Stereodifferentiation and base-pair selectivity in the binding of Δ and Λ cationic lanthanide complexes to $[(CG)_6]_2$, $[(AT)_6]_2$ and CT-DNA

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Metal- and ligand-based luminescence, absorption and circular dichroism difference spectroscopy signal the extent and nature of binding of enantiopure Δ and Λ cationic lanthanide complexes to $[(CG)_6]_2$ and $[(AT)_6]_2$.

Whilst there have been many reports probing the interaction of the aqua lanthanide ions with nucleotides and DNA using NMR[1] and luminescence methods,[2] no studies have appeared in which the binding of well defined chiral lanthanide complexes has been defined. Following the characterisation of the cationic, enantiomeric lanthanide complexes of ligands (*RRR*)-MeL[1a] and (*SSS*)-MeL[1b],[3] we report herein preliminary binding studies with calf-thymus DNA and the oligonucleotides $[(CG)_6]_2$ and $[(AT)_6]_2$.† These lanthanide complexes possess complementary properties allowing their interaction with chiral polyanions to be studied, including the absorption and fluorescence emission behaviour of the phenanthridinium unit and the intensity, polarisation and lifetime of the lanthanide emission.[4]

The absorption spectrum of each complex was monitored as a function of added oligonucleotide or DNA, at pH 7.4 in low added salt (Fig. 1 and Table 1). For the complexes with $[(CG)_6]_2$ and calf thymus (CT)-DNA isosbestic points were observed at 302 and 378 nm, with pronounced hypochromism in the bands at 320 and 370 nm and a red-shift, accompanied by formation of a long wavelength tail beyond 400 nm. For complexes with $[(AT)_6]_2$, changes in intensity were much smaller and the long wavelength tail and isosbestic point at 378 nm were absent. The observed hypochromism and concomitant red shifts are often associated with intercalative binding,[5] and are consistent here with a preferred charge transfer interaction involving the more electron-rich C and G bases and the positively-charged phenanthridinium unit.

The fluorescence of the *N*-alkylphenanthridinium moiety was monitored at 408 nm, as a function of added DNA, following excitation at the isosbestic point. Quenching of fluorescence was observed, as is often found when planar aromatic fluorophores bind to DNA, arising from charge transfer-catalysed non-radiative deactivation of the singlet excited state. Quenching of the long-lived europium emission (*e.g.* at 594 nm) was also observed and mirrored the decrease in the fluorescence intensity. No significant change in the lifetime of the metal emission was observed, from its value of 0.55 ms in water in the absence of DNA,[4] ruling out any direct quenching of the metal excited state by photoinduced electron transfer from the DNA bases.

Intrinsic binding constants, *K*, and site sizes *n*′ (per mole of duplex) were estimated using the method of McGhee and von Hippel[6] (Table 1). Values obtained for the model intercalators ethidium bromide and *N*-ethylphenanthridinium iodide were measured under the same conditions. Strongest binding of the Eu complexes was observed to $[(CG)_6]_2$ with one complex bound per three base pairs on average, and the Δ isomer bound slightly more strongly than the Λ. With Δ-$[EuL^{1a}]^{4+}$, binding affinity to $[(AT)_6]_2$ was over 50 times weaker than to $[(CG)_6]_2$ (with a reduced site density, suggestive of a weaker intercalative interaction) , and in this case the Λ isomer bound more strongly ($\Delta\Delta G$ = 4 kJ mol^{-1}). The effect of increasing the salt concentration (10, 50 and 100 mM NaCl) was also examined for

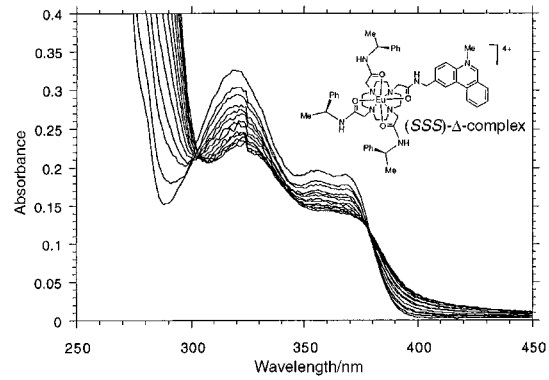

Fig. 1 UV-absorbance spectra for (*SSS*)-Δ-$[Eu(MeL^{1b})]^{4+}$ (23.6 μM) upon addition of increasing concentrations of $[CG)_6]_2$ (0–12.9 μM) in buffered aqueous solution (HEPES 10 mM, pH 7.4, NaCl 10 mM, 293 K).

