Metal Ion Assisted DNA-Intercalation of Crown Ether-linked Acridine Derivatives

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Crown ether linked DNA-intercalators have shown an enhanced binding ability to DNA in the presence of certain kinds of metal ions, due to a cationic property given by the co-ordination of the crown ether moiety to the metal ions.

Certain mutagenic aromatic compounds, natural or synthetic, possess structural moieties that seem to be able to complex with metal ions. The β-dicarbonyl structure in aflatoxin B_1 is an example. Some uncharged heteroaromatic compounds are only slightly mutagenic by themselves but show enhanced mutagenic activity when applied with metal ions.[1] Mutagens with such structures can permeate the cell membrane in the uncharged (uncomplexed) form and complex with metal ions in the cell to bear a cationic charge. This cationic charge is expected to increase the overall binding constant of the mutagens to the target DNA which is an anionic polymer (Figure 1).

The present paper describes the synthesis and DNA binding properties of (**1**) and (**2**) in the presence of various alkali metal ions. Both of these compounds contain an acridine-9-carboxylate structure which in solution stays electrically uncharged under neutral pH conditions [the pK_a values of compounds (**1**) and (**2**) in the protonated form are 3.6 and 4.0, respectively]. However, compound (**1**) possesses an added functional group, a crown ether structure (15-crown-5), which is also electrically uncharged but can form cationic complexes with alkali metal ions. There are several precedents which combine such functionalities into single molecules with the aim to probe DNA sequence and structure.[2] Compound (**1**) is a model compound for uncharged intercalators which can interact with metal ions.

Compounds (**1**) and (**2**) were synthesized by esterification of acridine-9-carboxylic acid with the corresponding alcohols.† Calf thymus DNA (Sigma) was sonicated and purified (MW 30 000).[3] Compounds (**1**) and (**2**) were dissolved in dimethyl sulphoxide (DMSO) and diluted to 1% (v/v) DMSO aqueous solution for spectral measurement. The solutions containing DNA and metal salt (0.1 M NaCl, KCl, LiCl, or NH_4Cl) were buffered by *N*-2-hydroxyethylpiperazine-*N'*-ethanesulphonic acid (HEPES) at pH 7.2.

The acridine derivatives (**1**) and (**2**) exhibited a characteristic red shift and hypochromicity in the visible spectra upon binding to DNA (Figure 2). The absorption showed a maximum at 360 and 363 nm for unbound and bound acridine derivatives, respectively. This indicates that the acridine moiety of (**1**) and (**2**) interacts with DNA by intercalation.[4] The binding ratio of the intercalator (percentage of the DNA-bound intercalator in the total intercalator added) was calculated in a standard manner by using the hypochromic effect.[5]

Figure 3 shows the plots illustrating the binding behaviour

† Compound (**1**): yellow viscous oil, ^1H NMR ($CDCl_3$): δ 3.3—4.2 (m, 21H), 4.5—4.9 (m, 1.8H), 7.6—8.5 (m, 8.0H). Compound (**2**): yellow solid, m.p. 112—113 °C, ^1H NMR ($CDCl_3$): δ 4.2 (s, 3.1H), 7.6—8.3 (m, 7.9H). Satisfactory elemental analyses were obtained.

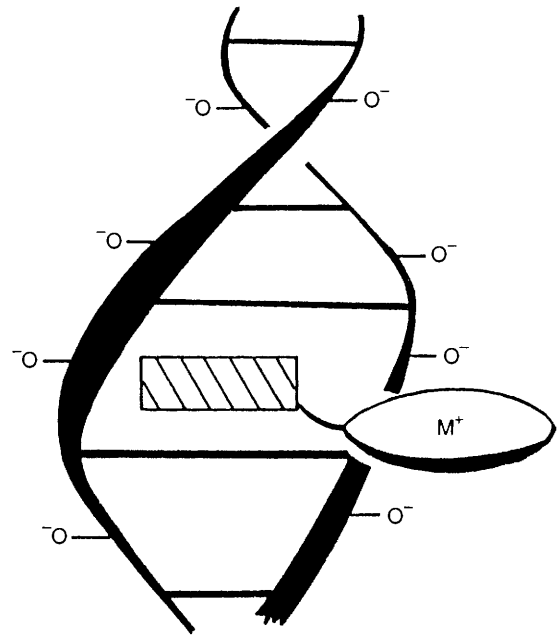
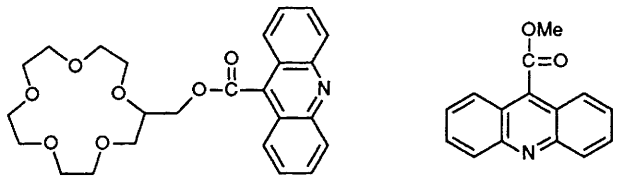


Figure 1. The concept of metal ion-assisted DNA-intercalation.



