Interaction of Platinum Compounds with Dinucleotides[1a]

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Abstract: Circular dichroism spectroscopy has been used to investigate the interaction of a number of platinum complexes with the dinucleotides A3′p5′A, A2′p5′A, A3′p5′C, A2′p5′C, and A3′p5′U. With these dinucleotides there is the possibility of a metal ion being chelated to form an interbase link within the dinucleotide. A cross link of this type can be detected from the temperature dependence of the CD spectra and the spectra at low pH as the link preserves the stacked configuration of the dinucleotide. *cis*-$Pt(NH_3)_2Cl_2$ and *cis*-$Pt(enim)_2Cl_2$ (enim = ethylenimine) form an interbase link with A3′p5′A and A3′p5′C whereas *trans*-$Pt(NH_3)_2Cl_2$ merely reacts to bring about unstacking of the dinucleotides. No cross links have been detected with the cis isomers and A2′p5′A, A2′p5′C, or A3′p5′U. From existing conformational data and the pH dependence studies, sites of cross linking of A3′p5′A and A3′p5′C have been suggested.

In recent years there has been considerable interest in platinum complexes that show antitumor activity.[2-9] These complexes are usually of the form *cis*-$Pt(A)_2Cl_2$, where A is an amino ligand. Trans complexes generally are inactive.

It appears that these complexes act by interfering with DNA replication[10] and one possibility is that they cross link the complementary strands of DNA,[11,12] thus preventing replication. It has been shown that both *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ can cross link DNA *in vitro*, but only the cis complexes can do so *in vivo*.[13] Clearly, in DNA there are many possible binding sites for metals and spectroscopic evidence indicates that the purine and pyrimidine bases are involved in this binding and the interaction of some platinum complexes with nucleosides has been investigated.[14-16]

The two replaceable chlorides on *cis*-$Pt(A)_2Cl_2$ are about 3–4 Å apart and therefore for cross linking there needs to be possible binding sites of the order of 3–4 Å apart. There are such sites on DNA, particularly in the wide groove, which meet these stereochemical requirements.

This present study was undertaken in order to investigate the effect on the binding of platinum ions to bases, of stacking the bases into a stereochemistry suitable for bidentate binding to a single metal ion. In particular, we have sought evidence for the formation of a complex between antitumor platinum compounds and dinucleotides in which a single platinum ion bridges two bases of the same dinucleotide.

The dinucleotides were selected as a model since various physical techniques have shown that they take up one of two possible conformations,[17] a stacked form, with both bases parallel and vertically above one another at an interplanar separation of 3–4 Å, or an unstacked form with the dinucleotide stretched out with the bases separated by a large distance. The stacked form of the dinucleotide is favored at low temperatures.[18,19] The circular dichroism (CD) spectra of the stacked form of the dinucleotides consists of a highly characteristic biphasic spectrum, while the unstacked form has a negligible contribution to the CD. Thus, it is possible to detect the stacked form even in the presence of a large amount of the unstacked dimer. If a platinum ion is able to link together two bases of the same dinucleotide when they are in a stacked conformation and, further, if the resulting complex is stable over a temperature range between 5 and 45°, the temperature dependence of the equilibrium, stacked $\rightleftharpoons$ unstacked, will be reduced or abolished. Thus the CD spectrum and a study of its temperature dependence allow one to detect the complex of interest.

Therefore we have studied the temperature dependence of the CD spectra of the dinucleotides A3′p5′A and A3′p5′C and their isomers A2′p5′A and A2′p5′C and the complexes they form with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ and *cis*-$Pt^{II}(enim)_2Cl_2$ (enim = ethylenimine). These were chosen as the conformation of the stacked form is known.[17]

Method. For a solution containing one optically active species the ellipticity θ is given by

$$\theta = [\theta]lc/100$$

where l is the path length, c the concentration in mol dm^{-3}, and θ the molecular ellipticity. For a solution with more than one optically active species present

$$\theta = \sum_i [\theta]_i l c_i/100$$

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Over the temperature range available for an aqueous solution $[\theta]_i$ will be independent of temperature, and therefore

$$d\theta/dT = \sum_i [\theta]_i l \, dc_i/dT$$

If the platinum complex reacts to form a "clipped" species with both bases bound to one metal ion, then this new complex should have a temperature-independent CD over the range of temperature covered. However, if the metal ion only attacks one of the bases, or if each of the bases is attacked, then the repulsion of the positive charges on the metal ions, as well as the stereochemical requirements, would cause the dinucleotide to unstack. Therefore, the only contribution to the CD will be from unreacted stacked dinucleotide and the "clipped" species. As a result

$$\theta \text{ (with Pt)} = x\theta \text{ (without Pt)} + \theta \text{ (clipped)}$$

and

$$d\theta/dT \text{ (with Pt)} = x \, d\theta/dT \text{ (without Pt)}$$

where x is the fraction of unreacted stacked dinucleotide present.

The magnitude of θ will depend on the amount of stacked and "clipped" dinucleotide present, but the temperature dependence results only from the amount of unreacted stacked dinucleotide present, and will be less with platinum present than with no platinum present, if the platinum complex reacts to form a temperature-independent species with a strong, biphasic CD signal.

Because it is only the unreacted dinucleotide which contributes to the temperature dependence of the CD, the ratio of the temperature dependence of the dinucleotide solutions with and without platinum is the fraction of unreacted stacked dinucleotide. Therefore at any one temperature the contribution of the unreacted dinucleotide is simply this fraction of the ellipticity when no platinum is present. If a "clipped" form is present then the experimental ellipticity will be greater than this fraction, but if no "clipped" form is present the measured ellipticity will be equal to this fraction and the platinum complex will have reacted to cause unstacking of the dinucleotide.

Information regarding the CD of the "clipped" and unstacked species can be obtained from pH-dependent studies. At pH 1 or lower the dinucleotides studied are unstacked due to protonation of the bases.[19,20] The repulsion of the positively charged protonated bases is the cause of the unstacking. If however, the bases are "clipped" by a platinum complex, then, provided that in the time required for measurement the protons do not replace the platinum, they will not unstack and the CD spectrum should be that of the "clipped" dinucleotide.

