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Label-free Biosensor Using Polyion-modified Gold Nanorods Adsorbed on a Glass Substrate

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Gold nanorods (NRs), which were modified with polyions, were fixed on polycation-modified glass substrates. Adsorption of antibodies and antigens on the glass substrates induced large peak shifts (44 nm) of longitudinal surface plasmon bands in the near-infrared region. The shifts were assignable to changes of refractive indices of polyion layers at the glass surfaces.

Gold nanorods (NRs) are rod-shaped nanoparticles that exhibit transverse and longitudinal surface plasmon (SP) bands in the visible and near-infrared (near-IR) regions, respectively.[1–3] The positions of the SP bands are tunable depending on the shape of the NRs and the refractive indices of their surroundings.[4–6] In previous papers, label-free optical sensors based on peak shifts of the longitudinal SP bands have been used to determine refractive indices of solvents[7] and to detect avidin–biotin conjugation[8] and antibody–antigen binding.[9,10] We also reported that longitudinal SP bands of NRs on a clean surface of a glass substrate showed peak shifts when they were immersed in some solvents.[7] However, the peak shift from water to chloroform (36 nm) was much smaller than the half bandwidth of NRs (205 nm). It was shown that the refractive indices of the surrounding medium did not perturb the local refractive indices of the NRs on a glass substrate.

In the present work, NRs were deposited on a glass substrate through electrostatic interaction between polyanions on NRs and polycationic-modified surfaces. Peak shifts of the longitudinal SP bands were monitored as a function of concentration of antigen in aqueous solution.

The NRs were synthesized by a photochemical method[11] in a joint research project between Mitsubishi Materials Corp. and Dai-Nihon-Toryo Co., Ltd. The initial ζ potential of the NRs was +20 mV, which originates from hexadecyltrimethylammonium bromide (CTAB) bilayers on the NR surfaces. A typical procedure for surface modification of NRs was as follows. As-prepared NRs were centrifuged twice at 15000 × g for 10 min and decanted to remove excess CTAB from the solution. The positively charged surfaces of the NRs were wrapped with poly(styrene sulfonate) (PSS, $M_w = 70000\,g\,mol^{-1}$, Aldrich) through electrostatic interaction.[12,13] The PSS-modified NRs are denoted PSS–NR. The ζ potential of PSS–NRs was typically −21.5 mV. The absorption spectrum of a PSS–NR solution shows double peaks that are typical for colloidal NRs, indicating modification by PSS of the NRs without formation of aggregates.

To deposit PSS–NRs on a glass substrate, water-soluble polymers were coated on the substrate layer-by-layer.[7] The resulting hydrophilic glass substrate was sequentially immersed in a poly(allylamine hydrochloride) (PAH, $M_w = 15000\,g\,mol^{-1}$, Aldrich) solution (1 mg mL^{-1}, 20 min), PSS solution (3 mg mL^{-1}, 20 min) and finally PAH solution again (1 mg mL^{-1}, 20 min) to obtain a multilayered polymer film (polyion–substrate). The outermost layer on the substrate was positively charged due to the presence of PAH. The polyion–substrate was then immersed in a PSS–NR solution for 24 h to deposit PSS–NR on the substrate surface. After immersion, the substrate was washed with water and again immersed in a PSS solution (2 mg mL^{-1}, 1 h). The NR-modified polyion–substrate is denoted NR + polyion–substrate. The additional PSS modification was effective in suppression of translocation of the NRs on the substrate surface. The NR + polyion–substrate was immersed in an anti-prostate specific antigen (anti-PSA) monoclonal antibody solution (67 μg mL^{-1} in PBS, pH 7.0). The NR + polyion–substrate was then immersed in a solution of albumin from bovine serum (BSA) (0.01 mg mL^{-1} in PBS, pH 7.6), followed by immersion in PSA solutions (0.1, 0.5, and 1 ng mL^{-1} in PBS, pH 7.4). In each step the immersion time was 1 h. All treatments were performed at room temperature.

Absorption spectra of NRs deposited on substrates were obtained with a spectrophotometer (V-570, JASCO), and ζ-potential measurements were performed using an ELSZ-2 (OTSUKA ELECTRONICS) instrument. Zeta potential was evaluated using a theoretical model for spherical particles. Thus, the value qualitatively indicated the sign and magnitude of the ζ potential of the NRs, which was sufficient for relative evaluation of surface charges of the NRs. A field emission scanning electron microscope (SEM, S-5000, Hitachi) was used for observation of NRs on the substrates.

Figure 1 shows an absorption spectrum and an SEM image of an NR + polyion–substrate. It should be noted that the spectrum was obtained in air, not in solvent. The characteristic longitudinal SP band at 815 nm indicates that the NRs were deposited on the substrate without forming aggregates. The SEM image for the surface supports this interpretation.