Table 1 Intrinsic binding constants (*K*), site sizes (*n*′) and degree of hypochromism measured for complexes of $[(CG)_6]_2$ and $[(AT)_6]_2$ with Δ$[LnL^{1a}]^{4+}$ and Λ$[LnL^{1b}]^{4+}$ (298 K, 10 mM HEPES pH 7.4, 10 mM NaCl)

Complex	10^{-5} K^a/dm^3 mol^{-1} duplex^{-1}	n'^a/(duplex)$^{-1}$	% hypochromism at 320 (370) nm
Δ-$[EuL^{1a}]^{4+}$/$[(CG)_6)]_2$	87	3.98	35 (27)[c]
Λ-$[EuL^{1b}]^{4+}$/$[(CG)_6]_2$[b]	36	3.96	33 (28)[c]
Δ-$[EuL^{1a}]^{4+}$/$[(AT)_6]_2$	1.6	1.52	13 (10)
Λ-$[EuL^{1b}]^{4+}$/$[(AT)_6]_2$	8.0	1.82	15 (12)
N-ethylphenanthridinium iodide/$[(AT)_6]_2$	< 0.3	n.d.	11 (10)
ethidium bromide/$[(CG)_6)]_2$	31	2.26	n.a.
N-ethylphenanthridinium iodide/$[(CG)_6)]_2$	6.8	3.88	32 (10)

[a] Typically [LnL] = 10–20 μM; data from luminescence intensity changes[d] with added oligonucleotide were analysed by the method of McGhee and von Hippel;[6] an approximate analysis of the more limited CD data gave similar values. [b] In 50 mM NaCl, $K = 7.2 \times 10^5$ and $n' = 2.0$ and in 100 mM NaCl, $K = 2.8 \times 10^5$ ($n' = 1.9$). [c] Very similar spectra were observed for Δ/Λ-Eu complexes binding to calf-thymus DNA (42% CG). [d] Limiting changes in fluorescence intensity ratio (I_{free}/I_{bound}) were 55 for Δ-Eu/$[(CG)_6]_2$ and 2 for Λ-Eu/$[(AT)_6]_2$.

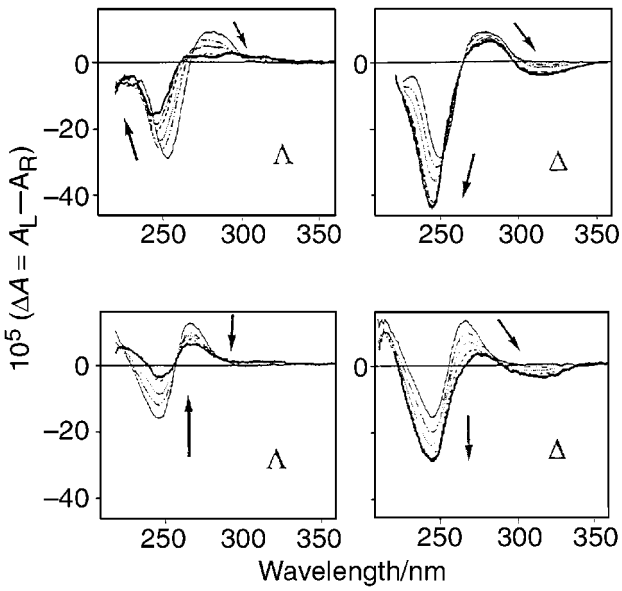

Fig. 2 Near-UV region CD spectrum of $[(CG)_6]_2$ (upper) and $[(AT)_6]_2$ (lower) in buffered aqueous. solution (10 mM HEPES, NaCl 10 mM) and in the presence of increasing ratios of Λ(0, 0.50, 0.99, 1.96, 2.91, 4.76, 7.41) and Δ-$[EuL^1]^{4+}$ (0, 0.49, 0.97, 1.92, 2.86, 3.77, 4.67) (left and right respectively). In each case, the CD spectrum of the complex itself at the same concentration has been subtracted.

Λ-$[EuL^{1b}]^{4+}$ /$[(CG)_6]_2$: only modest reductions in overall affinity were found (Table 1), ruling out the possibility of a predominantly electrostatic binding interaction.

