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Methyl green

A DNA major-groove binding drug

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Interaction and binding geometries of complexes of Methyl green with poly(dA–dT)$_2$, poly(dA) · poly(dT), and triplex poly(dA) · 2poly(dT) complexes have been studied by linear dichroism. For both of the complexes with double helical DNAs, the z symmetry axis of Methyl green is found to be approximately parallel to the DNA bases while the x symmetry axis lies at 40–44° relative to the local DNA helix axis, in agreement with a groove binding mode. However, in contrast to minor-groove binders (such as DAPI and Hoechst 33258) Methyl green is found to be excluded from binding to the triple helical poly(dA) · 2poly(dT) in which the major groove is filled by the third strand. While most so far studied groove-binding dyes bind in the minor groove of DNA, Methyl green thus appears to be an exception.

Methyl green; Triplex; Major groove binding; DNA; poly(dA) · poly(dT); poly(dA) · 2poly(dT); Linear dichroism

1. INTRODUCTION

DNA binding drugs tend to interact noncovalently with the host molecule through two general binding modes: through intercalation [1] which is stabilized electronically in the helix by π–π stacking and dipole–dipole interactions or minor-groove binding which is stabilized by electrostatic, hydrophobic and hydrogen-bonding interactions. A classical example of the intercalating molecule is ethidium, which is oriented with its planar moiety perpendicular to the helical axis in the intercalation pocket of DNA, and which unwinds and lengthens the helix. Hoechst 33258, distamycin and netropsin are well known examples of DNA minor-groove binding drugs. The structures of such minor-groove binding DNA complexes have in a few cases been characterized to atomic level [2–4]. These ligands generally consist of arc shaped, planar and unfused aromatic hydrocarbons with a positive electrostatic potential that attracts them to the electro-negative potential of the minor groove, the arc matching the curvature of the DNA double helix. The N2 amino group of guanine generally blocks binding of these drugs by steric hindrance but also by widening the minor groove, thereby decreasing the depth of the electro-negative potential. For these reasons, most minor groove binding drugs are AT-specific. There are few cases of proven major-groove binding. Tris-chelate metal complexes bound to DNA have been proposed, mainly from a steric argument, to bind preferentially in the major-groove [5,6], but recent NMR data indicate that they too are binding (non-intercalatively) in the minor groove [7]. There has been an attempt to explain a difference in spermine complexation to poly(dA–dT)$_2$ and poly(dG–dC)$_2$ in terms of a major-groove binding mode in the latter polynucleotide [8]. Some 15 days ago a major-groove binding geometry was proposed for the Methyl green–DNA complex on the basis of linear and circular dichroism results and structural considerations [9,10]. Nevertheless, solid evidence that there are any major-groove binding drugs have not been presented up to date.

In the triple helical form of poly(dA) · 2poly(dT), known from X-ray fiber diffraction studies [11], the second poly(dT) strand runs parallel to a B-form-like duplex of poly(dA) · poly(dT) along the major groove and bound via Hoogsteen base pairing. Taking advantage of the fact that the third strand fills the major groove of the double helix, we present the first strong indication for a major-groove binding mode: as an exception from minor groove binding ligands, the dye Methyl green (MG) is excluded from binding to triplex DNA.

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2. MATERIALS AND METHODS

The polynucleotides, purchased from Pharmacia, were dissolved in a buffer containing 100 mM NaCl, 5 mM cacodylate and 1 mM EDTA, pH 7.0, and dialyzed several times against 20 mM NaCl, 5 mM cacodylate buffer, pH 5.0 at 4°C. The triplex poly(dA) · 2(dT) was prepared by incubating the equimolar poly(dA)(dT)–poly(dT) mixture at 90°C for 30 min, followed by overnight cooling at room temperature in 2 mM $MgCl_2$, 20 mM NaCl, 5 mM cacodylate buffer, pH 5.0. The formation of the triplex is confirmed by its characteristic circular

