Surface modification of gold nanorods with synthetic cationic lipids

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Colloidal gold nanorods (GNRs), which were passivated with cationic cerasome-forming lipids having triethoxysilyl groups, were obtained in the aqueous phase by sonication of the mixture of lipids and GNRs.

GNRs show remarkable surface plasmon (SP) bands in the visible and near infrared (IR) regions.[1] The SP bands correspond to the absorption and scattering of irradiated light. The absorption associated with GNRs enables these colloids to act as photosensitizers for photo-thermal therapy,[2,3] or gene carriers activated by photoirradiation.[4,5] As well, the scattering of near-IR light enables GNRs to behave as markers for dark field (light scattering) observation of living cells or tissues.[2] To realize practical applications of nanorods in biochemistry, surface modification is a key technique, because the toxicity and affinity of these GNRs to living systems are affected by surface conditions. It is well known that hexadecyltrimethyammomiun bromide (CTAB), which is essential to synthesize GNRs, is toxic to every bio-system. Accordingly, as-prepared NRs were inappropriate for bioscience. Centrifugation to remove free CTAB not adsorbed on the nanorod surfaces was a practical approach to produce less-toxic GNRs;[2] however, if the CTAB would be completely removed from the solution, GNRs will lose stability in the colloidal dispersion and form aggregates. The replacement of CTAB with alternative amphiphilic molecules is a straightforward means for obtaining well-designed functional GNRs. Silica coating is one possible means of surface modification to produce biocompatible GNRs.[6] In our previous work, we used polyethylene glycol (PEG)[7] and egg phosphatidylcholine (PC)[3,4,8,9] as alternative stabilizing agents. PEG derivatives are widely used as biocompatible polymers for drug/gene delivery systems[7,8,10] while PC is a typical phospholipid contained in most biomembranes. We reported that the PEG- and PC-passivated GNRs were biocompatible.[7,8]

Lipids play important roles as matrices for fabricating biochemical functions in living systems.[11] Synthetic lipids have been studied to analyze the functionality of biosystems or to design a new biomimetic system having unique functions that cannot be realized with naturally-occurring lipids. A variety of characteristics of the synthetic lipids afford functionalities of novel biomimetic systems,[12] such as nano-reactors, drug/gene carriers, supramolecular devices, *etc.* Organoalkoxysilane lipids are a novel kind of synthetic lipid which form liposomal membranes, partially covered with silica (named Cerasome).[13] Due to the formation of a siloxane network on the surface of the Cerasome, the lipids formed extremely stable vesicles even under high ion concentration or in the presence of micellar-forming single-chain amphiphiles.

In our previous work, we prepared PC-passivated GNRs,[3,4,8,9] which were convenient nanoparticles useful as markers or photosensitizers; however, the PC is not a biofunctional lipid. If some of the PC molecules on the GNR surface could be replaced with synthetic lipids, a variety of functionalities will be given to the GNRs. Unfortunately, our previous method needed an excess amount of PC more than the estimated surface areas of the GNRs. In a typical case, 10 mg mL^{-1} of PC was dissolved in chloroform to obtain PC-passivated GNRs.[8,9] In addition, most of the PC was wasted after the preparation, because of CTAB contamination in the chloroform phase. In many cases, synthetic lipids having sophisticated functionalities are difficult to synthesize. It is true that our previous method is not a practical way to produce synthetic lipid-passivated GNRs. A new procedure for replacing PC with synthetic lipids has been expected. In this work, we tried to develop a practical way for preparing GNRs passivated with cationic cerasome-forming lipids (**1**) and cationic non-silylated lipids (**2**).

The preparation of the synthetic lipid (**1**,[14] **2**[15]) was described previously. All other chemicals were commercially available and used without further purification. GNRs were synthesized based on a photochemical method[16] in a joint research project of Mitsubishi Materials Corp. and Dai-Nihon-Toryo Co. Ltd. The colloidal GNR solution was kept in a refrigerator at 4 °C and the precipitated CTAB was removed by filtration. And then, the GNRs were extracted to a chloroform phase containing an appropriate amount of PC; 20 mL of GNR aqueous solution was shaken well with 10 mL of PC chloroform solution (2.5–10 mg mL^{-1}). The mixed solution was kept at 80 °C for two hours in a sealed tube, and then the phase-separated chloroform phase was collected. The GNR chloroform solutions were centrifuged twice at 1000 × g to remove the excess PC. In order to obtain surface–modified GNRs, the precipitated GNRs were redispersed in chloroform containing 32 μM of synthetic lipids (**1** or **2**) or dimyristoylphosphatidylcholine (DMPC), and then agitated with a vortex mixer for 10 min. The mixed solution was put into a vessel to evaporate the chloroform, and then dried in vacuum. The residue was sonicated (30 W, 1.5 min × 3) with a probe-type sonicator (Sonifier 250D, Branson Ultrasonic) with 3 mL of water. The zeta potentials of the GNRs were obtained using a Zetasizer nano ZS (Malvern Instruments). Transmission electron

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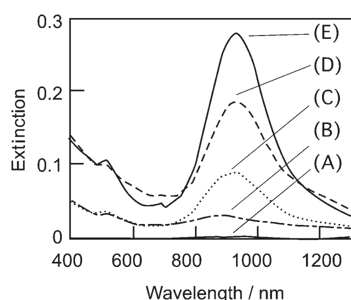

Fig. 1 Extinction spectra of chloroform phases after the extraction procedures. PC concentration in chloroform phases: (A) 0, (B) 2.5, (C) 5, (D) 7.5, (E) 10 mg mL^{-1}.

microscopic (TEM) observation was performed using a JEM-3100-FEF (JEOL).

