

LETTERS

Isolation and Selected Properties of a 10.4 kDa Gold:Glutathione Cluster Compound

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An unprecedented small thioaurite cluster compound (with metallic Au⁰ core) has been isolated in high yield by decomposition of polymeric Au(I)SG compounds, where GSH is the ubiquitous tripeptide glutathione, *N*- γ -glutamyl-cysteinyl-glycine. The Au:SG clusters appear to share the high stability and robustness of their hydrophobic *n*-alkyl analogues but are highly water soluble. The most abundant cluster produced by these methods can be easily separated from its homologues by gel electrophoresis. Its total molecular weight is ca. 10.4 kDa, and the mass of its strongly bound inorganic core is 5.6 kDa, suggesting the composition Au₂₈-(SG)₁₆. This composition is also consistent with the X-ray diffraction pattern of the crystalline molecular solid. Distinct features in the optical absorption spectroscopy are inherently different from either larger clusters or smaller gold cluster compounds. The compound is optically active, as evidenced by circular dichroism in the near-IR, visible, and near-UV regions. The ¹³C NMR spectra suggest that the bonding environment of the GS-adsorbate is similar to that of the *n*-alkyl-adsorbate clusters, and the nonsulfhydryl properties are retained. The cluster is thus envisioned as a large metallic-cluster compound with distinctive optical properties encapsulated by a bioactive peptide monolayer.

Large gold cluster compounds Au_N(SR)_M based on thio-group (-SR) adsorbate monolayers represent a significant advance in nanometer-scale chemistry and materials science.¹ Among their properties are (i) nanocrystalline (or quasicrystalline) metallic Au cores, with a minimum (critical) size for chemical, thermal, and photostability;² (ii) excellent handling and functionalization chemistry analogous to that of extended Au:SR self-assembled monolayers (SAMs);³ (iii) ease of preparation and separation by core size into distinct molecular fractions, which form well-ordered molecular crystalline solids;^{4,5} and (iv) well-quantized electronic properties as reflected in discrete

optical^{6,7} and charging (electrochemical and tunneling) phenomena.^{8,9} These properties are enhanced with decreasing Au core size, however, the smallest Au:SR cluster compounds (R = alkyl) that could be isolated showing good stability were those with gold core masses near 7.5 and 14 kDa. From steric considerations, it seemed to us that increasing the adsorbate bulk could improve the stability of the very small compounds against disproportionation into polymeric compounds and larger gold nanocrystal compounds.

The tripeptide glutathione (GSH = γ -Glu-Cys-Gly) is an especially bulky thiol and a highly abundant component of living cells, where, among other functions, it serves to maintain the cellular potential in a reducing state, thereby protecting biomolecular structures against oxidative damage.¹⁰ It is also the monomer unit of the γ -EC peptides which have been shown to limit the size of (encapsulated) CdS nanocrystals to ca. 1.8 nm.¹¹

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Furthermore, Aizawa et al. have shown that GSH forms a compact monolayer on polycrystalline gold surfaces, retaining its nonsulfhydryl biological activity.¹² Its reductive activity is formally represented by the half-reaction $\text{GSH} \leftrightarrow \frac{1}{2}\text{GSSG} + \text{H}^+ + \text{e}^-$. In the thiolate form (GS⁻) it can further act as a potent chelating agent. In redox reactions with ions of an electronegative metal, such as Au, one therefore expects the stoichiometric process $3\text{GSH} + \text{Au}^{3+} \leftrightarrow \text{Au}^0 + \frac{3}{2}\text{GSSG} + 3\text{H}^+$, wherein the Au⁰ is nucleated into metallic crystallites covered by a monolayer of GS or GSSG adsorbate groups. The aqueous reaction¹³ proceeds in two steps: first, a rapid initial formation of polymeric p-Au(I)SG,¹⁴ which is nonmetallic (transparent at $\lambda > 300$ nm). Then decomposition of the polymer is stimulated almost instantaneously at ambient temperature by addition of a strong reducing agent in great excess; in this case, a 2:3 H₂O:methanol medium is used to prevent uncontrolled reduction of the polymer.

