Photochemical deposition of noble metal ultrafine particles onto liposomes

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Composite particles consisting of liposomes loaded with silver or gold ultrafine particles have been prepared by UV irradiation ($\lambda = 253.7$ nm) of $AgClO_4$ or $NaAuCl_4$ aqueous solutions in the presence of L-α-dimyristoyl phosphatidylcholine liposomes. The diameter of the metal particles was <5–7 nm. The absorption peak of the composite particles was observed at $\lambda = 380$–390 (silver) or 540 nm (gold). The absorption spectra of the liposome/silver composite particles were broad and weak. The quantum yield of photoreduction of silver ions increased in the presence of liposomes. The usefulness of the liposome as a matrix for loading and stabilizing the noble-metal ultrafine particles has been demonstrated.

Ultrafine particles of noble metals, such as silver and gold have attracted considerable attention in the fields of photophysicochemistry[1–4] and material science related to non-linear optical materials.[5–9] We have studied the photochemical formation of colloidal metals in solutions in the presence of protective agents such as surfactants[10,11] or polyelectrolytes.[12,13] Agglomeration processes of metallic atoms produce clusters, ultrafine particles and finally, bulk precipitates of the metal. If a surfactant or polyelectrolyte is present, it adsorbs on the surface of the ultrafine particles, preventing further growth and aggregation. On the other hand, the preparation of the ultrafine metal particles in o/w microemulsions has been reported.[14] The particle growth and aggregation can also be controlled in microemulsions. When micelles, polymers or microemulsions are used as matrices, each ultrafine particle is well isolated and dispersed in the solution.

Some well dispersed solid particles can act as matrices loaded with many ultrafine metal particles and work as another type of protective agent on which particle growth and aggregation are suppressed. The special feature of this system is the high density of metal particles within the matrix. We have shown that the third-order non-linear optical susceptibility of densely gathered silver particles is much larger than that of separated particles.[7] It is clearly worthwhile to prepare stable dispersions of densely gathered metal particles. On the other hand, hybrid particles containing metal particles and organic functional molecules have attracted attention in relation to new optical materials.[1,8,9] Considering affinity with organic molecules, dispersibility in the solution and light scattering, desirable matrices to support ultrafine metal particles are organic small particles of diameter <100 nm.

Liposomes are small capsules of size of tens of nm[15–17] which are surrounded by a self-assembled bilayer lipid membrane. There have been many studies of liposomes loaded with semiconductor particles such as CdS.[18] Moreover, it has been reported that Ag_2O ultrafine particles can be created in the inner water phase of liposomes.[19] However, up to now, there have been only a few studies of noble metal particles supported on or within liposomes.[8,20] When we attempted to reduce metallic ions in the presence of liposomes by chemical reduction with hydrazinium sulfate the liposomes were destroyed and the solution became turbid. In this study, we attempted to fabricate liposomes loaded with ultrafine silver or gold particles by photoreduction.

Experimental

L-α-Dimyristoyl phosphatidylcholine (DMPC; Sigma, 99% purity) and $AgClO_4$, $NaAuCl_4$, $CHCl_3$, NaOH, NaCl (nacalai tesque, special grade) were used without further purification. DMPC liposomes were prepared in aqueous metallic salt solutions using a sonicator in the dark.[21] DMPC (20 μmol) in chloroform was slowly stripped of solvent in a round-bottomed flask *in vacuo* at 313 K using a rotary evaporator. Thin films of DMPC at the flask surface were then hydrated with *ca.* 10 cm^3 of an aqueous solution of a metallic salt at 313 K and the mixture shaken vigorously. The concentration of the metallic salt was 0.5 ($AgClO_4$) or 0.4 mmol dm^{-3} ($NaAuCl_4$). The solution was then sonicated with a 200 W bath-type sonicator, Model NS200–6U (Nippon Seiki Co.) at 313 K under N_2 bubbling until the solution was no longer turbid (20–30 min). The pH of the solution was adjusted to *ca.* 6 with 0.1 mol dm^{-3} NaOH. The liposomes prepared in this manner were small unilamellar vesicles, whose diameter was estimated to be 30–40 nm.[21]

Photolysis of liposome solutions in a $10 \times 10 \times 40$ mm^3 rectangular quartz vessel was performed with UV light ($\lambda = 253.7$ nm) using a 200 W low-pressure mercury lamp as described in previous papers.[10–13] The number of incident photons was 1.4×10^{16} cm^{-2} s^{-1}. Irradiation of the solution was carried out in air at room temperature. Absorption spectra of the solutions were measured using a Shimadzu spectrophotometer (UV-260). For the transmission electron microscopic (TEM) observations, the solution was placed on carbon coated copper grids and allowed to dry. A JEOL JEM-1200EX transmission electron microscope was used at an operating voltage of 80 keV. The amount of the reduced metal was determined by atomic absorption spectroscopy after purification of the metal particles by dialysis.

