Spatially controlled self-assembly of gold nanoparticles encased in α-helical polypeptide nanospheres†

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Gold nanoparticles (Au–NPs) are encased in aqueous nanospheres of α-helical poly(γ-benzyl L-glutamate)s (PBLG, number average degree of polymerization: n = 32), with spatially controlled self-assembly structures of solid core–shell nanospheres or double-layered hollow nanocapsules.

Biological systems synthesize unique organic/inorganic nanohybrids as exemplified by amorphous silica (diatoms),[1] calcite,[2] magnetite (magnetotactic bacteria)[3] and ferritin.[4] The iron-storage protein ferritin contains nanoparticles of ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$) encased in a stable polypeptide coat (apoferritin) that consists of 24 α-helix-rich protein subunits.[4] Inspired by the protein-regulated biomineralization process, formation of metal clusters in the interior of protein cages such as apoferritin[5–8] and viruses[9] have been attracting much interest. However, the preparative and synthetic procedures for these protein-caged systems are quite demanding and limited availability of the proteins also restricts their application. It is therefore a challenge to establish general and practical guidelines to produce biopolymer-based nanocapsules in which spatial organization of metal nanoparticles is finely controlled. α-Helical polypeptides are attractive as nanocapsule-forming molecules, since they are biocompatible and are amenable to functionalization. For example, they may serve as non-insulating cages, since properly designed α-helices are known to mediate transport of electrons[10,11] and ions.[12,13]

In this communication, we show that two typical spatial supramolecular organizations of gold nanoparticles (Au–NPs) are separately formed *via* the emulsion-templated interfacial self-assembly process. Au–NPs are either assembled in the central core of peptide nanospheres or accumulated on the inner wall of hollow capsules. Amphiphilic poly(γ-benzyl L-glutamate)s (PBLG, number average degree of polymerization: n = 12 and 32) **1** were synthesized according to the previous report (Fig. 1).[14] PBLG is a typical α-helical polypeptide that provides a hydrophobic and rigid rod structure. A diglycolic acid unit was introduced in the N-terminus as a hydrophilic group. As can be seen from the molecular models of **1**, these polypeptide rods (a, b) are much bulkier than didodecyldimethylammonium bromide (c), a typical synthetic bilayer-forming amphiphile.[15] The PBLGs **1** (n = 12, 32) are soluble in dichloromethane and the helicities determined by the molar ellipticity at 222 nm were *ca.* 68% for **1** (n = 12; $[\theta]_{222}$ = 2.3 × 10^4 deg. cm^2 dmol^{-1}) and 97% for **1** (n = 32; $[\theta]_{222}$ = 3.3 × 10^4 deg. cm^2 dmol^{-1}), respectively. On the other hand, these powdery polypeptides were poorly soluble in water even in the form of sodium salts. This is probably ascribed to the overwhelming hydrophobicity of the PBLG unit compared to the small hydrophilic group. To prepare aqueous nanospheres of **1**, dichloromethane solutions of **1** (5 unit mM) and aqueous solutions containing equimolar sodium hydroxide (CH_2Cl_2–water = 1 : 2 or CH_2Cl_2–methanol–water = 1 : 1 : 1, v/v) were vigorously mixed. Oil-in-water (o/w) microemulsions were obtained, from which the dichloromethane phase is successively removed by ultrasonication (Branson 1210J-MTH, *ca.* 30 min) and rigorous bubbling of nitrogen gas at room temperature. Homogeneous aqueous dispersions of polypeptides **1** were thus obtained.

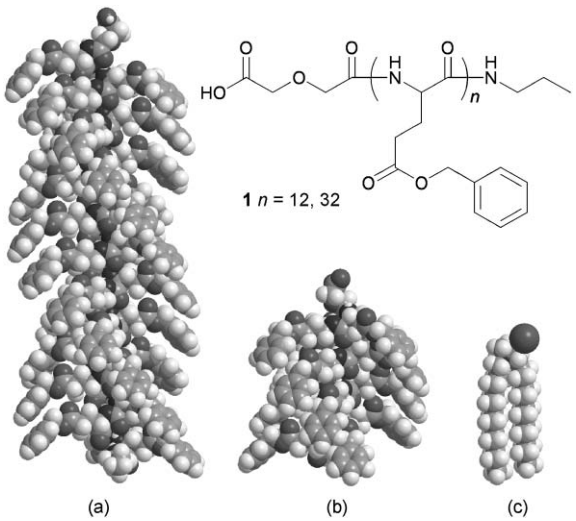

Fig. 1 Molecular models of (a) **1** (n = 32), (b) **1** (n = 12), and (c) didodecyldimethylammonium bromide. The polypeptide structures were energy-minimized by MM2 calculation.

