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The Influence of the Axial Ligands of a Series of Platinum(IV) Anti-Cancer Complexes on their Reduction to Platinum(II) and Reaction with DNA†

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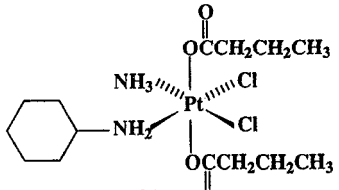
Abstract

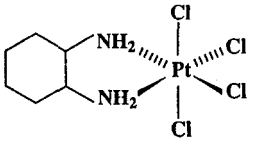
The electrochemical reduction and DNA binding have been studied for a series of platinum(IV) complexes with Cl^-, OH^-, and carboxylate anions as the axial ligands; $[Pt(en)Cl_4]$, $[Pt(en)Cl_2(OH)_2]$, and $[Pt(en)Cl_2(OC(O)R)_2]$, R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$. Cathodic reduction potentials vary by more than 650 mV with the tetrachloro complex reduced most readily and the dihydroxo least readily. The binding of the complexes correlates with the reduction potentials with the more readily reduced complexes binding more readily to DNA. The influence of the reducing agent glutathione on platinum binding to DNA was found to depend on whether it was added before or after Pt/DNA incubation. The results are consistent with octahedral platinum(IV) binding monofunctionally to DNA, and molecular modelling studies have been used to confirm that this is sterically feasible. The crystal structure of $[Pt(en)Cl_2(OC(O)CH_3)_2]$ has been determined by X-ray diffraction methods and refined to $R = 0{\cdot}028$ (977 F). The crystals are monoclinic, space group $C\,2/c$, a 15·569(6), b 8·104(1), c 13·188(1) Å, β 136·38(2)°.

Introduction

The recent development[1] of new, orally administrable, anti-cancer drugs such as JM221 has revitalized interest in the biological properties of platinum(IV) complexes. It has been known since the early studies of Rosenberg and his colleagues that platinum(IV) complexes exhibit substantial anti-cancer activity.[2,3]



JM221



tetraplatin

† Dedicated to the 'Golden Oldies' of ANZ Inorganic Chemistry, in particular Alan Sargeson and Hans Freeman.

[1] McKeage, M. J., Abel, G., Kelland, L. R., and Harrap, K. R., *Br. J. Cancer*, 1994, **69**, 1.
[2] Rosenberg, B., Van Camp, L., and Krigas, T., *Nature (London)*, 1965, **205**, 698.
[3] Rosenberg, B., Van Camp, L., Trosko, J. E., and Mansour, V. H., *Nature (London)*, 1969, **222**, 385.

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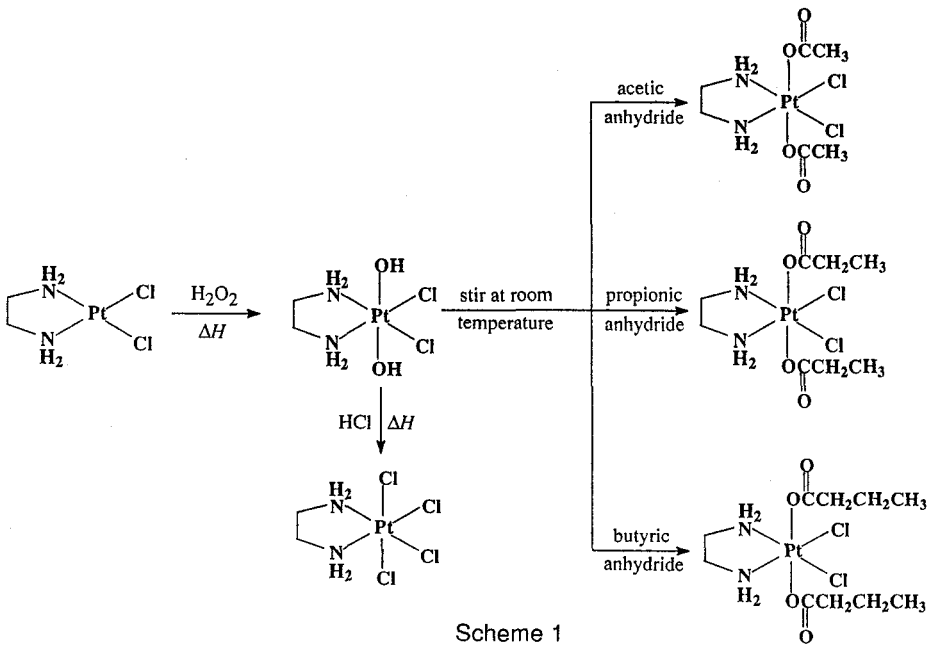
More recently, the platinum(IV) complex tetraplatin showed sufficient promise, particularly against tumours resistant to cisplatin, for it to be entered into clinical trials.[4] Unfortunately, tetraplatin was found to be too neurotoxic for the trials to continue and until the development of the new complexes with axial carboxylato ligands, interest in platinum(IV) compounds has not been as intense.

The mechanism of action of platinum(II)-based anti-cancer drugs almost certainly involves coordinative bonding of the platinum atom to DNA.[5,6] It has long been suspected that active platinum(IV) complexes do not themselves effect the anti-cancer action but are reduced intracellularly to their platinum(II) analogues.[7] In support of this are a number of studies which show that platinum(IV) complexes are readily reduced by both extracellular and intracellular reducing agents.[8–11] Further, it has been shown that platinum(IV) complexes do not unwind supercoiled plasmid DNA in the same way as their platinum(II) analogues.[7] Unwinding is known to be principally due to formation of the bifunctional adducts.[12] Thus, these results are consistent with platinum(IV) not forming the bifunctional adducts which are believed to be responsible for anti-cancer activity,[5,6] but the formation of monofunctional platinum(IV)/DNA adducts cannot be ruled out.

The majority of platinum(IV) complexes studied as potential anti-cancer agents have had either chloro or hydroxo ligands in the axial positions, but the new complexes, with carboxylato ligands in these positions, add an extra dimension to the family of platinum(IV) complexes. The kinetic stability of the platinum(IV)-axial ligand bonds in particular is known to strongly influence the reactions and reduction of platinum(IV) complexes[13] and, therefore, can be expected to exert an influence on their biological activity. In order to assess the effect of the axial ligands on the reduction to platinum(II) and on reaction with DNA we have undertaken electrochemical and other studies of a series of platinum(IV) complexes in which the axial sites are occupied by carboxylato, chloro or hydroxo ligands (Scheme 1). We have chosen $[Pt(en)Cl_2]$ (en = ethane-1,2-diamine) as the 'parent' platinum(II) complex for reasons of simplicity and to avoid any possibility of *cis/trans* isomerism. Numerous carboxylato ligands were tested during the development of JM221, and the activity was found to increase with increasing lipophilicity.[1] Therefore, we have studied a series of three complexes with carboxylato ligands bearing different sized aliphatic groups to assess whether this influences reaction with DNA or reduction to platinum(II). To investigate whether platinum(IV) complexes can bind to DNA we have carried out studies of binding to plasmid DNA both in the absence and presence of glutathione, a common intracellular reducing agent. We have also used molecular modelling to assess whether there are substantial steric barriers to six-coordinate platinum(IV) complexes binding to DNA.