Results and Discussion

Absorption spectra of $AgClO_4$ solutions in the presence of liposomes before and after UV irradiation are shown in Fig. 1. Before UV irradiation, only Rayleigh scattering[22] by the liposomes was evident. After UV irradiation, the solution remained homogeneous and transparent, while a broad absorption band appeared in the visible region. Such an absorption spectrum was observed even when the irradiation time was as short as one minute. The shape of the spectra was different from the

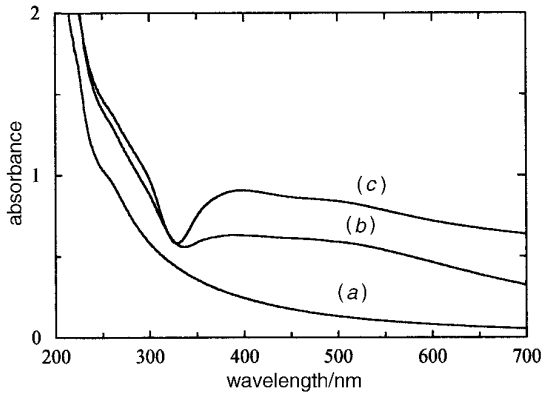

Fig. 1 Absorption spectra of the $AgClO_4$ aqueous solution in the presence of liposomes before and after UV irradiation. The solution contained 0.5 mmol dm^{-3} $AgClO_4$ and 2 mmol dm^{-3} DMPC. Irradiation time: (*a*) 0 min, (*b*) 1 min, (*c*) 10 min.

usual colloidal absorption band of silver.[3,4] The peak position and absorption intensity of colloidal silver obtained by the so-called 'photo-acetone method' were $\lambda = 380$–390 nm and >20 per mmol dm^{-3} of silver, respectively.[10] Although the peak position shown in Fig. 1 was not so different from this, the band shape was much broader and the absorption intensity lower.

The dependence of the amount of the reduced silver on irradiation time is shown in Fig. 2. The rate of photoreduction was high and the conversion efficiency of silver ion (2.5 μmol) was 70% after 10 min irradiation. The quantum yield of photoreduction of the silver ions was 0.12. We have previously shown that irradiation of $AgClO_4$ solutions with 253.7 nm light induces photo-oxidation of H_2O by excited Ag^+, resulting in the formation of silver atoms.[23]

$$Ag^+ + H_2O \xrightarrow{h\nu} Ag^0 + H^+ + OH^{\cdot} \quad (1)$$

In the absence of a protective agent, the quantum yield of photoreduction was only 0.008.[23] Therefore, the quantum yield is increased by a factor of *ca.* 15 in the presence of liposomes. The reason for the low quantum yield in the absence of protective agents is re-oxidation of silver atoms and clusters immediately after their formation. In the presence of liposomes, silver atoms are stabilized at the liposome surface and form ultrafine silver particles at these sites. Silver atoms and clusters trapped in liposomal membranes are resistant to re-oxidation.

A TEM photograph of liposomes loaded with silver ultrafine particles is shown in Fig. 3(*a*). Many black spherical ultrafine

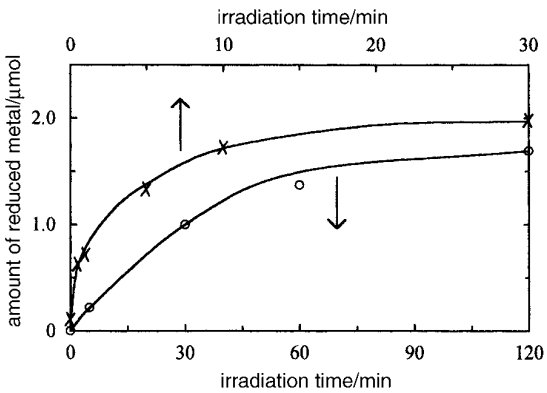

Fig. 2 Dependence of the amount of reduced metal on irradiation time: (×) 0.5 mmol dm^{-3} $AgClO_4$ and 2 mmol dm^{-3} DMPC; (○) 0.4 mmol dm^{-3} $NaAuCl_4$, 2 mmol dm^{-3} DMPC and 0.1 mol dm^{-3} NaCl. Amount of sample for analysis was 5 cm^3.

