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One step synthesis and phase transition of phospholipid-modified Au particles into toluene

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Abstract

We demonstrated that phospholipid (L-α-dipalmitoylphosphatidylcholine, DPPC)-capped gold nanoparticles could be synthesized with one step by reduction of $HAuCl_4$ in aqueous solution using sodium citrate and simultaneously accompanying with the phase transition across the water/toluene interface into the organic phase. Such a transition process has been observed by the color changes and verified by the UV–visual adsorption. Transition electron microcopy (TEM) image shows that the one-step synthesized gold nanoparticles obtained in the toluene phase has approximately 10 nm. Fourier transfer infrared reflectivity (FT-IR) study on the lipid-capped particles demonstrates that DPPC has adsorbed onto the gold nanoparticle surface. Extention of this work to other metals with even smaller sizes and use of different lipids would be possible.
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Keywords: Phospholipid; DPPC; Phase transition; Gold nanoparticles; Liquid/liquid interface

1. Introduction

With the recent development of biomaterials, using biological composites to bind the selected inorganic particles possesses the materials uniquely properties at nanometer scale and lead to novel engineering systems with the combination of biology and nanotechnology [1–3]. Meanwhile they can self or co-assemble into ordered nanostructures with novel mechanical or electronic properties [4]. Phospholipids are traditionally considered as a model system to study the biological membrane and its interaction with proteins due to their well-defined structure and property [5,6]. Typically, the combination of biological molecules (especially lipid) with metal particles has been widely applied as biosensors or for the drug delivery [7–10]. It is reported that "quantum dots" of encapsulated CdS or CdSe semiconductor nanocrystals by phospholipid acted as in vitro fluorescent probes to hybridize to specific complementary sequences when conjugated to DNA [11,12]. Gold and silver colloid particles modified by polymer-peptides have been employed as carriers for gene and drug [13,14]. On the other hand, biomolecules such as purple membrane and protein crystals could be labeled with gold particles to improve their regular arrays [15,16]. Lipid-capped gold particles may also form every typical lipid structure, like micelles, liposomes or bilayers, moreover, they possess a new feature and may serve as a biological probe to carry out various biological detections. However, phosholipid–gold conjugates were usually synthesized on the basis of chemical bonding on the gold particles surface [11]. Here we reported that phospholipid-capped gold particles with a nanosize can be synthesized by only one-step with the reduction of citrate sodium probably in organic phase and simultaneously transferred to the organic phase.

2. Materials

$HAuCl_4 \cdot H_2O$ (99.9+%) and sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99.0%) were purchased from Aldrich. L-α-Dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma. Toluene was received from Beijing Chemical Reagents, Co. All the chemicals are used without further purification. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and has a resistivity higher than 18.2 MΩ cm.

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3. Experimental methods

3.1. Preparation of phospholipid–gold conjugates

In a screw-capped 10 mL glass vessel, 0.9 mg DPPC was dissolved in 2 mL toluene and stirred vigorously for 15 min. Then we dropwised 1 mL 1.0×10^{-3} g/ml hydrogen tetrachloroaurate aqueous solution and following added 1 mL 1.0×10^{-2} g/ml sodium citrate aqueous solution into the stirred toluene solution. The reactants were continuously stirred for 8 h. Then we obtained a black milky emulsion. The emulsion underwent a phase separation process after centrifugation at a speed of 3500 rpm. The upper organic phase is clear ruby red while the lower aqueous phase is black. The separated phases were then subjected to UV–vis measurement. All the above experiments were carried out at a room temperature.

3.2. Characterizations of the samples

Transmission electron microscopy (TEM) was used to determine the size and morphology of the formed gold particles of the dispersion solution yielded from the organic phase. TEM images were obtained with a Hitachi H-8100 microscope operating at an acceleration voltage of 200 kV. Specimens of the phoshpolipid–gold conjugates were prepared by slow evaporation of a drop of the appropriately as-prepared gold–lipid solution deposited onto a copper mesh grid with Formvar films, followed by carbon sputtering. XPS measurements were conducted with an ESCLAB 220i-XL spectrometer (VG Co.) using Al Kα radiation as X-ray source. The charging calibration was performed by referring C 1s to the binding energy at 284.6 eV. A Philips energy-dispersive X-ray analyzer (EDAX) DX-4 system fitting on TEM was used to check the elements in the aqueous phase after reaction. All infrared spectra were acquired using a Bruker EQUINOX55 FT-IR spectrometer. For pure DPPC and citrate sodium solid samples, 600 mg KBr was ground in a mortar with a pestle, and enough DPPC or citrate sodium solid sample was ground with KBr to make a 1 wt.% mixture for preparation of a transparent KBr disk. However, the sample of the phoshpolipid–gold conjugates in toluene for FT-IR must be dropped on a KBr window and dried under vacuum for a while. The UV–vis absorbance spectra of phoshpolipid–gold conjugates were collected over the range of 350–700 nm with 2 nm resolution on a Hitachi U-3010 spectrophotometer. Photographs of the dispersion were taken with a Nikon digital camera.

