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Doughnut-Shaped Peptide Nano-Assemblies and Their Applications as Nanoreactors

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Abstract: Doughnut-shaped nanoreactors, peptide nano-doughnuts, were self-assembled from peptides and organic Au salts. We demonstrated that monodisperse Au nanocrystals were synthesized inside the cavities of peptide nano-doughnuts by the reduction of Au ions and the size of the Au nanocrystal was controlled by the cavity dimension. The Au nanocrystals inside the nano-doughnuts were extracted by destroying the nano-doughnuts via long UV irradiation (>10 h). These features may allow the peptide nano-doughnuts to be applied in the fields of nanomaterial syntheses, controlled release systems, and drug delivery.

Introduction

Nanomaterial synthesis is emerging as a crucial technology in nanotechnology to develop building blocks for nanometer-scale devices. To synthesize nanomaterials in controlled sizes and dimensions, various types of nanoreactors, nanometer-sized chemical reaction vessels, have been developed.[1−7] Because those nanoreactors are removed by means of external stimuli to extract products after nanomaterials are synthesized, the nanoreactors are applied not only in material syntheses but also in controlled release and drug delivery.

While dendrimers and block copolymers have been developed extensively as effective nanoreactors,[8−13] our development is focused on ring-shaped or doughnut-shaped nanoreactors because those nanoreactors introduce well-defined growth areas within the cavities, which offer straightforward size control of nanomaterials. Micelles and polyelectrolytes have convenient and flexible cavities that function as nanoreactors,[2,14−17] but more stable assemblies against broad ranges of precursors and chemical reagents are desirable to synthesize a variety of nanomaterials. For example, when micelles were used as nanoreactors to synthesize nanocrystals inside, micelle cavities were destroyed by reducing agents in the reduction processes.[14,18]

Here, we report a novel method to produce doughnut-shaped nanoreactors, peptide nano-doughnuts. The nano-doughnuts were self-assembled from peptides and organic Au salts. Previously, various shapes of peptide/protein assemblies have been produced in biomaterials,[19−31] but the doughnut-shaped nanoreactors assembled from synthetic peptides have not been reported yet, to our knowledge. We demonstrated that monodisperse Au nanocrystals were grown inside the cavities of peptide nano-doughnuts by the reduction of Au ions trapped in the cavities and the resulting Au nanocrystals were extracted by destroying the nano-doughnuts via long UV irradiation (Figure 1). Because

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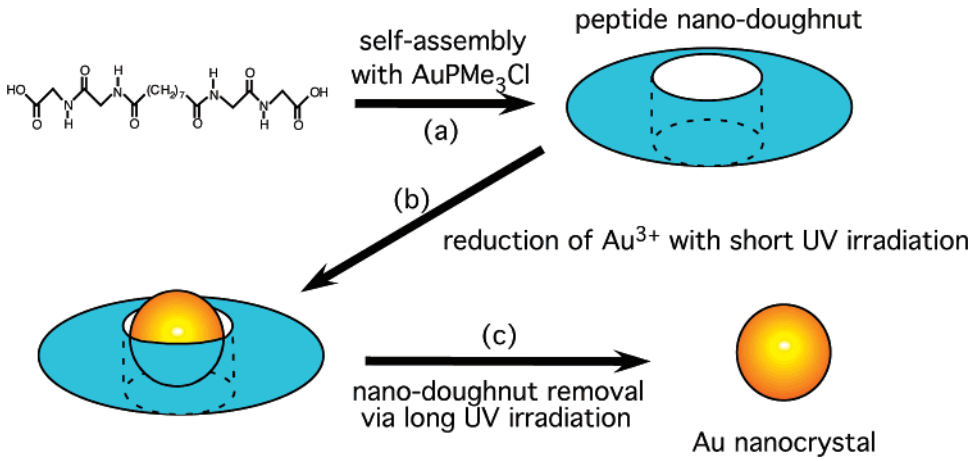

Figure 1. Scheme for the peptide nano-doughnut self-assembly and its application as a nanoreactor: (a) After the peptide monomers are self-assembled to the nano-doughnut in the presence of the organic Au salts, (b) Au ions in the cavity are reduced by short UV irradiation (<20 min). (c) Longer UV irradiation (>10 h) destroys the nano-doughnut to release the Au nanocrystal.

the peptide nano-doughnuts already contained Au ions inside the cavities, Au nanocrystal synthesis was completed in a simpler process as compared to that of micelle nanoreactors. Those features may allow peptide nano-doughnuts to be applied in the fields of nanomaterial syntheses, controlled release systems, and drug delivery.