(1) (2)

of (**1**) as a function of P/D ratio‡ in the solutions containing 0.1M NaCl, KCl, LiCl, or NH_4Cl. Similar plots obtained for (**2**) are shown in Figure 4. In both the figures, the fraction of the DNA-bound intercalators is low at low P/D ratios. On increasing the P/D ratio, the fraction of the bound intercalators increases to reach a plateau around P/D values of several hundred to one thousand, where all the intercalators in the solution become bound to DNA.

The important features of the DNA-binding properties of compounds (**1**) and (**2**) are summarized as follows. (i) The binding of (**1**) is strongly influenced by the nature of metal ions in the solution. This is not the case at all with (**2**). (ii) The DNA-binding affinity of (**1**) increases in the order of $K^+ > Na^+ > NH_4^+ >> Li^+$, which is in line with the order of metal complex stability order of 15-crown-5 in methanol.[6] (iii) The binding ability of (**1**) is higher than that of (**2**) in the presence of K^+ or Na^+ but becomes much lower than that of (**2**) in the presence of Li^+.

These observations are rationalized quite well by considering the picture of metal-assisted intercalation we postulated in the introduction. The crown ether structure in (**1**) is quite bulky and causes steric hindrance when the acridine moiety of (**1**) becomes intercalated to the DNA double helix. This means that without some extraneous stabilizing factor the intercalation ability of (**1**) should be much lower than that of (**2**). This is clearly indicated by the DNA–(**1**)–Li^+ system where Li^+ cannot interact at all with the crown ether function.

‡ P/D ratio. P, phosphate (DNA) concentration/D, acridine dye concentration.

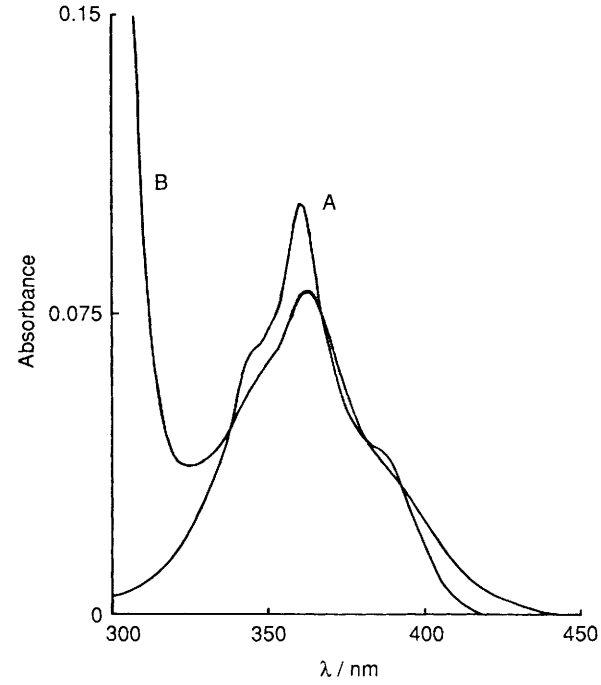


Figure 2. Visible absorption spectra of (**1**) in the presence and absence of calf thymus DNA in 0.1M NaCl solution. A: [(**1**)] = 1.0×10^{-5}M. B: [(**1**)] = 1.0×10^{-5}M, [DNA(phosphate unit)] = 5.8×10^{-3}M. As the DNA concentration increased from 0 to 580-fold the absorption of (**1**) decreased with a concurrent shift of the absorption maxima to a longer wavelength. A similar effect was observed for (**2**).