[4] Schilder, R. J., La Creta, F. P., and Perez, R. P., *Cancer Res.*, 1994, **54**, 709.
[5] Roberts, J. J., *Adv. Inorg. Biochem.*, 1981, **3**, 274.
[6] Pinto, A. L., and Lippard, S. J., *Biochim. Biophys. Acta*, 1985, **780**, 167.
[7] Blatter, E. E., Vollano, J. F., Krishnan, B. S., and Dabrowiak, J. C., *Biochemistry*, 1984, **23**, 4817.
[8] van der Veer, J. L., Peters, A. R., and Reedijk, J., *J. Inorg. Biochem.*, 1987, **26**, 3617.
[9] Eastman, A., *Biochem. Pharmacol.*, 1987, **36**, 4177.
[10] Gibbons, G. R., Wyrick, S., and Chaney, S. G., *Cancer Res.*, 1989, **49**, 1402.
[11] Chaney, S. G., Gibbons, G. R., Wyrick, S., and Podhasky, P., *Cancer Res.*, 1991, **51**, 969.
[12] Keck, M. V., and Lippard, S. J., *J. Am. Chem. Soc.*, 1992, **114**, 3386.
[13] Hartley, F. R., 'The Chemistry of Platinum and Palladium' (John Wiley: New York 1973).



Scheme 1

Experimental

Infrared spectra were recorded on BIO-RAD FTS (in the region 4000–400 cm^{-1}) and FTS-20 (in the region 550–100 cm^{-1}) Fourier-transform infrared spectrometers as KBr and polyethylene pellets respectively.

Dichloro(ethane-1,2-diamine)platinum(II)

The method of synthesis used here was adapted from that reported by Dhara.[14] A 10% excess of ethane-1,2-diamine (0·50 g, 8·3 mmol) was added to a solution of potassium tetraiodoplatinate(II), formed *in situ*, and the mixture was stirred for at least 5 min until yellow ethane-1,2-diaminediiodoplatinum(II) precipitated. This was filtered off, washed with water, ether and dried in a desiccator overnight. Yield 3·56 g, 7·0 mmol, 94%.

Ethane-1,2-diaminediiodoplatinum(II) (3·56 g, 6·99 mmol) was resuspended in water (50 ml), and silver nitrate (2·36 g, 14·0 mmol) in *c.* 3 ml of water was added. This mixture was heated to about 50°C and stirred for 1 h. A yellow precipitate of silver iodide formed and was removed by filtration. The filtrate was treated with a tenfold excess of sodium chloride (4·09 g, 69·9 mmol), and the solution was heated for 0·5 h, after which time dichloro(ethane-1,2-diamine)platinum(II) appeared as a bright yellow solid. The mixture was cooled to 4°C and the product collected at the pump. The solid was washed with ice-cold water, ethanol and ether. Yield 2·0 g, 6·1 mmol, 82%.

cis-*Dichloro(ethane-1,2-diamine)*-trans-*dihydroxoplatinum*(IV)

The complex was prepared by a combination of the methods described by Vollano *et al.*[15] and Tschugajeff *et al.*[16] A tenfold excess hydrogen peroxide (3% w/v, 69·5 ml, 61·3 mmol) was added to dichloro(ethane-1,2-diamine)platinum(II) (2·0 g, 6·1 mmol). The mixture was stirred for 1 h at 50°C and pale yellow *cis*-dichloro(ethane-1,2-diamine)-*trans*-dihydroxoplatinum(IV) precipitated. The solid was collected and washed with dimethyl sulfoxide, to remove any unreacted dichloro(ethane-1,2-diamine)platinum(II) complex, water and dried in a desiccator

[14] Dhara, S. C., *Indian J. Chem.*, 1971, **8**, 193.
[15] Vollano, J. F., Blatter, E. E., and Dabrowiak, J. C., *J. Am. Chem. Soc.*, 1984, **105**, 2732.
[16] Tschugajeff, V. L., and Chlopin, W., *Z. Anorg. Allg. Chem.*, 1925, **151**, 253.

overnight. Yield 1·86 g, 5·54 mmol, 90%. ν_{max} (KBr) 3490s cm^{-1} (OH). An infrared spectrum was run to ensure that the product was free from hydrogen peroxide.

Tetrachloro(ethane-1,2-diamine)platinum(IV)

Hydrochloric acid (0·1 M, 5·6 ml, 0·56 mmol) was added to *cis*-dichloro(ethane-1,2-diamine)-*trans*-dihydroxoplatinum(IV) (0·10 g, 0·28 mmol), and the mixture was stirred for 0·5 h at 50°C. Pale yellow solid tetrachloro(ethane-1,2-diamine)platinum(IV) formed. Yield 0·09 g, 0·23 mmol, 82%. ν_{max} (polyethylene) 256m cm^{-1} (*trans*)-Pt–Cl.

trans-*Dicarboxylato*-cis-*dichloro(ethane-1,2-diamine)platinum*(IV)

The synthesis was adapted from those reported by Khokhar *et al.*[17] and Giandomenico *et al.*[18] *cis*-Dichloro(ethane-1,2-diamine)-*trans*-dihydroxoplatinum(IV) (0·30 g, 0·84 mmol) was suspended in dichloromethane (50 ml), and a large excess of acetic anhydride (5 ml) was added. The reaction mixture was stirred at room temperature for approximately 2 weeks with periodic additions of additional acetic anhydride until the reaction was complete. The pale yellow *trans*-diacetato-*cis*-dichloro(ethane-1,2-diamine)platinum(IV) complex was recrystallized from water. Yield 0·20 g, 5·5 mmol, 51% (Found: C, 16·1; H, 3·0, N 6·1. $C_6H_{14}Cl_2N_2O_4Pt$ requires C, 16·2, H, 3·2; N, 6·3%). ν_{max} 3212s, 2976m, 2953m, 1645s, 1564s, 1429m, 1362m, 1285m, 1214m, 1031m, 935m, 707m cm^{-1}.

cis-Dichloro(ethane-1,2-diamine)-*trans*-dipropionatoplatinum(IV) and *trans*-dibutyrato-*cis*-dichloro(ethane-1,2-diamine)platinum(IV) were prepared in an analogous manner by replacing acetic anhydride with propionic and butyric anhydride respectively.