Experimental Section

Bolaamphiphile peptide monomers, bis(*N*-α-amido-glycylglycine)-1,7-heptane dicarboxylate molecules (10 mM), were dissolved in a pH 5.5 citric acid/NaOH solution, and an excess of trimethylphosphinegold chloride (Aldrich) was added to assemble the peptide nano-doughnuts for 5 days in the dark. The chemical process to synthesize and assemble the peptide monomers was described previously.[32,33] The nano-doughnut solution was washed with deionized water and centrifuged at 14.5 krpm. This process was repeated two times. To reduce Au ions in the peptide nano-doughnuts, the washed nano-doughnut solution was exposed to a UV light (14 mW/cm^2, 254 nm) for 20 min to 10 h. The reducing agent, hydrazine hydrate (2 M), was also used to produce Au nanocrystals from the peptide nano-doughnuts.

Scanning force micrographs (SFM) were recorded with a Nanoscope IIIa instrument (Digital Instruments, St. Barbara, CA) operating in the tapping mode at a resonance frequency of about 280 kHz. One drop of the diluted nano-doughnut solution (1% in volume) was placed on a freshly cleaved mica surface and spin cast. Transmission electron microscopy (TEM) was performed in the dark field mode on a HITACHI H-600 microscope operating at 80 keV. For IR studies, 50 μL of sample solution was deposited on a glass slide and air-dried. FTIR spectra were recorded with an IlluminatIR IR microspectrometer (SensIR, Danbury, CT).

Results

When peptide monomers were self-assembled in the presence of the water-insoluble trimethylphosphinegold chloride ($AuPMe_3Cl$) for 5 days in the dark (Figure 1a), the doughnut-shaped peptide assemblies were observed in the SFM image (Figure 2a). The average outer diameter of the nano-doughnut is 50 nm. Besides the peptide nano-doughnuts, elongated structures were observed in the SFM image, which correspond to the unincorporated Au-salt precipitates. The TEM image of the single nano-doughnut (Figure 2b) shows the ring-shaped peptide

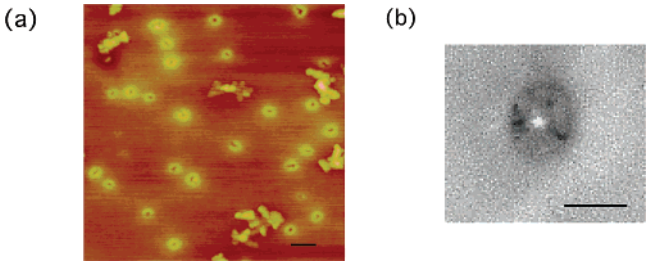

Figure 2. (a) SFM topographic image of the peptide nano-doughnuts. Scale bar = 100 nm. (b) TEM image of the peptide nano-doughnut. Scale bar = 50 nm.

assembly. It should be noted that the size of the nano-doughnut was not altered by the Au salt concentration in the peptide monomer solution.

To produce Au nanocrystals inside the cavities of peptide nano-doughnuts, Au ions in the nano-doughnuts were slowly reduced by UV irradiation (Figure 1b). After UV light was irradiated to the nano-doughnut solution for 20 min, Au nanocrystals were generated inside the cavities of nano-doughnuts (Figure 3a). The SFM image of these nano-doughnut complexes in the phase contrast mode (Figure 3b) showed that the particles in the middle of the cavity are much brighter than the rings, suggesting that these particles are harder Au nanocrystals as compared to the peptide-assembled rings. The TEM image of the nano-doughnut in Figure 3c also supplements the observation in the SFM images. In this TEM image, the Au nanocrystal appeared darker in the center of the nano-doughnut as compared to the cavity of the peptide nano-doughnut in Figure 2b. The particles in the cavities were also confirmed as Au nanocrystals by electron diffraction (Figure 3c). The histogram of Au nanocrystal diameters inside the cavities shows the narrow distribution in Figure 3d. From this histogram, the average diameter of the Au nanocrystal in the nano-doughnut is 14 nm. Those nano-doughnut−Au nanocrystal complexes were stable in the solution for weeks when the solution was stored in the dark.