Therefore, from temperature dependence of the CD spectrum, and the CD spectrum at low pH, it is possible to establish which platinum complexes form an interstrand cross link. Also from the pH studies possible sites of bidentate binding can be identified.

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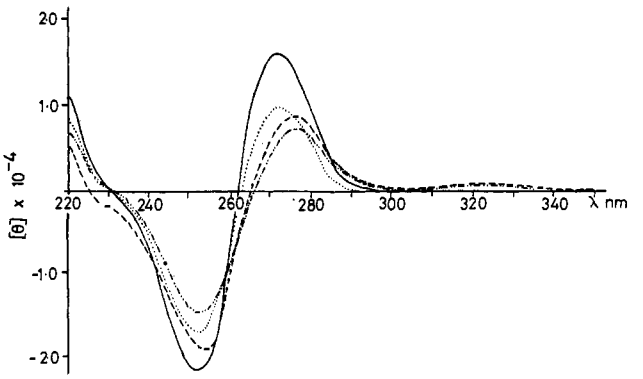

Figure 1. The effect of platinum complexes on the CD spectrum of A3'p5'A: Pt:P, 1:1; dinucleotide concentration, 4×10^{-5} mol dm^{-3}; temperature 37°. A3'p5'A (————), plus *cis*-Pt$(NH_3)_2Cl_2$ (- - - -), plus *trans*-Pt$(NH_3)_2Cl_2$ (·····), plus *cis*-Pt(enim)$_2$Cl$_2$ (-·-·-·-).

Experimental Section

A3'p5'C, A2'p5'A, and A2'p5'C were purchased from Sigma Limited. A3'p5'A and A3'p5'U were obtained from Miles-Seravac Limited. Circular dichroism spectra were recorded using a Cary 61 CD spectrometer with 1-cm cells in a thermostated compartment. Platinum complexes were prepared by standard methods.[21,22] Solutions of platinum complexes and dinucleotides were prepared as follows.

The first method, used for the majority of measurements, gave solutions with ratios of platinum to dinucleotide phosphorus (Pt:P) of 0:1, 1:1, 2:1, 3:1, 5:1, and 10:1. To 2 ml of a 10^{-4} mol dm^{-3} solution of dinucleotide in 0.1 mol dm^{-3} $NaClO_4$ was added respectively 0.0, 0.2, 0.4, 0.6, 1.0, and 2.0 ml of 10^{-3} mol dm^{-3} solution of the platinum complex in 0.1 *M* $NaClO_4$ and the resulting solution was made up to 5 ml with 0.1 *M* $NaClO_4$. These solutions were stoppered and stored in the dark at 37° for 14 days prior to measurements being taken. The pH of the solutions was checked at the time of measurement with wide-range pH paper and found to be in the range pH 6–7.

The second method, used for solutions of *cis*-Pt(enim)$_2$Cl$_2$ with dinucleotides, gave solutions at the higher concentrations of 10^{-4} *M* in both platinum and dinucleotide. Weighed amounts (0.352 mg) of the platinum complex were dissolved directly in 10-ml solutions of 10^{-4} *M* dinucleotide in 0.1 *M* $NaClO_4$. The platinum complex was weighed using an Oertling microbalance accurate to 0.1 μg.

The water used in all these solutions was doubly distilled from alkaline $KMnO_4$, under an atmosphere of nitrogen and stored under nitrogen.

The pH was altered to give solutions of high and low pH as follows. To 2 ml of equilibrated solution at pH 6–7 was added either 1 drop (0.02 ml) of 1 *M* NaOH to give pH 11–12 or 5 drops (0.1 ml) of concentrated $HClO_4$ to give a pH less than 1. pH was checked using wide-range indicator paper and spectra of these solutions were recorded immediately.

Results

1. 3',5'-Diadenosine Phosphate. This dinucleotide has a strong biphasic CD with a maximum at 272 nm and a minimum at 255 nm. In the presence of *cis*-Pt$(NH_3)_2Cl_2$ and *cis*-Pt(enim)$_2$Cl$_2$ at a Pt:P ratio of 1:1, this maximum is shifted to about 280 nm. The minimum is also red shifted but only slightly so. The intensity of the spectrum is reduced, as shown in Figure 1. *trans*-Pt$(NH_3)_2Cl_2$ diminishes the CD but does not shift the spectrum. This is also shown in Figure 1.

At higher ratios of Pt:P, both *cis*- and *trans*-Pt$(NH_3)_2Cl_2$ eventually cause unstacking of the dinucleotide. The trans complex does so at a ratio of 3:1, but

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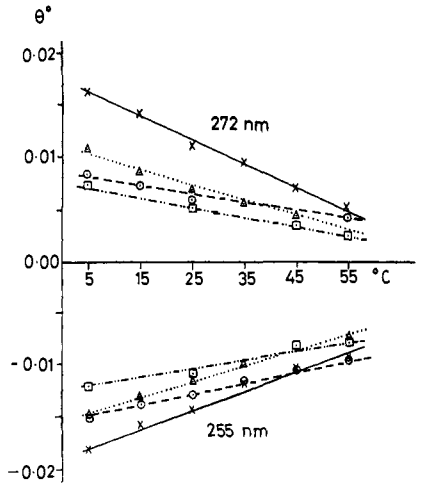


Figure 2. Temperature dependence of the CD spectrum of A3′-p5′A with platinum complexes: Pt:P, 1:1; dinucleotide concentration, 4×10^{-5} mol dm^{-3}. A3′p5′A (——×——), plus *cis*-$Pt(NH_3)_2Cl_2$ (----⊙----), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · · ·△· · · ·), plus *cis*-$Pt(enim)_2Cl_2$ (-· · ·-⊡-· · ·-·).

with the cis complex the biphasic CD characteristic of the stacked dinucleotide is observed up to a ratio of 5:1, although the spectrum is red shifted.

