Gold–Silver and Gold–Palladium Alloy Nanoparticles as Mass-Probes for Immunosensing

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Silver or palladium shelled gold nanoparticles were fused into alloy nanoparticles by pulsed-laser irradiation. The alloy nanoparticles could carry antibodies on their surfaces without affecting their immune functionalities and interact selectively with antigens on a blotting membrane. Silver or palladium ions desorbed from the alloy nanoparticles as reporter ions upon the UV laser irradiation in a mass spectrometer.

Keywords Alloy nanoparticle, mass probe, immune functional nanoparticle, core-shell nanoparticle

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Introduction

A mass probe, which is attached to antibodies, desorbs specific ions and shows the presence of target molecules. The desorbed ions act as reporter ions.1–3 Because mass spectrometry (MS) is a highly sensitive analytical method that can detect as low as $10^{-20}$ mol of ions, the main advantage of a mass probe is its sensitivity.

Gold nanoparticles were used as a mass probe.4–7 Ionization of gold nanoparticles to generate Au+, Au2+, and Au3+ ions is possible without matrix compounds. The limit of detection is estimated to be $10^{-18}$ mol (nanoparticle)/mm².5 Using antibody-modified gold nanoparticles as a mass probe, imaging mass spectrometry visualized the distribution of green fluorescent proteins (GFPs) in the section of a transgenic zebrafish.6 Gold nanoparticles have the potential to be used as a mass probe with high sensitivity; however, the gold nanoparticles give gold ions only. If several kinds of reporter ions simultaneously desorbed, a mass spectrometer could identify them in a one-time measurement.

Like gold nanoparticles, other metal nanoparticles seem to have potential for use as mass probes. Silver nanoparticles, for example, desorb silver ions (Ag+, Ag2+, Ag3+) upon pulsed-laser irradiation. However, the chemical stability of silver is not enough due to the oxidation by dissolved oxygen in water. To obtain a practical nanoparticle probe, antibodies should be deposited on their surfaces without affecting their immune functionalities. Generally speaking, nanoparticles consisting of pure metals, other than gold and platinum nanoparticles, are not suitable to carry antibodies on their surfaces due to their oxidation.

In the present work, we prepared gold-silver and gold–palladium alloy nanoparticles and assessed their modification with antibodies. Immune functionalities of antibodies on the alloy nanoparticles were evaluated by using a dot-blotting method.

Experimental

Preparation of nanoparticles

All chemicals were commercially available and used without further purification. Gold nanoparticles (“core” nanoparticles, 29 ± 4 nm) were prepared by the citrate reduction method (see Supporting Information). The gold nanoparticle solution (0.5 mM (Au atoms), 2 mL) was added to a citrate solution (1.3 mM, 30 mL). Under stirring, silver nitrate or tetrachloropalladium (10 mM, 100 μL) and L-ascorbic acid (100 mM, 500 μL) were added to the gold nanoparticle solutions. The L-ascorbic acid was the reducing agent to produce silver or palladium shells on the gold core nanoparticles. The reduction of Ag and Pd ions proceeded at 30 and 4°C, respectively.

The Au–Ag core-shell nanoparticles were fused by the two steps of pulsed-laser irradiation (10 ns, 20 Hz); 100 mW for 10 min and 120 mW for 15 min. For the Au–Pd core-shell nanoparticles, three steps of irradiation were applied: 100 mW for 10 min, 120 mW for 10 min, and 140 mW for 15 min. The laser-irradiated particles were centrifuged and re-dispersed in the 1.3-mM citrate solution. The absorbance of the final solution was set to be 0.3 cm⁻¹ at 355 nm. The surface modification with antibodies and the procedures of the dot blotting are shown in Supporting Information.

Apparatus

A Q-switched Nd-YAG laser (Polaris III, New Wave Research, USA) and transmission electron microscope (TEM, JEM-3010, JEOL, Japan) were used for the experiments. Mass spectrometry was performed using a MALDI-MS instrument (Autoflex Speed, Bruker, USA).
Results and Discussion

The solid line in Fig. 1(A) is the extinction spectrum of the solution in which silver ions were reduced. The two peaks at 374 and 503 nm are assignable to the surface plasmon (SP) bands of silver shells. The pulsed-laser irradiation at 532 nm decreased the peak intensities at 374 nm and shifted another peak from 504 to 465 nm. The TEM image in Fig. 2(A) indicates the formation of silver shells. The image after the laser irradiation shows spherical and monolithic nanoparticles that indicate the formation of the “AuAg alloy” nanoparticles.

The deposition of palladium decreased the SP band intensities of gold (Fig. 1(B)). The pulsed-laser irradiation almost quenched the SP band. The TEM images in Figs. 2(C) and 2(D) indicate the formation of the palladium shells and the fusion into spherical “AuPd alloy” nanoparticles.

Figure 3(A) shows an optical image of the blotting membrane on which four kinds of solutions of normal goat IgG (200, 20, 2.0, and 0.2 ng) were cast as dots. The rightmost spot was the control spot where 200 ng of bovine serum albumin (BSA) was cast. Contact with the AuAg alloy nanoparticles, which were modified with anti-goat IgG, clearly changed the color at the leftmost spot where 200 ng of the goat IgG was cast.

Figure 3(B) shows a mass spectrum obtained from the 200-ng spot of the goat IgG. The AuAg alloy nanoparticles provided Ag⁺, Ag₂⁺, AuAg⁺, Ag₃⁺, AuAg₂⁺, and Au₂Ag⁺ ions. The plot in Fig. 3(A) shows a line analysis of mass signal intensities of AuAg⁺ (m/z 304) on the blotting membrane. The signals were obtained in the spots where the goat IgG was cast, and the more concentrated solutions gave the larger mass signals. Even in the spot of the 2-ng spot of the goat IgG, distinguishable mass signals were found. Whereas the control spot (200 ng BSA) did not show any signals. This shows the specific interaction between the goat IgG on the membrane and the anti-goat IgG on the AuAg alloy nanoparticles.

Figure 4(A) shows the deposition of the AuPd alloy
nanoparticles in the spots where goat IgG was cast. Desorption of Pd\(^+\), Pd\(^2+\), AuPd\(^+\), AuPd\(^2+\), and Au\(^2\)Pd\(^+\) ions from the 200-ng spots is shown in Fig. 4(B). Since palladium shows five kinds of isotope peaks, the sum of the five ions are used for the line-analysis plot. (B): The mass spectrum obtained from the spot where 200-ng of goat IgG was cast.

Both alloy nanoparticles could carry the antibodies without affecting their immune functionalities. The contents of Ag in the AuAg alloy nanoparticles and Pd in the AuPd alloy nanoparticles were estimated as about 10 and 33\%, respectively (see Supporting Information). The alloy nanoparticles are gold-rich and chemically stable. The spectroscopic properties are retained at least for one month after the preparation. Even at one week after the antibody modification, the alloy nanoparticles could be used for dot blotting. The alloy nanoparticles are the practical mass probes that are chemically stable and can desorb silver or palladium ions as reporter ions.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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![Fig. 4](image-url)