After further reduction of Au ions in the nano-doughnuts with longer UV irradiation (Figure 1c), only Au nanocrystals were observed. Those Au nanocrystals were seen without the nano-doughnut shells after 10 h of UV irradiation (Figure 4a). The Au nanocrystals in Figure 4a appeared in the consistent contrast

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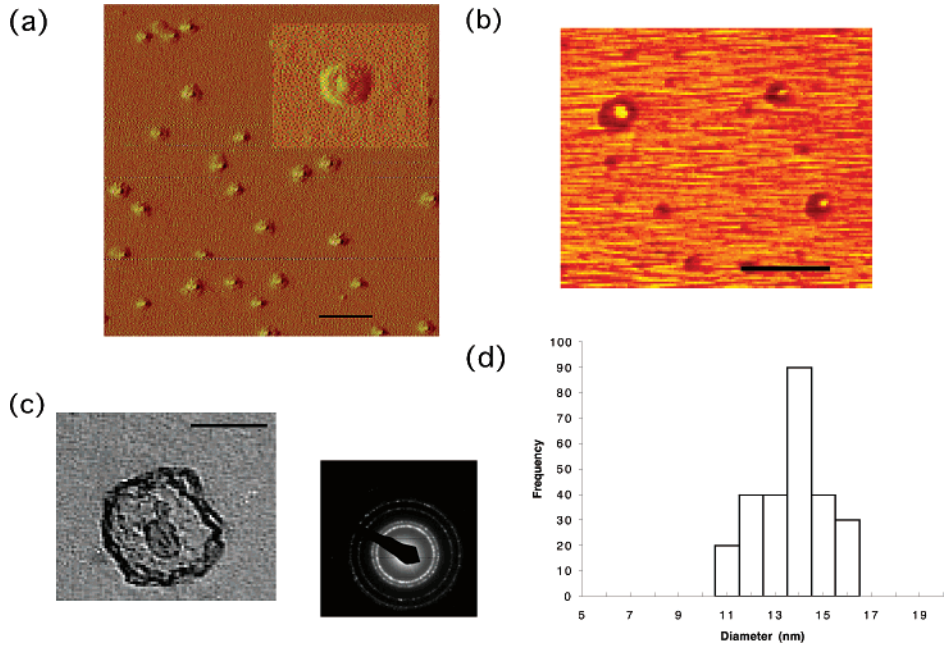

Figure 3. (a) SFM topographic image of the peptide nano-doughnuts with Au nanocrystals inside the cavities. Scale bar = 200 nm (inset: The magnified SFM image of the peptide nano-doughnut). (b) SFM phase image of (a). Scale bar = 100 nm. (c) TEM image (left) and electron diffraction (right) of the peptide nano-doughnut−Au nanocrystal complex. Scale bar = 50 nm. (d) The size distributions of the Au nanocrystals grown in the nano-doughnut cavities from the SFM images.

