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Interaction of Chromosomal Stains with DNA

An Electrofluorescence Study

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Abstract. A novel fluorescence procedure has been used to study the binding characteristics of DNA with three modern fluorochromes currently used in chromosome cytochemistry. The transient changes in the polarised components of fluorescence have been recorded for dye-tagged DNA solutions when subjected to short duration electric pulses. From these data, it has been inferred that, like ethidium bromide, berberine sulphate and quinacrine mustard both intercalate the DNA structure whilst the bi-benzimidazole derivative Hoechst 33258 binds with a distinctively different geometry, probably within the helical grooves.

Key words: Fluorescence – Chromosomal stains – DNA – Orientation

Introduction

Recently we have developed an extremely sensitive and rapid means for evaluating the gross binding geometry of fluorescent dyes to macromolecules in general and DNA in particular (Jennings and Ridler 1977; Ridler and Jennings 1980). The method is based upon a complex laser apparatus with which the polarised components of fluorescence are recorded for dilute dye-tagged macromolecular solutions. When subjected to a short duration, high voltage electric pulse, orientational order is induced in the macromolecular array. If the dye molecules are attached with high directional specificity to the macromolecules, then these too experience alignment. This results in changes in each of the accompanying fluorescence components. The signs and amplitudes of these changes enable estimates of the dye binding geometry to be evaluated (Ridler and Jennings 1978). The method has been particularly applicable to the study of DNA interactions at very low dye-phosphorus ratios. The binding characteristics of various chemotherapeutic agents and of covalently binding carcinogens with DNA have been published hitherto (Ridler and Jennings 1982a, b). Since it is

generally believed that most fluorochromes used in chromosome cytochemistry react with some part of the DNA molecule, we have used the method to study the interaction of four chromosomal stains with DNA. The objective has been twofold. Firstly, to reveal the geometry of interaction of these systems with DNA. Secondly, to indicate the potential of the method for characterising such dye-DNA moieties and thereby assist in an understanding of the mechanism of chromosomal staining procedures.

Materials and Methods

Materials

The fluorochromes studied were berberine neutral sulphate (Sigma Chemical Co., lot 35C-00761), quinacrine mustard (Sigma Chemical Co., lot 118C-0351) and Hoechst 33258. The chemical structures of these materials are shown in Fig. 1. In addition and for experimental comparison, data were recorded for ethidium bromide (BDH Chemicals Ltd.) since it is a renowned DNA intercalating compound. The DNA had a molecular weight of 5×10^6 daltons and was in the form of a Na salt after extraction from calf thymus (Messrs. Koch Light Ltd.). The fibrous materials was dissolved in deionised and doubly distilled water by slow agitation for 12 h. Aqueous solutions of the fluorochromes were added to the DNA solutions to give final solutions with a DNA concentration of 10^{-5} g · cm^{-3} and a DNA phosphorus-to-dye ratio of 300 : 1 for ethidium bromide, berberine sulphate and quinacrine mustard, and of 1000 : 1 for the Hoechst 33258 complex. Experiments were performed at 20° C at a pH of 6.4 ± 0.2.