Fig. 1 shows the extinction spectra of the chloroform phases after the extraction procedures in the absence (A) and presence (B–E) of PC in the chloroform phase. In the absence of PC (A), the extraction of GNRs into the chloroform phase was negligible. In contrast, the PC containing chloroform phases (B–E) after the extraction procedures contained a small amount of GNRs, which was indicated by the distinctive SP bands having two peaks in the visible and near-IR regions. More concentrated PC chloroform solutions gave larger SP bands. It is clear that the PC molecules assisted the extraction of the GNRs into the chloroform phases. At the present stage of this work, we do not have quantitative data to discuss the surface conditions of the GNRs in the chloroform phases; however, it is probable that hydrophilic interactions between the CTAB bilayers on the GNRs[17] and the PC molecules contribute to the colloidal distribution of the GNRs in the chloroform phase. In our previous papers,[8,9] the same extraction procedures gave PC-passivated GNRs in the aqueous phases. In these cases, the phase separated solutions were shaken moderately, and then the solutions were kept in a refrigerator (4 °C). The detailed behaviour of the extraction to obtain PC-passivated GNRs has already been reported in another paper.[9] In this work, in order to obtain the GNR-chloroform-solution, CTAB in the aqueous solution was removed first by precipitation and then by filtration. The aqueous GNR-solutions and PC-chloroform-solutions were shaken well, and then kept at 80 °C for two hours. These procedures were effective for the extraction of the GNRs into chloroform phases.

Fig. 2 indicates the extinction spectra of the aqueous solutions after sonication of the dried mixtures prepared with chloroform solutions of GNRs and the lipids. In the absence of lipids (A) and the presence of DMPC (B) in the GNR-chloroform-solutions, the sonication did not give colloidal GNRs in water. In the presence of **1** or **2**, the solutions after the sonication showed extinction peaks that were assignable to colloidal GNRs ((C) and (D)). In the case of **1** (C), the spectrum showed a somewhat broad and red-shifted SP band in comparison with that of **2**. This spectral change probably originated from the coupling of SP oscillations among a few numbers of GNRs;[18] that is, the redispersed GNRs with the aid of **1** formed smaller agglomerates in the aqueous phase than that of **2**. The zeta potentials of the nanorods were evaluated by using the solutions of (C) and (D) in Fig. 2. A CTAB-passivated GNR-solution (twice centrifuged and redispersed in water) was also used for zeta potential measurements. All zeta potentials were positive (CTAB: +67 mV, **1**: +29 mV, **2**: +47 mV). The zeta potentials suggested that the two synthetic lipids were adsorbed on the GNRs.

The TEM image (Fig. 3a) showed that the shape of the GNRs was retained after the sonication with **1** in a water phase. Electron energy-loss spectroscopy (EELS) mapping indicated the presence of Si just around the GNR surface (Fig. 3b). Energy dispersive X-ray spectroscopy (EDS), shown in Fig. 3c, also indicated the presence of Si in the sample sonicated in the presence of **1**; in contrast, no Si signal was observed in the case of **2**. Thus, we concluded that **1**-passivated GNRs were certainly obtained in aqueous solution as colloidal nanoparticles.

In conclusion, we could extract GNRs into a PC-chloroform-solution. With the assistance of a very small amount of synthetic lipids (**1** and **2**), the GNRs were redispersed in an aqueous phase. With this method, it will be possible to realize colloidal GNRs passivated with various biofunctional lipids. Furthermore, the rigid SiO_2 shells of the **1**-passivated GNRs contribute to the stabilization of the colloidal dispersion of GNRs. The additional surface modification of the SiO_2 shells by using silane coupling agents will realize a variety of biofunctional GNRs that will open up new applications of GNRs for biochemical fields.

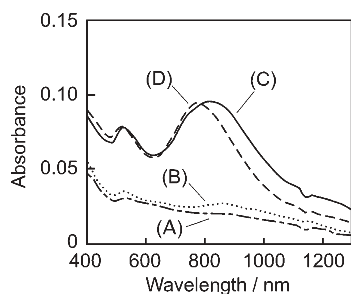

Fig. 2 Extinction spectra of aqueous solutions after the sonication in the absence (A) and the presence of the lipids (DMPC, B; **1**, C; **2**, D).

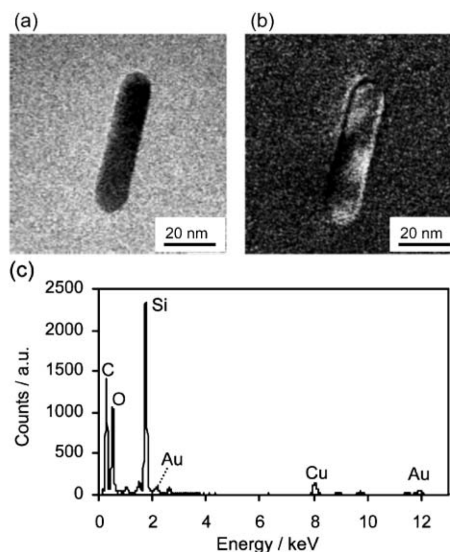

Fig. 3 TEM image (a) and Si mapping based on EELS (b) of **1**-passivated GNRs. The elemental analysis based on EDS (c).

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Notes and references

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