The reactions were monitored by infrared spectroscopy by periodically removing small samples from the reaction flask, drying them on a slide in a desiccator under vacuum and recording the spectrum. The reaction with propionic anhydride took approximately 5 weeks to complete while the reaction with the butyric anhydride took 6 weeks with the completion confirmed by the disappearance of the OH stretch and the presence of the carboxylate peaks. The *products* were recrystallized from boiling water giving similar yields. *cis*-Dichloro(ethane-1,2-diamine)-*trans*-dipropionatoplatinum(IV) (Found: C, 20·3; H, 3·6; N 5·7. $C_8H_{18}Cl_2N_2O_4Pt$ requires C, 20·4; H, 3·8; N, 5·9%). ν_{max} 3205s, 2985m, 2957m, 1636s, 1544m, 1350s, 1256s(sh), 1231vs, 1176m cm^{-1}. *trans*-Dibutyrato-*cis*-dichloro(ethane-1,2-diamine)platinum(IV) (Found: C, 22·6; H, 4·4; N, 5·7. $C_{10}H_{22}Cl_2N_2O_4Pt$ requires C, 24·0; H, 4·4; N, 5·7%). ν_{max} 3205s, 2964s, 2946s, 2887m, 1647s, 1554m, 1459m, 1376s, 1294m(sh), 1212vs cm^{-1}.

X-Ray Crystallography

A hexagonal crystal of *cis,trans*-[Pt(en)Cl_2($OC(O)CH_3$)$_2$] with dimensions 0·25 by 0·044 by 0·044 mm was mounted on a glass fibre, and 25 independent reflections were collected at 21°C on an Enraf Nonius CAD-4F four-circle diffractometer employing graphite-monochromatized MoKα radiation.

Crystal data.—Formula *cis,trans*-[Pt($NH_2CH_2CH_2NH_2$)Cl_2($OC(O)CH_3$)$_2$]; M 442·17, monoclinic, space group $C2/c$, a 15·569(6), b 8·104(1), c 13·188(1) Å, β 136·38(2)°, V 1147·93 Å^3, Z 4, D_c 2·558 g cm^{-3}, μ(Mo Kα) 127·5 cm^{-1}, λ(Mo Kα) 0·71069 Å, F(000) 824 e.

Intensity data were collected in the range $1 < \theta < 25°$ using an ω scan. The scan widths and horizontal counter apertures employed were $(1·05+0·35 \tan\theta)°$ and $(1·9+1·05 \tan\theta)$ mm respectively. Data reduction, and application of Lorentz, polarization, decomposition (<1%) and absorption (maximum transmission 0·426, minimum transmission 0·300) corrections were carried out with the Enraf–Nonius Structure Determination Package.[19] Of the 1138 independent non-zero reflections collected, 977 with $I > 2·5\sigma(I)$ were considered observed and used in the calculations.

[17] Khokhar, A. R., Deng, Y., Kido, Y., and Siddik, Z. H., *J. Inorg. Biochem.*, 1993, **50**, 79.
[18] Giandomenico, C. M., Abrams, M. J., Murrer, B. A., and Vollano, J. F., Sixth International Symposium on Platinum and Other Metal Coordination Compounds, San Diego, California, 1991, p. 58.
[19] Enraf–Nonius Structure Determination Package, Enraf Nonius, Delft, 1985.

The structure was solved by the heavy-atom method with SHELX-76,[20] and the solution was extended by difference-Fourier methods. All of the hydrogen atoms were successfully located and refined with group isotropic thermal parameters, and the non-hydrogen atoms were refined anisotropically. Full-matrix least-squares refinement of an overall scale factor, positional and thermal parameters converged (all shifts $< 0{\cdot}01\sigma$) with R 0·028,† R_w 0·028 and $w = 1{\cdot}0/(\sigma^2(F_o)+0{\cdot}0016F_o^2)$. Maximum excursions in a final difference map were +3·4 and −2·0 e Å^{-3}. Scattering factors and anomalous dispersion terms for Pt (neutral Pt) were taken from International Tables,[21] and for all other atoms those supplied in SHELX-76 were used. All calculations were carried out with SHELX-76, and plots were drawn with ORTEP.[22]

Table 1. Positional coordinates for non-hydrogen atoms of *cis,trans*-[Pt(en)Cl$_2$(OC(O)CH$_3$)$_2$]

Atom	x	y	z	Atom	x	y	z
Pt	0	0·21680(2)	0·25	C(2)	0·1548(1)	0·2743(6)	0·1978(7)
Cl(1)	0·1034(1)	0·0160(2)	0·4306(2)	C(3)	0·2783(7)	0·2505(12)	0·2535(9)
N(1)	0·0904(4)	0·4036(6)	0·4002(5)	O(1)	0·1496(4)	0·2097(4)	0·2855(6)
C(1)	0·0664(6)	0·5584(7)	0·3240(7)	O(2)	0·0719(4)	0·3520(6)	0·0860(5)

An ORTEP plot of *cis,trans*-[Pt(en)Cl$_2$(OC(O)CH$_3$)$_2$] is shown in Fig. 1. Positional atomic coordinates, bond lengths, and bond angles are presented in Tables 1–3. Observed and calculated structure factors, thermal parameters for non-hydrogen atoms, positional and thermal parameters of the hydrogen atoms, and close inter- and intra-molecular contacts are deposited as an Accessory Publication.‡

Electrochemistry

The platinum complexes were each dissolved in 5 ml of 0·1 M KCl solution. The final concentration of each solution with respect to the platinum complex was 2 mM. Each of the solutions was flushed with nitrogen to remove any traces of oxygen. Cyclic voltammetric experiments were performed on a BAS100 instrument at 20±1°C with a scan rate of 100 mV s^{-1}. The working electrode was a glassy carbon electrode, the reference electrode was Ag/AgCl, and the auxiliary electrode was a platinum wire.

