TECHNICAL MANUSCRIPT 152

EFFECT OF DEOXYRIBONUCLEIC ACID ON THE FLUORESCENCE OF ACRIDINE DYES

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EFFECT OF DEOXYRIBONUCLEIC ACID ON THE FLUORESCENCE OF ACRIDINE DYES

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ABSTRACT

The interaction of deoxyribonucleic acid and several acridine dyes has been studied fluorometrically. The data indicate that two complexes form when the acridine is substituted in the 2,7- and/or 3,6-positions by amino and/or alkylamino groups. A model is proposed for the two complexes, utilizing the intercalation and aggregation theories for deoxyribonucleic acid and acridine dye interaction.

I. INTRODUCTION

In a previous publication[1] the effect of deoxyribonucleic acid (DNA) on the absorption spectra of acridine orange (AO) was reported. The interaction of the DNA and AO demonstrated the formation of two complexes. The present study demonstrates the effect of DNA on the fluorescence of several acridine dyes and explains the nature of the observed interactions on the basis of structure of the dyes, using currently proposed theoretical models.

II. MATERIALS AND METHODS

Deoxyribonucleic acid, highly polymerized grade A salmon sperm (California Corporation for Biochemical Research Lot 59053) was utilized, without further treatment, throughout these studies. It had a molecular weight of 1.2×10^6 as determined by light-scattering techniques.

The acridine dyes shown in Table I were used as obtained from the manufacturer except that the AO was prepared as previously reported.[1] Concentrations of the dyes were selected to keep the monomer-dimer equilibrium adjusted in favor of the monomeric form as described for AO by Zanker.[2]

The solvent system for the studies was a sodium acetate - sodium barbital buffer with the pH and ionic strength adjusted as desired with 0.001 M HCl and/or 1.0 M NaCl.

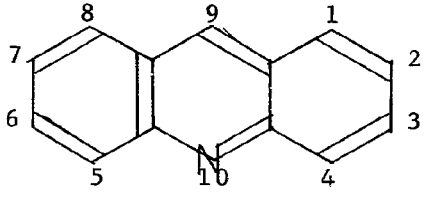
The stock solution of the buffer was prepared weekly and all other solutions were prepared daily. The DNA was dissolved in the buffer with the aid of magnetic stirring for approximately two hours.

The fluorescence measurements were made on a Farrand spectrophotofluorometer and/or a Photovolt fluorometer. All experiments were performed at room temperature.

TABLE I. ACRIDINE DYES

Dye	Activation Wave Length, mμ	Emission Wave Length, mμ	Source
Acridine[a/]	360	430	K & K Labs, New York Lot 194792
9-Aminoacridine	400	450	Winthrop Labs, New York Lot 75RJ
9-Nicotinylmethyl-acridine	347	470	Dr. Robert Levine Chem. Dept. Univ. of Pittsburgh Pittsburgh, Pa.
3,6-Diamino-N-methyl-acridine (acriflavine)	450	500	K & K Labs, New York Lot 21804
3,6-Diamino-2,7-dimethyl-acridine (acridine yellow)	445	505	K & K Labs, New York Lot 22730
3,6-Bisdimethylamino-acridine (acridine orange)	490	525	Allied Chemical & Dye Corp., New York CI No. 788

a. The structure of acridine and the numbering system used are:



III. RESULTS

The data obtained in this study were analyzed by the Stern-Volmer equation:

$$\frac{F_o}{F} - 1 = K_Q c \qquad (1)$$

where F_o is the fluorescence of the free dye, F is the fluorescence of the dye plus quencher, K_Q is the quenching constant, and c is the concentration of the quencher. The quenching of fluorescence of acridine dyes by DNA closely follows this equation to DNA concentrations of 0.1 mg/ml, above which deviations become appreciable.[3] Figure 1 shows some typical curves for each of the dyes studied. All the dyes conform to the equation, except that in curves D, E, and F the quenching reaches a maximum, begins to decrease, and in the case of AO falls below zero, indicating fluorescence enhancement. By applying Equation (1) to this fall-off portion of the curve the slope is determined, and is designated K_E, the enhancement constant. Since this enhancement occurs below the concentrations of DNA where Equation (1) does not hold, the calculation appears valid.

