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Preparation and Molecular Recognition of SERS Probe Based on Gold Nanoparticles Constructed from PEG–Oligoamine Copolymer Possessing a Coumarin Group between PEG and Oligoamine

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Novel gold nanoparticles (GNPs) as a SERS probe were prepared using two types of functional PEG/oligoamine polymers possessing biotin as biotag molecules at the PEG end or a coumarin moiety as a Raman probe molecule between the PEG and oligoamine segments. Eventually, the GNPs showed excellent dispersion stability under physiological conditions, as well as remarkable SERS signal resulting from molecular recognition between streptavidin and the biotin tag, even in the presence of bovine serum albumin (BSA).

Surface-enhanced Raman scattering (SERS) using gold nanoparticles (GNPs) has attracted much attention in the field of DNA- and immunosensors, because the enhancement of the Raman signal has been known to be as large as 10^{14}–10^{15} due to the combination of an electromagnetic mechanism and a chemical mechanism related to the charge transfer between the GNP and the Raman probe.[1,2] Most GNP-based SERS probes have been prepared by the adsorption of organic dye as Raman probes on the surface of GNPs through electrostatic interaction, followed by the immobilization of sulfanylated poly(ethylene glycol) (PEG) or sulfanylated DNA.[3] However, the Raman probes adsorbed on the GNP surface decrease the dispersion stability of the GNPs due to the decrease in the electrostatically repulsive force of the GNPs,[4] which prevents a large amount of Raman probes from being immobilized. The dissociation of the Raman probes from the gold surface under physiological conditions is also one of the problems. PEG or oligo-DNA is expected to improve the dispersion stability under physiological conditions. However, the Au–S bond is cleavable through the sulfanyl exchange reaction with sulfanyl-containing compounds such as glutathione, leading to the elimination of PEG or DNA, which causes the aggregation of GNPs. In order to construct a SERS-sensing surface, the aggregation of GNPs must be avoided before the immobilization of the GNPs on the sensor surface (Figure 1a). On the contrary, the SERS signal increased significantly when two or more GNPs were close to each other due to the enormous enhancement of the electromagnetic field, as shown in Figures 1b and 1c. In order to obtain a viable SERS sensing system, both the dispersion stability of GNP possessing sufficient amounts of Raman probe and a suitable molecular recognition function must be installed on the GNP surface. The objective of this study is to construct desirable GNPs functionality using two types of novel heterobifunctional PEGs. For the immobilization of the SERS probe, methoxy-ended

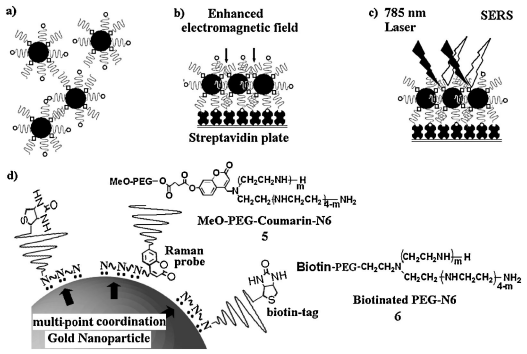

Figure 1. Schematic illustration of SERS. a) Dispersion of GNPs-based SERS probes, b) molecular recognition and formation of the enhanced electromagnetic field, c) SERS of the Raman probes on the GNPs, and d) illustration of GNP immobilized MeO–PEG–cumarin–N6 and biotin–PEG–N6.

PEG–coumarin–pentaethylenehexamine (MeO–PEG–coumarin–N6, **5**) was prepared. For the specific molecular recognition, biotin–PEG–N6 was prepared as shown in Figure 1d. Our idea was to assign two different roles, viz., installation of the SERS probe and specific molecular recognition, using two different PEG derivatives. Both PEGs improve the dispersion stability of the GNPs before molecular recognition as tethered chains on the GNP surface, because of the multi-coordination of the amino group in the N6 moiety[5] instead of the sulfanyl group.

Synthetic route of **5** is shown in Scheme S1 (see, the Supporting Information).[7] The introduction of coumarin molecules at the carboxylic acid group of monomethoxy PEG, **1** ($M_n = 4900$, $M_w/M_n = 1.05$), was carried out with **2** in the presence of carbodiimide at 30 °C for 48 h to obtain coumarin-conjugated PEG, **3**. The degree of functionality of the coumarin group in **3** was found to be 48%, as determined based on the ^{1}H NMR spectrum (Figure S3).[7] The obtained **3** was reacted with excess oligoamine, **4** (N6), to form **5**. After the purification of **5** using ion chromatography, the ^{1}H NMR spectrum of **5** was obtained as shown in Figure S4[7] with assignments. Peaks attributed to the coumarin moiety were observed at 7.7, 7.1, and 6.5 ppm, along with the peak at 3.7 ppm attributed to the ethylene protons of the PEG backbone. Additionally, broad peaks attributed to the ethylene protons of the N6 segment were also observed from 2.3 to 2.9 ppm. The degree of functionality of the N6 segment in **5** was determined to be 85%, based on the

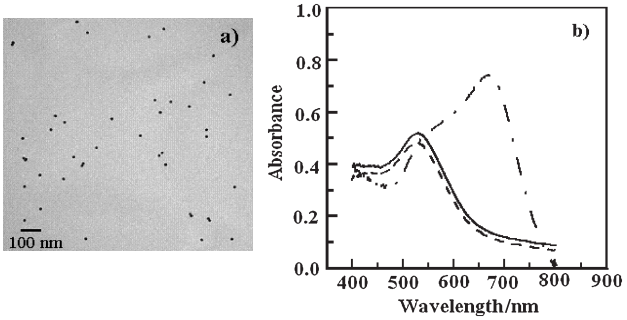

Figure 2. a) TEM image of GNP-based SERS probes and b) UV–vis spectra of GNP-based SERS probes in water (solid line), 150 mM NaCl solution after 12 h (dotted line) and coumarin-adsorbed GNPs in 150 mM NaCl(aq) after 1 min (dashed line).

