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COMMUNICATIONS

The binding of the benzimidazole dye H8208 to DNA and to polyinosinic-polycytidylic acid

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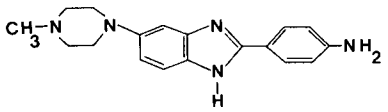
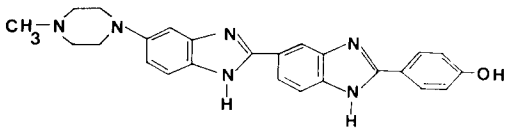
The benzimidazole dye H8208 (2-(4-aminophenyl)-5-(4-methylpiperazin-1-yl)benzimidazole) has been shown to intercalate into calf thymus DNA and into polyinosinic-polycytidylic acid (a model for A conformation DNA) by a variety of spectroscopic techniques. The binding affinity of the dye was found to be similar to both nucleic acids.

The benzimidazole dye H8208 (2-(4-aminophenyl)-5-(4-methylpiperazin-1-yl)benzimidazole, I) has been shown to stain mammalian metaphase chromosomes in-vitro in a comparable manner to another benzimidazole dye H33258 (2-(4-hydroxyphenyl)-5-[5-(4-methylpiperazin-1-yl)benzimidazol-2-yl]benzimidazole, II) (Schempp, personal communication).



H8208 (I)



H33258 (II)

The binding of H33258 to DNA has been investigated by Ridler & Jennings (1980), by a fluorescence polarization technique. They concluded that H33258 does not intercalate into the DNA helix but lies along its major groove. It would appear that the dimeric structure of H33258 is too bulky to bind by intercalation. In the present paper we present the results of an investigation into the binding of the monomeric dye H8208 to calf thymus DNA and to the synthetic nucleic acid polyinosinic-polycytidylic acid [poly(I.C.)].

Calf thymus DNA adopts the nucleic acid B conformation whilst poly(I.C.) adopts the nucleic acid A conformation which is also found in some viral DNAs, and also in double stranded RNA (Plumbridge & Brown 1977). Thus poly(I.C.) may be used to model the interaction between potential intercalating ligands and viral DNAs or double stranded RNA. The B conformation has been the most widely investigated (Arnott et al 1969). The A conformation possesses the same gross structure as the B conformation, the variations being in the dimensions. In particular, variations occur in the angles subtended by the base planes with respect to the helix axis and to each other. In B conformation DNA the bases are almost horizontal having a tilt of only $-2°$ and they are very slightly twisted with respect to each other, this angle being about 5°. Thus planar molecules can readily intercalate between adjacent base pairs on the B-DNA helix (Lerman 1961). Nucleic acids with the A conformation however, have a much greater angle of tilt by the bases, the value being of the order of 20° and the angle of twist being $-8°$ (Arnott et al 1969). There is therefore likely to be a far greater steric hindrance to intercalation into the A conformation helix than into the B conformation helix.

Materials and methods

The dye H8208 was used as the trihydrochloride salt and was a gift from Dr H. Loewe, Hoechst Aktiengesellschaft (Frankfurt). Solutions for spectrophotometric titration and for fluorescence polarization measurements were prepared in 0·008 M Tris HCl-0·05 M NaCl buffer, pH 7·0, and solutions for 'melting' temperature determination in 0·003 M Tris HCl-0·018 M NaCl buffer, pH 7·0, care being taken to avoid undue exposure of dye solutions to light. Solutions of DNA were prepared by dissolving calf thymus DNA (Sigma type 1) in buffer to give a solution of approximately 1 mg mL^{-1} and were assayed using the value $\varepsilon(p)_{260} = 6{\cdot}6 \times 10^{-3}$. Solutions

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of poly(I.C.) (Sigma) were prepared to a similar concentration and assayed using the value $\varepsilon(p)_{260} = 4{\cdot}85 \times 10^{-3}$ (Plumbridge & Brown 1977). Poly(I.C.) is known to exist in the A conformation at the ionic strength of the buffers used in this study (Arnott et al 1973).

