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ESMS and NMR investigations on the interaction of the anticancer drug cisplatin and chemopreventive agent selenomethionine †

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Received 25th October 2000, Accepted 22nd January 2001
First published as an Advance Article on the web 19th February 2001

The reactions of L-selenomethionine (L-Se-MetH) with cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, were investigated using electrospray mass spectroscopy (ESMS) and 2-D [^{1}H–^{15}N] NMR spectroscopy. The reaction intermediates and products identified were *cis*-$[PtCl(L\text{-}Se\text{-}MetH)(NH_3)_2]^+$, *cis*-$[Pt(L\text{-}Se\text{-}Met)(NH_3)_2]^+$, $[PtCl(L\text{-}Se\text{-}MetH)(NH_3)]^+$ and $[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)]^+$. Some binuclear adducts were also detected by ESMS during the reaction, although they existed in minor amounts. Similar reactions with L-MetH were also conducted under similar conditions for comparison. This work provides the first detailed studies on the reaction of a platinum-based drug with L-Se-MetH.

Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, is one of the most widely used anticancer drugs in the world. It is highly effective in the treatment of malignancies such as testicular and ovarian cancer.[1] Despite the undisputed fact that the major cellular target is DNA, sulfur-containing biomolecules may play an important role in the mechanism of action and metabolism of cisplatin.[2] The complex $[Pt(Met\text{-}S,N)_2]$ has been isolated from the urine of patients treated with cisplatin,[3] and its geometrical isomers have been separated and characterized.[4] Moreover, stable ring-opened complexes have been detected during the reaction of carboplatin $[Pt(NH_3)_2(CBDCA)]$ and its analogue [Pt(en)-(CBDCA)] (CBDCA = cyclobutane-1,1-dicarboxylate) with L-MetH and a similar species was found in the urine of animals treated with carboplatin.[5,6] Strong evidence shows that platinum thioether complexes may be potential intermediates for DNA platination.[7–9] Recent *in vivo* studies show that L-MetH reduces significantly cisplatin-induced toxicities.[10,11] Therefore, the interaction of platinum-based drugs with methionine and derivatives has been studied extensively in order to understand the chemistry and biochemistry of the reactions.[2,12–14]

Selenium is an essential micronutrient for mammalian animals. It is mainly present in the form of selenocysteine in the selenoenzyme glutathione peroxidase (GSH-Px).[15,16] Some selenoproteins incorporate selenomethionine in the place of methionine. Organoselenium compounds such as selenomethionine are on clinical trials as potential cancer chemopreventive drugs in reducing both environmentally and genetically determined cancer in the U.S.A.[17] It was reported recently that selenomethionine protects against cisplatin-induced renal and other toxicities in mice and rats.[18]

In contrast to many extensive and thorough investigations on methionine, studies on the interactions between Se-MetH and metal ions are rare.[19–22] ^{1}H NMR experiments showed that reaction of Se-MetH with Au^{III} gave rise to methionine-selenoxide and Au^{I}.[19] Faraglia and Fregona reported that palladium and platinum dihalides formed 1 : 1 complexes with *Se*,*N*-chelated D,L-Se-Met.[20] Similar adducts were obtained *via* reaction of $[PdCl_4]^{2-}$ and D,L-Se-Met.[21] Moreover, Se-Met was found to coordinate to copper(II) and zinc(II) in *N*,*O*-chelate mode.[22]

Here we report the first ESMS and [^{1}H–^{15}N] NMR studies on the interactions of L-Se-MetH with cisplatin. The 2-D [^{1}H–^{15}N] NMR technique has been proven to be very powerful for studying reactions of [^{15}N]cisplatin with molecules such as DNA and proteins[23,24] and allows detection of species at low concentrations and assignments of *trans* ligands in unknown species from the diagnostic ^{15}N chemical shift.[25,26] Recent examples have illustrated the successful application of ESMS in studies of inorganic and bioinorganic systems.[27,28]

Experimental

Materials and measurements

Cisplatin and [^{15}N]cisplatin were prepared and recrystallized according to the published procedure.[29] L-Selenomethionine (L-Se-MetH) and L-methionine (L-MetH) were purchased from Acros Organics.

Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ESMS. Finnigan) by loading 1.0 μl of solution into the injection valve of the LCQ unit and then injecting into the mobile phase solution (50% of aqueous methanol) which was carried through the electrospray interface into the mass analyser at a rate of 200 μl min^{-1}. The voltage employed at the electrospray needles was 5 kV, and the capillary was heated to 200 °C. A maximum ion injection time of 200 ms along with 10 scans was set. Positive ion mass spectra were obtained. Zoom Scan was used in the experiments. The predicted isotope distribution patterns for each of the complexes were calculated using the Isopro 3.0 program.[30]

NMR data were acquired on a 500 MHz Bruker DMX spectrometer. One-dimensional ^{1}H NMR spectra were typically acquired with 128 transients and 32 K data points over a spectral width of 10 kHz using standard pulse sequences. The ^{1}H chemical shifts are referenced to internal sodium 3-trimethylsilyl[$^2H_4$]propionate (*via* internal 1,4-dioxane). Two-dimensional [^{1}H–^{15}N] HSQC (Heteronuclear Single Quantum

† Abbreviations used: L-Se-MetH, L-selenomethionine; L-Se-Met, deprotonated L-selenomethionine; L-MetH, L-methionine; L-Met, deprotonated L-methionine.

Electronic supplementary information (ESI) available: isotopic distributions and 2-D [^{1}H–^{15}N] HSQC NMR spectra. See http://www.rsc.org/suppdata/dt/b0/b008611h/

DOI: 10.1039/b008611h

Table 1 Observed and calculated molecular masses of the complexes for the reaction of cisplatin and L-Se-MetH

Peak	Compound	Observed mass[a]	Calculated mass
1	$[PtCl(L\text{-}Se\text{-}MetH)(NH_3)_2]^+$	456.9–463.9	460.6
2	$[Pt(L\text{-}Se\text{-}Met)(NH_3)_2]^+$	421.9–427.9	424.1
3	$[PtCl(L\text{-}Se\text{-}MetH)(NH_3)]^+$	439.0–449.0	443.6
4	$[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)]^+$	582.1–590.9	586.3
5	$[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)(NH_3)_2]^+$	618.9–626.8	620.3
6	$[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)(NH_3)]^+$	600.9–610.9	603.3
7	$[Pt_2Cl(OH)(L\text{-}Se\text{-}Met)(NH_3)_4]^+$	702.7–709.8	705.7
8	$[Pt_2Cl_2(L\text{-}Se\text{-}Met)(NH_3)_4]^+$	718.9–729.9	725.2
9	$[Pt_2Cl_3(L\text{-}Se\text{-}MetH)(NH_3)_3]^+$	739.7–749.8	743.6
10	$[Pt_2Cl_4(L\text{-}Se\text{-}MetH + H^+)(NH_3)_2]^+$	754.7–765.6	762.4
11	$[Pt_2(OH)(L\text{-}Se\text{-}Met)_2(NH_3)_3]^+$	841.9–854.9	848.4
12	$[Pt_2Cl(L\text{-}Se\text{-}Met)_2(NH_3)_3]^+$	861.8–870.7	866.8
13	$[Pt_2Cl_2(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)(NH_3)_2]^+$	883.7–889.7	886.3
14	$[PtCl(NH_3)_2(H_2O)]^+$	279.0–283.9	282.5

[a] The peaks are separated by 1 *m/z* in the mass region indicated.

Coherence) NMR data were acquired and processed according to previously reported methods.[31] The ^{15}N chemical shifts are externally referenced to 1.5 M NH_4Cl in 1 M HCl. The indirect referencing method resulted in slightly different ^{15}N chemical shifts to those in previous reports for similar species, and no corrections have been made.

The pH measurements were carried out using a pHS-3C pH meter equipped with a Phoenix Ag–AgCl reference electrode, calibrated with phosphate buffer solutions at pH 4 and 10. The values reported are those measured after the reactions have finished.

Sample preparation

Samples for the following reactions were prepared using double-distilled water and studied by ESMS. No buffer solution was employed in these studies in order to avoid interference which increases background noise. (1) *cis*-$[PtCl_2(NH_3)_2]$ (3.3 mmol) and L-Se-MetH (3.3 mmol) were dissolved in 1 ml water and incubated at 310 K for 24 h in the dark. A clear solution was then obtained the pH of which was measured to be 4.4. (2) A solution containing *cis*-$[PtCl_2(NH_3)_2]$ (3.3 mmol) and L-Se-MetH (6.6 mmol) was prepared similarly and incubated for 24 h at 310 K. The pH of the resulting solution was measured to be 6.3. (3) *cis*-$[PtCl_2(NH_3)_2]$ (3.3 mmol) was mixed with L-Se-MetH in 1 ml water in a 1 : 1 molar ratio. The mixture was monitored by ESMS for a period of 24 h at 310 and 295 K respectively. (4) *cis*-$[PtCl_2(NH_3)_2]$ (3.3 mmol) was mixed with L-MetH in 1 ml water in a 1 : 1 molar ratio and the time dependence of the reaction followed by ESMS at 310 and 295 K, respectively. After 24 h the pH of the solutions was measured to be 4.6.