4. Results and discussion

The photograph (a) inset in Fig. 1 shows phase state of water (containing hydrogen tetrachloroaurate and sodium citrate)/toluene (containing DPPC) before mixing up. The upper phase of toluene solution is transparent while the lower aqueous phase displays obviously the color of aqueous solution of hydrogen tetrachloroaurate. After vigorous stirring, the aqueous phase changed from yellow to black shown in the inset (b) of Fig. 1. It is confirmed by TEM and EDAX spectra that the black substrates are the aggregated gold particles. The upper pink toluene phase, having a UV–vis absorption peak in Fig. 1, depicts a well-defined surface plasmon band with a maximum absorbance at about 527 nm, indicating the existence of gold nanoparticles in toluene phase [17,18]. Absorption spectra shows that the resulting smaller gold particles have middle leveled plasmon resonance band intensity and a comparable bandwidth. It also reveals that DPPC molecules were physically adsorbed onto gold surface rather than by chemical reaction [19].

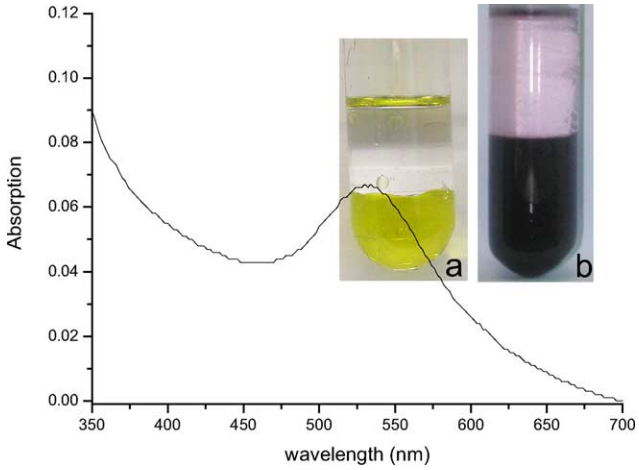

Fig. 1. The UV–vis absorption spectra of the phoshpolipid–gold conjugates in the toluene layer. Inset: photographs before (a) and after (b) reaction.

TEM images of the phoshpolipid–gold conjugates obtained from the toluene phase in Fig. 2 further prove that the phoshpolipid–gold conjugates are spherical and have less aggregation. The fact that the particles obtained from the organic phase did not fuse into larger particles indicates that phospholipid-capped nanoparticles are comparably sta-

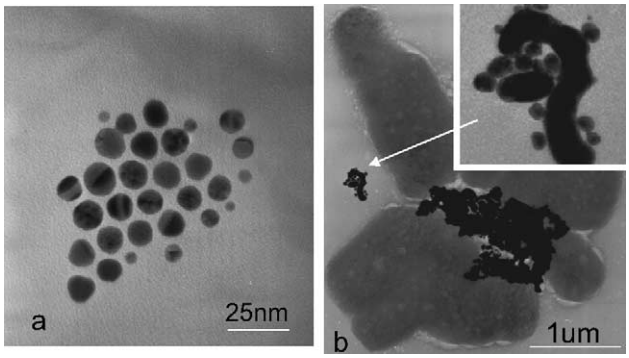

Fig. 2. (a) Typical TEM micrograph of the phoshpolipid–gold conjugates deposited on the carbon-coated copper grids, corresponding to the conditions shown for UV–vis absorption curve. (b) After reaction the TEM photograph of material in aqueous phase.

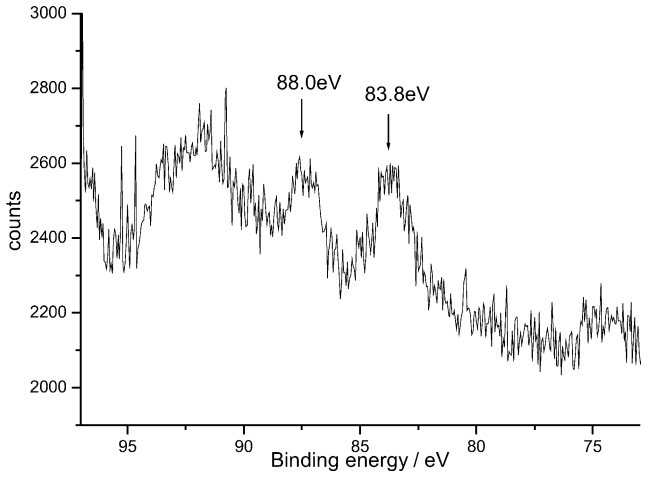

Fig. 3. XPS spectrum of Au 4f of the as-prepared phospholipid–gold conjugates deposited on a glass slide.

ble with alkanethiol-capped gold particles after solvent evaporation (Fig. 2a) [20]. The average particle-to-particle distance is about 4 nm, indicating that each particle is coated with a DPPC monolayer. It suggests that the alkyl chains of DPPC molecules of two adjacent gold particles may interdigitate into each other upon the solvent evaporation [11,21], like flexible DNA duplex without hybrids [22]. In contrast to the organic phase, the black clusters obtained from the aqueous phase in Fig. 2b are highly aggregated and could not enter the organic phase to perform the phase transition. The EDAX analysis shows that the black block aggregations are also gold particles, indicating that only DPPC modified gold particles with smaller size are able to enter toluene phase.