The binding of H8208 to the nucleic acids was measured by determination of isosbestic points, spectrophotometric titration (Bennett et al 1982), fluorescence polarization (Plumbridge & Brown 1977) and effect on 'melting' temperature (Zuino et al 1972), all of which are well documented methods of investigating the binding of organic ligands to nucleic acids. The binding parameters, K_a, the association constant, and n, the number of binding sites/mole nucleic acid, were estimated by means of a Scatchard plot (Scatchard 1949). From the spectrophotometric titration figures, 30 data points for r (number of moles ligand bound/mole nucleic acid) and r/c, where c = molar concentration of unbound ligand, could be obtained. These were calculated from the equations,

$$\alpha = \frac{\varepsilon_{unbound} - \varepsilon_{observed}}{\varepsilon_{unbound} - \varepsilon_{bound}} \quad \text{and} \quad r = \alpha C_t$$

where α = fraction of ligand bound, and C_t = molar concentration of ligand, using a simple computer program (Double & Brown 1975). The association constant, (K_a) and the number of binding sites/mole nucleic acid (n) were computed by linear regression analysis of the 12 data points on the intercalative section of the Scatchard plot. Spectrophotometric measurements were performed on a Perkin-Elmer SP 1750 UV spectrophotometer fitted with an SP876 Series 2 temperature programmer and spectrofluorimetric measurements on a Perkin-Elmer MPF-3 spectrofluorimeter.

Results and discussion

Determination of isosbestic points. Fig. 1 shows the effect of increasing nucleic acid ratios on the UV absorption spectra of a known concentration ($2{\cdot}5 \times 10^{-5}$ M) of H8208. Both DNA and poly(I.C.) produce a bathochromic shift of ~50 nm (330 → 376 nm) with an isosbestic point at 350 nm indicative of intercalation of H8208 into both nucleic acids.

Spectrophotometric titration. A similar decrease in extinction (75%) was observed on titrating H8208 with both DNA and poly(I.C.) Scatchard analysis of the extinction data obtained from spectrophotometric titration of H8208 ($2{\cdot}75 \times 10^{-5}$ M) against DNA ($2{\cdot}5 \times 10^{-3}$ M) and poly(I.C.) ($2{\cdot}19 \times 10^{-3}$ M) gave typical bilinear plots which can be interpreted as representing a strong intercalative mode of binding and a weaker external binding (Blake & Peacocke 1968). The binding constants, K_a the association constant and n, the number of binding sites/mole nucleic acid, for the intercalation of H8208 into both DNA and poly(I.C.) are presented in Table 1.

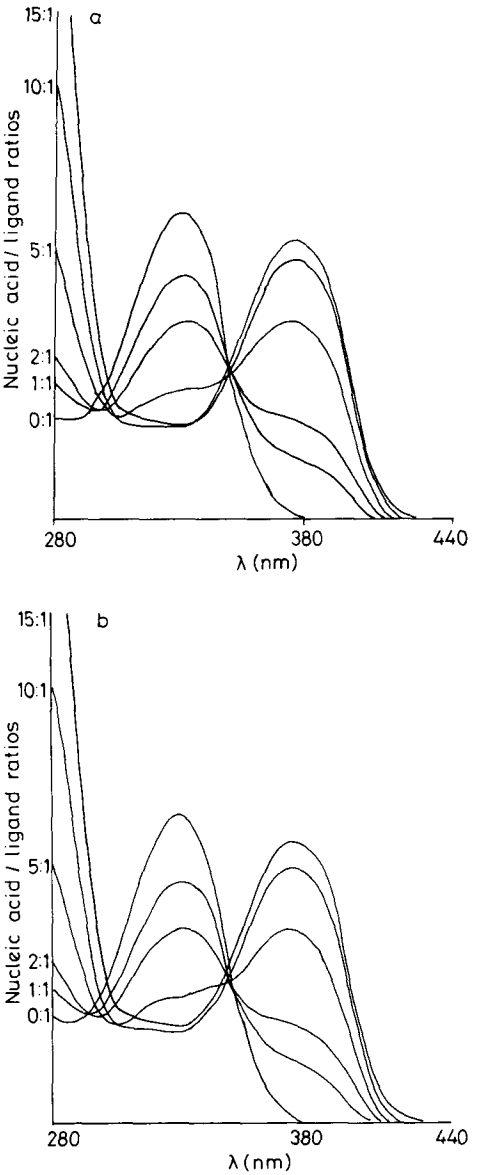

FIG. 1. UV absorption curves for H8208 ($2{\cdot}5 \times 10^{-5}$ M) in the presence of increasing ratios of (a) DNA and (b) poly(I.C.).