Plasmid-Binding Assays

Plasmid-binding experiments were adapted from methods described by Ushay *et al.*[23] Fresh stocks of the platinum complexes (10 mg) were prepared in H_2O (10 ml) immediately before use to avoid hydrolysis. The platinum solutions were filtered through 0·2 μm Millipore filters, and made up to the appropriate concentrations in the range 0–40 μM. Dialysed pGEM3Zf(−) DNA (5 μl, 85·3 μg/ml H_2O) and sterilized buffer (4 μl, 3 mM NaCl, 1 mM phosphate, pH 7·4) were added, and the volume in each tube was adjusted to 20 μl with sterilized H_2O.

The tubes were vortexed and pulse-centrifuged to facilitate mixing, and the reaction was allowed to proceed by incubation at 37°C for 3 h. The samples were then cooled to 0°C to prevent any further reaction from taking place. The platinum samples were incubated with 2 equiv. of glutathione for 20 h either before or after the addition of DNA. To each of the

† $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$, $R_w = \Sigma(w^{1/2}||F_o| - |F_c||)/\Sigma w^{1/2}|F_o|$.

‡ Copies are available on application to the Australian Journal of Chemistry, P.O. Box 89, East Melbourne, Vic. 3002. (The Accessory Publication includes force field parameters—see under Molecular Modelling.)

[20] Sheldrick, G. M., SHELX-76, A Program for X-Ray Crystal Structure Determination, University of Cambridge, 1976.
[21] 'International Tables for X-Ray Crystallography' Vol. 4 (Kynoch Press: Birmingham 1974).
[22] Johnson, C. K., ORTEP, A Thermal Ellipsoid Plotting Program, Oak Ridge National Laboratories, Oak Ridge, 1965.
[23] Ushay, H. M., Tulius, T. D., and Lippard, S. J., *Biochemistry*, 1981, **20**, 3744.

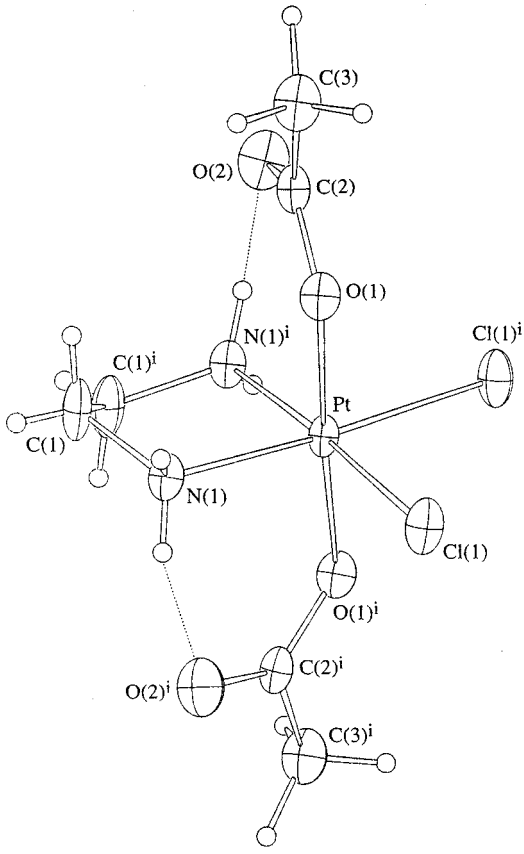


Fig. 1. ORTEP plot (30% thermal ellipsoids) of *cis,trans*-$[Pt(en)(OC(O)CH_3)_2]$ with atom numbering scheme.

Table 2. Bond lengths of *cis,trans*-$[Pt(en)Cl_2(OC(O)CH_3)_2]$

Superscript i denotes $-x, y, \frac{1}{2}-z$

Atoms	Distance (Å)	Atoms	Distance (Å)
Pt–Cl(1)	2·315(1)	Pt–O(1)	2·017(5)
Pt–N(1)	2·040(5)	C(1)–N(1)	1·476(7)
C(1)–C(1)^i	1·497(12)	C(2)–O(2)	1·218(8)
C(2)–C(3)	1·492(10)	C(2)–O(1)	1·325(8)

Table 3. Bond angles of *cis,trans*-$[Pt(en)Cl_2(OC(O)CH_3)_2]$

Superscript i denotes $-x, y, \frac{1}{2}-z$

Atoms	Angle (degrees)	Atoms	Angle (degrees)
O(1)–Pt–Cl(1)	85·1(1)	N(1)–Pt–Cl(1)	92·6(2)
N(1)–Pt–O(1)	85·5(2)	N(1)–Pt–N(1)	84·1(3)
Cl(1)–Pt–Cl(1)^i	90·7(1)	O(1)–Pt–O(1)^i	174·9(3)
O(1)–Pt–Cl(1)^i	92·7(2)	O(1)–Pt–N(1)^i	96·3(3)
N(1)–Pt–Cl(1)^i	176·5(2)	C(2)–O(1)–Pt	125·2(4)
C(1)–N(1)–Pt	108·2(3)	O(2)–C(2)–O(1)	125·5(6)
C(3)–C(2)–O(1)	113·0(6)	C(3)–C(1)–O(2)	121·5(6)
N(1)–C(1)–C(1)^i	108·4(6)		

tubes, 10X restriction buffer B (3 μl, 10 mM Tris–HCl, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM 2-mercaptoethanol, pH 8·0) and BamH1 (0·5 μl, activity 1 U/l) were added. Sterilized H_2O was added so that the final volume in each tube was 30 μl. The tubes were incubated at 37°C for 1 h to allow any DNA digestion to take place, then rapidly cooled to 0°C to prevent any further reaction from taking place.

Gel Electrophoresis

Tracking dye (6 μl, 0·25% bromophenol blue, 40% sucrose in water) was added to each tube, and 20 μl portions were loaded on an agarose gel and electrophoretically chromatographed. The agarose gels (1·5% w/v) were prepared with TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM boric acid, 2 mM edta, adjusted to pH 8·0 with 10 M HCl) containing ethidium bromide (0·5 g ml^{-1}). The agarose gel was contained in TBE buffer solution with ethidium bromide (0·5 g ml^{-1}). Electrophoresis was carried out at 20 V for 20 h, then 50 V for 1 h. Each gel was photographed under u.v. light through an orange filter, Polaroid type 55 film being used.

Molecular Modelling

A force field for modelling $[Pt(en)Cl_4]$, $[Pt(en)Cl_2(OH)_2]$ and $[Pt(en)Cl_2(OC(O)CH_3)_2]$ was generated by using the force constants described previously.[24,25] For those force constants that could not be found in the literature (Pt^{IV}–X), an estimate was made from the infrared stretching frequency according to established procedures.[26] The force field parameters developed in this work have been deposited. Hydrogen atom positions were calculated with the HPUT program.[27] Strain energy minimization calculations were carried out with the MOMEC-91 program.[28] Convergence was determined to have occurred when the shifts for the atomic coordinates were less than, or equal to, 0·002 Å.

