Enzymatic Synthesis of Gold Nanoparticles Wrapped by Glucose Oxidase

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An enzymatic synthesis route to protein-wrapped gold nanoparticles is developed. Glucose oxidase (GOD) reduces Au(III) ion in the presence of β-D-glucose, and stable gold nanoparticles with average diameter of 14.5 nm are formed. FT-IR spectra, zeta potential and CD spectra of purified nanoparticles indicate that they are stabilized by the adsorbed protein layer.

In nature, biological systems synthesize a wide range of organic/inorganic nanohybrids as exemplified by magnetite (magnetotactic bacteria),[1] amorphous silica (diatoms),[2] and minerals like calcite.[3] Biomediated synthesis of inorganic nanomaterials is emerging as an area of green nanochemistry, since it provides opportunity to create functional bioinorganic hybrids without employing toxic or environmentally risky reagents. To date, preparation of metal and semiconductor nanoparticles have been investigated by using bacteria[4] and fungal cells.[5] In these cellular systems, however, many proteins, carbohydrates and biomembranes contribute to the overall biological reactions. These bioderived inorganic nanoparticles have not been purified and their structural analysis is not conducted satisfactorily. It is desirable to develop more simple enzymatic routes to metal nanoparticles, especially those catalyzed by a single oxidoreductase. In this study, we demonstrate that aqueous solution of $AuCl_4^-$ can be reduced by glucose oxidase (GOD, EC 1.1.3.4), and it provides highly stable gold nanoparticles which are coated by GOD.

GOD is an acidic glycoprotein and it specifically converts glucose into gluconolactone, and this oxidation reaction is accompanied by reduction of the cofactor flavine adenine dinucleotide (FAD). To a phosphate buffer solution (Na_2HPO_4/KH_2PO_4, 0.1 M, 3.75 mL, pH 6.5) of $HAuCl_4$ (2.0 mM) and β-D-glucose (62.5 mM), GOD (from *Aspergillus niger*, Wako Pure Chemical Industries., 125 μg/mL in phosphate buffer, 0.25 mL) was added. The solution color immediately changed from pale yellow to dark blue, and black precipitates were formed in a few hours. The observed dark blue color is ascribed to agglomerated gold nanoparticles, as confirmed by transmission electron microscopy (TEM, supporting information). Atomic absorption analysis of the supernatant indicated that ca. 40 mol % of $AuCl_4^-$ ions has precipitated, whereas the rest of $AuCl_4^-$ ions (ca. 60 mol %) remained in the supernatant.

The formation of nanoparticles is ascribed to enzymatic reaction of GOD, since such sudden color changes were not observed when the reaction mixtures do not contain β-D-glucose or GOD.[6] In addition, the reaction is specific to β-D-glucose. No color change proceeded when β-D-glucose was replaced with the other carbohydrates such as galactose, mannose, or fructose. These observations clearly indicate that $AuCl_4^-$ ions are reduced in the enzymatic reaction of GOD.[7] This is reasonable, since the redox potential of Au^{3+} ($E^\circ = +1.52$ V vs SCE) is considerably higher than that of flavin adenine dinucleotide (FAD) in GOD (estimations for the $FADH_2/FADH_2^{\bullet+}$ and $FADH^\bullet/FADH^+$ couples are -70 and -90 mV vs SCE, respectively).[8]

To our surprise, soluble gold nanoparticles are formed succeedingly in the supernatant. After the initial precipitation of gold agglomerates, color of the supernatant gradually turned wine-red and an intense absorption peak centered at 527 nm immerged in ca. 24 h (Figure 1a). The observed 527 nm peak is characteristic of a plasmon resonance band for gold nanoparticles. A transmission electron micrograph of the wine-red colored solution is shown in Figure 1b. Gold nanoparticles with an average diameter of 14.5 nm are abundantly observed. These nanoparticles are stably dispersed without precipitation for more than several months.[9]

To purify these gold nanoparticles, the wine red-colored nanoparticle solution (4 mL) was freeze-dried and it was redissolved in pure water (0.5 mL). The concentrated solution was then applied to gel filtration chromatography (Sephadex G-75 column, manufacturer's exclusion limit 80 kDa for proteins). A fraction of gold nanoparticles with the plasmon absorption was

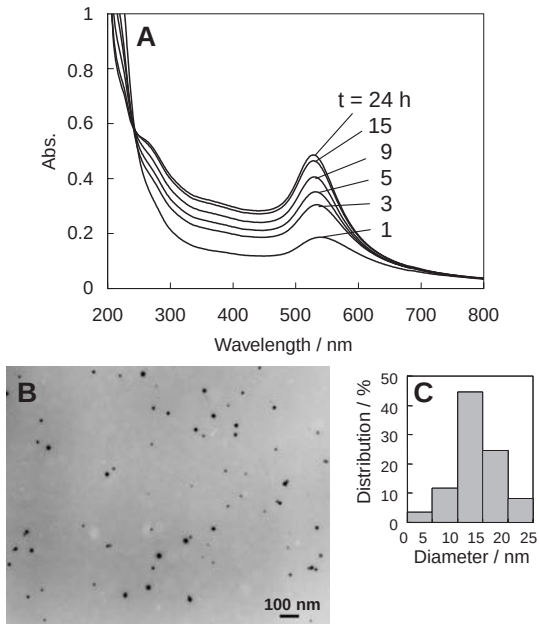

Figure 1. (A) Time dependence of UV–vis spectra. $[HAuCl_4] = 2.0$ mM. [GOD] = 125 μg/mL, [β-D-glucose] = 62.5 mM. The solutions were mixed at $t = 0$ and precipitates immediately formed were filtered after 30 min ($t = 0.5$) with a nitrocellulose filter (pore diameter, ca. 0.45 μm, Gelman Sciences Japan, Ltd.) Spectra of the supernatant were recorded. Temperature, 25 °C, in phosphate buffer, pH 6.5. (B) Transmission electron micrograph of gold nanoparticles and (C) their size distribution.

