The fragmentation of gold nanoparticles induced by small biomolecules†

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Spherical gold nanoparticles (3–5 nm) undergo a surprising fragmentation without extra energy imput and are converted into ultrasmall particles (less than 1.5 nm), which is a direct result of electron transfer between gold nanoparticles and cysteine.

Many of the physical and chemical properties of nanoscale materials, including luminescence, conductivity and catalytic activity, are under control of the size of nanoparticles.[1] It is generally accepted that metallic nanostructures are easier to be damaged by heat and light compared to their bulk solids.[2] The surface oscillations affected by heat energy induces mechanical instability of nanoscale particles, resulting in their fragmentation. The photoinduced fragmentation of nanoparticles has been employed to change the morphology of Ag nanoparticles.[3] A similar mechanism is operative in that case because thermal and photochemical changes continue to propagate.[4] In all of these cases, the changes in nanostructures are induced by thermal fluctuations. Additionally, gold nanorods have been successfully shortened by mild oxidation with O_2.[5] Recently, Novo's group demonstrated that $NaBH_4$ induced the fragmentation of gold nanorods.[6] They stated that they believe the fragmentation is due to electron injection. But, up to now, such an effect with biomolecules has not been reported for gold nanoparticles. The interaction between nanoparticles and biomolecules is significant in chemistry, biology and material sciences, because there has been growing interest in the application of nanotechnology in biomedicine and life sciences.

In our experiments, we found the biomolecule cysteine is able to fragment gold nanoparticles. During aging in cysteine solution, 3–5 nm gold nanoparticles spontaneously fragment into ultrasmall particles (less than 1.5 nm) without external energy induction. The fragmentation of gold nanoparticles is accompanied by cysteine oxidation and electron injection. The decrease in surface stress is caused by the increased surface charge density. When the surface charge density is beyond the Rayleigh limit,[7] the gold nanoparticles will undergo size changes.

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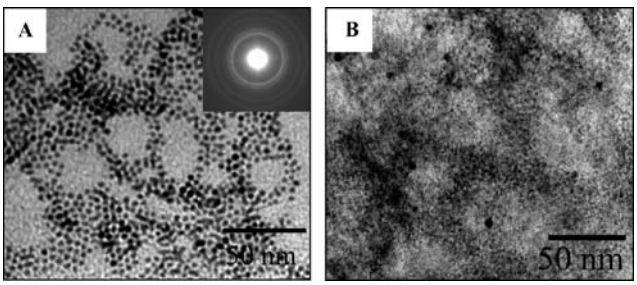


Fig. 1 TEM images of (A) gold colloid at the initial stages and (B) the same colloidal suspension after aging for 6 h. The inset in image (A) is an electron diffraction of image of large gold nanoparticles.

Briefly, 10.0 mL of distilled water, 0.2 mL of an aqueous 25 mM $HAuCl_4$ solution, and 1.0 mL of an aqueous 200 mM cysteine solution were mixed (cysteine : Au = 40 : 1). Next, excess $NaBH_4$ was added with stirring to prevent the presence of unreduced gold ions. No additional cleaning step was performed in subsequent aging process at room temperature.

At the initial stage (<5 min), the TEM image shows that 3–5 nm gold nanoparticles are present (Fig. 1A). The associated UV-Vis spectrum shows an absorption peak at 506 nm (Fig. 2), which is the surface plasmon absorption of small gold nanoparticles. Subsequently, we noticed that the red color of gold colloid suspension gradually faded (Fig. 2). Finally, significant optical features of the gold nanoparticles disappear after an elapsed time of 6 h. The suspension becomes transparent, which indicates that the fading of the solution is not due to the agglomeration of nanoparticles. Under TEM observation, these initial gold nanoparticles have been converted into <1.5 nm ultrasmall particles, and just few of initial gold nanoparticles remained as shown in Fig. 1B. The numbers of ultrasmall particles in a same limited area is greatly increased compared to initial stage, which can be attributed to the fact that one large nanoparticle has successfully been fragmented into several small ones.

The fragmentation process of gold nanoparticles was further monitored by the UV-Vis absorption method. Fig. 2 shows the time-dependent absorption spectra during the whole reaction process. The gold colloid suspension exhibits a slight red shift in the absorption peak and continuous decrease in the magnitude of absorption with the elapsed time.

