Hydrolysis Products of Cisplatin: pK_a Determinations *via* [^{1}H, ^{15}N] NMR Spectroscopy

Susan J. Berners-Price,[a] Tom A. Frenkiel,[b] Urban Frey,[c] John D. Ranford[c] and Peter J. Sadler*[c]

[a] *Division of Science & Technology, Griffith University, Nathan, Queensland 4111, Australia*
[b] *Biomedical NMR Centre, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*
[c] *Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratory, 29 Gordon Square, London WC1H 0PP, UK*

The pK_a values of *cis*-$[PtCl(H_2O)(NH_3)_2]^+$ (6.41) and *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ (5.37 and 7.21) have been determined at 300 K *via* the use of ^{15}N-edited ^{1}H NMR spectroscopy and [^{1}H, ^{15}N] heteronuclear multiple quantum coherence spectroscopy; this allows rapid measurements at low (millimolar) concentrations, and so avoids some of the problems associated with other methods.

Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$ **1**, is a widely used anticancer drug.[1] Its mechanism of action is thought to involve activation *via* hydrolysis inside cells where the Cl^- concentration is much lower (*ca.* 4 mmol dm^{-3}) than outside cells (*ca.* 104 mmol dm^{-3}).[2,3] Platinum(II)–OH_2 bonds are more reactive towards DNA (*e.g.* guanine N7) than either Pt–Cl or Pt–OH bonds,[4,5] and it is important therefore to determine the pK_a values of the coordinated water molecules in the cisplatin hydrolysis products *cis*-$[PtCl(H_2O)(NH_3)_2]^+$ **2** and *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ **3**. This is difficult to do because reactions of these species with base are complicated by the further hydrolysis of **2**, and by the slow formation of hydroxo-bridged polymers. These complicate the interpretation of potentiometric titration curves.[5–7] The pK_a values of species in mixtures can be determined by NMR spectroscopy provided they have suitable resonances which can be monitored as a function of pH.

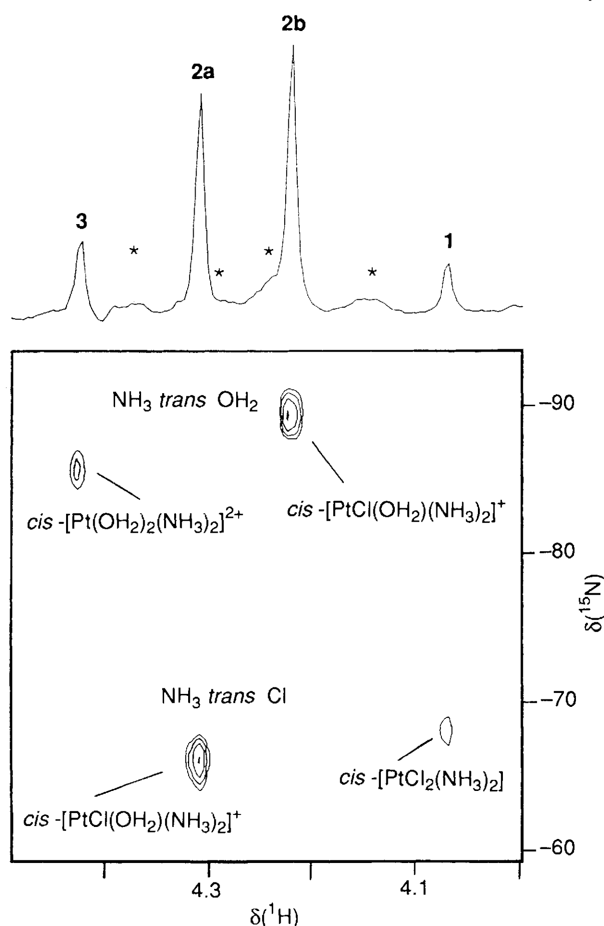

Fig. 1 500.13 MHz ^{1}H{^{15}N} spectrum of a 5 mmol dm^{-3} solution containing complexes **1**, **2** and **3** in 95% H_2O–5% D_2O, pH 4.72, and the corresponding [^{1}H, ^{15}N] spectrum (50.67 MHz ^{15}N). ^{195}Pt satellites are marked with an asterisk, $^2J(^1H, ^{195}Pt)$ *ca.* 64 Hz. The resonances are assigned to the Pt–NH_3 protons in: **1** *cis*-$[PtCl_2(NH_3)_2]$, **2** *cis*-$[PtCl(H_2O)(NH_3)_2]^+$ (**2a** NH_3 *trans* to Cl and **2b** NH_3 *trans* to H_2O) and **3** *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$.