The binding of $[Pt(en)Cl_4]$ to the N 7 position of guanine on double-stranded DNA with the sequence GG*GG:CCCC was modelled. The DNA starting models were constructed by using the HyperChem program.[29] The DNA force field used was that described previously.[30] Models with $[Pt(en)Cl_3\_]$ bound through axial and equatorial positions were set up. The energy was minimized by using the MOMEC-91 program.

Results and Discussion

Synthesis

Khokhar *et al.* reported that the synthesis of the diacetato complex, $[Pt(en)Cl_2(OC(O)CH_3)_2]$, took 2–3 days to reach completion.[17] In our hands the reaction took 2 weeks, and in the case of the dipropionato and dibutyrato complexes the reactions took up to 6 weeks. We considered the possibility that our efforts to remove all traces of platinum(II), so as to avoid interferences in the plasmid-binding studies, may have contributed to the slowness of the reactions since platinum(II) catalytically increases the rate of ligand exchange at platinum(IV) centres. However, addition of trace amounts of $[Pt(en)Cl_2]$ did not noticeably increase the reaction rate.

[24] Hambley, T. W., *Inorg. Chem.*, 1988, **30**, 937.
[25] Hambley, T. W., *Acta Crystallogr., Sect. B*, 1988, **44**, 601.
[26] Nakamoto, K., 'Infrared and Raman Spectroscopy of Inorganic and Coordination Compounds' 3rd Edn (Wiley–Interscience: New York 1978).
[27] Hambley, T. W., 'HPUT, Programme for Calculation of Hydrogen Atom Positions', University of Sydney, Australia, 1985.
[28] Hambley, T. W., 'MOMEC-91, Programme for Strain Energy Minimisation', University of Sydney, Australia, 1991.
[29] 'HyperChem, Structure Determination Package (SDP)', Enraf Nonius, Delft, Holland, 1985.
[30] Hambley, T. W., *Inorg. Chem.*, 1991, **30**, 937.

Description of the Structure

The crystal structure analysis confirms that the product sought, *cis,trans*-$[Pt(en)Cl_2(OC(O)CH_3)_2]$, was obtained. The platinum atom lies on a twofold rotation axis that also passes through the centre of the C–C bond of the ethane-1,2-diamine ring. The coordination geometry is similar to that in the closely related complex $[Pt(en)(OC(O)CH_3)_2(cbdca)]$ (cbdca = cyclobutane-1,1-dicarboxylic acid).[31] The Pt–O(carboxylate) and Pt–N(amine) bond lengths [2·017(5) and 2·040(5) Å] are indistinguishable from those in $[Pt(en)(OC(O)CH_3)_2(cbdca)]$ [2·008(6), 2·030(6) and 2·044(7) Å].[31] There are intramolecular hydrogen bonds between the terminal O atoms of the carboxylato ligands and H(amine) atoms of the ethane-1,2-diamine ligand (Fig. 1) which also occur in $[Pt(en)(OC(O)CH_3)_2(cbdca)]$. There are also intermolecular hydrogen bonds between the O atoms of the carboxylato ligands, the chloro ligands and H(amine) atoms of the ethane-1,2-diamine.

Electrochemistry

The cyclic voltammograms of the three dicarboxylato complexes are shown in Fig. 2*a*. All of the reduction processes are, as expected, irreversible, since reduction involves loss of the axial ligands. The cathodic potentials for $[Pt(en)Cl_2(OC(O)R)_2]$, R = CH_3, CH_2CH_3 and $CH_2CH_2CH_3$, occur at −546, −521 and −493 mV respectively. The variation in the cathodic potential follows the trend that would be expected if steric interactions between the carboxylato ligand and the remainder of the complex contributed to the value of the cathodic potential by promoting loss of the axial carboxylato ligands. Specifically, the bulkiest of the three complexes, R = $CH_2CH_2CH_3$, is the most readily reduced. However, the variation is small and is probably not significant in terms of the biological chemistry of these complexes. There is also a trend in the peak current, with that for R = CH_3 being highest and that for R = $CH_2CH_2CH_3$ being lowest, presumably as a result of a reduction in electromobility arising from increasing steric bulk.

The cyclic voltammograms of the complexes $[Pt(en)Cl_4]$, $[Pt(en)Cl_2(OH)_2]$ and $[Pt(en)Cl_2(OC(O)CH_3)_2]$ are compared in Fig. 2*b*. The tetrachloro complex is reduced at a substantially more positive cathodic potential, −224 mV, than the dihydroxo complex, −884 mV, and the diacetato complex falls midway between these two. This difference in reduction potentials is highly likely to influence the biological properties of the complexes. If, as suggested above, reduction of platinum(IV) to platinum(II) is essential for anti-cancer activity to be manifested, then the rate of reduction and the potential at which it took place would be important considerations. Too rapid or too ready reduction may be unfavourable as the side effects associated with the more reactive platinum(II) species are greater. Too slow reduction might lead to the platinum(IV) complex being passed through the body without effecting any anti-cancer activity.

Plasmid-Binding Studies

The plasmid DNA used, pGEM3Zf(−), has one BamH1 restriction site (5′-GGATCC-3′) containing two neighbouring guanines,[32] which are the principal binding site of platinum complexes. BamH1 cuts the plasmid DNA at this site, converting form I (closed circular DNA) into form II (nicked circular DNA) and

[31] Deng, Y., and Khokar, A. R., *Inorg. Chim. Acta*, 1993, **204**, 35.
[32] Lewin, B., 'Genes IV', Oxford University Press, 1990.

form III (linear DNA). Binding of platinum complexes at the BamH1 site will inhibit the cutting of the DNA by BamH1. Thus, the inhibition of cutting, indicated by the reduction in the intensity of the form III band, is a simple measure of platinum binding to DNA. Dichloro(ethane-1,2-diamine)platinum(II) is known to bind to DNA and, as expected, there is an increase in the intensity of the form II and form III bands on the electrophoretic gel with increasing concentrations of this complex, with total inhibition of cutting, occurring at concentrations of 10 μM and greater (Fig. 3*a*). The increase in the mobility of particularly form II of the DNA with increasing platinum concentration is indicative of the conformational change that occurs on the formation of bifunctional platinum adducts.[12]