Assuming[3,4] that the ratio of the quantum yields of fluorescence of the bound and free fluorescent molecules, p, is the same for all bound molecules, then the quenching can be expressed as

$$\frac{F_o}{F} - 1 = \frac{(1-p)\ C}{D\ + p\ C} \qquad (2)$$

where C is the concentration of the DNA-dye complex, D is the concentration of free dye, and F_o, F, and p have meanings previously described.

The values for K_{11}, the apparent equilibrium constant, and the extrapolation to k_1, the equilibrium constant, were obtained according to the method and assumptions of Oster[3] and Heilweil and VanWinkle.[4] Table II shows the values of K_Q, p, and K_{11}, with the extrapolated value of k_1. The extrapolation is shown graphically in Figures 2 and 3.

In general, studies over the range pH 5 to 9 confirmed previous observations that the fluorescence intensity decreased as the pH was lowered. This is attributed to the hydrogen ions' replacing the dye bound to DNA.[3]

In Table III, the effect of ionic strength on fluorescence is shown for the quenching and the equilibrium constants. The k_1 values were estimated from the data in Table II and calculated values of K_{11} were obtained from the experiments. The decreases in k_1 as a function of ionic strength for quenching are associated with electrostatic interaction. However, the enhancement data show an increase in the k_1 values as the ionic strength is increased. This indicates bonding associated with more than Van der Waal's or electrostatic bonding.