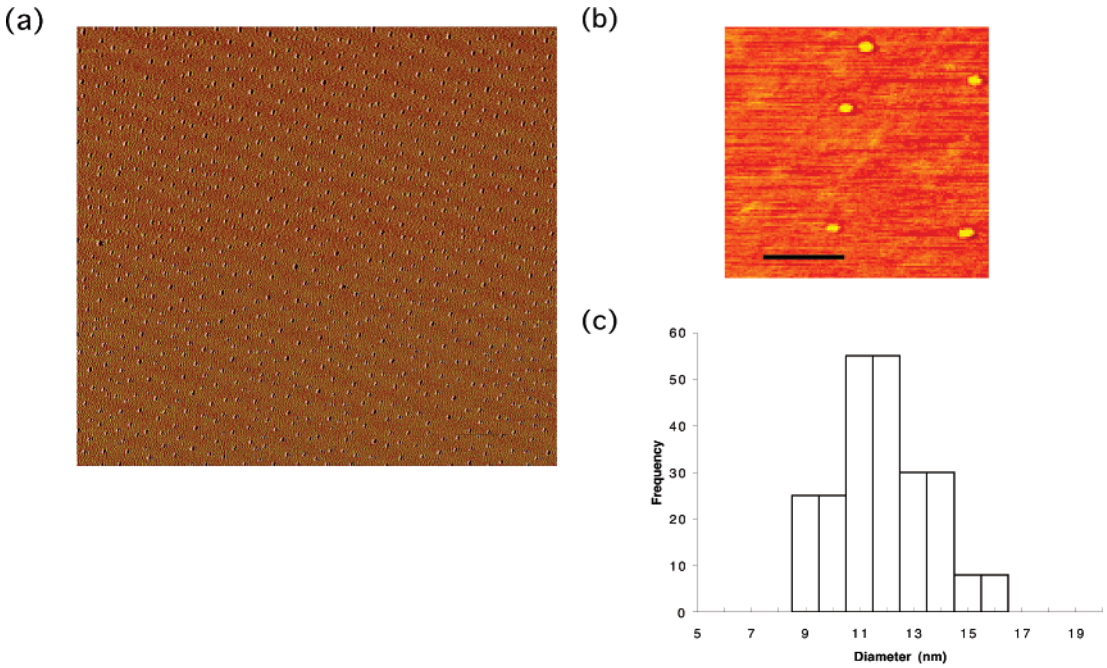

Figure 4. (a) SFM topographic image of Au nanocrystals after they were released from the peptide nano-doughnuts via longer UV irradiation (>10 h). Scale bar = 150 nm. (b) SFM phase image of (a). Scale bar = 100 nm. (c) The size distributions of the Au nanocrystals from the SFM images.

(Figure 4b) as compared to the Au nanocrystals inside the cavities (Figure 3b) in their SFM phase images. This comparison supports that the particles in Figures 3a and 4a are the same type of nanocrystals. Those nanocrystals were also confirmed as Au by the electron diffractions. The average diameter of the Au nanocrystal is 12 nm (Figure 4c), which is consistent with the diameter of the Au nanocrystal inside the nano-doughnut cavity before the longer UV exposure (Figure 3d). We observed no size change of the Au nanocrystals with UV irradiation longer than 10 h.

When the peptide nano-doughnuts were reduced with a stronger reducing agent, hydrazine hydrate, a striking difference was observed. Unlike the weak reduction with the short UV irradiation (Figure 3a), this stronger reduction produced Au nanocrystals after 20 min without forming the nano-doughnut−Au nanocrystal complexes (Figure 5a). The diameter of the Au nanocrystal produced with hydrazine hydrate is 23 nm in average, which is larger and slightly more polydisperse (Figure 5b) as compared to the one produced by UV irradiation (Figure 4c). The histogram in Figure 5b also indicates that the size of