The net result is a quantitative conversion of the insoluble colorless p-AuSG into intensely colored, highly water-soluble (~100 mg/mL, at pH = 8.5) Au:SG cluster compounds whose stability is indicated by its resistance to excess strong reducing agent. The reaction product is readily cleansed of small ions (GSH, GSSG, GSNa, etc.) by precipitation from dilute methanol, in which the smaller ions, but not the Au:SG cluster compounds, are soluble. The complete removal of such species is verified by ¹H and ¹³C NMR spectroscopy (see below). The cleansed material is then separated into distinct sizes by electrophoretic methods adapted to retain small molecular species.¹⁵ The electrophoresis shows four distinct bands (Figure S1, Supporting Information) corresponding to the lowest mass Au:SG clusters that are formed with this method and two other diffuse bands which correspond to the larger sizes. Each electrophoretically separated fraction is then precipitated from 2% acetic acid 3 times to produce a dark, highly hygroscopic powder. Each fraction is then analyzed by matrix-assisted laser desorption (MALDI) and electrospray ionization (ESI) mass spectrometry.¹⁶ The cluster compound that has the third highest electrophoretic mobility forms a distinct bright-orange band. This species was found to be the most abundant product, with a yield of ~40 wt %, under optimized conditions. This high abundance may indicate an exceptional stability and facilitates the investigation of its properties, on which this Letter therefore concentrates. The properties of larger and smaller cluster compounds will follow in a future report.

The negative ion MALDI-MS of this species is shown in Figure 1a. The MALDI mass spectra are calibrated against the negative ions of standard proteins (insulin, cytochrome-C) using the same matrix and instrumental conditions. The most abundant peak corresponds to a mass of 5340 amu, and the adjacent peaks (of lower abundance) are spaced by 197 and 32 amu, consistent with the inorganic core fragmentation and inconsistent with the retention of any glutathione (306 amu) adsorbate groups still attached. To overcome the fragmentation present in the MALDI-MS, electrospray ionization was used.¹⁶ The 5⁻ (*m/z* 2101), 6⁻ (*m/z* 1751), 7⁻ (*m/z* 1501), and 8⁻ (*m/z* 1313) peaks were detected in the ESI mass spectrum (Figure 1b), but surrounding these peaks on both sides were other less-intense peaks. When the mass spectrum is deconvoluted to "unit-charge" (Figure 1c), it was found that the parent peak consists of multiple peaks having spacing consistent with potassium and sodium ion adducts. The zero-charge spectrum indicates that the mass of the cluster is ~10 417 amu. From both the MALDI-MS and the ESI-MS, a proposed composition of Au₂₈(SG)₁₆ would be an obvious choice.

