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Stimuli induced Structural Control of Sequential Alternating Amphiphilic Peptide coated Gold Nanoparticle Assemblies

Kae Ushiba, Masahiro Higuchi,* and Masami Kawaguchi
Department of Chemistry for Materials, Faculty of Engineering, Mie University,
1577 Kurimamachiya-Cho, Tsu, Mie 514-8507
Fax: 81-59-231-9431, e-mail: higuchi@chem.mie-u.ac.jp

Gold nanoparticles having sequential alternating amphiphilic peptide chains, Phe-(Leu-Glu)$_8$, on the surface have been prepared. We described structural control of the amphiphilic peptide coated gold nanoparticle assembly by a conformational transition of the surface peptides. Under the acidic condition, the conformation of the surface amphiphilic peptide was converted to a β-sheet structure from an aggregated α-helix by incubation. The plasmon absorption maximum of the gold nanoparticles shifted to a shorter wavelength with the formation of the β-sheet assembly of the surface peptide. This suggests that the structure of the peptide coated gold nanoparticle assembly could be controlled by the conformational transition of the surface peptide. Furthermore, the core gold nanoparticle could be fixed in the β-sheet assembly in the state that stood alone. This system may be useful for novel molecular devices that exhibit quantized properties.

Key words: Sequential Alternating Amphiphilic Peptide, Peptide Coated Gold Nanoparticle Assembly, Conformational Transition, Structural Control, Plasmon Shift

1. INTRODUCTION

A design and assembly of ordered molecular nanostructures has become one of the key aims of new materials and molecular devices. Assemblies of nanoscale materials into specific structure composed of semiconductor and/or metal nanoparticles are predicted to exhibit quantized properties and have become a focus for novel functional devices [1-7] such as optoelectronics and medical diagnostics. These functions are closely related to the arrangement of nanoparticles in the assemblies. The formation of ordered arrangement of nanoparticles has been vigorously investigated. The nanoparticles having organic molecules such as peptides, [8] oligonucleotides, [9, 10] and oligosaccharides [11] as building blocks have been utilized to form ordered and periodicity arrangement of the nanoparticles. Especially, the structure of peptide assemblies can be controlled by the external stimuli such as pH. [12, 13] Therefore, the functions of peptide coated nanoparticle assemblies may be regulated by the stimuli induced rearrangement of the nanoparticles owing to the structural changes of the surface peptide chains.

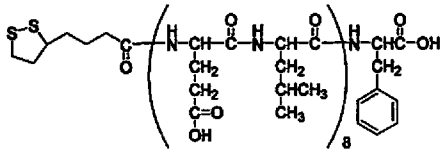
Here, we described preparation of peptide coated gold nanoparticles and structural control of the peptide-gold nanoparticle assembly by conformational transition of the surface peptides. We chose a sequential alternating amphiphilic peptide, whose amino acid sequence was Phe-(Leu-Glu)$_8$, as the surface peptide chain on the gold nanoparticle. The sequential alternating amphiphilic peptides have been observed the reversible helix-sheet conformational transition. [14-16] Under the basic condition, the sequential alternating amphiphilic surface peptide took an α-helical conformation containing a considerable amount of random coil conformation. On the other hand, the conformation of the surface peptide was converted to a β-sheet structure from an aggregated α-helix by incubation under the acidic condition. Interestingly, the plasmon absorption maximum of the gold nanoparticles shifted to a shorter wavelength with the formation of the β-sheet assembly. This suggested that the core gold nanoparticle could be fixed in the assembly in the state that stood alone. This system may be useful for the novel molecular devices that exhibit quantized properties.

2. EXPERIMENTAL

2-1. Peptide Synthesis

Phe-(Leu-Glu)$_8$ sequence was chosen as a β-sheet forming element. And thioctic acid for the Au binding site was introduced at the amino terminal of the sequential alternating amphiphilic peptide (Chart 1). The peptide, Phe-(Leu-Glu)$_8$-S, having thiocitic acid was synthesized by the conventional solid-phase method. [17] A Fmoc-Phe loaded CLEAR-Acid-Resin (0.4 meq/g,

Chart 1. Sequential Alternating Amphiphilic Sequential peptide.



