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Cisplatin Carbonato Complexes. Implications for Uptake, Antitumor Properties, and Toxicity

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Cisplatin is a potent cytotoxic agent used for the treatment of ovarian and testicular cancer and other malignancies.¹ The cytotoxicity of cisplatin is thought to be related to its ability to modify cellular DNA.² In plasma, where the chloride concentration is ~ 105 mM, cisplatin is believed to partially solvate to its aquated forms.³ Since aquated complexes can react with components present in blood and the cytosol, they may be important for the antitumor effects, resistance, and toxicity associated with cisplatin.⁴

Earlier, we used HSQC NMR and low concentrations of ^{15}N -labeled cisplatin, *cis*-Pt($^{15}\text{NH}_3$) $_2\text{Cl}_2$ (**1**, Figure 1), to identify the platinum species in culture media (RPMI + 10% fetal bovine serum) in the presence and absence of Jurkat cells.⁵ We interpreted the NMR measurements as showing that **1** hydrolyzes to the monoquo complex, **2**, which is in equilibrium with the monohydroxo species, **3**, as is known to occur in aqueous solution.³ We found that, with more than 5×10^6 cells present, **2** had disappeared by 0.5 h, the earliest time for which NMR measurements could be made. In the absence of cells, however, the NMR signal ascribed to **2** persisted to >20 h, which was unexpected because aquated species should react more quickly than **1** with components in the medium. To identify species having NMR signatures similar to that of **2** but with reduced reactivity, we studied the reaction of **1** in carbonate buffer⁶ and in culture media with and without carbonate ion. In this report, we show that, in the presence of carbonate, **1** reacts to form the carbonato complex **4**, which is the long-lived species observed in the cell culture studies. At long times in carbonate buffer, **4** reacts to form **5**. Since carbonate is present in blood at high concentrations and it may react with **1**, these observations have potentially important implications for the uptake, antitumor properties, and toxicity of cisplatin.

As we have earlier shown,⁵ [1] decreases with time in culture media approximately at the rate expected for the hydrolysis of **1** to **2**.³ However, the resonance assigned to **2** (**3**) was not observed in NMR studies in culture medium deficient in carbonate ion, whereas it was observed in studies in carbonate buffer alone. Thus, the species present in the earlier reported cell culture studies is not **2** but, rather, the carbonato complex, **4**.

The HSQC NMR spectrum of $65 \mu\text{M}$ cisplatin, in carbonate buffer, is shown in Figure 2. The peak for N *trans* to O of compound **4** is at $^1\text{H}/^{15}\text{N}$, $\delta = 3.61/-80.5$, while the peak for N *trans* to Cl of the compound is under the peak for **1**.⁵ The peak for N *trans* to O of **4** disappears with time, and a new compound forms, having a peak at $^1\text{H}/^{15}\text{N}$, $\delta = 3.70/-81.8$. This has been assigned to the biscarbonato complex **5**. The HSQC NMR data for **4** and **5** are consistent with assignments made for similar compounds.^{3b} The concentrations of the three compounds, **1**, **4**, and **5**, are shown as functions of time in Figure 3 and are discussed below.

A 1 mM solution of ^{14}N **1** and 5 mM $\text{NaH}^{13}\text{CO}_3$ was studied using 1D ^{13}C NMR.⁸ The spectrum showed resonances at ^{13}C , $\delta = 160.8$, 125.3, and 167.0 ppm, corresponding to HCO_3^- , $\text{CO}_2(\text{aq})$, and **5**, respectively. The chemical shifts of the carbonates in **5** are

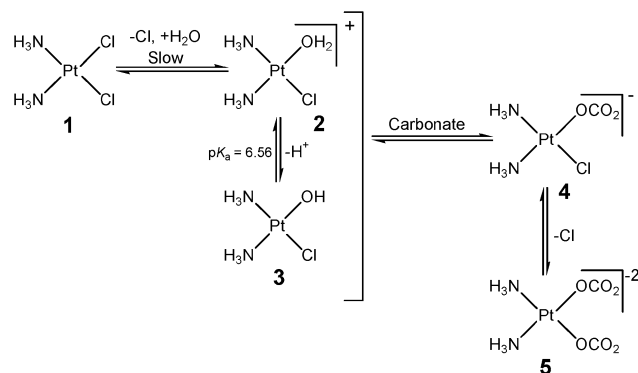


Figure 1. Reaction of cisplatin with carbonate.

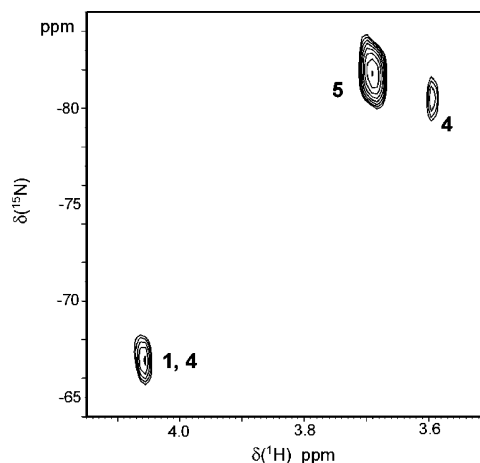


Figure 2. ^1H - ^{15}N HSQC NMR spectrum of $65 \mu\text{M}$ **1** in 5 mM HCO_3^- , $[\text{Cl}^-] = 3.3$ mM, 37°C , $t = 11.6$ h.

