Video Article

A Study of the Complexation of Mercury(II) with Dicysteinyl Tetrapeptides by Electrospray Ionization Mass Spectrometry

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Abstract

In this study we evaluated a method for the characterization of complexes, formed in different relative ratios of mercury(II) to dicysteinyl tetrapeptide, by electrospray ionization orbitrap mass spectrometry. This strategy is based on previous successful characterization of mercury-dicysteinyl complexes involving tripeptides by utilizing mass spectrometry among other techniques. Mercury(II) chloride and a dicysteinyl tetrapeptide were incubated in a degassed buffered medium at varying stoichiometric ratios. The complexes formed were subsequently analyzed on an electrospray mass spectrometer consisting of a hybrid linear ion- and orbi- trap mass analyzer. The electrospray ionization mass spectrometry (ESI-MS) spectra were acquired in the positive mode and the observed peaks were then analyzed for distinct mercury isotopic distribution patterns and associated monoisotopic peak. This work demonstrates that an accurate stoichiometry of mercury and peptide in the complexes formed under specified electrospray ionization conditions can be determined by using high resolution ESI MS based on distinct mercury isotopic distribution patterns.

Video Link

The video component of this article can be found at http://www.jove.com/video/53536/

Introduction

Current clinical drugs prescribed for chelation therapy of mercury poisoning[1] contain thiol group(s), which is/are responsible for binding and sequestering mercury ions[2,3]. However, studies have shown that these small thiol compounds [dimercaptosuccinic acid (DMSA) and dimercaptopropane-sulfonic acid (DMPS)] are not optimal for mercury chelation therapy[4-6]. Therefore, there is a need to understand the association and complex formation tendencies of mercury with thiols to enhance the rational drug design of thiol compounds for mercury chelation. Recently, we reported that *n*-alkyl and aryl dicysteinyl tripeptides with dithiol groups can serve as effective "double anchors" to accommodate the coordination sites of mercury(II) to form 1:1 mercury(II):peptide and 1:2 mercury(II):$(peptide)_2$ complexes[7]. Additionally, we studied the effect of increasing cysteinyl residues on complex type formations[8]. In this study, we investigate the association of mercury(II) with two dicysteinyl tetrapeptides, where the cysteinyl residues are separated by two amino acid residues. In order to evaluate the effect of auxiliary binding groups for mercury, the intervening amino acids are either two glycine (unsubstituted) residues or two glutamic acid (gamma-carboxylated) residues.

The reaction of cysteinyl peptide with mercury(II) requires conditions that will prevent the oxidation of the cysteinyl thiol groups to form disulfide bonds[9]. Moreover, the association of mercury(II) with cysteinyl peptides to form various types of mercury-peptide complexes is dependent on the initial ratio of mercury(II): peptide in the reaction mixture[7,8]. The types of mercury-peptide complexes formed in these reaction mixtures can be analyzed by soft-ionization mass spectroscopy, which is a sensitive analytical tool for determining species interactions between metal ions and peptides[10-14]. Accordingly, it will provide a profile of the various types of mercuriated peptide adducts that are formed under a specified electrospray ionization condition. Here, we will show how cysteinyl peptides and mercury(II) chloride solutions can be prepared in degassed ammonium formate buffer solution blanketed with argon to minimize oxidation. By reacting varying mole equivalents of mercury(II) with dicysteinyl tetrapeptides, we will show how the initial ratio of mercury(II):peptide has an effect on the types of complexes formed. We will also show how electrospray ionization (ESI) mass spectrometry can be used as a characterization tool to provide an accurate stoichiometry of mercury to peptide in the complexes formed. The associated video protocol will demonstrate the experimental conditions for preparing the mercury complexes, the procedure for analyzing the reaction mixtures under specified electrospray ionization conditions, and the characterization of the stoichiometries of mercury(II)-dicysteinyl tetrapeptide complexes, based on the distinct mercury isotope distribution patterns, by using the ChemCal program[15]. It is intended to assist those who are interested in using ESI orbitrap mass spectrometry to analyze various complexes formed by metal ions that exist in different isotopic forms.

Protocol

Note: Please consult all relevant material safety data sheets (MSDS) before use. Mercury chloride is a toxic chemical. Personal protective equipment (gloves, safety goggles, and lab coat) must be worn when handing it and all associated solutions. Dispose of solutions in clearly labeled chemical waste bottles designated for heavy metals.

1. Preparation of 5 mM Degassed Ammonium Formate Buffer, pH 7.5

1. Dissolve 0.1576 g of ammonium formate buffer in 450 ml of HPLC grade water. Adjust the pH of the above solution with 1 M formic acid and 1 M ammonium hydroxide to 7.5. Transfer this solution to a 500 ml volumetric flask and add HPLC water to the calibration line to make a 5 mM ammonium formate solution.
2. Degas the 5 mM ammonium formate buffer under a vacuum system for 10 min and purge with argon. Repeat twice and store solution under argon. On the day of use, filter the buffer solution through a 0.2 micron filter before use.

2. Preparation of Mercury(II) Chloride Solutions

1. Weigh out 0.2375 g mercury(II) chloride. Dissolve it in 25 ml of 5 mM ammonium formate buffer to produce a 0.035 M mercury(II) chloride solution.
2. Add 0.214 ml of 0.035 M mercury(II) chloride solution to 9.785 ml of 5 mM ammonium formate buffer to create a 7.5×10^{-4} M solution. Blanket the 7.5×10^{-4} M mercury(II) solution with argon gas.