Samples for NMR study were prepared as follows. *cis*-$[PtCl_2(^{15}NH_3)_2]$ (1.99 mmol) was dissolved in 440 μl water, to which 100 μl stock solution of L-Se-MetH (1.99 mmol) and 60 μl of D_2O were added. The final concentration of cisplatin and L-Se-MetH is 3.3 mM.

Results

(1) Reaction of L-Se-MetH with cisplatin at 1 : 1 molar ratio

After incubating L-Se-MetH and cisplatin for 24 h at 310 K the ESMS spectrum of the mixture solution was recorded (Fig. 1). All the isotopic peaks were separated by 1 unit and can be attributed to complexes with one positive charge. The assignments of the species, which were based on the molecular mass calculation and isotopic distribution, are listed in Table 1.

As can be seen from Fig. 1, the dominant product of the reaction was peak **3** which has a *m/z* value of 443.1 and can be assigned to the complex $[PtCl(L\text{-}Se\text{-}MetH)(NH_3)]^+$. The calculated molecular mass and the isotopic distribution match perfectly with the formula (see ESI Fig. S1). The *m/z* values and isotope distributions of peaks **4** (586.9) and **5** (620.8) corresponded to the masses of complexes containing two L-Se-MetH ligands, *e.g.* peak **4** to $[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)]^+$ (Table 1, ESI Fig. S2).

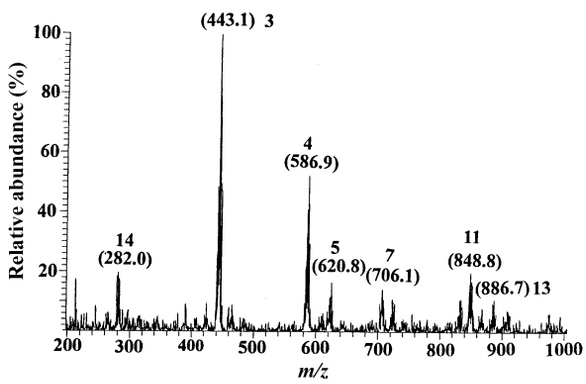


Fig. 1 The ESMS spectrum of a mixture of L-Se-MetH with cisplatin at 1 : 1 molar ratio after 24 h incubation at 310 K.

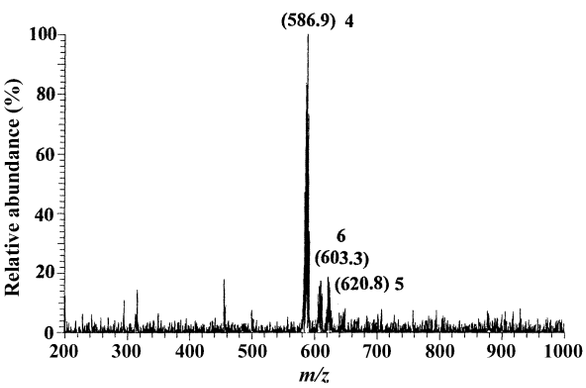


Fig. 2 The ESMS spectrum of a mixture of L-Se-MetH with cisplatin at 2 : 1 molar ratio after 24 h incubation at 310 K.

Interestingly, peaks **7**–**13** were also observed and their *m/z* values are 706.1, 724.8, 743.0, 762.0, 848.8, 866.5, 886.7, respectively, although they existed as minor products. These adducts can only be attributed to binuclear compounds which contain two platinum and two L-Se-Met moieties. For example, **11** corresponded to the molecular formula $[Pt_2(OH)(L\text{-}Se\text{-}Met)_2(NH_3)_3]^+$ (Table 1, ESI Fig. S3).