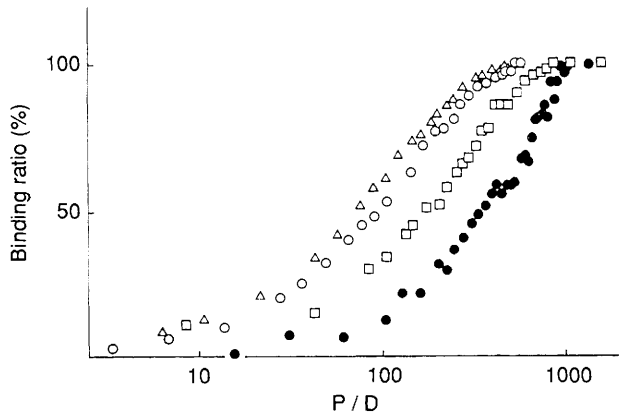


Figure 3. P/D binding ratio profiles for the intercalation of (**1**) to calf thymus DNA. [(**1**)] = 1.0×10^{-5}M, [calf thymus DNA] = 0–2.2×10^{-2}M, [metal ion] = 0.1M, [HEPES] = 5mM; 25 °C, pH 7.2. ●: Li^+, ○: Na^+, Δ: K^+, □: NH_4^+.

However, K^+ or Na^+ can form a cationic complex with the 15-crown-5 function, and (**1**), in spite of its stereochemical problem, indicates a strong affinity to DNA in the presence of these metal ions.

A detailed study on the mode of (**1**)–DNA interaction in the presence of metal ions, however, could not be made since the Scatchard analysis of the binding data indicated small binding constants (K, $\sim 3 \times 10^3$) which precluded the precise determination of the associated binding parameters.

The experimental observation in support of metal ion-assisted DNA-intercalation comes also from a filter-binding assay study.[8] Thus, by using similar crown ether-type intercalators it was shown that the crown ether derived intercalator

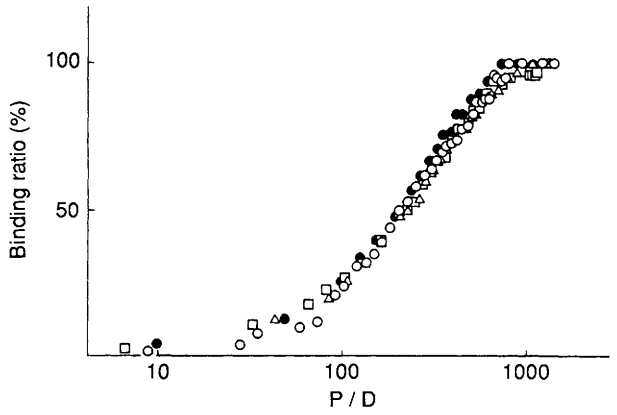


Figure 4. P/D binding percentage profiles for the intercalation of (**2**). Experimental condition are the same as those of Figure 3. ●: Li^+, ○: Na^+, Δ: K^+, □: NH_4^+.

interacts with DNA to a greater extent in the presence of Na^+ or K^+ than in the presence of Li^+ or NH_4^+, and as a result, the crown ether intercalator permeated the ultrafiltration membrane to a lesser extent in the presence of Na^+ or K^+ than in the presence of Li^+ or NH_4^+. Thus, it is quite reasonable that the metal binding functional group in the charge-neutral intercalator does assist the binding of the parent intercalator to DNA.

The concept of metal-assisted DNA-intercalation or, more generally, metal-assisted DNA–drug interaction is important because of the following reasons.

Firstly, it gives an idea of the chemical process involved in the action of certain types of mutagens which carry functional groups capable of interacting with metal ions. Secondly, it gives an idea for predicting possible mutagenic hazards caused by certain types of chemicals. Finally, it gives us a new chemical tool (introduction of metallophilic functional groups) in modifying the action of commonly known DNA-binding drugs for chemotherapeutic purposes. The metal-assisted drug binding suggests the possibility that the binding ability and the binding site selectivity of DNA-binding drugs (particularly those drugs not carrying net positive charge) can be substantially increased and altered, respectively, by introducing a metal binding site within the drug molecule. The importance of modifying the DNA-binding site selectivity is shown in the case of the antitumour drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP), which is commonly used with one or more of the intercalative drugs such as actinomycin in most chemotherapy treatments.[9] Lippard and co-workers and Malinge *et al.* reported that the presence of the intercalator ethidium bromide alters both the binding mode and binding site of *cis*-DDP on the DNA chain.[10]

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