Binding of 4',6-Diamidino-2-phenylindole to [Poly(dI-dC)]$_2$ and [Poly(dG-dC)]$_2$: The Exocyclic Amino Group of Guanine Prevents Minor Groove Binding

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Received July 20, 1993®

Abstract: Complexes of 4',6-diamidino-2-phenylindole (DAPI) with [poly(dG-dC)]$_2$ and [poly(dI-dC)]$_2$ were studied by optical spectroscopic techniques including linear dichroism (LD), circular dichroism (CD), and fluorescence measurements. The aim was to investigate the importance of the exocyclic amino group of guanine that protrudes into the minor groove of [poly(dG-dC)]$_2$ but is absent in [poly(dI-dC)]$_2$. When bound to [poly(dG-dC)]$_2$, DAPI exhibits a negative, and strongly wavelength-dependent, reduced linear dichroism (LD^r) in the DAPI absorption region, a weak positive CD, and a fluorescence behavior that is similar to that of free DAPI with accessibility to quenching by the aqueous solvent. These spectroscopic properties have been interpreted in terms of a major-groove binding geometry by Kim et al. (Kim, S. K.; Eriksson, S.; Kubista, M.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 3441–3447). By contrast, when bound to [poly(dI-dC)]$_2$, DAPI exhibits a strong positive CD in the 300–420-nm region, a positive (wavelength-independent) LD^r, a strong increase of the fluorescence intensity, and shielding to added quencher. These spectroscopic properties closely resemble those of the DAPI–[poly(dA-dT)]$_2$ complex, in which DAPI is situated deep in the minor groove of the polynucleotide. We conclude that the major groove binding geometry of DAPI when complexed with [poly(dG-dC)]$_2$ is a result of a decreased affinity to the minor groove of [poly(dG-dC)]$_2$ due to steric hindrance and decreased electronegative attraction caused by the amino group of guanine.

Introduction

It is now generally accepted that a number of compounds, such as 4',6-diamidino-2-phenylindole (DAPI) or the dye Hoechst 33258, bind to DNA preferentially at AT-rich regions and within the minor groove of B-DNA in solution.[2-4] Footprinting experiments at low binding ratios indicate that DAPI prevents cleavage of DNA at AT sequences of 3–4 base pairs, implying that DAPI binding occurs at sites with 3–4 nearest-neighbor AT base pairs.[5] Similar results were obtained from footprinting experiments for DNA complexed with netropsin and Hoechst 33258 as well.[6] In a crystal study of DAPI bound to the oligomer [d(CGCGAATTCGCG)]$_2$, Larsen et al.[7] confirmed that DAPI binds at the AATT sequence, and, in addition, they found that the ligand was situated in the minor groove of the double helix. Spectroscopic investigations support the minor groove binding geometry for the DAPI–[poly(dA–dT)]$_2$ complex: linear dichroism (LD) in the DAPI absorption wavelength region is positive, and the angle of the transition moment along the phenyl–indole bond is calculated from the reduced linear dichroism (LD^r) to be about 45° with respect to the DNA helix axis.[8] CD measurements of DAPI bound to the Dickerson dodecamer and [poly(dA–dT)]$_2$ indicate closely similar binding geometries and correlate with the crystal structure.

The results just described raise two immediate questions: what is the nature and geometry of binding of these substances to GC-rich regions of DNA, and why is there a strong binding preference to AT-rich regions? Concerning the first question, evidence including a negative LD^r signal and the ready accessibility to I_2 quencher of DAPI bound to DNA gives strong indication that DAPI bound to [poly(dG-dC)]$_2$ duplex is situated in the major groove of the double helix.[1] Concerning the second question, it is believed that the AT specificity of the binding of these ligands is due to the exocyclic amino group of guanine which, by protruding into the minor groove, prevents minor groove binding in GC rich regions. In this work we studied the binding of DAPI to [poly(dG-dC)]$_2$ and [poly(dI-dC)]$_2$. Since inosine lacks the amino group that in guanine points into the minor groove of the DNA helix, comparison of the binding geometries should provide evidence concerning the role of these amino groups in ligand–DNA interactions.

