Fluorescence Enhancement Due to Gap Mode of Gold Colloids Immobilized on a Hydrophilic Amino-terminated Glass Substrate

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The preparation and characterization of a novel plasmonic substrate that exhibits remarkable fluorescence enhancement due to the presence of gap mode and hydrophilicity have been studied.

The synthesis and application of metal colloids and nanorods have attracted considerable attention in the fields of chemistry and photonics.[1] Many studies have reported molecular sensing applications on the basis of the plasmon band shift observed in these materials.[2] However, more interesting properties of gold colloids and nanorods, such as the field enhancement effect, i.e., generation of an intense electromagnetic field between colloids separated by a distance of a few nanometers, called gap mode,[3] have not been studied thus far, particularly from the viewpoint of fluorescence spectroscopy.[4] In this paper, we report the preparation and characterization of a novel plasmonic substrate that exhibits remarkable fluorescence enhancement due to the presence of gap mode.

Solutions of gold colloids (20 and 40 nm in diameter, 0.0069 wt %) were purchased from Tanaka Kikinzoku Kogyo Co., Ltd. and used as received. Hydrophilic amino-terminated glass slides ($MAS^{(C)}$ coated) ($60 \times 26\,mm^2$) were purchased from Matsunami Glass Co. and used as received. This glass substrate facilitates the affinity between biological samples and gold colloids due to its strong hydrophilicity and adhesion properties; this affinity is much better than that achieved by a conventional amino-terminated glass substrate coated with aminopropyltrimethoxysilane (APTS).[2] The glass substrates were dipped in the colloidal gold solution for a series of dipping times at room temperature. After dipping the glass substrates for the allocated time, they were carefully washed with ultrapure water and dried in clean air.

Figures 1a and 1b show the changes in the absorption spectra of the glass substrates observed by dipping them in the 20- and 40-nm colloidal gold solutions, respectively, for dipping times up to 10 h. Intense absorption bands with maxima around 520 nm were observed in the spectra, which were consistent with the previously reported plasmon bands of the gold colloids.[5] As the dipping time increased, broad absorption bands appeared in the regions of wavelengths longer than those of the plasmon bands in the case of both solutions. These new bands are attributable to the gap mode, i.e., the optoelectronic interaction between two or more colloids separated by a small distance at a high immobilization density.[3] These observations suggest that the two colloids have different adsorption properties which may be due to the difference in the surface charge.

We selected 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-tetrasulfonic acid disulfuric acid tetrahydrate (TPPS) (Dojin Chemical Co.) as the fluorescence dye. The gold-colloid-immobilized substrates and a fresh MAS-coated glass substrate acting as a control were dipped in the 5 μM solution of TPPS for 10 min. Once removed, the substrates were carefully washed with ultrapure water and then dried in clean air. The absorption spectra of all the modified substrates showed intense Soret and Q bands characteristic of a free-base porphyrin. These bands indicate that the TPPS molecules were irreversibly immobilized by the amino groups on the surface to form ammonium. The intensities of Soret bands of all the substrates were comparable, suggesting that even after the immobilization of gold colloids, sufficient unreacted amino groups remained on the substrates.

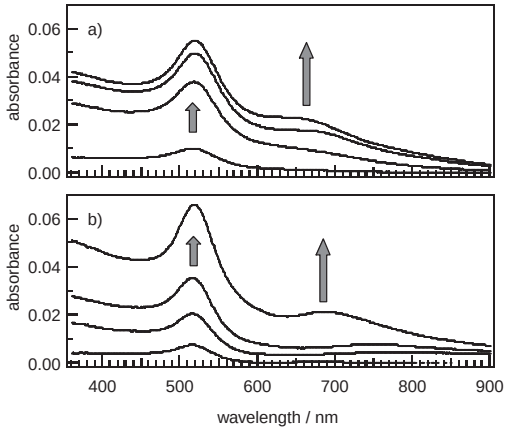

Figure 1. The changes in the absorption spectra of the glass substrates observed by dipping them in the 20- (a) and 40-nm (b) colloidal gold solutions for dipping times of 0.5, 1, 4, and 10 h.

Fluorescence measurement was carried out using an attenuated total reflection (ATR) configuration. The TPPS-immobilized glass substrate was attached to a glass prism using an index matching fluid, and the incident angle was set at 55° to ensure that the porphyrin molecules and gold colloids immobilized on the surface were effectively illuminated by evanescent light. The resulting fluorescence from the surface was monitored through free space.

