ELECTRICALLY INDUCED FLUORESCENCE AS A METHOD FOR STUDYING BENZO[a]PYRENE BINDING TO DNA

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1. Introduction

The interaction of benzo[a]pyrene (BP) with DNA has been extensively studied as it is a known chemical carcinogen. Although the native hydrocarbon binds to DNA, current interest [1] centres on the *trans*-7,8-dihydroxy-anti-9,10 epoxy-7,8,9,10-tetrahydrobenzo-[a]pyrene derivatives (BPDE). Of these, the (+) enantiomer shown in fig.1: (a) is the active metabolite which binds most extensively to DNA in living cells [2]; (b) binds covalently to DNA [3]; and (c) is far the most potent carcinogenic form [4].

The majority of in vivo covalent binding is between the N-2 of guanine and the 10 carbon atom position of the (+) conformer of BPDE [5]. Synthesised racemic BPDE, reacted with native DNA, gives predominantly the same adduct [6] but with a greater degree of binding, thereby making it more convenient for in vitro experimentation. Although the sites of interaction of BPDE with DNA are known, there is much current dispute as to the binding configuration of the covalent moiety. At least 4 different models exist. The first 2 are covalent intercalations [7,8] of the hydrocarbon into the DNA with or without extensive bending of the helix. The third is the base displacement model [9] in which the diol-epoxide chromophore displaces guanine bases from the DNA which then swing into the helical groove. The fourth is one in which diol epoxide binds externally to the DNA helix [10]. Each hypothesis implies a very different disposition of the hydrocarbon with respect to the DNA structure. A co-existence of models is also suspected [11].

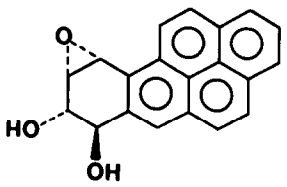

Fig.1. The (+) enantiomer of BPDE.

We have developed a new physical method for studying the binding geometry of fluorescent groups (fluorophores) to macromolecules [12]. The method is extremely sensitive to the presence of small amounts of bound dye and so is particularly relevant for the study of dye molecules on nucleic acids [13]. BP and its diol-epoxides are highly fluorescent when excited with near ultraviolet radiation. Here, we report studies on BPDE, native BP and proflavine, when independently bound to DNA. The last was included for comparative purposes as it is a known intercalating agent [14].

2. Experimental

The principle of the method: The absorption of light and its re-emission as fluorescence at longer wavelengths are processes associated with electronic transition moments that have fixed directions within the framework of any individual fluorescent molecule. Both the intensity and azimuth of polarisation of the fluorescence depend upon the orientations of these transition moments and the polarisation direction of the incident light beam. In dilute solution DNA molecules adopt a random orientational array. Alignment can be imposed by subjecting the solution to an external force field such as an electric field. Should the fluorophores have a high degree of directional order on the DNA, then alignment of the DNA molecules is accompanied by correspondingly increased order in

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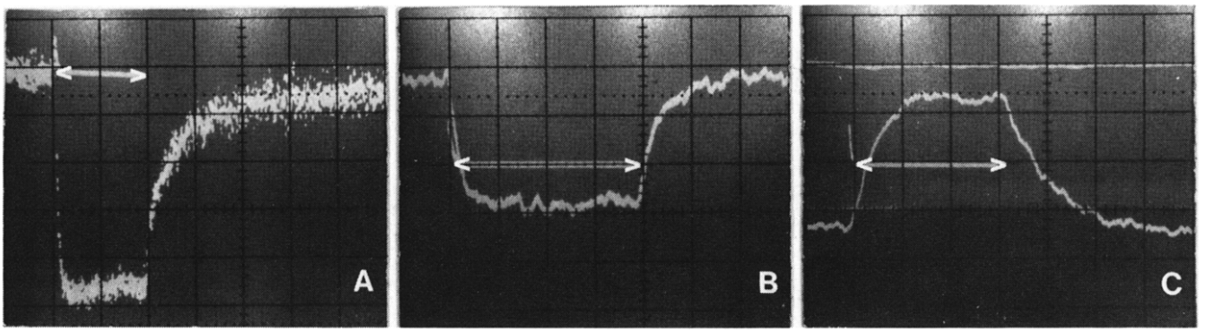

Fig.2. Transient change in fluorescence component V_v for (a) proflavine/DNA complex, (b) native BP/DNA complex, and (c) BPDE/DNA complex. E = 13 kV/cm and time scales are 200 μs/division. The pulse duration is shown as the horizontal arrow. Proflavine was excited by 458 nm and dected for $\lambda > 515$ nm. BP and BPDE were excited by a combination of 351 and 364 nm and detected for $\lambda > 400$ nm.

the fluorophore array. For a given direction of incident light polarisation, the application of the electric field is accompanied by changes in the intensity and polarisation of the fluorescent emission. By measuring these electrically induced changes the average orientation of the fluorophores relative to the major DNA axis may be estimated.

