Spontaneous Preparation of Highly Stable Gold Nanoparticle Stabilized with ω-Sulfonylated Alkylsulfanylaniline

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Abstract: Preparation, characterization, and stability evaluation of gold nanoparticles stabilized by ω-sulfonylated alkylsulfanylaniline have been described. The particle solution was obtained by the spontaneous reaction of HAuCl4 and ω-sulfonylated alkylsulfanylaniline in boiling water. It showed a deep red color, owing to surface plasmon resonance of the resulting gold nanoparticles. The size and shape of the nanoparticles were pH-dependent, and pH 8 was found to be the most suitable condition to prepare stable nanoparticles with an average size of 11.2 ± 5.9 nm. The resulting particle solution was stable for a wide range of pH (3–13) and in phosphate-buffered saline (PBS) solution. The nanoparticles were storable as dried powder for at least two weeks, and were redispersible in water or PBS to give almost the same absorption spectra as the freshly prepared solution. Nanoparticle modification was achieved by simply adding thiol molecules to the particle solution.

Key words: gold nanoparticle, ω-sulfonylated alkylsulfanylaniline

1 Introduction

Gold nanoparticles (AuNPs) have been recognized as one of the most common and popular nanomaterials, because they are easily modified by using various functionalized molecules such as DNAs, proteins, or fluorescent molecules. Functionalized AuNPs have found many applications in various fields; their biological and medicinal applications in particular have been studied most intensively1-12. Functionalized AuNPs are usually obtained by a ligand exchange reaction of thiol-terminated molecules with desired functional groups and preprepared nanoparticles. In most cases, citrate13 or ascorbate14-stabilized AuNPs were used as raw materials because they are easily prepared from inexpensive chemicals such as citric acid or ascorbic acid and they readily undergo ligand exchange reactions. However, the citrate- or ascorbate-stabilized AuNPs must be stored as diluted basic solutions, as they are unstable in acidic or neutral media and in saline solutions, which are the common conditions in biological and medicinal experiments.

In this article, the preparation and properties of ω-sulfonylated alkylsulfanylaniline-stabilized AuNPs, which can be an attractive alternative for citrate- or ascorbate-stabilized AuNPs, have been described. ω-Sulfonylated alkylsulfanylaniline 1 is a mild reducing reagent, similar to citric acid or ascorbic acid, and was reacted with HAuCl4 and coordinated with AuNPs by its amino group to form highly stable AuNPs spontaneously. The physical properties of resulting AuNPs were characterized by 1H NMR and absorption spectra, and transmission electron microscope (TEM) analysis. The surface modification of resulting AuNPs was examined with a thiol-terminated dye.

2 Experiments

2.1 Preparation of ω-sulfonylated alkylsulfanylanilines 1 and 2

Two ω-sulfonylated alkylsulfanylanilines 1 and 2 were prepared as shown in Scheme 1. Detailed protocol to prepare 1 is as follows: To a solution of 4-aminobenzene-thiol (10 mmol in 100 mL of MeOH), K2CO3 (10 mmol) and 1,6-dibromohexane (20 mmol) were added, and the mixture was stirred at room temperature for 12 h. After usual extrac-
tion procedure, unstable ω-bromodialkylsulfanylalanilin was obtained as a pale yellow oil, which was immediately treated with excess Na2SO3 (15 mmol) in aqueous EtOH (20 mL of EtOH and 20 mL of H2O) under reflux condition. After 4 h, the reaction mixture was concentrated, and the resulting white solid was washed with AcOEt (2 mL for two times) and H2O (2 mL for two times) to give 1 (yield 87%). Compound 2 was also prepared with the similar method in 93% yield.

Compound 1: 1H NMR (DMSO-d6) δ 7.02 (d, J = 8.5 Hz, 2H), 6.49 (d, J = 8.5 Hz, 2H), 5.06 (s, 1H), 2.62 (t, J = 7.8 Hz, 2H), 2.38 (t, J = 7.8 Hz, 2H), 1.35 (m, 8H); 13C NMR (DMSO-d6) δ 148.7, 133.9, 115.1, 87.2, 51.5, 29.1, 28.9, 28.3; IR (KBr pellet) 3360, 2941, 2855, 1686, 1604, 1498, 1427, 1257, 1136, 1014, 979, 621 cm⁻¹; HRFABMS m/z 292.0054, calcd for M⁺: 292.0049.