Materials and Methods

Chemical and Solutions. All chemicals used were of analytical grade. [Poly(dG-dC)]$_2$ (Lot. No. BH7010112, 805 base pairs) and [poly(dI-dC)]$_2$ (Lot. No. 2097880021, 1332 base pairs) were obtained from Pharmacia (Sollentuna, Sweden). All solutions were prepared using 5 mM cacodylate buffer of pH 7.0 at 20 °C. DAPI was purchased from Sigma and used without further purification. Concentrations were determined spectrophotometrically using base molar absorption coefficients of ϵ_{254} = 8400 M^{-1} cm^{-1} for [poly(dG-dC)]$_2$, ϵ_{251} = 6900 M^{-1} cm^{-1} for [poly(dI-dC)]$_2$, and ϵ_{342} = 27 000 M^{-1} cm^{-1} for DAPI in water.[9]

Under the conditions of the measurements, regarding the binding constants of DAPI complexed with polynucleotides,[2,10]

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• Abstract published in *Advance ACS Abstracts*, December 1, 1993.
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the amounts of free DAPI in the samples can be neglected and, accordingly, the mixing ratio, R, defined as the total number of added DAPI molecules per base, corresponds very closely to the amount of bound DAPI molecules per DNA base. This approximation is supported by the observation of an essentially mixing ratio-independent LD^r spectrum for both the DAPI–[poly-(dG-dC)]$_2$ mixtures and the DAPI–[poly(dI-dC)]$_2$ mixtures (see Results). The binding affinity of DAPI for [poly(dG-dC)]$_2$ is 1 order of magnitude weaker than that for [poly(dI-dC)]$_2$.[2,10] Still, the binding constant at the present ionic strength (5 mM Na^+) is greater than 10^6 M^{-1},[10] so in a typical mixture of DAPI (10 μM) and [poly(dG-dC)]$_2$ (100 μM), less than a few percent of the amount of DAPI will be unbound. This was also confirmed in a check experiment by analyzing the absorption spectrum in terms of free and bound fractions: at a somewhat higher ionic strength (10 mM NaCl), less than 4% of DAPI was found to be free.

Linear Dichroism (LD). LD(λ) is the differential absorption of light polarized parallel and perpendicular to the direction of an orienting field at a wavelength λ:

$$\mathrm{LD}(\lambda) = A_{\parallel} - A_{\perp} \tag{1}$$

The reduced dichroism (LD^r) is defined as

$$\mathrm{LD}^r(\lambda) = \mathrm{LD}(\lambda)/A_{iso}(\lambda) \tag{2}$$

where A_{iso} denotes the absorption spectrum of the isotropic sample. LD^r can be expressed as the product between an orientation factor, S, and an optical factor:

$$\mathrm{LD}^r(\lambda) = \frac{3S}{2}(3\langle\cos^2\alpha\rangle - 1) \tag{3}$$

where α is the angle between the transition moment responsible for the light absorption and the orientation axis and the brackets indicates an ensemble average. S depends on the stiffness of the DNA, on the flow rate, and on the viscosity of the medium.[11] It ranges from 0 for a randomly oriented sample to +1 for a perfectly aligned sample.

The polynucleotides were oriented in a Couette flow cell,[12,13] where the sample solution is placed in the space between two concentric cylinders, one of which is rotated while the other is static. In the apparatus, the generally used rotation speed of 1200 rpm corresponded to a shear gradient of 3770 s^{-1}.

The LD spectra were measured on a Jasco J-500A spectropolarimeter as described elsewhere;[14] an achromatic quarter wave device was used to convert the circularly polarized incident light to linearly polarized light.

Circular Dichroism (CD). An achiral molecule such as DAPI may acquire an induced CD upon chiral perturbation through binding to the chiral DNA helix. The origin of this CD is believed to be primarily due to coupling between nondegenerate transitions of the ligand and the nucleo bases of the host.[15,16] CD spectra were measured on a Jasco J-720 spectropolarimeter using a 1-cm quartz cell.

Absorption and Fluorescence Measurements. Absorption spectra were recorded on a Cary 2300 spectrophotometer and fluorescence on an Aminco SPF-500 spectrofluorometer in the quantum corrected mode. The emission spectrum of free DAPI was measured using 345-nm excitation. For the three DAPI–polynucleotide complexes, 360-nm excitation was utilized. The emission and excitation bandpass was 4 nm in all measurements. All spectra were corrected for the inner filter effect.

Quenching of the DAPI fluorescence by iodine (I_2) was measured by adding small volumes of freshly prepared I_2 solution into the DAPI-containing samples (up to 50 μL to 2.5 mL of solution); the measured intensities were corrected for volume changes. The excitation and emission wavelengths were at 360 and 460 nm, respectively, and the band-pass for both excitation and emission was 4 nm.

