A ^{1}H NMR Study of the Binding of Hg^{2+} with DL-seleno-methionine in Aqueous Solution

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Introduction

The binding of heavy metal ions such as Zn(II), Cd(II), Hg(II), Au(I) and Pt(II) with sulphur containing amino acids such as methionine and its derivatives has been studied by various research groups [1–4]. L-methionine [Met] is one of the constituents of almost all naturally occurring proteins and enzymes. Although extensive work has been done on the metal coordination of Met little information is available for DL-seleno-methionine (Se-Met), which is also a naturally occurring amino acid and can be found in various plants [5]. Recently, we have reported a study of the reaction of gold(III) with Se-Met by ^{1}H NMR spectroscopy [6].

In the present study, we report the preliminary results of a similar investigation, in which the binding of Hg(II) to Se-Met was followed by ^{1}H NMR spectroscopy in aqueous solution as a function of pH. The pK_1 and pK_2 constants of $-CO_2^-$ and $-NH_3^+$ have also been studied and compared with the corresponding Met constants.

Experimental

DL-seleno-methionine was obtained from K and K Labs., Plainview, New York. L-methionine and all the deuterated solvents were obtained from Aldrich Chemical Company. Analytical grade $Hg(NO_3)_2$ was obtained from BDH, England. These products were used as received.

^{1}H NMR spectra were measured at 200 MHz on a Varian XL-200 Spectrometer operating in the pulsed Fourier transform mode. The probe temperature was 22.0 °C. ^{1}H chemical shifts were measured relative to an internal tert-butanol reference and are compared with the methyl proton resonance of sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS). The methyl resonance of tert-butanol is at +1.23 ppm relative to DSS.

The binding of Hg(II) to Se-Met was studied in D_2O; the ionization constants were measured in doubly deionized distilled water using D_2O as an external lock.

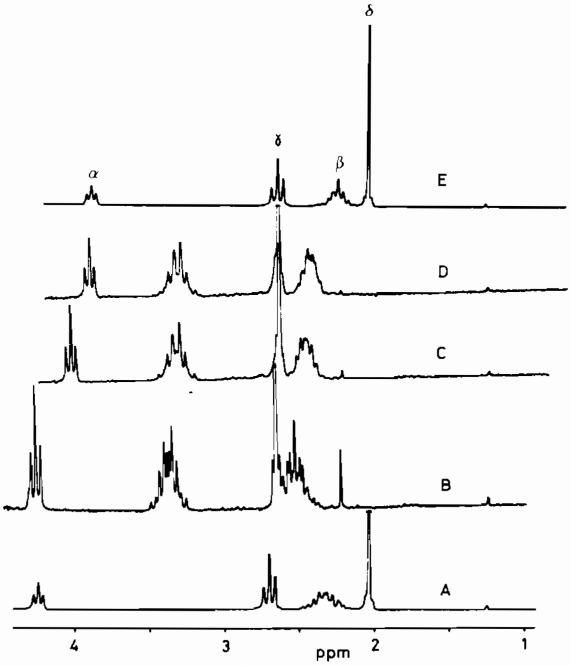

Fig. 1. (A) the 200 MHz ^{1}H NMR spectrum of 0.01 *M* Se-Met at pH* 1.04. Spectra (B) to (D) are of Hg^{2+}: Se-Met at 1:1 ratio (0.01 *M*) at (B) pH* 1.04, (C) pH* 2.60, and (D) pH* 3.40. Spectrum (E) is of 0.01 *M* Se-Met itself, pH* is 3.50.

pH Measurements were made at 22.0 ± 0.2 °C with a Fisher Accumet pH meter, model 620, equipped with a Fisher micro-probe combination pH electrode. pH* was used to indicate the actual meter reading for D_2O solutions with no correction for deuterium isotope effects [7].

Results and Discussion

The addition of 0.01 *M* $Hg(NO_3)_2$ to 0.01 *M* Se-Met(1:1) in D_2O solution at low pH* caused the formation of white precipitates which dissolved completely on shaking. The two methyl resonances at 2.215 and at 2.644 ppm for the ligand were observed at pH* 1.04. The methyl resonance in the absence of Hg(II) was at 2.024 ppm at the same pH*. The γ-CH_2 multiplets were shifted from 2.694 ppm to 3.367 ppm at the same pH* and at the same 1:1 ratio. Comparatively small chemical shift changes were observed for OC-CH and β-CH_2 resonances.

As the pH* was increased from 1.04 to 1.83 (not shown in Fig. 1), the methyl resonance at 2.215 ppm almost disappeared and shifted to 2.644 ppm. When

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TABLE I. Ionization Constants for L-Methionine and DL-Seleno-methionine in Aqueous Solution.