Peptide Institute Inc.) was used for the resin of peptide synthesis. A 0.5 g of the resin was swelled by 5 mL of dichloromethane for 1 day in the reaction vessel and then the resin was rinsed by 5 mL of pure dimethylformamide (DMF) 3 times. A solution of Fmoc-amino acid (0.7 mmol) in 3 mL of dimethylformamide (DMF), 1,3-diisopropylcarbodi-

imide (0.7 mmol) in 1 mL of DMF, and 1-hydroxy-7-azabenzotriazole (0.7 mmol) in 1 mL of DMF was added to the resin, and then the suspension was shaken for 2 hours to attach the Fmoc-amino acid to the amino group on the resin. After the reaction, the resin was rinsed by pure DMF and then the DMF solution containing 20 vol% piperidine was added to the vessel to remove the amino terminal Fmoc-protecting group for 1 hour. After the reaction, the resin was rinsed by pure DMF until the piperidine was completely removed. This reaction cycle were repeated successively to obtain the desired sequence. Then the thioctic acid was attached to the amino terminal of the peptide on the resin by the same protocol described above. After the coupling reactions were completed, the peptide-resin was dried under vacuum and then cooled in an ice bath. A 10 mL of cooled aqueous solution containing 95 vol% trifluoroacetic acid (TFA) was added to the cooled peptide-resin to remove the OBu^t protecting groups of Glu side chains and to cleavage the peptide from the resin support for 1 hour at room temperature. After the reaction, the reaction mixture was filtrated to separate the peptide solution from the resin support. The TFA solution of the peptide was concentrated to a volume of approximately 1-2 mL and then a 100 mL of cooled ether was added to the TFA solution to precipitate the peptide. Identification of the peptide was made by MALDI-TOF mass spectroscopy (SHIMADZU /KRATOS KOMPACT II Kratos Analytical). Molecular weight of Phe-(Leu-Glu)$_8$-S was 2288. These values was in fair agreement with calculated value, 2292

2-2. Preparation of Gold Nanoparticle having Sequential Alternating Amphiphilic Peptide on the Surfaces

Phe-(Leu-Glu)$_8$-S was dissolved in 40 mL of DMF. Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4$ $4H_2O$) was dissolved in 10 mL of purified water. Then the $HAuCl_4$ aqueous solution was slowly added to the Phe-(Leu-Glu)$_8$-S solution in a vessel to make desired concentration of Phe-(Leu-Glu)$_8$-S and $HAuCl_4$. The molar ration of $HAuCl_4$ to Phe-(Leu-Glu)$_8$-S was fixed to 2.3. The solution was vigorously stirred during the addition of 10 mL of ice-chilled aqueous sodium borohydride ($NaBH_4$) (10 times the molar concentration of $HAuCl_4$) at room temperature for 1 hr. The final concentration of $HAuCl_4$ was 0.2 mM. After the reaction, this reaction mixture was dialyzed overnight against 1 L of distilled water using a Spectra/Pore molecular porous membrane tube (Spectrum Medical Industries, Inc., MWMC 3500). After the dialysis, the solution was lyophilized to obtain the gold nanoparticle having Phe-(Leu-Glu)$_8$ on the surface (Phe-(Leu-Glu)$_8$-Au). The amount of surface peptide on the Au nanoparticle was determined from the absorbance at 250 nm of the trifluoroethanol (TFE) suspension of the Phe-(Leu-Glu)$_8$-Au on the basis of the molar extinction coefficient of the Phe residue of Phe-(Leu-Glu)$_8$-S in TFE. The amount of fixed sequential alternating amphiphilic peptide on the gold surfaces obtained was 0.18 mmol per 1 g of gold core nanoparticle.

2-3. Circular Dichroism (CD) Spectroscopic Measurements

The conformation of Phe-(Leu-Glu)$_8$ on the gold nanoparticle was investigated by means of circular dichroism (CD) spectroscopy. CD spectra were recorded on a J-820 spectropolarimeter (JASCO Ltd.) under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 5 mm path length over the range of 190-300 nm at ambient temperature. The concentration of Phe-(Leu-Glu)$_8$-Au nanoparticle was fixed at 0.25 mg / mL.

3. RESULTS AND DISCUSSION

3.1. Structural Study of Gold Nanoparticle having Sequential Alternating Amphiphilic Peptide on the Surfaces

The structure of the Phe-(Leu-Glu)$_8$-Au was investigated by UV-Vis absorption and CD measurements. Figure 1a shows the UV-Vis spectrum of the Phe-(Leu-Glu)$_8$-Au dispersion in TFE. The spectrum showed an absorption band at 512 nm, assigned to a gold nanoparticle plasmon band. We estimated the secondary structure of the surface peptide on the gold nanoparticle in TFE from the CD spectra (Figure 1b). The CD spectrum of the surface peptide showed a double minimum profile at 208 nm and 222