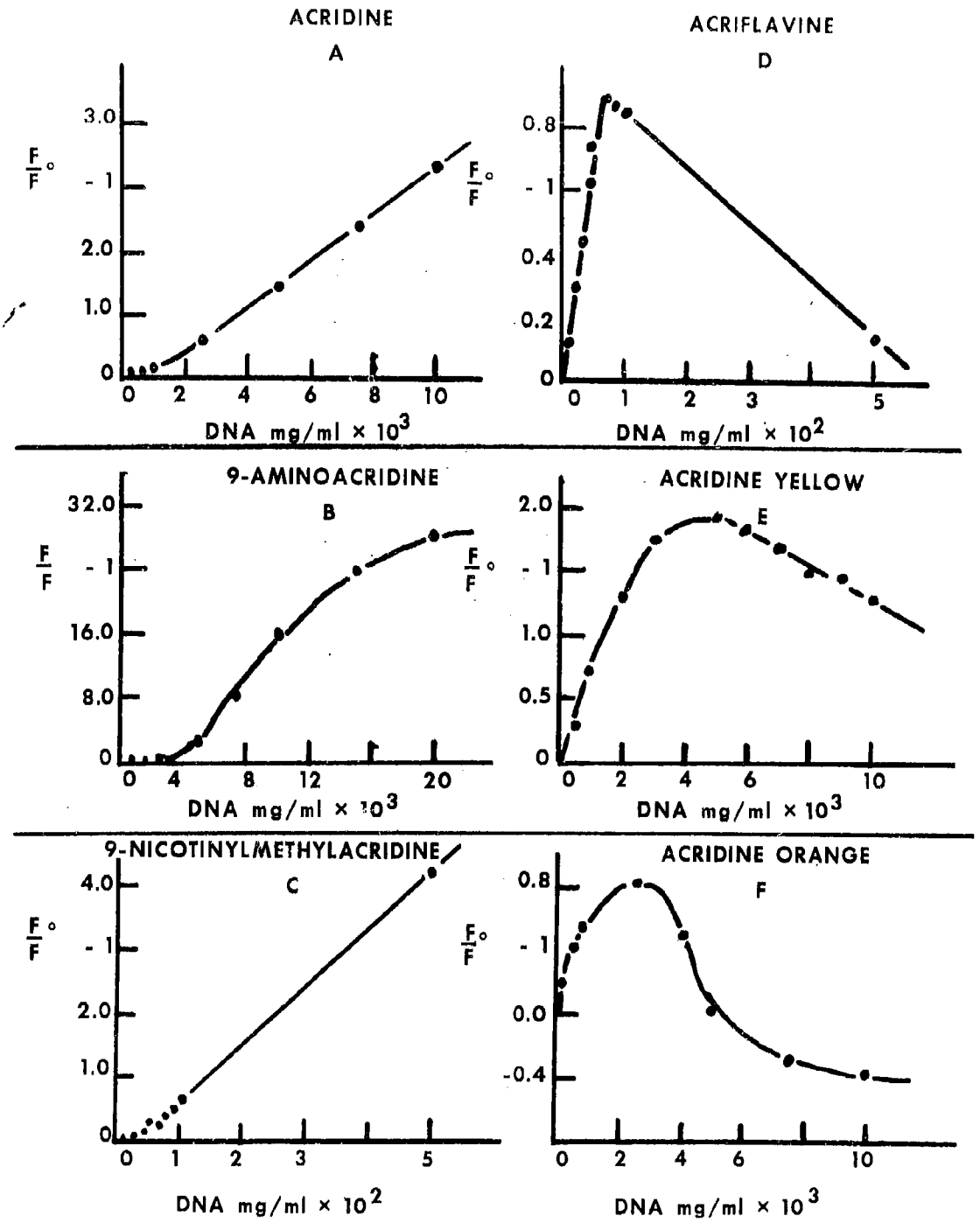


Figure 1. Effect of DNA on the Fluorescence of Various Acridine Dyes; Concentration = 3×10^{-6} M; pH = 7.0; μ = 0.005.

TABLE II. EFFECT OF DYE CONCENTRATION ON FLUORESCENCE

Dye[a]	Concentration M x 10^6	Quenching K_Q x 10^{-8}	P_Q	K_{11} x 10^{-8}	k_1 x 10^{-8}	Enhancement $-K_E$ x 10^{-8}	P_E	$-K_{11}$ x 10^{-8}	$-k_1$ x 10^{-8}
Acridine	0.50	0.080	0.940	1.33					
	1.00	0.074	0.801	0.37					
	3.00	0.440	0.048	0.46					
	5.00	0.200	0.529	0.42					
					1.5				
9-Amino-	0.50	24.8	0.031	25.6					
Acridine	1.00	26.0	0.026	26.7					
	3.00	28.8	0.026	29.5					
					25.0				
9-Nicotinyl-	0.50	1.44	0.167	1.73					
Methylacridine	1.00	1.15	0.179	1.40					
	3.00	1.10	0.188	1.35					
					2.0				
Acriflavine	0.50	2.9	0.662	8.58		0.570	0.971	1.966	
	1.00	1.3	0.720	4.64		0.097	0.995	1.940	
	3.00	1.7	0.527	3.59		0.210	0.887	1.860	
					8.0				2.0
Acridine	0.50	18.0	0.338	27.2		1.4	0.474	2.66	
Yellow	3.00	9.6	0.181	11.7		1.6	0.500	3.2	
					30.0				2.5
Acridine	0.50	16.8	0.810	88.0		3.0	2.33	2.25	
Orange	1.00	20.0	0.723	72.0		3.3	2.222	2.70	
	3.00	14.4	0.543	32.0		5.3	2.222	4.34	
					96.0				1.80

a. pH = 7.0; ionic strength = 0.005.

