A study of $[\text{Au}(\text{ethylenediamine})\text{Cl}_2]\text{Cl}$ interaction with L-methionine and DL-seleno-methionine by ^1H and ^{13}C NMR spectroscopy

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Abstract. The interaction of $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ with L-methionine (Met) and DL-seleno-methionine (Se-Met) was carried out in D_2O solution at $\sim\text{pH}$ 3.8. ^{13}C NMR spectra were interpreted by comparison with the spectra recorded after the reaction of Met or Se-Met with HAuCl_4 and also after oxidation by H_2O_2 . Reactions of $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ with Met and Se-Met were found to proceed through the exchange of chloride ligands from complex with Met or Se-Met. Gold(III) reduction takes place during the reaction and *en* ligand was released from $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ complex.

Keywords: L-methionine, DL-seleno-methionine, NMR spectroscopy

Abbreviations

<i>en</i> :	Ethylenediamine
Met:	L-methionine
Met-SO-CH ₃ :	L-methionine sulfoxide
Se-Met:	DL-seleno-methionine
Met-SeO-CH ₃ :	DL-methionine-seleno-oxide

1. Introduction

Gold complexes have been studied extensively as antiarthritic and anticancer agents during the last four decades [1–13]. Auranofin and Myocrisin are some examples of gold(I) drugs that have been used for the treatment of rheumatoid arthritis [7,13]. Gold(III) complexes, which are iso-electronic with anti-tumor platinum(II) complexes, have been evaluated for *in vitro* anticancer activity and are shown to have significant cytotoxic effects [2,4].

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Scheme 1. Met and Se-Met atoms numbering.

Ethylenediamine (*en*) is the most common chelating ligand and its gold complexes have been known since 1931 [16]. The crystal structure of $[Au(en)Cl_2]Cl$ has a square planar geometry about gold(III) and the anionic Cl^- ion is located in the equatorial positions [16]. The interest in this complex is due to the fact that similar complexes have shown significant cytotoxic effects [1]. Met is a thioether containing amino-acid and is a part of most of the proteins and enzymes in human body. Therefore studying its interaction with metal ions is of importance from bioinorganic chemistry point of view. Considering this importance, we have previously studied the interaction of Met with $AuCl_4^-$ [5] which shows that $AuCl_4^-$ acts as a strong oxidizing agent causing oxidation of Met to Met-SO-CH₃. In the present study, we have investigated the reactions of $[Au(en)Cl_2]Cl$ with Met and Se-Met in aqueous solution using ^{13}C NMR spectroscopy, in order to understand the interaction between the complex and the biologically important ligands. It will be interesting to see the difference in reactivity between two types of gold(III) complexes, one with and the other without *en* ligand. The structures of Met and Se-Met are shown in Scheme 1.

2. Experimental section

2.1. Chemicals

$HAuCl_4 \cdot 3H_2O$ was obtained from Stream Chemicals Co. D_2O was obtained from Fluka Chemicals Co. The ligands, Met- $^{13}C5$ and Se-Met- $^{13}C5$ were obtained from Isotech, USA.

2.2. Preparation of $[Au(en)Cl_2]Cl$

$[Au(en)Cl_2]Cl$ complex was prepared as described in previous work [16].

2.3. NMR measurements

All NMR measurements were carried out on a JEOL JNM-LA 500 NMR spectrophotometer at 297 K using 0.10 M solution of the complexes in D_2O . The ^{13}C NMR spectra were obtained at 125.65 MHz with 1H broadband decoupling. The spectral conditions were: 32k data points, 0.967 s acquisition time, 1.00 s pulse delay and 45° pulse angle.

Reactions were carried out by mixing D_2O solutions of $[Au(en)Cl_2]Cl$ (0.05 M) and Met as well as Se-Met (0.05 M). pH were adjusted by DCl and NaOD solutions to 3.8. The reactions were then monitored by NMR spectroscopy.

3. Results and discussion

3.1. $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ interaction with *L*-methionine

Met is an essential α -amino acid classified as nonpolar, and together with *L*-cysteine, constitute the two sulfur-containing proteinogenic amino acids [10]. It is also one of the most important bio-ligands that affect the efficacy of antitumor complexes. Interaction of Met with cisplatin (*cis*- $[(\text{NH}_3)_2\text{PtCl}_2]$) was studied by El-Khateeb et al. [3]. They reported that Met binds to cisplatin first by hydrolysis and water is later exchanged by Met-S ligation, which then rearranges to an N, S chelate complex. Thioethers, as thiols, are oxidized into sulfoxide forms by gold complexes, which have been found to be a source for toxicity of gold complexes [9,14].

