Electric Dichroism Evidence for Intercalation of Optically Active [Ru(bpy)$_2$(phi)]$^{2+}$ (phi = 9,10-phenanthrenequinone diimine, bpy = 2,2′-bipyridyl) by DNA

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Electric dichroism measurements on a solution containing DNA and enantiomeric [Ru(bpy)$_2$(phi)]$^{2+}$ (phi = 9,10-phenanthrenequinone diimine, bpy = 2,2′-bipyridyl) at pH 7.0 have revealed that the phi ligand of the bound metal complex orients perpendicularly to the helical axis of DNA, supporting a structure where the metal complex is bound to DNA with the phi ligand intercalated between the base pairs.

The binding of an optically active metal complex with double helical DNA has been studied intensively with the purpose of developing a novel reagent which recognizes DNA sites to induce the cleavage of a strand.[1] Recently it has been reported that [Rh(phen)$_2$(phi)]$^{2+}$ (phen = 1,10-phenanthroline; phi = 9,10-phenanthrenequinone diimine) and [Rh(bpy)$_2$(phi)]$^{2+}$ (bpy = 2,2′-bipyridyl) cleave a DNA chain in a site specific way under the illumination of UV light.[2] A model is postulated whereby the phi ligand is intercalated between the base pairs in the major groove, recognizing the local conformation of the DNA site.

Presently the interactions of enantiomeric [Ru(bpy)$_2$(phi)]$^{2+}$ with DNA have been studied with the method of electric dichroism measurements. Evidence has been presented that both enantiomers of the metal complex are bound with the phi ligands intercalated between the base pairs. The method has been previously applied for studying the binding state of [Ru(phen)$_3$]$^{2+}$.[3,4]

A racemic mixture of the complex is resolved by elution on a CM-sephadex column [35 × 1.8 cm (i.d.)] with a saturated potassium antimonyl tartrate solution. The δ-isomer is eluted first, being followed by the λ-isomer. Δε at 535 nm is determined to be 23 and −24 for the δ- and λ-enantiomers of [Ru(bpy)$_2$(phi)]Cl$_2$, respectively. Calf thymus DNA (Aldrich) is used without further purification. The pH of a sample solution is adjusted to 7.0 with 0.01 mol dm^{-3} sodium carcodylate.

The equilibrium shown in eqn. (1) is assumed in which M^{2+},

$$M^{2+} + n\,S \rightleftharpoons M^{2+}/n\,S, \quad (1)$$

S and n denote a metal complex, a base pair of DNA and the number of base pairs occupied by one bound metal complex, respectively. The intrinsic binding constant of an enantiomer (M^{2+}) is determined by absorption titrations at room temp. according to eqn. (2),[5] where [DNA] and [Ru] are the

$$[DNA]/n(\varepsilon_A - \varepsilon_F) = [Ru]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_A) \quad (2)$$

total concentrations of base pairs and metal complex, respectively. ε_A, ε_F and ε_B correspond to absorbance/[Ru], the extinction coefficient for the free ruthenium complex, and the extinction coefficient for the bound ruthenium complex, respectively. The absorbance at 535 nm is measured for various values of [DNA]/[Ru]. Possible values of K_b and n are searched until the plot of the left- and right-handsides of the above equations becomes linear. The binding constant (K_b) and n for [Ru(bpy)$_2$(phi)]$^{2+}$ are obtained as (1.00 ± 0.40) × 10^4 dm^3 mol^{-1} and 6.1 ± 0.2 and (0.86 ± 0.36) × 10^4 dm^3 mol^{-1} and 6.3 ± 0.2 for the δ- and λ-enantiomers, respectively. The results show that the metal complexes exhibit little stereoselectivity in the static binding properties.

Fig. 1 shows the circular dichroism (CD) spectra when each enantiomer of [Ru(bpy)$_2$(phi)]$^{2+}$ is fully bound by DNA. The absorption band around 520–550 nm is assigned to the metal-to-ligand charge transfer (MLCT) transition from a Ru^{II} to a phi ligand. Notably the λ-isomer changes the sign of the band from a negative to a positive one when it is bound to DNA. The δ-isomer shows a positive sign in the band whether it is free or bound to DNA. As a result, both isomers have the same positive sign on a DNA strand. The behaviours may be attributed to the induction of a positive CD by the asymmetric environments of the right-handed helicity. The results suggest that the phi ligands of both enantiomers penetrate deeply into the DNA chain.

