Binuclear ruthenium(II) phenanthroline compounds with extreme binding affinity for DNA

Per Lincoln and Bengt Nordén

Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, Sweden

Dimeric homochiral ruthenium complexes $[L_2Ru\{dppz(11\text{-}11')dppz\}RuL_2]^{4+}$ (L = 1,10-phenanthroline, 2,2′-bipyridyl; dppz = dipyrido[3,2-A:2′,3′-C]phenazine) bind extremely strongly to duplex DNA with different binding geometries for the ΔΔ and ΛΛ enantiomers of the phenanthroline complex but with similar geometries for the bipyridyl complex enantiomers.

Interactions between nucleic acids and transition-metal complexes have been studied extensively for several decades for various biotechnical or pharmaceutical purposes.[1–3] Trigonal complexes such as $[Fe(bipy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ generally show some enantiopreferentiality upon binding to DNA, however, for most compounds so far reported it has been modest.[1] Not even with $[Ru(phen)_2(dppz)]^{2+}$, which binds by intercalating the large dppz moiety between the base pairs of double helical DNA thereby fixing the two phenanthrolines in orientations which are different for the two complex enantiomers,[4] are there any substantial diastereomeric effects on binding affinity[5] although the photophysical properties[6] display striking variations between the enantiomers.[7]

By connecting two chiral centres into a dimeric complex one could hope to amplify the chiral discrimination. We here report on the DNA interaction of a novel type of dimeric compound: $[L_2Ru\{dppz(11\text{-}11')dppz\}RuL_2]^{4+}$ {L = 1,10-phenanthroline, 2,2′-bipyridyl; dppz = dipyrido[3,2-A:2′,3′-C]phenazine (Fig. 1)}. These were prepared from homochiral $[RuL_2(1,10\text{-phenanthroline-5,6-dione})]$[7] and 3,3′,4,4′-tetraaminobiphenyl (details of synthesis to be described elsewhere). Circular dichrosim, flow linear dichroism and normal absorption, together with displacement of intercalated $[Ru(phen)_2(dppz)]^{2+}$ (measured as emission quenching) have been used to characterise the mode of binding and to assess the binding strengths of their DNA complexes.

Fig. 2 shows absorption and flow linear dichroism[8] spectra of the new compounds, free as well as bound to calf-thymus DNA in 10 mmol dm^{-3} NaCl buffer, ratio [P]/[Ru] = 30. The hypochromic effect in the bi(dppz) ligand absorption band at 310–315 nm is relatively modest for all of the complexes compared to the cases of intercalating dppz or bdppz complexes.[4] While the LD spectra differ only slightly between the ΔΔ and ΛΛ bipy complexes [Fig. 2(*a*)], indicating rather similar binding geometries, the difference is striking [Fig. 2(*b*)] between the phen complex enantiomers. Note that the LD spectrum of ΛΛ phen closely resembles those of the bipy analogues.

The LD spectra may be analysed in terms of angles that the electric transition moments of the complexes make with the DNA helix axis to provide information about binding geometries.[8] The absorption spectrum around 300 nm is dominated by an intense transition of the bi(dppz) ligand polarized approximately along the line connecting the two metal centres. The angle between this direction and the helix axis was found to be −68 ± 3 ° for the ΔΔ and ΛΛ bipy compounds, and nearly the same (−64°) for the ΛΛ enantiomer for the phen compound, but +49 ± 3 ° for the ΔΔ phen compound. Above 400 nm metal-to-ligand charge transfer (MLCT) transitions contribute, whose polarisations analysed as in the parent monomer chromophore,

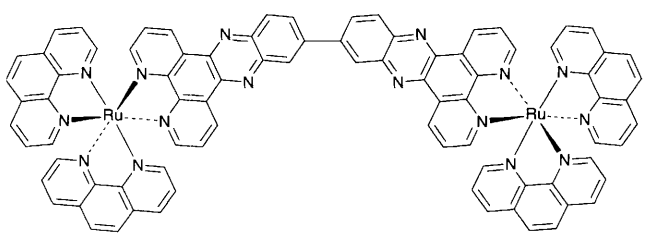

Fig. 1 Structure of ΔΔ-$[L_2Ru\{dppz(11\text{-}11')dppz\}RuL_2]^{4+}$ (L = 1,10-phenanthroline, dppz = dipyrido[3,2-A:2′,3′-C]phenazine)

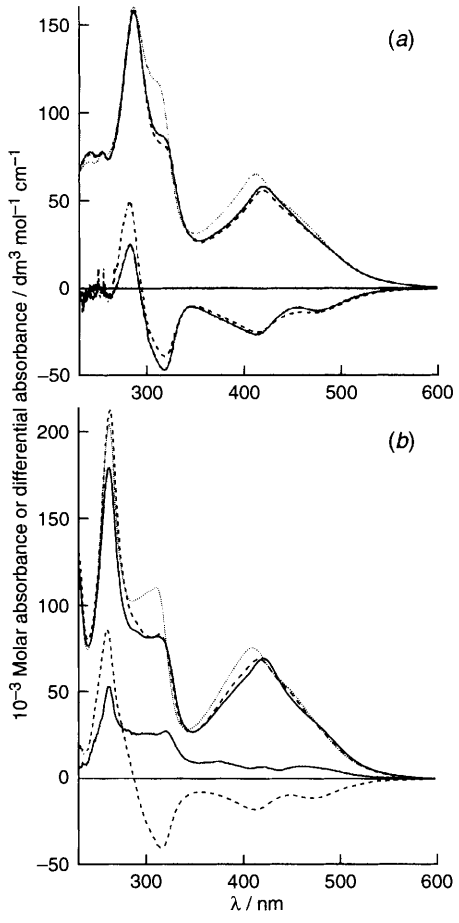

Fig. 2 Absorption (top) and flow linear dichroism (bottom) spectra of (*a*) the bipy and (*b*) the phen compound, free (···) and as complexes with DNA: ΔΔ (—) and ΛΛ (---). The linear dichroism has been normalised with respect to orientation (perfect orientation of DNA) and concentration, and contributions from DNA been subtracted. Flow linear dichroism was measured in a Couette flow cell with methylene blue as internal reference of DNA orientation.[4]

as described,[4] helped to solve the sign ambiguity when determining the angles from the LD of the bi(dppz) transition.

