Colloids and Surfaces A: Physicochem. Eng. Aspects 342 (2009) 92–96

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Controlling loading and optical properties of gold nanoparticles on liposome membranes

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ARTICLE INFO

Article history:
Received 11 November 2008
Received in revised form 9 April 2009
Accepted 10 April 2009
Available online 22 April 2009

Keywords:
Gold
Nanoparticles
Liposomes
Synthesis
Scattering spectra

ABSTRACT

Gold nanoparticle-loaded liposomes can serve as important biophysical and biochemical tools. A rapid, green, one-step synthetic method has been used for direct and controlled loading of gold nanoparticles onto liposomes. The effects of various synthetic parameters on nanoparticle loading as well as on optical properties of the liposome-anchored nanoparticles are discussed. Gold nanoparticle-loaded individual liposomes have been characterized by dark-field microscopy and optical spectroscopy.

1. Introduction

Rapidly increasing demand for nanomaterials with enhanced functionality and smart responsiveness is promoting development of various hybrids and composite nanosystems since it allows incorporation of desired multiple features into such systems [1]. Gold nanoparticle anchored-liposomes constitute one such class of composite materials. Gold nanoparticles are well known for their characteristic optical properties, unique surface chemistry, and high visibility in electron microscopy [2]. These features make gold nanoparticles well suited for widespread biomedical applications, such as spectroscopic markers, DNA-binding agents, transfection vectors or protein inhibitors [3]. As liposomes have numerous applications as biophysical and biochemical tools, by anchoring gold nanoparticles to liposomes one can get a composite system with new sets of properties that can find many applications in biochemical sciences. Liposomes, more specifically giant unilamellar vesicles (GUVs), are used as model systems for studying physical aspects of membranes due to their size and structure being comparable to typical biological cells [4]. Loading gold nanoparticles onto the liposomes provides several advantages concerning detection and imaging, thereby making such systems suitable for new applications such as liposome internalization or transport inside cells [5]. Moreover, these hybrid particles can be modified to conjugate with specific pharmacological agents for targeted and controlled drug delivery. Chiu et al. have reported that chemical transformations between the contents of individual phospholipid vesicles can be initiated by electroporation or electrofusion [6]. Recently, researchers have demonstrated that gold nanoparticles can enable UV or NIR light-induced, controlled content release from liposomes [7]. This shows that gold nanoparticle-loaded liposome systems are promising candidates for optically addressable delivery systems.

Earlier nanoparticle loading onto liposomes has been done either by mixing preformed nanoparticles with liposomes or by spontaneous in situ reduction of metal ions by one of the liposome forming components [5a,8,9]. Often the spontaneous precipitation methods of gold crystallites in liposomes/vesicles are practically very slow and some of them take days for completion [9,10]. Furthermore, in such cases the extent of loading and size of the nanoparticles on liposomes cannot be easily manipulated on demand. Chow et al. synthesized gold nanoparticles by the phospholipid membrane-bound Pd catalyzed reduction of Au^{3+} ions by hypophosphite [11]. Shelnutt and coworkers reported the synthesis of a diverse range of platinum nanostructures in liposomes [12]. This method, however, required light exposure and a light sensitive reductant along with a photocatalyst [12]. On the other hand, liposomes have been loaded with nanoparticles by mixing preformed particles with liposomes. In these cases, conventional synthetic strategies are used for the production of gold nanoparti-

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doi:10.1016/j.colsurfa.2009.04.014

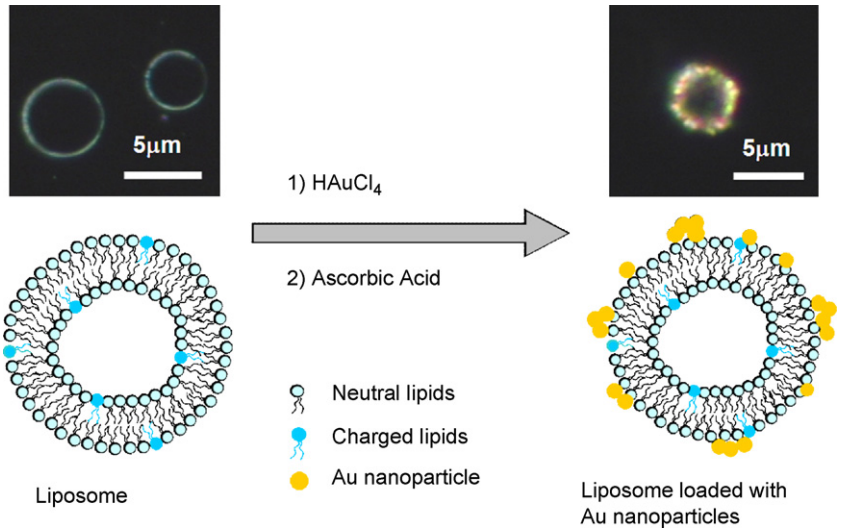

Fig. 1. Lower part: Scheme of the preparation of gold nanoparticle-loaded giant unilamellar vesicles (GUVs). Upper part: Typical dark-field micrographs of the naked (left) and gold nanoparticle-loaded (right) GUVs.