3. Preparation of CGGC Stock Solution

1. Dissolve 2.0 mg of the dicysteinyl tetrapeptide, CGGC, in 0.118 ml of HPLC grade acetonitrile and then add 1.0647 ml of 5 mM ammonium formate, pH 7.5 buffer that has been degassed in argon to yield a 5 mM CGGC stock solution.
2. Add 225 µl of the 5 mM CGGC stock solution to 1,275 µl of 5 mM ammonium formation pH 7.5 buffer to give a 7.5×10^{-4} M CGGC solution.

4. Preparation of Various Reaction Mixtures of Mercury(II) and CGGC

1. Preparation of 1:0.5 ratio of mercury(II):CGGC solution
    1. Place 255 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5×10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
    2. Vortex the solution for 10 sec. Then add 15 µl of 7.5×10^{-4} M CGGC solution into the 1.5 ml microcentrifuge. Vortex the solution for 10 sec. Let the solution stand for 10 min prior to injection into the mass spectrometer.

2. Preparation of 1:1 ratio of mercury(II):CGGC solution
    1. Place 240 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5×10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
    2. Vortex the solution for 10 sec. Then add 30 µl of 7.5×10^{-4} M CGGC solution into the 1.5 ml microcentrifuge tube. Repeat in a similar manner as described in section 4.1.

3. Preparation of 1:2 ratio of mercury(II):CGGC solution
    1. Place 210 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5×10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
    2. Vortex the solution for 10 sec. Then add 60 µl of 7.5×10^{-4} M CGGC solution into the 1.5 ml microcentrifuge tube. Repeat in a similar manner as described in section 4.1.

5. Preparation of CEEC Stock Solution

1. Dissolve 3.5 mg of the dicysteinyl tetrapeptide, CEEC, in 0.145 ml of HPLC grade acetonitrile to dissolve the peptide. Then add 13.067 ml of 5 mM ammonium formate, pH 7.5 buffer that has been degassed in argon to produce 0.5 mM CEEC solution.
2. Vortex the solution until all peptide is dissolved. Add 1.125 ml of 0.5 mM CEEC solution and 0.375 ml of 5 mM ammonium formate, pH 7.5 buffer to a 1.5 ml microcentrifuge tube to give a 7.5×10^{-5} M CEEC solution. Vortex until mixed.

6. Preparation of Various Reaction Mixtures of Mercury(II) and CEEC Solution

1. Preparation of 1:0.5 ratio of mercury(II):CEEC solution
    1. Place 255 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5×10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
    2. Vortex the solution for 10 sec. Then add 15 µl of 7.5×10^{-4} M CEEC solution into the 1.5 ml microcentrifuge tube. Repeat in a similar manner as described in section 4.1.

2. Preparation of 1:1 ratio of mercury(II):CEEC solution

1. Place 240 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5 x10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
2. Vortex the solution for 10 sec. Then add 30 µl of 7.5 x 10^{-4} M CEEC solution into the 1.5 ml microcentrifuge tube. Repeat in a similar manner as described in section 4.1.

3. Preparation of 1:2 ratio of mercury(II):CEEC solution
    1. Place 210 µl of 5 mM ammonium formate, pH 7.5 buffer into a 1.5 ml microcentrifuge tube. Add 30 µl of 7.5 x10^{-4} M mercury(II) chloride solution into the 1.5 ml microcentrifuge tube with the ammonium formate buffer.
    2. Vortex the solution for 10 sec. Then add 60 µl of 7.5 x 10^{-4} M CEEC solution into the 1.5 ml microcentrifuge tube. Repeat in a similar manner as described in section 4.1.

7. Analyzing the Reaction Mixtures of Mercury(II) and CGGC Samples by Orbitrap ESI Mass Spectrometry

1. Preparing the ESI mass spectrometer[16]
    1. Draw 100 µl of calibration standards into a 500 µl glass syringe.
    2. Place the syringe in the syringe cradle of the MS pump, attach the tubing, and inject into the mass spectrophotometer.
    3. Set up the file name for the run by selecting the file icon and typing the file name.
    4. Select acquire data button in the data acquisition module and collect 150 scans.
    5. Analyze the chromatogram to verify the calibration standards by opening data processing module of the software. Open the module, go to file menu and select "open", and select the file in the dialog box. Verify that the peaks in the chromatogram correlate to the mass to charge ratios of the standards.
    6. Clean the 500 µl glass syringe by drawing up 500 µl HPLC grade methanol and then dispense the methanol into a beaker.
    7. Draw up 500 µl of HPLC grade methanol into the glass syringe and flush the system as per step 7.1.2.
    8. Select the method setup module of the software to set the parameters. Choose the scan mode menu and identify the analyzer as FTMS, and then click on "OK". Then by clicking on the various icons on the real-time view spectrum page, set the following parameters: Sheath gas flow rate: 10, Source temperature: 0, Capillary voltage: 37 V, Tube lens: 95 V, Spray voltage: 4.20 kV, Flow rate 10.00 µl/min, Analyzer: FTMS, Number of scans: 150.

2. Running CGGC samples on ESI mass spectrometer
    1. Run the 5 mM ammonium formate pH 7.5 buffer.
        1. Place 500 µl of 5 mM ammonium formate buffer into the 500 µl glass syringe, place it into the syringe cradle of the MS pump, and attach the tubing.
        2. Run buffer through the tubing for 1-2 min.
        3. Set up the file name for the run by selecting the file icon and typing the file name.
        4. Select acquire data button in the module and collect 150 scans.
        5. Click the run button to stop collection after 150 scans are collected.
        6. Open the data browser module, then go to file menu and select "open", and select the file in the dialog box. Verify that no peaks at 483, 683, 1,163 and 1,363 m/z are present that resemble the peptide or mercury (II)-peptide complexes.

