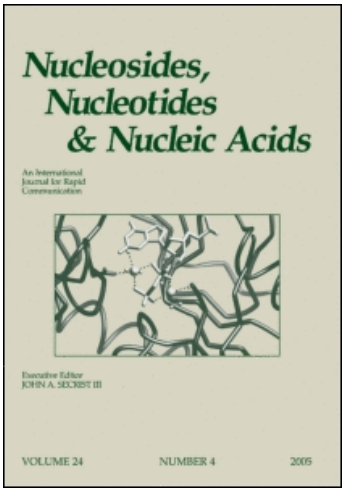

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Nordén, Bengt , Patel, Naina , Hiort, Catharina , Gräslund, Astrid and Kim, Seog Kyu(1991) 'DNA Interaction with Chiral Metal Complexes', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 195 — 205
To link to this Article: DOI: 10.1080/07328319108046446
URL: http://dx.doi.org/10.1080/07328319108046446

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 195-205 (1991)

DNA INTERACTION WITH CHIRAL METAL COMPLEXES

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Abstract: Despite extensive study of DNA interaction with propeller–shaped metal complexes, such as the Δ and Λ enantiomers of $[Ru(1,10–phenanthroline)_3]^{2+}$, the basis for their enantioselectivity, and even their binding modes, are not yet fully understood. ^1H NMR studies of the interactions with the self–complementary oligonucleotide $d(CGCGATCGCG)_2$ indicate that both enantiomers bind into the minor groove of the central AT–TA region, but with a rapid exchange between the bound and free states. Flow linear dichroism (FLD) and circular dichroism (CD) show different binding geometries for the two enantiomers. These two geometries are found in natural DNA as well as in a number of different B form polynucleotides, virtually independent of base composition and of methylation. The DNA interaction with the $[Ru(1,10–phenanthroline)_3]^{2+}$ complexes will be reconsidered in the light of NMR, FLD, CD and fluorescence results.

Introduction

To understand which interactions determine DNA–site recognition, not only the detailed structures of specific strong DNA complexes with small molecules and proteins should provide insight, but valuable information about the interplay of different interactions may also be gained from the study of weaker complexes. An interesting class of compounds in this context is the substitution inert tris(bidentate) metal complexes whose rigid structure and chiral shape could make some of them useful as DNA interaction probes. The tris(phenanthroline) and tris(bipyridyl) complexes of transition metal ions such as ruthenium(II) and iron(II) generally display significant enantiopreferentiality upon binding to DNA as evidenced from circular dichroism (CD) and linear dichroism (LD) spectroscopy (see Ref 1 which also reviews earlier work).

In addition it has been found that for the inversion labile Fe(II) complexes that DNA has a catalytic property, increasing the inversion rate compared to

racemization in free solution[2]. Despite extensive study of the DNA interaction with these propeller–shaped complexes, though, the basis for their enantioselectivity and even their binding modes is far from well understood.

Groove binding[1,3] as well as intercalation[4-8] have been proposed from optical spectroscopy and affinity studies, but conclusive evidence for either interaction mode is lacking. We here present results from high field proton NMR of the interactions of the Δ and Λ enantiomers of $[Ru(1,10\text{–phenanthroline})_3]^{2+}$ with the self–complementary oligonucleotide $d(CGCGATCGCG)_2$. In addition, the interaction of each of the enantiomers with the duplex polynucleotides $[poly(dA\text{–}dT)]_2$, $[poly(dA\text{–}dU)]_2$, $[poly(dG\text{–}dC)]_2$ and $[poly(dGme^5dC)]_2$ {below referred to as AT, AU, GC and metGC, respectively} has been studied using fluorescence, LD and CD techniques.

Materials and Methods

The decadeoxyribonucleotide $5'$–CGCGATCGCG–$3'$ was synthesized employing the phosphoamidite method, and purified by reverse HPLC (Pharmacia). Its concentration was determined using $\epsilon_{260} = 8040\ M^{-1}\ cm^{-1}$ (calculated on the basis of the nucleotide composition). The concentrations of the oligonucleotide for the titration studies ranged between 0.4–1.2 mM duplex.