(2) Reaction of L-Se-MetH with cisplatin at 1 : 2 molar ratio

Less reaction products were observed than in the above case

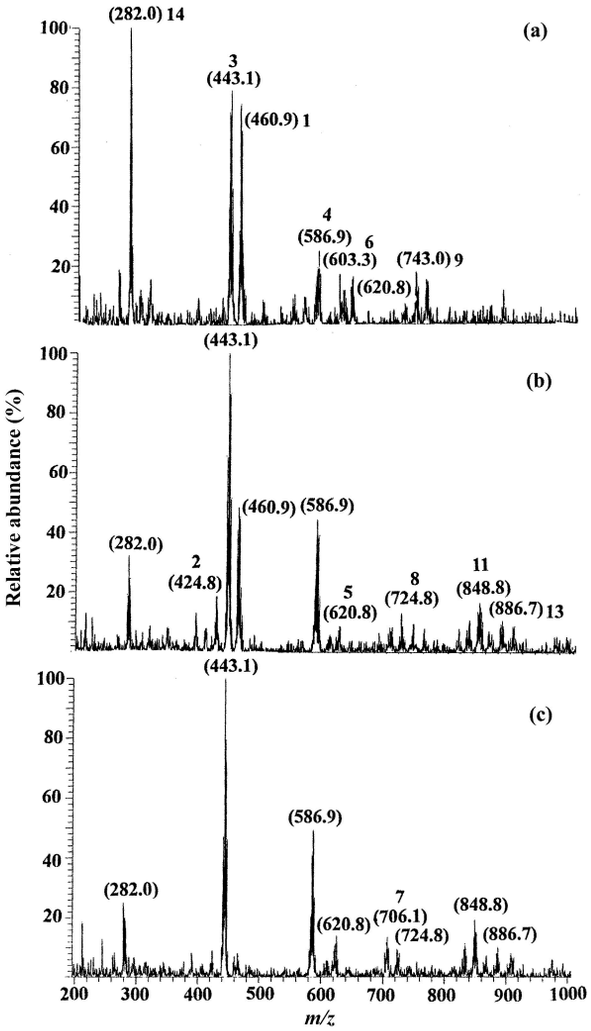


Fig. 3 The ESMS spectra of the reaction of L-Se-MetH with cisplatin (1 : 1, 310 K), recorded at different times: (a) 10 min; (b) 3 h; (c) 18 h.

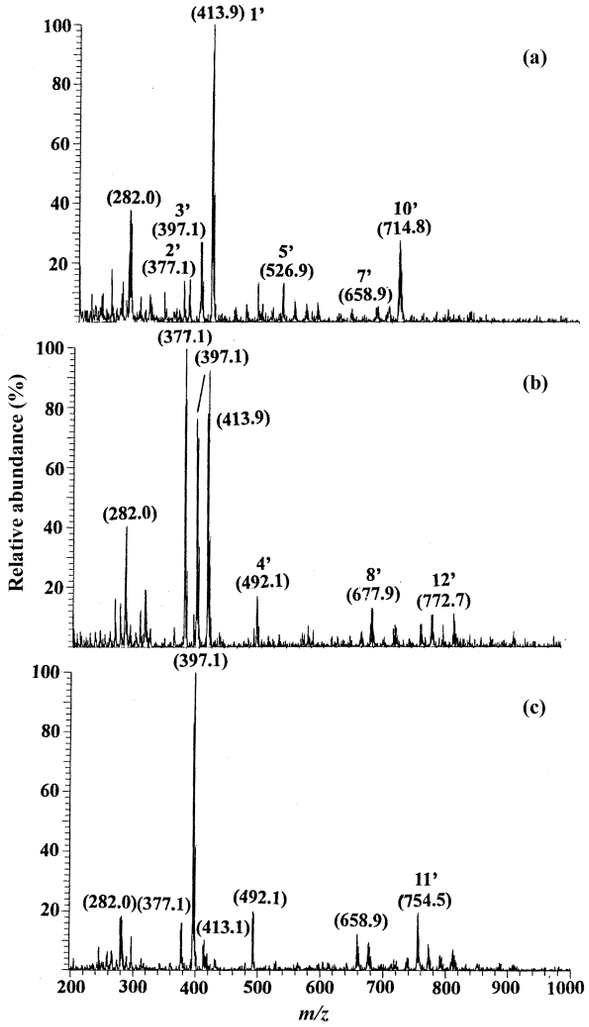


Fig. 4 The ESMS spectra of the reaction of L-MetH with cisplatin (1 : 1, 310 K), recorded at different times: (a) 13 min; (b) 7 h; (c) 24 h.

after 24 h incubation. As shown in Fig. 2, the major species is peak **4** which corresponds to $[Pt(L\text{-}Se\text{-}Met)(L\text{-}Se\text{-}MetH)]^+$. Other species such as complexes **5** and **6** which contain two L-Se-Met/MetH ligands were also observed. However, peaks corresponding to **3** and binuclear species were not observed.

