DNA binding studies of cationic lanthanide complexes bearing a phenanthridinium group

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The binding of tetracationic Λ- and Δ-europium complexes of tetraamide and triamide ligands incorporating an *N*-methylphenanthridinium group to calf-thymus DNA, poly(dGdC) and super-coiled plasmid DNA has been monitored by absorption, luminescence and CD difference spectroscopy. With the tetraamide complexes, preferential binding to (CG) base-pairs was signalled by pronounced hypochromism, a small red shift and substantial fluorescence quenching with a limiting base-pair complex ratio of 2 : 1, consistent with an intercalative interaction and charge neutralisation. For the triamide Eu complex, a lower base-pair : complex ratio was found and changes in the form and polarisation of the Eu emission spectrum were consistent with coordination of a nucleobase phosphate group to the hydrated Eu centre.

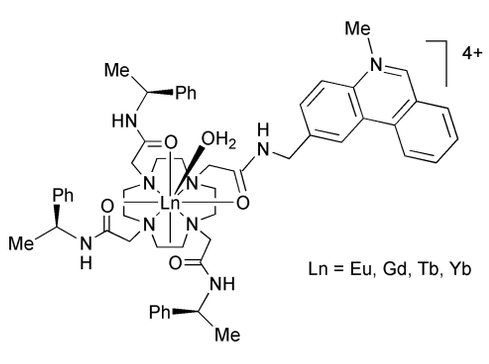
Over the past twenty years there has been intense interest in studying the interaction between well-defined chiral octahedral complexes of the d-block elements and nucleic acids.[1,2] The structural rigidity and chemical diversity of the ground and excited states of these cationic complexes, such as $[Ru(phen)_3]^{2+}$, makes them attractive systems to probe the site- and stereo-selectivity of the binding with the different nucleic acid types, *e.g.* A, B and Z-DNA. Their utility is enhanced by the sensitivity of the strong metal–ligand charge transfer bands to the local solvation and electronic environment.[3,4] With many complexes that bind to high MW DNA, only the amount of bound complex is readily determined by spectroscopic methods. However if the *bound* complex is chemically reactive, then biochemical methods may be applied to determine the position of DNA scission and, by inference, the local site of binding. Examples include complexes which sensitise singlet oxygen formation, although the rather long mean-free path of this reactive species and its tendency to lead to selective oxidation of GG states[5] restrict the utility of this method. Complexes which promote the hydrolytic cleavage of nucleic acids may be of greater intrinsic interest as the site of cleavage is expected to trace faithfully the site of complex binding.[6]

Although the aqua lanthanide ions have been examined in detail as luminescent and NMR probes[7–10] for nucleic acids, until very recently[11] the absence of structurally rigid, well-defined kinetically stable cationic complexes meant that very little work had been reported on the binding of chiral f-block complexes to nucleic acids. Lanthanide complexes offer considerable diversity of function in this respect by simple variation of the Ln ion. Thus, redox-active (Ce, Eu), luminescent (Eu, Tb, Nd, Yb), relaxation (Gd) or chemical shift (Dy, Tm) probes may readily be obtained. The introduction of cationic tetraamide complexes—analogues of which were first studied by Morrow and co-workers as potential nucleic acid cleavage agents[12]—represents the first step in the development of a new family of nucleic acid probes. The preceding paper[13] introduced $[Ln\cdot\mathbf{1}]^{4+}$, for which the Δ- and Λ-isomers are available, possessing approximate C_4 symmetry about the Ln centre in a mono-capped square-antiprismatic coordination geometry. Here, we report the binding properties of this complex to calf-thymus DNA (42% GC), poly(dGdC) and super-coiled plasmid DNA (53% GC), and also examine the interaction of the 'coordinatively unsaturated' analogue $[Ln\cdot\mathbf{2}]^{4+}$, which possesses up to two displaceable water molecules in aqueous media.[14] This complex has been shown to bind reversibly to hydrogenphosphate anions to generate a ternary complex in which the phosphate acts as a monodentate ligand and in which a proximate water molecule remains bound to the Ln centre.[14] Such features offer scope for hydrolytic activity,[6] in principle.