The electric dichroism is measured by applying an electric field pulse of 3 kV × 400 μs on a solution of metal complex and

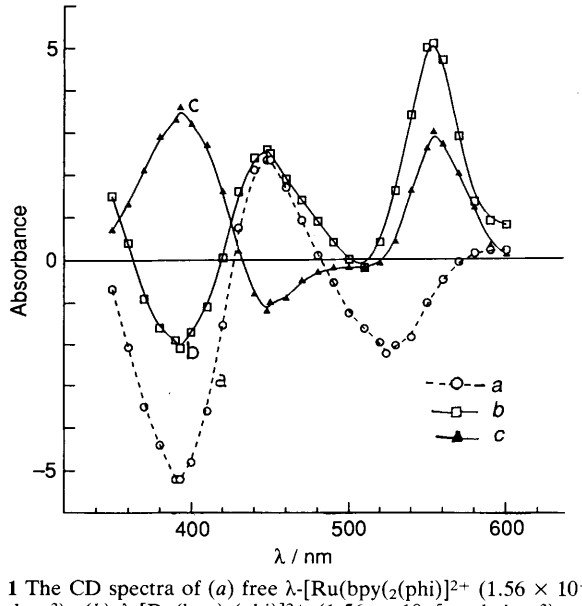

Fig. 1 The CD spectra of (*a*) free λ-[Ru(bpy(2(phi)]$^{2+}$ (1.56 × 10^{-5} mol dm^{-3}); (*b*) λ-[Ru(bpy)$_2$(phi)]$^{2+}$ (1.56 × 10^{-5} mol dm^{-3}) and DNA (1.04 × 10^{-4} mol dm^{-3}); (*c*) δ-[Ru(bpy)$_2$(phi)]$^{2+}$ (1.66 × 10^{-5} mol dm^{-3}) and DNA (1.42 × 10^{-4} mol dm^{-3})

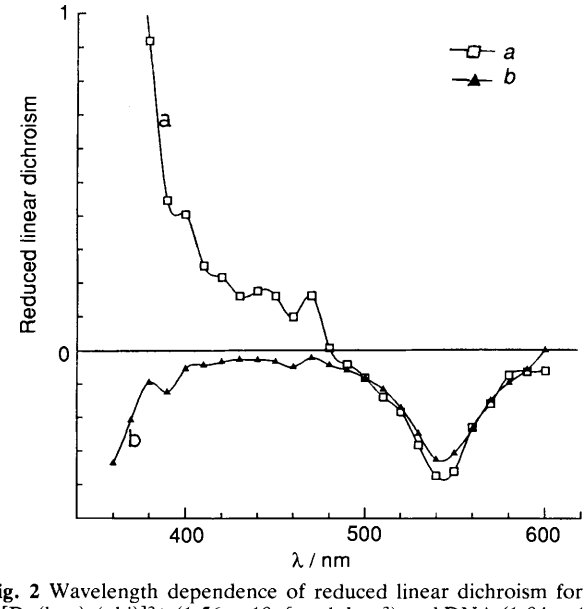

Fig. 2 Wavelength dependence of reduced linear dichroism for (*a*) λ-[Ru(bpy)$_2$(phi)]$^{2+}$ (1.56 × 10^{-5} mol dm^{-3}) and DNA (1.04 × 10^{-4} mol dm^{-3}); (*b*) δ-[Ru(bpy)$_2$(phi)]$^{2+}$ (1.66 × 10^{-5} mol dm^{-3}) and DNA (1.42 × 10^{-4} mol dm^{-3})

DNA. The induced change of absorbance follows the equation of orientational dichroism;[6] $\Delta A/A = (\rho/6)(1 + 2\cos 2\theta)$, in which $\Delta A/A$ and θ denote the relative absorbance change and the angle between the electric field and the polarization of monitoring light, respectively. ρ is the reduced linear dichroism defined by $\rho = (\varepsilon_{\parallel} + \varepsilon_{\perp})/\varepsilon$, in which ε, $\varepsilon_{\parallel}$ and $\varepsilon_{\perp}$ are the extinction coefficients for non-polarized, perpendicularly-polarized and parallel-polarized lights, respectively.

Fig. 2 shows the wavelength dependence of ρ. For both isomers, ρ at 543 nm is largely negative. Since the transition moment of the band directs along the twofold symmetry (C_2) axis of a phi ligand, ρ at 543 nm is expressed by the angle between the C_2 axis and the helical axis of DNA, φ, as shown in eqn. (3) in which $\Phi(E)$ is the orientation function

$$\rho = (3/4)(1 + 3\cos 2\varphi)\,\Phi(E), \qquad (3)$$

representing the degree of the fraction of DNA polymers aligned in the electric field direction. $\Phi(E)$ is estimated to be 0.81 from the field-strength dependence of ρ. The angle, φ, is determined to be 60 and 57° for the δ- and λ-isomers of $[Ru(bpy)_2(phi)]^{2+}$, respectively. Thus both enantiomers direct their phi ligands roughly perpendicularly to the helical axis. The results are consistent with the previously proposed binding model that the phi ligand is intercalated between the base pairs.[2]

The absorption band at 400–500 nm is owing to the MLCT transition from Ru^{II} to two bpy ligands. The band consists of two transitions: the one around 450 nm directs along the C_2 axis of the coordinated bpy ligand and the other around 420 nm perpendicular to the C_2 axis.[7] From Fig. 2, it can be seen that these two transitions have the same value of ρ which is 0.20 and −0.02 for the λ- and δ-isomers, respectively. Applying these values to eqn. (3), it is concluded that the transition moments of these bands make an angle of 51 and 56° with respect to the helical axis of DNA for the λ- and δ-isomers, respectively. Although the difference in the angles is small, it is certain that there is stereoselectivity in the orientation of bpy ligands. This may arise from the steric interactions of the bpy ligands with the direction of the major groove of DNA.

Received, 14th July 1992; Com. 2/03726B

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