The temperature dependence at 272 and 255 nm was studied at a Pt:P ratio of 1:1 and a dinucleotide concentration of 4×10^{-5} *M*. Measurements were repeated with *cis*-$Pt(enim)_2Cl_2$, 1:1 ratio and concentration 10^{-4} *M*. The results at the lower concentration are shown in Figure 2. With *trans*-$Pt(NH_3)_2Cl_2$, θ is reduced slightly and $d\theta/dT$ is negligibly altered. However, with the two cis platinum(II) complexes, θ is reduced considerably as is $d\theta/dT$. Table I lists the ratios

Table I. Ratio of Temperature Dependence and Ellipticities for A3′p5′A with Various Pt Complexes

Wavelength	Compound	Ratio of temp dependence with and without Pt ion, x	$x\theta_{ApA}$ at 5°	Exptl θ at 5°
272 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.369	0.0061	0.0085
	trans-$Pt(NH_3)_2Cl_2$	0.652	0.0108	0.0110
	cis-$Pt(enim)_2Cl_2$	0.413	0.0068	0.0075
255 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.579	−0.0106	−0.0150
	trans-$Pt(NH_3)_2Cl_2$	0.816	−0.0149	−0.0148
	cis-$Pt(enim)_2Cl_2$	0.579	−0.0106	−0.0120

of the temperature dependence of the solutions containing platinum to that without platinum, *i.e.*, the fraction of temperature-dependent species contributing to the CD, the product of this fraction and the ellipticity of the A3′p5′A solution at 5°, and the experimental ellipticity at 5°. The close agreement between the values in columns 4 and 5 for *trans*-$Pt^{II}(NH_3)_2Cl_2$ shows that there is no temperature-independent species contributing to the CD spectrum. On the other hand, the presence of such a species is clear in the case of the cis isomers. The nonagreement between the fraction of unreacted dinucleotide at 272 and 255 nm is explained by the pH-dependent studies which reveal that contrary to

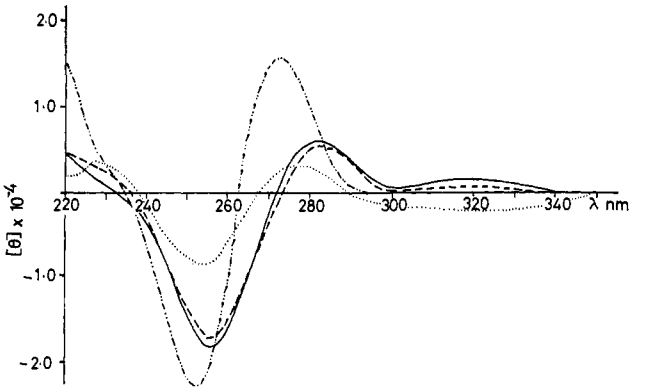


Figure 3. pH dependence of the CD spectrum of A3′p5′A with *cis*-$Pt(enim)_2Cl_2$: Pt:P, 1.1; dinucleotide concentration, 1×10^{-4} mol dm^{-3}; temperature 37°. pH 6–7 (————), pH 11–12 (- - - -), pH <1 (· · · · · · · · ·), A3′p5′A at pH 6–7 (-· · -· · -· ·).

our original assumptions, the unstacked form of the dinucleotide does possess a weak negative CD in the 250-nm region. At the higher concentrations of 10^{-4} *M* with *cis*-$Pt(enim)_2Cl_2$ a temperature-independent shoulder is observed in the CD spectrum at approximately 283 nm.

These temperature-dependent studies are consistent with a "clipped" platinum–dinucleotide complex being formed with the *cis*-platinum(II) complexes, this "clipped" complex having a temperature-independent CD at slightly higher wavelengths than the unreacted A3′p5′A.

At low pH A3′p5′A is unstacked due to protonation of the bases but remains unstacked at high pH.[18,20] With platinum present it is only the solutions of A3′-p5′A with the two *cis*-platinum(II) complexes that exhibit a biphasic CD, although this is much reduced. The results for *cis*-$Pt(enim)_2Cl_2$ are shown in Figure 3. At high pH the CD spectrum is virtually the same as at intermediate pH. The pH was not taken above pH 12, as above this precipitation of the platinum occurs. The residual spectrum at pH less than 1 has a maximum at approximately 278 nm and a minimum at *ca.* 258 nm. This supports the evidence from the temperature-dependence measurements that the dinucleotide can act as a bidentate ligand toward *cis*-$Pt(A)_2Cl_2$ complexes and that once bound to the platinum is not easily unstacked.

2. 3′-Adenosine 5′-Cytidine Phosphate. This dinucleotide also possesses a strong biphasic CD but the spectrum is not as symmetrical as that of A3′p5′A. The maximum comes at 275 nm and the nimimum at 238 nm.

In the presence of the two *cis*-platinum(II) complexes there is a considerable change in the CD spectrum. At a Pt:P ratio of 1:1 a strongly red-shifted CD spectrum is observed with two minima, at 260 and 238 nm, a maximum in the 280-nm region, and a shoulder at about 290 nm. The complex *trans*-$Pt(NH_3)_2Cl_2$ causes a reduction in the ellipticity, especially at 275 nm, but does not shift the position of the maximum and minimum. This can be seen in Figure 4.

At higher ratios of Pt:P with *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ the biphasic CD diminishes. Both the complexes cause unstacking of the dinucleotide at a ratio of 5:1 and higher. With *cis*-$Pt(NH_3)_2Cl_2$ at a ratio of 3:1 the intensity in the 290- and 260-nm region has in-

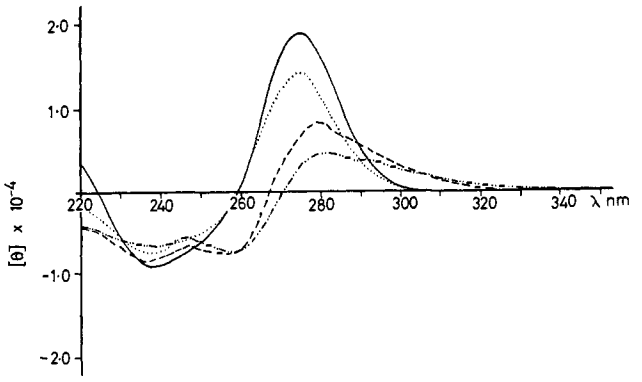


Figure 4. The effect of platinum complexes on the CD spectrum of A3′p5′C: Pt:P, 1:1; dinucleotide concentration, 4×10^{-5} mol dm^{-3}; temperature 37°. A3′p5′C (———), plus *cis*-Pt$(NH_3)_2Cl_2$ (- - - -), plus *trans*-Pt$(NH_3)_2Cl_2$ (· · · · · · ·), plus *cis*-Pt(enim)$_2Cl_2$ (—·—·—·—).