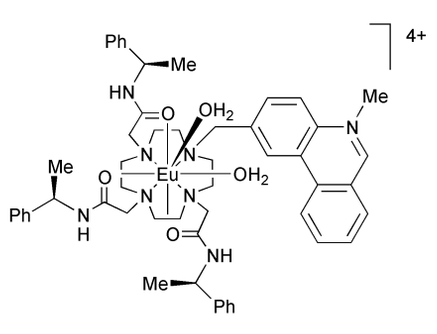
Results and discussion

Absorption and emission spectroscopy

Changes in the absorption spectrum of Δ- and Λ-$[Ln\cdot\mathbf{1}]^{4+}$ (Ln = Eu, Tb) were monitored as a function of added calf-thymus



(*SSS*)-Δ-$[Ln\cdot\mathbf{1}]^{4+}$



(*RRR*)-Λ-$[Eu\cdot\mathbf{2}]^{4+}$

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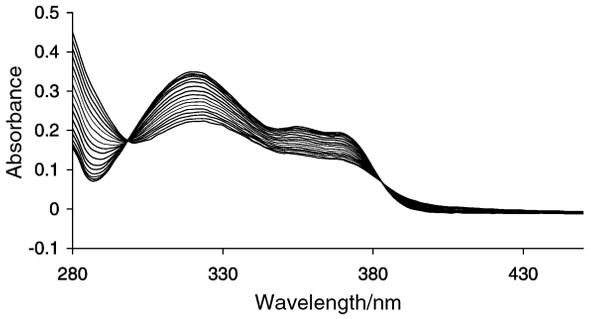

Fig. 1 Absorption spectra for Λ-[Eu·**1**]$^{4+}$ following addition of poly-(dGdC) showing the hypochromism (370, 320 nm), modest red shift and isosbestic points (383 and 298 nm), [pH 7.4, 295 K, 10 mM HEPES, 10 mM NaCl].

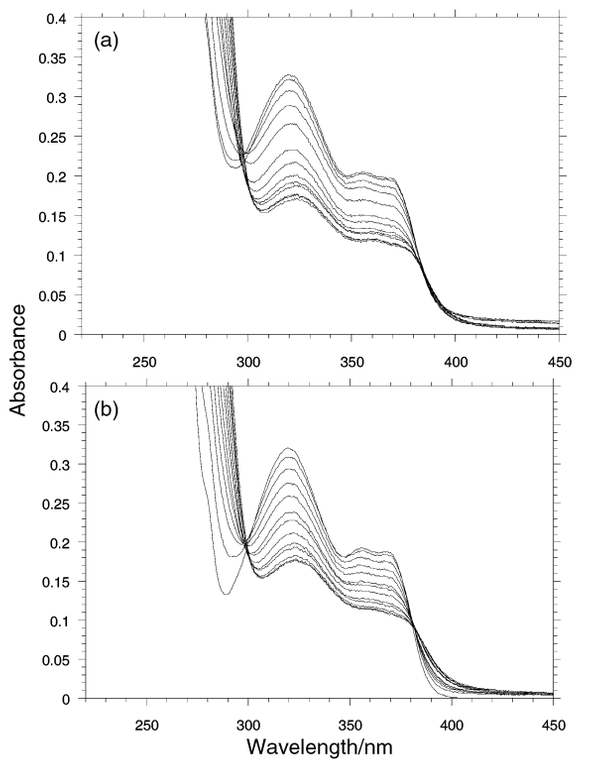

Fig. 2 Changes in the absorption spectra of Δ-[Eu·**1**]$^{4+}$ (a) and the Λ-isomer (b) following addition of super-coiled plasmid DNA (53% GC) (pH 7.4, 10 mM HEPES, 10 mM NaCl, 295 K).

DNA. The bands at 320 and 370 nm shifted slightly to the red and decreased in intensity by 34 and 28% (Δ) and 31 and 29% (Λ) respectively. A significant tailing absorption band appeared in the range 390 to 420 nm and well-defined isosbestic points were apparent at 378 and 303 nm. Very similar behaviour was observed with poly(dGdC) with slightly lower % hypochromism (Fig. 1). The variation of observed hypochromism was plotted as a function of the number of base-pairs per complex and reached a limiting value at a base-pair : complex ratio of 2 : 1 for poly(dGdC) and closer to unity for calf-thymus DNA (42% GC rich). With the plasmid DNA (pEG1acZ containing 53% GC base pairs),[15] more marked hypochromism was obtained. With the Δ-isomer, a clearly defined isosbestic point near 380 nm was not discerned and the long-wavelength tail was also not so apparent. The percentage hypochromism was *ca.* 50% at 370 nm and 41% at 320 nm; for the Λ-isomer a very similar degree of hypochromism was noted (Fig. 2), but isosbestic points at 298 and 379 nm were much more well-defined. These absorbance changes resemble those observed with $[(CG)_6]_2$:[13] in each case examined, the Λ-isomer gave the better defined isosbestic point at *ca.* 378 nm and gave rise to the more intense long-wavelength tail absorption. Such behaviour suggests that