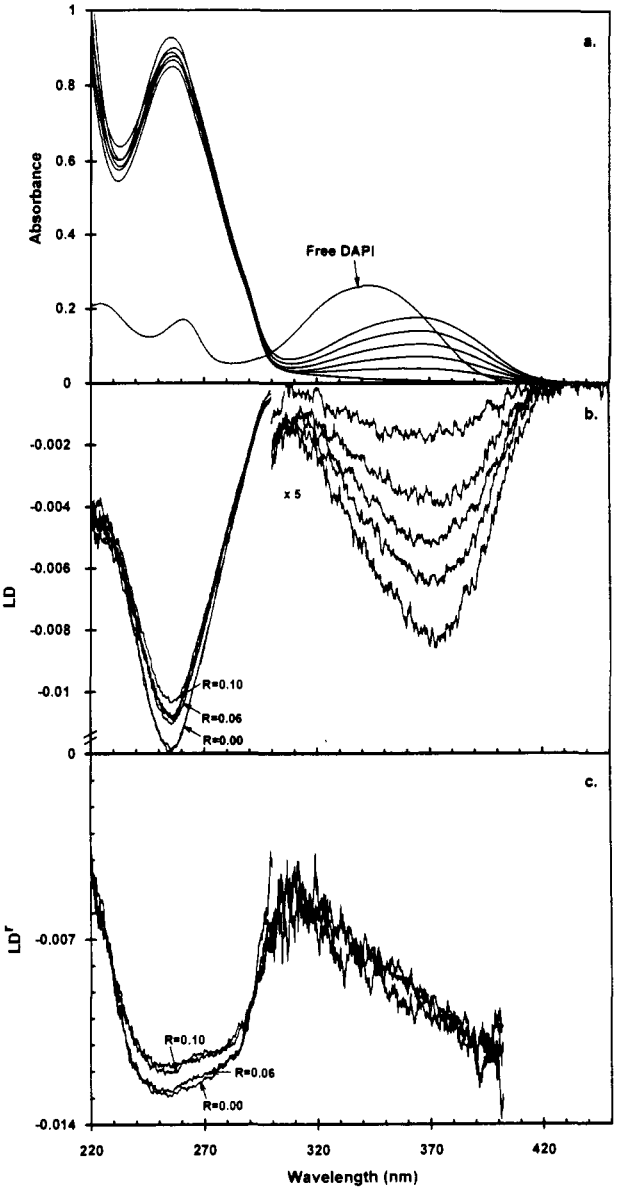

Figure 1. Absorbance (a), linear dichroism (LD, b), and reduced linear dichroism (LD^r, c) spectra of DAPI in the presence of [poly(dG-dC)]$_2$. R values from bottom to top in the 300–420-nm region of the absorption spectra are 0.00, 0.02, 0.04, 0.06, 0.08, and 0.10. Polynucleotide concentration was 100 μM, and the concentration of free DAPI was 10 μM. The LD spectra were recorded with an outer rotating Couette cell, and the flow speed was 1200 rpm.

Results

Absorption, LD, and LD^r. In Figure 1 are shown the relevant spectra of the DAPI–[poly(dG-dC)]$_2$ complexes at R values of 0.02, 0.04, 0.06, 0.08, and 0.10. In addition, Figure 1a includes the absorption spectrum of free DAPI at a concentration corresponding to that at R = 0.10. Compared with free DAPI, the species bound to [poly(dG-dC)]$_2$ exhibits a substantial hypochromism of near 40% in the long wavelength band and a

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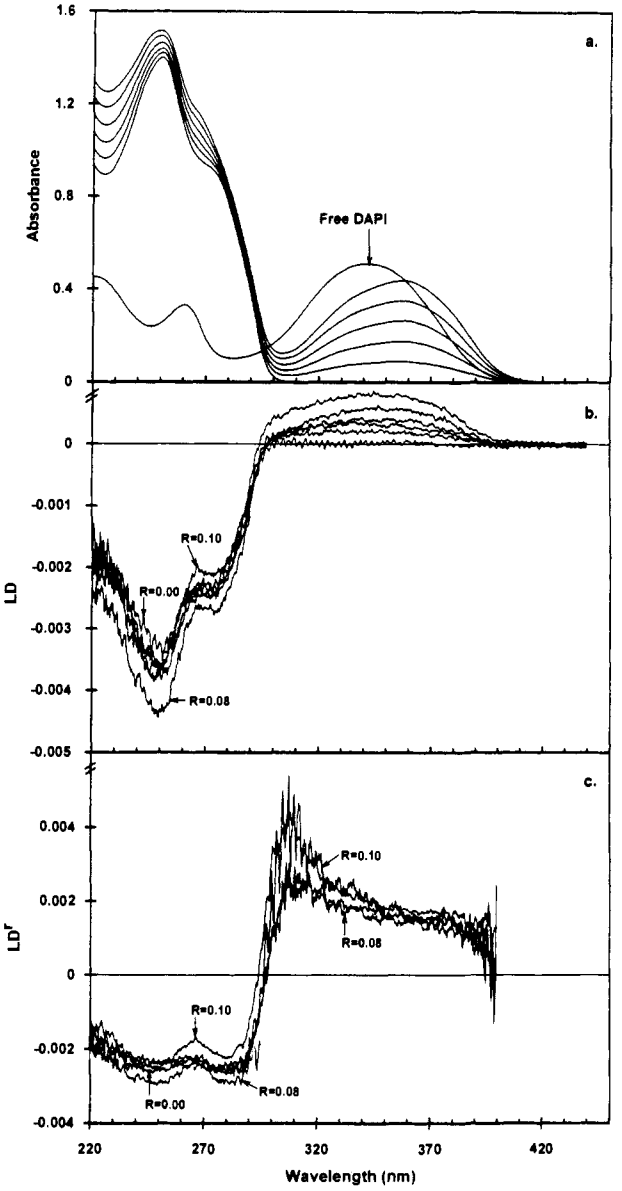

