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UV/Vis Spectroscopy

Gas-Phase Synthesis and Intense Visible Absorption of Tryptophan–Gold Cations**

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Gold nanoparticles (GNPs) have received considerable attention because of their strong surface plasmon resonance in the visible part of the spectrum, which can be tuned by changing the particle size and surface environment, and by the formation of hybrids with molecular units.[1–3] GNPs are commonly prepared by "wet chemistry" procedures, in which clusters of gold atoms are formed in the presence of a surface-capping ligand.[1,4] The hybridization of molecular units with GNPs can further introduce novel chemical and biochemical functionalities. For example, the application of GNPs linked to biomolecules to produce efficient optical probes in the fields of biodiagnostics and biomolecular imaging has become widespread.[3] In this context, the conjugation of GNPs with peptides or proteins has been used for cell-targeting applications.[5] Such functionalization of GNPs not only changes the surface plasmon resonance, but also favors the occurrence of energy-transfer phenomena.[6] This results in specific emissive properties ranging from complete fluorescence quenching of "biomolecules" to strong metal-enhanced fluorescence.[7] Although these studies have opened up an exciting new field spurred by the unique optical properties of nanohybrids, the large number of noncontrollable parameters involved makes complete understanding of the fundamental molecular mechanisms of these phenomena difficult.

Recently, new alternative approaches for the synthesis of functionalized metal nanoparticles through the direct use of peptides or proteins containing aromatic amino acids as a reducing agent have been reported.[8–10] For example, when Au^{III} ions were added to aqueous bovine serum albumin solutions,[10] highly fluorescent gold nanoclusters were formed by the entrapment and reduction of Au ions. The strong fluorescence of such nanohybrids containing small nanoclusters makes them highly attractive for applications. Despite the technological importance of GNPs conjugated with biomolecules, attempts to control their shape, size, and properties have met with limited success. One difficulty is that little is known about the mechanism and the precursor stage involved in their formation. Specifically, it is still unclear how a precursor salt in the presence of biomolecules is reduced to neutral atoms, which then subsequently aggregate to form nanohybrids. A molecular study of the nature of the precursor capable of acting as a nucleation seed for forming nanoclusters with optical properties would make it possible to control and predict the formation and properties of nanohybrids at the nanoscale.

In this joint experimental and theoretical contribution, we present the synthesis of the smallest possible precursor consisting of a single gold cation bound to tryptophan and report on its unique optical properties—a strong absorption band in the visible spectral region attributed to charge-transfer excitations.

$[TrpAu]^+$ cations were synthesized in a quadrupole ion trap by collision-activated dissociation (CAD) of tryptophan–gold complex precursor ions. Tryptophan, hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4{\cdot}3\,H_2O$), and ascorbic acid ($C_6H_8O_4$) were dissolved in acetonitrile/water (1:1) at a ratio of 1:1:1 to provide a final concentration of approximately 500 μM. Ascorbic acid exhibits keto–enol tautomerism, and its enol form is susceptible to ionization in aqueous solution and has been used as a versatile reducing agent for a variety of metal ion precursors to prepare metal nanoparticles.[11] In this case, it acts as a weak reducing agent to reduce Au^{III} ions to Au^{I} ions [Eq. (1)]. The Au^{I} ions are then

$$Au^{3+} + C_6H_8O_4 \rightarrow Au^{+} + C_6H_6O_4 + 2\,H^{+} \qquad (1)$$

complexed by tryptophan molecules. The use of stronger reducing agent leads to aggregated gold particles. Electrospraying of this solution immediately after its preparation results in the formation of $[(Trp)_2Au]^+$ complex cations (Figure 1a). The presence of $[(Trp)_2Au]^+$ cations instead of $[TrpAu]^+$ cations may be due to better solvation of Au^{I} in solution by two molecules of tryptophan. Such ditryptophan compounds are assumed to be involved in the formation of gold and silver nanoparticles by using tryptophan-based peptides.[8] This finding is also supported by our observation of $[(Trp)_2Au_3]^+$ cations in a higher *m*/*z* range (see Figure S1 in the Supporting Information). Such trimers may act as a nucleation seed in the formation of larger metal nanoparticles, as recently proposed by Xia and co-workers.[12]

