Silver Particle and Silver Thiolate-Based Molecular Sensing/Recognition Units Operating via Surface-Enhanced Raman Scattering

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ABSTRACT

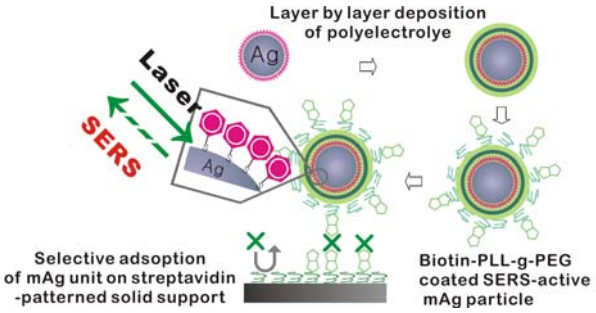
We demonstrate that commercially available 2-μm-sized silver (μAg) powders can be used as a core material for constructing molecular sensing/recognition units operating via surface-enhanced Raman scattering (SERS). This is possible because μAg powders are very efficient substrates for both the infrared and Raman-spectroscopic characterization of molecular adsorbates prepared in a similar manner on silver surfaces. The agglomeration of μAg particles in a highly concentrated buffer solution can be prevented by the layer-by-layer deposition of cationic and anionic polyelectrolytes such as poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) onto SERS-marker molecules assembled initially on μAg particles. The outermost PAA layer can be derivatized further, for instance, with biotin-derivatized poly(L-lysine). On the basis of the nature of the SERS peaks of marker molecules, the biotinylated μAg powders are readily confirmed to selectively bind to streptavidin arrays.

Keywords: surface-enhanced Raman scattering, SERS, silver powder, molecular sensing/recognition, biotin-streptavidin interaction

1 INTRODUCTION

Noble metallic nanostructures exhibit a phenomenon known a surface-enhanced Raman scattering (SERS) in which the Raman scattering cross-sections are dramatically enhanced for the molecules adsorbed thereon [1]. When the adsorbed molecules are subjected to resonance Raman scattering, it is called a surface-enhanced resonance Raman scattering (SERRS) by which even single molecule detection is known to be possible, suggesting that the enhancement factor can reach as much as 10^{14}-10^{15} [2,3]; the effective Raman cross sections are then comparable to the usual fluorescence cross sections. In recent years, there has also been considerable interest in the application of SERS/SERRS in biomolecular detection [4,5]. Although fluorescence is currently the principal detection method in bioassays, it has inherent drawbacks such as photobleaching, narrow excitation with broad emission profiles, and peak overlapping in multiplexed experiments. The latter limitations can be overcome by means of SERS/SERRS. Several groups have thus developed various types of SERS-active tagging materials that can be used in diagnostic bioassays [4-7].

We have previously reported that commercially available 2-μm-sized silver (μAg) powders are effective substrates for the infrared and Raman-spectroscopic characterization of molecular adsorbates prepared in a manner similar to those on silver surfaces [8]. On one hand, we can obtain an infrared spectrum of organic molecules adsorbed on μAg particles with a very high signal-to-noise ratio by diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), and the DRIFT spectral pattern is minimally different from the reflection-absorption infrared spectral pattern taken for the same molecules on a vacuum-evaporated thick silver film. On the other hand, the Raman spectrum of organic monolayers on powdered silver is an SERS spectrum, exhibiting little difference from that obtained from the thin, rough vacuum-evaporated silver films.



Scheme 1: Fabrication of μAg particle-based SERS/SERRS unit for ready application to biomolecular sensing and recognition

We demonstrate in this work that μAg powders can be used as a core material for constructing molecular sensing/recognition units operating via SERS/SERRS. The basic strategy is schematically drawn in Scheme 1. A dye molecule called rhodamine B isothiocyanate (RhBITC) is used as a SERS/SERRS marker molecule in this specific example. RhBITC-adsorbed μAg particles are protected by poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) layers. After depositing biotin-derivatized poly(L-lysine) onto the PAA layer, the modified μAg particles are finally confirmed to interact very favorably with strepavidin arrays formed on a separate biotinylated substrate. According to a dose response curve drawn based on the measured SERS/SERRS marker peaks,

the detection limit is as low as sub-nanograms per milliliter for strepavidin molecules.