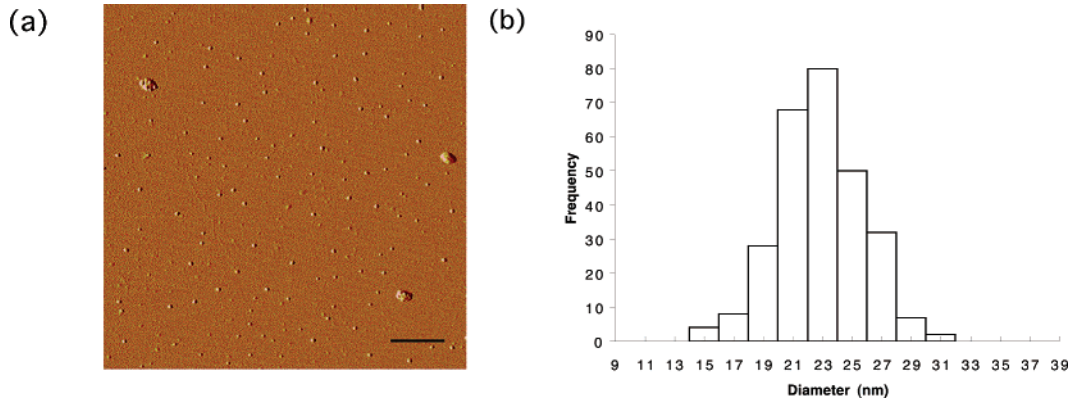

Figure 5. (a) SFM topographic image of Au nanocrystals after reducing the peptide nano-doughnuts by hydrazine hydrate. Scale bar = 150 nm. (b) The size distributions of the Au nanocrystals from the SFM images.

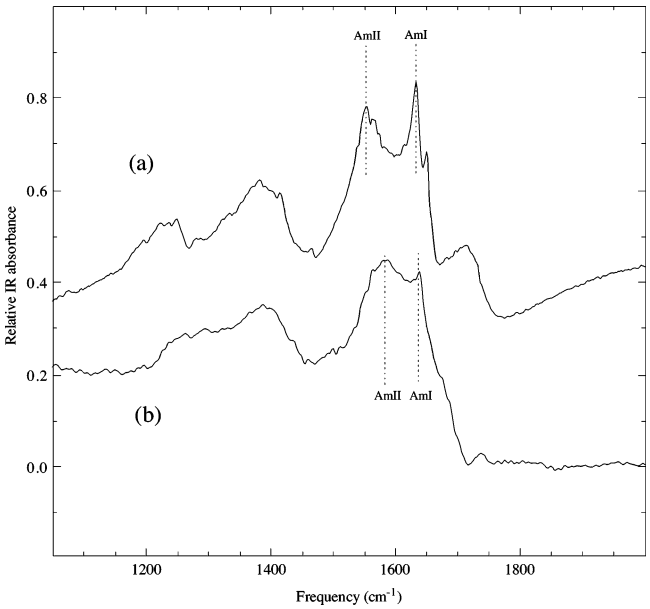

Figure 6. FTIR spectra of (a) nanotubes self-assembled from the peptide monomers without the organic Au salts, and (b) nano-doughnuts self-assembled from the peptide monomers in the presence of organic Au salts. AmI and AmII correspond to amide I and amide II, respectively.

the Au nanocrystal produced by hydrazine hydrate is larger than the cavity of the peptide nano-doughnut in Figure 3a.

Discussion

When the peptide monomers were self-assembled in the growth solution without the organic Au salts, peptide nanotubes were formed instead of the peptide nano-doughnuts.[33] Those nanotubes were assembled via intermolecular hydrogen bonds between amide and carboxylic acid groups. Therefore, the addition of the organic Au salts to the peptide monomer assembly likely has a significant influence on those chemical interactions to alter the assembled structure. Because the nano-doughnuts were not observed when an inorganic Au salt, $HAuCl_4$, was incubated in the peptide solution instead of $AuPMe_3Cl$, the ligand of this organic Au salt may play an important role in the assembly of the nano-doughnuts. To understand the binding scheme between the peptide and the organic Au salt in the nano-doughnuts, IR spectra of the peptide nano-doughnuts and the peptide nanotubes were compared. In Figure 6a, the amide I and amide II peaks of the peptide nanotubes are observed at 1631 and 1551 cm^{-1}, respectively. While the amide I peak also appears at 1631 cm^{-1} in the nano-doughnut spectrum in Figure 6b, the amide II peak shows a significant red-shift to 1573 cm^{-1}. Previously, a similar trend in the amide peak shifts was observed in amide-containing self-assembled monolayers and dendrimers, respectively, after Au salts were bound to their amide groups.[34−36] Therefore, this IR investigation suggests that the organic Au salts are incorporated in the peptide self-assemblies and contribute to the nano-doughnut formation.