The color of metal nanoparticles originates from an absorption band of the characteristic surface plasmon.[8] When the size of gold colloids decreases to <1.5 nm, they do not possess a plasmon absorption band in the visible range.[9] The absence of the plasmon band for ultrasmall metallic particles is attributed to quantum-size effects which lead to the formation of

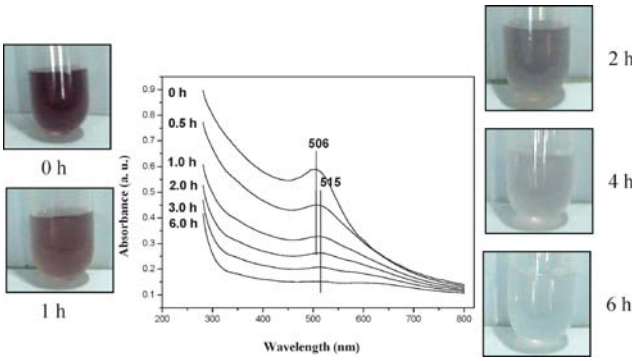


Fig. 2 Photographs of aging of the gold colloid suspension at room temperature for the indicated times, and time-dependent UV-Vis spectra of the gold colloid suspension during the aging process.

quantized energy states.[9] The complete loss of color in the experiments reported rather indicates that the nanoparticles completely disintegrate to molecular sized species. While conceivably particle fragments observed by TEM could be a result of Au reduction under the electron beam, taking into account the fact that the final suspension is colorless and the absorbance at the plasmon peak is reduced by >96%, the amount of initial nanoparticles that do not fragment are a very small portion of initial gold particles.

The initial gold nanoparticles exhibit a polycrystalline structure, as determined by the microscopic electron diffraction (ED) pattern (inset of Fig. 1A). However, for the ultrasmall particles, no ED pattern was observed. The change in crystal structure was confirmed by powder XRD (Fig. 3). The initial gold nanoparticles have a typical diffraction peak corresponding to (111) planes of face-centered cubic (fcc) gold. However, the (111) diffraction peak of fcc gold in the XRD pattern of the nanoparticle fragments disappear, which is similar to that of noble metals from large nanoparticles to atomic-scale clusters.[10]

What is the driving force for the fragmentation of the gold nanoparticles? In FT-IR spectroscopy experiments, we observed that gold nanoparticles catalyze cysteine oxidation (Fig. S1, ESI†), which is identified by comparable experiments between gold nanoparticles suspensions and pure cysteine solutions. As for the cysteine solution, the small peak at 2549 cm^{-1} corresponding to the S–H stretching vibration modes is still seen after aging for 8 h. In the case of the gold

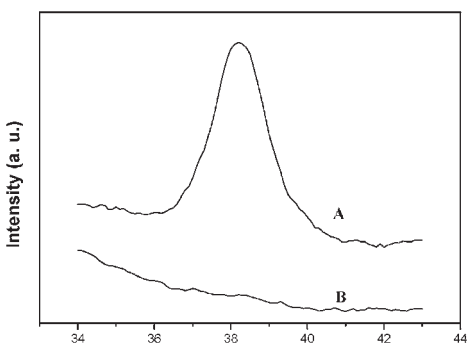
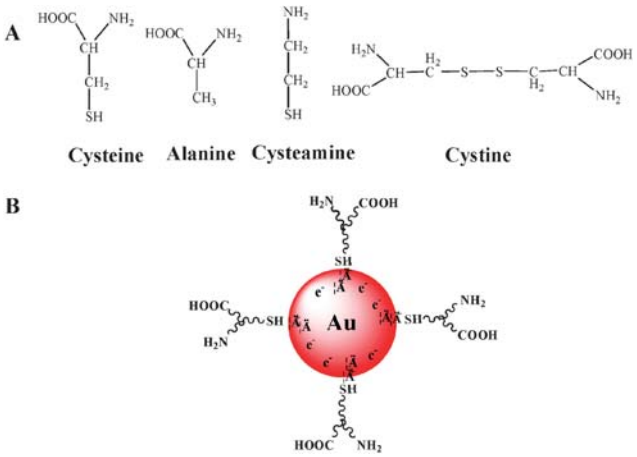


Fig. 3 X-Ray diffraction patterns of (A) initial gold nanoparticles and (B) nanoparticle fragments.



Scheme 1 (A) The structures of cysteine, cystine and related molecules. (B) Schematic presentation of surface complexation by cysteine molecules and charge distribution on gold nanoparticles.