Figure 2. Absorbance (a), LD (b), and LD^r (c) spectra of DAPI complexed with [poly(dI-dC)]$_2$. The polynucleotide concentration was 200 μM, and that of free DAPI was 20 μM. From bottom to top at 360 nm in the absorption spectra: R = 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, and free DAPI. The LD measurements were performed with an inner rotating Couette flow cell at a shear gradient of 3770 s^{-1}.

red shift of the absorption maximum by about 30 nm (≈2300 cm^{-1}); these values are relatively large and indicate that the interaction between DAPI and the nucleotide bases is strong.

The LD spectrum, shown in Figure 1b, is expanded by a factor of 5 in the long wavelength range for the sake of clarity. The signals in this region are negative, indicating that the transition moments are tilted more perpendicular to the flow lines than parallel to them.[12,13] In the predominantly DNA absorption region around 260 nm, the LD is negative, in agreement with expectation, since the planes of the bases in B-DNA are essentially perpendicular to the helix axis. However, compared to the uncomplexed polynucleotide, the LD intensity in this region is diminished by 10–20% upon DAPI binding. The corresponding decrease in LD^r (Figure 1c) in the low-wavelength DNA absorption region suggests that the ability of the polynucleotide duplex to orient along the flow lines is significantly impaired by the presence of DAPI.

Another feature of the reduced linear dichroism is its significant wavelength dependence at the red end of the spectrum. In the

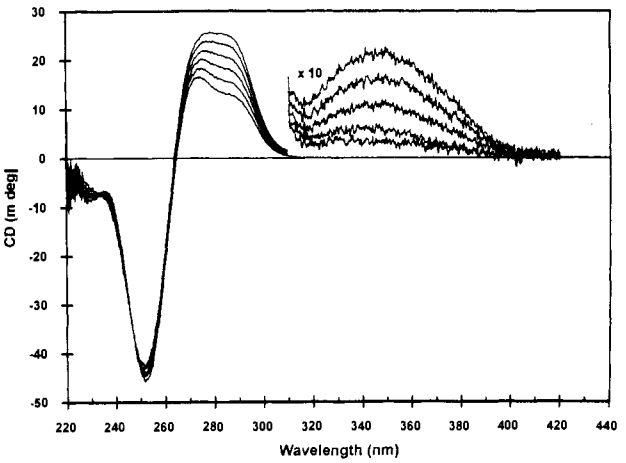

Figure 3. Circular dichroism (CD) spectra of DAPI complexed with [poly(dG-dC)]$_2$. The polynucleotide concentration was 200 μM, and the R values were the same as in Figure 1. Optical path length was 1 cm.

300–400-nm region, DAPI is known to have two electronic transitions polarized near the long axis of the molecule.[15] On the basis of eq 3 and using methods outlined elsewhere,[11,13] orientation angles of the DAPI transition moments relative to the DNA helix can be calculated from the LD^r data. Assuming an effective angle of the DNA base transition moments relative to this axis of 86°,[17,18] the average angles of the transition moments around 340 and 380 nm are calculated to be 69 ± 1° and 83 ± 1°, respectively. The angles calculated from all R ratios fall into the same range, suggesting the existence of a single binding mode of DAPI to [poly(dG-dC)]$_2$.

The absorption, LD, and LD^r spectra of the DAPI–[poly(dI-dC)]$_2$ complexes are shown in Figure 2; the complex spectra refer to the same R values as those in Figure 1. The hypochromism shown by DAPI upon binding is now only about 15%, and the red shift of the maximum of the long wavelength band is 17 nm (≈1400 cm^{-1}); both these values are significantly smaller than those observed for DAPI bound to [poly(dG-dC)]$_2$. The LD^r spectra in the DAPI absorption region are positive, and one calculates transition moment directions relative to the DNA helix of near 45° and 50°, respectively, of the two electronic transitions of DAPI in this region.

