The interaction of benzimidazole compounds with DNA: Intercalation and groove binding modes

Yukio Kubota*, Takayuki Iwamoto and Toshimasa Seki
Department of Chemistry, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan

ABSTRACT
Benzimidazole compounds (Fig. 1) have been synthesized to study their DNA-binding properties. Results obtained with spectroscopy and viscosity measurements indicate that the binding mode varies from intercalation to groove-binding, depending on the number of benzimidazole rings (conformation and size of compounds).

INTRODUCTION
The bis-benzimidazole drug Hoechst 33258 (H33258; Fig. 1) has been widely used as a fluorescent DNA stain and is well known to bind in the minor-groove of B-DNA, preferentially at AT-rich regions.1 In contrast to groove-binding of H33258, 2-phenylbenzimidazole compounds were found to intercalate between successive DNA base pairs.2 This family of drugs is important as a starting compound for rational drug design and provides a good model system to investigate the molecular basis for DNA sequence recognition. In order to obtain further information, we have synthesized several benzimidazole compounds and studied the interaction of these compounds with DNA using spectroscopic (absorption, CD, and fluorescence) and viscosity measurements.

EXPERIMENTAL
Benzimidazole compounds (1~4; Fig. 1) were synthesized according to the method described in the literature3 and purified by repeated recrystallizations.4 Calf thymus DNA (DNA) was purchased from Sigma. Absorption, CD, and fluorescence spectra were measured with a Hitachi U-3300 spectrophotometer, a Jasco J-720 spectropolarimeter, and a Hitachi MPF spectrophotofluorometer, respectively, as a function of the molar ratio of DNA base to drug (P/D). Flow dichroism and viscosity measurements were carried out as described elsewhere.5 Sonicated DNA (molecular weight of 2.5 x 106) was used for viscosity measurements. All measurements were made in 5 mM phosphate buffer (pH 6.9, 25°C) with NaCl added to give desired ionic strength.

RESULTS AND DISCUSSION
Absorption spectra of 1 and 2 upon binding to DNA showed a red-shift with a clear isosbestic point. Binding isotherms obtained from absorbance titrations were well-fitted by the site exclusion model,6 the binding site sizes were 3 and 4 base-pairs for 1 and 2, respectively, and the binding constants (K) were found to be 1.2 x 104 M-1 and 2.9 x 104 M-1 for 1 and 2, respectively, in 5 mM phosphate buffer containing 0.04 M NaCl. On the other hand, absorption spectra of 3 and 4 bound to DNA showed a progressive increase in absorption intensity and a continued red-shift with increasing P/D until there is no further change at a high P/D value. The intensities of the induced CD of 1 and 2, upon binding to DNA, were weak (Δε=4~15 M-1 cm-1), whereas those of

Fig. 1. Chemical structures of benzimidazole compounds.
Fig. 2. Absorption (—) and flow dichroism (○) spectra of (a) DNA-2 and (b) DNA-3 complexes in 5 mM phosphate buffer. The drug concentration and PD were (6.3-13) × 10⁻⁵ M and 20-30, respectively. ΔΔ is defined by ΔΔ = Δmax - Δmin, where Δmax and Δmin are the absorbances of the solutions measured with the polarization vector of the light beam oriented parallel or perpendicular to the direction of flow.

3 and 4 were strong (ΔΔ>60 M⁻¹ cm⁻¹); all the CD spectra at high PD values were very similar in shape to corresponding absorption spectra. The absorption and CD spectral behavior suggests that 1 and 2 interact with DNA by intercalation, while 3 and 4 by groove binding.

In order to obtain more details on the binding interaction, we measured the flow dichroism, viscosity, and fluorescence energy transfer. The results are summarized in the following. The flow dichroism of all DNA-drug complexes was negative in the absorption region of DNA bases (220-300 nm), while negative for DNA-1 and DNA-2 complexes and positive for DNA-3 and DNA-4 complexes in the drug absorption band (300-450 nm) (Fig. 2). The intrinsic viscosity of sonicated DNA in the presence of 1 or 2 increases with increasing the binding ratio, while that in the presence of 3 or 4 only slightly increases. An intercalation binding mode results in very short base to drug distances. Thus, the use of fluorescence energy transfer experiments gives an insight into the binding mode of drug. The enhancements of relative fluorescence yields due to energy transfer to bound drug from DNA bases were observed for DNA-1 and DNA-2 systems, but not for DNA-3 and DNA-4 systems. These results clearly indicate that 1 and 2 are intercalators, but 3 and 4 are groove-binders.

Semi-empirical (AM1) and ab initio (Gaussian 98) calculations show that the ring systems of 1 and 2 are nearly co-planar, whereas those of H33258, 3 and 4 do not adopt planar conformations. In conclusion, the binding mode of benzimidazole compounds varies from intercalation to groove binding, depending upon the number of benzimidazole rings (conformation and size of compounds).

REFERENCES
4. The compounds 1-4 gave analytical data (C, H, N) in agreement with calculated values and gave spectroscopic data ('H NMR and MS) consistent with the assigned structures. We are grateful to Ube Research Laboratory, Ube Industries, Ltd. for MS and elemental analyses.