Evidence for Stereospecific Binding of Tris(1,10-phenanthroline)-ruthenium(II) to DNA is provided by Electronic Dichroism

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Electronic dichroism measurements on a solution of DNA and enantiomeric $Ru(phen)_3^{2+}$ (phen = 1,10-phenanthroline) have revealed that the Δ- and Λ-isomers of $Ru(phen)_3^{2+}$ are bound to DNA stereospecifically with their three-fold symmetry axes approximately parallel and vertical to the helical axis of DNA, respectively.

Differences in the biological activities of the enantiomers of an optically active metal complex have been noted with respect to toxicity and the inhibition of enzymatic activities.[1] It would be useful to understand such an effect in terms of the molecular interactions between a chiral metal complex and biological substances. Direct evidence is given herein for the stereospecific binding of optically active chelates to DNA based on electronic dichroism measurements. This work was prompted by the recent observation of Barton *et al.* of the enantiomeric selectivity on binding tris(1,10-phenanthroline)-zinc(II) [$Zn(phen)_3^{2+}$] to DNA.[2]

$Ru(phen)_3(ClO_4)_2$ was synthesized and resolved by literature methods.[3] A DNA stock solution was prepared by dissolving 2.3 mg of calf thymus DNA (Worthington Biochemical Corporation) in 5 ml of distilled water at pH 8.3 (adjusted by 10 mmol/l of hexamethylenetetramine). In a typical electronic dichroism measurement, the solution was adjusted to contain 1×10^{-5} mol/l $Ru(phen)_3(ClO_4)_2$, 20 μg/ml DNA, and 2 mmol/l hexamethylenetetramine (pH 6.5). An electric field pulse was produced as a square-wave with an amplitude of 7.5 kV/cm and a duration of 1 msec. The build-up of dichroism was monitored by the change in intensity of transmitted linearly polarized light (360—600 nm).

$Ru(phen)_3^{2+}$ had an intense absorption band in the wavelength region 350—500 nm [Figure 1(a)]. When DNA was added, the spectrum was only slightly different with an isosbestic point at 462 nm [Figure 1(b)]. There was no further change in the absorbance at concentrations of DNA above 40 μg/ml. There was no detectable difference between the DNA solutions of Λ- and Δ-$Ru(phen)_3^{2+}$ enantiomers. When an electric field pulse was imposed on the solution, an electronic dichroic effect was observed which rose within 10 μsec and decayed unexponentially with a half-life of 200 μsec. The stationary amplitude of the signal obeyed the expression for orientational dichroism, equation (1),[4] where $\Delta A/A$ is the relative absorbance change, θ the angle between the electric field and the polarization of a monitoring light, and ρ the

$$\Delta A/A = (\rho/6)(1 + 3\cos 2\theta) \quad (1)$$

reduced linear dichroism defined as $\rho = (\epsilon_{\parallel} + \epsilon_{\perp})/\epsilon$.† Curves (c) and (d) in Figure 1 show the dependence of the dichroism amplitude at $\theta = 0°$, ΔA, on wavelength for solutions of Λ- and Δ-$Ru(phen)_3^{2+}$, respectively. The dependence of ΔA on wavelength was different for the two enantiomers when

† ϵ = isotropic molar extinction coefficient, $\epsilon_{\parallel}$ = molar extinction coefficient for light polarized parallel to the electric field and $\epsilon_{\perp}$ = molar extinction coefficient for light polarized perpendicular to the electric field.