    2. Run the 1:0.5 mercury(II):CGGC ratio solution.
        1. Place 250 µl of 1:0.5 mercury(II):CGGC ratio of the sample into the syringe.
        2. Place the syringe into the syringe cradle of the MS pump, attach the tubing, and prime the apparatus.
        3. Select a file name for the run by selecting the file icon and typing the file name.
        4. Press the acquire data button in the data acquisition module and collect 150 scans and click the run button to stop the collection.
        5. Open the module, go to file menu and select "open", and select the file in the dialog box. Verify that the chromatogram contains peaks including the one for the CGGC peptide alone.
        6. Wash the syringe by aspirating with 500 µl ammonium formate buffer and then dispensing the ammonium formate buffer into a beaker.
        7. Select the waste button on the MS and flush the tubing three times with 500 µl ammonium formate buffer.
        8. Wash the syringe by aspirating with 500 µl methanol and then dispensing the methanol into a beaker.
        9. Flush the tubing one time with 500 µl methanol.
        10. Select load detector button on the MS.
        11. Add 500 µl of ammonium formate buffer to the syringe.
        12. Place the syringe into the syringe cradle of the MS pump, attach the tubing, and prime the apparatus.
        13. Select a file name for the buffer run by selecting the file icon and typing the file name.
        14. Press the acquire data button and collect 150 scans and then click the stop run button.
        15. Open the data browser module, go to file menu and select "open", and select the file in the dialog box. Verify that the chromatogram is void of peaks from the previous Hg:CGGC run.

    3. Run the 1:1 mercury(II):CGGC ratio solution.
        1. Place 250 µl of 1:1 mercury(II):CGGC ratio sample into the syringe.
        2. Repeat in a similar manner as described for Steps 7.2.2.2 to 7.2.2.15.

    4. Run the 1:2 mercury(II):CGGC ratio solution
        1. Place 250 µl of 1:2 mercury(II):CGGC concentration sample into the syringe.

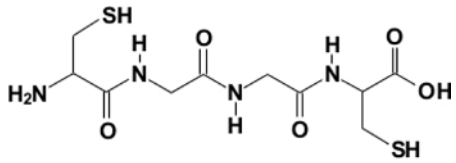
2. Repeat in a similar manner as described for Steps 7.2.2.2 to 7.2.2.15.

8. Analyzing the Reaction Mixtures of Mercury and CEEC Samples by Orbitrap ESI Mass Spectrometry

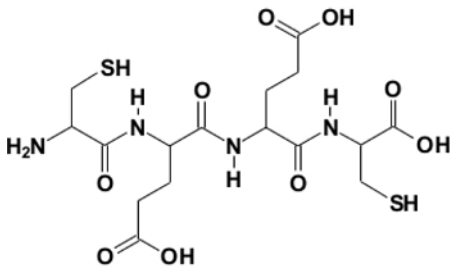
1. Running CEEC samples on ESI mass spectrometer
    1. Repeat analysis procedure (Steps 7.1 to 7.2) using CEEC samples and reaction mixtures of mercury(II) and CEEC at various stoichiometric ratios.

Representative Results

A study was performed to characterize the possible mercury-peptide complex composition for two tetrapeptides, CGGC and CEEC (**Figure 1**) by ESI mass spectrometry. Complexes of mercury(II) with CGGC or CEEC were investigated by reacting the mixtures of mercury(II) and peptide solutions at three different molar ratios: 1:0.5, 1:1, and 1:2 (mercury(II): peptide). The concentration of mercury(II) was 7.5 x 10^{-6} M and the peptide concentration varied accordingly.



Cys-Gly-Gly-Cys (CGGC)



Cys-Glu-Glu-Cys (CEEC)

Figure 1. Dicysteinyl peptide structures. Chemical structures of the dicysteinyl tetrapeptides, CGGC and CEEC. Please click here to view a larger version of this figure.

