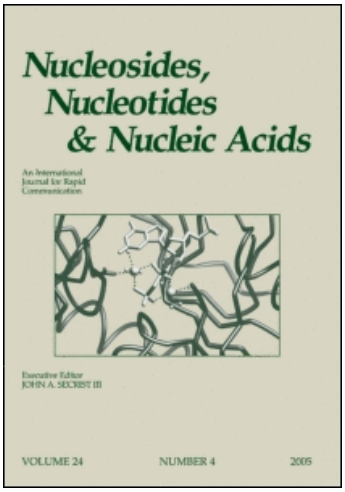

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ENANTIOSELECTIVE DNA BINDING GEOMETRIES OF Δ AND Λ RU(PHENANTHROLINE)$_3^{2+}$ STUDIED WITH LINEAR DICHROISM

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Abstract: Flow dichroism shows that the Δ and Λ enantiomers of Ru(phen)$_3^{2+}$ both bind to DNA, but with different binding geometries. The same two types of binding geometries are also observed with poly(dG–dC) and poly(dA–dT), indicating that the enantiomerically specific geometry is determined by the "texture" (helical pitch and sense) of the double–helix, irrespective of base composition.

The propeller–shaped trigonal metal complexes ML$_3^{2+}$, with M=Fe(II), Zn(II) or Ru(II) and L=2,2'–bipyridyl (bipy) or 1,10–phenanthroline (phen) are interesting DNA ligands. They are too bulky to be intercalated and the stereoselectivity they exhibit upon binding to B DNA (first revealed for the iron complexes)[1] can be assumed to arise from the texture of the double helix, experienced by the complex upon binding to a groove, rather than from local chirality on a base–pair level. The chiral discrimination observed with B DNA has inspired to numerous attempts to use this type of complexes for probing the handedness of DNA, e.g. Z DNA, but the results are controversial.[2-4] The Δ enantiomer is the isomer that is generally observed to prefer B DNA, and Barton[5] has reported that with Ru(4,7–diphenylphenanthroline)$_3^{2+}$ the Λ isomer is completely precluded from binding.

Linear dichroism (LD) is a sensitive detector of DNA interaction when intense ligand absorption bands are available. The appearance of an LD signal implies that the ligand chromophore is no longer free and randomly oriented, proving the association to the macroscopically aligned biopolymer. The sign and magnitude of the LD signal also provide evidence about the binding geometry. In earlier studies the LD spectra of the intense charge–transfer band of Fe(bipy)$_3^{2+}$ and Fe(phen)$_3^{2+}$ are characterized by a negative (A_2) band at longest wavelength and a

positive (E) band at shorter wavelength, upon DNA binding.[1-6] Circular dichroism shows that these inversion–labile complexes are preferentially converted to Δ form in the presence of B DNA.

We show here that Δ–Ru(phen)$_3^{2+}$ displays essentially the same LD spectrum as the Δ forms of the iron complexes when interacting with DNA, while the spectrum of the Λ form indicates a quite different binding geometry. The interactions with DNA, poly(dA–dT) and poly(dG–dC) are geometrically very similar for each of the enantiomers, despite different binding affinities. This demonstrates that the enantioselectivity is indeed related to the textural properties of the double helix.

Results

FIG. 1 shows the LD spectra of DNA solutions containing Δ, Λ and racemic Ru(phen)$_3^{2+}$. Upon interaction with DNA the Δ enantiomer displays two LD bands in the charge–transfer region, one negative and one positive, with maxima at 470 nm and 380 nm, respectively. The Λ enantiomer shows only one strong positive band centered at 425 nm and a small negative indication at 480 nm. The LD spectrum of the racemate can be simulated as a linear combination of the Δ and Λ spectra, demonstrating that both enantiomers bind to DNA, but without influencing each others binding geometries significantly. Practically the same two LD spectra are obtained with poly(dG–dC) and poly(dA–dT), showing that Δ and Λ Ru(phen)$_3^{2+}$ also bind to the polynucleotides with, which is most important, effectively the same respective binding geometries as to DNA.

The CD spectra of the pure enantiomers are affected upon binding to DNA and the strongest perturbation is observed for the long–wavelength (470 nm) positive CD band of the Λ enantiomer, which is increased by some 25 %.

A similar behavior is observed with Λ+poly(dA-dT), which form a strong complex as evidenced from LD, and also with Λ+poly(dG-dC) where though, a very weak LD indicates that binding is weak or random. That the 470 nm CD band is prone to asymetric perturbation is also clearly seen when adding racemic Ru(phen)$_3^{2+}$ to DNA: a 470 nm is the result of an inbalance between the enantiomers owing to different extents of perturbation of their respective CD. Dialysis experiments confirm that.