(3) Time dependent reaction of L-Se-Met and cisplatin at 1 : 1 molar ratio (310 K)

The first ESMS spectrum was recorded 10 min after mixing the two reactants, in which peaks **1** and **3** were the major species which have the *m/z* values of 460.9 and 443.1, respectively (Fig. 3a). Based on the *m/z* values, peak **1** can be assigned to $[PtCl(L\text{-}Se\text{-}MetH)(NH_3)_2]^+$ in which the L-Se-MetH is likely coordinated to Pt *via* selenium only, as observed similarly by NMR spectroscopy during the reaction of cisplatin with L-Met.[32] The intensity of peak **1** decreased markedly after 3 h (Fig. 3b). Peak **2** started to appear after 1 h and can be observed for the next 8 h, however its intensity is very weak. This peak corresponds to the complex $[Pt(L\text{-}Se\text{-}Met)(NH_3)_2]^+$. Both peaks **1** and **2** disappeared completely after 18 h (Fig. 3c). Similar to the case of Fig. 1, the dominant species in the ESMS after 18 h was complex **3**. Complexes **5**, **6** and binuclear compounds existed as minor adducts. No further changes in the ESMS spectra were observed afterwards, which indicated the reaction was finished within 18 h.

The same reaction was also conducted at 295 K. At this temperature peak **2** was observable during the whole reaction course (24 h), and its intensity in ESMS is stronger than that observed at 310 K.

(4) Time dependent reaction of L-MetH and cisplatin at 1 : 1 molar ratio (310 K)

The reaction was conducted in order to make a comparison between L-Se-MetH and L-MetH. The time course was followed for 26 h by ESMS. As shown in Fig. 4, after mixing the reactants for 10 min, the dominant species is peak **1′** which can be assigned to the complex $[PtCl(L\text{-}MetH)(NH_3)_2]^+$ (*m/z* = 413.7). Peaks **2′**, **3′** and **4′** which are assignable to $[Pt(L\text{-}Met)(NH_3)_2]^+$ (*m/z* = 377.3), $[PtCl(L\text{-}MetH)(NH_3)]^+$ (*m/z* = 396.8) and $[Pt(L\text{-}Met)(L\text{-}MetH)]^+$ (*m/z* = 492.5), respectively, were also observed. Peaks **5′** and **6′** which contain two methionine moieties appeared at the same time. The intensity of peaks **2′** and **3′** increased gradually with time, and after 7 h peaks **1′**, **2′** and **3′** had similar intensity (Fig. 4b). After 24 h the main product of the reaction was peak **3′** and **2′** was still observable. The long lifetime of the latter suggests that complex **2′** is more stable than **2** under similar conditions. Species with high *m/z* values (peaks **7′**–**13′**) were also observed during the reaction and were assigned to binuclear complexes. Their *m/z* values and assignments are listed in Table 2. As an example, ESI Fig. S4 shows the ESMS spectrum of peak **11′** (*m/z* = 754.3) and the simulated spectrum based on the molecular mass of $[Pt_2(OH)_2(L\text{-}Met)(L\text{-}MetH)(NH_3)_2]^+$.

When the same reaction was conducted at 295 K, however, peaks **2′** and **3′** were the major adducts even after 26 h, showing the higher stability of complex **2′** at lower temperature.

Table 2 Observed and calculated molecular masses of the complexes for the reaction of cisplatin and L-MetH

Peak	Compound	Observed mass[a]	Calculated mass
1′	$[PtCl(L\text{-}MetH)(NH_3)_2]^+$	412.0–415.9	413.7
2′	$[Pt(L\text{-}Met)(NH_3)_2]^+$	376.1–380.0	377.3
3′	$[PtCl(L\text{-}MetH)(NH_3)]^+$	395.1–399.2	396.8
4′	$[Pt(L\text{-}Met)(L\text{-}MetH)]^+$	491.1–495.1	492.5
5′	$[Pt(L\text{-}Met)(L\text{-}MetH)(NH_3)_2]^+$	524.8–528.8	526.5
6′	$[PtCl(L\text{-}MetH)_2(NH_3)]^+$	544.1–548.1	545.9
7′	$[Pt_2Cl(OH)(L\text{-}Met)(NH_3)_4]^+$	656.1–662.9	658.9
8′	$[Pt_2Cl_2(L\text{-}Met)(NH_3)_4]^+$	674.7–679.8	677.3
9′	$[Pt_2Cl_3(L\text{-}MetH)(NH_3)_3]^+$	693.7–700.7	696.7
10′	$[Pt_2Cl_4(L\text{-}MetH + H^+)(NH_3)_2]^+$	710.7–718.7	715.2
11′	$[Pt_2(L\text{-}Met)(OH)_2(L\text{-}MetH)(NH_3)_2]^+$	751.1–761.0	754.3
12′	$[Pt_2Cl(L\text{-}Met)(OH)(L\text{-}MetH)(NH_3)_2]^+$	769.9–776.9	774.0
13′	$[Pt_2Cl_2(L\text{-}Met)(L\text{-}MetH)(NH_3)_2]^+$	787.7–793.7	792.0

[a] The isotopic peaks are separated by 1 *m*/*z* in the mass region indicated.