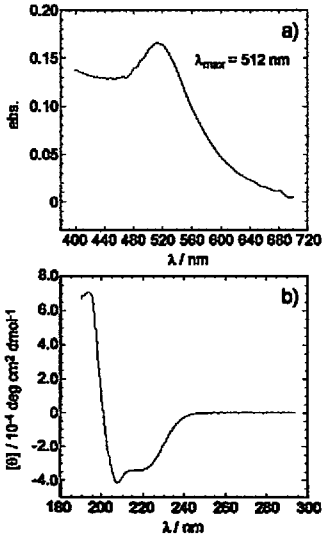


Figure 1. a; UV-Vis and b; CD spectra of Phe-(Leu-Glu)$_8$-Au in TFE.

nm, typical of a stable, right-handed α-helix[18]. This result suggested that the Phe-(Leu-Glu)$_8$-Au formed stable nanoparticle whose surface peptide chains took an α-helical conformation in TFE.

3.2. pH-Induced Conformational Transition of Surface Peptide on the Gold nanoparticle

We demonstrated the regulation of the surface peptide conformation on the gold nanoparticle. Figure 2 shows pH-induced CD spectral changes of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% TFE and the fraction of the secondary structure, which was calculated by a curve-fitting method using a linear combination of typical CD spectra for α-helical, β-sheet, and random

coil conformation. The CD measurements were carried out within 5 minutes after adjustment of the pH of the sample solution. At pH 9.2, the surface Phe-(Leu-Glu)$_8$ chains on the gold nanoparticle showed a negative maximum at 223 and 206 nm, indicating the existence of a right-handed α-helical conformation with a considerable amount of random coil and β-sheet

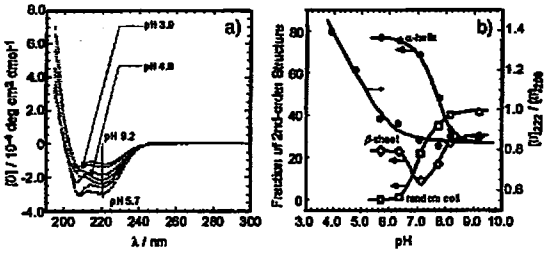


Figure 2. a); pH-induced CD spectral changes of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% TFE. b); pH dependence of $[\theta]_{222}/[\theta]_{208}$ and fraction of 2 nd-order structure of the surface Phe-(Leu-Glu)$_8$ chains estimated from the CD-curve fitting method and the. The CD measurements were carried out immediately after adjustment of the pH for the sample solution.

structure. It is clear that the random coil conformation of the surface Phe-(Leu-Glu)$_8$ chain changed to the α-helical conformation by decreasing the pH of the aqueous solution. On the other hand, CD spectra of Phe-(Leu-Glu)$_8$-Au in acidic aqueous solution below pH 5, did not fit by a linear combination of typical CD spectra for dispersed α-helical, β-sheet, and random coil conformation. The ratio of molar ellipticity of the CD band at 222 nm to that at 208 nm, $[\theta]_{222}$ / $[\theta]_{208}$, was increased by decreasing the pH under the acidic condition below pH 5. It is well known that the CD spectra of aggregated α-helical peptides were red shifted of a 222 nm band and flattened of a 208 nm band,[19] compared with typical dispersed α-helical peptides. This result implied that surface Phe-(Leu-Glu)$_8$ chains took an α-helical conformation and the surface peptide chains formed aggregate under the acidic condition owing to the decreasing the electrostatic repulsion

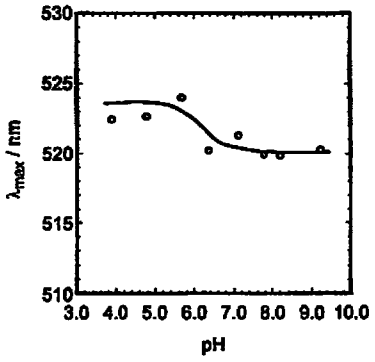


Figure 3. pH dependence of the plasmon absorption maximum of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% of TFE. The measurements were carried out immediately after adjustment of the pH for the sample solution.

between the Glu residues. Figure 3 shows the pH dependence of the plasmon absorption maximum of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% of TFE. The UV-Vis spectra were measured within 5 minutes after adjustment of the pH of the sample solution. The absorption maximum of Phe-(Leu-Glu)$_8$-Au, whose surface peptide took aggregated α-helical structure under the acidic solution, was shifted to higher wavelength. It has been reported that the formation of gold nanoparticle assemblies cause a red shifting of the peak wavelength of the plasmon absorption spectrum. [20] This result suggested that the α-helical surface peptide on the gold nanoparticle, which was formed under the acidic condition, connected the peptide coated gold nanoparticles owing to the peptide – peptide interaction.