Stock solutions of the pure metal complex were made up in distilled water. For the oligonucleotide titration studies aliquotes were freeze–dried, and redissolved using the oligonucleotide solution. Solutions of the oligonucleotide were made up in either deuterated (D_2O) or undeuterated (H_2O) 10 mM phosphate buffer (pH(D) 7.0) and used without further dilutions.

One dimensional ^1H NMR spectra were recorded on an AM 500 Bruker NMR spectrometer interfaced with an Aspect 3000 computer. Sample volumes were kept between 400–500 μl.

In the D_2O studies solvent suppression was achieved by using a 1331 pulse sequence as described elsewhere[9]. The internal standard was 2,2–dimethyl–5–sulphonate (DSS).

The LD, CD and fluorescence measurements were carried out in a buffer containing 0.01 M NaCl and 0.001 M sodium cacodylate adjusted to pH 7.

Results

NMR ***The iminoprotons.*** We have recorded the imino proton resonances as a function of total concentration of added, enantiomerically pure, metal complex at 2 and 25 ^0C. The data shows that the $[Ru(phen)_3]^{2+}$/oligonucleotide system is in fast

Table 1: Change in oligonucleotide chemical shifts(ppm) when adding $[Ru(phen)_3]^{2+}$ to $d(CGCGATCGCG)_2$.
The metal complex/duplex ratio (R) was 2.0.

	Oligonucleotide proton	δ at R = 0	isomer	Change in δ	upfield/ downfield
Imino protons	GC1, GC2	13.05	Δ	0.08	u
			Λ	0.11	u
	GC3	12.92	Δ	0.13	u
			Λ	0.10	u
	GC4	12.75	Δ	0.26	u
			Λ	0.12	u
	AT5	13.57	Δ	0.58	u
			Λ	0.33	u
Aromatic protons					
(Major groove)	A5H8	8.18	Δ	0.12	d
			Λ	0.06	d
	T6H6	7.14	Δ	0.04	u
			Λ	0.03	d
(Minor groove)	A5H2	7.75	Δ	0.31	u
			Λ	0.62	u
Sugar protons					
(Minor groove)	A5H1′	6.24	Δ	0.11	u
			Λ	0.45	u
	G2H1′	6.16	Δ	0.16	u
			Λ	0.32	u

chemical exchange on the NMR time scale, at the higher temperature. Thus only one set of the oligonucleotide signals was observed in the titration experiment. At 2 °C, though, all the imino proton resonances are resolved. Henceforth, only the measurements performed at 25 °C, with a metal complex/duplex ratio of 2.0, will be discussed.

For both enantiomers the AT imino proton resonances are the most markedly perturbed (Table 1), with Δ showing a significantly larger upfield shift than Λ (0.58 ppm and 0.33 ppm, respectively). At this mixing ratio we also observed an upfield chemical shift of the GC4 imino proton (0.26 ppm for Δ, 0.12 ppm for Λ). Upfield shifts of about 0.1 ppm for the GC1, GC2 and GC3 imino protons, and some broadening of the GC1 resonances are also observed.

Both enantiomers of $[Ru(phen)_3]^{2+}$ clearly interact with the oligomer, particularly at the central AT base pairs, and Δ seems to be closer to the core of the oligonucleotide helix. However, the upfield shifts and the broadening of the imino

proton resonances are quite moderate (intercalators typically give large upfield shift of 1.0 ppm[10] and broadening of the resonances of the protons near the binding site) and could be accounted for by a binding model where the metal complexes reside in one of the grooves near the hydrogen bonds of the base pairs. Using only the imino proton spectra it is difficult to identify in which groove the interaction is taking place.