A full description of the apparatus and its manipulative procedures is in [14]. A few ml of the solution is held in a glass cell and a pulsed electric field applied vertically. An argon-ion laser beam is vertically polarised and incident on the sample. The fluorescence at 90°C is analysed into 2 polarised components, designated by V_v and V_h, where the capital letter indicates the direction of polarisation of the incident light and the subscripts those of the emitted light. As the DNA is oriented in response to the short duration, rectangular electric-pulsed field, transient changes are recorded in V_v and V_h. The fluorescence components build up to a steady value in the field and decay to their pre-field value once the external field is removed. The amplitudes of the changes ΔV_v and ΔV_h are recorded and used directly to calculate the DNA/adduct binding geometry.

Racemic BPDE, reacted with calf thymus DNA in vitro, was provided by Dr M. R. Osborne (Pollard's Wood Research Station, Royal Cancer Hospital). The preparation was that of [6] with the addition that BPDE/DNA solution was passed through a 25 cm Sephadex LH20 column. In addition repeated extraction from the aqueous phase with ether was performed. These two procedures ensured that unreacted and non-covalently bound BPDE or BP tetraols were absent. The resulting material had a composition of ~1 BPDE molecule/100 DNA basepairs. This was dissolved in freshly distilled, deionised water to give working solutions with 10 mg reacted DNA in 100 ml water at 23°C. The native BP was a commercial sample. This was gradually stirred into an aqueous DNA solution for a 24 h. The non-dispersed material was removed by centrifugation. The supernatant was then diluted to the required concentration. The commercial proflavine sample was dissolved in water and added to a DNA solution as in [13].

3. Results and discussion

For all 3 systems, regular transient responses were recorded for the amplitude changes ΔV_v and ΔV_h. Representative transients are shown in fig.2. In all cases these effects tend to constant values at high fields (fig.3). Fig.2 and table 1 contain representative data under this high field condition.

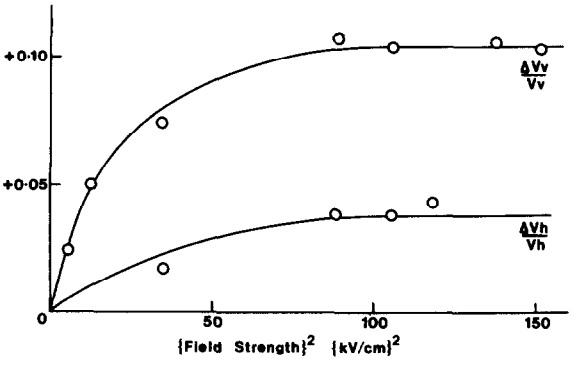

Fig.3. Field strength dependence of the polarised component changes for BPDE/DNA complex.

Table 1
Relative changes in polarised components of fluorescence

	$\Delta V_v/V_v$	$\Delta V_h/V_h$	V_v/V_h
Proflavine/DNA	−0.71	−0.46	2.2
Native BP/DNA	−0.22	−0.14	1.4
BPDE/DNA	+0.10	+0.04	1.3

Proflavine is well known to intercalate DNA [15]. The data recorded herein support this. In a vertical applied field, the DNA molecules orient with their long axes towards the vertical [16]. This is accompanied by reductions in both V_v and V_h (see table1), consistent with the absorption and emission transition moments being predominantly in the horizontal plane [12]. With planar ring compounds, both the absorption and emission moments are generally constrained to the plane of the rings. Hence the proflavine plane associates predominantly perpendicular to the DNA axis.

Fig.2 and table 1 indicate that the native BP interacts with the DNA helix in essentially the same way as proflavine. True intercalation or external binding with the plane of the BP heterocycle approximately perpendicular to the DNA long axis is indicated.

A different situation exists for the BPDE/DNA complex. All polarised component changes are of opposite sign to those for the intercalating system (see fig.2 and table 1). The essentially different nature of binding is evident immediately. The actual directions of the absorption and emission moments, assumed to be at angles ψ and ψ' to the long DNA axis respectively, can be specifically evaluated [17]. Using the data at high electric field strength, the following were obtained:

$$\psi = 52 \pm 3^\circ; \quad \psi' = 45 \pm 6^\circ$$

It is evident from the foregoing that the pyrene fluorophore of BPDE does not intercalate the DNA helix, at least in the way that proflavine or BP do. In principle, knowledge of the 2 transition moments should enable one to define the plane of the heterocycle in space. Assuming that both moments are parallel and lie within the pyrene plane and along its long axis, then the present data indicate that the BPDE molecule is oriented with its axis at ~50° to the core of the DNA helix. Finally it is known that the N-2 guanine site associates with the carbon 10 position of the BPDE. It has been instructive to note that by using space-filling models and associating these relevant sites, the BPDE rings are able to fit readily into the minor groove [18] of the double helix with the approximate inclination indicated above.

Although not excluding the presence of other types of binding geometry, our studies can be interpreted in terms of either external binding [10] or the highly kinked DNA intercalation [8].

In conclusion, the electro-fluorescence method appears to be an effective indicator of binding geometry for polycyclic hydrocarbons on nucleic acids. It can be used for low ligand-to-phosphate ratios, is very fast, and of high sensitivity.

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