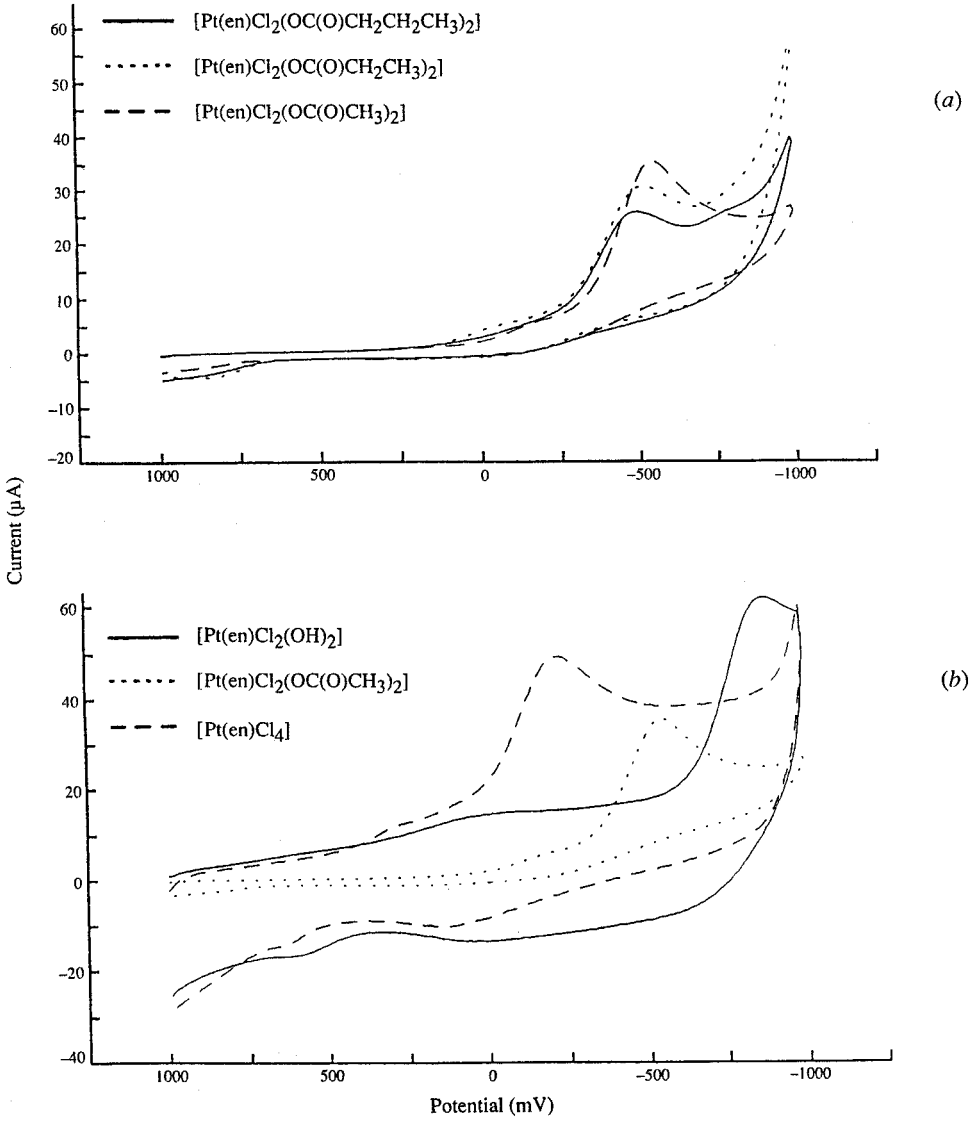
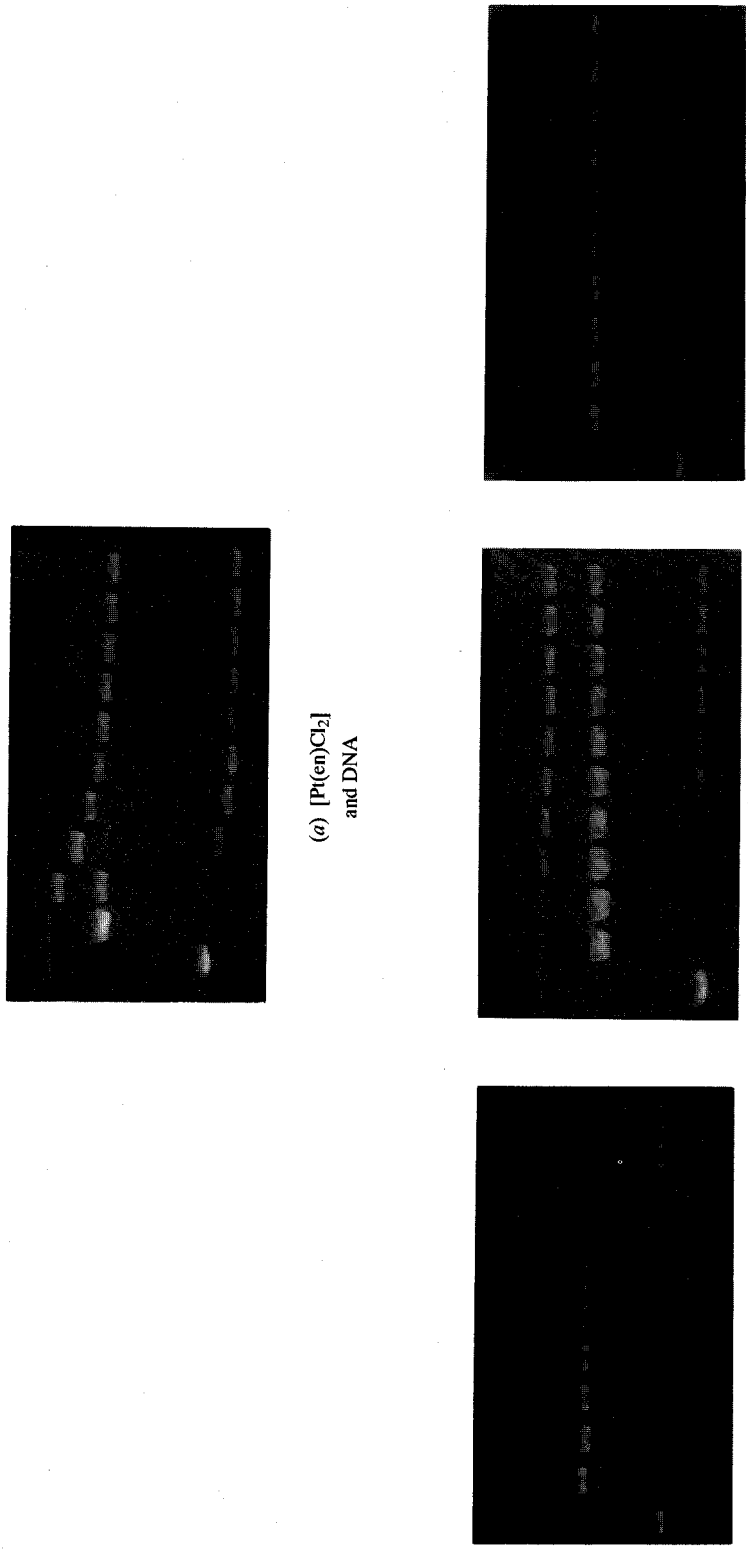


Fig. 2. Cyclic voltammograms for (*a*) $[Pt(en)Cl_2(OC(O)R)_2]$, R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$; (*b*) $[Pt(en)Cl_4]$, $[Pt(en)Cl_2(OH)_2]$, $[Pt(en)Cl_2(OC(O)CH_3)_2]$.