Induced CD. The CD spectrum of DAPI in the long wavelength band on [poly(dG-dC)]$_2$ (enlarged by a factor of 10 to simplify comparison) is positive (Figure 3). The induced CD is weak and its shape independent of the mixing ratio up to at least R = 0.10, indicating only one type of binding. However, the shape of the CD spectrum of the DAPI–[poly(dI-dC)]$_2$ complex (Figure 4) varies significantly with mixing ratio. At low binding ratios the CD spectrum consists of a positive feature with a maximum at 342 nm. With increasing R ratio, the maximum shifts to 365 nm. The same dependence of the CD spectra on mixing ratio has been observed in both the DAPI–DNA and the DAPI–[poly(dA-dT)]$_2$ complexes.[8,19]

Fluorescence Measurements. The fluorescence emission spectra of DAPI bound to [poly(dG-dC)]$_2$, [poly(dI-dC)]$_2$, and [poly(dA-dT)]$_2$ are shown in Figure 5 together with that of free DAPI (those of free DAPI and DAPI–[poly(dG-dC)]$_2$ complex are multiplied by a factor of 5 for easier comparison with the spectra for the [poly(dI-dC)]$_2$ and [poly(dA-dT)]$_2$ complexes). Upon binding of DAPI to [poly(dG-dC)]$_2$, the fluorescence intensity of the emission spectrum of DAPI decreases somewhat, in agreement with the finding of Kim et al.[1] It is also blue-shifted and narrower compared to that of free DAPI. The spectral half-

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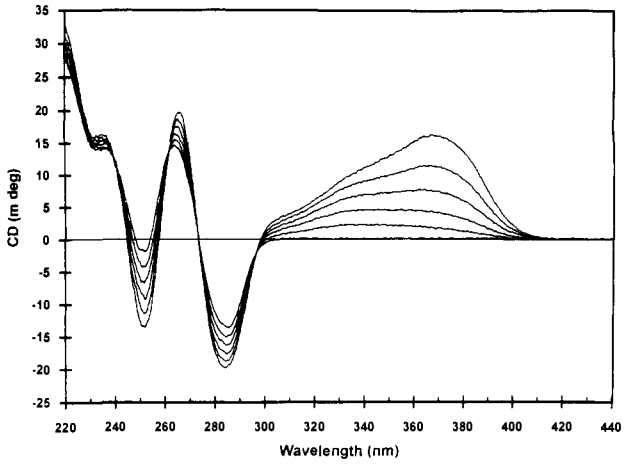

Figure 4. Circular dichroism of DAPI bound to $[poly(dI\text{-}dC)]_2$ measured at an optical path length of 1 cm. Polynucleotide concentration and R values were the same as in Figure 2.

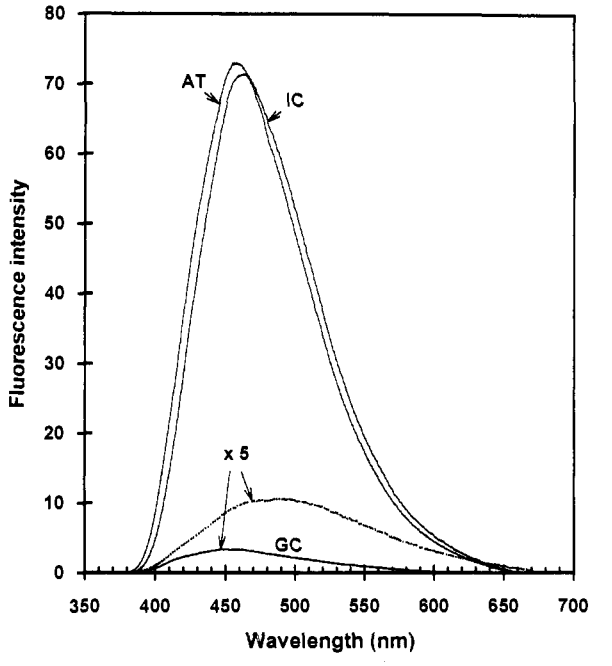

Figure 5. Fluorescence emission spectra of free DAPI (dotted curve) as well as of that complexed with $[poly(dG\text{-}dC)]_2$, $[poly(dI\text{-}dC)]_2$, and $[poly(dA\text{-}dT)]_2$. Polynucleotide concentrations were 40 μM, and the concentration of free DAPI was 4 μM. The R ratio was 0.10, and the spectral bandwidths were 4 nm.

width of free DAPI of 139 ± 2 nm (about 5700 cm^{-1}) is reduced to 106 ± 2 nm (about 4500 cm^{-1}) in its complex with $poly[(dG\text{-}dC)]_2$.

