INTERACTIONS OF METHYLATED ACRIDINES WITH DNA

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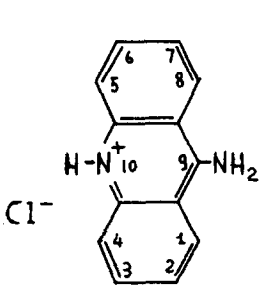
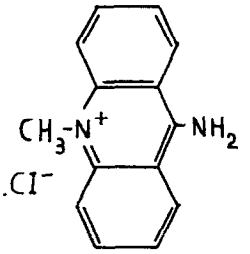
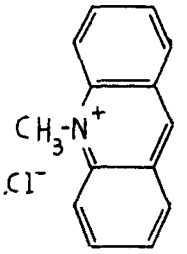
The observation by Lerman (1964) that methylation of the ring nitrogen in acridines abolishes their mutagenic effect, suggested a comparative study of *in vitro* complexes of DNA with 10-methylacridines and with non-methylated ones. The following acridines were studied:



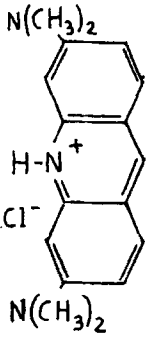
9-Aminoacridine hydrochloride (9AA)



9-Amino 10-Methyl-acridinium chloride ($9AA\text{-}N\text{-}CH_3$)



10-Methyl-acridinium chloride ($A\text{-}N\text{-}CH_3$)



Acridine Orange (AO)

The extent of intercalation of acridines between DNA bases in the strong complex, at low $r = \frac{\text{bound acridines}}{\text{nucleotides}}$ values, was studied by measuring the double helix stabilization against thermal denaturation, and the hypochromic effect of complex formation as a function of r. In addition, the weak (external) complex between 9AA and DNA was compared with the weak

9AA-N-CH_3—DNA complex, to test any difference in this alternative mode of binding.

EXPERIMENTAL

DNA was obtained by B. subtilis and purified according to the method of Marmur (1961). The purification of acridines (purchased from B.D.H., Ltd.) and the methylation of the ring nitrogen were carried out by Prof. E. Testa (Lepetit Corporation, Milan). Acridine-DNA complexes were obtained by mixing varying amounts of acridine and DNA solutions, in order to reach the desired r ratios. All measurements were carried out in 5.0 mM NaCl and 0.5 mM sodium citrate solutions (pH 7). Under these ionic strength conditions and with an excess of DNA, the reaction (acridine + DNA $\rightleftharpoons$ acridine-DNA) is almost completely displaced on the side of the complex. Denaturation measurements were carried out in a Beckman D.U. thermoregulated spectrophotometer, in airtight quartz cuvettes. The temperature inside the cuvettes was measured by a thermocouple in a parallel cuvette.

Equilibrium dialysis measurements on the weak complex were carried out in 15 mM NaCl and 0.15 mM sodium citrate solution, according to the technique described by Peacocke and Skerrett (1956).

RESULTS

Strong complex:

a) Stabilizing effect. All the acridines examined stabilize DNA towards thermal denaturation. The increase of T_m for the four acridines at the different r values are shown in Fig.1. It was already observed (Riva et al., to be published) that the linear increase of T_m as a function of r (at low r values) can be attributed to the intercalation of acridines between adjacent

bases (strong complex). It can be seen that for a given r value the degree of stabilization decreases in the following order:

$$9AA = 9AA\text{-}N\text{-}CH_3 > A\text{-}N\text{-}CH_3 > AO$$

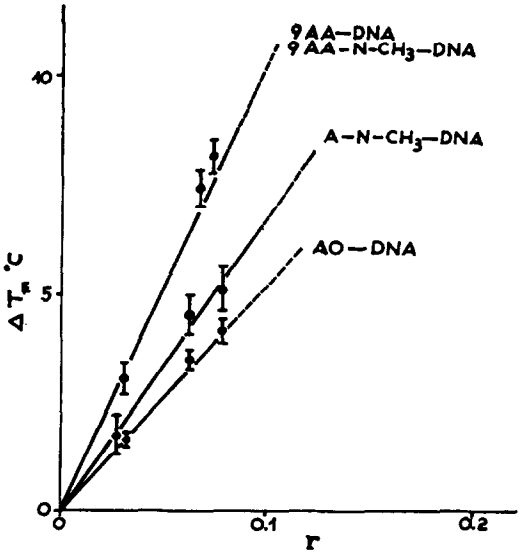


Fig. 1 Increase of T_m as a function of r for the four acridines.

This observation suggests that the methylation of acridines does not inhibit their capacity to stabilize DNA and that therefore, the extent of intercalation is not appreciably reduced.

b) Hypochromic effect. The binding of acridines to DNA causes a hypochromic effect, that is, the $O.D._{260\ m\mu}$ of acridine--DNA complex is less than the sum of the $O.D._{260\ m\mu}$ of acridine and of DNA. Very likely, this effect too is due to intercalation. It was already shown (Walker, 1965) that the hypochromicity can be ascribed to a decrease in the acridine and not in the DNA absorption spectrum as a consequence of the strong binding. The magnitude of the effect shows a linear increase with r (at low r values) as reported in Fig. 2. The degree of hypochromicity, for a given r value, is also dependent on the acridine tested, as shown by Fig. 2, in the following order:

$$9AA = 9AA\text{-}N\text{-}CH_3 > A\text{-}N\text{-}CH_3 > AO$$

In addition this observation indicates that methylation does not reduce the extent of intercalation of acridines in DNA.