All substrates showed fluorescence bands characteristic of the free-base porphyrin under excitations of 420, 520, and 600 nm; these bands are consistent with the Soret, $Q_y(0–1)$, and $Q_x(0–1)$ bands, respectively. On comparing the observed fluorescence intensities of the gold-colloid-immobilized substrates with those of the control substrate, it was found that the fluorescence in the case of 40-nm colloids was clearly enhanced, while the fluorescence of 20-nm-colloid-immobilized substrates showed either small enhancements or partial quenching, depending on the experimental conditions. Because the excitation wavelengths overlap with the absorption bands of gold colloids

and the evanescent light has a weak photon flux, it was speculated that the observed enhancement of the fluorescence intensities could be attributed to the masking of the excitation light by gold colloids. Therefore, we must correct the fluorescence intensity at each excitation wavelength using the absorption efficiency, i.e., the absorbance ratio between the gold colloids and porphyrin molecules immobilized on the surface, as shown in the following equations:

$$\mathrm{Abs_{por}} = \mathrm{Abs_{total}} - \mathrm{Abs_{colloid}} \quad (1)$$

$$I_{\mathrm{F\ corr}} = I_{\mathrm{F\ obs}}/(\mathrm{Abs_{por}}/\mathrm{Abs_{total}}) \quad (2)$$

where $\mathrm{Abs_{total}}$, $\mathrm{Abs_{por}}$, and $\mathrm{Abs_{colloid}}$ are absorbances of the substrate, porphyrin, and gold colloids, respectively, and $I_{\mathrm{F\ corr}}$ and $I_{\mathrm{F\ control}}$ are the corrected fluorescence intensity and the fluorescence intensity of the control substrate, respectively. The enhancement factor E_{F} can be defined as the ratio between $I_{\mathrm{F\ corr}}$ and $I_{\mathrm{F\ control}}$.

$$E_{\mathrm{F}} = I_{\mathrm{F\ corr}}/I_{\mathrm{F\ control}} \quad (3)$$

The enhancement factor E_{F} calculated at different excitation wavelengths is plotted against the corresponding dipping times, as shown in Figure 2.

In the case of both solutions, the E_{F} corresponding to the excitation at 420 nm was less than three or close to unity. In contrast, the E_{F} at 520 and 600 nm increased significantly since these excitation wavelengths are exactly or partially consistent with the plasmon and the gap mode bands, respectively. The intensity of the gap mode band of 40-nm colloids increased significantly as the dipping time increased beyond 8 h; correspondingly, the E_{F} value under 600 nm excitation increased remarkably after 10 h. The small E_{F} value in the case of 40-nm colloids under 600 nm excitation before 8 h is attributable to the ineffective overlap of the excitation wavelength with the gap mode band. In contrast, the gap mode band of the 20-nm gold colloids effectively overlapped with the excitation wavelength at 600 nm; however, the values of E_{F} in this case are smaller than those of 40-nm colloids. The dependence of plasmonic effect on the colloid size is expected to be responsible for the striking difference between the enhancement effects of the two colloids.[6] It has been reported that small gold colloids primarily quench the photoexcited states of fluorophores and thus enhance the fluorescence of only those object dyes that have very low fluorescence quantum yields due to the generation of alternate emission pathway by plasmons.[7] On the other hand, gold colloids with large sizes have been reported to enhance the fluorescence of a dye that has a large fluorescence quantum yield.[7] On the basis of these two observations, we can explain the present results as follows. In the case of 40-nm colloids, the fluorescence enhancement is significant only after the intensity of the gap mode band increases significantly; in contrast, in the case of 20-nm colloids, the fluorescence quenching competes with fluorescence enhancement even as the intensity of the gap mode band increases.

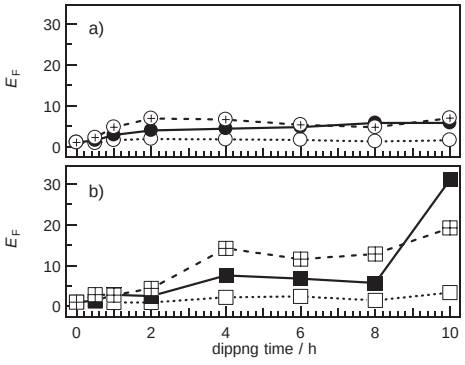

Figure 2. The increases in the fluorescence enhancement factors (E_{F}'s) for 20- (a) and 40-nm (b) gold colloids with increasing the dipping time under excitation of 420 (dotted line), 520 (dashed line), and 600 nm (solid line).

It is worth mentioning that the substrates immobilized with 40-nm colloids after dipping for a prolonged time showed a new fluorescence band at around 770 nm.[8] The origin of the new band is still unclear. However, the new band was not observed under the rather insufficient aggregation condition[9] and the wavelength region coincides with that of the fluorescence of a porphyrin J-aggregate.[10] Accordingly, the gaps formed between 40-nm colloids were probably more suitable for facilitating the aggregation of porphyrin molecules or for effective excitation of the aggregates formed on the colloid surfaces by the enhanced electromagnetic field. This result suggests that for the practical applications of the gap mode, "gap friendly" molecular designs must be developed.

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