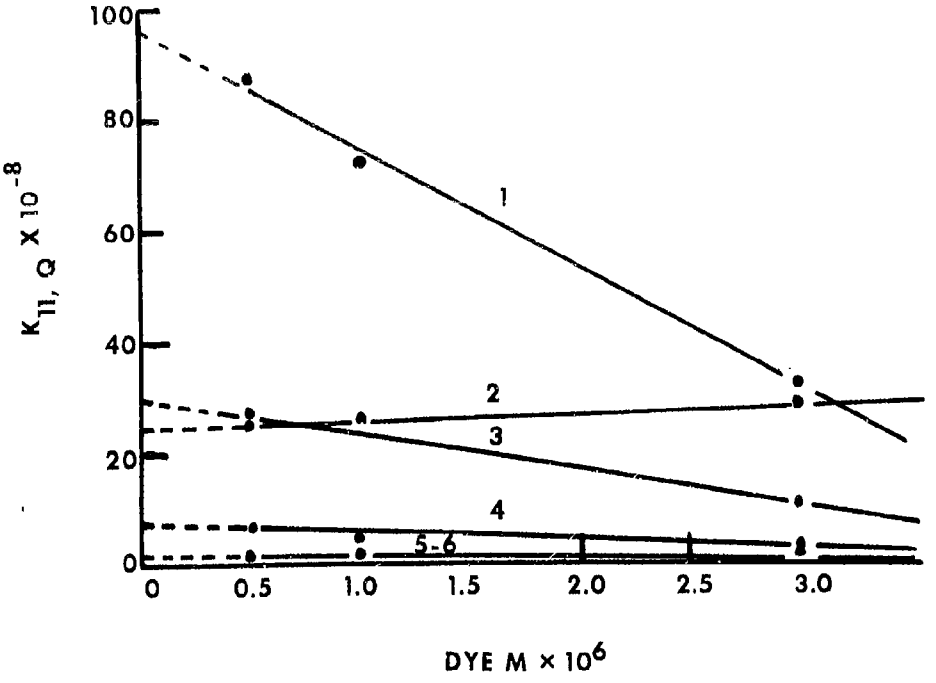


Figure 2. K_{11} as a Function of Dye Concentration for Quenching; pH = 7.0; μ = 0.005
1 = acridine orange; 2 = 9-aminoacridine;
3 = acridine yellow; 4 = acriflavine;
5 = 9-nicotinylmethylacridine; 6 = acridine.

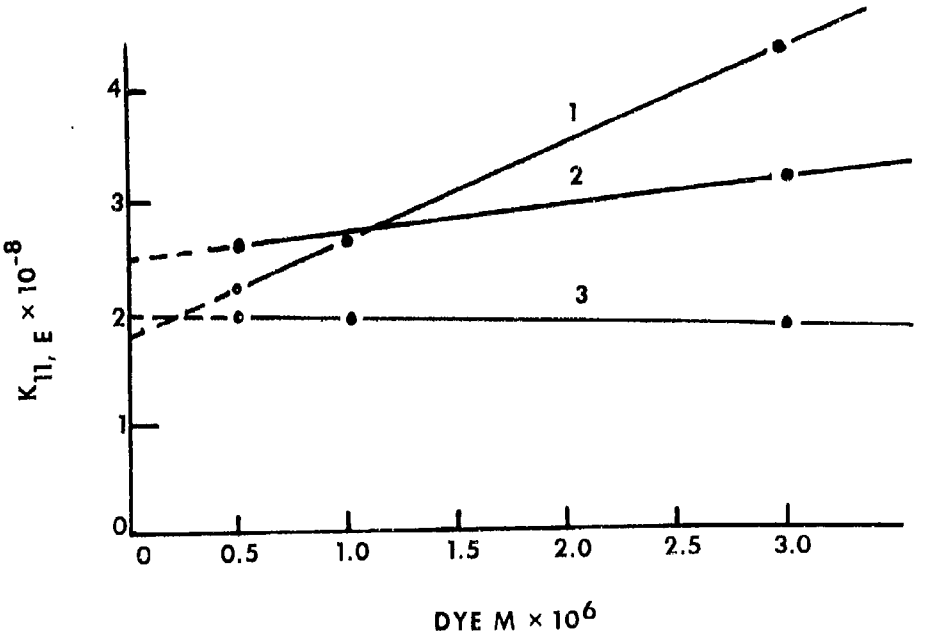


Figure 3. K_{11} as a Function of Dye Concentration for Enhancement; pH = 7.0; μ = 0.005
1 = acridine orange; 2 = acridine yellow;
3 = acriflavine.