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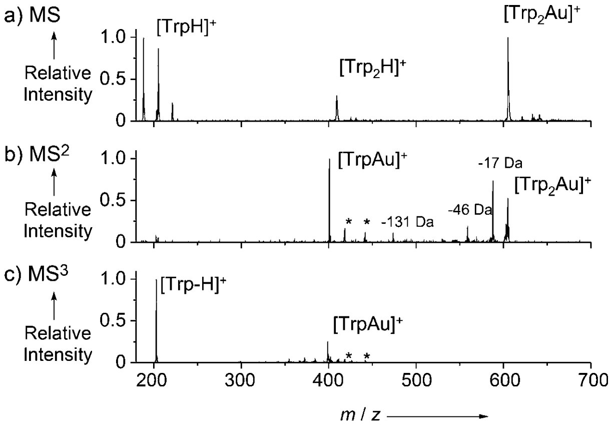

Figure 1. a) Positive-mode mass spectrum of a solution of tryptophan, hydrogen tetrachloroaurate(III) trihydrate, and ascorbic acid. b) MS^2 CAD spectrum of $[Trp_2Au]^+$ cations. c) MS^3 CAD spectrum of $[TrpAu]^+$ cations. The signals marked with * are due to adducts on $[TrpAu]^+$ (hydroxy groups and acetonitrile).

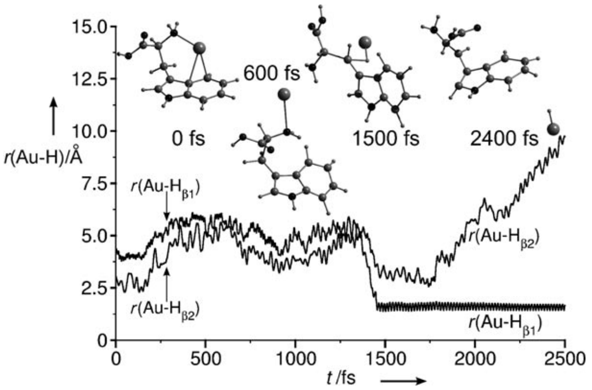

Figure 2. Snapshots of the MD simulation "on the fly" in the electronic ground state from selected trajectories at $T=1800$ K, illustrating the main fragmentation channel leading to the loss of a AuH molecule (hydrogen is abstracted from the C_β carbon atom). Black and gray curves represent the distance between the gold atom and β_1 and β_2 hydrogen atoms calculated along the trajectory.

$[TrpAu]^+$ was obtained by collisional activation and fragmentation of the $[(Trp)_2Au]^+$ complexes (Figure 1 b). Stability and fragmentation of the complex was investigated by collisional activation dissociation. The results are displayed in Figure 1 c. The main fragmentation channel corresponds to the loss of an AuH molecule, leading to the formation of $[Trp\text{-}H]^+$ species.[13] Losses of small neutral fragments are also observed. The same fragment ions are observed after laser activation. The loss of AuH requires reorganization of the complex with the formation of the AuH bond prior to dissociation. It is interesting to note that this fragmentation pattern is different from that observed for silver–tryptophan cations, for which fragmentation of tryptophan and loss of silver atoms occur.[14]

To address the structural and dynamical properties of the $[TrpAu]^+$ cation, additional theoretical work was performed. The lowest-energy isomer of the $[TrpAu]^+$ complex corresponds to a charge-solvated structure in which the Au^+ interacts with carbon atoms of the six-membered ring of the indole and the nitrogen atom of the amine group (see initial structure in Figure 2). The fragmentation of the complex was investigated theoretically by ab initio molecular dynamics (MD) simulation "on the fly" in the electronic ground state using density functional theory (DFT). The loss of the AuH molecule was observed in 6 out of 20 propagated trajectories in the temperature range from 1700 to 2500 K. Other trajectories showed either the fragmentation of the organic part (10 trajectories) or no fragmentation within the propagation time. The loss of the AuH molecule was observed on a timescale of approximately 1.5 ps (Figure 2). The fragmentation channel leading to the formation of AuH is more favorable energetically than the loss of a bare gold atom. The difference between dissociation energies (Au loss vs. AuH loss) is 0.32 eV. It should be noted that in the case of $[TrpAg]^+$, the situation is the exact opposite. The AgH fragmentation channel is not observed experimentally[14] and, theoretically, is unfavorable by 0.36 eV relative to the loss of an Ag atom. This difference is due to the higher binding energy of AuH (3.02 eV) relative to AgH (2.34 eV). However, loss of AgH was recently observed with other amino acids.[15]