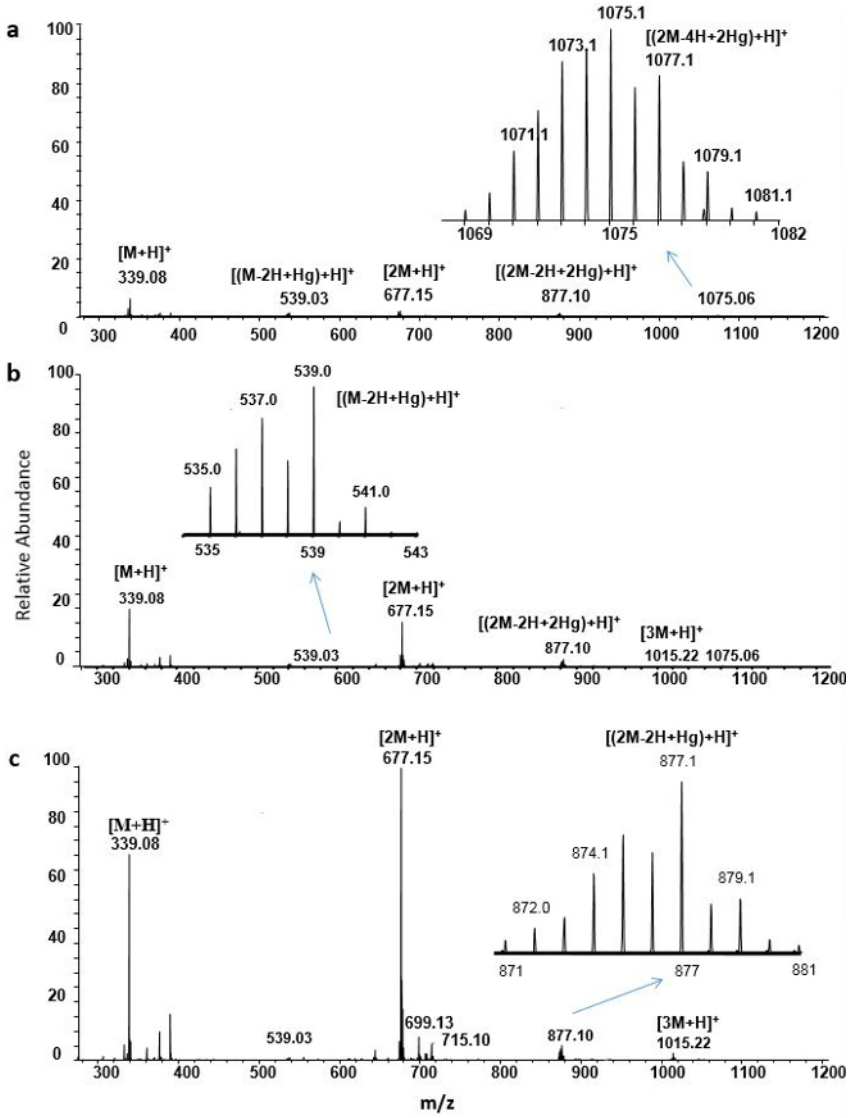


Figure 2. ESI MS of mercury(II) and CGGC. Electrospray ionization orbitrap mass spectra from a solution containing 7.5 x 10^{-6} M Hg^{2+} in ammonium formate buffer, pH 7.5 containing varying Hg^{2+}: CGGC stoichiometric ratios: (**A**) 1:0.5 ratio, (**B**) 1:1 ratio, and (**C**) 1:2 ratio. Insets show the mercury isotopic patterns of the indicated mercury-peptide complexes. Please click here to view a larger version of this figure.