Thus the pK_a values of **2** and **3** have been determined by ^{15}N NMR studies of ^{15}N-labelled complexes,[8] and for **2** these values are usually cited as the only reliable data in the literature. However, direct observation of ^{15}N peaks necessitated the use of high concentrations of Pt (*ca.* 100 mmol dm^{-3}). For **3** polymerization was still a problem, and the measurements had to be carried out at 278 K.[8] We report here studies of complexes **2** and **3** by ^{15}N-edited ^{1}H NMR spectroscopy and [^{1}H, ^{15}N] heteronuclear multiple quantum coherence (HMQC) spectroscopy,[9] which allow the rapid determination of their pK_a values, at low concentrations. These [^{1}H, ^{15}N] studies also provide a basis for detecting cisplatin hydrolysis products during reactions of platinum drugs with nucleotides and other important biomolecules under conditions of physiological relevance.

A solution containing **2** was prepared by incubating ^{15}N-labelled **1** (7.6 mg, 25 μmol) with $AgNO_3$ (4.2 mg, 25 μmol) in 50 μl of [$^2H_7$]DMF (dimethylformamide) at 310 K for 19 h, followed by removal of the AgCl precipitate, and dilution to 5 ml with 95% H_2O–5% D_2O. Dioxane (0.2 mmol dm^{-3}) was added as an internal ^{1}H chemical shift reference [δ 3.767 ppm rel. to $Me_3SiCD_2CD_2CO_2Na$ (TSP)] and the final Pt concentration was 5 mmol dm^{-3} in a solution which contained 1% DMF. Measurements of pH were made at *ca.* 298 K directly in the NMR tube, before and after recording spectra, using a Corning 240 meter equipped with an Aldrich micro combination electrode calibrated with Aldrich buffer solutions at pH 4, 7 and 10.

One-dimensional ^{15}N-edited ^{1}H NMR spectra were recorded on a Bruker AM500 spectrometer in 5 mm tubes at 300 K using a spin-echo difference sequence[10] optimized for ^1J(N,H) = 73 Hz. The intense H_2O resonance was preirradiated for 1.5 s by means of a DANTE sequence.[11] Typically, 32–64 transients were acquired over a period of 2–4 min. Two-dimensional [^{1}H, ^{15}N] HMQC spectra were acquired using a standard sequence,[9] modified to include a pair of purge pulses[12] for improved H_2O suppression. The TPPI method[13] was used to give 2D spectra with absorption mode line shapes with sign discrimination in F_1. ^{15}N-Decoupling was achieved by means of the GARP-1 sequence.[14] Typically, 2D spectra were the result of 8 scans and 32 t_1 increments (*ca.* 12 min in total). ^{15}N shifts are referenced to 1.5 mol dm^{-3} NH_4Cl in 1 mol dm^{-3} HCl (external).

The ^{1}H{^{15}N} spectrum of the above solution (pH 4.72) contained four major peaks corresponding to four types of coordinated NH_3 ligands, Fig. 1. These are assignable *via* correlation of ^{1}H and ^{15}N chemical shifts, and the known ^{15}N chemical shifts of cisplatin and its hydrolysis products.[8] In particular, the ^{15}N shifts for NH_3 ligands *trans* to oxygen are *ca.* 20 ppm to high field of those for NH_3 *trans* to N or Cl. The mono-aqua complex **2** therefore predominates, but there are also small amounts of unreacted cisplatin **1** and the diaqua complex **3** present in the solution.† The pH of one aliquot of

† In a separate experiment, the peak assigned to **3** predominated, as expected, after reaction of **1** with *ca.* 2 mol. equiv. of $AgNO_3$.

Table 1 ^1H and ^{15}N NMR chemical shifts, and pK_a values of complexes **2** and **3**

Complex		δ		pK_a		
		^1H	^{15}N	^1H[a]	^{15}N[b]	Other
cis-[$PtCl_2(NH_3)_2$]	**1**	4.06	−68.7			
cis-[$PtCl(H_2O)(NH_3)_2$]$^+$	**2a**	4.33[e]	−66.8	6.42 ± 0.02	(6.85)	(6.3)[c]
cis-[$PtCl(OH)(NH_3)_2$]		4.05[e]	−67.9			
cis-[$PtCl(H_2O)(NH_3)_2$]$^+$	**2b**	4.25[e]	−90.0[e]	6.40 ± 0.03		
cis-[$PtCl(OH)(NH_3)_2$]		3.60[e]	−81.1[e]			
cis-[$Pt(H_2O)_2(NH_3)_2$]$^{2+}$	**3**	4.51[e]	−87.2[e]	5.37 ± 0.09	(5.93)	(5.5)[c] (5.5)[d]
cis-[$Pt(OH)(H_2O)(NH_3)_2$]$^+$		3.98[e]	—	7.21 ± 0.09	(7.87)	(7.1)[c] (7.2)[d]
cis-[$Pt(OH)_2(NH_3)_2$]		3.59[e]	−79.9[e]			

[a] This work. [b] Ref. 8. [c] Ref. 5. [d] Ref. 7. [e] Value obtained by fitting eqn. (1) or (2).