Table 1 shows ^1H NMR chemical shift data for the reaction of Met with $[\text{Au}(\text{en})\text{Cl}_2]^+$ and HAuCl_4 . Significant deshielding was observed of Met protons upon reaction, H4 and H5 resonances show the largest downfield shift in the chemical shift values indicating bonding through sulfur. After 18 h, $\sim 86\%$ of *en* ligand was dissociated from $[\text{Au}(\text{en})\text{Cl}_2]^+$.

Figure 1 shows the ^{13}C NMR spectra for Met- $^{13}\text{C}5$ reaction with $[\text{Au}(\text{en})\text{Cl}_2]^+$, signal at 14.7 ppm, which is due to $^{13}\text{C}5$ was disappeared and three other signals appeared in spectrum, 17 min after mixing (Fig. 1b), corresponding to three different forms of Met in solution, which are assigned to $\text{Au(III)}\text{-Met}$ at 23 ppm, $[(\text{Met-S,N})\text{Au}(\text{en})]\text{Cl}_3$ at 33 ppm and the third at 37 ppm due to overlapping of methionine sulfoxide and free ethylenediamine dissociated from $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ complex.

Several experiments on the reactions of Met with H_2O_2 and HAuCl_4 were also carried out and results were as follows to prove our assignments.

The signal at 23 ppm corresponding to $[(\text{Met-S})\text{Au}(\text{en})]^{3+}$, was decreasing until it completely disappeared after 1 h. The signals at 33 ppm and 37 ppm were attributed to $[(\text{Met-S,N})\text{Au}(\text{en})]$ and Met-SO-CH_3 , respectively. The signal at 33 ppm decreased in intensity and that at 37 ppm became dominant after 7 h, indicating complete oxidation of Met to Met-SO-CH_3 along with Au(III) reduction into Au(I) . The chemical shift assignments are shown in Table 2. Table 2 shows that *en* ligand was found to be released from $[\text{Au}(\text{en})\text{Cl}_2]^+$ complex after reaction indicating Au(III) reduction.

An experiment, where Met ^{13}C -labeled at C5 was oxidized by H_2O_2 , was carried out. The disappearance of signal at 15 ppm and the appearance of two signals at 23 and 37 ppm corresponding to C5 represented the oxidation products of Met (Met-SO-CH_3). The resonances at 23 and 33 ppm were not observed indicating that these signals were due to Met-gold complex.

Figure 2 shows the ^1H NMR spectra for Met reaction with HAuCl_4 at $\text{pD} = 3.8$. Higher pH causes the hydrolysis of $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ complex [16]. Downfield shifts were observed in H5, H3 and H2 after ligation to gold metal center. Polymeric $[\text{Au}(\text{Met})]_n$ complex was precipitated out of solution after few days.

Table 1
 ^1H NMR chemical shifts for Met interaction with $[\text{Au}(\text{en})\text{Cl}_2]^+$ in D_2O at 25°C , $\text{pD} = 3.8$

	<i>en</i>	H5	H4	H3	H2
Met		1.987	2.486	1.960	3.724
Met + $[\text{Au}(\text{en})\text{Cl}_2]^+$	3.115	2.375	2.706, 2.602	2.196	3.829
Met + HAuCl_4	–	2.552	2.225	2.932, 2.875	4.080

Note: Atoms numbering according to Scheme 1.

Figure 1. ^{13}C NMR spectra of (a) free $\text{Met-}^{13}\text{C}_5$ while spectra (b)–(f) are for $\text{Met-}^{13}\text{C}_5$ after reaction with $[\text{Au}(\text{en})\text{Cl}_2]^+$, (b) after 17 min, (c) after 34 min, (d) after 44 min and (e) after 7 h, in D_2O at 25°C , $\text{pD} = 3.8$.

Table 2
¹³C NMR chemical shifts of Met reaction with [Au(en)Cl₂]⁺ in D₂O at 25°C, pD = 3.8

	en	C1	C2	C3	C4	C5
Met	–	174.97	54.64	30.44	29.62	14.73
[(Met)Au(en)] ³⁺	37.29	173.69	53.65	32.52	49.95	24.40

Figure 3 shows the ¹³C NMR spectra for Met that are recorded before and a long period of time after reaction with HAuCl₄ (2 days). Figure 3b shows the signal at 37 ppm attributed to Met–SO–CH₃ indicating Met oxidation by gold(III), that is reduced to gold(I). Polymeric [Au(Met)]_n was precipitated out of solution, and when Na₂S₂O₃ was added to solution, reduction of Met–SO–CH₃ took place. Reaction could be described by Eqs (1) and (2).