The aromatic protons. The oligonucleotide was titrated with Δ– and Λ–$[Ru(phen)_3]^{2+}$ in deuterated phosphate buffer (pD 7.0) at 25 °C. Again we found only one set of proton resonances. In the aromatic region of the spectra (6.8–8.6 ppm) the A5H2 resonance (located in the minor groove) is the most significantly perturbed for both enantiomers (Table 1). On the other hand, the non–exchangeable protons falling in the major groove experience relatively small perturbations, typically around or below 0.1 ppm. This suggests that the interaction between the metal complexes and the oligomer takes place in the minor groove. The resonances of the A5H2 protons are more strongly upfield shifted for the Λ enantiomer than for Δ (0.62 and 0.31 ppm, respectively). The major groove protons also show differences for the two enantiomers: e.g. T6H6 is shifted 0.04 ppm upfield for Δ and 0.03 ppm downfield for Λ, showing that even if they both reside in the minor groove their binding modes are different.

The sugar protons. The changes in the sugar proton H1′ resonances are interesting. In the presence of Λ, the A5H1′ and G2H1′ protons are more upfield shifted (0.45 and 0.32 ppm, respectively) than when Δ is present (0.11 and 0.18 ppm, respectively). Since the H1′ protons fall in the minor groove this is again evidence that both Δ and Λ bind in the minor groove of the oligonucleotide. The difference between Δ and Λ might indicate that the Δ enantiomer is associated somewhat deeper into the groove than the Λ enantiomer.

Flow linear dichroism, LD. In an earlier paper we have shown that both enantiomers of $[Ru(phen)_3]^{2+}$, when added to natural DNA as well as polynucleotides, exhibit LD in the visible region of the spectrum[1]. This observation demonstrates that the metal complexes become oriented through association with DNA. Changing the nucleotide concentration in a sample with a fixed $[Ru(phen)_3]^{2+}$ concentration results in isosbestic points in the LD[1], indicating that over a wide range of binding ratios a single effective binding geometry predominates for each of the enantiomers. A similar isosbestic behaviour was found for salt titration when the amount of associated complex was successively decreased by increasing the ionic strength. The same spectral features were found for each of the Δ and Λ adducts with calf thymus DNA, AT and GC.

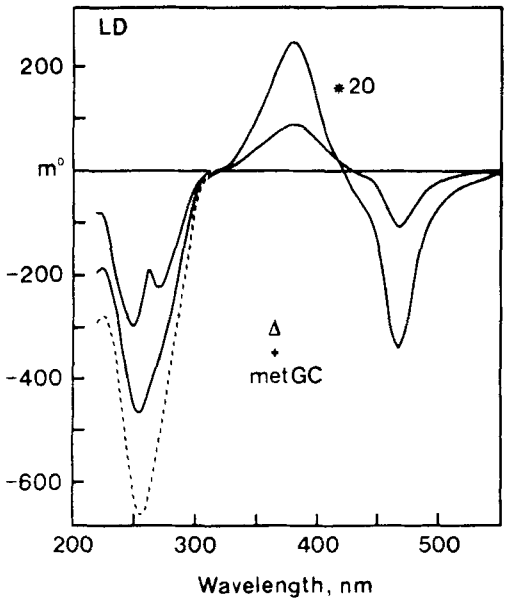

Figure 1. LD spectra of Δ– and Λ–$[Ru(phen)_3]^{2+}$ in the presence of $[poly(dG–me^5dC)]_2$ (full lines). The broken curve show the LD of pure polynucleotide. The total concentrations of metal complex were 10 μM (lower intensity in the visible region) and 40 μM (structured spectrum in the UV region). The polynucleotide phosphate concentration was 500 μM. 1 m° corresponds to $3.03 \cdot 10^{-5}$ absorbance units. Optical path length 0.1 cm.

From the reduced linear dichroism, $LD^r = LD/A_{iso}$, geometric information was obtained about these binding modes, corresponding to Δ being oriented with its three–fold symmetry axis at on the average 70 degrees with respect to the DNA helix axis, and Λ at 54 degrees.