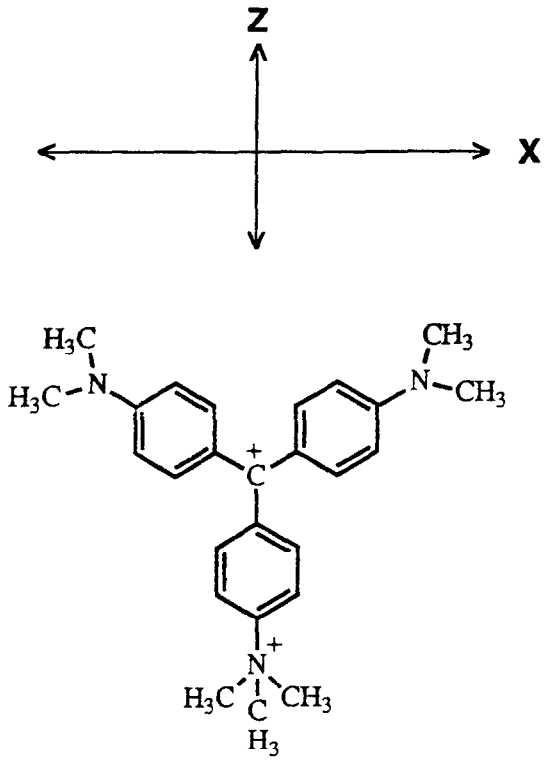

Fig. 1. Molecular structure of Methyl green with its x and z pseudo-symmetry axes.

dichroism spectrum (CD). Methyl green perchlorate was obtained and purified as described by Bengtsson [12]. The CD and flow linear dichroism spectra (LD) were measured on a Jasco J-720 and J-500A spectropolarimeter, respectively, as described elsewhere [13]. Concentrations were determined spectrophotometrically using the molar extinction coefficients: ε_{262} = 6600 $M^{-1} \cdot cm^{-1}$, ε_{260} = 6000 $M^{-1} \cdot cm^{-1}$, ε_{264} = 8520 $M^{-1} \cdot cm^{-1}$ and ε_{638} = 85,300 $M^{-1} \cdot cm^{-1}$, respectively, for poly(dA–dT)$_2$, poly(dA)(dT), poly(dT) and Methyl green.

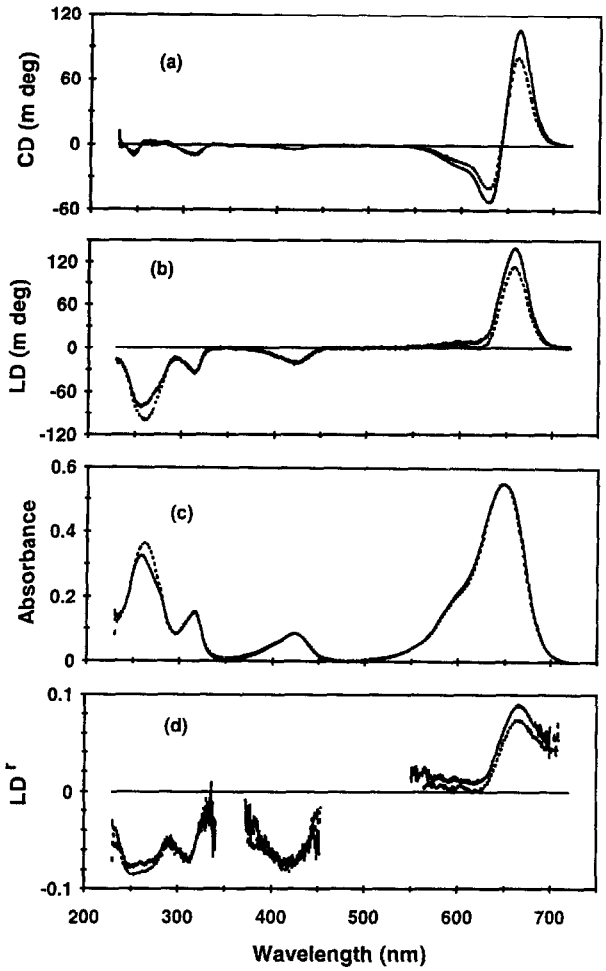

Fig. 2. CD (a), LD (b), absorption (c) and LD^r (d) spectra for the Methyl green–poly(dA–dT)$_2$ complex (dotted curves) and the Methyl green–poly(dA) · poly(dT) complex (solid curves). Concentrations are 50 μM (base pairs) polynucleotide and 5 μM Methyl green. Optical path length 1 cm for CD and absorption spectra and 1 mm for LD. Shear gradient for LD is 3000 s^{-1}.