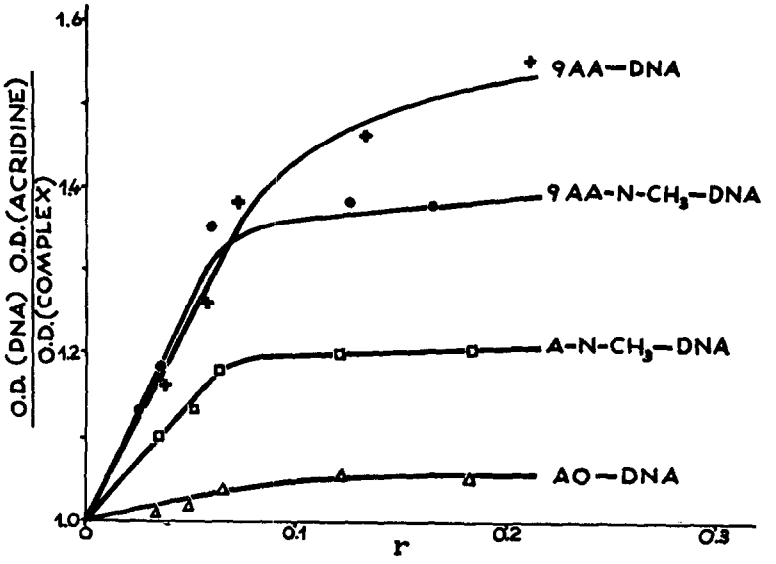


Fig. 2 Hypochromic effect at 260 mμ as a function of r for the four acridines at 20°.

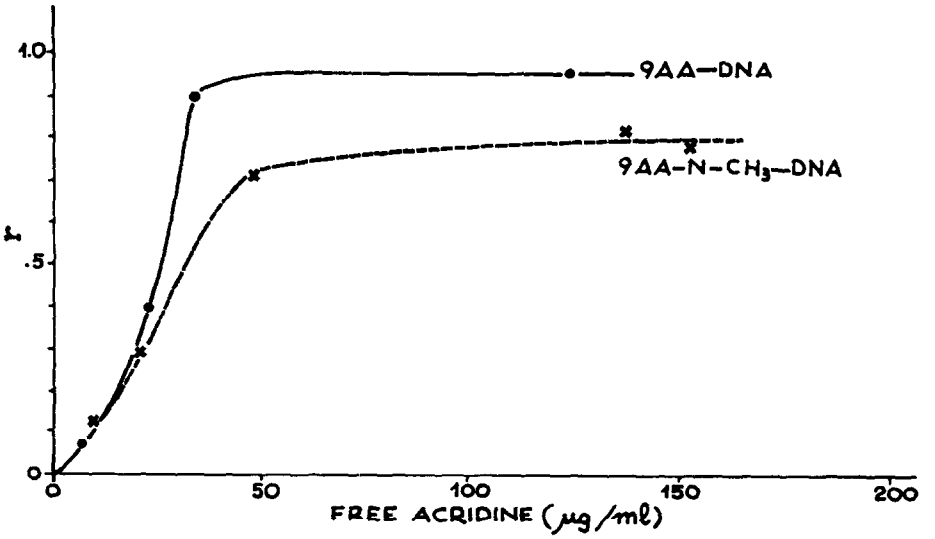


Fig. 3 Binding of the two 9-aminoacridines with DNA; r vs. free acridine concentration. Equilibrium dialysis data at 20°. DNA concentration = 1 mg/ml.

Weak complex

Fig. 3 shows $\underline{r}$ values plotted against free acridine concentration in the solution, obtained by equilibrium dialysis measurements for 9AA and 9AA-N-CH_3. In both cases it is possible to attain (with a large excess of acridine) $\underline{r}$ values very close to the saturation value ($\underline{r}$ = 1) of the weak complex, showing a large extent of weak binding formation. Indeed the $\underline{r}$ values obtained, 0.95 for 9AA and 0.80 for 9AA-N-CH_3 are much higher than the theoretical maximum value for the intercalated complex ($\underline{r}$ = 0.5).

DISCUSSION

As Fig. 1 and Fig. 2 show, there is a direct relationship between stabilization and hypochromic effect. Very likely, both phenomena are due to Van der Waals interactions between the planar acridine molecules and the bases with which they are intercalated in "sandwich" fashion. It is possible, in fact, that such interactions, besides modifying the acridine absorption spectrum, cause a decrease in the stacking free energy of DNA which is an appreciable fraction of the total free energy of the double helix (Crothers and Zimm, 1964). Both phenomena can be taken as an index of the extent of interaction of different acridines with DNA, so far as the intercalation is concerned. They therefore show that, at low $\underline{r}$ values (strong complex), there is no appreciable difference between 9AA and 9AA-N-CH_3, in spite of the great difference in mutagenicity. It is therefore impossible to account for the different mutagenic action observed, with a model based on intercalation only. On the other hand, the methylation of the ring nitrogen does not seem to inhibit the formation of the weak complex either, as Fig. 3 shows. The above observations are in agreement with the idea that neither the intercalation (strong binding) nor the weak interaction are directly correlated with the mutagenic action of acridines (Lerman, 1964). We may therefore conclude

that the types of acridine-DNA complexes measured by the parameters used so far are irrelavant to the mechanism of mutation induced by these agents.

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