Design of chelate ring-opening platinum anticancer complexes: reversible binding to guanine

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Chelate ring-opening in bis(aminophosphine) complexes of platinum(II) can be controlled by the substituents on N and P under biologically relevant conditions of pH and Cl^- concentration; selective and reversible binding to the DNA base guanine can be achieved as well as cytotoxicity towards cancer cell lines.

Two platinum(II) complexes (cisplatin and carboplatin) have already been approved for clinical use and several others are on clinical trials.[1] Current attention is focused on the development of new agents which can overcome the problem of resistance and those which are active against a wider range of types of cancer.

In the present work we have synthesized complexes containing aminophosphine ligands[2] since they combine the presence of two *cis* nitrogens, a feature found in all current Pt agents in clinical use,[1] with that of phosphine ligands. Diphosphine ligands and metal diphosphine complexes have previously been found to exhibit anticancer activity *via* a mechanism of action different from that of platinum am(m)ine complexes.[3] Because both phosphines and amines bind very tightly to Pt^{II}, bis-chelate aminophosphine complexes of platinum(II) might not be expected to be antitumour-active since they cannot readily bind to DNA bases by inner-sphere interactions. We show here that due to the high *trans* influence of P, and with appropriate choice of substituents on both P and N, it is possible to achieve selective and reversible binding to the DNA base guanine *via* a novel chelate ring-opening mechanism which can be controlled by conditions of biological relevance (pH and chloride concentration).

First we synthesized a complex containing a primary amino–tertiary phosphine ligand, *cis*-[Pt{$H_2N(CH_2)_2PPh_2$}$_2$]Cl_2 **1**, from [$PtCl_2$(cod)] (cod = cycloocta-1,5-diene) and 2 mol equiv. of the ligand. ^{195}Pt and ^{31}P NMR spectroscopy of **1** in water and X-ray crystallography[4] show that it has a bis-chelated structure in solution and in the solid state. In these systems the $^1J(^{195}Pt–^{31}P)$ coupling is diagnostic of the *trans* ligand (Pt *trans* to Cl *ca.* 3500–3800 Hz, N *ca.* 3200–3300 Hz, P *ca.* 2400–2700 Hz).[5] Remarkably, complex **1** is soluble in both organic solvents and in water; such partitioning properties can be advantageous for drug administration and uptake by cells.

When the same preparation was carried out with the tertiary amino–tertiary phosphino ligand $Me_2N(CH_2)_2PPh_2$ a crystalline product was obtained which can be formulated as *cis*-[PtCl{$Me_2N(CH_2)_2PPh_2$-*N*,*P*}{$Me_2N(CH_2)_2PPh_2$-*P*}]Cl **2a** on the basis of ^{31}P and ^{195}Pt NMR spectra. The peaks in the ^1H and ^{13}C NMR spectra of **2a** were surprisingly broad, suggesting the possibility of chemical exchange reactions (with **2**).

When complex **2a** was dissolved in D_2O the pH* (pH meter reading) was alkaline (8.6), and the ^{31}P NMR spectrum [Fig. 1(*a*)] showed evidence for the existence of both ring-closed (**2**) and -opened (**2a**) forms, in a ratio of 2 : 1. This was confirmed by the ^{195}Pt NMR spectrum [Fig. 1(*b*)] which contained a doublet of doublets (for **2a**) as well as a triplet (for **2**). The equilibrium between **2** and **2a** was found to be pH dependent: as the pH was lowered the ring-opened complex **2a** predominated, whereas at high pH* the reverse was true. The effects were reversible with change in pH*. A plot of the relative amounts of each species *vs.* pH gave a pK_a of 6.9, which can be associated with protonation of the dangling-arm amino group. Thus chelate ring-opening can be controlled within the physiological pH range.

As expected, the equilibrium between ring-closed and -opened complexes **2** and **2a** is dependent on the Cl^- concentration. Thus when all the Cl^- was removed from complex **2a** by precipitation with $AgNO_3$, only the ring-closed complex **2** was observed in D_2O or $CDCl_3$ solution by NMR. For *cis*-[Pt{$Me_2N(CH_2)_2PPh_2$}$_2$][NO_3]$_2$ **2**, it was necessary to lower the pH much further to *ca.* 2 in order to observe chelate ring opening (and protonation of the dimethylamino group). Conversely in the presence of added Cl^- (*e.g.* 10 mol equiv.) it was possible to maintain the ring-opened complex even at high pH (*e.g.* > 9).