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collected. Purification of the nanoparticle was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), which showed the absence of free GOD molecules in the bulk aqueous phase.

FT-IR spectrum of the purified gold nanoparticles was measured by casting the solution on a BaF_2 plate (apparatus, Nicolet Magna IR 860). Peaks assignable to the polypeptide $\nu_{C=O}$ and δ_{N-H} vibrations are observed at 1653 and 1550 cm^{-1} respectively, similarly to those observed for a cast sample of GOD ($\nu_{C=O}$ at 1653 cm^{-1}, δ_{N-H} at 1570 cm^{-1}). Zeta potential of the purified nanoparticle solution (apparatus, MALVERN, Zetasizer nanoZS) showed a pH dependence with an isoelectric point of ca. 3.9, which is in good agreement with that reported for GOD (pI, 4.2).[10] These results show that GOD molecules are bound to gold nanoparticles and stabilizing the nanoparticle structures in water. Circular dichroism (CD) spectra of the GOD/Au nanoparticle solution showed a peak at 207 nm, which is altered from that of GOD in native form (CD peak at 222 nm, apparatus, JASCO J-725G). Thus, the ternary structure of GOD molecules adsorbed on gold nanoparticles is altered from that of the native GOD. The observed unfolding is reasonable since GOD molecules are conjugated directly to the nanoparticle surface without any surface modifiers.[11] It is widely known that thiol groups bind to the gold surface via a covalent bond, and Cys206, Cys164, or Cys521 residues of a GOD molecule may be involved in the adsorption to gold nanoparticles.

Stability of GOD-capped gold nanoparticles is further compared with citrate-reduced gold nanoparticles (diameter, ca. 13 nm).[12] Both of the GOD-capped and citrate-capped nanoparticles are anionic in phosphate buffer (pH 6.5). In Figure 2, effects of added NaCl on the plasmon resonance absorption of nanoparticles are shown. When aqueous NaCl is added to the citrate-reduced nanoparticles in the concentration range of 0–30 mM, a decrease in plasmon absorption at 530 nm and an increase in absorbance above 560 nm are observed (Figure 2b). Upon addition of 40 mM NaCl, wine-red color of the solution turned dark blue, which was accompanied by a spectral change to broad, red-shifted plasmon resonance absorption. This spectral change is typical to the aggregation of gold nanoparticles,[12] which occurred owing to the salt-induced shielding of anionic surface charges. On the other hand, in the case of GOD-capped nanoparticles, the addition of NaCl in the concentration range of 0–200 mM caused no appreciable spectral changes (Figure 2a). This observation clearly demonstrates that GOD-coated nanoparticles are highly stably dispersed in water. GOD have a net negative charge of −77 at neutral pH,[13] and their accumulation on nanoparticle surfaces not only suppresses their growth to bulk agglomerates but also affords highly hydrophilic protein layers. A GOD molecule possesses approximate dimensions of ca. $6 \times 5.2 \times 3.7$ nm (without surface carbohydrate units),[14] and a gold nanoparticle with a diameter of 14.5 nm is estimated to be surrounded by ca. 20–30 GOD molecules.

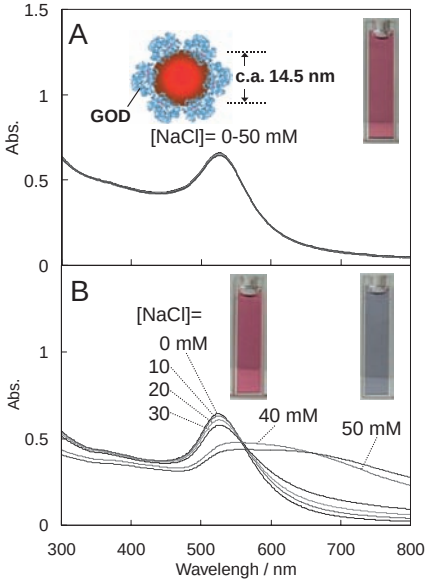

Figure 2. Pictures of nanoparticle dispersions and dependence of plasmon resonance absorption on NaCl concentration. (A) GOD-wrapped gold nanoparticles, (B) Citrate-reduced gold nanoparticles in pure water, 25 °C.

In summary, the biomediated synthesis of Au nanoparticles stabilized by GOD is developed by utilizing its own enzymatic reaction. GOD serves both as reducing agents and stabilizers. In situ preparation of gold nanoparticles by the use of oxidoreductase is a greener route that does not require toxic reducing agents or detergents. We envisage the use of protein-wrapped inorganic nanoparticles as versatile platforms in nanobiochemistry.

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