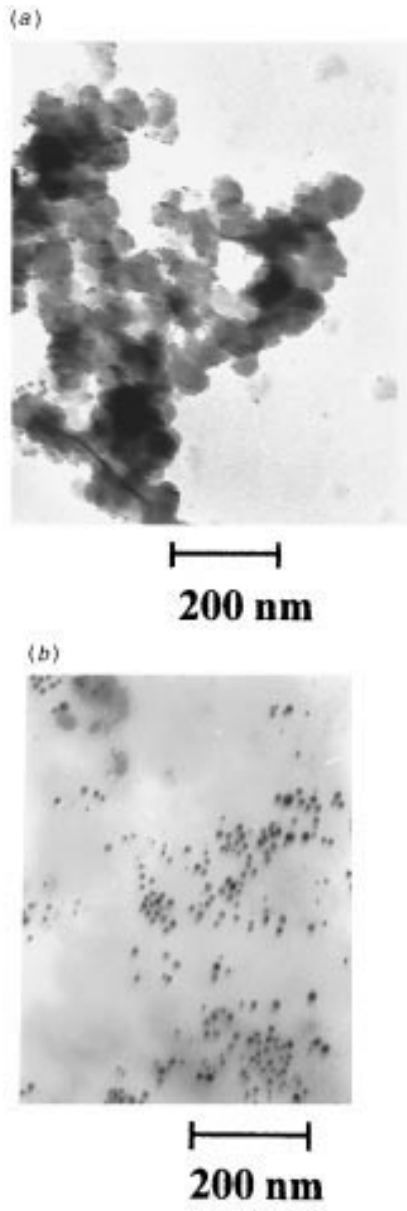

Fig. 3 Transmission electron micrographs of noble metal ultrafine particles on liposomes: (*a*) 0.5 mmol dm^{-3} $AgClO_4$ and 2 mmol dm^{-3} DMPC, irradiation time 1 min; (*b*) 0.4 mmol dm^{-3} $NaAuCl_4$, 2 mmol dm^{-3} DMPC and 0.1 mol dm^{-3} NaCl, irradiation time 60 min

particles, with diameters <5 nm, are seen on the surface of liposomes of 50 nm diameter (lighter shading). The large dark regions also correspond to liposomes overlapping with each other during sample preparation for TEM observation. The ultrafine silver particles did not aggregate into bulk silver precipitates in the solution, since the ultrafine particles were trapped on the liposome having large surface area and many trap sites. A particle size distribution of the silver ultrafine particles on the liposomes is shown in Fig. 4(*a*). More than 300 individual particles were measured to determine the distribution. The mean particle diameter was 4 nm and the percentage of particles <2 nm was *ca.* 25%. The plasmon band of silver particles is affected by the size, shape and aggregation state of the particles and the surrounding medium.[1] If the silver particles are <5 nm, the plasmon band is gradually weakened and broadened with decreasing particle size, owing to the size-dependent relative permittivity of silver.[24] On the other hand, the plasmon band of aggregated particles is very

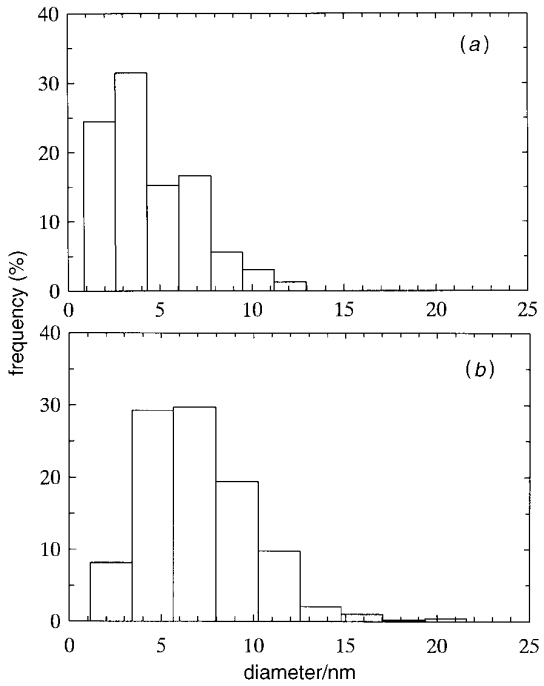

Fig. 4 Particle size distributions of noble metal ultrafine particles on liposomes: (*a*) 0.5 mmol dm^{-3} $AgClO_4$ and 2 mmol dm^{-3} DMPC; irradiation time 1 min; (*b*) 0.4 mmol dm^{-3} $NaAuCl_4$, 2 mmol dm^{-3} DMPC and 0.1 mol dm^{-3} NaCl, irradiation time 60 min