The increase in fluorescence quantum yield of DAPI upon binding to DNA and $[poly(dA\text{-}dT)]_2$ is well known.[3,8,9,20,21] In the figure, a very strong increase in fluorescence quantum yield is observed upon binding to $[poly(dI\text{-}dC)]_2$ as well as to $[poly(dA\text{-}dT)]_2$, an indication that the nature of interactions between DAPI and these polynucleotides differs from that with $[poly(dG\text{-}dC)]_2$. A blue-shift and a 42-nm ($\approx$1300 cm^{-1}) narrowing of the emission spectrum is observed upon binding to $[poly(dI\text{-}dC)]_2$. The shape of the spectra is virtually independent of the R ratio, and the narrowing of the spectral bandwidths in the emission spectra is observed independent of the base content and sequence.[22]

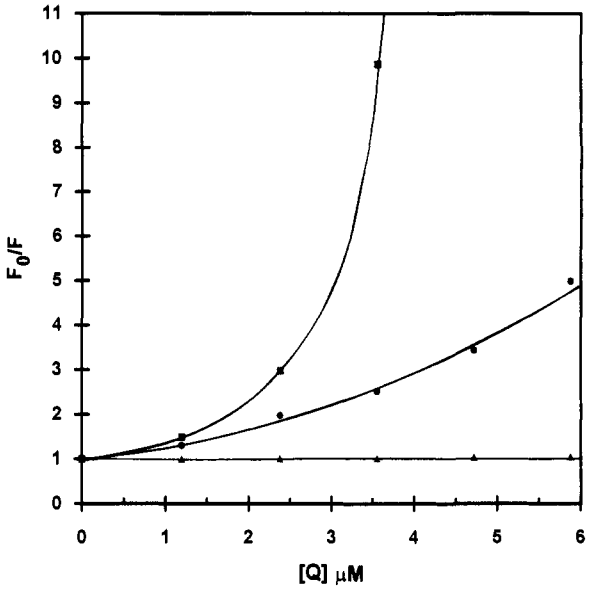

Figure 6. Fluorescence quenching of free DAPI (■), DAPI–$[poly(dG\text{-}dC)]_2$ complex (●), and DAPI–$[poly(dI\text{-}dC)]_2$ complex (▲) by I_2. The DAPI concentration was 2 μM, and the polynucleotide concentration was 200 μM. Excitation and emission wavelengths were 360 and 460 nm, respectively, and the bandwidths were 4 nm. Lines have been drawn in the figure for better lucidity.

In Figure 6, the ratio of the intensity of the fluorescence in the absence of the quencher to that in its presence is shown as a function of the I_2 quencher concentration. Free DAPI (squares) is very accessible to the quencher; the fluorescence of 2 μM DAPI is almost fully quenched in the presence of 3 μM I_2. However, when DAPI is complexed with $[poly(dG\text{-}dC)]_2$, it shows a lower, but still significant, accessibility to I_2 quencher (circles). Both plots exhibit upward bending curves, which implies that the fluorophore is quenched both by collisions (dynamic quenching) and by complex formation (static quenching) with the I_2 quencher.[23] In the DAPI–$[poly(dI\text{-}dC)]_2$ complex (triangles), fluorescence is not quenched at all, a behavior also shown by the DAPI–DNA and DAPI–$[poly(dA\text{-}dT)]_2$ complexes,[1] where DAPI is known to bind in the minor groove of the DNA.

Discussion

Absorption Spectra. Hypochromicity and red shifts of the absorption spectra are common features of ligands upon binding to DNA. However, those observed for DAPI upon binding to $[poly(dG\text{-}dC)]_2$ (Figure 1a) are unusually large and suggest a strong interaction between DAPI and the polynucleotide bases. When DAPI is bound to $[poly(dI\text{-}dC)]_2$, hypochromicity and red shift (Figure 2a) are similar to those observed with $[poly(dA\text{-}dT)]_2$. The larger hypochromicity and red shift in the case of $[poly(dG\text{-}dC)]_2$ implies that the excited states of the DAPI chromophore have suffered a greater perturbation by interaction with the transition moments of the nucleic acid bases than in the case of $[poly(dI\text{-}dC)]_2$, which confirms that the relative geometries and/or distances between DAPI and perturbing bases differ significantly. This behavior would be consistent with intercalation of DAPI between the base pairs. However, as we shall see, detailed analysis of linear dichroism spectra, together with fluorescence quenching properties, contradicts a classical intercalative binding geometry.

Linear Dichroism. The shape of the LD spectrum of the DAPI–$[poly(dG\text{-}dC)]_2$ complex in the 300–420-nm region (Figure 1b) is quite different from that of the absorption spectrum, which is clearly visualized by the LD^r spectrum depicted in Figure 1c; the

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wavelength dependence of LD^r in the 320–390-nm region is obvious. The variation can be explained either by the different orientations of the two transition moments[15,19] or by the presence of different binding geometries.