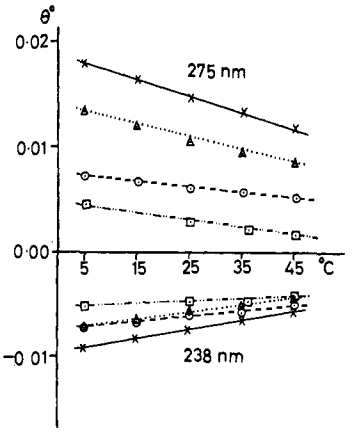


Figure 5. Temperature dependence of the maximum and minimum in the CD spectrum of A3′p5′C with various platinum complexes: Pt:P, 1:1; A3′p5′C concentration, 4×10^{-5} mol dm^{-3}. A3′p5′C (——×——), plus *cis*Pt$(NH_3)_2Cl_2$ (——⊙——), plus *trans*-Pt$(NH_3)_2Cl_2$ (· · · ·△· · · ·), plus *cis*-Pt(enim)$_2Cl_2$ (—·—·□·—·—).

creased relative to the 275- and 238-nm region. The trans complex at 3:1 has merely diminished the CD without shifting the spectrum.

Figure 5 shows the temperature dependence at 275 and 238 nm for A3′p5′C with the platinum complexes, and as with A3′p5′A two sets of results are observed, those for the two *cis*-platinum(II) complexes being different from the trans. The two *cis*-platinum(II) solutions have a much reduced ellipticity and $d\theta/dT$ whereas the trans solution has a slightly reduced ellipticity and the $d\theta/dT$ expected if the solution only has unreacted dinucleotide contributing to the CD. This is shown in the analysis of the temperature dependence given in Table II.

Table II. Ratio of Temperature Dependence and Ellipticities for A3′p5′C with Various Platinum Concentrations of Pt:P of 1:1 and Nucleotide Concentration 4×10^{-5} mol dm^{-3}

Wavelength	Compound	Ratio of temp dependence with and without Pt ion, x	$x\theta_{ApC}$ at 5°	Exptl θ at 5°
275 nm	*cis*-Pt$(NH_3)_2Cl_2$	0.352	0.00629	0.00725
	trans-Pt$(NH_3)_2Cl_2$	0.781	0.01398	0.01350
	cis-Pt(enim)$_2Cl_2$	0.469	0.00859	0.00450
238 nm	*cis*-Pt$(NH_3)_2Cl_2$	0.643	−0.00595	−0.00725
	trans-Pt$(NH_3)_2Cl_2$	0.786	−0.00727	−0.00725
	cis-Pt(enim)$_2Cl_2$	0.286	−0.00264	−0.00525

The spectrum attributable to the "clipped" species has been red shifted to such an extent that the CD at 275 nm is negative. This is seen clearly in the spectrum at low pH which is due solely to the "clipped" species as the unreacted dinucleotide has been unstacked by protonation. The shoulder observed in the 290- to 300-nm region of the CD spectrum when a *cis*-platinum(II) complex is present is temperature independent, as expected if it is due to a "clipped" species.

Again, with A3′p5′C as with A3′p5′A, we see the breakdown of the assumption that the unstacked dinucleotide does not contribute to the CD. A3′p5′C in its unstacked form has a weak positive CD in the 270-nm region, as revealed by the low pH measurements. This accounts for the discrepancy between the fraction of unreacted dinucleotide at 275 and 238 nm.

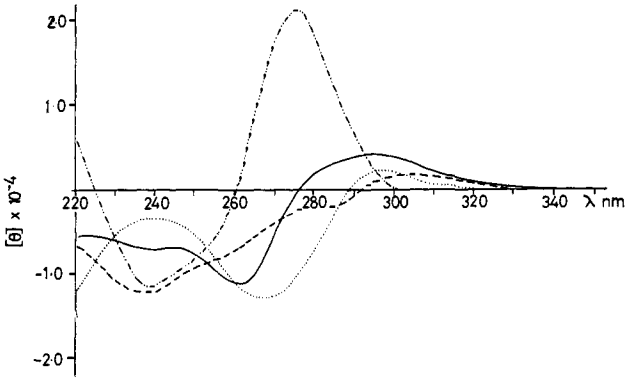


Figure 6. pH dependence of the CD spectrum of A3′p5′C with *cis*-Pt(enim)$_2Cl_2$: Pt:P, 1:1; dinucleotide concentration, 1×10^{-4} mol dm^{-3}; temperature 37°. pH 6–7 (———), pH 11–12 (- - - -), pH <1 (· · · · · · · ·). A3′p5′C at pH 6–7 (—·—·—·—).

Figure 6 shows the CD spectrum at high, intermediate, and low pH for A3′p5′C with *cis*-Pt(enim)$_2Cl_2$ at the higher concentration of 10^{-4} *M*. The spectrum at pH 6–7 can be seen to be a composite of the spectrum at pH less than 1, which should be solely due to the "clipped" species, and the spectrum of a residual amount of unreacted dinucleotide. The spectrum at pH 11–12, however, shows that at this pH the "clipped" species has been attacked by the hydroxide ions and become unstacked.

These results show that complexes of the form *cis*-Pt$(A)_2Cl_2$ are able to bind the two bases of A3′p5′C, the chelated dinucleotide having a characteristic spectrum in the stacked configuration. However, this linkage is unstable in alkaline solution.

3. 2′,5′-Diadenosine Phosphate. In a similar fashion to the (3′p5′) isomer, A2′p5′A has a strong symmetrical biphasic CD with a maximum at 272 nm and a minimum at 252 nm, but the temperature dependence of the spectrum is rather low.

Both *cis*-Pt$(NH_3)_2Cl_2$ and *cis*-Pt(enim)$_2Cl_2$ reduce the intensity of this CD and shift the maximum and minimum to longer wavelengths (Figures 7 and 8).

