REARRANGEMENT OF A PLATINUM (II) COMPLEX IN DNA FROM INTERCALATION OUTER-SPHERE POSITION TO NON-INTERCALATION COORDINATION

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1. Introduction

The binding of the anti-cancer drug *cis*-$[Pt(NH_3)_2Cl_2]$ to DNA has drawn considerable interest but so far only very few mechanistic and structural conclusions have been possible [1–8] and the mechanism through which this and analogous Pt complexes exhibit anti-cancer activity has not been established. The neutrality, which would facilitate passage through the cell membrane, and the displaceable ligands (enabling strong binding to DNA bases) have been pointed out as crucial properties. The fact that the *trans* isomer is inactive [1] could suggest that coordination via two adjacent nucleotide base nitrogens is required. Recent studies have shown that the substitution-inert [Pt(ethylenediamine) (2,2′-dipyridine)]$^{2+}$ is intercalated between adjacent base pairs whereas the non-planar [Cu(2,2′-dipyridine)$_2$$(H_2O)_2$]$^{2+}$ is not intercalated and most probably binds to base nitrogens [9]. Intercalation appears to be the rule with maximally coordinated planar complexes and is therefore a feasible intermediate stage in the binding of these platinum compounds.

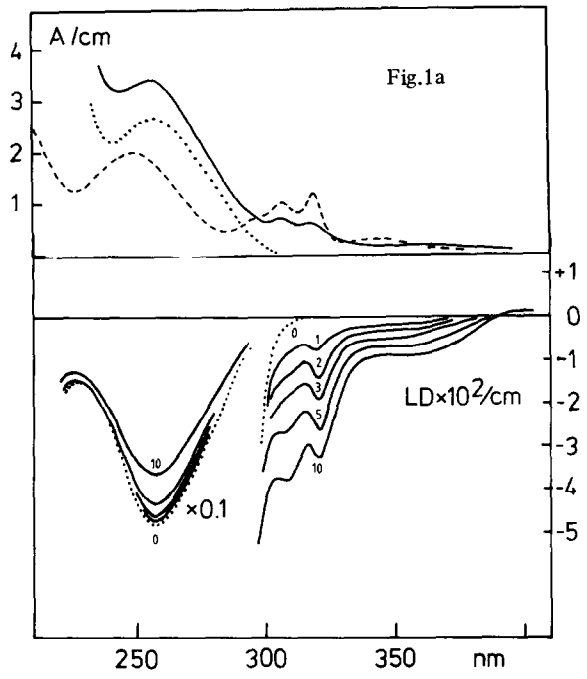
In this report it is shown that [Pt dipy$(H_2O)_2$]$^{2+}$ forms an initial complex with an angular orientation of the Pt–dipy plane that indicates intercalation. It then shifts to an orientation which is incompatible with intercalation but consistent with coordination to vertically-positioned nitrogens belonging to adjacent bases on one of the DNA strands. Consideration of the bond distances and platinum (II) chemistry indicates that this would probably be the most stable coordination site on DNA.

