ESI FT-MS and DFT studies of aqueous Au(I) complexes with cysteine and its disulfide form cystine

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A B S T R A C T

Aqueous complexes of $[Au(CySSCy)(CySH)_n]^+$ containing gold(I), cysteine (CySH) and the disulfide cystine (CySSCy) have been examined by electrospray ionization (ESI) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry, and the identities of individual Au complexes have been confirmed by infrared multiphoton dissociation (IRMPD)- and collision-induced dissociation (CID) experiments. Results from positive-mode ESI–MS experiments show that the aqueous Au(III)/cysteine system has a strong tendency to auto-oxidize toward cystine and form aurous-disulfide complexes of the type $[Au(CySSCy)]^+$ and $[Au(CySSCy)(CySH)_n]^+$. An examination of the concentration- and time-dependent complexation behavior of Au(I) with CySSCy and CySH shows that the abundance of $[Au(CySSCy)(CySH)]^+$ and $[Au(CySSCy)(CySH)_2]^+$ increases with increasing ESI solution concentration, consistent with the aqueous ionization of cysteine at $pK_{a1} = 1.92$. In general, the response of individual ESI mass spectra of $[Au(CySSCy)(CySH)_n]^+$ to changes in solution concentration is indicative of a direct correlation between gas-phase and aqueous Au speciation, a feature that is likely rooted in gold complexation processes occurring in solution prior to electrospraying. Finally, equilibrium geometries of Au(I) complexes with cystine and cysteine have been predicted using M06 and PCM/TD-DFT calculations, and these results have been employed to predict stepwise complexation energies of $[Au(CySSCy)(CySH)_n]^+$ and to pinpoint the most important UV–vis absorption features in aqueous solution.

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1. Introduction

The speciation and stability of gold-thiolate complexes is a major theme of research in oncotherapy [1], pharmaceutical [2,3] and physical chemistry [4–9], primarily because these molecular species exhibit strong Au-S bonds. Gold-sulfur interactions also play an important role in the mobility of Au in the Earth's crust, and are therefore of great interest in aqueous geochemistry [10]. For instance, there is a growing body of experimental data which shows that gold complexes with sulfur ligands are involved in Au transport in both aqueous solution [11,12], and water vapor [13], and thus, implicated in the formation of large-scale ore deposits [14,15]. It is also well-known that thiol-containing amino acids, such as cysteine [16], are capable of reducing Au(III) to Au(I) in aqueous media, with the disulfide cystine being the major oxidation product [17]. These observations were found to be consistent with recent ESI–MS studies of aqueous gold-cysteine [18], which showed that reduction of Au(III) results in the formation of disulfides, however, with no indication for the presence of Au(I)-cysteine

or Au(I)-cystine complexes. These findings were further supported by solution UV and NMR spectroscopic experiments [18].

From a solution chemistry perspective, ESI–MS studies of aqueous gold-thiol systems can provide valuable information on the distribution and abundance of molecular Au complexes in solution, and by doing so, deepen our understanding of gold speciation in natural systems. Moreover, there are a growing number of ESI–MS studies that have established a direct link between species present in solution, and those observed in the gas phase, and as discussed by Schröder [19], these type of mass spectrometric experiments can serve as a complement to results obtained from solution spectroscopic studies. Of particular relevance here are recent ESI–MS studies of $AuCl_3$ [20], $PdCl_2$ [21], $NiCl_2$ [22], and $CuCl_2$ [23], that point toward a correlation between gas-phase ESI mass spectrometry and solution phase data. At present, there are no ESI–MS data available for Au(I)-cysteine or Au(I)-cystine complexes, however, long chain, insoluble oligomers of $[AuCyS]_n$ have been observed in aqueous solution [24], but with no information available on the stability and structure of these species. It is this knowledge gap and the growing body of ESI–MS work on aqueous gold complexes [18,20,25], which has been the motivation behind our interest in studying gold- complexes. Herein we report results from ESI mass spectrometric experiments of dilute aque-

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ous $AuCl_3$:cysteine solutions in the concentration range and ratio 0.5:1.0 mM–3.0:6.0 mM and pH 1.9–3.0. These experiments have been complemented by M06 structure and energy calculations for $[Au(CySSCy)]^+$, $[Au(CySSCy)(CySH)]^+$ and $[Au(CySSCy)(CySH)_2]^+$ as well as TD-DFT (CAM-B3LYP) calculations, which have been applied to estimate theoretical UV–vis spectra in the gas phase and in solution using PCM solvation.