cles, e.g. metal ion reduction via $NaBH_4$ or citrate. However, $NaBH_4$ or citrate synthetic protocols are not suitable for particle loading on biofunctional liposomes. $NaBH_4$ is a toxic and strong reducing agent that can modify biological functional groups. Additionally, the high temperature required for the citrate reduction method destroys the liposomes. In fact, there is hardly any synthetic method reported in the literature for the controlled loading of gold nanoparticles onto liposomes suitable for biochemical applications. Here, we report a 'green' and efficient methodology that can load liposomes with gold nanoparticles in a simple, rapid, and controlled manner. It allows better control than the lipid-reduced particle formation methods over the particle size and number of nanoparticles per liposome as well as their degree of aggregation at the liposome surfaces.

2. Experimental/materials and methods

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (chloride salt) (DOPC+), and 1,2-dioleoyl-*sn*-glycero-3-phosphopropanol (sodium salt) (DOPP) were purchased as 25 mg/ml solutions in chloroform from Avanti Polar Lipids. $HAuCl_4{\cdot}3H_2O$ (99.99%) and L(+)-ascorbic acid (99%) were obtained form Alfa Aesar and Sigma–Aldrich, respectively. All the chemicals were used without further purification. Milli-Q water was used in all experiments.

The growth of giant unilamellar vesicles (GUVs) was carried out in a chamber based on the design by Bagatolli and Gratton [13b]. Nine separate samples can be prepared simultaneously. To produce GUVs, the phospholipids were diluted with chloroform down to concentrations of 1 mg/ml. For the cationic or anionic lipid-doped liposomes, the solutions were prepared on a basis of 10% m/m of the ionic dopant lipid and 90% m/m DOPC. 20 μl of solution per chamber was dispersed on top of the platinum wires. The chambers were then filled with sucrose solutions (10, 300 or 700 mM) and the platinum wires connected to a function generator (Hewlett-Packard 33120A). An AC-voltage of 10 V at 10 Hz was applied for 2 h, followed by 5 minutes of 10 V at 1 Hz to remove the GUVs from the wires. The GUV yield is extremely high, with an average diameter of around 20 μm. GUVs are stable for several weeks at 4 °C. In order to produce doped GUVs, 10% of 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (DOPC+) or 1,2-dioleoyl-*sn*-glycero-3-phosphopropanol (DOPP) was mixed with 90% of DOPC. Then, the preparation of the doped GUVs followed the same procedures as for the undoped GUVs. The yield and size distribution of the doped and undoped GUVs were similar.

The gold nanoparticle loading was carried out as follows. Typically, 0.5 mL of the GUV solution were added to a solution of sucrose and $HAuCl_4$. Then ascorbic acid (1.6 times the concentration of gold ions) was added and mixed to reduce the gold ions. The concentration of $HAuCl_4$ was varied between 4×10^{-6} M and 5×10^{-5} M. The growth of the gold nanoparticles was monitored by observing the extinction of the particle-plasmon with a Varian Cary 5000 UV-Vis-NIR spectrophotometer. The entire growth process takes 1–2 h depending on the gold ion concentration. The GUVs, both before and after gold nanoparticle loading, were analyzed by dark-field microscopy and spectroscopy. For this a Zeiss Axiovert 100 microscope was used with a 100×, 1.0NA water immersion objective. A halogen lamp was used for illumination through a high-aperture dark-field condenser.

3. Results and discussion

We prepared GUVs via the electroformation method from 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) using aqueous sucrose solutions as growth medium [13]. The resulting GUVs are mainly unilamellar, spherical and range in size between 4 and 60 μm, with an average size/diameter around 20 μm. The growth of the gold nanoparticles was monitored by extinction spectroscopy. The entire growth process takes 1–2 h depending on the precursor concentration. In the presence of liposomes, the gold nanoparticles nucleate and grow preferentially on the liposomes. Fig. 1 shows the scheme of the preparation of gold nanoparticle-loaded giant unilamellar vesicles. Control experiments show that the lipids themselves do not reduce gold ions. When aqueous NaCl or $CaCl_2$ is used instead of sucrose as the growth medium, no gold particle formation occurs in the absence of ascorbic acid.