2 EXPERIMENTAL

The poly(allylamine hydrochloride) (PAH, *M*w ~70 kDa), poly(acrylic acid) (PAA, *M*w ~450 kDa), poly(L-lysine hydrobromide) (PLL-HBr, *M*w ~20 kDa), rhodamine B isothiocyanate (RhBITC, 97%), and silver powder (μAg, 99.9+% purity) with a nominal particle size of 2-3.5 μm were purchased from Aldrich and used as received. Other chemicals, unless specified, were reagent grade, and highly pure water, of resistivity greater than 18.0 MΩ·cm (Millipore Milli-Q System), was used in making aqueous solutions. The synthesis of PLL-*g*-PEG and biotinylated-PLL-*g*-PEG was based on the protocols described by Spencer et al. [9] and the detailed processes were reported in our recent publication [10].

For the self-assembly of RhBITC on silver, 0.050 g of silver powder was placed in a small vial into which 2 mL of 0.5 mM ethanolic RhBITC solution was subsequently added. After 12 h, the solution phase was decanted and then rinsed with highly pure water. The remaining solid particles were left to dry in a vacuum for 2 h. Subsequently, polyelectrolyte layers were formed by the sequential dipping of the RhBITC-modified μAg powders into the PAA and PAH solutions (0.1 mg/mL) for 10 min at room temperature. In the interim, to change the polyelectrolyte solution, μAg powders were intensively rinsed with water. At the final stage, the PAA-derivatized μAg particles were electrostatically reacted with biotinylated-PLL-*g*-PEG [11].

To construct a dose-response curve for strepavidin, a glass slide was initially soaked in a piranha solution to assume negatively charged surfaces. The glass slide was subsequently dipped in PLL-g-PEG (20 μg/mL) solutions for 10 min. After washing with water and then drying in a nitrogen atmosphere, 1.5 μL of biotinylated-PLL-*g*-PEG (100 μg/mL) was pipetted onto it to obtain a 5-mm domain. The glass slides coated with biotinylated-PLL-*g*-PEG were soaked in a streptavidin solution at various concentrations for 10 min, followed by extensive rinsing with a PBS solution and drying by a nitrogen stream. The streptavidin-attached slides were subsequently immersed in a solution containing μAg particles (1 mg/mL) derivatized consecutively with RhBITC, PAA/PAH, and biotinylated-PLL-*g*-PEG. After 10 min, the glass slides were washed with water, and the dried slides were finally subjected to Raman spectral measurements.

For microarray spotting, biotinylated-PLL-*g*-PEG dissolved in a PBS buffer (~20-100 μg/mL) was dispensed into a thin-walled polycarbonate plate comprising 96 wells with a volume of 15 μL per well. The spotting was subsequently carried out on glass slides in a linear, as well as array, mode by using a Spotarray 24 (Perkin-Elmer) instrument. The spacing between the spots was maintained at 500 μm; further, for each spot, the spotting was repeated 5-20 times. The spotted slides were immediately incubated in PBS for 30 min, followed by thorough washing with distilled water and air-drying; it was then stored under N_2.

Infrared spectra were measured using a Bruker IFS 113v Fourier transform IR spectrometer equipped with a globar light source and a liquid N_2-cooled wide-band mercury cadmium telluride detector. Raman spectra were obtained using a Renishaw Raman system Model 2000 spectrometer equipped with an integral microscope (Olympus BH2-UMA). The 514.5 nm line from a 20 mW Ar^+ laser (Melles-Griot Model 351MA520) or the 632.8 nm line from a 17 mW He/Ne laser (Spectra Physics Model 127) were used as the excitation source. The SERS/SERRS peak intensities were normalized with respect to the peak intensity of a silicon wafer at 520 cm^{-1}.