2. Methods

2.1. Experimental approach

ESI–MS experiments were conducted using a Bruker 7T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer with the ESI source set to positive-ion mode. ESI stock solutions of aqueous $AuCl_3$:cysteine were electrosprayed at a flow rate of 120 μL/h with ESI capillary temperature set to 180 °C. The ESI source parameters for the capillary exit, skimmer 1 and skimmer 2 were 120, 20 and 10 V, respectively. Ion mass spectra were obtained by electrospraying binary solutions of cysteine (Sigma-Aldrich, CAS: 52-90-4, $C_3H_7NO_2S$, purity 98%) and gold(III) chloride (Sigma-Aldrich, CAS: 13453-07-1, $AuCl_3$, purity 99.99%), for which the gold-to-cysteine concentration ratio was held constant at a value of 1:2. Five aqueous solutions of *i*) 0.5:1 mM (pH 2.98), *ii*) 1:2 mM (pH 2.96), *iii*) 2:4 mM (pH 2.09) and *iv*) 3:6 mM (pH 1.96) were electrosprayed; the pH of each solution was determined using an Orion 8220BNWP pH meter, and all solutions were prepared from Millipore H_2O (18 MΩ cm). The identities of disulfides and gold-cystine complexes were determined by comparison with theoretical mass spectra, which were generated using the Bruker Data Analysis software. For instance, for $[Au(CySSCy)]^+$, i.e. $AuC_6H_{12}N_2O_4S_2$, the ion mass spectra appear as a triplet signal at m/z 436.9, 437.9 and 439.9 due to monoisotopic Au and the relatively high natural abundance of ^{14}N (99.9%), ^{16}O (99.7%) and ^{32}S (95.0%). Accordingly, a disulfide ion, such as $[(CySSCy)H]^+$, appears as a triplet in ion mass spectra at m/z 241.0, 242.0 and 243.0. The fragmentation patterns of Au(I)-disulfide complexes were further studied by IRMPD-MS experiments using a continuous wave CO_2 laser (Synrad model J48-2, 25 W) and each spectrum presented here represents an average of 100 scans at 256k data points per scan. Collision induced dissociation (CID) experiments have been undertaken, in which the fragmentation of $[Au(CySSCy)]^+$ was accomplished by collision with pulsed Ar gas at 99% purity (pulse duration: 250–500 ms). A summary of our IRMPD results and those derived from CID experiments is given further below. We also conducted a series of time-dependent ESI–MS experiments in which aqueous solutions of $AuCl_3$:cysteine (2:4 mM, pH 2.09) were electrosprayed over a time period of 12 h. The primary goal of these time-series ESI–MS experiments was to explore trends in speciation of Au-free and Au(I)-disulfide species, which occur as the solution approaches steady state composition. A separate set of ESI–MS experiments was also undertaken to explore the influence of ESI source conditions on the abundance and distribution of disulfide and Au(I)-disulfide complexes. These effects were studied by recording ESI mass spectra as a function of cone voltage V_c in the range 80–130 V. Ion intensities were fine-tuned by generating voltage-spectra maps where V_c was systematically varied as mass spectra were recorded. A maximum in the gold-cystine complex abundance was observed at $V_c \approx 120$ V, and results from these ESI–MS experiments are presented in Fig. 1.

