2) to reveal the effects of the dye structure on these parameters, and (3) to obtain some information on the location of the intercalative acridine dyes.

Experimental

Materials. Calf thymus DNA was purchased from Worthington Biochemical Corporation. The concentration of DNA was calculated from the absorbance at 260 nm, using the molar extinction coefficient per nucleotide residue (ε= 6600 M^{-1} cm^{-1}).[8]

Acridine orange and proflavine were obtained from Chroma and British Drug Houses, respectively. 3,6-Bis(methylamino)-acridine (Ac[NHMe]$_2$), 3,6-bis(ethylamino)acridine (Ac[NHEt]$_2$), and 3,6-bis(diethylamino)acridine (Ac[NEt_2]$_2$) were the same as previously reported.[9] 10-Alkylderivatives of acridine orange (R=methyl, propyl, pentyl, isopropyl, and benzyl) were prepared according to the method of Miethke and Zanker;[10] these are hereafter abbreviated as AO-methyl, AO-propyl, AO-pentyl, AO-isopropyl, and AO-benzyl. These dyes were purified by repeating crystallization and chromatography. Any trace of impurity was not detected by thin-layer chromatography on silica gel for each dye.

Methods. All dialysis experiments were made at ionic strengths of 0.01 and 0.11; these conditions were such that the Donnan effect could be neglected.

Both DNA and dye were dissolved in 0.005 M phosphate buffer (pH 6.9) and ionic strength was controlled by addition of NaCl solution. The DNA solutions (5 cm^3) were sealed in dialysis bags prepared from constant length (9 cm) of cellulose tubing (1.5 cm in diameter; Visking Co.) which had been cleaned by being boiled in 1% sodium hydrogencarbonate. These bags were immersed in 50 cm^3 brown flasks containing 30 cm^3 of dye solutions; the flasks had been coated with silicone to reduce the adsorption of dye. The initial dye concentrations ranged from 8×10^{-6} to 1.5×10^{-5} M except for Ac-[NEt_2]$_2$, AO-isopropyl and AO-benzyl at ionic strength of 0.11 ($(4—7)\times10^{-5}$ M). The flasks were mechanically rocked for 60 h in a thermostat.

For the determination of equilibrium dye concentration, both outer and inner solutions were analyzed because of the large adsorption of dye on the dialysis bag. The concentration of free dye (C_f) was determined from the absorbance of the outer solution at an appropriate wavelength for each system. Inner solutions were analyzed spectrophotometrically after dissociation of the DNA–dye complexes with an equal volume of 0.2 M LiCl in methanol.[11] Then the concentration of the bound dye was taken as a difference between the dye concentration of the inner solution and that of the outer solution.

All spectrophotometric measurements were made with a Shimadzu QV-50 spectrophotometer using the expanded scale whenever necessary.

For a single binding process to equivalent and independent binding sites, the following equation can be derived:[12]

$$r/C_f = K(n-r) \tag{1}$$

where r is the number of bound dye molecules per DNA phosphate, K is the association constant between a dye molecule and a binding site, and n is the maximum number of binding sites per DNA phosphate. By rearranging Eq. 1 one can obtain:

$$1/C_f = K(n/r-1). \tag{2}$$

Accordingly, a plot of $1/C_f$ against $1/r$ should give a straight line crossing the axes at $1/r=n$ and $1/C_f=-K$. If m independent binding processes are present, Eq. 2 holds for each binding and therefore one can obtain:

$$1/C_f = \sum_{i=1}^{m} K_i(n_i/r_i-1) \tag{3}$$

Then the plot of $1/C_f$ against $1/r$ cannot be linear.

Results and Discussion

Typical plots of $1/C_f$ against $1/r$ at various temperatures are shown in Figs. 1 and 2. A linear relationship

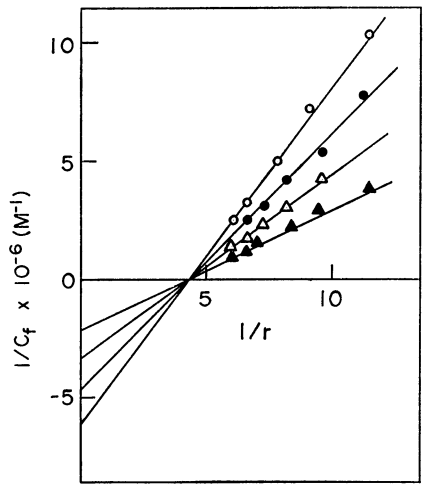

Fig. 1. Plots of $1/C_f$ against $1/r$ for the DNA–Ac[NHEt]$_2$ system at ionic strength of 0.01.
(○) 25 °C, (●) 31 °C, (△) 38 °C, (▲) 45 °C.

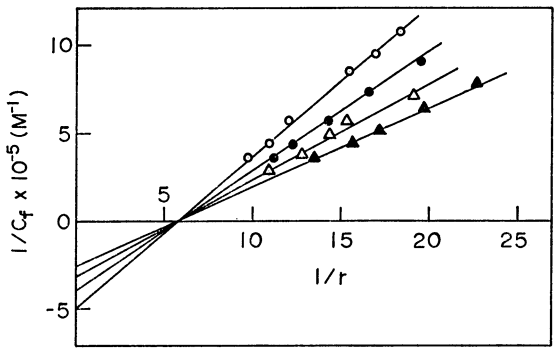

Fig. 2. Plots of $1/C_f$ against $1/r$ for the DNA–Ac[NEt$_2$]$_2$ system at ionic strength of 0.01.
(○) 25 °C, (●) 31 °C, (△) 38 °C, (▲) 45 °C.

between $1/C_f$ and $1/r$ was pretty good. From such plots, both K and n were determined by extrapolating the linear portion to $1/C_f$ and $1/r$ axes, respectively. As can be seen in Figs. 1 and 2, the K values are dependent on the temperature, while the n values seem to be constant within the experimental errors in the temperature ranges examined. In general, the $1/C_f$ *vs.* $1/r$ curves deviated from the linearity at low $1/r$ values ($r>0.2$); presumably due to the weak binding process.[4,5] Armstrong *et al.* proposed that, at low ionic strength, a binding of dye dimers takes place at high r values by binding of a second dye molecule to those already intercalated.[11] To avoid complications for the analysis of data,[11,13] we restrained our study to the range of strong binding ($r<0.15$) at relatively high ionic strengths.