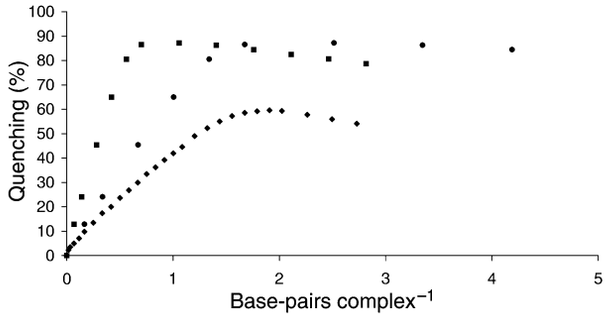

Fig. 3 Degree of quenching of phenanthridinium fluorescence (λ_{exc} 378 nm, λ_{em} 406 nm) as a function of DNA base pairs per Eu-complex for Λ-[Eu·**1**]$^{4+}$, following addition of CT-DNA (■) and poly(dGdC) (◆). The circles (●) represent values for CT-DNA after correcting for 42% (GC) content (295 K, pH 7.4, 10 mM HEPES, 10 mM NaCl).

the phenanthridinium moiety is undergoing a strong charge-transfer interaction with the GC base-pairs, presumably *via* an intercalative interaction[2] in which the Λ-isomer binds in a more 'well-defined' manner.

With the model oligonucleotides $[(CG)_6]_2$ and $[(AT)_6]_2$, it was shown that charge-transfer mediated deactivation of the phenanthridinium singlet excited state occurred up to 20 times more efficiently for GC base-pairs. Therefore, variations in the observed fluorescence quenching will tend to be dominated by the interaction of the complex with GC base-pairs. Incremental addition of CT-DNA or poly(dGdC) to Λ-[Eu·**1**]$^{4+}$ gave rise to 90 and 59% quenching of phenanthridinium fluorescence (I_o/I), in the limit, respectively. Parallel variations in Eu-emission intensity were observed, with no change in the form of the spectrum nor in the measured emission lifetime (0.55 ms, H_2O, λ_{exc} 397 or 579 nm). Plotting the % quenching *versus* base-pairs per complex, (Fig. 3), revealed a limit at a ratio of 2 : 1 for poly(dGdC). This limit was the same for CT-DNA after allowing for the 42% GC content, *i.e.* disregarding any quenching contribution from AT base-pairs. Such a limit is consistent with charge neutralisation and with the neighbour exclusion principle that states that an intercalator can bind at most to alternate base-pair sites on DNA. A similar limiting ratio was reached for the Δ-isomer. The differing limits of quenching for CT-DNA *versus* poly(dGdC) suggest that the quenching of the phenanthridinium singlet excited state is strongly influenced by the presence of AT base-pairs. In related work described in the preceding paper, it was shown that in the model oligonucleotide $[(AT)_6]_2$, the Λ-isomer was apparently bound more strongly[13] whereas in the Drew–Dickerson oligonucleotide, $[CGCGAATTCGCG]_2$, NMR studies had revealed that the Δ-isomer bound more avidly to the central (AT) region.

Circular dichroism

Circular dichroism difference spectra were recorded following incremental addition of Δ- or Λ-[Eu·**1**]$^{4+}$ to CT-DNA and poly(dGdC). Spectra were corrected for dilution and the CD spectrum due to the free complex at the same concentration was subtracted to reveal changes associated with the bound complex alone. With CT-DNA, the observed CD difference spectra were very similar to those obtained with $[(CG)_6]_2$,[13] consistent with preferential localisation of the complex in proximity to CG base pairs. Similar behaviour was observed in binding to poly(dGdC) (Fig. 4). The DNA retained its B-form, although the positive band at 280 nm diminished in intensity. The binding of the Δ-isomer led to a 60% increase in intensity of the negative near-UV band, which also shifted by 6 nm to lower wavelength. A new negative band also appeared at 320 nm. This feature was not apparent with the Λ-isomer; in addition, the 250 nm band *decreased* in intensity by 40% and shifted to the blue by 5 nm, while the positive band at 280 nm decreased slightly in intensity and shifted to *ca.* 266 nm. Thus, significant