2.2. Theoretical calculations

Density functional theory calculations were undertaken using the M06 functional [26]. The M06 functional was applied to pre-
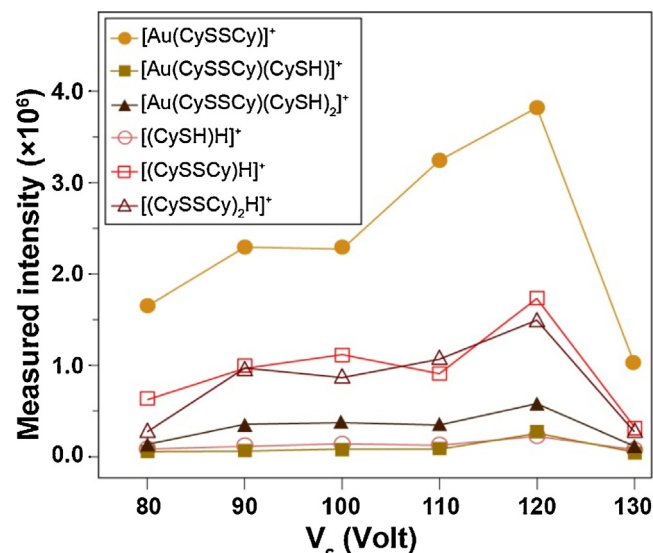

Fig. 1. Changes in the abundance of cysteine, disulfides and $[Au(CySSCy)(CySH)_n]^+$ (n = 0–2) complexes as a function of capillary exit potential V_c.
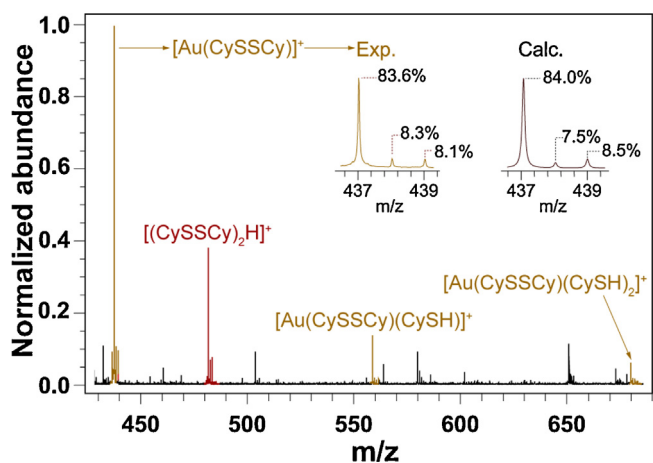

Fig. 2. Positive-ion mode ESI mass spectra of aqueous $AuCl_3$:cysteine (2:4 mM, pH = 2.09); inset showing experimental and calculated ion mass spectra and isotopic distributions for $[Au(CySSCy)]^+$.

dict equilibrium geometries and energies of $[Au(CySSCy)(CySH)_n]^+$ (n = 0–2) and disulfides species using cc-pVDZ-(PP) [27] for Au, and cc-pVDZ [28] basis sets for C, H, N, O and S. Vibrational frequency analyses were performed to ensure that all complexes were true minima on the potential energy surface (PES). Time-dependent density functional theory (TD-DFT) calculations were undertaken at the CAM-B3LYP level of theory together with PCM solvation to predict solution-phase UV–vis spectra for $[Au(CySSCy)]^+$, $[Au(CySSCy)(CySH)]^+$ and $[Au(CySSCy)(CySH)_2]^+$, and all calculations have been performed using the Gaussian09 (Rev.C.01) [29] software package.

3. Results and discussion

3.1. Experimental results: ESI–MS and IRMPD-MS

Positive-ion mode ESI mass spectra for electrosprayed aqueous solutions of $AuCl_3$:cysteine (2:4 mM, pH 2.09) are presented in Fig. 2. Three major classes of Au-free species in the mass range m/z 120–820 have been identified: *i*) protonated cysteine $[(CySH)H]^+$ (m/z 122.0), *ii*) protonated cystine $[(CySSCy)H]^+$ (m/z 241.0), and *iii*)