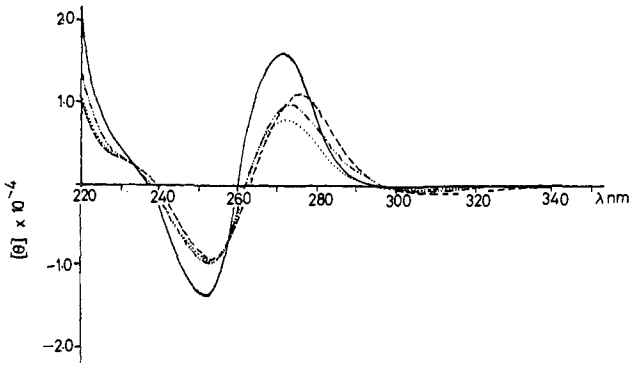


Figure 7. The effect of platinum complexes on the CD spectrum of A2′p5′A: dinucleotide and platinum concentrations, 4×10^{-5} mol dm^{-3}; temperature 37°. A3′p5′A (————), plus *cis*-$Pt(NH_3)_2Cl_2$ (- - - -), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · · · · · · · ·), plus *cis*-$Pt(enim)_2Cl_2$ (-·-·-·-·-).

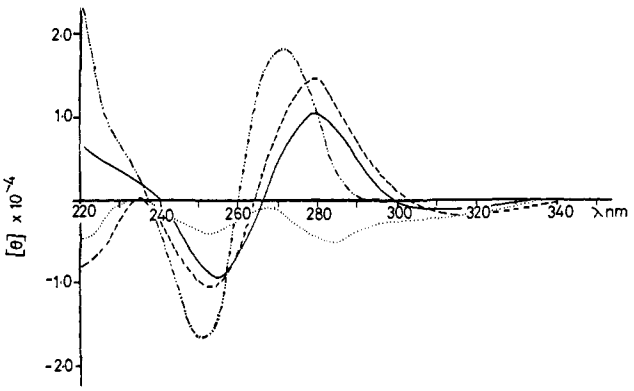


Figure 8. Effect of change of pH on a 1:1 solution of A2′p5′A with *cis*-$Pt(enim)_2Cl_2$: A2′p5′A concentration, 1×10^{-4} mol dm^{-3}; temperature 37°. pH 6–7 (————), pH 11–12 (- - - -), pH <1 (· · · · · · ·). A2′p5′A at pH 6–7 (-·-·-·-).

trans-$Pt(NH_3)_2Cl_2$ diminishes the CD and red shifts the spectrum as well.

With *cis*-$Pt(NH_3)_2Cl_2$ a biphasic CD, showing that a stacked form is present, is observed at a Pt:P ratio of 1:1, 2:1, and 3:1. At 5:1 and higher no stacked form is present. *trans*-$Pt(NH_3)_2Cl_2$ causes complete unstacking of the dinucleotide at a ratio of 2:1.

At low pH (pH <1) the CD spectrum of A2′p5′A shows no biphasic CD with *cis*-$Pt(enim)_2Cl_2$, or any other of the platinum complexes investigated. Figure 8 also reveals that the reaction between *cis*-$Pt(enim)_2Cl_2$ and A2′p5′A is favored at high pH.

The temperature dependence at 272 and 252 nm for solutions of this dinucleotide and platinum complexes is seen in Figure 9. The results shown in this figure strongly suggest that all the platinum complexes are reacting in a similar fashion. The analysis of the temperature dependence, given in Table III, reinforces the conclusion that no temperature-independent stacked species have been identified.

4. 2′-Adenosine 5′-Cytidine Phosphate. This dinucleotide, unlike the (3′p5′) isomer, has a symmetrical CD spectrum, with a maximum at 280 nm and a minimum at 255 nm. The effect of all the platinum complexes is to cause a decrease in the ellipticity with no shifting of the spectrum, the two *cis*-platinum(II) complexes causing the greatest reduction. The results for a Pt:P ratio of 1:1 are shown in Figure 10 and suggest

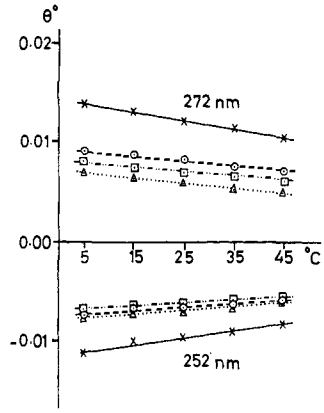


Figure 9. Temperature dependence of the maximum and minimum of the CD spectrum of 1:1 solutions of A2′p5′A with various platinum concentrations: A2′p5′A concentration, 4×10^{-5} mol dm^{-3}. A2′p5′A (—×—), plus *cis*-$Pt(NH_3)_2Cl_2$ (---⊙---), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · ·△· · ·), plus *cis*-$Pt(enim)_2Cl_2$ (-·-·⊡·-·-).

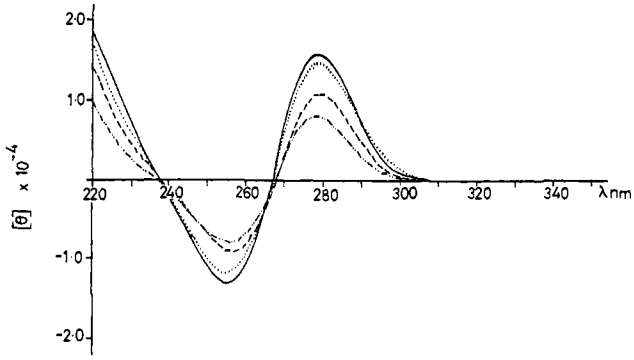


Figure 10. The effect of different platinum complexes on the CD spectrum of A2′p5′C: Pt:P, 1:1; concentration of dinucleotide, 4×10^{-5} mol dm^{-3}; temperature 37°. A2′p5′C (————), plus *cis*-$Pt(NH_3)_2Cl_2$ (- - - -), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · · · · · · · ·), plus *cis*-$Pt(enim)_2Cl_2$ (-·-·-·-·-).