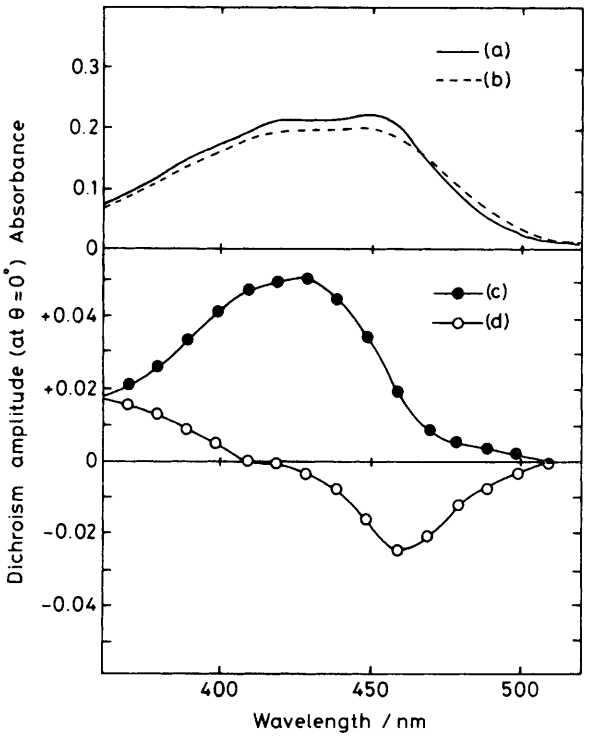

Figure 1. (a) The electronic spectrum of a solution of 1.0×10^{-5} mol/l $Ru(phen)_3(ClO_4)_2$, (b) as (a) but with the addition of 22 μg/ml DNA, (c) the dependence of the stationary amplitude of the electronic dichroism signal on wavelength at $\theta = 0°$ [equation (1)] for a solution of 1.0×10^{-5} mol/l Λ-$Ru(phen)_3(ClO_4)_2$ and 22 μg/ml DNA, and (d) as (c) but for 1.0×10^{-5} mol/l Δ-$Ru(phen)_3(ClO_4)_2$.

bound to DNA. Generally, ρ or ΔA is a function of both the electric field intensity (E) and the angle of the transition moment of a bound chromophore with respect to the orientated polymer axis.[4] Curves (c) and (d) in Figure 1 were obtained at the same value of E, 7.5 kV/cm. Moreover, since the spectra of free and bound $Ru(phen)_3^{2+}$ are almost identical, for both enantiomers [curve (b)], the electronic structures of the enantiomers of $Ru(phen)_3^{2+}$ are unchanged on binding to DNA. Thus, the above results demonstrate unambiguously that there exists a structural difference between the Λ- and Δ-enantiomers of $Ru(phen)_3^{2+}$.

The visible absorption of $Ru(phen)_3^{2+}$ was assigned to the charge-transfer transition from a Ru^{2+} ion to three phenanthroline ligands.[5] The allowed transitions were theoretically predicted to be doubly degenerate with their moments perpendicular to the three-fold rotational axis (C_3) of the chelate.[5,6] Such degeneracy would be removed by the perturbations present in a real chelate. In fact, more rigorous calculations on $Fe(phen)_3^{2+}$ predicted several absorption bands in the visible region.[7] Figure 1(d) could be interpreted as indicating the presence of more than one transition at 400—500 nm. By considering the transition at the longest wavelength, *ca.* 460 nm, ρ was found to be -0.36 and $+0.28$ for the Δ- and Λ-enantiomers of $Ru(phen)_3^{2+}$, respectively. When the transition moment concerned was uniaxially fixed with respect to the orientated helical axis of DNA, ρ was given by equation (2)

$$\rho = (3/4)(1 + 3\cos 2\psi).\Phi(E) \quad (2)$$

where ψ is the angle between the transition moment and the helical axis of DNA and $\Phi(E)$ the orientation function representing the degree of the orientation at a given electric field strength.[4] Inserting $\rho = -0.36$ [Δ-$Ru(phen)_3^{2+}$] or $+0.28$ [Λ-$Ru(phen)_3^{2+}$] and $\Phi(E) = 0.6$ at $E = 7.5$ kV/cm,[8] into equation (2), gave ψ as 63 and 49° for Δ- and Λ-$Ru(phen)_3^{2+}$, respectively. Thus the transition moments at the longest wavelength of the enantiomers of $Ru(phen)_3^{2+}$ are orientated in different directions with respect to the helical axis of DNA. Undoubtedly this stereospecific binding is caused by the interplay between the right-handed helicity of DNA and the propeller-like chirality of $Ru(phen)_3^{2+}$.

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8 $\Phi(E) = 0.6$ at $E = 7.5$ kV/cm, from Figure 18 in N. C. Stellwagen, *Biopolymers*, 1981, **20**, 399.