3 RESULTS AND DISCUSSION

One difficulty when using micro silver powders in SERS-based bioassays is associated with their aggregation characteristics, even in water at neutral pH. The extent of aggregation increases with the ionic strength of the medium. The tendency of aggregation can be significantly reduced by coating the silver particles with charged polymers. In our previous work, amine group-derivatized Ag particles could be dispersed in water and electrolytic solutions by the layer-by-layer (LbL) deposition of PAA and PAH thereon [10]. The first thing to do in this work would then be to confirm whether PAA and PAH could be deposited also onto the RhBITC-derivatized μAg powders. For that purpose, we took infrared spectra after the repeated deposition of PAA and PAH onto the μAg powders.

In fact, aliphatic polymers are strong infrared absorbers, therefore the IR peaks due to RhBITC are weakly identified as a result of the deposition of PAA and PAH polyelectrolytes (data not shown). Strong peaks appear at 1713, 1567, and 1397 cm^{-1} due to the C=O stretching band of the carboxylic group (-COOH) and the antisymmetric and symmetric stretching bands of the carboxylate group ($-COO^-$) of PAA, respectively, while a comparatively weak peak appears at 1592 cm^{-1} due to RhBITC on μAg particles. The peak intensities of the ν(C=O) and $\nu_{as}(COO^-)$ bands increase linearly as a function of the deposition cycles of PAA and PAH, indicating that the PAA/PAH bilayers are formed homogeneously from the beginning, probably due to hydrophobic interaction between RhBITC and PAA/PAH.

Having confirmed that PAA and PAH can be deposited consecutively on the RhBITC/μAg powders, we have subsequently examined whether the Raman spectral feature of RhBITC is maintained after the layer-by-layer deposition of PAA/PAH bilayers. Figures 1(a) and (b) show the SERRS spectra of RhBITC/μAg taken using 514.5-nm radiation as the excitation source respectively before and after the deposition of five PAA/PAH bilayers. Similarly, Figures 1(c) and (d) show the SERS spectra of RhBITC/μAg taken using 632-nm radiation respectively before and after the deposition of five PAA/PAH bilayers.

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We notice that the spectral patterns in Figures 1(a) and (b) are nearly the same as each other. The peak positions, as well as the peak intensities of RhBITC, in Figures 1(c) and (d) are also hardly different from each other. As can be seen in Figure 1(e), the intensity of a characteristic band of RhBITC at 1647 cm^{-1} is largely independent of the number of PAA/PAH bilayers. It is thus concluded that RhBITC molecules are retained on the surfaces of μAg powders even after the deposition of polyelectrolytes. The absence of peaks due to PAA and PAH in Figures 1(b) and (d) can be understood by recalling the facts firstly, that aliphatic polyelectrolytes are intrinsically weak Raman scatterers and, secondly, that the SERS signal must derive mostly from the adsorbates that are directly in contact with SERS-active particles in accordance with the electromagnetic and chemical enhancement mechanisms in SERS [1].