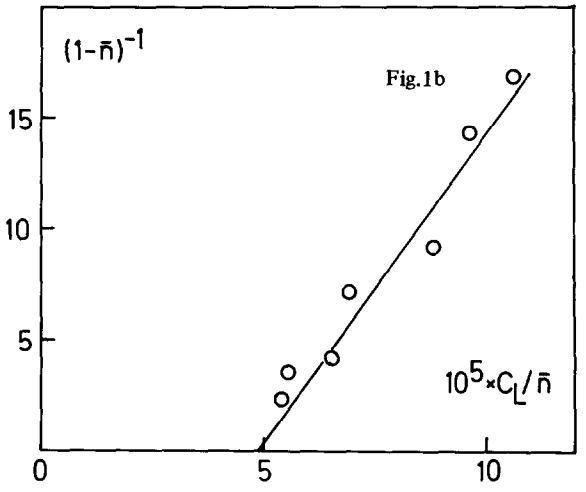
2. Experimental

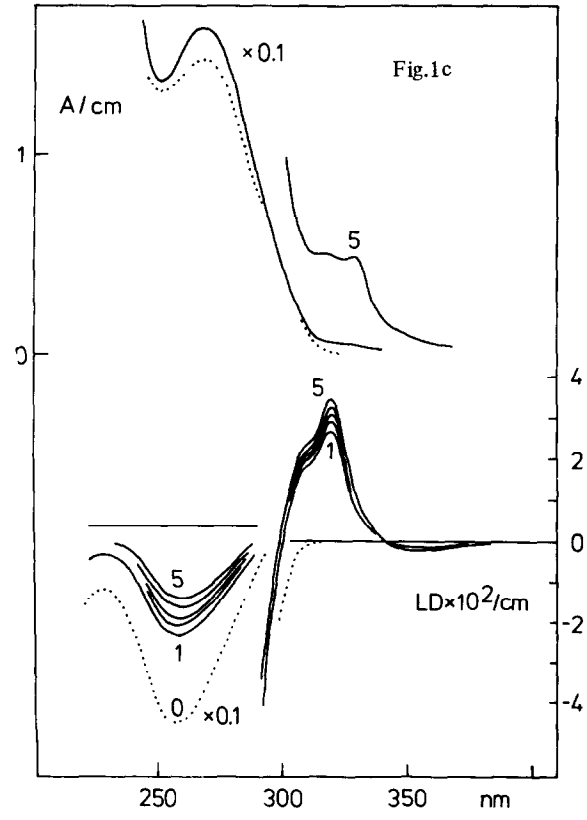
The linear dichroism technique has been described [10,15]. The preparation of platinum (II) amine–dipyridyl complexes has been described [7,13]. [Pt dipy$(H_2O)_2$]$^{2+}$ was obtained by dissolving the very weak nitrate complex in water. Calf-thymus DNA, Sigma Ltd., type 1, was used without further purification. The metal complexes were added slowly as 1 mM solutions to the DNA solution in the measurement Couette cell.

3. Results and discussion

Figure 1a shows a series of linear dichroism (LD) spectra recorded on flow-oriented calf thymus DNA solutions containing different concentrations of [Pt dipy$(H_2O)_2$]$^{2+}$ (obtained by dissolving the very weak nitrate complex in water). The linear dichroism band centered at 257 nm is due to the intrinsic DNA chromophores; the negative bands at 308 nm and 320 nm arise from transitions within the Pt–dipy moiety and give direct evidence on the binding. A Scatchard type plot [10] shows (fig.1b) that the LD is consistent with a single complex with the stability constant $K = 3 \times 10^5$ M^{-1} and the occupation density $n = 0.2$ (in 0.005 M $NaNO_3$). The LD/A_r (where A_r is the random absorbance) determined for the complex is −0.15 which is close to the value for the intrinsic base chromophore (−0.19). The transition moments of the strong bands are polarized in the Pt–dipy plane [11] and this is thus very nearly parallel to the base pair plane. The orientation and







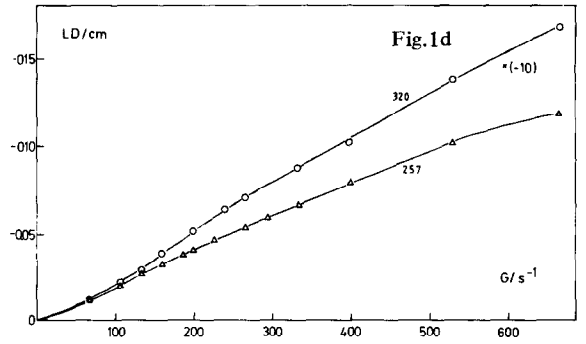
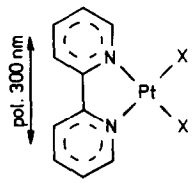