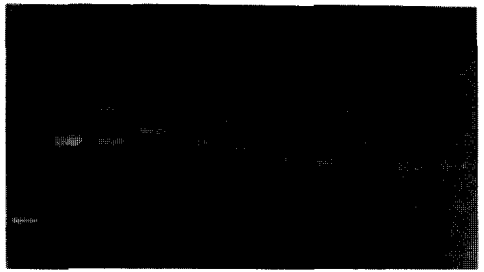


(*a*) [Pt(en)Cl_2] and DNA

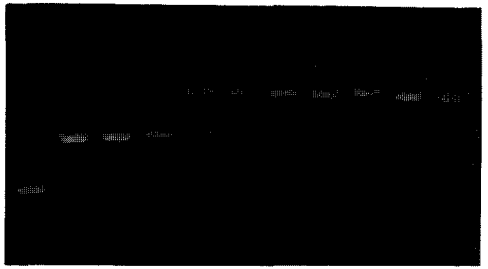
(*b*) [Pt(en)Cl_4] and DNA

(*c*) [Pt(en)Cl_2(OC(O)CH_3)$_2$] and DNA

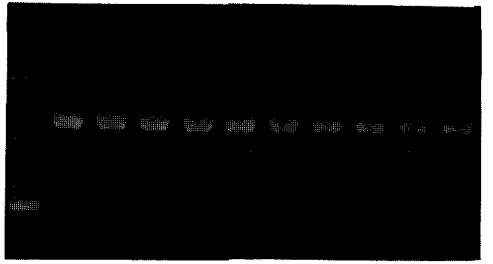
(*d*) [Pt(en)Cl_2(OH)$_2$] and DNA



(*e*) [Pt(en)Cl_4]
and DNA then glutathione

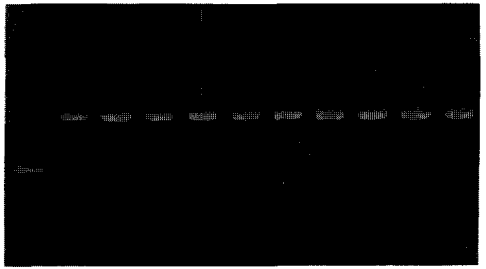


(*f*) [Pt(en)Cl_2(OC(O)CH_3)$_2$]
and DNA then glutathione

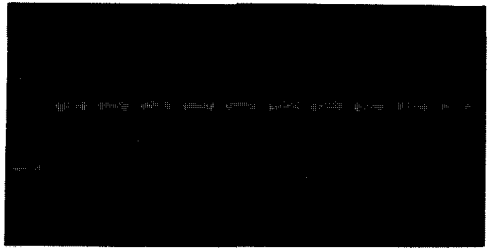


(*g*) [Pt(en)Cl_2(OH)$_2$]
and DNA then glutathione

(*h*) [Pt(en)Cl_4]
and glutathione then DNA



(*i*) [Pt(en)Cl_2(OC(O)CH_3)$_2$]
and glutathione then DNA



(*j*) [Pt(en)Cl_2(OH)$_2$]
and glutathione then DNA

Fig. 3. Agarose gels showing the electrophoretic mobility of plasmid DNA treated with the reagents indicated and BamH1 restriction enzyme. In each gel, the 11 lanes correspond to DNA only; DNA and BamH1; and DNA, BamH1 and the platinum complex at concentrations of 1, 5, 10, 15, 20, 25, 30, 35 and 40 μM. The three bands (from the top down) correspond to form II (open circular, least mobile), form III (linear) and form I (super coiled, most mobile).

In the absence of glutathione, none of the platinum(IV) complexes caused complete inhibition of BamH1 at concentrations up to 40 μM, and the degree of inhibition varied substantially. $[Pt(en)Cl_2(OH)_2]$ did not cause any observable inhibition in this range, $[Pt(en)Cl_4]$ caused substantial inhibition, and the carboxylato complexes produced intermediate inhibitions. The results for the three dicarboxylato complexes were little different and, therefore, only the gel for $[Pt(en)Cl_2(OC(O)CH_3)_2]$ is presented. There is relatively little variation in the mobility with increasing platinum concentration, even when inhibition is observed. Monofunctional adducts inhibit the restriction enzyme as effectively as bifunctional interactions but they cause less unwinding.[33] Therefore, the results are consistent with platinum(IV) complexes forming principally monofunctional adducts but probably at a lower frequency than does the platinum(II) complex. The variation in inhibition, and therefore in DNA binding, follows the same order as the cathodic reduction potentials. The most readily reduced complex, $[Pt(en)Cl_4]$, binds most and the least readily reduced complex, $[Pt(en)Cl_2(OH)_2]$, binds least.

The influence of the common intracellular reducing agent glutathione was investigated by either (i) reacting the platinum(IV) complex with the DNA for 3 h, then adding the glutathione and incubating for 20 h, or (ii) incubating the platinum(IV) complexes with glutathione for 20 h, then reacting with DNA for 3 h. When 2 equiv. of glutathione were added following the platinum(IV)/DNA reaction, the inhibition increased substantially over that observed for the glutathione-free experiments. $[Pt(en)Cl_4]$ (Fig. 3*e*) showed a similar inhibition pattern to that of the platinum(II) complex, with total inhibition occurring at concentrations greater than 10 μM. $[Pt(en)Cl_2(OC(O)CH_3)_2]$ (Fig. 3*f*) and $[Pt(en)Cl_2(OH)_2]$ (Fig. 3*g*) showed increased, but not complete, inhibition. These results are consistent with two possible mechanisms; either the platinum(IV) complexes bind to DNA and then, on addition of glutathione, are reduced to the platinum(II) complex, a process allowing formation of bifunctional adducts to take place, or, alternatively, unbound platinum(IV) complexes are reduced by the glutathione, and the platinum(II) complexes that result then bind to the DNA. A combination of these two mechanisms is also possible.