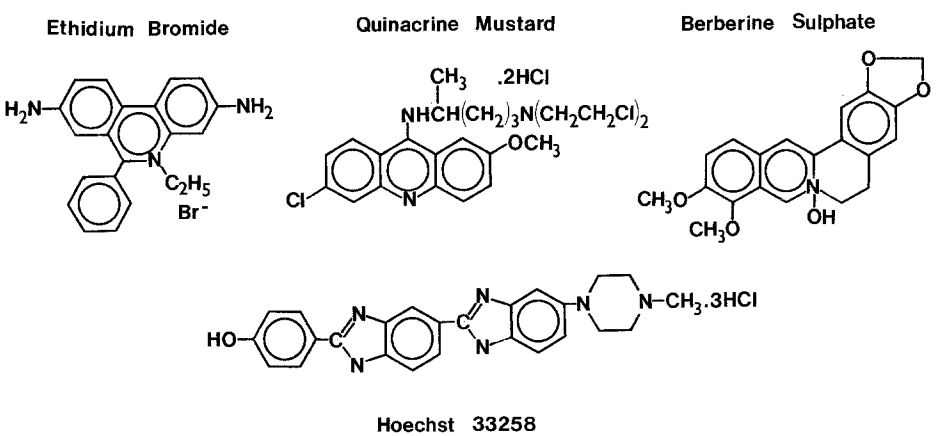

Fig. 1. Chemical structure of the fluorochromes

Method

At any instant in time the absorption of light and its remission as fluorescence at longer wavelengths are processes associated with electronic transition dipole moments that have specific directions within a fluorochrome. Plane polarised incident light is strongly absorbed and hence the fluorescence greatest when the fluorochrome is oriented so that its absorption moment is parallel to the incoming light polarisation direction. The emitted fluorescence is plane polarised parallel to the emission transition moment. Hence, for incident light of well defined polarisation state, a measurement of the intensity and direction of polarisation of fluorescence from a molecule of known orientation containing an active fluorochrome can lead to the determination of the orientation of the absorption and emission transition moments and hence to the specific orientation of the fluorochrome within the molecule. In dilute solution, the many composite DNA molecules adopt a random orientational array so that, even if added fluorescent dye molecules bind to the DNA with a highly specific geometry, the fluorochromes are randomly distributed and oriented throughout the medium. In the presence of a suitable electric field, DNA molecules align parallel to the field direction as an induced electric dipole moment along the long axis couples with the applied field (Fredericq and Houssier 1973). Such alignment imposes orientational order on the appended fluorochromes. This is accompanied by changes in both the intensity and polarisation of the fluorescence. Practically, electric fields are applied in the form of short duration pulses of sufficient duration to allow DNA orientation. Changes are recorded in both the vertically (V_v) and horizontally (V_h) polarised components of the fluorescence for vertically polarised incident light. The changes are transient in nature owing to the finite rotary relaxation time of the nucleic acid molecules. From the steady-state changes in the polarised components, designated ΔV_v and ΔV_h, the average spatial directions of the absorption and emission transition dipoles can be determined with respect to the induced dipolar electric axis of the DNA. As these transition dipoles generally lie in the plane of these ring compounds, the fluorochrome disposition relative to the DNA axis is estimated.

Apparatus

An argon-ion laser with several selectable optical lines in the blue and ultra-violet region of the spectrum acts as the light source. After suitable wavelength selection and beam attenuation, the light is rigorously polarised in the vertical direction prior to falling on the sample cell. This is a rectangular glass structure which holds some 0.5 ml of solution between a pair of horizontal, parallel, stainless steel electrodes. Application of a potential difference between these electrodes generates a vertical electric field. The two polarised components of the fluorescence are recorded simultaneously by means of two optical detection limbs laterally placed to the cell and at 90° in the horizontal plane to the incident beam direction. Each limb has an optical cut-off filter, a

high precision polariser and a fast recording photomultiplier for the detection of the rapid transient changes in the fluorescence components V_v and V_h. Application of the pulse results in gradual changes in these components up to equilibrium conditions $(V_v + \Delta V_v)$ and $(V_h + \Delta V_h)$. Termination of the pulse is accompanied by a field-free decay back to the pre-field values as the solute molecules relax back to a random array. Fields of up to 25 kV · cm^{-1} are applied for durations of up to 400 μs. The fast, transient photodetected responses are fed to a transient digitiser, and either displayed on an oscilloscope screen and photographed or fed directly to a data analysing system from which ΔV_v, ΔV_h are obtained. From these experimental parameters, the average azimuthal angles ψ and ψ' of the absorption and emission transition moments relative to the DNA orientation axis are evaluated directly.

Results

For all fluorochrome-modified-DNA complexes, regular transient responses were observed (Figs. 2 and 3). The existence and magnitude of these effects indicates that, in each system, fluorochrome binding to the DNA is highly regular. Random binding would have given no transient response. Relative values of ΔV_v and ΔV_h under high field conditions, where they were found to be invariant with field strength, were deduced as shown in Table 1, together with calculated values of ψ and ψ'.