Fig. 2 shows dynamic light scattering (DLS, Otsuka Electronics DLS-7000DL) and scanning electron microscopy (SEM, Hitachi S-5000) of the aqueous polypeptide dispersions. Aggregates with DLS diameters of *ca.* 120 ± 35 nm are observed for **1** (n = 32). Consistently, nanospheres with diameters of *ca.* 100–200 nm are abundantly seen in the SEM picture. In the case of **1** (n = 12), peptide nanospheres (DLS diameter: 160 ± 60 nm, SEM: 100–200 nm particles) were similarly observed. These diameters observed are considerably larger than the molecular lengths of **1** (n = 32, *ca.* 6 nm; n = 12, *ca.* 2.5 nm), and these nanospheres are

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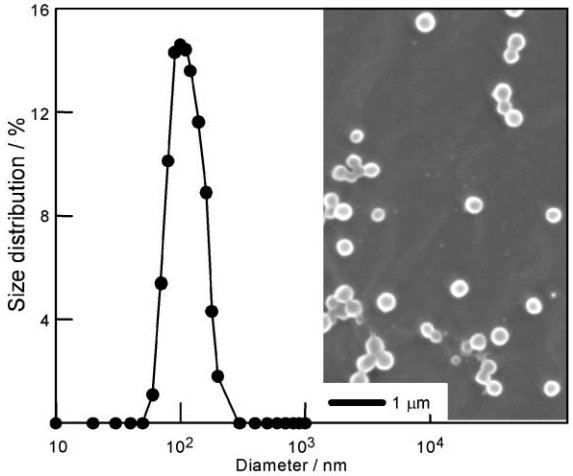

Fig. 2 DLS size distribution of polypeptide assemblies **1** (n = 32) dispersed in water. Inset: SEM image of polypeptide nanospheres **1** (n = 32). The peptide amphiphiles (5 unit mM) were dissolved in CH_2Cl_2 and were emulsified with water at the CH_2Cl_2–water ratio of 1 : 2 (v/v).

self-assembled from component peptides during the ultrasonic removal of dichloromethane from o/w emulsions.

X-ray diffraction experiments (XRD, MacScience Micro-focus X-ray diffractometer M18XHF, Cu Kα, λ = 1.54 Å) were conducted for the polypeptide nanospheres. Samples were corrected from aqueous dispersions by centrifugation at 10 000 rpm. The XRD diffraction of **1** (n = 32) displayed a Bragg reflection peak that corresponds to a spacing of 1.35 nm, which is consistent with the intermolecular distance reported for PBLG helices.[16,17] IR spectra of the specimens also supported the maintenance of α-helical structures in nanospheres.† These nanospheres are thermally stable, since heating of aqueous dispersions in aqueous urea (2 M, temperature up to 60 °C) caused no changes in the particle size as confirmed by DLS measurement.

As these aqueous polypeptide nanospheres are formed by way of o/w emulsions, they can encapsulate hydrophobic metal clusters. Dodecanethiol-stabilized Au–NPs (average diameter, 2 nm) were dissolved in dichloromethane solution of **1** (concentration, 5 unit mM) at varied mixed ratios. The mixed solutions are emulsified with water as described above (CH_2Cl_2–methanol–water = 1 : 1 : 1). Brown-colored aqueous dispersions were obtained after evaporation of the organic phase, indicating successful entrapment of water-insoluble Au–NPs in the polypeptide assemblies. Fig. 3 shows transmission electron microscopy (TEM) of aqueous dispersions without staining (JEOL JEM-2010, acceleration voltage, 120 kV). In the absence of Au–NPs, polypeptide nanospheres with a diameter of *ca.* 80–120 nm are observed (Fig. 3a), consistent with the SEM observation (Fig. 2 inset). On the other hand, when Au–NPs are added in the dichloromethane phase, nanospheres with core–shell structure were observed. In these nanospheres, Au–NPs are spherically assembled in the core and they are surrounded by the shell of polypeptides (Fig. 3b–e, the polypeptide layer and Au–NPs are shown by black and white arrows, respectively). The diameter of Au–NPs(core)@polypeptide(shell) nanospheres is comparable to the original polypeptide nanospheres. Each of Au–NPs assembled in the core is maintaining its integrity, as clearly seen from the electron microscopy (Fig. 3c–e).