Fig. 3 shows the oxidation state of gold nanoparticles determined by X-ray photoelectron spectroscopy. The XPS spectrum of the phoshpolipid–gold conjugates reveals that the Au $4f_{7/2}$ and $4f_{5/2}$ doublet have the binding energies of 83.8 and 88.0 eV, respectively, which is a very reasonable value for metal Au^0 [23].

To further prove that gold particles transferred into toluene phase were in deed modified by phospholipids, we performed the measurements of FT-IR for the obtained gold particles from the organic phase. It indicates that the effective limiting frequency range of the phoshpolipid–gold conjugates in the toluene layer was measured in the range of 500–3750 cm^{-1} in the transmission mode. Fig. 4 shows the IR spectra of phospholipid-capped gold, pure phospholipid and pure citrate sodium. By comparing spectroscopic curves a, b and c, one can see that the curve a is similar to curve b, but much different with the curve c, which means that the gold particles are capped by phospholipids rather than by citrate sodium although it is usually considered as a traditional stabilizer for gold particles in aqueous phase. This is because one can readily observe the characteristic vibration peaks of $-CH_2-$ as well as $-CH_3$ in the phospholipid-capped gold particles. Meanwhile, the peaks of the symmetric and asymmetric stretching vibrations of $-CH_2-$ have been used as a sensitive indicator of the ordering of the alkyl chains. The two peaks at 2850 and 2914 cm^{-1} for the phoshpolipid-gold conjugates are almost the same as those of DPPC in the solid state, implying that in the solid state highly ordered all-*trans* alkyl chains may surround the particles surface [23]. The headgroup vibration peaks of the phoshpolipid–gold conjugates are consistent with those of pure DPPC, indicating that lipid remains an ordered status [24]. The selected spectrum of curves a and b in Fig. 4 shows that the methylene rock vibration of pure DPPC lies at 1179 cm^{-1}, whereas the methylene rock vibration of the phoshpolipid–gold conjugates appears at 1167 cm^{-1}. The energetic shift of methylene rock vibration indicates a lower density of gauche defects for phoshpolipid–gold conjugates than the pure DPPC. There-

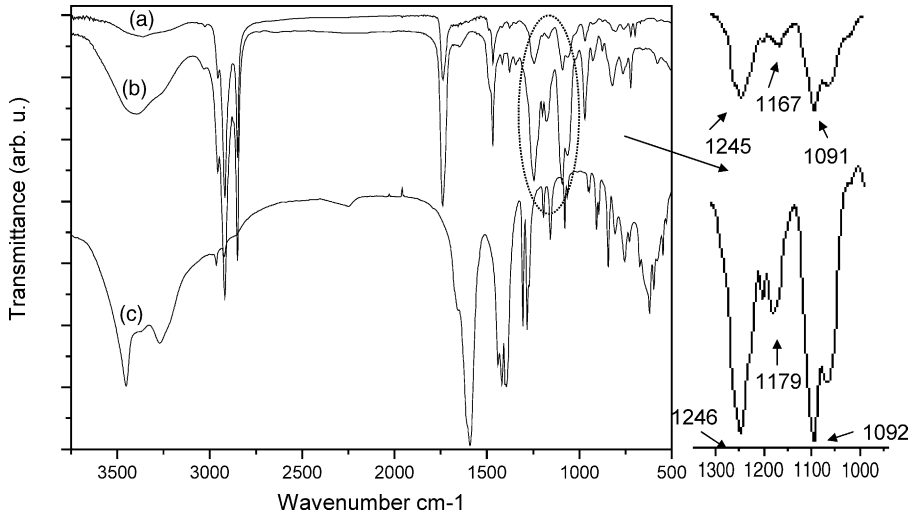

Fig. 4. FT-IR spectra of (a) phoshpolipid–gold conjugates in the toluene layer; (b) DPPC molecules; (c) sodium citrate molecules. All spectra were acquired in the form of KBr plates, either by mixing the solid with KBr powders (b and c) or by dropping the toluene solution on a KBr window and drying under vacuum (a).

fore, in respect of the above FT-IR spectral studies it confirms that DPPC molecules in deed adsorbed on the gold particles and may have an ordered solid state.

5. Conclusion

In summary, nanosize gold particles can be synthesized in one-step from $HAuCl_4$ in the presence of phospholipid at a water/toluene interface. Meanwhile the phospholipid-capped conjugates can be transferred readily into organic phase across the water/toluene interface. The preparation of lipid-capped gold nanoparticles may provide a method to produce metal quantum dot arrays based upon liposome as a template. It should be readily applicable to a wide range of combination of biological molecules with other bio-compatible nanoparticles.

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