In order to investigate the reaction between glutathione and the platinum(IV) complexes, glutathione was incubated with the platinum(IV) complexes prior to reaction with DNA. When this was done the inhibition was not increased. In the case of $[Pt(en)Cl_4]$ and $[Pt(en)Cl_2(OH)_2]$ inhibition was not noticeably altered compared to that observed when no glutathione was added (see Figs 3*h* and 3*j*) but that of $[Pt(en)Cl_2(OC(O)CH_3)_2]$ (Fig. 3*i*) was decreased to the point that no inhibition was observed in the concentration range studied. Glutathione is known to form insoluble complexes with cisplatin,[34] and reduces the DNA binding of $[Pt(en)Cl_2]$.[33] Thus, the decrease in inhibition by $[Pt(en)Cl_2(OC(O)CH_3)_2]$ following preincubation with glutathione may be due to formation of such inactivated complexes. However, it is not clear why the same does not occur for $[Pt(en)Cl_4]$, unless the rates and/or products of glutathione reduction differ depending on the nature of the axial groups.

[33] Er, H. M., Honours Thesis, University of Sydney, 1992.
[34] Berners-Price, S. J., and Kuchel, P. W., *J. Inorg. Biochem.*, 1990, **38**, 305.

Molecular Modelling

The aim of the molecular modelling study was to establish whether or not there was a substantial steric barrier to formation of monofunctional platinum(IV)/DNA adducts. Two models of $[Pt(en)Cl_4]$ binding were established: one in which an axial chloro ligand is lost and the platinum binds to an N7(guanine) atom through this site, and the other in which an equatorial chloro ligand is lost and interaction with the DNA is through this equatorial site. Fig. 4 is a schematic diagram showing these two interactions. In both cases the models were easily established and only a small number of short non-bonded contacts between the complex and DNA were observed. Energy minimization relieved these interactions and at the cost of only moderate deformation in the DNA structure. Thus, it appears that monofunctional binding of octahedral platinum(IV) to DNA through N7(guanine) is feasible.

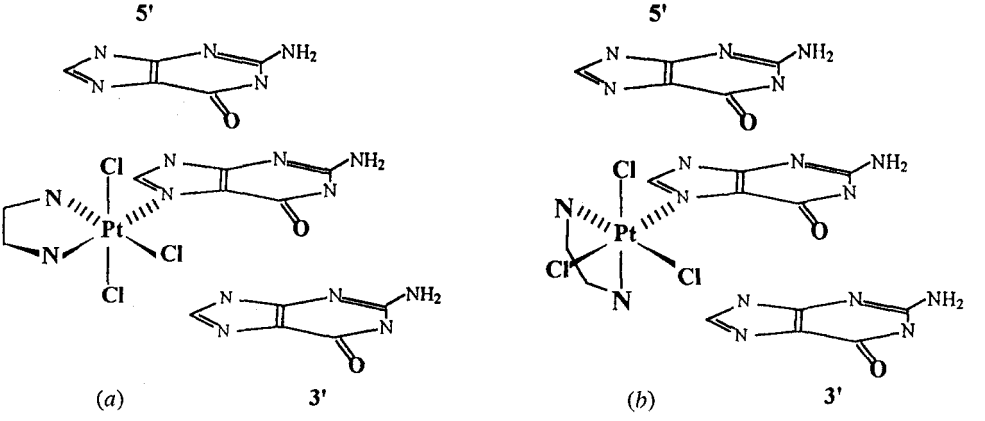


Fig. 4. Schematic diagram showing $[Pt(en)Cl_{3-}]$ bound to DNA through: (*a*) an equatorial site; (*b*) an axial site.

Conclusions

From the plasmid-binding assays, it appears that the platinum(IV) complexes did not bind to DNA as effectively or in the same way as the platinum(II) analogue. However, the degree of binding of the platinum(IV) complexes was found to depend strongly on the nature of the axial ligands and in a way which correlated with the cathodic reduction potentials. The cathodic reduction potentials are, to some extent at least, a reflection of the lability of the axial ligand. Thus, there are two possible explanations for this correlation. The first is that the more readily reduced complexes are partially reduced to platinum(II) by the elements of the reaction media, and these platinum(II) complexes then bind to the DNA inhibiting the restriction enzyme. However, the mobility of the plasmid DNA does not vary as much as would be expected if platinum(II) binding was responsible for the inhibition. Therefore, a more plausible explanation is that the platinum(IV) complex binds directly to DNA following loss of one of the axial ligands. The more readily an axial ligand is lost the greater the binding. Molecular modelling studies have shown that monofunctional binding of octahedral platinum(IV) is feasible.

The addition of glutathione, following incubation of the platinum(IV) complexes with DNA, enhanced the binding, and the inhibition and mobility patterns became more indicative of platinum(II), particularly for $[Pt(en)Cl_4]$. Incubation of the $[Pt(en)Cl_2(OC(O)CH_3)_2]$ with glutathione prior to reaction with DNA decreased inhibition, possibly as a result of the formation of an insoluble and deactivated platinum(II)/glutathione complex. These results are consistent with glutathione reducing platinum(IV) bound monofunctionally to DNA. Reduction of the unbound platinum(IV) complexes followed by binding of the platinum(II) complex cannot be ruled out; however, given that inactivation is observed in the case of the acetato complex, this is less likely.

It is questionable whether or not similar mechanisms would operate in biological systems since there are numerous intracellular and extracellular species that can reduce platinum(IV) complexes prior to their interactions with DNA.[8–11] However, it is worth noting in this context that most of the studies of the reduction of platinum(IV) complexes by biochemicals have related to tetrachloro complexes. We have shown here that the tetrachloro complexes are reduced more readily than complexes with other axial ligands and, therefore, it should not be assumed that the carboxylato or hydroxo complexes are reduced as readily by the same biochemicals. Depending on the rate of reduction, some of the platinum(IV) complexes with carboxylato and hydroxo ligands might survive to the point where they can interact directly with DNA. The improved uptake of JM221 by cells resistant to cisplatin[1] is probably associated with the axial carboxylato ligands. If so, the survival of JM221 in the platinum(IV) state to the point of cellular uptake would be crucial, and the lower reduction potential of the carboxylato complexes may be an important factor in determining their effectiveness.

Acknowledgments

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