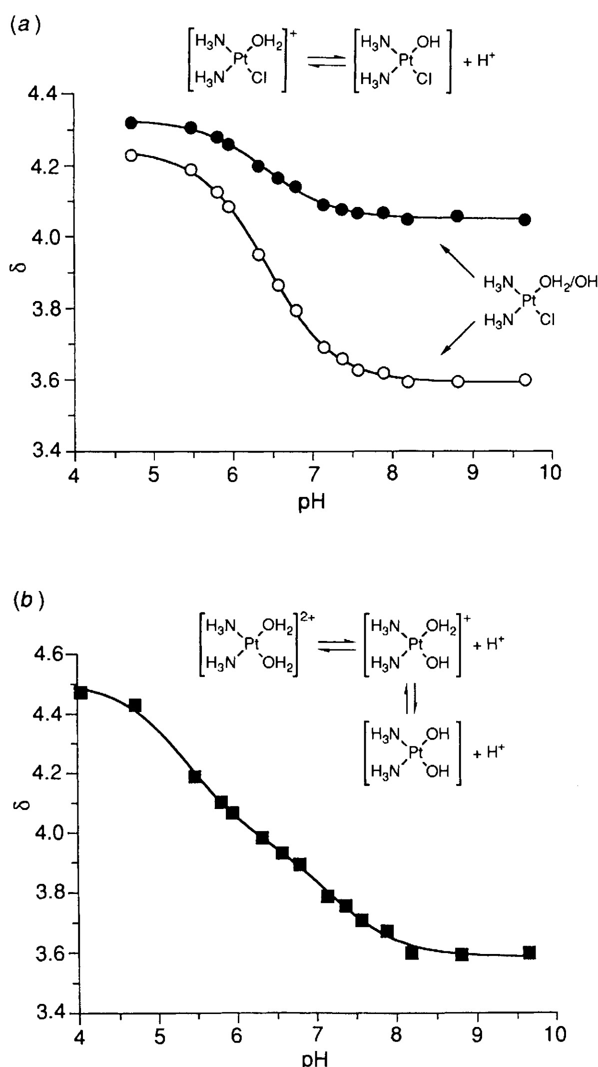

Fig. 2 Plots of ^1H NMR chemical shifts *vs.* pH for (*a*) complex **2** and (*b*) complex **3**. The resulting δ and pK_a values are listed in Table 1. The point at pH 4.04 for complex **3** was obtained from a separate 5 mmol dm^{-3} solution of the diaqua complex. Proton exchange between acidic and basic forms is fast on the (^1H and ^{15}N) NMR time-scale and average peaks are seen.

this solution was then raised to 9.65 and 1D and 2D spectra were recorded; the pH of another aliquot was raised in stages using microlitre aliquots of 0.2 mol dm^{-3} NaOH. Additional 2D spectra were recorded at pH 7.16 and 8.19.

The pH titration curves for the peaks of complexes **2** and **3** are shown in Fig. 2. Eqns. (1) and (2) were fitted to the experimental data for complexes **2** and **3**, respectively,‡ and the δ and pK_a values obtained are listed in Table 1. The ^1H NMR chemical shifts of **3** and **2b** are very close over much of the pH range. Assignments of these were aided by the unambiguous assignment of **2a**, and therefore a predicted titration curve for **2b**, and by the use of ^1H and ^{15}N shifts for the peak assignment. Over the course of the titration (*ca.* 5 h in total), the intensity of the peak for **3** increased at the expense of **2**, and, towards the end, small peaks assignable to hydroxo-bridged complexes (<10% of total intensity, ^1H δ 3.9–4.0 ^{15}N δ −83.9 to −85.6) were present in the spectrum.

Table 1 compares the pK_a values obtained here with those reported previously. Our values are close to those previously determined for **3** by potentiometry,[6,7] and to those calculated or estimated by Martin.[5] They are significantly lower than those determined previously by ^{15}N NMR studies at higher concentration, and for **3** at a lower temperature (277 K).[8]

As an example of the further use of these new NMR methods, we studied the hydrolysis of ^{15}N-cisplatin (9.3 mmol dm^{-3}) in 95% H_2O–5% D_2O at 310 K. At equilibrium (40 h), the integration of ^1H NMR peaks gave a ratio for **1**:**2**:**3** of 0.64:0.35:0.01, respectively. From these data, an equilibrium constant of 2.72 (pK_1) for the first stage of cisplatin hydrolysis was calculated. This can be compared with values of *ca.* 2.0–2.5 obtained previously by other methods, under slightly different conditions.[15,16] This agreement is reasonable when the errors in the NMR determination are considered (*e.g.* peak integration, possible cross-saturation of peaks close to H_2O). Our equilibrium solution also contained a small peak at δ 3.92(^1H)/δ −85.0(^{15}N) assignable to a hydroxo-bridged species. This was ignored in the calculation.