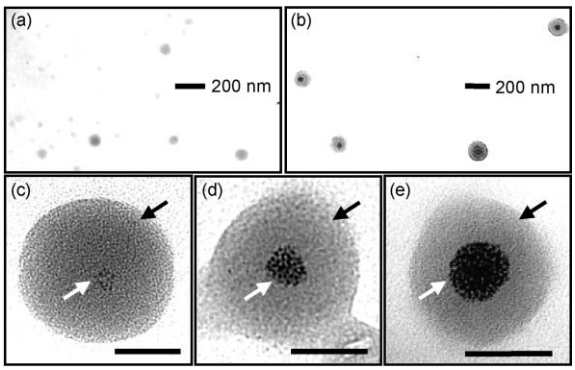

Fig. 3 TEM images of the gold nanoparticles (Au–NPs)-encased peptide nanospheres. The polypeptide **1** (n = 32, 5 unit mM) and Au–NPs (a, 0 μg ml^{-1}; b and d, 36 μg ml^{-1}; c, 3.6 μg ml^{-1}; e, 180 μg ml^{-1}) were dissolved in dichloromethane and the mixtures were emulsified with water at the CH_2Cl_2–MeOH–water ratio of 1 : 1 : 1 (by volume). The aqueous polypeptide dispersions were obtained by ultrasonication-promoted stripping of dichloromethane phase from emulsions. Black and white arrows indicate polypeptide layers and aggregates of Au–NPs, respectively. Scale bars: 50 nm. Samples are not negatively stained.

It is important to note that the core size, *i.e.*, the number of aggregated Au–NPs can be controlled depending on their initial concentration. When Au–NPs are mixed in dichloromethane at a concentration of 36 μg ml^{-1}, average diameter of the Au–NPs assemblies in the hybrid nanospheres was *ca.* 28 nm (27% of the total diameter of gold@polypeptide nanospheres, Fig. 3b,d). On the other hand, when fivefold Au–NPs are added (concentration, 180 μg ml^{-1}), the diameter of Au–NPs assemblies was increased to *ca.* 36 nm (45% of the total diameter, Fig. 3e). Decrease in the concentration of Au–NPs to 3.6 μg ml^{-1} leads to a smaller number of Au–NPs located in the core (Fig. 3c). From these results, it is apparent that the core size of hybrid nanospheres is tunable at nanometer-level, and the emulsion-templated self-assembly is indispensable to regulate spherical self-assembly of Au–NPs in polypeptide nanospheres.

Interestingly, when dichloromethane was evaporated from o/w emulsions without ultrasonication, hollow nanocapsules with larger diameter of 200–500 nm were obtained (Fig. 4, [**1**] = 5 unit mM, CH_2Cl_2–methanol–water = 1 : 1 : 1, v/v). The inset in Fig. 4b shows a broken nanocapusle contained in the sample, confirming their hollow structures. In contrast to the regular

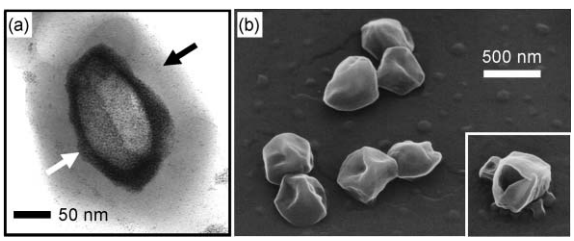

Fig. 4 TEM (a) and SEM (b) images of double layered hollow assemblies of polypeptides and Au–NPs. The polypeptide **1** (n = 32, 5 unit mM) and Au–NPs (180 μg ml^{-1}) were dissolved in dichloromethane and the mixtures were emulsified with water at the CH_2Cl_2–MeOH–water ratio of 1 : 1 : 1 (by volume). The hollow nanocapsules were obtained by keeping the emulsions on carbon-coated TEM grids, and the inner dichloromethane phase was spontaneously evaporated from the emulsion without applying ultrasonication.