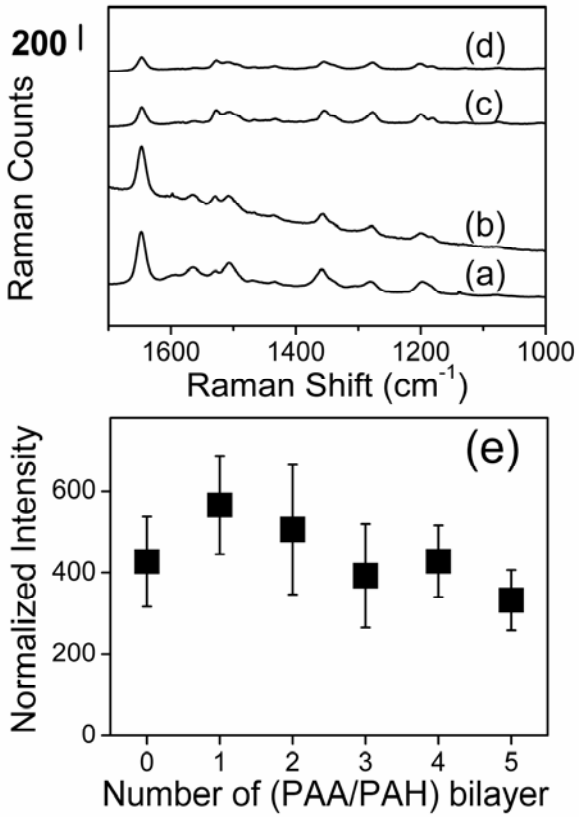

Figure 1: SERRS spectra of RhBITC/μAg taken using 514.5-nm radiation as the excitation source (a) before and (b) after the deposition of five PAA/PAH bilayers. Similar but SERS spectra taken using 632.8-nm radiation (c) before and (d) after the deposition of PAA/PAH bilayers. (e) SERRS intensity of the characteristic band of RhBITC at 1647 cm^{-1} drawn versus the number of deposition cycles of the PAA and PAH polyelectrolytes.

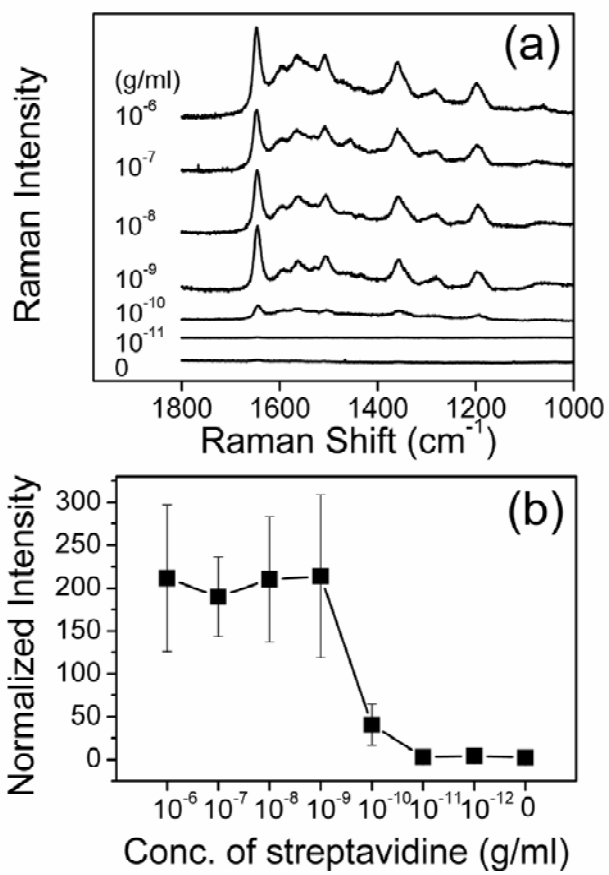

Figure 2: (a) A typical SERRS spectrum of RhBITC measured after biotinylated μAg powders containing RhBITC were allowed to interact via streptavidin with other biotinylated layers on glass. (b) SERRS intensity of the characteristic band of RhBITC at 1647 cm^{-1} measured as a function of streptavidin concentration; the amount of Ag particles used was 1 mg/mL, and the SERRS intensities were the average of 10 different measurements with the error bars denoting their standard deviation.

We subsequently evaluated the sensitivity of the biotinylated RhBITC/μAg powders to recognize streptavidin molecules by constructing a dose-response curve. After fabricating biotinylated glass slides, SERS/SERRS spectra were obtained as a function of the strepavidin concentration ranging from 10^{-4} to 10^{-12} g/mL. A typical spectrum obtained using 514.5-nm radiation at 10 s integration time is shown in Figure 2(a); the spectral feature is the same as that in Figure 1(b). Figure 2(b) shows the normalized SERRS intensity of the characteristic band of RhBITC at 1647 cm^{-1}; all the SERRS peaks were normalized with respect to the peak intensity of a silicon wafer at 520 cm^{-1}. A very intense SERS/SERRS spectrum is obtained as long as the streptavidin concentration is above 10^{-9} g/mL. At 10^{-10} g/mL, the number of Ag particles adsorbed on the biotinylated substrates decreases, resulting in a lowering of the SERS/SERRS intensity.