Under biologically relevant conditions inside cells where [Cl] = 4 mmol dm^{-3} and pH = 7.4, we calculate that 20% of the cisplatin will be present in solution as reactive aqua complexes: 5% as *cis*-[$PtCl(H_2O)(NH_3)_2$]$^+$ **2**, 15% as *cis*-[$Pt(OH)(H_2O)(NH_3)_2$]$^+$, and 0.1% as **3**. Attempts to detect these aqua complexes as intermediates during reactions of cisplatin with polynucleotides by ^{195}Pt NMR spectroscopy (using enriched ^{195}Pt) have not been successful.[17] In contrast, peaks for **2** are readily detectable by ^1H NMR spectroscopy during the early stages of reactions of cisplatin with 5′-GMP.[18] The methods described here will therefore allow more detailed investigations of the pathways of reactions of cisplatin under biologically-relevant conditions to be made, and should lead to the elucidation of the aqueous solution chemistry of a

‡
$$\delta = (\delta_A[H^+] + \delta_B K_a)/([H^+] + K_a) \quad (1)$$
$$\delta = (\delta_{AB} + \delta_{AA}[H^+]/K_{a1} + \delta_{BB}K_{a2}/[H^+])/(1 + [H^+]/K_{a1} + K_{a2}/[H^+]) \quad (2)$$
where K_a is the acid dissociation constant for complex **2**, and K_{a1} and K_{a2} for complex **3**, and δ_A, δ_B, δ_{AA} and δ_{BB} are the chemical shifts of [$PtCl(H_2O)(NH_3)_2$]$^+$, [$PtCl(OH)(NH_3)_2$], [$Pt(H_2O)_2(NH_3)_2$]$^{2+}$, [$Pt(OH)(H_2O)(NH_3)_2$]$^+$ and [$Pt(OH)_2(NH_3)_2$], respectively. For fitting the program KaleidaGraph (Synergy Software, Reading, PA, USA) was used.

wide range of ammine and amine complexes of other metal ions.

We are grateful to the UK MRC, the Australian National Health and Medical Research Council, The Royal Society, the UK SERC, University of London Intercollegiate Research Service, and the Wolfson Foundation for their support for this work.

Received, 5th March 1992; Com. 2/01184K

References

1 M. P. Hacker, E. B. Douple and I. H. Krakoff, *Platinum Coordination Complexes in Cancer Chemotherapy*, Martinus Nijhoff, Boston, 1984.
2 B. Rosenberg, *Metal Ions Biol. Syst.*, 1980, **11**, 127.
3 A. F. LeRoy, R. T. Lutz and R. L. Dedrick, *Cancer Treatment Rep.*, 1979, **63**, 59.
4 M. Howe-Grant and S. J. Lippard, *Metal Ions Biol. Syst.*, 1980, **11**, 63.
5 R. B. Martin, *Am. Chem. Soc. Symp. Ser.*, 1983, **208**, 231.
6 K. A. Jensen, *Z. Anorg. Chem.*, 1939, **242**, 87.
7 A. A. Grindberg, A. I. Stetsenko, N. D. Mitkinova and L. S. Tikhonova, *Russ. J. Inorg. Chem.*, 1971, **16**, 137.
8 T. G. Appleton, J. R. Hall, S. F. Ralph and C. S. M. Thompson, *Inorg. Chem.*, 1989, **28**, 1989.
9 A. Bax, R. H. Griffey and B. L. Hawkins, *J. Magn. Reson.*, 1983, **55**, 301.
10 R. Freeman, T. H. Mareci and G. A. Morris, *J. Magn. Reson.*, 1981, **42**, 341.
11 G. A. Morris and R. Freeman, *J. Magn. Reson.*, 1978, **29**, 433.
12 G. Otting and K. Wütrich, *J. Magn. Reson.*, 1988, **76**, 569.
13 D. Marion and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 1983, **113**, 967.
14 A. J. Shaka, P. B. Barker and R. Freeman, *J. Magn. Reson.*, 1985, **64**, 547.
15 K. W. Lee and D. S. Martin, *Inorg. Chim. Acta*, 1976, **17**, 105.
16 S. E. Miller, K. J. Gerard and D. A. House, *Inorg. Chim. Acta*, 1991, **190**, 135, and other papers in this series cited therein.
17 D. P. Bancroft, C. A. Lepre and S. J. Lippard, *J. Am. Chem. Soc.*, 1990, **112**, 6860.
18 S. J. Berners Price, T. A. Frenkiel, J. D. Ranford and P. J. Sadler, submitted for publication.