Compound 2: 1H NMR (DMSO-d6) δ 7.02 (d, J = 8.5 Hz, 2H), 6.49 (d, J = 8.5 Hz, 2H), 3.33 (s, 1H), 2.70 (t, J = 7.8 Hz, 2H), 2.50 (t, J = 7.8 Hz, 2H), 1.70 (tt, J = 7.8 Hz, 7.8 Hz, 2H); 13C NMR (DMSO-d6) δ 148.7, 133.9, 115.2, 87.2, 50.5, 25.4; IR (KBr pellet) 3360, 1604, 1495, 1427, 1191, 1066, 978, 814, 629 cm⁻¹; HRFABMS m/z 292.0054, calcd for M⁺: Na⁺: 292.0049.

2.2 Preparation of AuNPs stabilized with ω-sulfonylated alkyldisulfanylalanilin

The typical preparation method for stabilized AuNPs using 1 is as follows: Freshly prepared aqueous solution of HAuCl₄ (1.0 mM, 10 mL) was added to an aqueous solution of disodium succinate (10 mM, 10 mL). The pH of the solution was controlled by adding a few drops of aqueous NaOH (1.0 M) or HCl (1.0 × 10⁻⁴ M). The volume of the solution was adjusted to 50 mL by adding water, and the solution was heated until boiling. An aqueous solution of 1 (2.0 mM, 10 mL) was added dropwise to the boiled solution, and the whole mixture was left for 48 h at 90°C. After cooling to room temperature, the resulting red solution was used as 1-AuNP (0.17 mM based on Au).

Powdered 1-AuNP was obtained by concentrating the solution in a rotary evaporator, followed by drying using a vacuum pump. The powdered 1-AuNP was dispersed in water (60 mL) or PBS (pH 7.4, 60 mL).

The ¹H NMR spectrum of 1-AuNP was measured after purification. Freshly prepared solution of 1-AuNP (60 mL) was concentrated to approximately 10 mL that was dialyzed to remove inorganic salts and free ligands. The purified solution was concentrated to dryness with a vacuum pump, and the resulting 1-AuNP was dissolved in DMSO-d6 (0.7 mL) to measure ¹H NMR.

2.3 Ligand exchange reaction with thiol

An aqueous solution of sodium 2-(3-mercaptopropanamido)naphthalene-1-sulfonate (3, 0 to 0.80 mM, 2.0 mL) and aqueous NaOH (1.0 × 10⁻³ M, 2.0 mL) were added to an aqueous solution of 1-AuNP (0.40 mM based on Au, 4.0 mL, pH 8.0) at 50°C, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was cooled to room temperature, and poured into dialysis tube (Spectra/ Por 3) that was put in a beaker filled with water (500 mL) at room temperature. The water was slowly stirred, and replaced with every 8 h. After 24 h, excess 3 and the free ligand were removed, and the resulting solution was diluted with water to 16 mL to measure its absorption spectrum.

3 Results and discussion

3.1 pH effect on preparation of stabilized gold nanoparticles (1-AuNP)

Preparation of AuNPs in nearly neutral media is favorable, particularly in biological applications. The reaction of 1 and HAuCl₄ successfully proceeded in a weakly basic solution (pH 8) that is acceptable for most of the biological applications, to give stable 1-AuNP solution in 48 h. The TEM image showed the majority of the particles were spherical with a diameter of 10–15 nm and an average size of 11.2 ± 5.9 nm (Fig. 1A and B).

The reaction also proceeded in a neutral solution (pH 6.5) or in a weakly acidic solution (pH 4.5), and completed much faster (24 h and 8 h, respectively). It is well known that the reaction of Au⁺ to Au° accelerates in a weakly acidic solution, and our results have supported the pH effect. The resulting AuNPs from these reactions were much larger and their shapes were ununiformed (Fig. 1C).
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It can be explained by the protonation of the aniline moiety in 1. As shown in Scheme 2, the Au$^{3+}$ ion is easily reduced by 1 to form Au$^0$. The resulting Au$^0$ starts growing into nanoparticles. Since an excess of 1 is used for this reaction, the unreacted 1, which is more coordinative than the oxidized 1, coordinates to the surface of the nanoparticles. This stops the growth of the gold nanoparticles, thereby forming stable 1-AuNP in alkaline solution. In the acidic or neutral medium, a considerable amount of 1 is protonated and is less coordinative; thus, the nanoparticles grow to become larger and uneven particles.