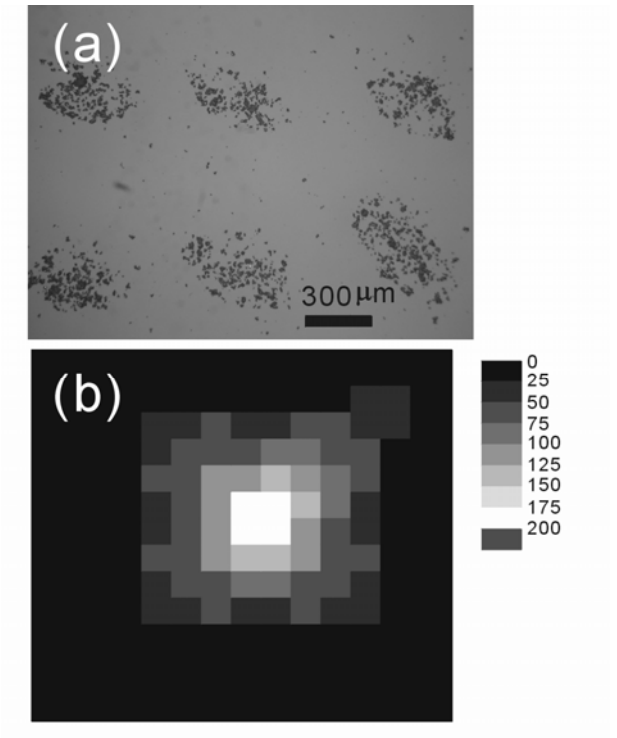

Figure 3: (a) Optical image of biotinylated μAg powders in contact with a patterned streptavidine layer on glass. (b) A typical Raman mapping constructed using the SERS/SERRS peak intensity of the characteristic band of RhBITC at 1647 cm^{-1}, measured after biotinylated μAg powders in contact with one spot of streptavidine arrays on glass.

Having confirmed that biotinylated RhBITC/μAg powders interact via the mediation of streptavidin with other biotinylated layers on glass, we have examined the same interaction using patterned streptavidin layers. For that purpose, biotinylated-PLL-*g*-PEG was spotted on a glass slide using a microarray spotter (see the Experimental section). After drying under an ambient condition, the glass slide was dipped in PLL-*g*-PEG solutions (20 μg/mL) for 10 min. The glass substrate, patterned with biotinylated-PLL-*g*-PEG and PLL-*g*-PEG, was then soaked in a streptavidin solution (20 μg/mL) for 10 min, followed by extensive rinsing with a PBS solution and drying by a nitrogen stream. The streptavidin-attached glass slide was immersed again in a PBS solution, into which aliquots of biotinylated RhBITC/μAg particles dispersed in the PBS solution were added. After 10 min, the glass slide was washed with the PBS solution and dried under ambient conditions. Figure 3(a) shows the optical image of the glass slide. Orthogonally adsorbed μAg particles are clearly identified; these conform to the patterned streptavidin. Figure 3(b) shows a typical Raman mapping constructed on one spot of streptavidin arrays on glass, by referring to the peak intensity of the characteristic band of RhBITC at 1647 cm^{-1}.

In summary, we confirmed, by DRIFT spectroscopy, that the layer-by-layer deposition of PAA and PAH can be conducted irrespective of the kind of SERS/SERRS-marker molecules. In a similar manner, biotinylated-PLL-*g*-PEG can be electrostatically bound onto the outermost PAA layer of μAg powders. The deposition of polyelectrolytes onto Ag particles restrains the μAg powders from agglomerating in highly concentrated buffer solutions. These biotinylated μAg powders were confirmed via SERS/SERRS to recognize selectively the streptavidin arrays assembled on glass substrates. According to a separate dose response curve drawn, based on the measured SERRS peaks of RhBITC, the detection limit was as low as 10^{-10} g/mL for strepavidin molecules. The fabrication method employed in this work is very simple, but the resulting SERS/SERRS signal can be easily detected using a low power laser. In addition, a number of different molecules can be used as SERS/SERRS-marker molecules; therefore, μAg powders should be regarded as a prospective material for biomolecular sensing/recognition, particularly via SERS and SERRS.

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