The bis-chelated and ring-opened complexes exhibit contrasting cytotoxicities towards cancer cells. Thus the ring-closed complex **1** is relatively non-cytotoxic to LXFL non-small cell lung carcinoma and OVXF ovarian adenocarcinoma cells in

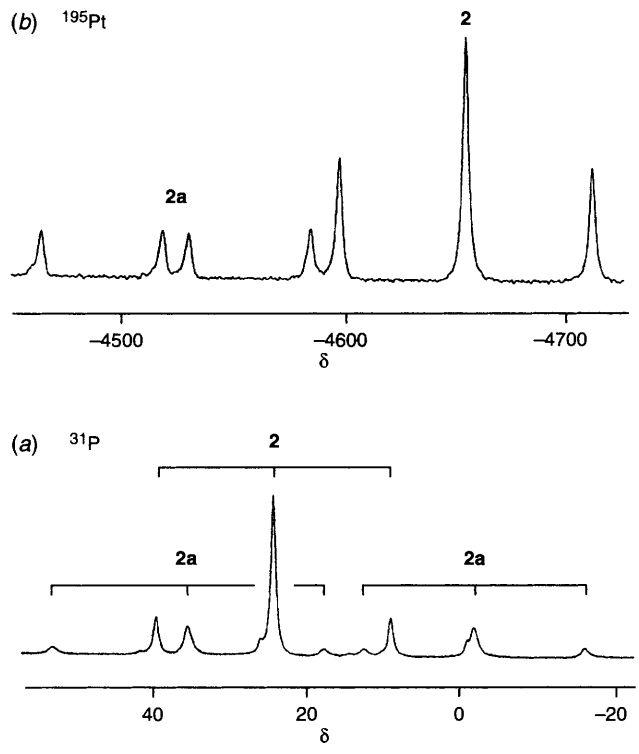


Fig. 1 Equilibrium between ring-closed and -opened complexes. NMR spectra of a solution of *cis*-[PtCl{$Me_2N(CH_2)_2PPh_2$-*N*,*P*}-{$Me_2N(CH_2)_2PPh_2$-*P*}]Cl **2a**, 20 mmol dm^{-3} in D_2O, pH* 8.6, at ambient temperature. (*a*) 109.25 MHz $^{31}P\{^1H\}$ and (*b*) 57.94 MHz $^{195}Pt\{^1H\}$, showing the presence of ring-closed (**2**) and ring-opened (**2a**) complexes. ^{31}P NMR peaks are broadened by exchange and sharpen at low pH* where ^2J couplings are well resolved (17 Hz). As expected,[6] the ^{31}P shift for a chelated ligand (*i.e.* P *trans* to Cl) is to low field of that for the ring-opened complex (P *trans* to N).

culture, whereas the ring-opened complex **2a** is only slightly less potent than cisplatin. In contrast, both **1** and **2a** are active against A2780 cells and are as potent as cisplatin. However complex **2a** has a much lower resistance factor against an A2780 cell line which has acquired resistance to cisplatin, than either cisplatin itself or complex **1**. Therefore it seems likely that ring-closed and ring-opened aminophosphine complexes can act *via* different mechanisms in different types of cells, involving attack on DNA by Pt, interference with mitochondrial membrane potentials (acting as lipophilic cations[3]), and/or redox reactions of the phosphine ligand.

We have investigated the binding of these complexes to DNA bases. The ring-closed complex **1** does not undergo facile reactions with 5′-GMP, but both **2** and **2a** react rapidly (minutes). Reaction of **2** (nitrate salt) with 5′-GMP at equimolar ratios (10 mmol dm^{-3}) in D_2O at pH* (meter reading) 5.5 gave rise to two products (>90% yield based on ^{31}P NMR peak intensities) in a *ca.* 2:1 molar ratio which can be formulated as diastereomers of *cis*-[PtCl{$Me_2N(CH_2)_2PPh_2$-*N*,*P*}{$Me_2HN(CH_2)_2PPh_2$-*P*}(5′-GMP-N^7)]$^{3+}$ (**2b**, charge on 5′-GMP ignored), on the basis of ^{31}P NMR shifts and coupling constants, Fig. 2(*a*). The 5′-GMP H^8 ^1H NMR resonances of the two isomers of **2b** are shifted to low field by *ca.* 0.6 ppm compared to free 5′-GMP which is typical for N^7 coordination by 5′-GMP.[7]

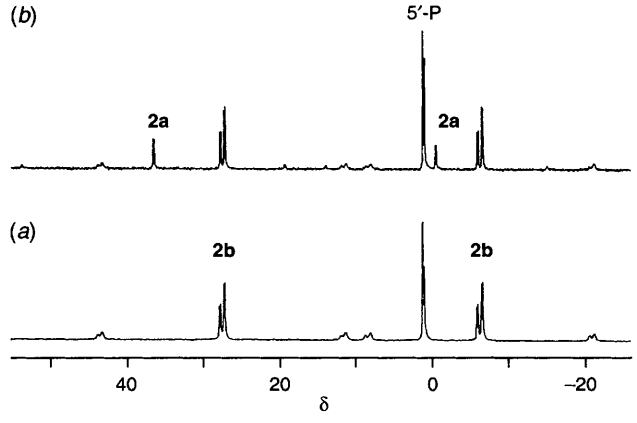
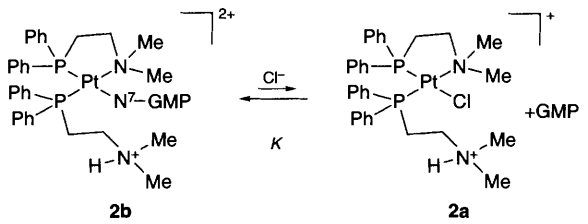


