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9-AMINOACRIDINE BINDING TO DEOXYRIBONUCLEIC ACID: A FLUOROMETRIC ANALYSIS

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9-AMINOACRIDINE BINDING TO DEOXYRIBONUCLEIC ACID: A FLUOROMETRIC ANALYSIS

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ABSTRACT

By using fluorescence quenching and a modification of a spectrophotometric calculation, the number of binding sites for 9-aminoacridine on deoxyribonucleic acid was determined to be 650. That number remained constant although the intrinsic association constant varied from 7.0×10^{8} to 0.17×10^{8} as a function of ionic strength, pH, and dye concentration.

9-AMINOACRIDINE BINDING TO DEOXYRIBONUCLEIC ACID: A FLUOROMETRIC ANALYSIS

The binding of dyes to polymers has been described by the Langmuir absorption isotherm:

$$r = \frac{nc}{K + c} \tag{1}$$

where r is the number of molecules of dye bound to a molecule of polymer, n is the number of binding sites, c is the equilibrium concentration of the free dye, and K is the intrinsic dissociation constant of the complex. There are many experimental techniques for arriving at the value of K for a given system.[1] One such method is the spectrophotometric method described by Clark and Shack[2,3] modified by Irvin, Irvin, and Parker[4] and further modified by Peacocke and Skerrett.[5]

This paper presents a further modification that utilizes fluorescence quenching measurements.

Let P equal the molar concentration of the polymer, DNA in this case, I the initial molar concentration of the ligand 9-aminoacridine, c the equilibrium concentration of the ligand, F_i the fluorescence of I, F_b the fluorescence of the bound I at maximum quenching (in the presence of excess polymer), and F the fluorescence of the mixture at any other given value of P. Then:

$$F = \frac{cF_i}{I} + F_b \frac{rP}{I} \tag{2}$$

Defining α as the fraction of total dye bound, then:

$$\alpha = rP/I = (F_i - F)/(F_i - F_b) \tag{3}$$

Knowing α, then:

$$r = \alpha I/P \tag{4}$$

and

$$c = I\,(1-\alpha) \tag{5}$$

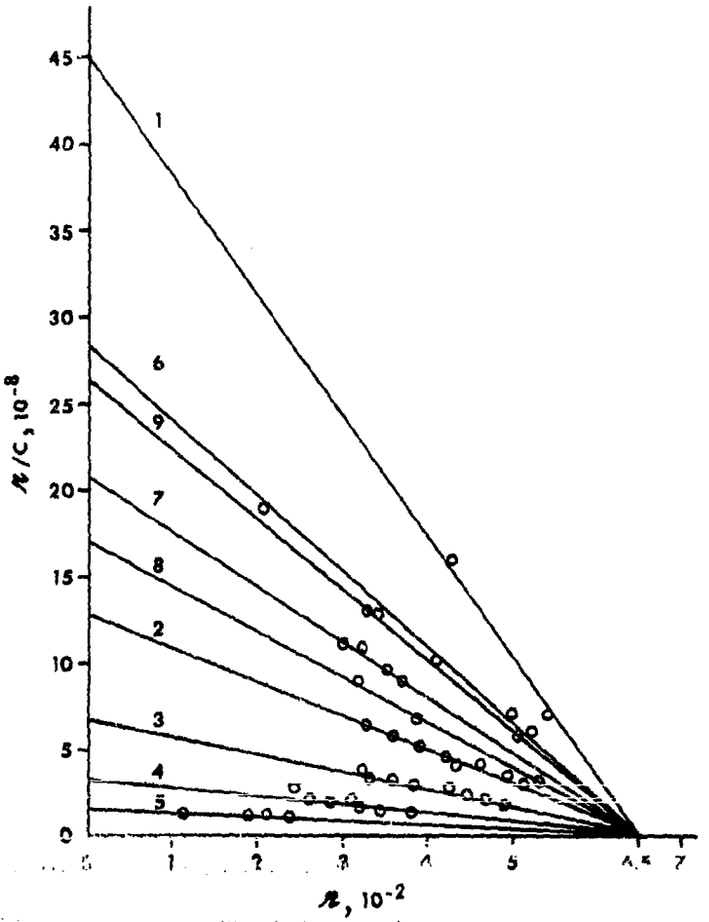
From these values and the values of P and I, the constants K and n can be evaluated from the graphs of r/c versus r, where the intercept on the ordinate is Kn, on the absissa is n, and the slope of the line is -K.

The 9-aminoacridine used in this study was obtained through the courtesy of Professor Adrian Albert of the Australian National University and was used without further treatment. It had an absorption maximum at 400 mμ and an emission maximum at 500 mμ. The absorption maximum was determined on a Beckman DK2 spectrophotometer and all fluorescence measurements were made on a Farrand spectrophotofluorometer at room temperature with 1-cm quartz cells. Highly polymerized grade A salmon sperm DNA (California Corp. for Biochemical Research) was used throughout these studies without further treatment. It had a molecular weight of 1.2×10^6 as determined by light-scattering studies. The solvent system was sodium acetate - sodium barbital buffer with pH and ionic strength adjusted as desired with HCl or NaCl.

Results of plotting r/c versus r as a function of ionic strength, pH, and dye concentration are shown in Figure 1, with the values of K and n.

The homogeneity of the binding is indicated by the constancy of the number of binding sites. The variations in the intrinsic association constants are in complete agreement with previously reported studies,[5-8] although the absolute values do differ. This might be due to the degree of polymerization of the deoxyribonucleic acid. The low value for K at pH 9.0 is probably associated with depolymerization or denaturation or both.

Application of the method used here is suggested for dye-binding studies in dilute solution where the binding is homogeneous, and for elimination of the errors associated with dye losses or surface of material such as viscose tubing used in binding studies.



Curve	Dye Concentration, 10^{-6} M	pH	Ionic Strength, M	K, 10^{6}	n, 10^{3}
1	3	7.0	0.005	7.00	6.5
2	3	7.0	0.010	2.00	6.5
3	3	7.0	0.025	1.04	6.5
4	3	7.0	0.050	0.53	6.5
5	3	7.0	0.100	0.17	6.5
6	1	7.0	0.005	4.38	6.5
7	0.5	7.0	0.005	3.20	6.5
8	3	5.0	0.005	2.62	6.5
9	3	9.0	0.005	3.82	6.5

Figure 1. Binding of 9-Aminoacridine to DNA, r/c versus r.

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