I. Ethidium Bromide

This chromosome stain (Hecht et al. 1974) has long been known to react specifically with DNA by intercalation between the base pairs (Le Pecq and Paoletti 1967). For the vertically polarised incident light of 488 nm wavelength and the vertically directed electric field used herein, the magnitudes of the field-induced decreases in both ΔV_v and ΔV_h (Fig. 2) are compatible with the plane of the phenanthridinium ring of the ethidium structure binding approximately at 70° to the orientation axis and thus being characteristic of an intercalating compound. These data have been discussed elsewhere (Jennings and Ridler 1977). They are consistent with results from related electric dichroism experiments (Hogan et al. 1979) and indicate the suitability of the experimental method for these studies.

II. Berberine Sulphate

This is an alkaloid derived from the plant *berberis aristata* which has chemotherapeutic properties. The compound produces fluorescent bands in chromosomes which are used for their identification (Moutschen et al. 1973). It reacts readily with DNA, although the mechanism of action and the sites of binding are as yet unclear (Moutschen 1976). The fluorescence from the complex

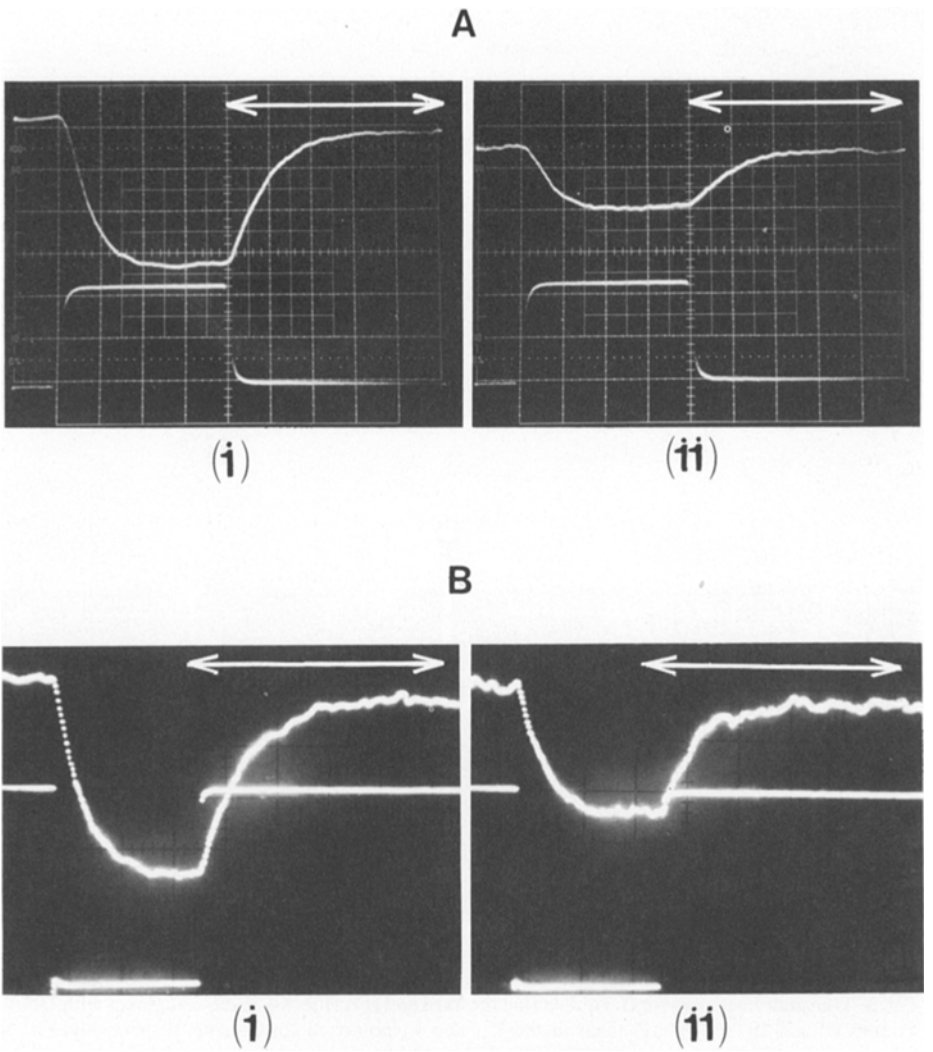

Fig. 2. Transient responses for (A) ethidium bromide, and (B) berberine sulphate complexes with DNA. Frames (i) and (ii) refer to the ΔV_v and ΔV_h polarised components respectively. The rectangular trace indicates the applied field pulse of 4 and 20 kV · cm^{-1} for A and B respectively. Time runs from left to right with the arrow indicating 0.5 ms