The high coordination of gold with Trp also has an influence on the optical properties of the complex. Figure 3 a compares the experimentally recorded photodissociation

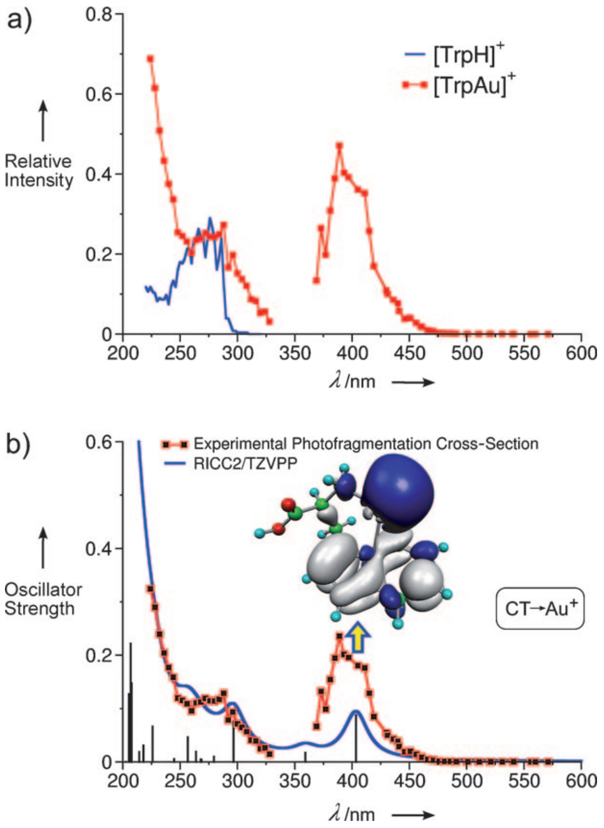

Figure 3. a) Fragmentation yields of $[TrpAu]^+$ and protonated tryptophan cations ($[TrpH]^+$). b) Comparison of the theoretical absorption spectrum of $[TrpAu]^+$ (blue line) with the experimental photofragmentation cross-section (red line). The inset shows the electronic density difference between the ground state and excited states for the excitation band at around 400 nm.

spectra for $[TrpAu]^+$ and $[TrpH]^+$. For $[TrpH]^+$, a band centered at around 275 nm is observed. It is ascribed to a π–π* excitation within the indole ring. For $[TrpAu]^+$, the fragmentation yield shows a first band between 250 and 310 nm and the onset of a second particularly pronounced band below 250 nm. In contrast, whereas no fragmentation is observed above 295 nm for $[TrpH]^+$, $[TrpAu]^+$ exhibits an intense fragmentation band in the visible part centered at around 400 nm. The spectrum of $[TrpAu]^+$ calculated by using the approximate coupled-cluster method (RI-CC2; Figure 3b) is in full agreement with the experimental findings. The transition located at 400 nm is mainly due to HOMO–LUMO excitation and, as illustrated by the insert in Figure 3b, is dominated by a charge-transfer (CT) excitation from the π indole system to an s-type orbital of the gold atom. Analogous CT excitations have already been observed for $[TrpAg]^+$ complexes (ca. 330 nm).[14] However, in marked contrast to silver, binding of a single gold cation to the tryptophan molecule gives rise to the band in the visible part of the spectrum, thereby making the gold cation highly attractive for applications.