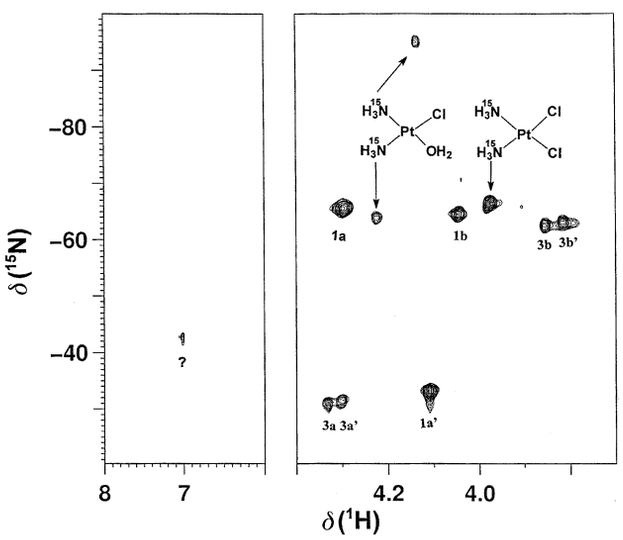


Fig. 5 The 2-D [^{1}H–^{15}N] HSQC NMR spectrum of the reaction of [^{15}N]cisplatin with L-Se-MetH at 1 : 1 molar ratio and at 310 K, recorded after 5 h incubation.

(5) NMR investigation of the reaction of L-Se-MetH and cisplatin at 1 : 1 (310 K)

On reaction of L-Se-MetH with cisplatin at 1 : 1 molar ratio the CH_3 singlet (with ^{77}Se satellites) of L-Se-MetH at δ 2.03 decreased in intensity, and a new singlet at δ 2.47 appeared, together with other singlets at δ 2.51 and 2.58. These can be assigned to the CH_3 signals of Se-bound L-Se-MetH similar to those observed during the reaction of cisplatin and L-MetH.[32] However, due to the complex nature of the spectrum, no attempt has been made to follow the time course of the reaction by ^{1}H NMR. After 24 hours the CH_3 singlet (with ^{77}Se satellites) of free L-Se-MetH disappeared completely and the dominant CH_3 signals were those at δ 2.51 and 2.58.