broad and has a longer-wavelength tail.[1,25] The densely gathered silver ultrafine particles on the liposomes seem to be analogous to aggregated particles. Furthermore, the position and shape of the plasmon band of silver-coated dielectric nanoparticles are predicted to vary widely with alteration of the ratio of thickness of coated silver to the diameter of the dielectric core particles.[1,9] The particle size distribution of liposomes would introduce a distribution of silver particle densities on the liposomes and the ratio of silver thickness to core diameter. Although rarely existing large particles whose diameter is similar to the liposomes might affect the absorption spectra, we assume that the above mentioned factors also lead to broad absorption spectra of the liposomes loaded with ultrafine silver particles.

Fig. 5 shows the change of the absorption spectra upon UV irradiation of liposome solutions containing $NaAuCl_4$. The absorption peak at $\lambda = 320$ nm prior to UV irradiation was

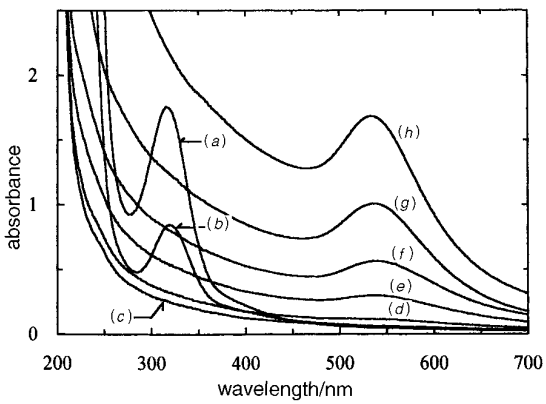

Fig. 5 Absorption spectra of the $NaAuCl_4$ aqueous solution in the presence of liposomes before and after UV irradiation. The solution contained 0.4 mmol dm^{-3} $NaAuCl_4$, 2 mmol dm^{-3} DMPC and 0.1 mol dm^{-3} NaCl. Irradiation time: (*a*) 0 min, (*b*) 2 min, (*c*) 5 min, (*d*) 10 min, (*e*) 20 min, (*f*) 30 min, (*g*) 60 min, (*h*) 120 min.

assigned to the LMCT (ligand to metal charge transfer) transition of $AuCl_4^-$.[26] After 5 min irradiation, the LMCT band disappeared. After an induction period of *ca.* 20 min, an absorption peak of colloidal gold developed at *ca.* $\lambda = 540$ nm. A typical TEM photograph and a particle size distribution of the gold ultrafine particles on the liposomes are shown in Fig. 3(*b*) and 4(*b*), respectively. Although liposomes are not clearly seen, the gold particles are assumed to be located on the liposome surface. The mean particle diameter of the gold ultrafine particles was 7 nm, which was somewhat larger than the silver particles in Fig. 3(*a*) and 4(*a*). Although the plasmon band of gold particles < 3 nm almost disappears because of the size-dependent relative permittivity, the plasmon band of gold particles of *ca.* 7 nm diameter is not significantly reduced in intensity.[27–29] The absorption spectra of the liposomes loaded with gold ultrafine particles shown in Fig. 5 were not as broad as those with silver ultrafine particles. We anticipate that the difference of the size of metal particles, particle density on the liposomes and relative permittivities of the metals would result in different features of these plasmon bands. The position of the plasmon band of the gold ultrafine particles on the liposomes ($\lambda = 540$ nm) was somewhat red-shifted from that of gold particles of similar size in aqueous solution ($\lambda =$ 520 nm).[28,30] It has been reported that the plasmon band of gold particles was red-shifted by *ca.* 20 nm upon changing the refractive index of the surrounding medium from 1.3 to 1.6.[30] On the other hand, the plasmon band of gold particles (9 nm in diameter) protected by hexadecyltrimethylammonium chloride (CTAC) in aqueous solution was located at $\lambda = 540$ nm.[31] CTAC has a same head group as DMPC. It is plausible that the red-shift of the plasmon band of gold ultrafine particles in the bilayer lipid membrane of DMPC liposomes is introduced by the change of refractive index of the surrounding medium and adsorption of trimethylammonium.

