Bis-9-acridinyl Derivative Containing a Viologen Linker Chain: Electrochemically Active Intercalator for Reversible Labelling of DNA

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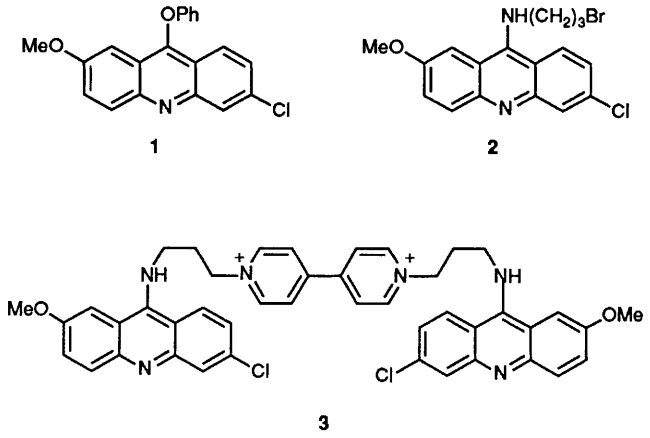
A new synthetic bis-9-acridinyl derivative containing a viologen linker chain binds strongly to DNA and shows a typical cyclic voltammogram, indicating a potential for use as a reversible electochemical labelling agent for DNA.

Electrochemical studies of DNA-binding ligands have a potential importance in probing the DNA–ligand interaction and in electrochemical detection of DNA. For probing the interaction with DNA, several studies have been reported on redox active DNA-binding ligands such as intercalating dyes[1–3] and metal coordination compounds.[4,5] However, studies on electroanalytical non-covalent (reversible) labelling of DNA have not been made because of the limited availability of DNA-binding ligands which possess redox activity.



By connecting two intercalating ligands by an aliphatic chain, bis-intercalating agents have been obtained which show high affinity and specificity to DNA.[6] Methylviologen is redox-active and binds efficiently to DNA,[7] suggesting that a bis-intercalating agent with a connecting viologen bridge would be a prime candidate for non-covalent, reversible labelling of DNA for electroanalytical detection. We report the synthesis and the properties of a bis-9-acridinyl derivative **3** which contains a viologen linker.

Treatment of 2-methoxy-6-chloro-9-phenoxyacridine **1** with 3-bromopropylamine hydrobromide in phenol afforded **2** in good yield. The reaction of **2** with 4,4′-bipyridine in DMF (dimethylformamide) at 95 °C for 20 h gave **3** in 11% yield after recrystallization.† Spectrophotometric pH-titration indicated that **3** possessed two proton dissociation constants in the pH range 3–11 (pK_{a1} = 4.0, pK_{a2} = 7.5). The dissociation constants are unusually large for a 9-aminoacridine derivative (ordinary pK values, 8.0–10), reflecting the electrostatic effect of the dicationic viologen linker unit. The compound **3** exists essentially in dicationic form (9-acridinyl units being unprotonated) at pH above 8.

The bis-9-acridinyl derivative **3** exhibited peculiar hypochromic and bathochromic shifts in the absorption spectra upon binding to DNA, characteristic of DNA intercalation. The concentrations of the bound and the free **3** could be determined from the spectra assuming the formation of single association complex species. Spectral changes were used to construct Scatchard plots,[8] which were well fitted by the site exclusion model of McGhee and von Hippel[9] affording binding constant (K) and site size (n) parameters. In a 0.1 mmol dm^{-3} tris-HCl buffer with 0.1 mmol dm^{-3} NaCl (pH 8.0), the constants are K, 1.7×10^5 dm^3 mol^{-1} and n, 3.8 which are similar to those obtained for bis-acridine derivatives containing a hexa-or nona-methylene chain.[10] However, a difference exists in the mode of interaction between these two types of bis-intercalators. Compound **3** interacts with DNA in dicationic form with the two acridine units remaining essentially unprotonated. On the other hand, the bis-acridine derivative containing a polymethylene linking unit carries two cationic charges on the acridine rings when it becomes bound to DNA.