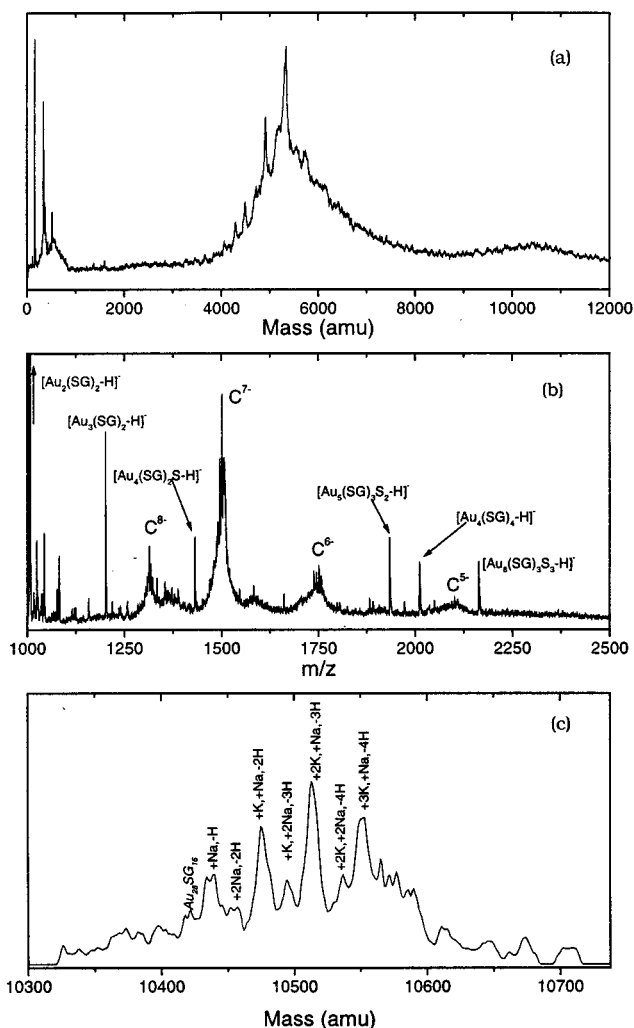


Figure 1. Mass spectra of the most abundant species in the mixture, corresponding to the cluster compound having the third highest electrophoretic mobility. The MALDI mass spectrum (a) indicates that the cluster core is fragmenting extensively in the desorption/ionization process. The ion spacing of 197 and 32 is consistent with various Au_{*n*}S_{*M*} cluster ions produced by laser-desorption experiments on *n*-alkane Au:SR cluster compounds. The electrospray data (b) shows the various charge states of ions, corresponding to a cluster compound with a molecular weight of ~10417. Even under ideal electrospray conditions, some fragmentation is still evident, as seen in the low-mass ions labeled in b. The peak spacing seen in the unit-charge spectrum (c) corresponds to various K⁺ and Na⁺ adducts to the cluster compound.

The powder X-ray diffraction (Figure 2) is due primarily to the heavier Au atoms, and the large-angle scattering suggests that the core geometry is loosely associated with bulk fcc-Au, however, the reflections are severely broadened due to the limited number of Au lattice planes in the cluster core. From the Debye–Scherrer formula ($D_{\text{core}} = \sim 0.9/\Delta s$), the estimate (derived from the full-width at half-maximum of the first reflection (111)) for the gold core diameter is ~0.9 nm (consistent with Au₂₈). The peak positions in the small angle (small *s*) region indicates that the interplane distance is ca. 1.7 nm, with several Bragg diffraction peaks indicating a well-defined molecular crystalline solid with one metal core per lattice site. Such solid-state properties are similar to that of the hydrophobic Au:SR compounds that have been studied extensively.¹⁷ The entire diffraction pattern (peak positions, intensities, widths) is consistent with dense globular cores of ~0.9-nm equivalent diameter, spaced by a nearest-neighbor distance of

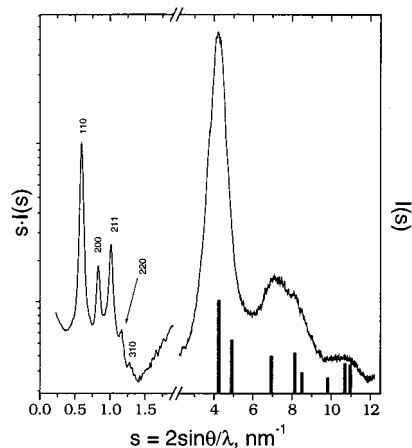


Figure 2. X-ray diffraction pattern for the 10 kDa compound obtained using the methods discussed in the text. The LA-XRD reflections are loosely related to the reflections of bulk *fcc* gold (bars), however, due to the size of the gold cores, the features are broadened severely. In the small-angle region, five distinct peaks are observed and indicate that, even in the mixtures, the small crystallites are being oriented in an ordered lattice, with nearest-neighbor distances of ~ 2.0 nm.

~ 0.2 nm, arranged in a *bcc*-(super)lattice. This implies a very compact arrangement for the monolayer-adsorbed GS-groups.

Positive identification of *intact* adsorbed tripeptide GS-groups is provided by high-resolution NMR spectra of the cluster-compound mixture in D_2O under near-saturation conditions (~ 100 mg/mL). Correlation of the ^{13}C resonances to those of the free GSH is provided in Figure 3 (1H and ^{13}C NMR pH-dependent shifts are provided as Supporting Information, Table S1, Figure S2). All 10 carbon groups, as well as four nonexchanging 1H sites, were still present and retained the correct functionality in the cluster materials as compared to those of the free thiol counterpart. As with the hydrophobic counterparts,^{18,19} the resonances for the carbon groups closest to the cluster surface (cys- α -CH, 6; cys- β -CH, 7) were severely broadened and shifted compared to the thiol counterpart, indicating a similar adsorption mechanism through the sulfur. The assignments in both the aliphatic and carbonyl region were confirmed by ^{13}C - 1H coupling (not shown) and comparison to the reported chemical shifts of each carbon group at different pH values.^{20,21}