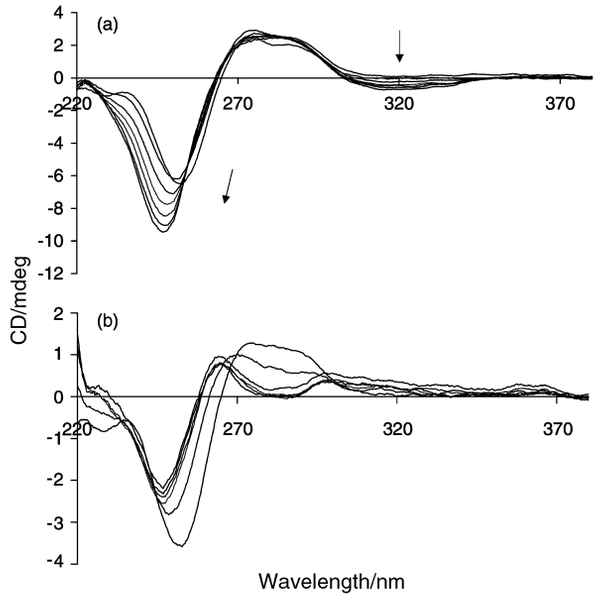

Fig. 4 CD difference spectra for poly(dGdC) following addition of Δ- (a) or Λ- (b) $[Eu{\cdot}\mathbf{1}]^{4+}$ (0.11, 0.22, 0.33, 0.44, 0.55, 0.66 complex per base-pair; 295 K, pH 7.4, 10 mM HEPES, 10 mM NaCl).

changes in the local helicity of the DNA host accompany complex binding.

Fluorescence lifetime and anisotropy measurements

Depolarisation of emission is primarily caused by rotational diffusion of fluorophores. Anisotropy measurements reveal the average angular displacement of the fluorophore that occurs between absorption and emission. For small fluorophores in non-viscous solutions, the rate of rotational diffusion is faster than the rate of emission. Consequently the emission is depolarised and the steady-state anisotropy tends to zero. If the fluorophore is associated with a more slowly tumbling macromolecule, its rotational motion decreases and the emission may retain some degree of polarisation, $r > 0$. Such behaviour has been used to substantiate intercalative binding, whereas no retention of polarisation is observed for surface-bound molecules.[16] The complex $[Eu{\cdot}\mathbf{1}]^{4+}$ was dissolved in increasing ratios of glycerol–H_2O and the emission intensities I_{VV}, I_{VH}, I_{HV} and I_{HH} (H and V refer to horizontally and vertically polarised excitation and emission respectively) were measured at 295 K. The resultant anisotropy factor r was calculated from eqn. (1).

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \qquad \text{where } G = \frac{I_{HV}}{I_{HH}} \qquad (1)$$

Values of r ranged from 0 (0% glycerol) to 0.13 (99.5% glycerol). At −60 °C in a frozen glass (75 : 25 glycerol–H_2O) a value of 0.34 was measured for the immobilised phenanthridinium fluorophore. However, near zero anisotropy was measured at 295 K for $[Eu{\cdot}\mathbf{1}]^{4+}$ bound to excess CT-DNA or poly(GC). The excited state lifetimes of the phenanthridinium fluorophore were also measured in the absence and presence of added poly(dGdC), varying the base-pair : complex ratio from 0 to 4. The decay curve fitted well to a single mono-exponential decay in each case, and gave values of the singlet lifetime of 2.9 ns for the free complex which reduced only very slightly to 2.5 ns at a base-pair : complex ratio of 4.

Thus, it is apparent from the fluorescence decay characteristics, from the absence of anisotropy in the bound species and from the lack of variation observed in the singlet state lifetime measurements that the bound complex is *not* emissive, and that the observed emission is due to residual unbound complex. This precludes further study of this system and means

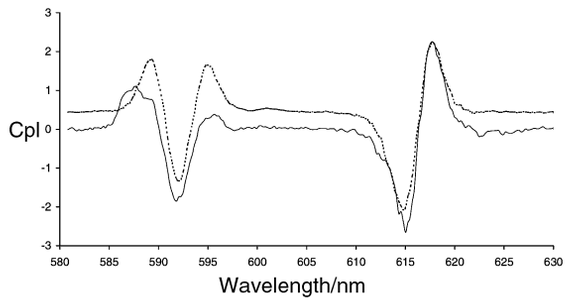

Fig. 5 Circularly polarised luminescence spectra for Λ-$[Eu{\cdot}\mathbf{2}]^{4+}$ in the presence of 10 equivalents of NaH_2PO_4 (---) and in the presence of poly(dGdC) [2 base-pairs per complex], showing the three components of the $\Delta J = 1$ transition around 590 nm and the $\Delta J = 2$ manifold around 615 nm (pH 7.4, 10 mM HEPES, 10 mM NaCl, 295 K).