was found to be greatly enhanced compared with that from the free dye. Two electrofluorescence studies were conducted, one using near u.v. and the other blue visible light excitation. The results were identical. Table 1 given data for 457 nm wavelength exciting light and fluorescence in excess of 515 nm. A direct comparison with the results from both Fig. 2 and Table 1 for the ethidium bromide complex shows the similarity of berberine sulphate binding with DNA.

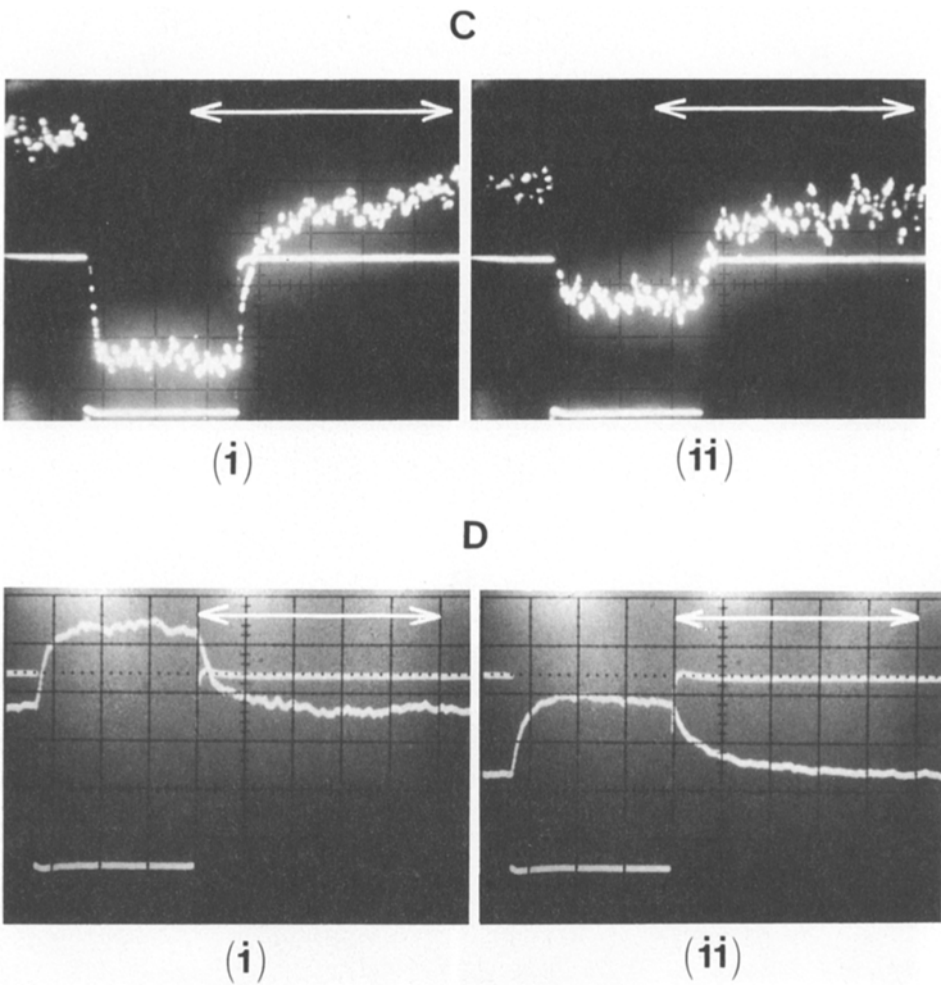

Fig. 3. Transient responses for (C) quinacrine mustard and (D) Hoechst 33258 complexes with DNA. Frames (i) and (ii) indicate changes in the V_v and V_h polarised components respectively. Other information as in Fig. 2 save that E = 20 kV · cm^{-1} in each case