If DAPI had been intercalated between the DNA base pairs, the two transition moments in the 300–420-nm region should have appeared at the same effective angle, near 90° with respect to the DNA helix axis. If the two transitions had had orthogonal polarizations in the plane of DAPI, the effective angles 70° and 83° relative to the DNA helix could be consistent with intercalation, recalling that the DNA bases may display anisotropic tilt. However, since the two transitions are separated by only a small angle (about 15°),[15] the LD^r variation observed means that the plane of the chromophore has to be nearly parallel to the DNA axis, i.e., to exhibit an inclination that is totally incompatible with an intercalative binding geometry. Additional evidence against intercalation comes from the LD^r behavior around 260 nm. Classical intercalators, such as ethidium bromide, produce unwinding, elongation, and stiffening of the DNA helix, which should result in an increase of the LD^r magnitude. For the DAPI–[poly(dG-dC)]$_2$ complex, LD^r decreases, suggesting some less negative contribution from the bound DAPI or on impaired orientation of the polynucleotide. Therefore, the LD^r behavior suggests that DAPI is not intercalated between the base pairs of [poly(dG-dC)]$_2$.[1]

The interaction of DAPI with [poly(dI-dC)]$_2$ differs markedly from that with poly[(dG-dC)]$_2$. The LD measurements performed on the former complex show a strong positive, and wavelength-independent, LD^r (Figure 2c) in the DAPI absorption region, 320–390 nm, associated with the two long-axis-polarized $\pi \rightarrow \pi^*$ transitions. A first important conclusion about a nonintercalative binding of DAPI to [poly(dI-dC)]$_2$ comes from this positive LD, which immediately implies an angle less than 55° between the long axis of DAPI and the polynucleotide helix axis. In fact, the average angles of the transition moments in the DAPI long wavelength range are calculated to be 45–50°. The calculated angles from all R ratios fall into this range, suggesting a rather homogeneous binding mode of DAPI to [poly(dI-dC)]$_2$.

The spectroscopic properties for the DAPI–[poly(dI-dC)]$_2$ complex are all very similar to those obtained for the DAPI–[poly(dA-dT)]$_2$ complex and are strong indications of a minor groove binding mode also in this case.

Induced Circular Dichroism. The induced CD of an achiral, planar chromophore is expected to be relatively weak when nondegenerate coupling with the chirally arranged DNA transitions is the main source of the rotational strength.[16,24,25] The CD per bound DAPI in the DAPI–[poly(dG-dC)]$_2$ complex is constant for a wide range of binding ratios, confirming the conclusion of a single binding geometry and showing that the ligands are too far apart to give any significant excitonic CD. By contrast, the DAPI–[poly(dI-dC)]$_2$ complex shows a CD spectrum that is not constant in shape but changes markedly as the binding ratio becomes larger than 0.04 (see Figure 4). This observation indicates that at higher R values, DAPI molecules come so close to each other that they give rise to exciton coupling, which, due to chiral orientations of the DAPI molecules relative to one another, generally is associated with strong CD-inducing powers.

Changes in the CD in the 260-nm region scale exactly with the binding ratio when DAPI binds to either of the two polynucleotides and, thus, reveal no change of the intrinsic CD and the conformation of the polynucleotides. The induced CD of an electric dipole-allowed transition of a DNA intercalator in the (dAdT) and (dGdC) miniduplexes has been calculated by Lyng et al. (1987).[16] According to that calculation, the CD intensity depends on the angle, γ, which is defined as the azimuthal angle, in the plane of the intercalation pocket, between the electric dipole transition moment of an intercalated molecule and the pseudodyad axis. If the angle γ is 90°, i.e., the long axis of the intercalator lies parallel to the long axis of the intercalation pocket, then the induced CD is expected to be negative. This is concluded not to be the case in either the DAPI–[poly(dG-dC)]$_2$ complex or in the DAPI–[poly(dI-dC)]$_2$ complex since both complexes exhibit positive induced CD in the 300–420-nm region. Strongly positive CD is instead predicted by calculations for groove binding geometries,[16] as is also confirmed from CD measurements on various minor groove DNA complexes,[25] DAPI–[poly(dA-dT)]$_2$ being the most relevant example in this context.