We varied the synthetic parameters systematically in order to explore their roles in controlling particle loading. First, three different concentrations of sucrose solutions (10 mM, 300 mM and 700 mM) were used as growth media. No difference in GUV formation and particle loading was observed. Next, we investigated the influence of the concentration of $HAuCl_4$. For a given concentration of GUVs, a higher concentration of $HAuCl_4$ leads to a larger amount of gold nanoparticles on the GUVs. This can be clearly seen in Fig. 2, where typical dark-field microscopy images of loaded GUVs obtained with the three concentrations of $HAuCl_4$ are shown.

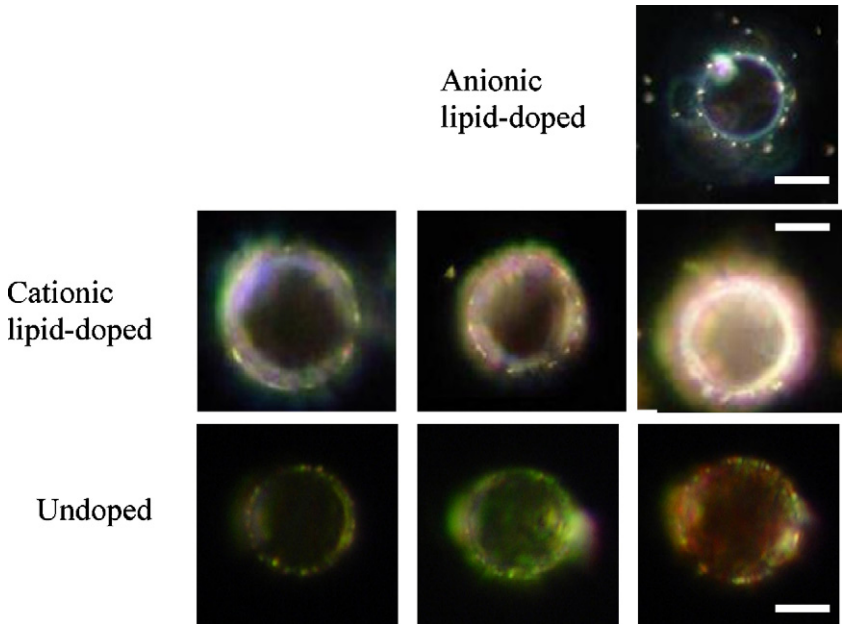

Fig. 2. Controlling loading of the gold nanoparticles onto the liposomes. Dark-field micrographs show the effects of concentration variation of $HAuCl_4$ and the nature of dopant lipid on gold nanoparticle loading. The concentration of $HAuCl_4$ increases from left to right (8×10^{-6} M, 2×10^{-5} M, 4×10^{-5} M corresponding to first, second, and third column, respectively) for three different types of liposomes (shown in each row). Ascorbic acid concentration was 1.6 times that of gold. At lower $HAuCl_4$ concentrations, mostly unloaded liposomes are observed for anionic lipid-doped liposomes. The scale bar equals to 5 μm and is the same for each row.

It has been reported that introduction of ionic surfactants or charged lipids into liposomes changes the liposome properties in several ways [14]. Robertson et al. studied the effects of surfactant charge on phospholipid-reduced particle formations by preparing mixed phospholipid-surfactant vesicles using ionic surfactants, cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulfate (SDS) [10]. These authors, however, reported that particle formation was extremely slow, especially in the case of CTAB, taking weeks to complete. In addition to DOPC liposomes, we studied a cationic and an anionic lipid-doped DOPC liposome systems. Doped GUVs were produced by mixing 10% of 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (DOPC+) or 1,2-dioleoyl-*sn*-glycero-3-phosphopropanol (DOPP) with 90% of DOPC. The cationic lipid DOPC+ and the anionic lipid DOPP have the same chain lengths as DOPC. This kind of doping offers a simple way to further fine tune the particle loading at the liposomes without any addition of dissimilar chemicals, need of pH change, etc.