Table 1. High field induced relative changes in the polarised components of fluorescence

	$\frac{\Delta V_v}{V_v}$	$\frac{\Delta V_h}{V_h}$	$\frac{V_v}{V_h}$	ψ	ψ'
Ethidium bromide/DNA	− 0.71	− 0.42	1.92	67 ± 3°	62 ± 8°
Berberine sulphate/DNA	− 0.63	− 0.35	2.08	66 ± 3°	61 ± 10°
Quinacrine mustard/DNA	− 0.63	− 0.38	1.90	66 ± 3°	62 ± 8°
Hoechst 33258/DNA	+ 0.40	+ 0.25	2.30	48 ± 2°	46 ± 6°

All data are for E = 20 kV · cm^{-1}. ψ and ψ' are the directions of the absorption and emission transition moments respectively to the DNA orientation axis

III. Quinacrine Mustard

The quinacrine mustard compound produces highly specific fluorescent banding patterns particularly in human chromosomes (Caspersson et al. 1971). These are of utility both for chromosome identification, and for the recognition of chromosome abnormalities and hence the diagnosis of hereditary disorders (Caspersson and Zech 1973). In 1969, Caspersson et al. suggested that quinacrine mustard interacts with DNA in a manner similar to that proposed for the amino acridines, namely, by helix intercalation. Furthermore, they invoked the existence of covalent bonding to the N_7 site of guanine. However, Weisblum and de Haseth (1972) have shown that fluorescence is enhanced by *A-T* rich DNA.

The electrofluorescence data are recorded in Fig. 3 and the Table 1 for the same conditions of optical exciatation as were used for the berberine sulphate. The normalised changes in each of the fluorescent components are again seen to be remarkably similar to those found for berberine and ethidium.

IV. Hoechst 33258

This is a bi-benzimidazole derivative 2-[2-(4-hydroxyphenyl)-6-benzimidazole]-6-(1-methyl-4-piperazyl)-benzimidazole-trihydrochloride. It is a new fluorescent probe which produces curious banding patterns and is increasingly being adopted both in chromosome identification and as a marker in time dependent studies of DNA replication (Latt 1973). Studies have shown that the fluorescence is enhanced on binding to double stranded DNA and that the enhancement is greater for *A-T* than for *G-C* rich regions (Weisblum and Haenssler 1974; Comings 1975). Unlike the quinacrine mustard compound, Hoechst 33258 fluoresces brightly in C band heterochromatin (Hilwig and Gropp 1972).

Figure 3 shows for near UV excitation transient fluorescence responses completely different to those of the aforementioned compounds. From the data of Table 1 its is seen that the angles ψ and ψ' differ markedly from those hitherto discussed. We conclude therefore that the fluorophore does *not* intercalate the DNA helix, but binds with its transition moments and long axis at $48° \pm 4°$ to the axis of the DNA. This is very close to the projection angle of the DNA grooves. Although the present method cannot differentiate between association with the major or minor groove, we note with interest the suggestion by Comings (1975) that Hoechst 33258 has an association with the base pairs in the major groove. A more complicated interaction cannot however be ruled out. The sheer length of the molecule may mean that part of it is intercalated, with part of it, the fluorophore, binding on the outside of the DNA.

Conclusions

Like ethidium bromide, the two fluorescent chromosomal stains berberine sulphate and quinacrine mustard dihydrochloride both intercalate the DNA helix according to current models for this type of binding (Hogan et al. 1979). The probe Hoechst 33258 appears to adopt a different binding characteristic more closely associated with the helical grooves. All three systems are important current fluorescent probes used for chromosomal identification, so that knowledge of their binding geometry with DNA should help our understanding of the nature of their interactions with chromosomes. Finally, the method is fast, of high sensitivity and is applicable to low dye-to-phosphorus ratios. It should find increasing application in this area of research.

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