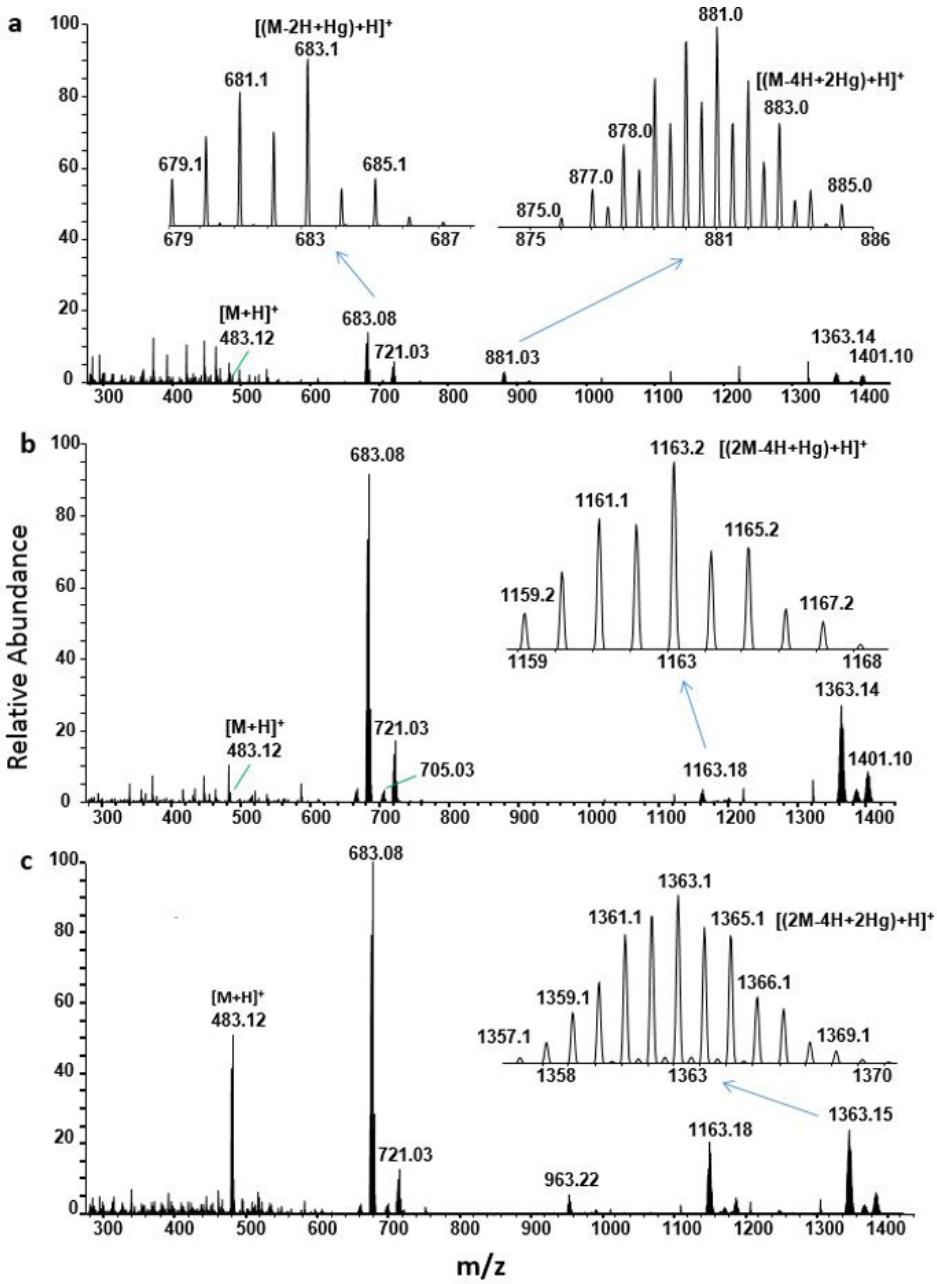


Figure 3. ESI MS of mercury(II) and CEEC. Electrospray ionization orbitrap mass spectra from a solution containing 7.5 x 10^{-6} M Hg^{2+} in ammonium formate buffer, pH 7.5 containing varying Hg^{2+}: CEEC stoichiometric ratios: (**A**) 1:0.5 ratio, (**B**) 1:1 ratio, and (**C**) 1:2 ratio. Insets show the mercury isotopic patterns of the indicated mercury-peptide complexes. Please click here to view a larger version of this figure.

Electrospray ionization orbitrap mass chromatograms were collected for mercury(II) complexing with CGGC (**Figure 2**) and CEEC (**Figure 3**) at various mercury(II) to peptide stoichiometric ratios (1:0.5, 1:1, and 1:2). The observed mercury-peptide complex types show distinct mercury isotopic peaks (insets), which are used to determine the number of mercury ions in the complex as well as the number of deprotonations. For example, **Figure 1b** inset shows the mercury isotopic signature in the peptide-mercury adduct, which corresponds to the seven main naturally occurring isotopes of mercury: ^{196}Hg (0.146%), ^{198}Hg (10.02%), ^{199}Hg (16.84%), ^{200}Hg (23.13%), ^{201}Hg (13.22%), ^{202}Hg (29.80%), ^{204}Hg (6.85%), with percent natural abundances indicated in parentheses. The two major isotopes ^{200}Hg and ^{202}Hg show a distinct relative intensity ratio of 2.3:3. Accordingly the most intense isotopic peak of this one-mercury isotope cluster constitutes the monoisotopic mass for the adduct (m/z = 539). It correlates with a two-coordinate complex, which is formed by the deprotonation of two cysteinyl thiols to form the $[(CGGC-2H+Hg)+H]^+$ adduct. This analysis is made as follows:

m/z value for $[(CGGC-2H+Hg)+H]^+$ is equal to (338 - 2 + 202 +1) = 539.

Figure 1A inset shows the mercury isotopic signature in the peptide-mercury adduct, which corresponds to a two-mercury complex as calculated by using the ChemCal program for $[(2CGGC-4H+2Hg)+H]^+$ (**Figure 4**). The theoretical protonated monoisotopic mass corresponds to an m/z value of 1077.061, which is the ninth isotopic peak in the calculated isotopic cluster. **Figure 1A** inset shows an isotopic peak corresponding to an m/z value of 1077.1, which is also the ninth peak in the observed isotopic cluster. Therefore, the originating adduct for this isotopic cluster can be assigned for $[(2CGGC-4H+2Hg)+H]^+$.

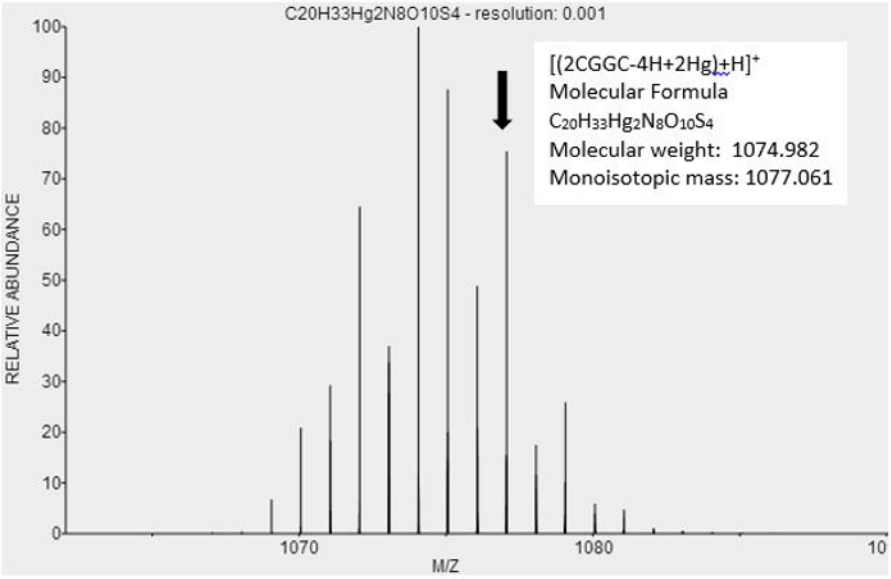


Figure 4. Theoretical isotopic patterns for $[(2CGGC-4H+2Hg)+H]^+$. The theoretical isotopic patterns for $[(2CGGC-4H+2Hg)+H]^+$ as calculated by using the ChemCal program. Arrow indicates monoisotopic peak. Please click here to view a larger version of this figure.