It was observed that the charge of the lipid dopants had strong influence in controlling the particle loading onto the liposomes. Fig. 2 displays representative dark-field micrographs of gold nanoparticle-loaded undoped DOPC GUV as well as GUVs doped with DOPC+ and DOPP. Visual inspection under the dark-field microscope demonstrates that for the same precursor concentration, more particles are attached to the GUVs doped with cationic lipids and less to the GUVs doped with the anionic lipids. Except for anionic lipid-doped liposomes at lower $HAuCl_4$ concentrations, always most of the liposomes were loaded with gold nanoparticles and the number of unloaded liposomes was $<\sim$10% of the total population of liposomes, depending on the nature of the lipid dopants, and concentration of the precursor. It is not completely clear how gold nanoparticles anchor to the liposome surface. However, variation in the number of liposome-anchored nanoparticles with the nature of the lipid dopant suggests that electrostatic interaction might be responsible for the surface-anchoring. Possibly ammonium group, $>N-^{(+)}$, in the lipid molecule tends to bind to the negatively charged precursor, $[AuCl_4]^-$ and/or negatively charged gold nanoparticles. This binding tendency is modified by the presence of the cationic or anionic lipid dopants, leading to the variation in the number of surface-anchored particles. Additionally, it can be observed that the apparent color of the loaded GUVs under the dark-field microscope depends on the doping of the GUVs. This is an indication that the particles attached to different GUVs have different sizes, morphologies and degree of aggregation.

To obtain better images of the particles' size and morphology, transmission electron microscopy (TEM) was used. Though TEM has a much higher resolution than DFM, it suffers from the facts that GUVs cannot survive the TEM sample preparation step and it is not clear which of the viewed particles were actually attached to the GUVs. However, it was observed that nearly spherical particles formed at low precursor concentrations, whereas at higher precursor concentrations mainly non-spherical and aggregates of gold nanoparticles appeared (Fig. 3). Relatively large sized gold nanoparticles were observed in the anionic lipid-doped GUV systems. This could be attributed to the difference in the interactions between the dopant lipid and the precursor, or gold nanoparticles. Possibly the favorable interaction of the cationic lipids with the precursor anion $[AuCl_4]^-$ results in a higher number of nucleation centers on cationic lipid-doped GUVs, unlike anionic lipid-doped GUVs. The more the nucleation centers, the more the number of particles formed, and hence the less the quantity of gold per particle, thereby forming smaller particles in the cationic lipid-doped GUV systems.

Further insight is obtained by spectrally resolving the scattered light from individual liposomes, as shown in Fig. 4. The spectra from different GUVs exhibit some dispersion but a clear trend can be identified as depicted by the exemplifying spectra shown in Fig. 4. All the spectra can be satisfactorily fitted with three Gaussian peaks. It can be noted here that the spectral behavior of the liposome-bound gold nanoparticles were significantly different from those generated in liposome free solutions [15]. For the particle-loaded liposomes, a first band centered between 425 and 445 nm is observed in all spectra and corresponds to scattering by the liposomes themselves, as corroborated in control measurements. A second band centered between 525 and 535 nm is observed in the spectra of all nanoparticle-loaded GUVs. This corresponds to isolated, nearly spherical gold nanoparticles [16]. Finally, a third,

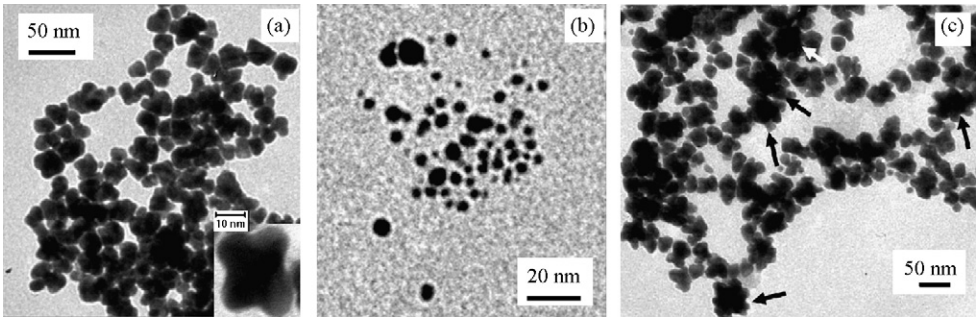

Fig. 3. TEM images of gold nanoparticles produced in DOPC liposomes doped with cationic lipid (a and b) and anionic lipid (c). Images in (a) and (b) show the effects of two different concentrations of $HAuCl_4$. $HAuCl_4$ concentration decreases from 4×10^{-5} M in (a) to 8×10^{-6} M in (b). $HAuCl_4$ concentration remains the same in (a) and (c). Arrows in (c) show relatively large sized gold particles. The concentration of ascorbic acid was 1.6 times that of $HAuCl_4$. Inset in (a) shows a typical tetrapod with scale bar.