The results of viscometric titrations of closed circular superhelical DNA (8800 base pairs) by **3** are shown in Fig. 1. Compound **3** gives a viscosity maximum which is typical of intercalating ligands.[11] This relaxation of supercoiled DNA by **3** could also be monitored on 1% agarose gel electrophoresis.

† Satisfactory spectral data and elemental analyses were obtained.

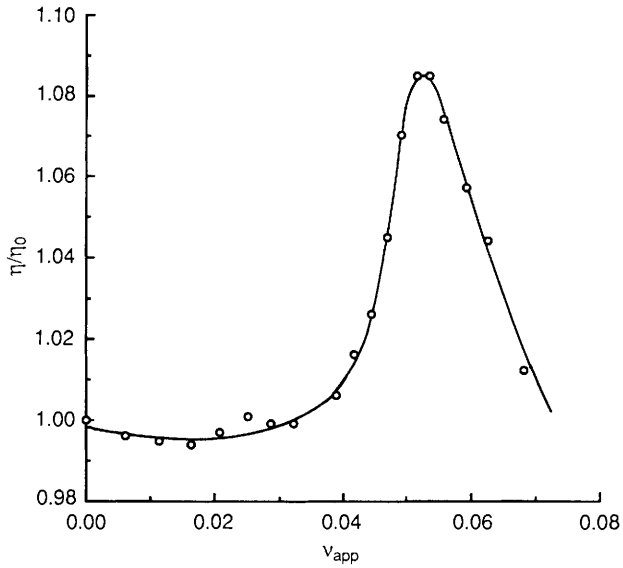


Fig. 1 Viscometric titration of closed circular superhelical DNA by **3**. The ratio of reduced specific viscosity (η/η_0 where $\eta = \eta_{sp}/C$) is plotted as a function of mole **3** added per mole nucleotide phosphate (ν_{app}). η_0 and η are the reduced specific viscosities of DNA alone and in the presence of **3**, respectively. Titrations were conducted at 25 °C in 1 mmol dm^{-3} tris-HCl, 1 mmol dm^{-3} NaCl, and 10 μmol dm^{-3} ethylenediaminetetraacetic acid (pH 8.0)

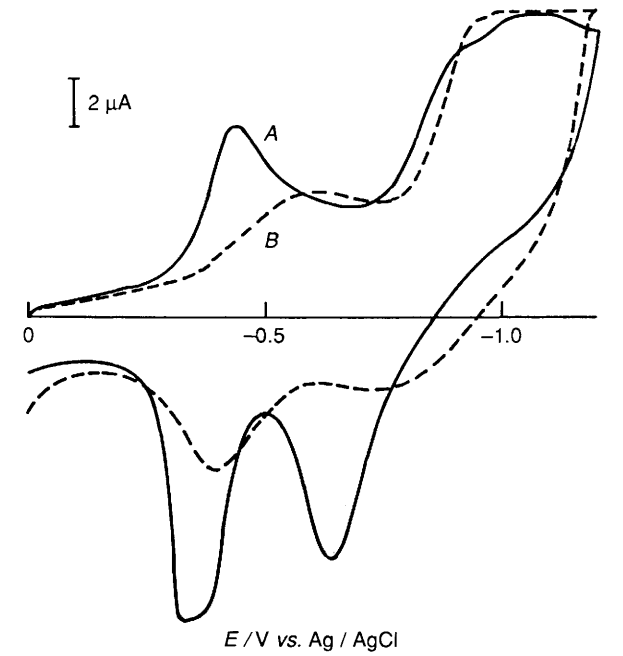


Fig. 2. Cyclic voltammograms of 1.0×10^{-4} mol dm^{-3} **3** (*A*) in the absence and (*B*) in the presence of 1.0×10^{-3} mol dm^{-3} DNA (nucleotide phosphate unit). Sweep rate: 120 mV s^{-1}. Supporting electrolyte: 5×10^{-2} mol dm^{-3} KCl and 10^{-2} mol dm^{-3} containing 10% dimethyl sulphoxide cyclohexylaminoethane sulphonic acid pH 9.0