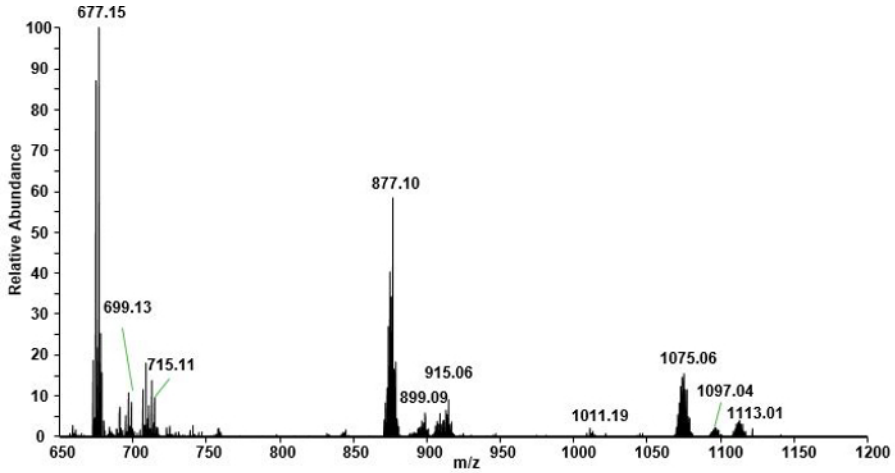


Figure 5. Cationized adducts. Some cationized sodium and potassium adducts associated with the mercury-peptide complexes. Please click here to view a larger version of this figure.

Figure 5 shows some cationized sodium and potassium adducts associated with the mercury-peptide complexes formed by CGGC. Sodiated adducts are 22 mass units larger than the corresponding protonated mercury-CGGC complexes, whereas the potassium adducts are 38 mass units larger. The dominant protonated CGGC dimer (m/z = 677) also forms cationized species with sodium (m/z = 699) and potassium ions (m/z = 715). This further confirms the formation of CGGC dimers without the oxidation of the cysteinyl thiol groups to form disulfides, which would have resulted in a decrease of two mass units for protonated or cationized adducts.

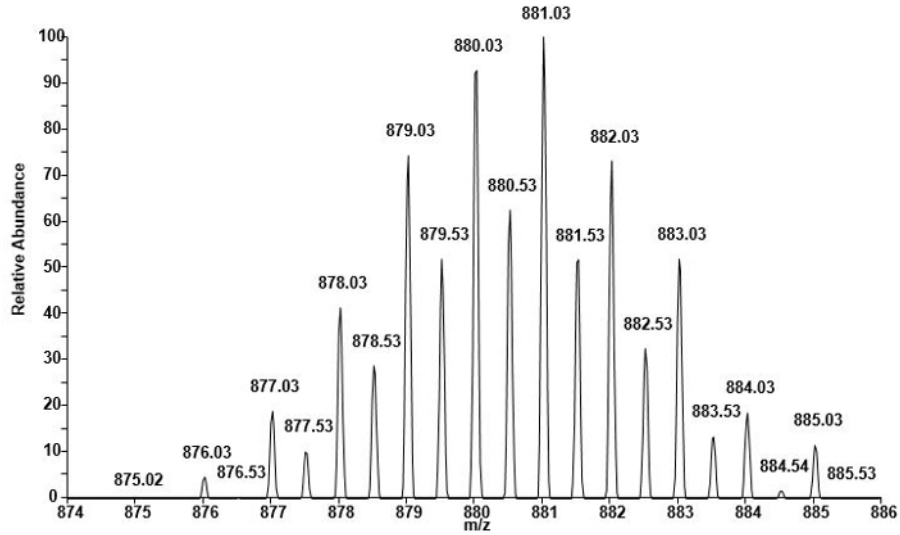


Figure 6. Overlapping +1 and +2 charge states. Overlapping peaks associated with mercury-peptide ions $[(CEEC-4H+2Hg)+H]^+$ in the +1 and +2 charge states. Please click here to view a larger version of this figure.

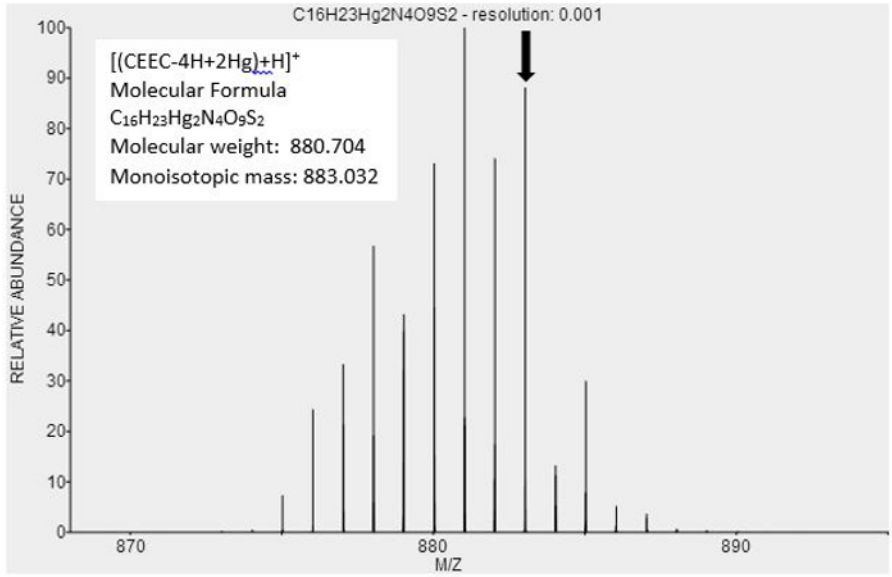


Figure 7. Theoretical isotopic patterns for $[(CEEC-4H+2Hg)+H]^+$. The theoretical isotopic patterns for $[(CEEC-4H+2Hg)+H]^+$ as calculated by using the ChemCal program. Arrow indicates monoisotopic peak. Please click here to view a larger version of this figure.