at ~ 1 ppm higher field than that of the corresponding bidentate carbonate in *cis*-bis(triethylphosphine)carbonato platinum(II) and at ~ 4 ppm higher field than that of a bridging carbonate in a dinuclear complex of Zn^{2+} .⁹

By analysis of UV absorption data, it was determined that compound **5** has a *d-d* absorption band at 268 nm, which is shifted to higher energy relative to the main *d-d* band for **1**. Since the crystal field strength of CO_3^{2-} is greater than that of Cl^- , this shift to higher energy for **5** is expected.¹⁰

Compounds **1** and **5** each have a single HSQC NMR peak, but **4** has two equal peaks, one overlapping the peak for **1**. The true intensity of **1** is obtained by subtracting the apparent intensity of **4** from the apparent intensity of **1**, and the true intensity of **4** is double the apparent intensity of **4**. These true intensities are proportional to species concentrations, so, given the total Pt concentration, one can convert intensities to concentrations, giving the points of Figure 3. The curves in Figure 3 are best-fit curves for the reaction scheme shown in Figure 1. The rate constants for conversion of **1** to **2** and

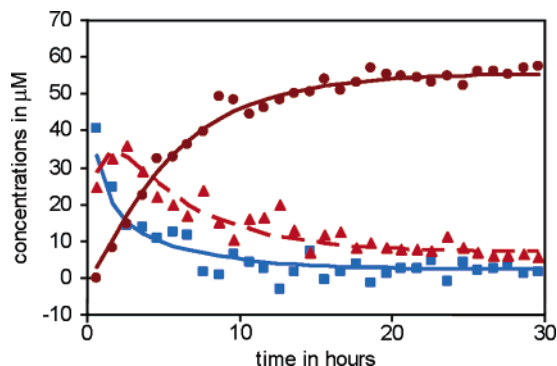


Figure 3. Concentrations of **1** (blue, squares), **4** (red, triangles), and **5** (brown, circles) from NMR measurements, with fits to the model of Figure 1 (smooth curves).

the reverse are known from previous work.^{3a,b} By analogy with other metal complexes,^{6,11} compound **4** can be formed by reaction of CO_2 with **3** and/or reaction of CO_3^{2-} with **2**. The rate constants for conversion of **4** to **5** (by reaction with carbonate) and the reverse are parameters in the model. The fit is excellent, confirming that, in 5 mM carbonate buffer, **1** disappears to form **4**, which then yields **5**. In 0.5 mM carbonate, the conversion of **1** to **4** occurs at the same rate as that in 5 mM carbonate, but **5** is formed much less rapidly. This shows that the rate of conversion of **4** to **5** is carbonate-dependent.

Although **5** is observed in carbonate buffer, it is not observed in culture media.⁵ This may be due to the high chloride content of the medium which suppresses the formation of **5** and/or rapid reaction of the compound with the many possible nucleophiles present in the medium. Low concentrations of the compound would be more difficult to detect with HSQC NMR.

It is known that hydrating patients with hypertonic saline solutions reduces the renal toxicity of cisplatin.^{4a,b} Since increased chloride concentration in blood would shift the equilibria in Figure 1 to the left, reducing the concentration of carbonate species, it may be that the latter are, in part, responsible for some of the toxic effects observed with cisplatin. However, work with relevant renal cells will be necessary in order to show if this is the case.

The fate of **4**, which quickly disappears from the HSQC NMR spectrum when Jurkat cells are present, is unknown.⁵ However, it is possible that this species is selectively taken up by the cell. It is important to note that the heavy metal ion Cd^{2+} forms carbonate complexes which are taken up by cells and that anion exchangers found in the plasma membrane may be involved in its transport.¹² Work in progress will determine if a similar uptake mechanism is involved with carbonate complexes of cisplatin.

In this work, we use low concentrations of **1**¹³ to show that the earlier detected cisplatin metabolite that forms in culture media, and is taken up/modified by cells,⁵ is the carbonate complex, **4**. In carbonate buffer, **4** slowly reacts to form the biscarbonate complex,

5. Since the carbonate concentrations in culture media, plasma, and the cytosol are similar, platinum carbonate complexes may be present in therapy, and they could be the species reacting with components in the cell. Despite the large body of work on cisplatin and its analogues, little attention has been paid to the potential role of carbonate ion in these reactions.¹⁴ Hopefully, this report will stimulate new inquiry on the role of carbonate in the mechanism of action of platinum anticancer drugs.

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Supporting Information Available: Absorption data and analysis, the kinetic model for the scheme shown in Figure 1, a ¹³C NMR spectrum of **5** in carbonate solution, and an HSQC NMR spectrum showing peaks for **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (8) A Bruker DRX500 Avance spectrometer with a Nalorac 10 mm broadband observe probe was used to acquire the one-dimensional ¹³C spectrum. The spectrum was acquired with a 31450 Hz spectral window (digital resolution of 2 Hz/point), 42k scans with a 6 s repetition time. Chemical shifts are referenced to external, 3% CHCl₃ in acetone.
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