Thermodynamic parameters, the change of free energy ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$) were calculated using the following equations.

$$\Delta G° = -RT\ln K \quad (4)$$

$$\Delta H° = -Rd\ln K/d(1/T) \quad (5)$$

$$\Delta S° = (\Delta H° - \Delta G°)/T \quad (6)$$

A plot of log K against $1/T$ for each system gave a straight line in the temperature ranges examined (25—45 °C).

Table 1. Thermodynamic parameters at 25 °C

Dye	Ionic strength	n	$k\times10^{-5}$ (M^{-1})	$-\Delta G°$ (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal deg^{-1} mol^{-1})
Proflavine	0.01	0.22	48	9.1	8.5	2.0
	0.11	0.20	3.1	7.5	8.7	−4.0
Ac[NHMe]$_2$	0.01	0.24	61	9.3	9.0	1.0
	0.11	0.22	6.7	7.9	8.7	−2.7
Ac[NHEt]$_2$	0.01	0.23	60	9.2	8.8	1.3
	0.11	0.21	3.9	7.6	8.8	−4.0
Ac[NEt$_2$]$_2$	0.01	0.17	4.9	7.8	5.2	8.7
	0.11	0.15	0.17	5.8	5.3	1.7
Acridine orange	0.01	0.22	55	9.2	8.8	1.3
	0.11	0.20	2.6	7.4	8.6	−4.0
AO-methyl	0.01	0.23	51	9.2	9.0	1.0
	0.11	0.21	4.4	7.7	9.2	−5.0
AO-propyl	0.01	0.23	54	9.2	8.5	2.3
	0.11	0.20	3.4	7.5	8.8	−4.4
AO-pentyl	0.01	0.24	53	9.2	8.5	2.3
	0.11	0.20	3.7	7.6	8.6	−3.4
AO-isopropyl	0.01	0.17	15.6	8.4	6.5	6.4
	0.11	0.17	0.61	6.5	6.2	1.0
AO-benzyl	0.01	0.18	18.4	8.5	6.9	5.4
	0.11	0.18	0.63	6.5	6.5	0

The thermodynamic parameters calculated from the data of equilibrium dialysis are listed in Table 1. These results show that the thermodynamic parameters depend on the dye structure and can be summarized as follows. (1) The n values for all acridine dyes do not exceed 0.25 ($0.15<n<0.25$); this is consistent with the findings reported earlier.[13–16] (2) The value of the association constant for all 3,6-diaminoacridines, except Ac[NEt$_2$]$_2$, are of the same order of magnitude. The K and $\Delta G°$ values for the DNA–proflavine and DNA–acridine orange complexes are in good agreement with the values in the literatures.[14,15,17] (3) The thermodynamic parameters for AO-methyl, AO-propyl and AO-pentyl are almost the same as those for acridine orange. (4) The K and n values for AO-isopropyl, AO-benzyl and Ac[NEt$_2$]$_2$ are much smaller than those for the other dyes. A marked increase in the $\Delta S°$ value is observed for these dyes when compared to the $\Delta S°$ value for the other dyes. The results imply that the bulky substituents of these dyes may exert some steric effect on the binding.

Due to the presence of bulky substituents such as diethylamino and isopropyl on the acridine ring, the original intercalation model of Lerman[6,7] may be modified.[18,19] According to the model proposed by Pritchard *et al.*,[18] the positively charged nitrogen of the acridine ring associates closely with the negatively charged oxygen of the phosphate group. Therefore, it can be predicted that any substituent on the ring nitrogen strongly affects the binding. However, the results of AO-methyl, AO-propyl and AO-pentyl show no dependence on the length of the alkyl chains attached to the ring nitrogen (Table 1). On the other hand, the results of AO-isopropyl, AO-benzyl and Ac[NEt$_2$]$_2$

indicate that the introduction of the bulky groups to the 10- or 3,6-positions of the acridine ring leads to a remarkable decrease in the K and n values. Further, the smaller K value for $Ac[NEt_2]_2$ in comparison with those for AO-isopropyl and AO-benzyl (Table 1) suggests that the bulky groups attached to the 3,6-positions have more pronounced effect on the binding than the bulky groups on the ring nitrogen do. In agreement with the findings of Löber and Achtert,[20] our results may be interpreted to show that the 10-position is not so important for the strong binding and that the acridine dyes examined in this study are intercalated in such a way that the 3,6-diamino groups are directed towards the DNA phosphate groups or very close to them. In such a location, the bulky substituents at the 3,6-positions would show significant steric effect. Even in such a location, however, it is expected that the bulky groups such as isopropyl and benzyl on the ring nitrogen still produce some steric hindrance to the binding. Therefore, such bulky groups may lie in the groove of the DNA helix so as to minimize the steric hindrance. In an attempt to elucidate further the effect of the dye structure on the strong binding, flow polarized fluorescence and viscosity studies are currently in progress.

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