Figure 6 shows overlapping peaks associated with mercury-CEEC adducts in the +1 and +2 charge. It shows isotopic peaks that are associated with mercury-peptide ions $[(CEEC-4H+2Hg)+H]^+$ in the +1 charge and an m/z value of 883. This is in agreement with a two mercury complex as calculated for $[(CEEC-4H+2Hg)+H]^+$ by using the ChemCal program (**Figure 7**). The theoretical protonated monoisotopic mass corresponds to an m/z value of 883.032.

The above observed $[(CEEC-4H+2Hg)+H]^+$ adduct with a monoisotopic peak of 883.03 overlaps with another adduct containing corresponding peaks showing an additional 0.5 mass units. With the extremely high resolution achieved by the orbitrap mass spectrometry instrument, it can be postulated that these overlapping peaks correspond to adducts with a charge of +2. Accordingly, the monoisotopic mass of the overlapping complex being ionized can be calculated as follows. **Figure 8** shows that the m/z difference between the isotopic peaks is 0.5 and the mass difference between them is 1 amu. Therefore, the charge state is +2. To calculate the mass of the mercury-peptide complex, the m/z for the monoisotopic peak is multiplied by the charge state, and subtracted from the mass of two protons, which made the complex ion positively charged.

Calculations for the +2 adduct:

m/z difference between isotopic peaks is 0.5

Mass difference between isotopic peaks is 1 amu (1 neutron)

z = 1 divide by 0.5 = 2

m/z for protonated monoisotopic peak is (883.53 x 2) - 2 = 1765.06

The above *m/z* value for the protonated monoisotopic peak, $[(2CEEC-8H+4Hg)+H]^+$, is consistent with the theoretical value as calculated by the ChemCal program as 1765.056 (**Figure 8**).

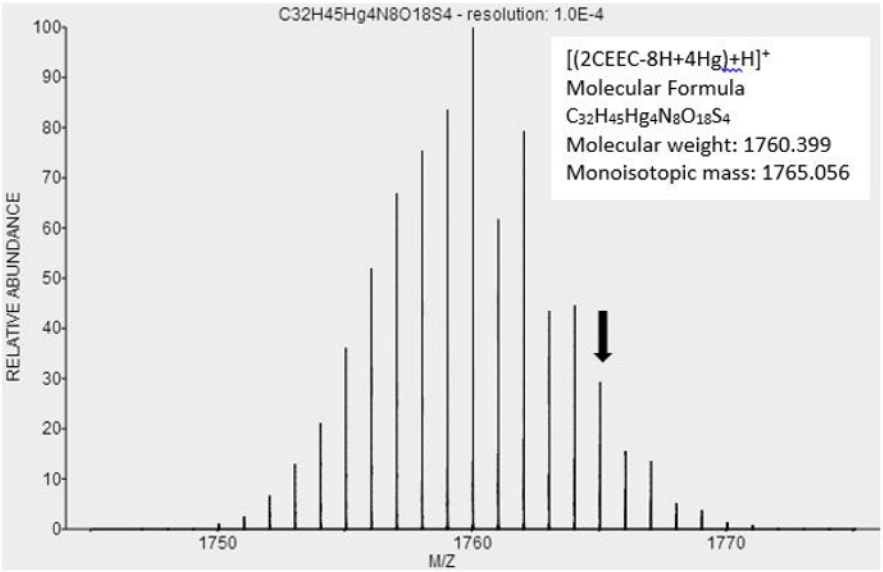


Figure 8. **Theoretical isotopic patterns for $[(2CEEC-8H+4Hg)+H]^+$.** The theoretical isotopic patterns for $[(2CEEC-8H+4Hg)+H]^+$ as calculated by using the ChemCal program. Arrow indicates monoisotopic peak. Please click here to view a larger version of this figure.

The advantage of analyzing mercury-peptide complexes with an ESI orbitrap mass spectrometer is that the charge of every ion can be readily assigned as shown above. Peptides containing basic amino-terminus can readily stabilize positive charges. When using electrospray ionization and a high resolution mass analyzer such as the orbitrap, the charge state of peptide ions with greater than +1 charge can be determined more readily compared to the lower resolution iontrap mass analyzer.

The overlapping peaks associated with mercury-CEEC adducts (**Figure 3A** and **Figure 6**), as described above, were also analyzed by tandem MS. It did not show any MS-MS fragmentation, which indicated that the obtained signals belong to the expected compound as discussed above, and are not clustered artifacts formed at higher concentrations of mercury-to-peptide ratios.

Discussion

The hydrophobic dicysteinyl tetrapeptide CGGC ($C_{10}H_{18}N_4O_5S_2$; MW = 338) (**Figure 1**), forms complexes with mercury(II) as shown in **Figure 2** and **Table 1**. Additionally, it forms peptide dimers and trimers incrementally as the amount of peptide increases in the reaction mixture. As shown by the m/z values of the associated dimers $[(2M+H)^+ = 677]$ and trimers $[(3M+H)^+ = 1015]$, the thiol groups of CGGC did not oxidize to form disulfides under the experimental conditions. The formation of these associated CGGC species could be due to the hydrophobicity of this tetrapeptide. CGGC forms two types of complexes with mercury corresponding to 1:1 mercury(II):peptide and 1:2 mercury (II):$(peptide)_2$ complexes as previously reported for dicysteinyl tripeptides[7]. However, in the presence of excess or equivalent mercury(II), it also forms a 2:2 $[mercury(II)]_2$:$(peptide)_2$ complex.

