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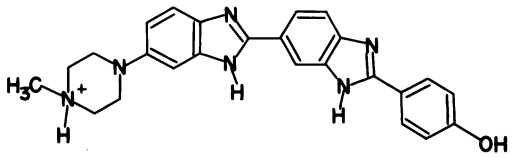
The Benzimidazole Dye Hoechst 8208 Intercalates into DNA

Yukio KUBOTA* and Hiroyuki NAKAMURA
Department of Chemistry, Faculty of Science, Yamaguchi University,
Yoshida, Yamaguchi 753

The interaction of the benzimidazole dye Hoechst 8208 (H8208) with DNA has been studied by optical, hydrodynamic, and ^{1}H NMR techniques. All of the results clearly indicate that H8208 intercalates between successive DNA base pairs.

The bi-1*H*-benzimidazole dye Hoechst 33258 (H33258; Fig. 1) has widely been used as a fluorescent cytological stain for DNA. Recent X-ray crystallographic studies of the complex of H33258 with B-DNA dodecamers have shown that the dye binds in the minor groove of the B-DNA double helix.[1] On the other hand, spectroscopic and hydrodynamic studies of DNA complexed with H33258 have suggested that the binding nature might be rather complicated in aqueous solutions.[2,3] In order to obtain further information, we have investigated the physical properties of the binding of the benzimidazole dye Hoechst 8208 (H8208; Fig. 1) to DNA by absorption, fluorescence, flow dichroism, circular dichroism (CD), viscosity, and ^{1}H NMR measurements. It is interesting to compare the results of H8208 having one benzimidazole ring with those of H33258 which contains two benzimidazole rings. We present here unambiguous evidence supporting that H8208, as well as intercalators such as acridine dyes, intercalates between successive DNA base pairs.



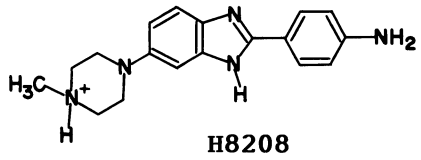


Fig. 1. Chemical structures of H33258 and H8208.

H8208 was a gift from Dr. H. Loewe (Kelkheim) and Dr. M. Schorr (Hoechst Aktiengesellschaft, Frankhurt am Main). The following DNAs and synthetic polynucleotides were commercial products: *Clostridium perfringens* DNA (CP DNA; Sigma), calf thymus DNA (CT DNA; Sigma), *Micrococcus lysodeikticus* DNA (ML DNA; Sigma), poly(dA-dT)·poly(dA-dT) (Pharmacia), and poly(dG-dC)·poly(dG-dC) (Pharmacia). Unless otherwise stated, all the

measurements were made in 5 mM phosphate buffer (pH 6.9, 25°C) with NaCl added to give the desired ionic strength (1 M=1 mol dm^{-3}). Viscosity and flow dichroism measurements were performed as described elsewhere.[4] ^1H NMR spectra were obtained on a Hitachi R-250H spectrometer in a D_2O buffer (2.5 mM Na_2HPO_4, 2.5 mM $NaHPO_4$, 0.1 M NaCl, pH 6.9) at 62°C.

The interaction of H8208 with DNA results in large spectral shifts and a significant induced CD. The absorption spectrum of the DNA-H8208 system showed a red shift of about 45 nm (λ_{max} of free dye:327 nm) with an isosbestic point at 348 nm. The binding isotherms (Fig. 2) obtained from the absorption changes were well-fitted by the site exclusion model of McGhee-von Hippel.[5] All DNAs studied had similar binding constants (K) with 3 base pairs per binding site, regardless of the GC content of DNA (Table 1). Results of the pH spectrophotometric titration reveal that the pK_a value of the benzimidazole ring of free H8208 is 5.4, whereas 8.1 upon binding to DNA; this means that bound H8208 exists as a dication at pH 6.9. A plot of log K vs. -log [Na^+] (Fig. 2) has a slope of 1.83, suggesting that two ion pairs contribute mainly to the complex formation between H8208 and DNA. This slope and the K values are similar to those observed for the intercalation of the dicationic acridine dye, quinacrine.[6]

There are a narrowing and a blue shift of the fluorescence band of the DNA-H8208 complex. This behavior and a red shift of the absorption band were very similar to those observed when the dye is solubilized into the micelle of sodium dodecyl sulfate. It therefore appears that the bound dye lies in the hydrophobic environment. The fluorescence of H8208 was quenched when bound to DNA, but the fluorescence quantum yield (Φ_F) showed no strong dependence on the base composition (Table 1).

The CD spectral behavior of DNA-H8208 complexes as a function of the molar ratio of DNA phosphate to dye (P/D) was very similar to that obtained with proflavine (PF; 3,6-diaminoacridine), one of typical intercalators.[7] At P/D=2—6, there are a positive CD band at 388 nm and a negative CD band at 350 nm due to exciton interactions between two bound dyes.

The DNA-H8208 complexes showed a negative flow dichroism for both the absorption region of DNA bases (220—280 nm) and the dye absorption band (320—400 nm) (Fig. 3). The reduced dichroism at a perfect orientation was estimated from the dependence of the reduced dichroism on the velocity gradient and found to be -0.60 at 260 nm and -0.74 at 375 nm, respectively. This result suggests that the bound dye, as well as DNA bases, orients more or less perpendicularly to the DNA helix.[4]

The intrinsic viscosity of sonicated CT DNA in the presence of H8208 increased with an increase in the binding ratio r (moles of H8208 bound per mole of DNA base pair) until it reached a plateau due to saturation of

binding sites. This increase in the viscosity (about 50% at r=0.2), which is very similar to that of PF, can be ascribed to the lengthening of the DNA helix through the dye binding.[8]