Figure 4 shows the optical absorption spectrum and circular dichroism for the most abundant species ($Au_{28}(SG)_{16}$). The absorption spectrum is well structured with an onset near 950 nm (1.3 eV), a shoulder just below 800 nm, and a first strong maximum at 675 nm ($7500 M^{-1} cm^{-1}$), followed by a broad minimum near 600 nm that gives rise to the distinctly orange tint. The absorption strength rises strongly in the blue-green and near-UV regions but continues to show pronounced structure, with a value near $130\,000 M^{-1} cm^{-1}$ at 250 nm, as expected of fully reduced metallic Au(0) compound and a many electron system.²² The discreteness of the spectrum (as compared to other, larger Au:SR cluster compounds) has two far reaching implications: (i) the implied homogeneity of the cluster system and (ii) the emergence of molecular-like quantum levels due to the limited number of electrons in the system. The absorption onset at 1.3 eV is presumed to be the largest optical gap yet deduced among the large Au:SR cluster compounds, distinctly blue-shifted from the ~ 0.9 eV onset for the 7.5 kDa (core) cluster. Interestingly, this optical "fingerprint" matches almost identically the spectrum of a very low abundance Au:SC6 fraction, which was not sufficiently stable to allow a complete characterization. The circular dichroism (top, Figure 4) of this

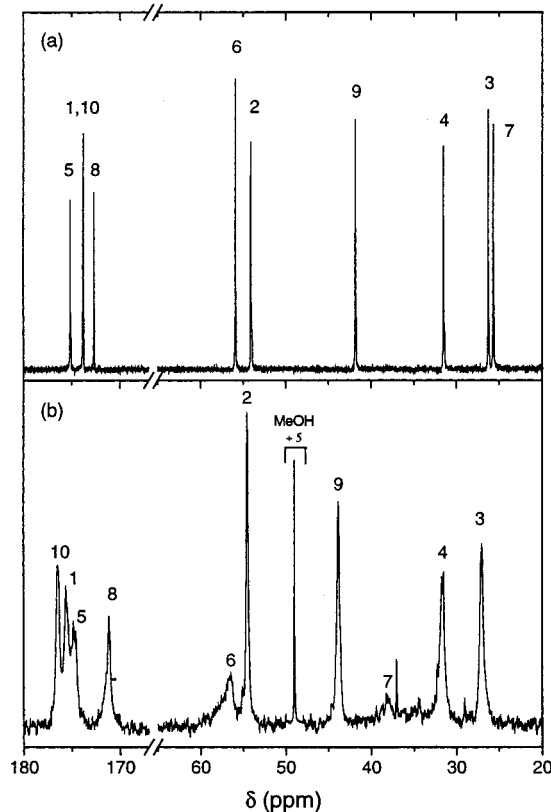
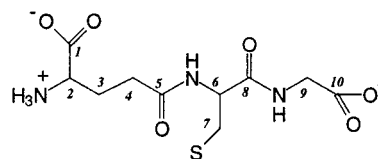


Figure 3. ^{13}C NMR of GSH at pH = 2.3 (a), and the GS:Au clusters in D_2O at pH = 8.5 (as prepared) (b), with the numbers referring to the structure above the spectra. The peak widths and shifts are consistent with previous results on the hydrophobic gold clusters, where the carbon resonances closest to the gold surface (6,7) are severely broadened. The other shifts seen in the aliphatic and carbonyl region are due to pH effects in glutathione, which have been well documented.^{18,19} A complete assignment table at various pH values is provided as Supporting Information.

compound shows clear optical activity in the region of the gold absorption bands (the GSH as well as the AuSG polymer are transparent and inactive below ~ 300 nm). The fine features evident (both positive and negative Cotton effects) suggest a rich spectrum of energy levels, perhaps dominated by the interband (d to conduction sp) transitions. The exact origin of the chiroptical effects has yet to be determined for this class of compounds.

These results show that very small Au nanocrystals (large Au clusters) can be obtained in a robust hydrophilic form through the assembling of a chelating tripeptide adsorbate monolayer. The size control (absence of larger clusters/colloids of 1.5 nm or greater diameter core size) is remarkably strong and may indicate that the maximum core size is dictated by the severe steric requirements of the peptide monolayer, analogous to that found in models of the self-limiting CdS: γ EC nanocrystal complexes by Dameron and Dance.^{11,23} The Au core appears to retain the properties expected of the metallic state (bond length and optical characteristics), including the interesting level-quantization and chiroptical properties. Furthermore, the peptide exterior confers a highly charged (polyelectrolyte) character to the assembly as a whole. The possibility of the glutathione