3-3. Structural Changes of Peptide Coated Gold Nanoparticle Assembly

We investigated the dynamics of pH-induced conformational transition for Phe-(Leu-Glu)$_8$ surface peptide of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% TFE. First, the pH of Phe-(Leu-Glu)$_8$-Au in aqueous solution was adjusted to basic (pH 9.2; α-helical conformation with considerable amount of random coil and β-sheet structure, Figure 2). Under this condition, the CD spectra of the Phe-(Leu-Glu)$_8$-Au did not change after the incubation of 3days (Figure 4a). The pH of the Phe-(Leu-Glu)$_8$-Au solution was rapidly changes to acidic (pH 3.7; aggregated α-helical structure, Figure 2) and then the CD spectral changes of the Phe-(Leu-Glu)$_8$-Au with incubation time were measured (Figure 4b). Under the this condition, pH 3.7, the CD spectrum of the Phe-(Leu-Glu)$_8$-Au was rapidly altered to the typical pattern of β-sheet structure, with a single negative maximum around 218 nm from the pattern of

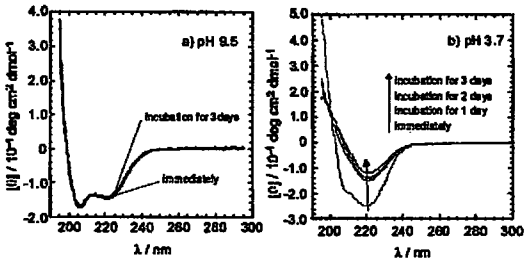


Figure 4. CD spectral changes of Phe-(Leu-Glu)$_8$-Au in aqueous solution containing 40 vol% TFE after the pH of the solution was rapidly changed a) to pH 9.5 and b) from pH 9.5 to pH 3.7.

the aggregated α-helical conformation. The single negative maximum of the CD spectra was shifted to shorter wavelength and the value of $[\theta]_{208}$ decreased towards 0 with progress in incubation time (Figure 5a). These CD spectral changes of Phe-(Leu-Glu)$_8$-Au under acidic conditions implied that the aggregated α-helical rods of surface Phe-(Leu-Glu)$_8$ chains on the gold nanoparticles changed in the β-sheet structure.

We investigated the structural changes of Phe-(Leu-Glu)$_8$-Au under the acidic condition by the shift of the plasmon absorption maximum. We have

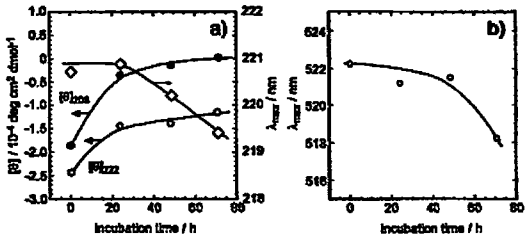


Figure 5. a); Time dependence of molar ellipticities at 222 nm and 208 nm, and CD negative maximum of Phe-(Leu-Glu)$_8$-Au. b); Time dependence of the plasmon absorption maximum of Phe-(Leu-Glu)$_8$-Au. The measurements were carried out within 5 minutes after the pH of the solution was rapidly changed from pH 9.5 to pH 3.7.

shown that plasmon absorption maximum of the Phe-(Leu-Glu)$_8$-Au, whose surface peptide chains formed the aggregated α-helical structure, was shifted to higher wavelength under the acidic condition (Figure 3). The wavelength of the plasmon absorption maximum was decreased with incubation time under the acidic condition (Figure 5). In other word, the conformational transition to the β-sheet structure from the aggregated α-helix of the surface peptide of the Phe-(Leu-Glu)$_8$-Au induced the blue shift of the plasmon absorption maximum. This result suggests that the core gold nanoparticles could be fixed in the state that stood alone in the β-sheet assembly of the sequential alternating amphiphilic peptides

In summary, the conformation of surface peptide composed of sequential alternating amphiphilic peptide, Phe-(Leu-Glu)$_8$, on the gold nanoparticle could be controlled by the external stimuli. Under the acidic condition, the surface peptide formed the aggregated α-helical structure immediately after the pH adjustment of the solution. The conformation of the surface peptide on the gold nanoparticle changed to the β-sheet structure and the peak wavelength of the plasmon adsorption spectrum shifted to shorter wavelength with progress in incubation time. This suggests that the core gold nanoparticles cold be fixed in the state that stood alone in the β-sheet assembly of the surface peptides. This system may be useful for novel molecular devices that exhibit quantized properties. We now study the morphological regulation of the peptide coated gold nanoparticle assembly induced by the external stimuli.

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