3. RESULTS AND DISCUSSION

Triphenyl methane dyes are non-planar by sterical hindrance [14]. However, MG (Fig. 1) can be considered as having a pseuco-C_{2v} symmetry with its absorption intensity polarized along the orthogonal x (absorption band ca. 630 nm) and z (absorption band ca. 420 nm) axes [15]. Poly(dA) · poly(dT) has been reported to adopt various conformations in solution [16,17]. To determine whether the conformation of bound MG to poly(dA) · poly(dT) is the same as that in the B-form DNA, the spectroscopic properties of the MG-poly(dA) · poly(dT) and MG–poly(dA–dT)$_2$ complex ([MG]/[DNA] = r = 0.10) may be compared (Fig. 2). Both linear and circular dichroism (CD and LD) spectroscopic properties for the two complexes agree closely with the corresponding spectra of the MG–DNA complex [9,10] at r = 0.23, indicating very similar DNA conformations and binding geometries. The complexes all exhibit an excitonic CD spectrum around 600–700 nm wavelength region due to dye–dye interaction. LD, defined as the difference in the absorption spectra measured with the polarization vector of the incident light beam oriented parallel and perpendicular relative to the shear flow direction, is positive in the 550–700 nm region and negative around 400–450 nm for both complexes. The CD and LD signals for the MG–poly(dA–dT)$_2$ complex is somewhat lower than those for the MG–poly(dA) · poly(dT) complex in these regions. The absorption spectra are the same. Reduced linear dichroism spectrum (LD^r) is defined as:

$$\mathrm{LD}^r(\lambda) = \frac{\mathrm{LD}(\lambda)}{A_{iso}} = 3S\frac{(<3\cos^2\alpha> - 1)}{2} \quad (1)$$

where A_{iso} is the absorption spectrum of the sample at rest, α is the orientation angle between a transition moment of the bound ligand and the local DNA helix axis, and S is the orientation factor which reflects the degree

of the orientation [13]. Assuming an angle of 86° for the DNA base plane relative to the flow direction, the angle of z axes of the MG are calculated to be 79 ± 2° and 82 ± 3°, respectively for poly(dA–dT) and poly(dA)· poly(dT) complex relative to the flow direction. Corresponding angles for the x axes are 44° and 40°, respectively. These values are in good agreement with the angles reported for the MG–DNA complex [9,10]. Therefore, MG is likely to sit in a similar mode and environment in DNA, poly(dA–dT)$_2$ and poly(dA) · poly(dT). The possibility of complete intercalation can be directly ruled out from the 40–50° angles, which instead corresponds to the pitch of a groove; hence, minor groove vs. major groove binding geometries have to be considered.