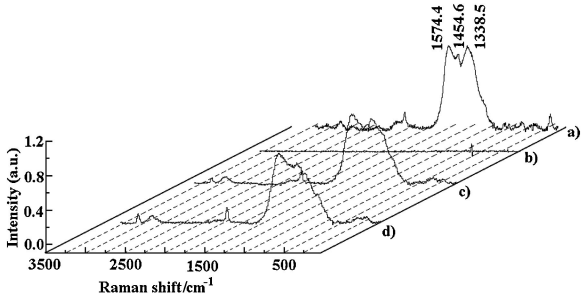

Figure 3. SERS spectra of GNP-based SERS probes in the well of the streptavidin-coated plate after evaporating of the buffer. Molecular recognition was carried out a) in phosphate buffer, b) with incubation excess free biotin to block the molecular recognition cites of streptavidin before the addition of GNP-based SERS probes, c) in the presence of BSA (6 g dL^{-1}) in phosphate buffer, and d) in phosphate buffer with 150 mM NaCl.

integral ratio between the peaks of the ethylene proton of N6 and the peak of the PEG backbone. Biotinated PEG–N6, **6**, was also synthesized according to the Scheme S2 (see, the Supporting Information).[7] The degree of functionality of the biotin and the N6 segment in **6** was determined to be 68% and 55% by the HABA biotin quantitative method and based on the ^{1}H NMR spectrum (Figure S9),[7] respectively. To prepare the SERS probe based on PEGylated GNP, **6** was initially added to GNP (diameter 15 nm) aqueous solution, followed by the addition of **5**. To remove the unimmobilized **5** and **6**, centrifugation was carried out repeatedly, and the solvent was finally substituted with PBS (pH 7.4). Figure 2a shows the transmission electron microscopy (TEM) image of the obtained GNPs. These GNPs did not show any aggregation.

The dispersion stability of the obtained GNPs was evaluated at high salt concentration (150 mM) by the measurement of the change in the UV–vis spectra, because the surface plasmon band (SPB) at 520 nm is known to shift the absorption peak toward a longer wavelength when coagulation occurs. The large shift of SPB of the GNPs was not observed even after incubation for 12 h in 150 mM NaCl(aq) (Figure 2b), clearly indicating that hardly any aggregation of the GNPs occurred, due to the steric stabilization of the PEG chains surrounding each GNP through multi-point coordination between the GNP and the oligoamine segment.[5] In sharp contrast, coumarin-adsorbed GNPs without **5** and **6** showed an immediately increasing absorbance at >650 nm (within 1 min), caused by the coagulation of the GNPs.

To clarify the molecular recognition ability of the GNPs with **5** and **6**, a streptavidin-coated plate was used as a model for the protein array. GNP solution was added to each well, and the plate was incubated for 6 h at 4 °C, followed by washing with water. The SERS signals from the GNPs after evaporating of the water were obtained by means of a Raman spectrometer (Senterra conforcal Raman system, Bruker Optics Inc., laser, 785 nm; power, 2 mW; integration, 5 min) as shown in Figure 3. As seen in Figure 3a, three broad SERS signals were observed by the aggregation of the GNPs through the molecular recognition between biotin and streptavidin after evaporating of the solvent. The broadening of the SERS signals is presumably due to the evaporating of the water, since broadening of the SERS signals of coumarin (at ca. 1380, 1540, and 1590 cm^{-1}) immobilized on the silver surface was also observed after evaporating of solvent.[6] These facts suggest that three broad SERS signals at 1338.5, 1454.6 and 1574.4 cm^{-1} were attributed to the C=C (benzene ring), C=C (olefin), and C=O bond of the coumarin molecules, respectively. In sharp contrast, when excess free D-biotin was incubated in the well of the streptavidin-coated plate before the addition of the GNP solution, no SERS signal was observed due to the blocking of the recognition site of streptavidin by the free D-biotin (Figure 3b). These facts strongly indicate that the observed SERS signal of the GNPs (Figure 3a) arises from specific molecular recognition between the biotin molecules located at the PEG-chain ends of the GNPs and streptavidin immobilized on the plate. It was worth noticing that the SERS spectra of the GNPs could be observed even in the presence of BSA (6 g dL^{-1}) (Figure 3c) and 150 mM NaCl (Figure 3d), without aggregation of GNPs in the molecular recognition process (in the solution), indicating that the highly dense PEG chains surrounding the GNP surface significantly reduced the nonspecific absorption of BSA. Thus, enhancing the dispersion stability of the GNPs facilitates the specific SERS signals through the molecular recognition process.

In conclusion, we described here a novel GNP-based SERS probe constructed from PEG–coumarin–N6, **5**, and biotin–PEG–N6, **6**. The obtained GNPs showed excellent dispersion stability as well as remarkable SERS signal resulting from the molecular recognition between streptavidin and biotin under various conditions without false detection. It is thought that these results were achieved by preventing the aggregation of GNPs and the elimination of the Raman probe. Therefore, the GNPs obtained here seem to be suitable as a platform for the SERS-based sensor.

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7 Supporting Information is available electronically on the CSJ-Journal Website, http://www.csj.jp/journals/chem-lett/.