Fluorescence polarization measurements. The polarization of the fluorescence of H8208 (1×10^{-7} M) was measured in the presence of increasing ratios of both DNA and poly(I.C.). The results are presented in Table 2, and show a quenching of the fluorescence and a rise in fluorescence polarization of the ligand/nucleic acid complex with increasing nucleic acid with both DNA and poly(I.C.) indicating intercalation of H8208 into both DNA and poly(I.C.).

Table 1. Binding parameters for the binding of H8208 to DNA and poly(I.C.).

Nucleic acid	Decrease in extinction (%)	[a] $k_a \times 10^{-5} m^{-1}$	[a] n	Melting temp. (T_m)	[b] ΔT_m
DNA	75%	16·45[c]	0·22	79·3 °C	6·5 °C
Poly(I.C.)	75%	13·07[c]	0·23	57·5 °C	8·75 °C

[a] k_a and n are the association constant and the number of binding sites per mole of nucleic acid, respectively.
[b] $\Delta T_m = TM_{nucleic\ acid\ +\ ligand} - Tm_{nucleic\ acid\ alone}$
T_m DNA = 72·8 °C; T_m poly(I.C.) = 49·75 °C.
[c] A mean of three determinations.

Effect on 'melting' temperature. The effect on the 'melting' temperatures of DNA and poly(I.C.) of H8208 was determined. For DNA measurements the temperature was raised from 50–100 °C at a rate of 0·25 °C min^{-1} and for poly(I.C.) measurements from 35–70 °C at a rate of 0·25 °C min^{-1}. Blank determinations were carried out for each nucleic acid and the results are presented in Table 1. The T_m is the temperature at which a 50% change in the absorbance of the nucleic acid occurs. An increase in the T_m of both nucleic acids was observed in the presence of H8208 which is indicative of intercalative binding.

Table 2. Fluorescence polarization of H8208 and proflavine at fixed ratios of nucleic acid.

Nucleic acid/ ligand ratio	% Quenching of fluorescence (DNA)	P_{DNA}	% Quenching of fluorescence poly(I.C.)	$P_{poly(I.C.)}$
H8208				
P	100	0·02	100	0·00
10	73	0·05	67	0·02
20	67	0·07	51	0·03
50	54	0·14	32	0·05
100	50	0 21	20	0·09
150	45	0·21	15	0·09
Proflavine				
0	—	0·00	—	0·00
10	—	0·12	—	0·01
20	—	0·25	—	0·01
50	—	0·31	—	0·04
100	—	0·32	—	0·06
150	—	0·32	—	0·08

Discussion

It has thus been shown that the benzimidazole dye H8208 intercalates into both the A and B conformations of nucleic acids and that the interaction is qualitatively and quantitatively similar for both nucleic acid conformations. This can be seen in the similarities in bathochromic shifts of H8208 produced by both DNA and poly(I.C.) (Fig. 1), and the essentially equal binding affinities and melting temperature elevations for the interaction of H8208 with both DNA and poly(I.C.) recorded in Table 1. The fluorescence of H8208 is also quenched by similar amounts by both DNA and poly(I.C.) and although the numerical values for fluorescence polarization of H8208 are much lower for poly(I.C.) than for DNA (Table 2), a similar picture is observed with proflavine, a compound known to intercalate into the A conformation of DNA (Alden & Arnott 1977).

The 2-(4-aminophenyl)benzimidazoyl grouping on H8208 is essentially planar and is sufficiently unhindered to intercalate between the base pairs of the helix of both A and B conformation nucleic acids, with the more bulky methylpiperazinyl ring system which would be protonated at pH 7·0 (pK_a 10·08), binding electrostatically to the ribose phosphate backbone.

H8208 is too toxic for clinical use (Loewe, personal communication) but is of interest as a compound which can intercalate with equal effectiveness into both A and B conformations of nucleic aids unlike classical intercalating compounds such as ethidium, which intercalates only very weakly into A conformation nucleic acids and daunomycin and mepacrine which do not intercalate at all into A conformation nucleic acids (Plumbridge & Brown 1977). It may therefore serve as a new lead molecule in the search for effective antitumour or antiviral drugs.

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