The rotational flexibility around the central pivot bond makes structure analysis based solely on LD data rather speculative. A planar *syn* conformation of the bi(dppz) ligand seems likely in complex with DNA. The assumption of an approximately planar, thus achiral, geometry is justified by the lack of any strong induced circular dichroism (data not shown). It is further reasonable that the bi(dppz) ligand has its concave side facing DNA as the other alternative would effectively prevent the outer dentate ligands from contact with DNA. For both the ΔΔ and ΛΛ bipy complexes as well as the ΛΛ phen complex the LD data are consistent with a binding geometry in which the concave bi(dppz) ligand embraces the sugar–phosphate backbone placing one RuL_2 moiety in each groove. By contrast, for the ΔΔ phen complex, the data would allow both metal centres to be placed in the same groove.

All four complexes, which are themselves nonluminescent in aqueous solution as well as in the presence of DNA, were found to very efficiently quench the luminescence of DNA-bound Δ-[Ru(phen)$_2$(dppz)] (whose luminescence is totally quenched in aqueous solution[6,7]). The lifetimes of partially quenched samples were somewhat shorter (from 80% 730 ns, 20% 160 ns at I/I_0 = 1 to 70% 510 ns, 30% 65 ns at I/I_0 = 0.1 with ΔΔ bipy) but the main cause of the quenching is clearly a displacement of Δ-[Ru(phen)$_2$(dppz)] from DNA. Fig. 3 shows titrations in which the new compounds are added to DNA in the presence of an excess of Δ-[Ru(phen)$_2$(dppz)]. The relative luminescence intensity, corrected for excitation light absorption and lifetime quenching, is expressed as a binding ratio of Δ-[Ru(phen)$_2$(dppz)]. Also shown are theoretical curves calculated for the noncooperative competitive binding of two different DNA ligands. For Δ-[Ru(phen)$_2$(dppz)], the site coverage parameter n was taken equal to 2 and K to 5×10^7 dm^3 mol^{-1} as reported.[7] The data fall between the curves for $n = 3$, $K = 5 \times 10^{11}$ dm^3 mol^{-1} and $n = 4$, $K = 10^{13}$ dm^3 mol^{-1}, and suggest the following order of the binding constants: ΛΛ bipy ≈ ΔΔ bipy > ΔΔ phen > ΛΛ phen, *ca.* 5×10^{11} dm^3 mol^{-1}.

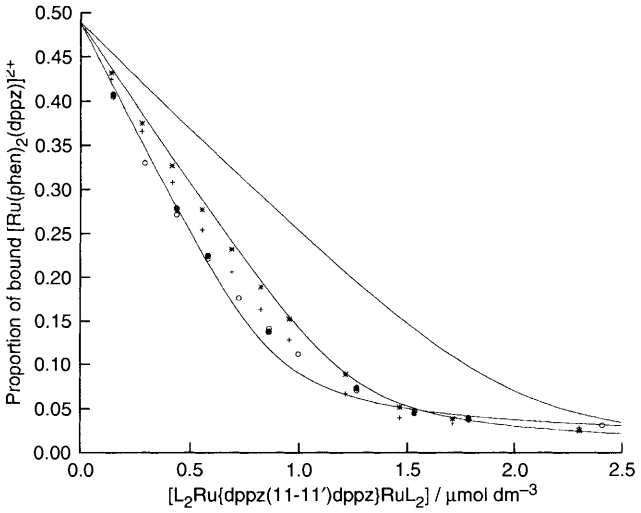

Fig. 3 Displacement isotherms based on quenching of DNA-bound [Ru(phen)$_2$(dppz)]$^{2+}$ by the ΔΔ (○) and ΛΛ (●) bipy compounds and the ΔΔ (+) and ΛΛ (*) phen compounds. Vertical axis shows bound [Ru(phen)$_2$(dppz)]$^{2+}$ per DNA base pair and horizontal axis shows concentration of added dimer compound in μmol dm^{-3}. Concentration of (calf thymus) DNA was 4 μmol dm^{-3} base pairs and [Ru(phen)$_2$(dppz)]$^{2+}$ was 10 μmol dm^{-3}. Theoretical isotherms represent $K = 10^{10}$ dm^3 mol^{-1}, $n = 2$ (top curve), 5×10^{11} dm^3 mol^{-1}, $n = 3$ (middle curve) and 10^{13} dm^3 mol^{-1}, $n = 4$ (bottom curve).

In conclusion all four dimeric complexes are found to bind with extremely high affinities to DNA ($K \approx 10^{12}$ dm^3 mol^{-1}). As judged from strong deviations of the bi(dppz) plane from perpendicularity and only modest hypochromicity, none of the complexes is intercalated. Exchanging the bipyridyl chelate ligands to phenanthrolines seems to enhance enantioselectivity while the affinity for DNA is somewhat reduced. The linear dichroism data are consistent with a novel binding geometry for three of the compounds, in which the dimeric complex in *syn* conformation embraces the sugar–phosphate backbone, placing one RuL_2 moiety in each groove. The ΔΔ enantiomer of the phen complex binds in a distinctly different way, possibly with both Ru(phen)$_2$ moieties residing in the same groove.

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