When the peptide nano-doughnuts were weakly reduced by UV irradiation in 20 min, Au nanocrystals were observed inside the doughnut cavities (Figure 3). The particles in the center of the doughnut cavities are identified as Au nanocrystals from the SFM phase images, the TEM images, and the electron diffractions in Figure 3b and 3c. In fact, the incorporation of the Au nanocrystal increases the mechanical strength of the peptide nano-doughnut. This was shown by the comparison between SFM images, Figures 2a and 3a. After those samples were dried on mica surfaces, the peptide nano-doughnuts without Au nanocrystals collapsed and displayed a deformed ring shape, whereas the peptide nano-doughnuts with Au nanocrystals inside the cavities showed a monodisperse and isotropic ring shape. The organic Au salts seem to be trapped inside the cavities before the reduction to produce Au nanocrystals in the center of the nano-doughnuts, and this may be explained by the capillary effect of the nano-doughnut cavity. Previously, we observed a similar observation that the organic Au salts were trapped inside peptide nanotubes due to their capillary effects.[19]

Further reduction by UV irradiation (>10 h) removed Au nanocrystals from the peptide nano-doughnuts, and the nano-doughnut shells disappeared (Figure 4). Because the size of the released Au nanocrystal (Figure 4c) is consistent with the size of the Au nanocrystal in the nano-doughnut cavity (Figure 3d), it seems that the longer reduction destroyed the nano-doughnuts. Because the nano-doughnuts are stable over weeks in solution with no light, the UV irradiation triggers the disappearance of the peptide nano-doughnut. It is reasonable to observe this phenomenon if the nano-doughnuts are assembled via the chemical interactions between the peptide monomers and the Au salts, which is supported by IR spectroscopy (Figure 6). The Au salt can no longer function as a glue to sustain the

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doughnut structure after the reduction, and therefore the nano-doughnuts disappear during the long UV exposure. The self-assembled nanotubes from the same peptide monomers without the Au salts were stable under UV irradiation and hydrazine hydrate incubation, and this observation also supports that the reduction of Au salt plays a role in the nano-doughnut breakage. It should be noted that a similar phenomenon was observed previously in Au salt−polymer composites.[37] When Au-salt-loaded polymer-brushes were exposed to UV light for 36 h, the polymer shells disappeared due to the reduction of Au salt.

When the Au salts in the peptide nano-doughnuts were reduced by the stronger reducing agent, hydrazine hydrate, Au nanocrystals without the nano-doughnut shells were observed (Figure 5). Because the sizes of these Au nanocrystals were larger as compared to the size of the nano-doughnut cavity, the strong reduction seems to produce Au nanocrystals without using the nano-doughnut templates. One possible mechanism is that the peptide nano-doughnuts are destroyed by the reduction of Au ions binding the peptide monomers before Au nanocrystals are formed. Because there is no template to regulate the size of the Au nanocrystal in this case, the resulting Au nanocrystals are larger and more polydisperse, which was observed in Figure 5. Therefore, the rate of Au ion reduction in the peptide nano-doughnuts must be carefully controlled to produce monodisperse Au nanocrystals in the well-defined size.

Conclusion

Doughnut-shaped nanoreactors, peptide nano-doughnuts, were produced from synthetic peptides and organic Au salts via self-assembly. Monodisperse Au nanocrystals were synthesized inside the cavities of peptide nano-doughnuts by the reduction of Au ions trapped in the cavities. The Au nanocrystals inside the nano-doughnuts were extracted by destroying the nano-doughnuts with long UV irradiation (> 10 h). When Au ions in the nano-doughnuts were reduced rapidly by the stronger reducing agent, Au nanocrystals were grown without the nano-doughnut templates and the size of the Au nanocrystal was not controlled by the cavity dimension. Therefore, the reduction rate of Au ion must be controlled carefully to synthesize monodisperse Au nanocrystals using the nano-doughnuts. Peptide self-assemblies have shown potential as building blocks for application in nanodevice fabrications via their molecular recognitions for their geometric alignment[38−40] and physical property control.[19−21] Unlike other peptide self-assemblies, these peptide nano-doughnuts can be destroyed by UV irradiation. This feature may allow the peptide nano-doughnuts to be applied in the fields of controlled release systems and drug delivery.

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