Fig. 1 shows the LD spectrum recorded for Δ–$[Ru(phen)_3]^{2+}$ bound to metGC. If Fig. 1 is compared to Fig. 4a of Ref. 1, where the corresponding spectrum of Δ–$[Ru(phen)_3]^{2+}$ bound to GC was presented, it is obvious that the methyl–group in the major groove of GC does not significantly affect the shape of the LD spectrum. Therefore, the effective binding geometry of the metal complex is the same with the two polynucleotides. Interestingly, the fluorescence intensities of the

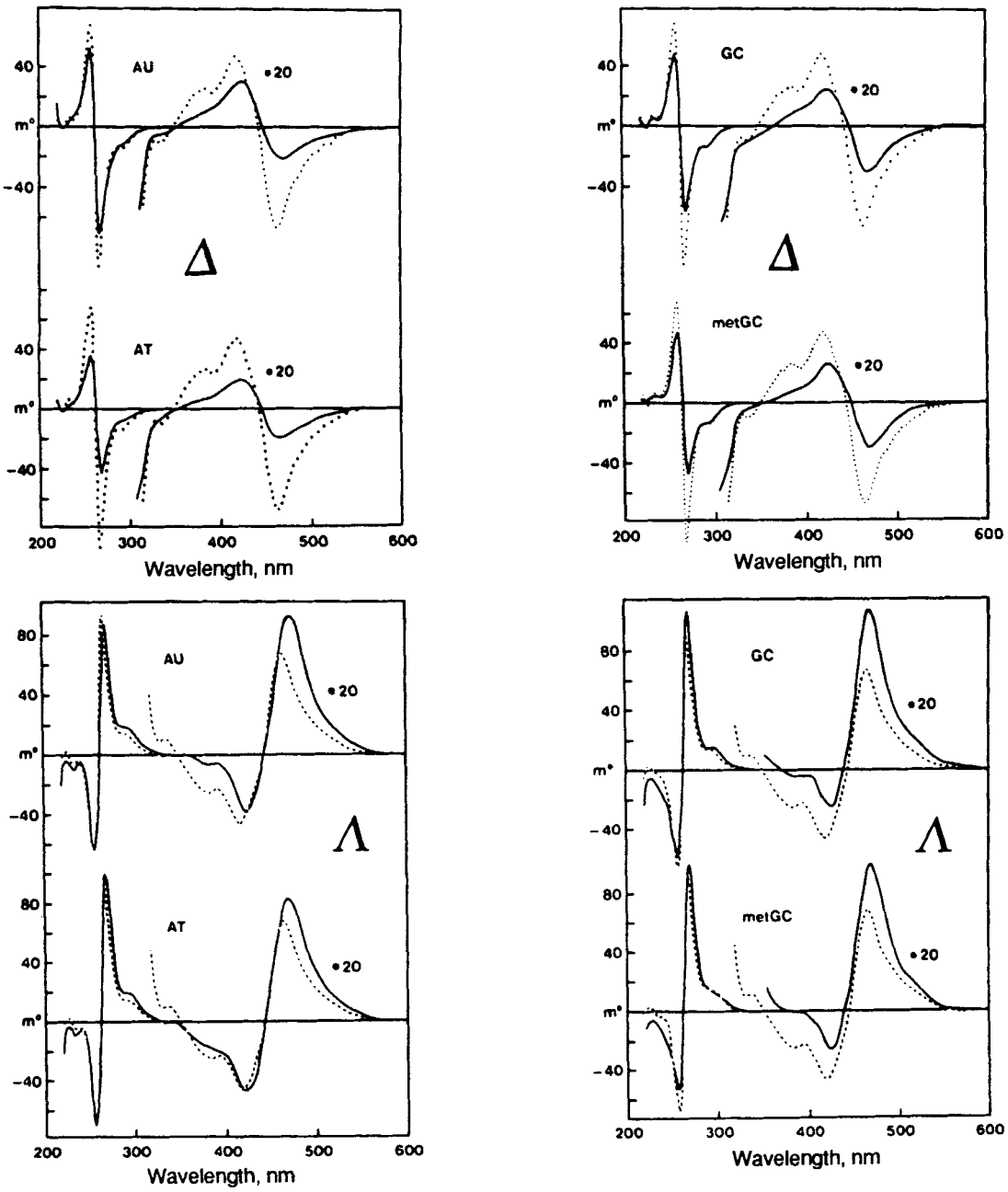

Figure 2. CD spectra of Δ— and Λ—$[Ru(phen)_3]^{2+}$ with different polynucleotides (full curves). Indicated in the figures are the respective combinations of enantiomer and polynucleotide. Contributions from DNA have been subtracted. The total concentrations of $[Ru(phen)_3]^{2+}$ and polynucleotide phosphate were 10 and 500 