The dependence of the amount of the reduced gold on the UV irradiation time is shown in Fig. 2. The rate of photoreduction was much slower than that of $AgClO_4$, while the conversion efficiency of gold ions (2.0 μmol) was 85% after 120 min irradiation. The quantum yield of photoreduction of gold ions was 0.005, which was smaller than that of aqueous solutions of $AuCl_4^-$ in the absence of protective agents (0.013).[12] When UV light of 253.7 nm was used the photo-decomposition of $AuCl_4^-$ is initiated by the excitation of the LMCT band. The following reactions then lead to Au^0.[32]

$$AuCl_4^- \xrightarrow{h\nu} AuCl_3^- + Cl^\cdot \quad (2)$$

$$2AuCl_3^- \longrightarrow AuCl_4^- + AuCl_2^- \quad (3)$$

$$AuCl_2^- \xrightarrow{h\nu} Au^0 + Cl^\cdot + Cl^- \quad (4)$$

For photolysis of $AuCl_4^-$ in the presence of liposomes, an induction period prior to growth of the colloidal absorption band was seen. Judging from disappearance of the LMCT band after 5 min irradiation (Fig. 5), one-electron reduction [eqn. (2)] may be complete within 5 min. It is plausible that $AuCl_3^-$, $AuCl_2^-$, Au^0 or small gold clusters are stabilized in liposomal membranes during the induction period. If $AuCl_3^-$ is trapped and stabilized, the induction period would reflect the suppression of the disproportion reaction [eqn. (3)]. However, the absorption band of $AuCl_3^-$ reported in the literature[32] is not evident in the absorption spectrum after 5 min of irradiation. Although Torigoe and Esumi[31] reported the existence of a long-lived transition state during photoreduction of $AuCl_4^-$–cationic surfactant complexes, the nature of the stabilized species in the induction period is unclear at present.

We have previously prepared colloidal silica loaded with

ultrafine silver particles by a photochemical method.[12] The colloidal silica employed consisted of monodisperses spherical particles of 13.5 nm diameter. Ultrafine silver particles of 2–3 nm diameter were loaded on the colloidal silica surface in the same manner as on the liposome surface. The quantum yield of photoreduction was 0.053. Because of the small particle size and large specific surface area, colloidal silica improves the quantum yield of photoreduction as do DMPC liposomes. The absorption peak of colloidal silica loaded with silver particles was observed at $\lambda = 410$–420 nm, and the shape of the absorption band was very broad. As the relative permittivity of colloidal silica is similar to that of phospholipid membranes, it is reasonable that the absorption spectra of the similarly sized insulator particles, *i.e.* DMPC liposomes and colloidal silica, loaded with ultrafine silver particles resemble one another.

In contrast to colloidal silica, liposomes are capable of incorporating organic functional molecules containing a long conjugated system, such as dyes. We have made initial attempts to prepare liposomes loaded with both noble metal ultrafine particles and organic dye molecules. When DMPC liposomes loaded with silver particles were added to an aqueous solution of a cyanine dye, 5,5′-dichloro-3,3′-diethyl-9-phenylthiacarbocyanine chloride, the cyanine dye molecules were adsorbed on the composite liposomes. On the other hand, ultrafine silver particles were deposited on DMPC liposomes containing *N*-ethyloctadecylrhodamine B by the photolysis of an $AgClO_4$ solution in the presence of the liposome containing dye molecules. The fabrication of hybrid particles containing metals and organic materials is an interesting subject in connection with new optical materials[1,8,9] and research along this line is now in progress.

Conclusion

Novel composite particles, DMPC liposomes loaded with ultrafine silver or gold particles, have been prepared by UV irradiation ($\lambda = 253.7$ nm) of $AgClO_4$ or $NaAuCl_4$ solutions in the presence of liposomes. The absorption peaks of these particles were observed at $\lambda = 380$–390 nm (silver) and 540 nm (gold), respectively. The shape of the absorption spectra of the liposome/silver composite particles and their absorption intensity were broad and weak, respectively. The quantum yield of photoreduction of silver ions in aqueous solutions increased in the presence of liposomes. The liposome surface is not only the reaction center for metal deposition but also plays a role as a trapping site at which re-oxidation is suppressed. Like colloidal silica, liposomes are useful matrices for loading and stabilizing ultrafine noble metal particles.

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