Table III. Ratio of Temperature Dependence, Predicted Ellipticities, and Experimental Ellipticities for A2′p5′A with Platinum Complexes[a]

Wavelength	Compound	Ratio of temp dependence with and without Pt ion, x	$x\theta_{ApA}$ at 5°	Exptl θ at 5°
272 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.543	0.00746	0.00900
	trans-$Pt(NH_3)_2Cl_2$	0.571	0.00786	0.00700
	cis-$Pt(enim)_2Cl_2$	0.571	0.00786	0.00800
252 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.533	−0.0060	−0.0075
	trans-$Pt(NH_3)_2Cl_2$	0.417	−0.00469	−0.00750
	cis-$Pt(enim)_2Cl_2$	0.483	−0.00544	−0.00675

[a] Pt:P, 1:1; A2′p5′A concentration, 4×10^{-5} mol dm^{-3}.

that any reduction of the ellipticity is due solely to the unstacking of the dinucleotide by the platinum complexes.

At higher Pt:P ratios *cis*-$Pt(NH_3)_2Cl_2$ continues to unstack the dinucleotide and by 5:1 has completely done so. With *trans*-$Pt(NH_3)_2Cl_2$, however, there is still a small amount of stacked dinucleotide present at 5:1, but this has vanished by 10:1.

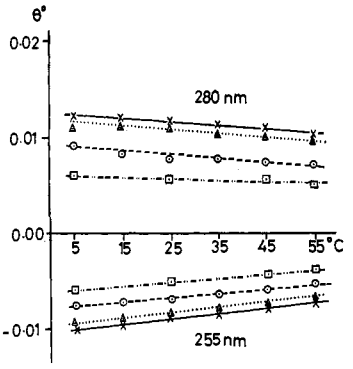


Figure 11. Temperature dependence at 280 and 255 nm of the CD spectrum of A2'P5'C with platinum complexes: Pt:P, 1:1; dinucleotide concentration, 4×10^{-5} mol dm^{-3}. A2'p5'C (————), plus *cis*-$Pt(NH_3)_2Cl_2$ (– – –○– – –), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · · △ · · ·), plus *cis*-$Pt(enim)_2Cl_2$ (– · · –□– · ·).

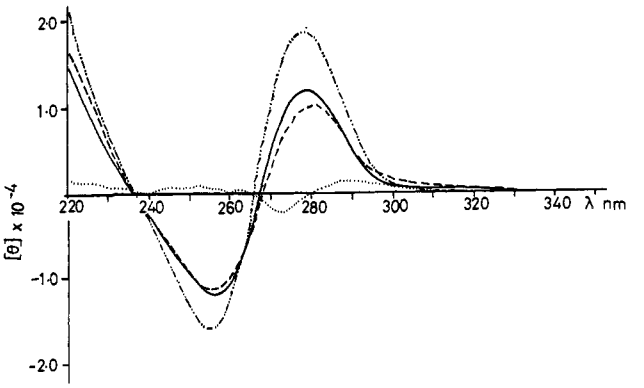


Figure 12. Effect of pH on the CD spectrum of A2'p5'C with *cis*-$Pt(enim)_2Cl_2$ at a concentration of 1×10^{-4} mol dm^{-3} and a Pt:P ratio of 1:1; temperature 37°. pH 6–7 (————) pH 11–12 (- - - - -), pH <1(· · · · · · · · ·). A2'P5'C at pH 6–7 (– · · – · · – · ·).

The temperature dependence of the CD of solutions of A2'p5'C with the platinum complexes is given in Figure 11 and reveals that the two *cis*-platinum(II) complexes have been allowed to react to the greatest extent. The analysis of the temperature dependence given in Table IV, however, suggests that the CD of

Table IV. Ratio of Temperature Dependence and Ellipticities for A2'p5'C with Various Platinum Complexes[a]

Wavelength	Compound	Ratio of temp dependence with and without Pt ion, x	$x\theta_{ApC}$ at 5°	Exptl θ at 25°
280 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.875	0.01094	0.00876
	trans-$Pt(NH_3)_2Cl_2$	1.000	0.1250	0.1175
	cis-$Pt(enim)_2Cl_2$	0.500	0.00625	0.00700
255 nm	*cis*-$Pt(NH_3)_2Cl_2$	0.750	−0.00769	−0.00775
	trans-$Pt(NH_3)_2Cl_2$	0.833	−0.00854	−0.00925
	cis-$Pt(enim)_2Cl_2$	0.583	−0.00598	−0.00575

[a] Pt:P, 1:1; dinucleotide concentration, 4×10^{-5} mol dm^{-3}.

these solutions is due solely to unreacted stacked dinucleotide, as it is with the solutions of the other platinum complexes. That this dinucleotide does not act as a bidentate chelate toward *cis*-platinum(II) complexes is supported by the temperature dependence of the 1:1 solution of *cis*-$Pt(enim)_2Cl_2$ and A2'p5'C at 10^{-4} *M*. No temperature-independent section of the spectrum is observed near the maximum or minimum and the analysis of the temperature dependence reveals that the CD is due to unreacted dinucleotide.

The effect of change of pH is seen in Figure 12. The spectrum of A2'p5'C at pH 11–12 is virtually the same as at pH 6–7 and at low pH has a small negative CD in the 250-nm region, but no positive CD. Thus, we conclude that no "clipped" species between A2'p5'C and either of the cis isomers have been identified.