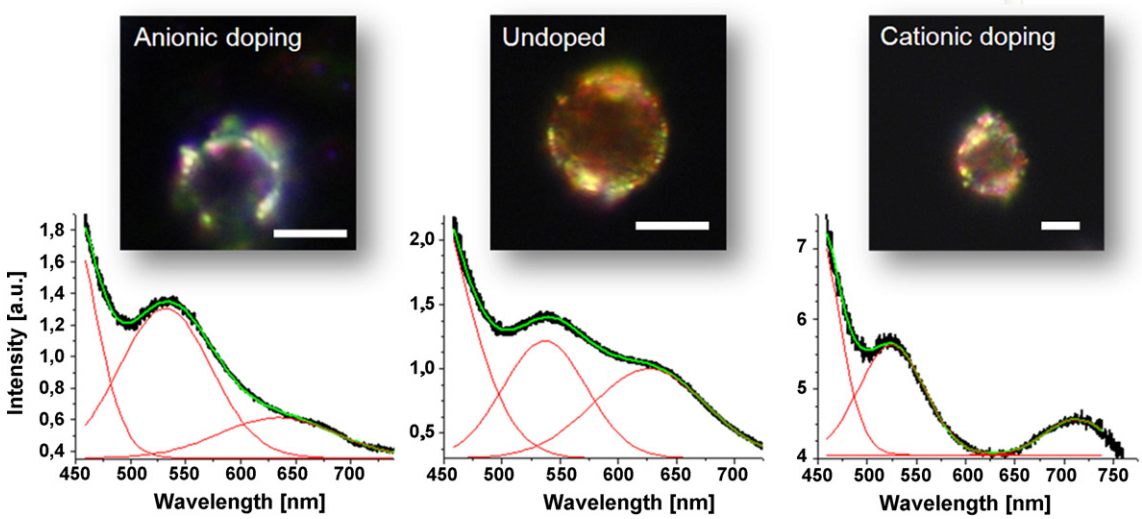

Fig. 4. Effects of GUV doping with ionic lipids on the particle loading and their optical properties. Upper part: representative dark-field micrographs of anionic lipid-doped (1/10 s acquisition time), undoped (1/40 s) and cationic lipid-doped (1/100 s) GUVs loaded with gold nanoparticles. Lower part: representative scattering spectra of single anionic lipid-doped, undoped and cationic lipid-doped GUVs with corresponding three-Gaussian fits. The concentration of $HAuCl_4$ was 5×10^{-5} M and ascorbic acid concentration was 1.6 times that of gold. The scale bar equals to 5 μm.

red-shifted band is found that is assigned to larger, non-spherical particles or aggregates of gold nanoparticles [16,17]. For the anionic lipid-doped and undoped GUVs, this third band is centered between 625 and 640 nm. Comparing the relative intensity of these two bands it can be clearly seen that less particle aggregates are formed on the GUVs doped with anionic lipids, which is clear from the DFM images in Fig. 2 (top panel). This can be owed to the charge repulsion among the anionic lipid, the precursor ($[AuCl_4]^-$) and slightly negative gold nanoparticles. In the case of the cationic lipid-doped GUVs, the third band is further red-shifted to 710–740 nm, corroborating a higher degree of aggregation of the particles at the liposome surface [16,17]. The positively charged lipids can favorably interact both with the negatively charged precursor, $[AuCl_4]^-$ and negatively charged gold nanoparticles, and favor the formation of more surface-anchored particles and higher degree of aggregation.

4. Conclusion

In conclusion, a room temperature and biocompatible methodology with practical implications for the controlled loading of gold nanoparticles at liposome membranes is presented. The size and number of gold nanoparticles formed, and their degree of aggregation at the liposome surfaces can be controlled by changing the ratio of liposome to precursor concentrations or by doping the liposomes with ionic lipids. Negatively charged lipids lead to the formation of isolated nanoparticles at the liposome surface. Positively charged lipids induce a higher degree of aggregation of nanoparticles at the liposome surface. The degree of control over the number and degree of aggregation of gold nanoparticles is of fundamental importance for the function of the loaded GUVs. Clear prospects of applications for these liposome-nanoparticle hybrids include the optical generation of enhanced electric fields at the liposome surface for localized photochemistry or optical spectroscopy, and the optical generation of localized heating for controlled diffusion through the lipid membrane [18].

Acknowledgments

We gratefully acknowledge financial support of the DFG via the Excellence Research Cluster *Nanosystems Initiative Munich* (NIM), the LMU*excellent* program, and the Alexander von Humboldt Foundation (TKS).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfa.2009.04.014.

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