^{1}H NMR technique was applied to monitor the chemical shifts of aromatic protons of bound H8208. It was found that all proton signals of H8208 shift significantly upfield and broaden upon addition to sonicated CT DNA; this evidence is in strong support of an intercalation binding mode.[9]

Finally, the molecular mechanics calculations[10] on H8208 predicted its minimum energy conformation in which the 2-(4-aminophenyl)-1*H*-benz-

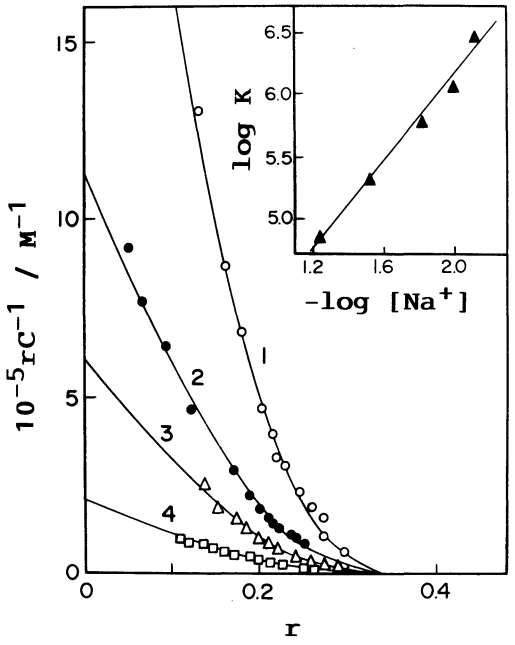


Fig. 2. Binding isotherms for the CT DNA-H8208 interaction in 5 mM phosphate buffer (pH 6.9) at 25°C: The symbol C on the ordinate denotes the concentration of free H8208. [Na^+]: (1) 7.5 mM, (2) 10 mM, (3) 15 mM and (4) 30 mM (data at [Na^+]=57.5 mM are not shown). The lines represent nonlinear least-squares best-fits to the data. The inset shows plots for log K vs. -log [Na^+].

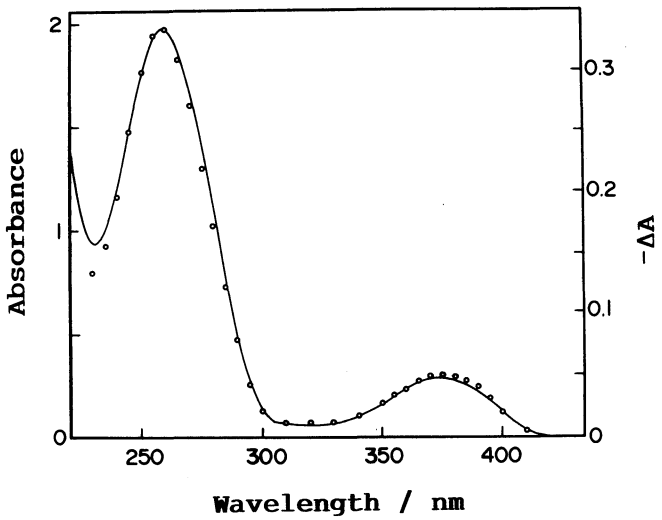


Fig. 3. Absorption (—) and flow dichroism spectra (o) of unsonicated CT DNA complexed with H8208 in 5 mM phosphate buffer. ΔA is defined by $\Delta A=A_{\parallel} - A_{\perp}$, where $A_{\parallel}$ and $A_{\perp}$ are the absorbances of the solutions measured with the polarization vector of the light beam oriented parallel or perpendicularly to the direction of flow. The light-path lengths were 0.2 cm and 0.1 cm, respectively, for absorption and flow dichroism measurements. The dye and DNA phosphate concentrations were 49 μM and 1.49 mM.

Table 1. Binding constants and fluorescence quantum yields[a]

DNA	GC/%	$Kx10^{-6}/M^{-1}$	Φ_F[b]
Poly(dA-dT)·poly(dA-dT)	0	3.06	0.34
CP DNA	30	2.55	0.44
CT DNA	42	2.94	0.39
ML DNA	72	2.69	0.35
Poly(dG-dC)·poly(dG-dC)	100	2.59	0.32

a) The solvent was 5 mM phosphate buffer, pH 6.9, 25°C. b) The Φ_F values were obtained at P/D>200. The Φ_F value of free H8208 was 0.50.

imidazoyl group is almost coplanar. Accordingly, this group can sterically fit between adjacent base pairs at the intercalation site. The bulky and cationic piperazine ring, like that of H33258, would locate in the groove of the DNA helix and associate with the DNA phosphate group by electrostatic interactions.[1)]

Bardsley et al.[11)] have claimed that their spectroscopic data are in harmony with the intercalative binding of H8208 to DNA and poly(I)·poly(C). All of the results presented here clearly demonstrate that H8208 binds to DNA by intercalation with binding strength in the range typical of intercalators.[6)] On the contrary, H33258 complexed with DNA shows a positive flow dichroism for the dye absorption region, only slight increases in viscosity, and no CD bands due to the exciton interactions;[3)] these results are consistent with the groove-binding mode of H33258.[1)] Typical intercalators are fused-ring, planar aromatic molecules. Very recently, Wilson et al.[12)] have found that 4',6-diamidino-2-phenylindole (DAPI), a classical groove-binding molecule, can bind to GC-rich regions of DNA by intercalation. It is interesting that H8208, which is similar in structure to DAPI, also belongs to intercalators with unfused ring systems and that H8208 exhibits no base-sequence specificity upon binding to DNA (Table 1), in contrast with DAPI[12)] or H33258 showing a strong specificity for AT base pairs.[1,2)] Thermodynamic and kinetic studies are in progress to obtain more details on the binding interaction of H8208 with DNA.

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