Absorption spectra of the NR + polyion–substrates after each surface-modification step are shown in Figure 2. The longitudinal SP bands of the as-prepared NR + polyion–substrates are found at 815 ± 2.6 nm (a). After immersion in

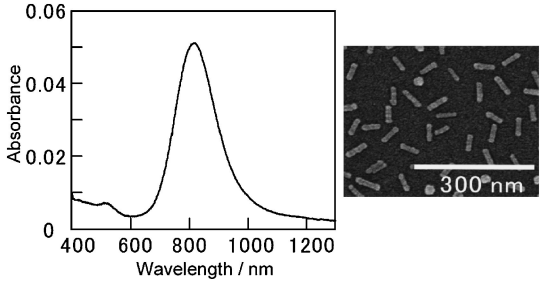

Figure 1. Absorption spectrum and SEM image of PSS–NRs deposited on a glass substrate.

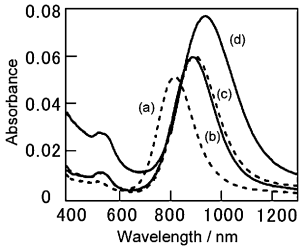

Figure 2. Absorption spectra of the NR + polyion–substrate before (a) and after immersion in the antibody solution (b), the BSA solution (c), and the PSA solution (1 ng mL^{-1}) (d). All spectra were measured in air.

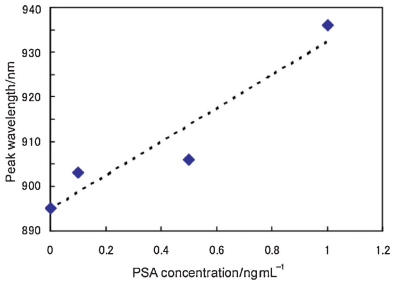

Figure 3. Plasmon band peak position of the antibody-modified NR plate immersed in the antigen solutions (PSA concentrations: 0.1, 0.5, and 1 ng mL^{-1}). All spectra were measured in air.

antibody solution (b), the SP band shifted to 889 ± 1.5 nm, which corresponds to a 74 nm red shift. This peak shift is assignable to the adsorption of the antibodies on the substrate surface. The substrates were then immersed in BSA solutions. The second immersion induced a further peak shift to 894 ± 2.3 nm (c), corresponding to a 5-nm red shift from the previous step: nonspecific adsorption of BSA on the substrates probably induced this peak shift. Finally, the BSA–NR–substrates were immersed in PSA solutions (antigens). When the substrates were immersed in a 1 ng mL^{-1} PSA solution, the peak shifted to 938 nm (d). The peak shift from the previous step was thus about 44 nm. In the every step of the surface treatment, the band width of the longitudinal SP band was retained. In addition, SEM observation indicated that significant aggregation was not induced by the surface treatments (data not shown). Thus, the peak shifts are assignable to changes of refractive indices around the NRs.

Figure 3 shows the peak positions of the longitudinal SP band after immersion in solutions with different PSA concentrations. The peak shifted to longer wavelength as the concentration increased. Control experiments using BSA solutions did not change the peak positions (data not shown). The linear relationship between PSA concentration and SP band position indicates that the NR + polyion–substrate can be a sensor plate for immunosensing of peptides. Even after immersion in a 0.83 ng mL^{-1} PSA solution, a 34-nm peak shift was observed. PSA levels between 4 and 10 ng mL^{-1} are considered to be potentially indicative of prostate cancer.[14] Thus the NR + polyion–substrate shows sufficient sensitivity to be a practical sensor plate for PSA detection.

The total peak shift of the longitudinal SP bands, which was induced by the adsorption of antibodies and antigens, was about 123 nm. This peak shift is much larger than that observed in the previous work[10] and difficult to explain in terms of simple refractive index changes around the NRs.[4] Structural changes of the polyion layers should be taken into account. In the previous work,[10] NRs were fixed on a glass substrate with alkanethiols. Molecular assemblies of the hydrophobic alkanethiols were not affected by adsorption of the hydrophilic polypeptide. It is probable that the outer polymer layers are responsive to the adsorption of the polypeptides (IgG, BSA, and PSA) on the polymer layers. The NRs probably detected changes of optical properties of the polymer layers.

In conclusion, we have found that the longitudinal SP bands of NRs on polyion layers are sensitive to the adsorption of polypeptides. The large peak shifts of the NRs are assigned to the changes of refractive indices of the polyion layers. The longitudinal SP bands in the near-IR regions are advantageous to avoid interference from absorption of organic molecules. Optimization of the polyion layers will improve the response of the longitudinal SP bands and will realize a new optical sensing method for biofunctional molecules.

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