In conclusion, the strongly positive induced CD of DAPI bound to [poly(dI-dC)]$_2$ is consistent with a minor groove binding geometry, just as with [poly(dA-dT)]$_2$, and inconsistent with intercalation. The CD of DAPI bound to [poly(dG-dC)]$_2$ is 1 order of magnitude weaker than those of the complexes with [poly(dA-dT)]$_2$ and [poly(dI-dC)]$_2$, excluding a "normal" groove geometry (pitch 45°). A weak induced CD may suggest intercalation; however, the positive sign is not consistent with an orientation parallel to the long axis of the intercalation pocket.

Fluorescence Measurements. The enhancement and reduction, respectively, of the fluorescence intensity as well as, in both cases, a narrowing of the emission spectra of DAPI upon binding to the dI-dC and the dG-dC polynucleotides can be understood as different populations of two forms of protonation in the DAPI molecule.[26] The strong increase of the fluorescence intensity and the large narrowing of emission spectra upon binding DAPI to [poly(dI-dC)]$_2$ can be explained as reducing the intramolecular proton-transfer process by shielding the DAPI molecule from the aqueous solvent. This behavior is in conformity with that of the DAPI–[poly(dA-dT)]$_2$ complex, which is known to exhibit a minor groove binding mode. On the other hand, when DAPI binds to [poly(dG-dC)]$_2$, a decrease of intensity but still a narrowing of emission profile is observed. If DAPI is bound in a major groove binding mode to [poly(dG-dC)]$_2$, as has been proposed,[1] it would be exposed to solvent, facilitating proton transfer in harmony with the observed decrease of the fluorescence intensity.

The fluorescence quenching technique can be used to study solvent accessibility. If a fluorophore is protected from the solvent, it will not be quenched by an external quencher. When the dye DAPI is free, it is effectively quenched by I_2. The quenching effect is significantly lower for the DAPI–[poly(dG-dC)]$_2$ complex and almost ineffective for DAPI bound to [poly(dI-dC)]$_2$ (Figure 7). Quenching studies performed by Kim et al.[1] on DAPI bound in the minor groove of DNA as well as of [poly(dA-dT)]$_2$ show no accessibility to I_2 quencher. From this, one can conclude that DAPI is protected from contact with I_2, probably by being situated in the minor groove of the polynucleotide, when bound to [poly(dI-dC)]$_2$. By contrast, when complexed with [poly(dG-dC)]$_2$, the data suggest that DAPI is situated near the surface of the polynucleotide.

Conclusions. The spectroscopic properties of DAPI in complex with [poly(dI-dC)]$_2$ are the following: 15% hypochromicity and a 17-nm red shift in the absorption spectrum, a strong, positive, R-dependent CD in the 300–420-nm wavelength region, a manyfold increase of the fluorescence intensity, and essentially no accessibility to I_2 quencher. Furthermore, the LD^r spectrum is positive and independent of wavelength. If DAPI had been intercalated, the LD^r would have been negative as the transition moments should have been perpendicular relative to the DNA helix axis. In a case of surface binding, in which the positively charged DAPI molecules are associated to the phosphate groups at the outside of DNA by electrostatic interaction, they are likely to have a poor average orientation and hence a weak LD signal. Obviously, this is not the case for the DAPI–[poly(dI-dC)]$_2$

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complex, but its spectroscopic properties are very similar to those of the DAPI–[poly(dA-dT)]$_2$ complex, in which DAPI is situated in the minor groove of the polynucleotide.

By contrast, the DAPI–[poly(dG-dC)]$_2$ complex is confirmed to exhibit a negative, markedly wavelength-dependent, LD^r in the DAPI absorption region, a weak, positive CD, a 30-nm red shift and 40% hypochromicity absorption, and an intermediate accessibility to I_2 quencher. These spectroscopic properties have been interpreted in terms of a major groove binding geometry by Kim et al.[1]

Considering the exocyclic amino group of guanine that protrudes into the minor groove of [poly(dG-dC)]$_2$, it is conceivable that it plays an important role in determining the binding site of DAPI, possibly by steric hindrance to binding in the minor groove. When the amino group is removed, such as in [poly(dI-dC)]$_2$, a behavior very similar to that of [poly(dA-dT)]$_2$ is observed, with a restoration of the minor groove binding mode. The situation is probably more complex; in addition to steric effects, the ability of hydrogen-bonding and other electrostatic attraction effects involving the electronegative carbonyl group of thymine are likely to be important. The amino group, by making the minor groove less electronegative, could significantly reduce the binding affinity of DAPI to [poly(dG-dC)]$_2$. In other words, the nonminor groove binding geometry of DAPI observed when DAPI is complexed with [poly(dG-dC)]$_2$ is concluded to be a result of steric hindrance and decreased electronegative potential in the minor groove by the amino group of guanine.

Acknowledgment. Dr. T. Kurucsev is greatly acknowledged for valuable discussions. This project has been supported by the Swedish Natural Science Research Council (NFR).