The energy of the CT excitation can be correlated to the difference between the ionization potential of the tryptophan molecule (IP_{Trp}) and the electron affinity of the metal cation ($-IP_{atom}$), and thus to the HOMO–LUMO gap. Therefore, we expect that different metal cations interacting with tryptophan may lead to different optical spectra correlated with their IP values. The calculated HOMO–LUMO gaps for complexes of alkali, silver, and gold cations for the lowest energy structures are presented in Figure 4. In all cases, the most stable isomer assumes the charge-solvated (CS) type of structure, in agreement with previous experimental and theoretical findings.[16] The obtained results confirm the qualitative relation between the energy of the CT excitation and the ΔIP value. $[TrpAu]^+$ has the smallest HOMO–LUMO gap in the series that was explored, thus confirming the unique role of gold for providing intense transition in the visible part of the spectrum for metal–tryptophan complexes.

In brief, we have developed a new route for producing small gold biomolecule complexes. The complexation with a metal cation drastically changes the optical properties of tryptophan. An intense absorption band in the visible range is observed and theoretically confirmed for gold. The present work opens the way for using gold atoms as efficient protein labeling agents for absorption spectroscopy in the visible range.

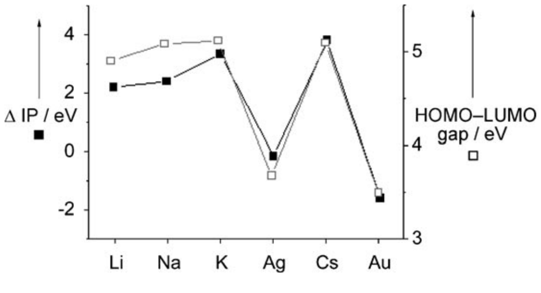

Figure 4. Comparison of the calculated HOMO–LUMO gaps and ΔIP values for $[TrpM]^+$ complexes with M = Li, Na, K, Ag, Cs, and Au.

Experimental Section

Stock solutions of 2 mM hydrogen tetrachloroaurate(III) trihydrate (Carl Roth), 2 mM ascorbic acid (Sigma), and 1 mM tryptophan (Carl Roth) were each prepared in H_2O/CH_3CN (1:1, v/v). The gold and ascorbic acid solutions (750 μL each) were mixed until the solution became transparent (reduction of Au^{3+} to Au^+). Then, the tryptophan solution (1.5 mL) was added. Fresh solutions were stable for 8–10 h before gold nanoparticles appeared. The experiments were performed using an ion-trap mass spectrometer equipped with an electrospray source and coupled to an optical parametric oscillator (5 ns pulse width, 20 Hz repetition rate).[17] The solution was introduced in the ESI chamber at a flow rate of $25\,\mu L\,min^{-1}$, a spray voltage of 4–5 kV, and a capillary temperature of 200 °C. The distance between the ESI needle and the stainless steel capillary was found to be critical, and the optimal distance for the generation of $[Trp_2Au]^+$ complexes was around 3 cm. Nitrogen was used as a sheath gas and helium (purity > 99.9999 vol %) as a collision and damping gas in the ion trap. For the photodissociation measurements, the laser light was injected at the center of the trap and the trapped ions were irradiated for 500 ms (10 laser shots). Mass spectra obtained after laser irradiation were recorded. The yield of fragmentation (σ) was measured as a function of the laser wavelength ($\sigma = \ln((\text{parent}+\Sigma\,\text{frag})/\text{parent})/\phi$), where ϕ is the laser fluence, parent is the intensity of the parent signal, and Σfrag represents the total intensity of the photofragment signals).

The structural and electronic properties of the systems studied were calculated at the B3LYP level[18,19] of density functional theory. For the Au atom, the 19 electron relativistic effective core potential of the Stuttgart group[20] together with the (9s7p5d1f)/[7s5p3d1f] atomic basis set,[21] which has been optimized for the description of excited electronic states,[21] was employed. The organic part was treated by employing valence triple zeta quality (6-311G**) basis sets. The fragmentation channels in the ground electronic states were investigated in the framework of the ab initio molecular dynamics "on the fly" using a nonhybrid BLYP functional[19,22] together with the resolution-of-the-identity approximation (RI) and split valence plus polarization basis sets (SVP)[23] for C, O, N, and H atoms. The absorption spectrum of $[TrpAu]^+$ was calculated with the approximate coupled-cluster method CC2 by employing the resolution-of-the-identity (RI) approximation and by using TZVPP basis set for C, O, N, and H atoms.[24]

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