The $^1$H NMR spectra of 1 and 1-AuNP are shown in Fig. 2.

Fig. 1 A: TEM image of the particles prepared in a solution of pH 8.0. B: Distribution of particle diameters in a solution of pH 8.0. C: TEM image of the particles prepared in a solution of pH 6.5. D: TEM image of the particles prepared in a solution of pH 4.5.
The solution of 1-AuNP was tolerant to a wide range of pH. The absorption spectrum of a solution of pH 13, prepared by adding 1.0 M aqueous NaOH to the freshly prepared 1-AuNP solution, showed the same $\lambda_{\text{max}}$ (526 nm) as the original 1-AuNP solution of pH 8 (Fig. 4). In the acidic condition of pH 3, achieved by adding 1.0 M aqueous HCl, the spectrum was slightly broader and $\lambda_{\text{max}}$ shifted to 531 nm, although the profile did not change for several weeks. Highly acidic condition was detrimental to the nanoparticles. The absorption spectrum of the 1-AuNP solution of pH 2 showed a weak and broad signal, and a black precipitate was formed within a few hours.

The pH dependent stability can be explained by the pKa of compound 1. Since the pKa of protonated anilinium ion is known as 5.217, most of the –NH$_2$ groups 1-AuNP are supposed to be protonated at pH 3 or lower. The protonated 1 is released from AuNP, and the resulting naked AuNPs aggregate to form larger particles or precipitates. In alkali condition, on the other hand, all of the –NH$_2$ groups aren’t protonated, and 1-AuNP has not been changed at all.

### 3.3 Ligand exchange with thiol molecule

Since 1-AuNP was formed by coordination between the AuNP and aniline’s –NH$_2$ group, it can be modified by ligand exchange reaction with a thiol that can firmly bind to the AuNP. A given amount of thiol 3 and 1-AuNP were stirred for 1 h at 50°C, and the resulting solution was dialyzed to remove excess 3. The absorption spectra of the resulting solutions are shown in Fig. 5. Because molar absorption coefficient of 3 is much higher than that of 1 (compound 3: $\lambda_{\text{max}}$ at 246 nm, $\varepsilon$ = $3.4 \times 10^4$ M$^{-1}$cm$^{-1}$, compound 1: $\lambda_{\text{max}}$ at 260 nm, $\varepsilon$ = $1.2 \times 10^3$ M$^{-1}$cm$^{-1}$), a dose-dependent increase of characteristic absorption of 3 has been clearly observed, which suggests that the expected ligand exchange reaction proceeded well.
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Fig. 3  A: Absorption spectra of 1-AuNP; a: freshly prepared solution, b: redispersed solution of 1-AuNP powder in water, and c: redispersed solution of 1-AuNP powder (stored for two weeks) in water. B: Absorption spectra of 2-AuNP; a: freshly prepared solution, b: redispersed solution of 2-AuNP powder in water, and c: redispersed solution of 2-AuNP powder (stored for two weeks) in water. C: TEM image obtained for the redispersed solution of 1-AuNP powder in water. D: Distribution of particle diameters of the redispersed 1-AuNP powder in water.

Fig. 4  Absorption spectra of 1-AuNP solutions in different pH.

Fig. 5  Absorption spectra of the ligand exchange reaction of 1-AuNP and 3.
4 Conclusion

New reductive stabilizers 1 and 2 have been developed. Gold nanoparticles, highly stable and storable for a long time, were prepared by the spontaneous reaction of HAuCl₄ and 1 in an aqueous solution of pH 8. The reaction yielded spherical 1-AuNP with an average size of 11.2 ± 5.9 nm. The resulting 1-AuNP solution was stable over a wide pH range of 3–13. 1-AuNP is storable in its powdered form for a long time at ambient temperature in air and is dispersible in water or PBS solution to give almost the same absorption spectra as the freshly prepared solution. Surface modification of 1-AuNP was easily achieved by ligand exchange reaction with thiol 3. These advantages suggested that 1-AuNP can be an attractive alternative for citrate- or ascorbate-stabilized AuNPs, particularly in biological and medicinal applications.

Acknowledgments

This work was supported by the Grants-in-Aid for Scientific Research (No. 23550160 and No. 26410096) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by the Cooperative Research Program of the “Network Joint Research Centre for Materials and Devices” (No. 2011287 and 2014440).

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