The carboxylated dicysteinyl tetrapeptide CEEC ($C_{16}H_{26}N_4O_9S_2$; MW = 482) (**Figure 1**) form complexes with mercury(II) as shown in **Figure 3** and **Table 1**. It did not form CEEC dimers as readily as that observe for the more hydrophobic CGGC. Comparable to CGGC, it forms complexes with mercury corresponding to 1:1 mercury(II):peptide and 1:2 mercury (II):$(peptide)_2$ complexes. However, with the auxiliary carboxylate groups, it forms the 2:2 $[mercury(II)]_2$:$(peptide)_2$ complex more readily. Moreover, in excess mercury, it forms the 2:1 $[mercury(II)]_2$:peptide complex and the 4:2 $[mercury(II)]_4$:$(peptide)_2$ peptide complex, which were not observed for CGGC.

The summary of the observed signals for the complexes formed as m/z values are shown in **Table 1**.

Cysteinyl Peptides	Hg(II) : peptide molar ratio in mixture[a]	Peptide molecular ion *(m/z)*	Complexes *(m/z)* Hg (II) : peptide
CGGC		339.08 $[M+H]^+$	
	1:0.5	339.08 $[M+H]^+$	
		677.15 $[2M+H]^+$	
			539.03 (1:1)
			877.10 (1:2)
			1077.06 (2:2)
	1:1	339.08 $[M+H]^+$	
		677.15 $[2M+H]^+$	
		1015.22 $[3M+H]^+$	
			539.03 (1:1)
			877.10 (1:2)
	1:2	339.08 $[M+H]^+$	
		677.15 $[2M+H]^+$	
		1015.22 $[3M+H]^+$	
			539.03 (1:1)
			877.10 (1:2)
CEEC		483.12 $[M+H]^+$	
	1:05	483.12 $[M+H]^+$	
			683.08 (1:1)
			883.03 (2:1)
			1365.14 (2:2)
			1765.06 (4:2)
	1:1	483.12 $[M+H]^+$	
			683.08 (1:1)
			1163.18 (1:2)
			1365.14 (2:2)
	1:2	483.12 $[M+H]^+$	
			683.08 (1:1)
			1163.18 (1:2)
			1365.15 (2:2)

[a]The concentration of Hg (II) in the sample mixture is fixed at 7.5×10^{-6} M

Table 1. Summary of mercury-peptide complexes signals. Mercury-peptide complexes signals in the LTQ/Orbitrap MS chromatograms in ammonium formate buffer, pH 7.5.

We have demonstrated that the reaction of mercury(II) and two dicysteinyl tetrapeptides form complexes that are dependent on the initial ratios of mercury(II):peptide as well as the presence of auxiliary binding groups in the dicysteinyl tetrapeptide. Moreover, accurate stoichiometry of mercury and peptide in the complexes formed under specified electrospray ionization conditions can be determined by using high resolution ESI mass spectrometry based on distinct mercury isotopic distribution patterns.

In reacting cysteinyl peptides with mercury(II), precautions must be taken to prevent the oxidation of cysteinyl thiol groups to form disulfide bonds. Within the described protocol, the buffer solutions were carefully degassed and stored under argon. In addition, all reaction samples are prepared immediately before analysis by ESI mass spectrometry.

Due to differences in solubility between the two tetrapeptides, CEEC and CGGC, different concentrations were used to prepare the stock solutions. The freezer stock of CGGC peptide was wetted with acetonitrile and was easily dissolved followed by 5 mM ammonium formate buffer, pH 7.5 to produce a 7.5×10^{-4} M CGGC solution. The CEEC was prepared at a lower concentration, 7.5×10^{-5} M, prior to the mercury(II):peptide reaction mixture steps because of its lower solubility. The optimal dilution for analyzing the mercury(II) complexes was deemed to be 10^{-5} M because of the solubility of the peptide and to allow for removal of residues in the mass spectrometer. In contract to the CGGC solutions, CEEC residues adhere to the tubing, which necessitates occasional tubing replacement.

The significance of using ESI mass spectrometry for the analysis of mercury-peptide complexes lies in its soft ionization of analytes. This facilitates the analysis of molecular ions with negligible fragmentation. As shown in this work, it can be used to characterize the stoichiometries of mercury-peptide complexes based on the signature mercury isotopic distribution patterns. However, a volatile buffer system is necessary for analysis by ESI mass spectrometry. This may limit its practical use for identifying analytes that require less volatile solvents or buffering media for dissolution.

As we have previously mentioned[7,8], ESI mass spectrometry provides a sensitive analytical tool for an accurate determination of the stoichiometry of mercury and peptide in the mercury-peptide complexes under the specified electrospray ionization condition. However, it is necessary to use additional methods (for example, ^{1}H, ^{13}C, ^{199}Hg NMR spectroscopy, extended X-ray absorption fine structure, or potentiometry[17-18]) to provide a more accurate determination of the content of complexes in solution.

We have shown that ESI with an orbitrap mass analyzer can be used to analyze mercury-peptide complexes. We expect that this technique can be applied toward the analysis of other metal ions and their complexes with various small compounds. It will be especially useful for analyzing complexes formed by other metal ions that can exist in different isotopic forms.

Disclosures

There is no disclosure for the work reported.

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