Ligand	pK_1 (CO_2^-)	pK_2 (NH_3^+)	Medium	Temp.	Method	Ref.
Met	2.17	9.20	(0.15 *M*) $NaClO_4$	20 °C	gl	a
Met	2.24	9.17	(0.1 *M*) KCl	25 °C	gl	b
Met	2.35	9.15	(0.154 *M*) NaCl	22.0°C	NMR	c
Se-Met	2.30	9.15	(0.154 *M*) NaCl	22.0°C	NMR	c

[a] C. J. Hawkins and D. D. Perrin, *Inorg. Chem., 2,* 843 (1963). [b] B. S. Sekhon, P. P. Singh and S. L. Chopra, *Ind. J. Chem., 9,* 485 (1971). [c] This work, 0.02 *M* ligands were dissolved in doubly deionized distilled water.

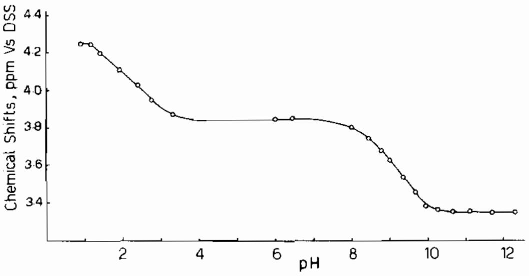


Fig. 2. pH dependence of the α-CH proton chemical shifts of 0.02 *M* Se-Met in 0.154 *M* NaCl aqueous solution.

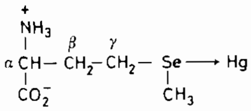
the pH* was increased from 1.83 to 3.40 no change was observed except that the OC-CH and β-CH_2 resonances shifted to lower field. Very small shifts were observed for the γ-CH_2 and δ-CH_3 resonances.

The OC-CH chemical shift was not affected by the presence of Hg(II) and the resonance shifted to high field as the pH* was increased. This is due to the deprotonation of the carboxylate group. At pH* 3.40, the Hg^{2+}–Se-Met complex gave white precipitates which did not dissolve even at high pH*.

Fairhust and Rabenstein [1] have studied the binding of methyl mercury(II) with Met and report that it binds exclusively to the thioether group in acidic medium. However, at pH > 7, the methyl mercury(II) binds more strongly to the amino group. Natush and Porter [8, 9] have shown that Hg^{2+} binds to sulphur in acidic solution but that in alkaline solution it binds via the amino and the carboxylate groups. Similar observations were made with Zn^{2+} and Cd^{2+} [10, 11].

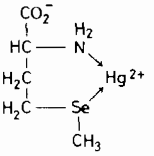
The pK_1 and pK_2 values for $-CO_2^-$ and $-NH_3^+$ in Se-Met are similar to those of Met as indicated in Table I. The selenium is far from both the ionizing groups, therefore Se-Met does not have pK values significantly different from those of Met. As shown from the plot (Fig. 2) of chemical shifts *vs.* pH, the carboxylate group was completely ionized at pH 3.8. As soon as $-CO_2^-$ was formed (pH* 3.4) white precipitates resulted. This suggests that the $-CO_2^-$ group is not binding to Hg^{2+} in solution.

The following structures are possible for a 1:1 ratio of Hg^{2+}:Se-Met in solution:

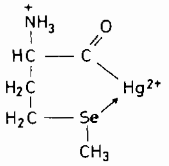


$$\alpha\,\underset{CO_2^-}{\overset{\overset{+}{NH_3}}{CH}}-\overset{\beta}{CH_2}-\overset{\gamma}{CH_2}-\underset{CH_3}{Se}\longrightarrow Hg^{2+}$$

(I)



(II)



(III)

The methyl resonance at 2.215 ppm may be due to the 1:1 Hg^{2+}–Se-Met complex formation as shown in (I). The methyl resonance at 2.644 ppm may be due to the formation of (II) or (III). Since the chemical shift of α-CH resonance remains unchanged throughout the pH* titration in the presence of Hg^{2+}, structure (III) can be ruled out. Therefore structure (II) is more probable in aqueous solution.

Comparative studies have been done on the complexation of selenosemicarbazide and thiosemicarbazide with Zn(II), Cd(II) and Hg(II) [12]. It has been reported that Hg(II) behaves as a typical class 'b' metal ion with the order of stability being Se > S > O [12, 13].

From this preliminary study it is clear that although the ionization constants for Met and Se-Met remain unchanged, their binding property to Hg^{2+} is different. Further studies are in progress to identify the Hg^{2+}–Se-Met complex in the solid state, as well as Se-Met complexes with other heavy metal ions.

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