The CD, LD and absorption spectra of MG in the presence of poly(dA) · poly(dT) duplex and poly(dA) · 2poly(dT) are compared in Fig. 3. It is clearly seen that MG does not exhibit any CD or LD signal in the presence of the triplex poly(dA) · 2poly(dT). Further, the absorption spectrum of the MG in the presence of the triplex is exactly the same as that of the free dye. The possibility of indirect extrusion from the minor groove binding by the filling up of the major groove of poly(dA) · poly(dT) was checked by using the AT-specific minor groove binder 4′,6-diamidino-2-phenylindole (DAPI) [18]. The spectroscopic properties of the DAPI–poly(dA) · poly(dT), DAPI–poly(dA–dT)$_2$ complexes and the DAPI–triplex poly(dA) · 2poly(dT) complex are similar, showing that DAPI remains bound in the minor groove of the triplex DNA (manuscript in preparation). The interaction of ethidium with the poly(dA) · poly(dT) duplex and the poly(dA) · 2poly(dT) triplex has been investigated [19]. The fluorescence energy transfer, quencing, viscometric and CD properties of the ethidum–triplex poly(dA) · 2poly(dT) complex indicate that drugs can be bound to the triplex via intercalation, indeed, binding to the triplex by ethidium is some 30 times stronger than to duplex [19]. We conclude that neither the binding of a minor groove binder nor an intercalator should be affected by the formation of triple helix. Finally the presence of Mg^{2+}, needed for stabilizing the triplex, does not seem to significantly affect the binding geometry of MG as judged from the fact that the spectroscopic properties of the MG–poly(dA– dT)$_2$ complex in the presence and absence of $MgCl_2$ are not different (data not shown).

To sum up, we here present solid evidence for a major-groove binding geometry of MG bound to poly(dA) · poly(dT) duplex: in the triplex poly(dA) · 2poly(dT), where a strand of poly(dT) runs along the major groove of the duplex poly(dA) · poly(dT) and sterically hinders binding of MG. As judged from very similar dichroic spectra, the MG–DNA and MG–poly(dA–dT)$_2$ complexes have the same binding geometries as the MG–poly(dA) · poly(dT). The discovery of a major-groove binding small molecule seems important regarding the role of the major groove in protein recognition. Finally, the complete exclusion of Methyl green from binding to poly(dA) · 2poly(dT) makes the dye useful as to monitor triplex formation.

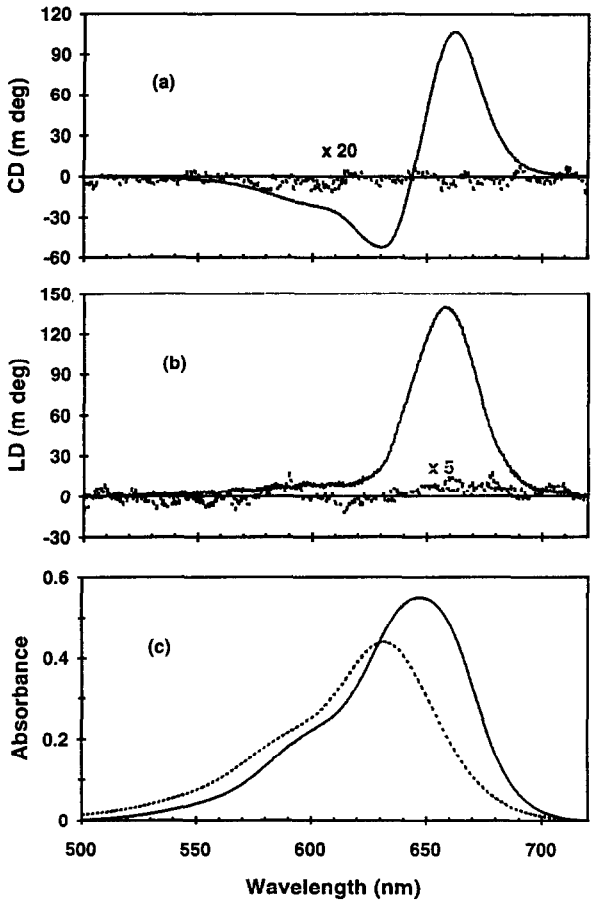

Fig. 3. CD (a), LD (b) and absorption spectra (c) of Methyl green in the 500–720 nm region in the presence of triplex poly(dA) · 2(dT) (dotted curves) and duplex poly(dA) · poly(dT). The CD and LD signal for the MG–triplex mixture is expanded by ×20 and ×5, respectively. Concentrations are the same as in Fig. 2. The absorption spectrum of the free Methyl green coincides with that of the triple helix mixture through the entire wavelength region (220–720 nm) after correction for nucleotide absorption.

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