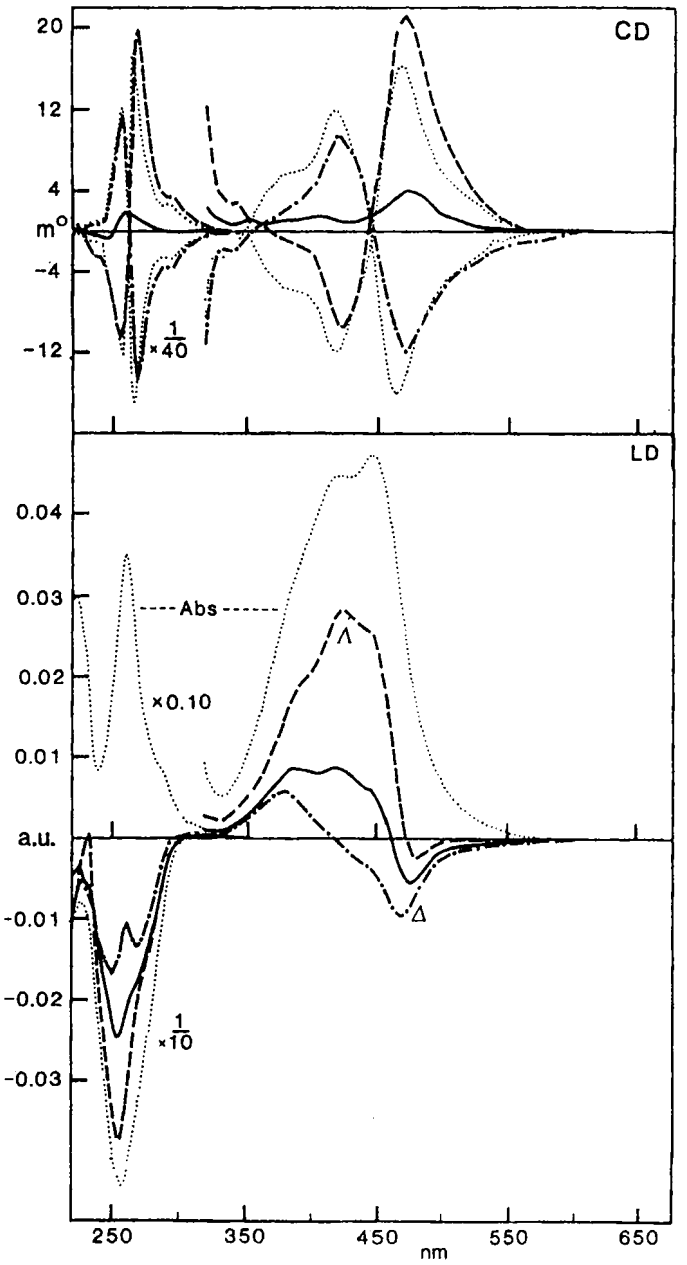

Figure 1. : LD and CD spectra of 25 μM Δ (—·—), Λ (---) and racemic (———) $Ru(phen)_3^{2+}$ with 0.36 mM DNA.
LD of pure DNA, absorbance of free $Ru(phen)_3^{2+}$ and CD of Δ and Λ–$Ru(phen)_3^{2+}$ denoted by dotted curves.

Λ–Ru(phen)$_3^{2+}$ displays a preferential, strong binding to poly(dA–dT), but that Δ is the enantiomer which predominates upon binding to DNA and poly(dG–dC). The following binding preference was obtained :

$$\Lambda + AT > rac + AT > \Delta + GC > rac + GC > \Delta + AT > \Lambda + GC$$

Discussion

LD provides evidence of the textural chiral discrimination by right–handed B DNA. This interaction may be regarded as steric (repulsive) and seems to determine binding geometry uniquely, irrespective of base sequence. The primary interaction is electrostatic, and is the interaction explaining base–specificity, for example, Λ + AT > Λ + GC. The LD results show that Λ still binds in the same geometrical way (different from that of Δ) to both AT and GC owing to the texture of the B DNA helix.

It has been speculated whether the Δ enantiomer might be partially intercalated with one of its propeller wings inserted between base–pairs. However, the relatively modest CD changes show that the geometry of the metal complex is not strongly perturbed by the DNA interaction.

A sharp isosbestic point at 466 nm in the absorption spectrum of Δ–Ru(phen)$_3^{2+}$, when titrated with DNA, is consistent with a distinct binding geometry for this isomer as compared to Λ which, despite a comparable change in absorption, does not display any isosbestic point. Also LD spectra obtained at different binding ratios support the impression that the Λ–DNA complex is of a more heterogeneous nature compared to the Δ complex.

Finally, the sign and magnitude of the LD, contains information regarding the orientation of the metal complex relative to DNA. (We consider the absorption at 350–400 nm which is less perturbed than the band at longer wavelength. From crystal studies this band is known to be polarized in the xy–plane, perpendicular to the C_3–axis of Ru(phen)$_3^{2+}$). For both isomers the positive LD in this region indicates a preferential orientation of the metal complex with its xy–plane parallel to the helix axis; the smaller amplitude with Δ indicates a larger tilt of this complex.

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