μM, respectively, in all cases. Broken lines show the CD spectra of the free metal complex at the same total concentration. Optical path length 0.5 cm.

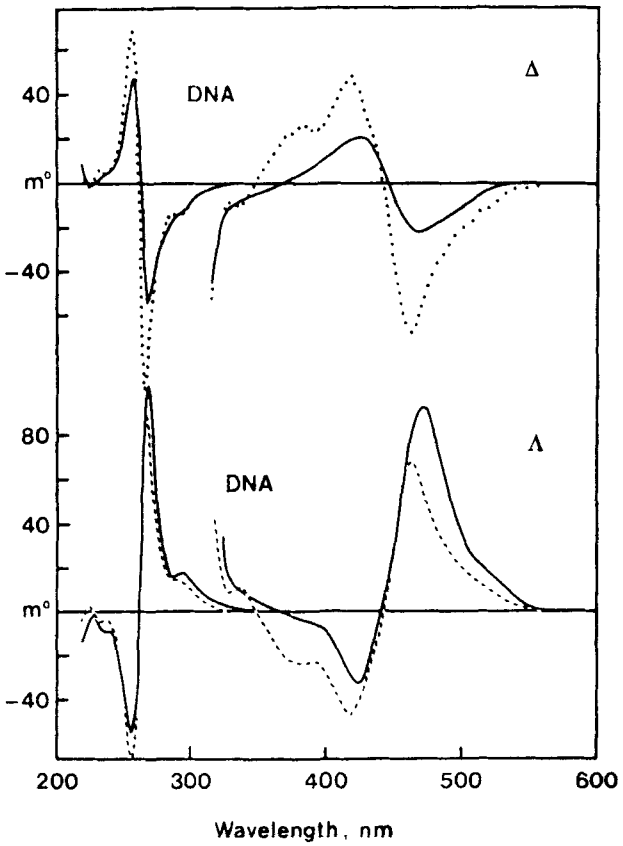

Figure 3. CD spectra of Δ— and Λ—$[Ru(phen)_3]^{2+}$ in the presence of calf thymus DNA. All concentrations and other conditions were as in Figure 2.

corresponding complexes with methylated and normal GC are markedly different (vide infra).

Circular dichroism, CD. The CD in the charge–transfer bands of the metal complexes change as a result of structural or electronic perturbation by the DNA. In Figures 2 and 3 we have subtracted the DNA–CD from the measured CD, assuming that the interaction between DNA and ligand is sufficiently weak for this to be feasible. 2D NOESY results indicate that the oligomer is in B–form conformation also after adding the metal complex.