5. 3'-Adenosine 5'-Uridine Phosphate. It is not expected that this dinucleotide should form a "clipped" species with platinum(II) complexes, as studies with nucleosides have revealed that uridine and thymidine do not react with *cis*- and *trans*-$Pt(NH_3)_2Cl_2$.[14] Figure 13 shows the CD of a solution of A3'p5'U and 1:1 solu-

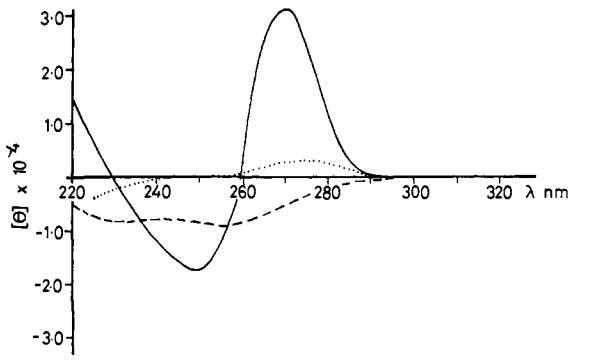


Figure 13. The effect of *cis*- and *trans*-$Pt(NH_3)Cl_2$ on the CD spectrum of A3'p5'U at a Pt:P ratio of 1:1; temperature 37°. A2'P5'U (————), plus *cis*-$Pt(NH_3)_2Cl_2$ (- - - - -), plus *trans*-$Pt(NH_3)_2Cl_2$ (· · · · · ·).

tions of A3'p5'U with both *cis*- and *trans*-$Pt(NH_3)_2Cl_2$, revealing that this dinucleotide has a CD characteristic of a stacked dimer, but in the presence of platinum complexes A3'p5'U is unstacked.

Discussion

The results obtained show that the cis isomers of $Pt(NH_3)_2Cl_2$ and $Pt(enim)_2Cl_2$ react with the dinucleotides A3'p5'A and A3'p5'C to form a link between the two bases of the dinucleotide producing a species with a temperature-independent, biphasic CD spectrum. No such species has been unambiguously identified in the reaction with A2'p5'A and A2'p5'C. By contrast, *trans*-$Pt(NH_3)_2Cl_2$ reacts with all four dinucleotides to give a CD spectrum with no temperature-independent biphasic signal.

In choosing the technique of CD spectroscopy to identify such species we anticipated that the CD spectrum of the resulting "clipped" dimer would dominate the spectrum yielding a CD spectrum totally independent of the temperature. This proved not to be the result since the extent of reaction of the platinum complexes with the dinucleotides was not complete at a mole ratio of metal to dimer of 1:1 and since the unreacted dinucleotide has an intense CD signal. However, by measuring the temperature dependence over as wide a range as possible to estimate the extent of reaction, evidence is obtained for the presence of a temperature-independent species in those cases noted above.

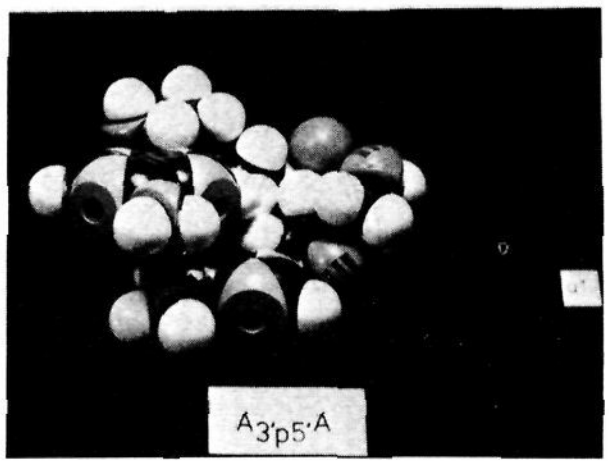


Figure 14. CPK model of A3′p5′A showing the relative positions of the 6-amino groups and their suitability for cross linking.

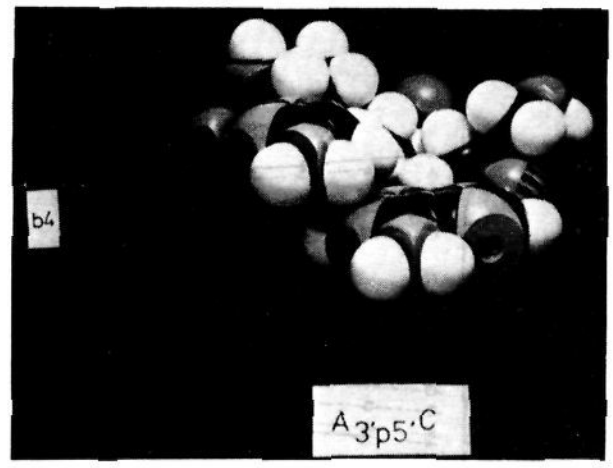


Figure 15. CPK model of A3′p5′C, showing the 4-NH_2 of cytidine and N-1 of adenosine.

In order to confirm that these species are stacked it is necessary to show that they have a biphasic CD spectrum. We therefore have taken advantage of the effect of low pH on the CD signal of the unreacted dimer. All the dimers studied are rapidly unstacked below pH 3[18,20] since the bases become protonated, positive charges being placed on the rings. The consequent repulsion leads to the unstacking. Thus a low pH abolishes the intense biphasic CD signal due to the unreacted dinucleotides. Provided that the platinum ion is not displaced from its bridging position by protons, the CD signal of the "clipped" species would be visible. This proved to be so for the case of the cis isomers with A3′p5′A and A3′p5′C but not for A2′-p5′A or A2′p5′C. No evidence for a stacked species was found with *trans*-$Pt(NH_3)_2Cl_2$ and any of the dinucleotides at low pH. Thus our technique proves useful when used in conjunction with the pH dependence studies and when a comparison is made between cis and trans isomers.

The difference between the cis and trans isomers clearly arises from their stereochemistries since their reactivities are not very different. The cis complexes have replaceable chloride ions 3–4 Å apart on the same side of the molecule[23] and the conformational data indicate that the interbase separation in the dinucleotides is 3–4 Å. Hence, potential liganding groups such as ring N atoms or amino groups are stereochemically well positioned to form a bidentate chelate. On the other hand, in the trans isomer the replaceable chloride ions are over 4 Å apart and on opposite sides of the molecule. Hence, bidentate chelation to a dinucleotide is stereochemically impossible. However, monofunctional binding at one nucleotide may take place and will place a positive charge on the ring leading to an unstacking of the dinucleotide and a weak CD spectrum.

Uridine and thymine do not appear to form simple complexes with *cis*-$Pt(NH_3)_2Cl_2$[14] and so we do not expect an interbase cross link to be formed with A3′-p5′U. The fact that both *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ cause complete unstacking of this dinucleotide agrees with this and also confirms our assumption that if one base is attacked by the platinum complex, then the dinucleotide will become unstacked. It also rules out the possibility that the results we have observed are due to a cross link between adjacent dinucleotides rather than the dinucleotide acting as a bidentate chelate, as we have postulated.