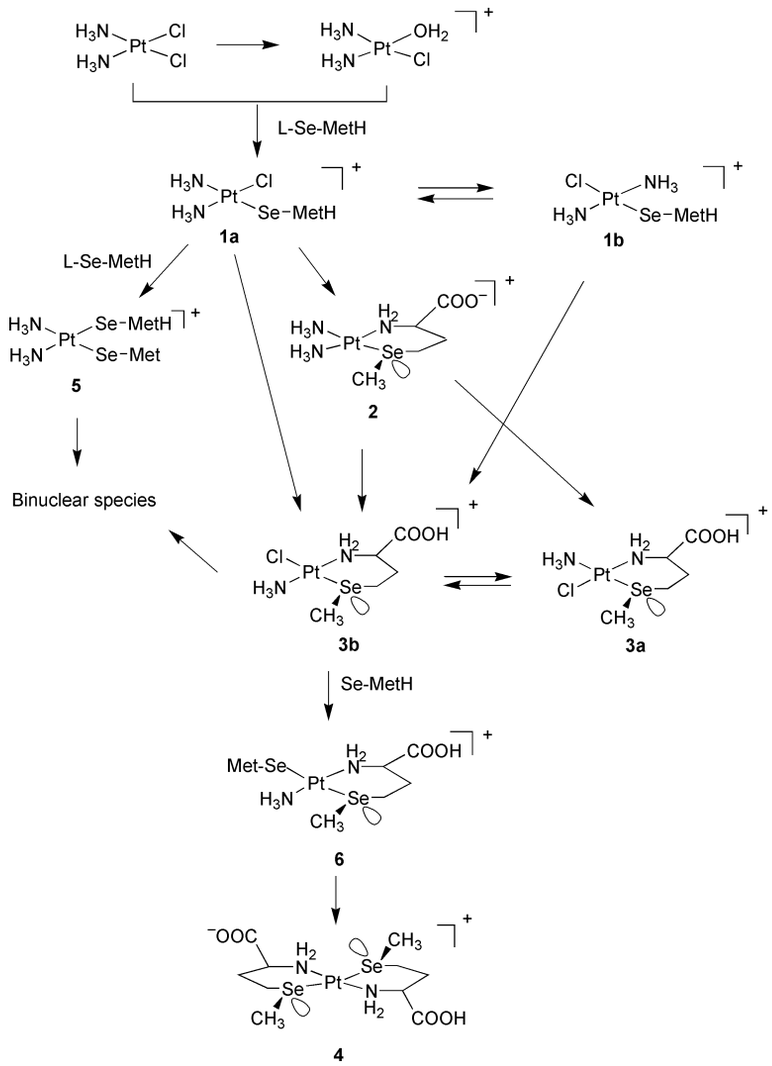
The same reaction was followed by 2-D [^{1}H–^{15}N] HSQC which demonstrated a rather clear reaction pathway. A typical spectrum recorded after 5 h of reaction is shown in Fig. 5. After *ca*. 30 min reaction of [^{15}N]cisplatin with L-Se-MetH, apart from the cross-peak of cisplatin at δ 3.98/−64.6, a pair of new cross-peaks appeared at δ 4.30/−63.9 (**1a**) and 4.11/−39.8 (**1a′**). The former can be assigned to the NH_3 *trans* to Cl and the latter to the NH_3 *trans* to Se. Therefore, the data suggested the formation of *cis*-$[PtCl(L\text{-}Se\text{-}Met\text{-}Se)(NH_3)_2]^+$ **1a**, which agrees well with the mass spectroscopy result. After 1 h, cross-peaks **1a** and **1a′** increased in intensity and new cross-peaks appeared at δ 4.04/−63.2 (**1b**), 4.33/−37.9 (**3a**) and 4.30/−38.3 (**3a′**). Peak **1b** has a ^{15}N chemical shift in the region of NH_3 *trans* to Cl or N, and peaks **3a** and **3a′** in the region of NH_3 *trans* to Se. Cross-peak **1b** can only be assigned to a species which contains a monodentate L-Se-MetH because Se,N chelation would generate a pair of cross-peaks due to slow inversion of *R*,*S* configurations at the chiral Se atom, similar to these observed for L-MetH at the chiral S atom.[32] Therefore, cross-peak **1b** is assigned to the geometric isomer of **1a**, *trans*-$[PtCl(L\text{-}Se\text{-}Met\text{-}Se)(NH_3)_2]^+$. After 2 h (ESI Fig. S5), cross-peaks **1b**, **3a** and **3a′** increased in intensity and a pair of new cross-peaks appeared at δ 3.85/−61.6 (**3b**), 3.82/−61.9 (**3b′**). In the following 2 h, cross-peaks **3b** and **3b′** increased in intensity more quickly than **3a** and **3a′**, so that after 5 h **3b** and **3b′** had comparable intensities as shown in Fig. 5. After 7 h, cross-peaks **3b** and **3b′** became much stronger than **3a** and **3a′** (ESI Fig. S6). Cross-peaks **3a** and **3a′** disappeared after *ca*. 12 h, while **3b** and **3b′** were observable even after 24 h. These two pairs of cross-peaks can be related to peak **3** in ESMS recorded under the same conditions. Therefore, they can be assigned to the geometric isomers of $[PtCl(L\text{-}Se\text{-}Met\text{-}Se,N)(NH_3)]^+$ **3a** and **3b**. As noted in Fig. 5, a cross-peak (**?**) at δ 7.01/−42.9 was observed, which has a ^{15}N chemical shift in the region of NH_3 *trans* to Se, but the ^{1}H chemical shift is rather unusual for a Pt–NH_3 signal, and it is unassigned. Cross-peaks at δ 4.14/−86.2 and 4.23/−62.8 are known and can be assigned to *cis*-$[PtCl(NH_3)_2(H_2O)]^+$.

Cross-peaks for complexes **1a** and **1b** disappeared completely after 18 h, while those for **3** (**3b** and **3b′**) are observable even after 24 h, together with peaks for unchanged cisplatin and *cis*-$[PtCl(NH_3)_2(H_2O)]^+$.

Discussion

Selenium-containing amino acids have attracted considerable current attention due to their anticancer and antioxidant activities. Selenomethionine has been shown to prevent cisplatin-induced drug resistance in human ovarian tumours *in vivo*.[33] However, it is surprising that there appears no report on the interaction of cisplatin with Se-MetH.