The changes are numerically similar but of different signs for the different bands in the two isomers: for Δ the CD intensity of both the short (420 nm) and

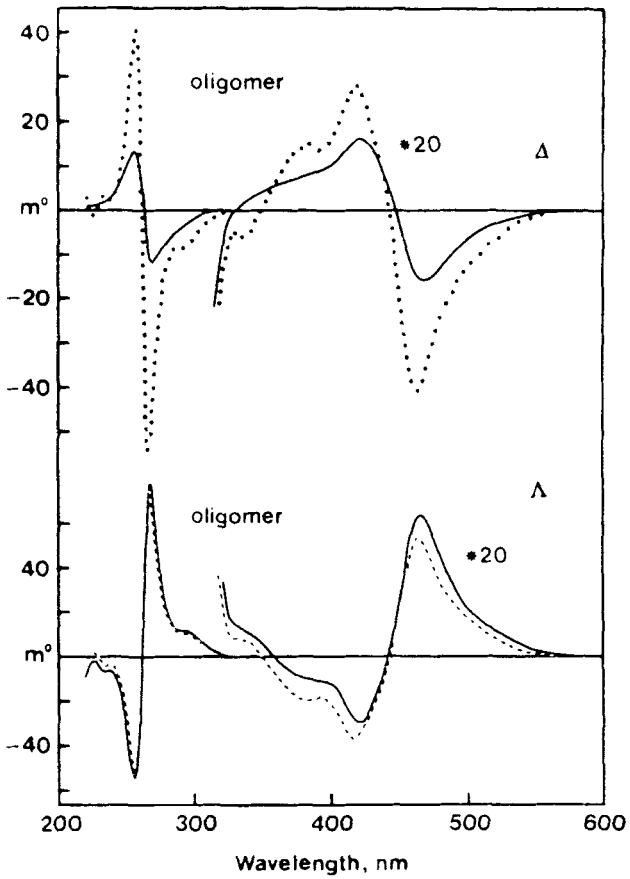

Figure 4. CD spectra of Δ− and Λ−$[Ru(phen)_3]^{2+}$ with the oligomer $(CGCGATCGCG)_2$. The oligomer phosphate concentration was 580 μM and the total concentrations of Δ− and Λ−$[Ru(phen)_3]^{2+}$ were 30 and 40 μM, respectively. Optical path length 0.2 cm. Other conditions were as in figure 2.

long (460 nm) wavelength bands decreases upon binding to natural DNA, AT, GC as well as AU and metGC, while the Λ isomer displays a decrease (420 nm) and a more marked increase (460 nm) when binding to any of the polynucleotides. Importantly, the same behaviour is observed when the isomers are bound to the studied oligonucleotide (Fig. 4).

The CD behaviour supports the conclusion from the LD experiments about two, somewhat different binding geometries for the two enantiomers of the metal complex when associated to DNA, and that the same two effective

binding geometries are found in the different double–helical polynucleotides, as well as with the oligonucleotide subject to NMR investigation. The latter link between oligonucleotide and polynucleotide is important as this indicates that the conclusion from the NMR results should apply to the polynucleotide systems as well.

For the pure oligonucleotide the CD was effectively unchanged in the concentration range 0.03 to 0.6 mM with no indication of hair–pin formation.

Fluorescence. Figure 5 shows the F/F_0 ratio for the two enantiomers of $[Ru(phen)_3]^{2+}$ (where F_0 is the fluorescence intensity in the absence of polynucleotide) at increasing GC and metGC concentrations. The Λ enantiomer shows essentially the same behaviour when interacting with GC or metGC. It is known[1] that Λ binds less strongly to GC and the results in Figure 5 support that this is also the case with metGC.

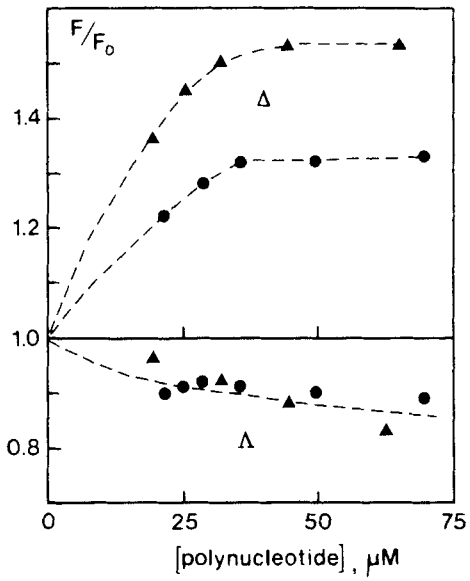

Figure 5. Changes in emission intensities for Δ– and Λ–$[Ru(phen)_3]^{2+}$ when titrating with $[poly(dG–dC)]_2$ (▲) or $[poly(dG–me^5dC)]_2$ (●). The total concentration of metal complex was 2 μM. λ_{exc} = 460 nm, λ_{em} = 600 nm, slit widths 5 nm.