Vujačić et al. [15] have studied the Met reaction with HAuCl₄ which shows that Met-S binding on Au(III) metal center take place via exchange of one chloride ligand which then rearranges to chelating Met-S,N mode. This binding is consistent with our finding regarding the formation of three species of Met that convert finally into Met–SO–CH₃.

While [Au(en)Cl₂]⁺ react with Met according to Eq. (3).

From proceeding discussion we may suggest that reaction proceed through the following reaction mechanism (Eqs (4)–(7)).

Equations (4)–(7) describe possible mechanism for Met reaction with [Au(en)Cl₂]⁺ complex. The reaction starts with [Au(en)Cl₂]⁺ hydrolysis, as expected by comparison with reaction with cisplatin complex, as shown by El-Khateeb et al. [3]. In the first step one Cl[–] ligand is exchanged by Met-S ligand that rearranges into chelating mode, Met-S,N (Eq. (5)). Reduction of Met takes place in the next stage concurrent with en ligand dissociation and gold(I) formed in solution (Eq. (6)). The reaction stops finally by the precipitation of Au(I) with non-oxidized Met as polymeric [Au(Met)Cl]_n.

3.2. [Au(en)Cl₂]Cl interaction with DL-Se-methionine

Selenium compounds have received more attention in the recent decades and are found to be an essential element in mammals [12]. It is known that seleno-ligands bind more strongly to gold than thio-ligands [6,8].

Figure 2. ^1H NMR spectra of (a) Met, spectra (b) after mixing with HAuCl_4 (white ppt formed in solution), (c) after $\text{Na}_2\text{S}_2\text{O}_3$ added to the solution, (d) after 10 days in D_2O at 25°C , $\text{pD} = 3.8$.

Figure 3. ^{13}C NMR spectra of (a) Met, spectra (b) after mixing with $\text{H[AuCl}_4]$ (white ppt formed in solution and clear solution decanted), (c) after $\text{Na}_2\text{S}_2\text{O}_3$ added to solution, in D_2O at 25°C , $\text{pD} = 3.8$.

DL-selenomethionine (Se-Met) interaction with $[\text{Au}(\text{en})\text{Cl}_2]^+$ was studied in this work and ^{13}C NMR spectra were recorded for the reaction.

Figure 4. ^{13}C NMR spectra for Se-Met- $^{13}\text{C}5$ before reaction (a) and after reaction with $[\text{Au}(\text{en})\text{Cl}_2]^+$ at 1:1 ratio (b), in D_2O at 25°C and $\text{pD} = 3.7$.

Reaction of $[\text{Au}(\text{en})\text{Cl}_2]^+$ with Se-Met were carried out using Se-Met labeled $^{13}\text{C}5$. The ^{13}C NMR spectrum was recorded for the resulting solution (Fig. 4). Fast and complete shift of signal at $\delta = 4$ ppm, corresponding to C5, to 32 and 34 ppm, attributed to $[(\text{Se-Met})\text{Au}(\text{en})]^{3+}$ (C5' signal) and Met-SeO-CH_3 (C5'' signal), respectively, was observed. These signals were assigned by comparison with the spectrum resulting from Se-Met- $^{13}\text{C}5$ oxidation experiment by H_2O_2 .

The mechanism suggested for Met is also applicable for Se-Met. Hence the reaction mechanism could be described as chloride ligand exchange by Se-Met followed by Se-Met oxidation. Gold(III) is reduced to gold(I) along with *en* ligand dissociation from the reduced gold(III) center and precipitation of white polymeric $[\text{Au}(\text{Se-Met})\text{Cl}]_n$ at the bottom of the tube.

4. Conclusion

^{13}C NMR was employed to study the reaction mechanisms of $[\text{Au}(\text{en})\text{Cl}_2]\text{Cl}$ reactions with Met and Se-Met. The reactions were found to proceed through; the exchange of chloride ions, gold(III) reduction, precipitation of polymeric gold(I)-Met and gold(I)-Se-Met complexes along with the release of *en* ligand from the gold complex. Met oxidation was found to be reversible in solution.

Acknowledgement

The authors would like to acknowledge the support by the Deanship of Scientific Research at King Fahd University of Petroleum & Minerals for funding this work through project No. CY/GOLD/374 (IN070374).

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