Fig.1a. Absorbance and linear dichroism (*LD*, $G = 2000\ s^{-1}$) of calf thymus DNA solutions (3.7×10^{-4} M nucleotide; $I = 0.005$ M $NaNO_3$) immediately after the addition of Pt–dipy$(NO_3)_2$ (concentrations: 0, 1, 2, 3, 5 and 10×10^{-5} M). Dotted and dashed curves refer to pure DNA and metal complex. For synthesis see [13]. Fig.1b. $(1-\bar{n})$ versus $C_L/\bar{n}$ plot based on the $LD/LD_{saturation}$ ratio observed immediately after mixing ($C_N = 2.4 \times 10^{-4}$ M). Fig.1c. Absorbance and LD spectra after 30 h at room temperature. Notations as in fig.1a. Fig.1d. LD_{257} and LD_{320} versus flow gradient of DNA (2.4×10^{-4} M) + Pt dipy$(NO_3)_2$ (4.0×10^{-5} M) 6 h after mixing.

the binding data are consistent with binding according to the intercalation model in [12].



The intercalated complex is labile and slowly transforms into a more stable complex characterized by a positive *LD* in the Pt–dipy absorption (fig.1c). The rearrangement and the new orientation, with the Pt–dipy plane practically parallel to the helix axis, can be explained as a replacement by base nitrogens of the weak water ligands. Two different polarisations in the Pt–dipy plane can be recognized [11] but their directions have not yet been unambiguously assigned and we cannot further specify the orientation on DNA. That the final complex is of inner-sphere type is supported by the observation that the *LD* signal is not diminished on adding $NaNO_3$ (the negative *LD* of the intercalation complex can however be strongly reduced in this way).

There is a slow decrease in the *LD* of DNA (257 nm) indicating that alteration of higher structure occurs after some time in solutions with high platinum content. Comparison of the *LD* versus flow gradient curves obtained at 257 nm and 320 nm (fig.1d) shows a slight difference in shape which can be interpreted in terms of a greater stiffness in the binding site fragments than in the entire DNA chain. If the platinum had been found to differently oriented single strands (to explain the *LD* sign) the opposite behaviour would have been expected. A saturation tendency in the *LD* from the final complex suggests that the metal to nucleotide ratio is only slightly lower than for the intercalation site.

It is of interest that $[Pt(dipy)Cl_2]$ slowly combines with DNA giving a complex which according to the *LD* spectrum (fig.2) has an inner-sphere coordination structure very similar to that formed with $[Pt(dipy)(H_2O)_2]^{2+}$. The absence of an immediate *LD* on addition of $[Pt(dipy)Cl_2]$ indicates that this neutral complex does not form the labile intercalated complex found with $[Pt(dipy)(H_2O)_2]^{2+}$.

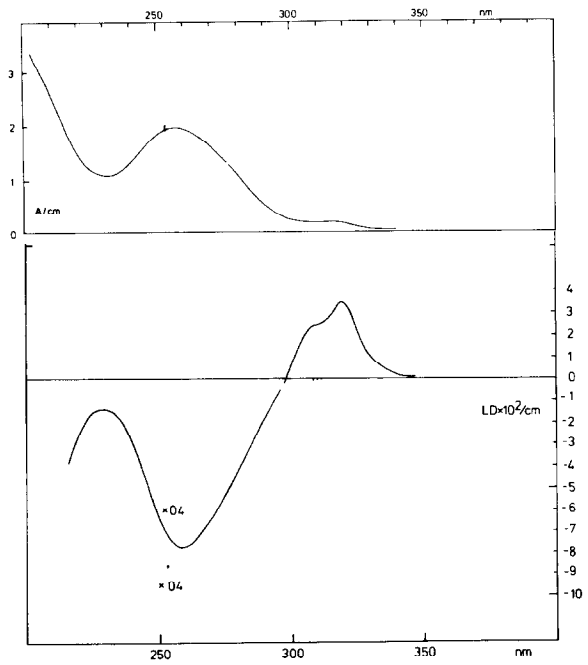


Fig.2. DNA (0.29 mM) + $[Pt(dipy)Cl_2]$ (0.04 mM) after 30 h. $(LD/A_r)_{319} = +0.2$ and $(LD/A_r)_{257} = -0.096$ correspond to an average orientation ($\theta = 0$ in eq. (2) of [14]) of the transition dipole (i.e., the Pt–dipy plane) parallel to the helix axis.

Acknowledgement

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