The interaction of the europium complexes with the oligonucleotides has also been examined by circular dichroism difference spectroscopy, adding the complex to a fixed concentration of the oligonucleotide. In the near-UV region, the CD spectra of $[(CG)_6]_2$ and $[(AT)_6]_2$ show the features characteristic of *B*-DNA;[7] these gross features persisted even after addition of a substantial excess of the Eu complexes (Fig. 2) ruling out the $B \rightarrow Z$ transition which can occur in the presence of a highly charged metal complex.[2] In the 220–260 and 290–340 nm regions, the CD changes for a given oligonucleotide were markedly dependent upon the chirality of the complex, and changed in the opposite sense for each pair of diastereoisomeric complexes. In particular, in the 290–340 nm region, the observed changes for the Λ isomer titration were at least 50% less than those for the Δ isomer. Control experiments, examining CD difference spectra following addition of the tripositive C_4 symmetric parent complexes (*i.e.* lacking the phenanthridinium moiety) to $[(CG)_6]_6$ and $[(AT)_6]_2$, revealed only *very* small changes in ellipticity (< 6% of the change seen with $[EuL^{1a}]^{4+}$). The difference CD spectra obtained with $[EuL^{1a}]^{4+}$ and CT-DNA were almost identical to those obtained with $[(CG)_6]_2$.

In the visible region (Fig. 3), the CD signals arise exclusively from the phenanthridinium chromophore. For the unbound enantiomeric Eu complexes, weak, mirror image CD spectra were observed in this region. Upon binding to both $[(CG)_6]_2$ and to $[(AT)_6,]_2$, a positive induced CD at *ca.* 370 nm was observed for each enantiomer and qualitatively similar changes were noted with increasing oligo concentration. This suggests that the chirality of the oligonucleotide binding site must be similar for both $[(CG)_6]_2$ and $[(AT)_6]_2$ as the induced CD is *independent* of complex handedness.

The stoichiometry of the oligonucleotide–Eu complex binding was further investigated using the method of continuous variation, by plotting the CD intensity at a single wavelength *vs.* the mole fraction of the complex. Discontinuities at 0.5 and 0.67

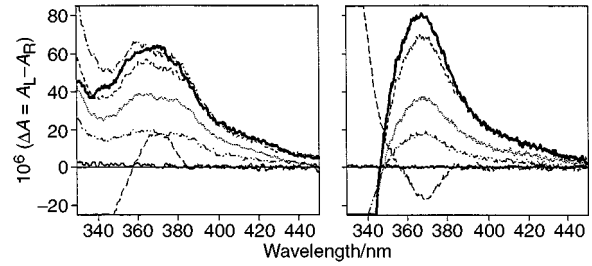

Fig. 3 Visible region CD spectra of $[(CG)_6]_2$ in the presence of increasing ratios of the Λ (left) and Δ (right) enantiomers of the Eu complex (Λ ratios shown are 0.50, 0.99, 1.96, 2.81 and 7.1; Δ: 0.49, 0.98, 1.92, 2.83), showing the long wavelength region where signals arise only from the phenanthridinium group. The CD spectrum of the complex at the same concentration has been subtracted in each case; spectra of the complexes in the absence of DNA are shown for reference (---------).

were found, consistent with stepwise formation of 1:1 and 2:1 complexes for both $[(AT)_6]_2$ and $[(CG)_6]_2$ binding.

In conclusion, the absorbance and fluorescence changes of the phenanthridinium moiety upon binding to $[(CG)_6]_2$ and CT-DNA, together with the relatively weak induced CD at 370 nm, are all consistent with a binding interaction predominantly involving intercalation. Binding to $[(AT)_6]_2$ is over 50 times weaker with Δ-$[EuL^{1a}]^{4+}$ and any intercalation is much less pronounced; the very similar spectral (CD, absorbance) changes observed with CT-DNA (42% CG) accord with this base-pair selectivity. AT-Rich sequences are believed to favour metal complex binding at the minor groove when the handedness of the complex is complementary to that of the right-handed helix.[8] Thus the stereodifferentiation observed in the interaction of the Eu complexes with $[(AT)_6]_2$ could arise from preferential minor-groove binding of the **Λ** isomer.

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Notes and references

† $(CG)_6$ is an abbreviation for $(dC–dG)_6$, which exists as a duplex in aqueous media.

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