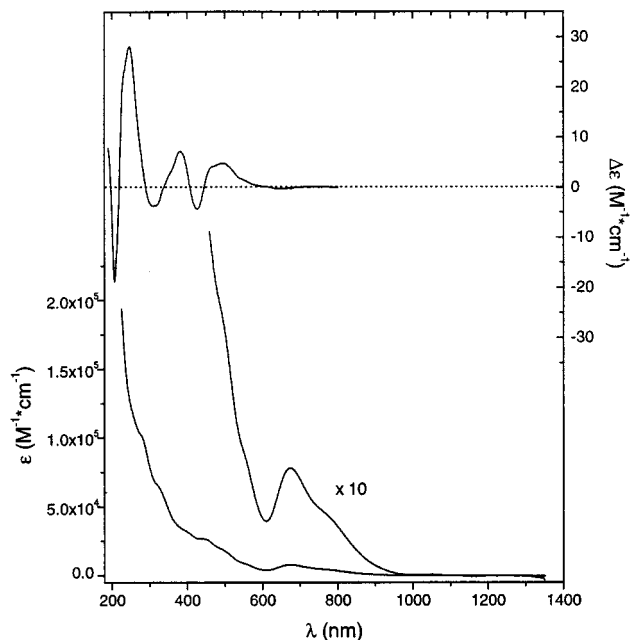


Figure 4. Optical absorbance spectrum and circular dichroism for the most abundant cluster compound separated by electrophoresis showing that indeed discrete electronic transitions are responsible for the shape of the absorbance spectrum. All of the features seen in the CD of the cluster compound can be associated with the discrete steps seen in the absorbance spectrum.

retaining its biological activity after adsorption seems promising due to the preliminary NMR studies and a recent study performed by Aikawa et al., where a glutathione monolayer on gold was used to immobilize a glutathione S-transferase: calmodulin fusion protein.¹²

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Supporting Information Available: Figures of the electrophoresis of Au:SG cluster compounds and ¹H peak assignments of the GS:Au clusters and table of complete assignment of pH values (3 pages). Ordering information is given on any current masthead page.

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- Polymer: 120 mL of 1.1 mM HAuCl₄ in methanol is mixed with an aqueous solution of GSH (80 mL, 5.0 mM), pH of ~2, resulting in a change from clear yellow to cloudy white, indicating the formation of the polymer; suspension is stirred for an additional 15 min. Clusters: a large excess of NaBH₄ in water (10 mL, 0.6 M) is added to the stirring suspension, causing a rapid color change to opaque brown/black, and the solution is stirred for an additional 30 min. The resulting solution is evaporated to near dryness, and the cluster compounds are precipitated with *N,N*-dimethylformamide or dilute methanol to remove the diborane and excess GSH/GSSG. Removal of starting materials and byproducts is determined by NMR. The polymer conversion to clusters was quantitative, i.e., no insoluble (bulk) Au or unreacted polymer.
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- Electrophoresis: The cleansed product is redissolved ~4 mg/mL in water with 5% glycerol and purified by PAGE using a Bio-Rad PROTEIN II system. Approximately 30 μ L of the sample was loaded onto a 20 cm \times 1 mm acrylamide/bis gel (24% T; 7% C) and eluted with 5 mM Tris, 39 mM glycine, pH 8.8 at 150 V for 6 h. The bands formed by electrophoresis were cut out and placed into distilled water. The resulting solution was evaporated to near dryness, an equal volume amount of 2% acetic acid was added, and then precipitation was induced by methanol. This was repeated twice to form a fine black powder. Yield of band 3 (most abundant) was ~40% (by weight) of all clusters made.
- MALDI-MS: The acetate salt of each separated cluster compound was dissolved at a concentration of 3 mg/mL in water and then mixed (1:1, v:v) with a 0.07 M solution of 2,5-dihydroxybenzoic acid (DHB). Four microliters of that solution was vacuum-dried on a polished stainless steel tip, and the 3rd harmonic of a Nd:YAG (355 nm) was used for desorption/ionization into a reflectron time-of-flight mass spectrometer. ESI-MS: The acetate salt of the most abundant species was dissolved in water:methanol (60:40) to a concentration of 0.24 mg/mL and sprayed at a flow rate of 3 μ L/min into a Sciex API 165 LC/MS quadrupole mass spectrometer.
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