We have estimated a duplex unwinding angle of 28° for **3** by using the technique described by DeLeys and Jackson.[12] This unwinding angle is consistent with values typical of bis-intercalating ligands,[10] indicating that compound **3** interacts with DNA by bis-intercalation. A full intercalation by the two

acridine units in **3** also assures a full interaction of the viologen linker unit along the DNA groove. Thus, it seems that the acridine units (uncharged) and the viologen unit (doubly charged) in compound **3** are effectively integrated into a sort of single function to bind to DNA with high affinity.

Typical cyclic voltammograms of **3** are shown in Fig. 2 (1.0×10^{-4} mol dm^{-3}) in the absence (curve *A*) and in the presence (curve *B*) of sonicated calf thymus DNA (1.0×10^{-3} mol dm^{-3} phosphate unit). In the absence of DNA (Fig. 2*A*), the reduction of $Viol^{2+}$ (Viol: compound **3** as represented by the viologen unit) species to $Viol^{\cdot+}$ occurred at $E_{pc} = -435$ mV and the reoxidation at $E_{pa} = -340$ mV (rate of potential scan, $v = 120$ mV s^{-1}), giving $E_{1/2} = -388$ mV. The peak potential separation was 95 mV, indicating a fairly reversible electron transfer. In the presence of 10^{-3} mol dm^{-3} DNA(Fig. 2*B*), E_{pc} shifted to -600 mV and E_{pa} to -395 mV, yielding $E_{1/2} = -498$ mV ($\Delta E_p = 205$ mV). Thus, the apparent $E_{1/2}$ shifted to more negative potential by 110 mV in the presence of DNA. The net shift in $E_{1/2}$ can be used to estimate the ratio of binding constants to DNA of $Viol^{2+}$ and $Viol^{\cdot+}$ species as shown by Carter *et al.*[5] The ratio of binding constants $Viol^{\cdot+}/Viol^{2+}$ is 0.014, and the $Viol^{2+}$ species is bound *ca.* 70 times more strongly than the $Viol^{\cdot+}$ species.‡ This indicates the importance of electrostatic interactions between **3** and DNA.

‡ Binding equilibria[5] are given by:

$$\begin{array}{ccc} & \overset{K_f}{\rightleftarrows} & \\ Viol^{2+} + DNA + e^- & & Viol^{\cdot+} + DNA \qquad E_f^{0\prime} \\ \uparrow\downarrow K_{2+} & & \uparrow\downarrow K_{\cdot+} \\ & \underset{K_b}{\rightleftarrows} & \\ Viol^{2+}DNA + e^- & & Viol^{\cdot+}DNA \qquad E_b^{0\prime} \end{array}$$

where $E_f^{0\prime}$ and $E_b^{0\prime}$ are the formal potentials of the $2+/\cdot+$ couple, in the free and bound forms, respectively and K_{2+} and $K_{\cdot+}$ are the corresponding binding constants for the 2+ and $\cdot+$ species to DNA. The relation between the formal potentials and equilibrium constants is given by:

$$E_b^{0\prime} - E_f^{0\prime} = 0.059 \log (K_{\cdot+}/K_{2+}).$$

Electrochemical detection is potentially a sensitive and versatile method. However, DNA is redox-inactive under ordinary conditions, and its labelling or derivatization for electrochemical detection has attracted only little attention so far. The methodology developed in this study provides a new possibility in analytical techniques for DNA, and, in addition, the facile introduction of redox sites into DNA is expected to give a new tool in functionalizing DNA for studying its chemistry.

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