This work shows that ESMS and 2-D [^{1}H–^{15}N] HSQC NMR techniques could provide valuable information on the mechanism of reaction of cisplatin with L-Se-MetH and L-MetH. The 2-D [^{1}H–^{15}N] NMR technique has been shown to be powerful for elucidating reactions of [^{15}N] cisplatin with a variety of biomolecules.[25,26] However, when applied in the reactions of cisplatin with sulfur-containing ligands, the strong *trans* effect of sulfur promotes labilization of ammine and limits the potential of the 2-D NMR method. ESMS can provide precise molecular masses and structural information for the intact ions formed during the reaction. However, for the *S*,*N*- or *Se*,*N*-chelated methionine–platinum species, it is well known that slow inversion of the methyl group at the chiral sulfur or selenium gives rise to different diastereoisomers which could not be distinguished by ESMS. This, on the other hand,



Scheme 1 Reaction pathway of cisplatin with L-Se-MetH in 1 : 1 mole ratio at pH 4.4. Complexes **2** and **4** were observed by the ESMS method, but not by NMR due to their low abundance.

can be differentiated by 2-D [^{1}H–^{15}N] NMR if the *trans* $^{15}NH_3$ is not detached from platinum.

Based on the ESMS and 2-D [^{1}H–^{15}N] NMR results, the reaction pathway of cisplatin with L-Se-MetH is summarized in Scheme 1. As can be noted, cisplatin reacts directly with L-Se-MetH to give rise to complex **1a** which can be isomerized to **1b**, both of which gave only one cross-peak in the 2-D [^{1}H–^{15}N] HSQC spectra due to fast inversion at the monodentate chiral Se. These two isomers can chelate to form complexes **2**, **3a** and **3b** or react with a second L-Se-MetH to give **5**. Complexes **2** and **5** are observed only by ESMS due to their low abundance in solution. It is noted in the NMR spectra that the formation of **3a** is faster than that of **3b** while the disappearance of **3a** is also faster, illustrating the differences in kinetic and thermal stabilities between the two isomers. Both **3a** and **3b** have two diastereoisomers due to slow inversion of the *R* and *S* configuration at the chiral Se as shown by NMR spectroscopy (Scheme 1). This work demonstrated that the mechanism of reaction of cisplatin with L-Se-MetH parallels that of cisplatin with L-MetH.[32] However, complex **2** in the L-Se-MetH reaction is much less stable and less abundant than **2′** in the L-MetH reaction, as shown by the ESMS data.

The presence of the binuclear complexes during the 1 : 1 reactions was notable. It appears to be the first time that such species have been identified in the reaction of cisplatin and L-MetH. These species were present in minute amounts and could exist in many different isomers, therefore it is impractical to carry out further characterization.

The dominant product of reaction of cisplatin with L-Se-MetH at 1 : 2 molar ratio at 310 K is the bis-*Se*,*N*-chelated platinum complex **4**. Other species such as [PtCl(L-Se-MetH-*Se*,*N*)(NH_3)]$^+$ **3** and binuclear adducts were not observed, suggesting that their formation was unfavourable when L-Se-MetH is in excess.

Conclusion and biological relevance

This work has shown that ESMS and NMR can be complementary methods in the understanding of the reaction pathways of cisplatin with selenomethionine and methionine. The major intermediates identified for the reaction of cisplatin with L-Se-MetH in 1 : 1 molar ratio are *cis*-[PtCl(L-Se-MetH-*Se*)$(NH_3)_2$]$^+$ **1** and *cis*-[Pt(L-Se-Met-*Se*,*N*)$(NH_3)_2$]$^+$ **2**, which undergo further reactions to give *cis*-[PtCl(L-Se-MetH-*Se*,*N*)(NH_3)]$^+$ (**3a** and **3b**, *cis*-[Pt(L-Se-Met-*Se*)(L-Se-MetH-*Se*)$(NH_3)_2$]$^+$ **5**, [Pt(L-Se-Met-*Se*,*N*)(L-Se-MetH-*Se*,*N*)]$^+$ **4** and minor binuclear products. Under similar conditions, the reactions of cisplatin with L-MetH gave similar adducts.

Recent *in vivo* studies demonstrated that both selenomethionine and methionine are able to inhibit cisplatin-induced toxicities and resistance, but do not affect the cytotoxicity of the drug. In the presence of an excess of L-Se-MetH or L-MetH the predominant platinum(II) species are likely to be the bis-chelated Se,N- or S,N-chelated adducts. These suggest that Pt^{II}–(Se)MetH adducts may still be active towards the target molecule DNA. We are currently investigating the DNA

binding properties of platinum-based drugs in the presence of selenomethionine. On the other hand, it is unknown whether cisplatin could interact with glutathione peroxidase whose active centre contains selenocysteine residues.

Acknowledgements

We thank the financial supports from the National Natural Science Foundation of China for the Distinguished Young Scientists Fund (Grant No. 29925102) and the Major State Basic Research Development Program (Grant No. G200007750). We thank Professor P. J. Sadler (Edinburgh) for helpful comments on the script and for access to his NMR spectrometer.

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