We have further shown that the "clipped" species formed between the cis isomers and A3′p5′A is stable at low pH and high pH whereas the species formed with A3′p5′C is only stable at low and intermediate pH, the cross link being lost in solutions of high pH. We now use this information, together with out knowledge of the binding sites of the platinum complexes on the rings of mononucleotides, to suggest the sites that may be involved in bidentate binding to platinum. With the aid of models built from published conformations of the dinucleotides, these sites are shown to be stereochemically reasonable.

Studies of the interaction between adenosine and *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ led to the conclusion that the cis isomer forms a bidentate complex either with the 6-NH_2 and N-1, or 6-NH_2 and N-7, whereas the trans isomer binds to N-1 or N-7.[14] In dinucleotides other sites of binding arise from groups on different bases. With A3′p5′A the obvious sites for bidentate binding of the *cis*-platinum(II) complexes are the 6-NH_2 groups of the adenosines, which, as shown in Figure 14, are directly above each other. Another possibility, involving some relative movement of the bases, is 6-NH_2-(5′) to N-1(3′) or N-7(5′) to 6-NH_2(3′).

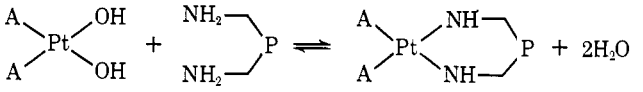
The dinucleotide A3′p5′C stacks somewhat differently, as shown in Figure 15. The bases are not directly above each other but there are still sites suitable for cross linking by *cis*-platinum(II) complexes. Previous studies with *cis*-$Pt(NH_3)_2Cl_2$ and cytidine have suggested bidentate binding of the platinum to N-3 and 4-NH_2.[14] The most obvious sites for a cross link in A3′p5′C are either the 4-NH_2 on cytidine and the N-1 of adenosine or NH_2 to NH_2, although this latter possibility would involve movement of the bases toward each other. Stereochemically the favored link would be 4-NH_2(5′) to N-1-(3′).

In aqueous solution the platinum complexes under discussion are hydrolyzed[24] and the platinum amine complexes react with the dinucleotides as the aquo or hydroxy complexes. The conformational data for these dinucleotides suggest the involvement of at least one amino group in the bidentate binding to platinum. The relevant equilibria at high pH can then be formulated as follows.

(23) G. H. W. Milburn and M. R. Truter, *J. Chem. Soc. A*, 1609 (1966).

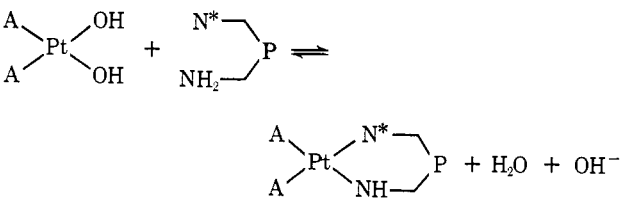
(24) F. Basolo and R. C. Pearson, "Mechanism of Inorganic Reactions," 2nd ed, Wiley, New York, N. Y., 1967.

(1) Assuming an NH_2 to NH_2 cross link, at high pH



This reaction is not suppressed at high pH.

(2) Assuming an NH_2 to N* link, where N* is either N-1 or N-7 of adenine or N-3 of cytosine, at high pH



This reaction should be displaced to the left at high pH.

Reaction of type 1 is observed with the dinucleotide A3′p5′A, in which the "clipped" species persists at high pH. However, type 2 reaction is seen with A3′-p5′C. At high pH the CD of the solutions of A3′p5′C with *cis*-platinum(II) complexes indicates that the dinucleotide is completely unstacked. The hydroxide ion is successfully competing with one of the nucleic acid bases for the platinum, thus leaving a monofunctional platinum bound to one of the nucleosides. As with A3′p5′U, this situation causes the dinucleotide to unstack.

The pH dependence and models suggest, then, that with A3′p5′A, a cross link caused by the dinucleotides acting as a bidentate chelate toward *cis*-platinum(II) complexes involves the NH_2 groups on the nucleosides. With A3′p5′C the measurements at high pH show that the cross link is from an amino group to a ring nitrogen atom. This could involve the 4-NH_2 group of cytosine and either N-1 or N-7 of adenosine or the 6-NH_2 group of adenosine and the C-N atom of cytosine. The models suggest 4-NH_2 of cytosine (5′) and N-1 of adenosine (3′) as the stereochemically favored link.

Our inability to identify a cross link with A2′p5′A and A2′p5′C may arise from the rather feeble temperature dependence of the free dinucleotide themselves. However, this is made less likely by the results of experiments carried out at low pH which also failed to reveal unambiguously a "clipped" species. Models of these dimers show that the two rings do not lie vertically above one another. Thus, to form a cross link between the two amine groups in A2′p5′A would require a lateral movement of the bases. It is not apparent from models that this would involve any steric strain. However, links between sites other than the amino groups would cause considerable steric strain. Thus, it seems likely that the affinity of both A2′p5′A and A2′p5′C toward a bidentate link with the *cis*-platinum isomers is considerably reduced compared with the 3′,5′ dinucleotides.

These studies are of interest when considering the mode of action of the platinum complexes that show antitumor activity. Only complexes of the general form *cis*-$Pt(A)_2Cl_2$ show this activity and it has been suggested that this is due to their ability to cross link the complementary strands of DNA. The results in this paper show that complexes of the form *cis*-$Pt(A)_2Cl_2$ are able to cross link the bases of dinucleotides and that it would appear that the amine groups of these bases are involved, at least with adenosine and cytidine. In DNA the sequence AT in the 3′,5′ direction places the 6-amino groups of adenosine on opposite strands vertically above each other with a separation of 3–4 Å.

There are other sites in DNA, both in the wide and the narrow groove, where amino and other reactive sites on the bases from opposite strands are separated by 3–4 Å and are therefore suitable for crosslinking by metal ions. As we have shown, it is only platinum(II) complexes with cis replaceable ligands that are able to cross link the adjacent bases of dinucleotides. It is these same complexes which possess antitumor activity.

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