By contrast, upon interaction with the two polynucleotides Δ shows markedly different fluorescence intensities. This result suggests that Δ has a lower binding affinity to metGC or that it is bound in a less polar environment in this polynucleotide.

Discussion

The NMR results indicate similar modes of binding for the two enantiomers of the $[Ru(phen)_3]^{2+}$ complex to the duplex $d(CGCGATCGCG)_2$, with preference for the central AT base pair. The observation of overall smaller chemical shifts for protons located in the major groove than for protons in the minor groove, is a strong indication that the binding preferentially occurs in the minor groove. We cannot, however, exclude that this may be an indirect effect of conformational changes of the oligomer upon association of the metal complex in the major groove. The AH2 proton displays a stronger upfield shift for the Λ enantiomer than for Δ, but the

difference is difficult to relate to variations in binding geometry. We may apply what we know from the LD results about the orientation of the enantiomers and transfer the suggested binding geometries[1], for location in the major groove, to the minor groove. If we ignore asymmetry due to base sequence, DNA has a 2–fold rotation axis perpendicular to the helix axis. A likely preferred binding geometry is then one where a 2–fold axis of the metal complex aligns with this DNA dyad axis. There are two possible binding geometries consistent with this symmetry: one with one wing in the groove and the other with two wings in the groove. One possibility is that Δ adopts one of these binding geometries and Λ the other as suggested in Ref. 1.

The behaviour of the Λ enantiomer is essentially that of a minor groove binder, with preference for AT regions and with a stabilizing effect on the DNA helix. The binding of the Δ enantiomer seems to require a more widened groove, giving rise to increased DNA flexibility as evidenced from the LD[1].

The fluorescence results can be interpreted to suggest that Δ displays some major groove binding in GC since the binding is affected by the introduction of the methyl group into the major groove. However, the fact that the LD and CD spectra for Δ with GC, metGC, AT and calf thymus DNA indicate essentially the same binding geometry, irrespective of base pair composition or methylation, suggests that Δ binds exclusively in one of the grooves. Another feasible explanation of the fluorescence results is then that the presence of the methyl group indirectly, through a slightly changed DNA structure, decreases the affinity of the Δ enantiomer for metGC. Λ shows less binding affinity in both cases (metGC and GC), again a behaviour consistent with minor groove binding for this enantiomer. Unfavourable steric interactions with the exocyclic amino groups of guanine in the minor groove decreases the binding affinity for Λ to this polynucleotide.

Acknowledgement This study was supported by the Swedish Natural Science Research Council, Magnus Bergwalls Stiftelse and Biokemifonden at the University of Umeå.

References

1. Hiort, C.; Nordén, B.; Rodger, A. *J. Am. Chem. Soc.* **1990**, *112*, 1971–1982
2. Härd, T.; Nordén, B. *Biopolymers* **1986**, *25*, 1209–1228
3. Nordén, B.; Tjerneld, F. *FEBS Lett.* **1976**, *67*, 3, 368–370
4. Barton, J. K. *J. Biomol. Struct. Dyn.* **1983**, *1*, 621–632
5. Barton, J. K.; Danishefsky, A. T.; Goldberg, J. M. *J. Am. Chem. Soc.* **1984**, *106*, 2172–2176

6. Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518–5523
7. Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 2081–2088
8. Barton, J. K. *Science* **1986**, *233*, 727–734
9. Hore, P. J *J. Magn. Reson.* **1983**, *55*, 283–300
10. Feigon, J.;Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450–465