Fig. 2 Displacement of 5′-GMP from Pt^{II} by chloride. 109.25 MHz ^{31}P {^1H} NMR spectra of (*a*) complex **2b** *cis*-[PtCl{$Me_2N(CH_2)_2PPh_2$-*N*,*P*}{$Me_2HN(CH_2)_2PPh_2$-*P*}(5′-GMP-N^7)]$^{3+}$ (two isomers present in a 1:2 ratio), and (*b*) after addition of 20 mmol dm^{-3} KCl, pH* 5.5, showing formation of the chloro complex **2a**. The peaks for **2a** and **2b** are doublets due to ^{31}P–^{31}P coupling, together with ^{195}Pt satellites. The singlet for 5′-GMP is overlapped with the two singlets for 5′-phosphate of the isomers of **2b**.



Scheme 1 Displacement of GMP by chloride

Addition of KCl led to the displacement of coordinated 5′-GMP from **2b** and formation of the ring-opened chloride adduct **2a**, Scheme 1, Fig. 2(*b*). From NMR peak intensities over the titration range 1–10 mol equiv. KCl at pH* 5.5, an equilibrium constant K = ([**2b**][Cl])/([**2a**][GMP]) = 0.14 ± 0.01 was calculated. The reaction of **2** with 5′-GMP was similar at pH* 7, except that the isomer ratio for **2b** was 3:1. The equilibrium constant for displacement of 5′-GMP by chloride appeared to have a similar value at pH* 7 although a full titration was not carried out. No reaction between complex **2** or **2a** with 5′-adenosine monophosphate (5′-AMP) was observed under similar conditions, even over a wide pH range and after standing at ambient temperature for several weeks.

The facile displacement of N^7-bound GMP from Pt^{II} by chloride appears to be unprecedented, although Yang *et al.*[8] have recently observed a chloride-induced migration of platinum from an intra- to an inter-strand GG crosslink. The driving force for chelate ring-opening in complex **2** is probably the relief of steric interactions caused by the substituents on *cis* N and P atoms, coupled with the high *trans* influence of P which leads to a weakening of the Pt–N bond.[9] Also the amino group in the dangling arm may be involved in hydrogen-bonding interactions with coordinated Cl^- or GMP, as indicated by preliminary molecular modelling.[10] Surprisingly, even *N*-acetyl-L-methionine, a thioether and potential S-donor, did not cause ring-opening of complex **2** and did not displace chloride from complex **2a**. This suggests that the environment created around Pt by the hindered aminophosphine ligands exerts a high degree of electronic and steric control over the recognition of incoming ligands. It remains to be seen whether ring-opening complexes of the type described here will exhibit antitumour activity *in vivo*.

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References

1 J. Reedijk, *Chem. Commun.*, 1996, 801.
2 Previous reports of the use of related ligands include: Von K. Issleib and A. Kipke, *Z. Anorg. Allg. Chem.*, 1980, **464**, 176; G. K. Anderson and R. Kumar, *Inorg. Chem.*, 1984, **23**, 4064; M. Bassett, D. L. Davies, J. Neild, L. J. S. Prouse and D. R. Russell, *Polyhedron*, 1991, **10**, 501.
3 S. J. Berners-Price and P. J. Sadler, *Struct. Bonding (Berlin)*, 1988, **70**, 27.
4 A. Habtemariam, B. S. Potter, R. E. Palmer and P. J. Sadler, to be submitted.
5 P. S. Pregosin, *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*, VCH, New York, 1987, vol. 8, p. 465.
6 P. E. Garrou, *Chem. Rev.*, 1981, **81**, 229.
7 S. J. Berners-Price, U. Frey, J. D. Ranford and P. J. Sadler, *J. Am. Chem. Soc.*, 1993, **115**, 8649.
8 D. Yang, S. S. G. E. van Boom, J. Reedijk, J. H. van Boom and A. H.-J. Wang, *Biochemistry*, 1995, **34**, 12912.
9 M. L. Tobe, *Inorganic Reaction Mechanisms*, Nelson, London, 1972.
10 A. Habtemariam, T. W. Hambley, N. Margiotta and P.J. Sadler, unpublished work.

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