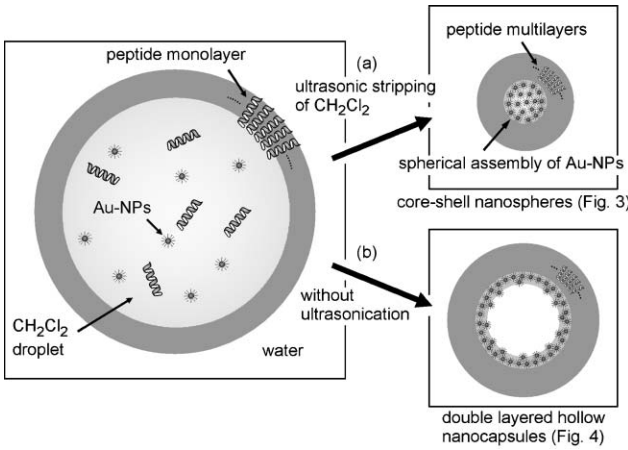

Fig. 5 Schematic illustration of the emulsion-templated self-assembly process for **1** (n =32) and Au–NPs. o/w microemulsions containing Au–NPs and the amphiphilic peptides are converted to (a) aqueous core@shell nanospheres or to (b) layered hollow nanocapsules, depending on the stripping condition of the organic phase.

core–shell nanospheres obtained under ultrasonication (Fig. 3b–e), the hollow nanocapsules show bumpy structures with Au–NPs accumulated on the inner surface (Fig. 4a). It is noteworthy that both of the solid core–shell and hollow inorganic/polypeptide layered structures are easily prepared, by way of the emulsion-templated self-assembly. Though metal nanoparticles have been adsorbed on polymer capsules prepared by the layer-by-layer technique,[18,19] the versatile control on stereo-architecutres is a unique feature of this approach.

Fig. 5 schematically illustrates the self-organization process of Au–NPs/polypeptide nanohybrids. The core–shell nanoarchitectures would be formed by phase separation of dodecanethiol-capped Au–NPs from the polypeptide **1** (n = 32) during the stripping of dichloromethane from o/w emulsions. It is probable that monolayers of amphiphilic polypeptides are first adsorbed at the CH_2Cl_2–water interface to stabilize emulsions. Increase in the concentration of polypeptides caused by the removal of dichloromethane allowed deposition of polypeptide molecules at the o/w interface, resulting in the growth of polypeptide shells. The dodecanethiol-capped Au–NPs are less miscible with the polypeptide layer. They are concentrated in the core and left as spherical aggregates after the depletion of dichloromethane (Fig. 5a). This intra-nanosphere phase separation mechanism is supported by the irregular assembly of Au–NPs formed in nanospheres of **1** (n = 12).† The shorter peptide **1** (n = 12) displays lower α-helical content and it makes the assembly less exclusive to Au–NPs. On the other hand, the layered hollow nanocapsules are formed by spontaneous stripping of CH_2Cl_2 from emulsions without ultrasonication. Accumulation of **1** (n = 32) at the CH_2Cl_2–water interface causes segregation of Au–NPs into the inner CH_2Cl_2 phase. After depletion of CH_2Cl_2, Au–NPs are deposited on the inner surface of polypeptide shells (Fig. 5b).

In summary, we have demonstrated controlled formation of Au–NPs@polypeptide superstructures by way of the emulsion-directed self-assembly. Significance of the present study is twofold. First, the diglycolic acid-derived α-helical polypeptides give stable nanospheres or hollow nanocapsules in water, *via* o/w emulsion-templated assembly. Second, control of the spatial organization of metal nanoparticles is achieved. The ultrasonic stripping of the organic phase allows formation of aqueous nanospheres, in which spherical assemblies of lipophilic Au–NPs are encapsulated in the core. The size of Au–NPs assemblies can be regulated, depending on preparative conditions. Hollow nanocapsules with double-layered Au–NPs/polypepeptide structures are also obtainable. To date, polymer capsules,[18,19] peptide tubules[20–22] and fibers[23–25] have been employed as templates to accumulate metal nanoparticles on their surfaces. Gold nanoparticles capped by thiol-containing polypeptides and their aggregation behavior have also been reported.[26–28] However, controlled spatial organization of metal-nanoparticle-assemblies as shown in Fig. 5 has not been achieved. This approach is simple, and would be widely applicable to the spatially controlled organization of hydrophobic nanomaterials.

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