TABLE III. EFFECT OF IONIC STRENGTH ON FLUORESCENCE

Dye [a]	Ionic Strength	Quenching				Enhancement			
		K_Q x 10^{-8}	P_Q	K_{11} x 10^{-8}	k_1 x 10^{-8}	$-K_E$ x 10^{-8}	P_E	$-K_{11}$ x 10^{-8}	$-k_1$ x 10^{-8}
Acridine	0.005	0.44	0.048	0.46	2.86				
	0.010	0.094	0.721	0.34	2.74				
	0.025	0.070	0.725	0.25	2.65				
	0.050	0.048	0.810	0.25	2.65				
	0.100	0.016	0.924	0.21	2.61				
9-Amino-Acridine	0.005	28.8	0.026	29.5	34.3				
	0.010	3.6	0.182	4.4	9.2				
	0.025	2.24	0.183	2.7	7.5				
	0.050	2.33	0.089	2.6	7.4				
	0.100	1.44	0.036	1.5	6.3				
9-Nicotinyl-Methylacridine	0.005	1.1	0.188	1.35	3.75				
	0.010	0.54	0.309	0.78	3.18				
	0.050	0.36	0.409	0.61	3.01				
	0.100	0.19	0.559	0.43	2.83				
Acriflavine	0.005	1.7	0.527	3.59	7.79	1.68	0.327	2.5	2.38
	0.010	-	-	-	-	-	-	-	-
	0.025	1.8	0.456	3.31	7.51	0.196	0.659	0.57	0.45
	0.050	1.98	0.405	3.33	7.53	0.22	0.525	0.46	0.34
	0.100	2.4	0.186	2.95	7.15	-	-	-	-
Acridine Yellow [b]	0.025	4.33	0.544	9.50	25.85	0.75	1.087	8.6	9.2
	0.005	1.90	0.599	4.73	21.08	0.18	0.965	5.0	5.6
	0.010	2.2	0.596	5.44	21.79	0.29	0.991	0.32	0.92
	0.050	1.1	0.343	1.67	18.02	0.13	1.960	0.85	1.45
	0.100	0.68	0.361	1.06	17.41	0.72	-	-	-
Acridine Orange	0.005	14.4	0.543	31.5	52.5	4.8	2.222	4.34	6.84
	0.010	7.2	0.687	23.0	44.0	2.0	2.035	1.93	4.45
	0.050	1.2	0.933	17.1	38.1	0.64	2.310	0.49	3.01
	0.100	-	0.957	-	-	0.43	2.039	0.41	2.93

a. Dye concentration = 3 x 10^{-5} M, pH = 7.0.
b. pH = 9.0.

IV. DISCUSSION

The results of these experiments show that acridines substituted in the 2,7- and/or 3,6-positions with amino or alkylamino groups can have their fluorescence quenched by DNA and that the interaction of these substituted dyes with DNA can enhance fluorescence. This is especially evident with acridine orange, where the fluorescence of the interaction becomes greater than that of the free dye.

Contemporary models[6-12] have been proposed for the mechanism of the interaction of DNA and acridines comprising base displacement, base tilting, aggregation, and intercalation. Recent studies[11,13,14] and a personal communication* on the interaction by polarization of fluorescence and small-angle X-ray scattering have given strong support to the intercalation model. However, this model only explains quenching and does not account for enhancement.

In a previous publication,[1] the formation of two complexes was proposed as a result of the alteration of the absorption spectra of acridine orange by DNA. The variation of p in Equation (2) and the ability to calculate the enhancement constant further amplifies this concept. However, the two-complex concept is only valid for those dyes properly substituted.

Based on the above evidence, a model of interaction is proposed consisting of intercalation of the dye in the DNA helix that causes quenching and aggregation to the free sugar phosphates to give enhancement. This latter can only occur when the dye is substituted in the 2,7- and/or 3,6-positions with amino and/or alkylamino groups.

All acridine dyes, except those showing obvious steric hindrance, can be intercalated, with quenching resulting.

This model, intercalation with aggregation, might well be compared with a model reported by Bersohn and Isenberg[15] for the quenching of phosphorescence of DNA by metal ions, and with the studies of McRae and Kasha.[16]

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* I. Blei, Melpar, Inc., Falls Church, Virginia.

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