colloid suspension, a new band appearing at 3364 cm^{-1} may be attributed to B–OH vibrations, which is originated from the product of decomposed borohydride solution.[11] After aging for 8 h, the S–H vibrational band disappears suggesting the formation of Au–S or S–S linkages. Moreover, electron impact (EI, Fig. S2 in ESI†) mass spectra provides strong evidence that all the cysteine (M = 121, formula: Scheme 1A) has been oxidized with the aid of gold nanoparticles and the main final product is cystine (M = 240, formula, Scheme 1A). As is well known, free mercapto (R–SH) groups are unstable in the oxo-aqueous environment. They are easily oxidized by reactive oxygen species such as H_2O_2 to form disulfide (R–S–S–R′) and other organic sulfur oxidation products.[12] However, in our experiments, we observed that the fragmentation of gold nanoparticles and the oxidization of cysteine still occured in deaerated aqueous solution, oxygen not being necessary for the oxidization of cysteine. Gold nanoparticles instead of active oxygen species remove electrons from cysteine, and result in the oxidization of cysteine. It has been shown previously that colloidal particles can store a great number of electrons.[13] Pakiari *et al.* also reported that the gold nanoparticles tend to oxidize amino acids and excess electrons exist in the gold nanoparticles.[14] Therefore, in the present system, it is reasonable to conclude that the nanoparticle fragmentation is a direct result of electron transfer between gold nanoparticles and cysteine.

The surface charges of gold nanoparticles play a predominant role in stabilizing the nanoparticles. A droplet becomes unstable when the charge on the droplet reaches a critical value.[15] This condition is known as ''Rayleigh instability'',[16] of the critical value of charges, Q_R, that exist on a droplet of radius, R, when electrostatic repulsion is balanced by surface tension. This is given by eqn (1), where ε_0 is the permittivity of the surroundings and γ is the surface tension:

$$Q_R = 8\pi(\varepsilon_0 \gamma R^3)^{1/2} \quad (1)$$

$$\sigma_R = \frac{Q_R}{4\pi R^2} = 2(\varepsilon_0 \gamma R^{-1})^{1/2} \quad (2)$$

Here σ_R is surface charge density corresponding to a particle with charges Q_R. During oxidization of cysteine, the gold

colloids gradually collected electrons from cysteine. The cohesive force provided by the surface tension is offset by the electrical stress created by charge distribution on the nanoparticles.[6] As the charges approach and exceed this critical value (Q_R), the disturbances due to shape oscillations grow, leading to nanoparticle fragmentation. Eqn (2) predicts smaller size nanoparticles can suffer from higher surface charge density. Higher surface charge density produces larger repulsion for electrons and makes electrons injection difficult. Thereby, the nanoparticle fragments are more stable than large nanoparticles.

To further verify the above-mentioned mechanism of the nanoparticle fragmentation, a series of control experiments were performed. First, the effect of $NaBH_4$ was assessed. The freshly prepared cysteine-capped gold nanoparticles were purified by four cycles of centrifuging the suspension and discarding the supernatant. The purified gold nanoparticles were separately redispersed in $NaBH_4$ and cysteine aqueous solution. The fading phenomenon only occurred in cysteine rather than $NaBH_4$ solution. The performance of cysteine was also found valid for gold nanorods and for gold nanoparticles as large as $\sim$20 nm (Fig. S3, ESI†), suggesting the fragmentation was independent of the morphology and promising a suitable range of nanoparticle size. Second, for alanine (formula, Scheme 1A) aqueous solutions, the nanoparticles remained intact. That indicates the mercapto (–SH) group is necessary in the nanoparticle fragmentation. Third, cysteamine (formula, Scheme 1A), an –SH bearing molecule, was employed to replace cysteine during the experimental process, and again no fragmentation of the gold nanoparticles was observed. The only difference between cysteine and cysteamine is that cysteine possesses an additional carboxyl group, which is favored for the formation of hydrogen bonding. The hydrogen bonding between the cysteine adsorbed on the nanoparticle surfaces and the free cysteine in suspension can increase the local concentration around the gold nanoparticles.[17] The distance between the free cysteine and gold nanoparticles is distinctly shortened, which is beneficial for free cysteine to inject electrons into gold nanoparticles.[18] Because cysteamine lacks a carboxylic group, free cysteamine is far away from gold nanoparticle surfaces. Thereby, it is difficult to transfer electrons between gold nanoparticles and molecules. Based on the same reason, the strong reducing agent $NaBH_4$ can not inject electrons into gold nanoparticles either. Hence, $NaBH_4$ does not participate in the gold nanoparticle fragmentation. All the results indicate that not only the mercapto group but also the carboxyl group in the cysteine molecule are key elements for the gold nanoparticles fragmentation.

In conclusion, we present an initial example of gold nanoparticle fragmentation induced by the small cysteine biomolecule. An observed increase in the rate of the oxidation of cysteine in the gold colloid suspension leads to excess electron injection into gold nanoparticles from cysteine, as evidenced by the formation of S–S bonds. The accumulating charges in the nanoparticles are a prerequisite for the efficient fragmentation of the gold nanoparticles. Because particles with ultrasmall dimensions show fluorescence,[19] this procedure can and will be used to prepare fluorescent metallic particles with potential applications in biological labels and light emitting sources in nanoscale electronics.

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