that the terbium analogue, $[Tb{\cdot}\mathbf{1}]^{4+}$, notwithstanding the fact that it sensitises singlet oxygen formation with an overall quantum yield of 20%[17] (λ_{exc} 355 nm, 295 K, pH 7.4, 10 mM HEPES, 10 mM NaCl), cannot be used as a 'reactive' probe complex for DNA.

Behaviour of the triamide complex, $[Eu{\cdot}\mathbf{2}]^{4+}$

The spectroscopic behaviour of the triamide complex Λ-$[Eu{\cdot}\mathbf{2}]^{4+}$, possessing a phenanthridinium chromophore held much closer[18] to the metal centre and in which reversible anion ligation[14] may occur at the Ln centre, was studied in the presence of CT-DNA and poly(dGdC). Neither significant hypochromism nor a measurable red shift was observed in absorption spectral studies. Addition of DNA caused an almost complete (*ca.* 95%) quenching of the phenanthridinium fluorescence, with an apparent saturation limit of about 2.2 base-pairs per complex for CT-DNA (42% GC) and one base-pair per complex for poly(dGdC). Such behaviour is somewhat different from that found with Δ- or Λ-$[Eu{\cdot}\mathbf{1}]^{4+}$, for which a 2 : 1 limiting ratio was observed in each case, after correcting for GC content. However, the extent of quenching observed for $[Eu{\cdot}\mathbf{2}]^{4+}$, is again proportional to the GC content, suggesting that a *non-intercalative* charge transfer interaction is occurring with the more electron-rich G and C base-pairs. Observation of the form of the Eu emission spectrum revealed a significant change in the presence of DNA, that was highlighted in the corresponding CPL spectra (Fig. 5). The total emission and CPL spectrum (particularly the $\Delta J = 1$ and $\Delta J = 2$ patterns) closely resembled those obtained following addition of NaH_2PO_4[14] (pH 7.4, 295 K, 10 mM HEPES), strongly suggesting that the Eu ion is binding to the oligonucleotide phosphate group, and that this binding is associated with a concomitant non-intercalative binding interaction with CG base-pairs.

Summary and conclusions

The Δ- and Λ-tetraamide complexes, $[Ln{\cdot}\mathbf{1}]^{4+}$, bind preferentially to CG tracts in CT-DNA. The phenanthridinium chromophore is engaged in an intercalative interaction, but with little apparent stereoselectivity in binding affinity. In the bound form, the luminescence of the complex is completely quenched by a rapid charge-transfer mediated deactivation of the phenanthridinium S_1 state, limiting further developments of complexes based on this chromophore. With the related triamide europium complex, $[Eu{\cdot}\mathbf{2}]^{4+}$, intercalation of the phenanthridinium moiety is not apparent and the complex binds to the DNA *via* reversible ligation of the nucleotide phosphate group. The phenanthridinium fluorescence is also quenched efficiently by being brought into proximity with the GC base-pairs.

Therefore, in formulating improved luminescent lanthanide NMR and reactive probes for nucleic acids, attention is now focussed on complexes that possess a sensitising chromophore

that is not deactivated by charge-transfer from the more electron-rich base pairs. Such work is underway.

Experimental

Calf-thymus DNA and poly(dGdC) of the highest available purity were obtained from Sigma and were used as received. The super-coiled plasmid DNA was obtained from Dr Cath Caterall (Celltech Chiroscience, Slough, UK) [15] and was used as received. The lanthanide complexes were obtained as described in ref. 13 and absorption, luminescence, CPL and CD spectra were obtained as described in ref. 13.

Steady state fluorescence anisotropy measurements were made using a Perkin-Elmer LS-50B equipped with polarising accessory. The instrumental factor '*G*' was determined from the spectra obtained using horizontally polarised excitation. The low temperature spectra and anisotropy measurements were recorded using an Oxford Instruments DN-1704 cryostat and ITC-6 temperature controller. Details of experiments involving singlet oxygen quantum yields are given in ref. 17.

Acknowledgements

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