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Biomimetic synthesis of gold nanoparticles and their aggregates using a polypeptide sequence

Zheng Wang[1], Jinchun Chen[1]*, Peng Yang[2] and Wantai Yang[2]

[1]College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, People's Republic of China
[2]College of Material Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, People's Republic of China

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The polypeptide sequence MS14 (MHGKTQATSGTIQS) was used to explore a new method for biomimetic preparation of gold nanoparticles and their aggregates. Self-congregation of gold nanoparticles into aggregates in MS14 aqueous solution and self-assembly of gold crystallites onto the designed complex of MS14-PET film [protonated poly(ethylene terephthalate)] proved the specific gold-binding characteristic of the single-copy peptide MS14 *in vitro*. In aqueous solution MS14 could recover Au(III) to Au(0), tested by means of TEM, EDX and XPS. Further research suggested that the pH of the solution and the concentration of Au(III) influenced the morphology and size of the gold nanoparticles formed. In addition, extra reducing agent, sodium citrate, was introduced into the $HAuCl_4$–MS14 system and uniformly dispersed nanoparticles under neutral condition were obtained. Finally, we discuss the possible mechanism of this biomimetic synthesis. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: inorganic-binding peptide; gold nanoparticles; biomimetic synthesis

INTRODUCTION

Research on dispersed gold particles has attracted great attention in recent years as nanogold has been widely used as catalyst and biosensor and in immunofluorescent labeling and cellular imaging.[1,2] Since Faraday's work in the nineteenth century,[3] many different routes to produce colloidal gold have been reported, most processes involving the reduction of gold compounds in aqueous or nonaqueous media. Numerous reducing agents including borohydrides, aminoboranes, hydrazine, formaldehyde, hydroxylamine, alcohols, citric and oxalic acids, polyols, sugars, hydrogen peroxide, sulfites, carbon monoxide, hydrogen, and acetylene have been used for this purpose.[4] Using these chemical methods carried out under stringent conditions, we have synthesized size- and morphology-controlled gold particles effectively.

Comparatively, biomimetic synthesis carried out at neutral pH and room temperature might be an ideal alternative. Proteins and peptides[3,5–8] that adhere specifically to inorganic surfaces have been identified by phage display (PD)[9,10] and cell-surface display (CSD)[11] methods. The peptides possess limited selectivity for binding to metal surfaces such as Au, Ag, Pt, Pd or metal oxide surfaces such as GaAs, ZnO, SiO_2, Cr_2O_3, Fe_2O_3 and $CaCO_3$.[12] According to Mehmet Sarikaya and Stanley Brown,[6,13–16] an *E. coli* polypeptide GBP1 with at least three tandem repeats ([MHGKTQATSGTIQS]$_3$), identified by CSD technology, binds to gold interfaces with high affinity in the cell surface environment. Based on their discoveries, gold-binding peptide GBP1 and even its single copy might be utilized for the biomimetic synthesis of gold nanoparticles *in vitro*. However, we have not seen other reports on whether GBP1 or the single peptide MHGKTQATSGTIQS, which is referred to as MS14, could perform such a function.

We are interested in the single peptide MS14, which consists of only 14 amino acid residues and is easy to synthesize by chemical methods and easy to link with certain macromolecules like immunoglobulins or signal peptides to form bifunctional composites. We attempted to synthesize gold nanoparticles with defined shape and size because the application of nanoparticles is often affected by its size, shape, composition, crystallinity and structure. Manifestly, the key

*Correspondence to: Jinchun Chen, Beijing University of Chemical Technology, College of Life Science and Technology, Beijing 100029, People's Republic of China.
E-mail: jingchunchen@hotmail.com
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step for manufacture of these advanced materials in large scale is to learn about the controllable biomimetic synthesis by MS14. Here we report that the single peptide MS14, either immobilized on PET film or free in solution, could function for the synthesis of gold nanoparticles and their aggregates under ambient conditions and attempt to discuss the mechanism of the process.

EXPERIMENTAL

Reagents

MS14 (HGKTQATSGTIQS) was synthesized by Symphony, Protein Technologies Inc., USA. It was purified by RP-HPLC. All the reagents, such as tetrachloroauric(III) acid and sodium citrate, were of analytical grade.

MS14 immobilization on PET film

Commercial PET film was modified by incorporating tetra-amine functionality to poly(ethylene terephthalate) (PET) surface via UV-induced surface aminolysis reaction (USAR) at first. Sequentially, PET-N$(CH_3)_2$ was further protonated by soaking it in hydrochloric acid (pH 2.0) for 30 min to form PET-N^+ $(CH_3)_2$ film. The method is shown in 'Facile preparation of a patterned, polymer surface by UV-light-induced surface' by Yang *et al*.[17]

Two milligrams of MS14 peptide were dissolved into 20 ml ddH_2O (double-distilled water) and the pH was adjusted to 9.0 with NaOH at room temperature. PET film was soaked into the solution containing MS14 peptide for 1 h. MS14 peptide was adsorbed on the surface of the film through electrostatic interaction to form the MS14-PET complex, followed by stringent washing with ddH_2O to remove the unadsorbed MS14 peptide. The control was a blank PET film treated with the same procedures as MS14-PET film except MS14 peptide treatment.

The MS14-PET film was first soaked into 2.0 mM l^{-1} $HAuCl_4$ solution for 24 h, and then was put in a desiccator and transferred for sample drying for 24 h at room temperature and at atmospheric pressure.

Preparation of gold nanoparticles

The peptide MS14 was dissolved into tetrachloroauric(III) acid ($HAuCl_4$) aqueous solution in a clean Eppendorf minicentrifuge tube. The ultimate concentration of Au(III) in the reaction is 1 mM with 50 μM MS14. In repeating experiments, the concentration of Au(III) was increased to 10 and 100 mM to prepare larger particles, and sodium citrate 1 mM was added to accelerate the recovery and dilute HCl or NaOH to adjust the pH of the solution. All the samples were incubated for 24 h at room temperature to prepare for characterization.

Characterization

Scanning electron microscopy (SEM) was carried out with an SP, 250MK3 (Cambridge Co., UK), with an acceleration voltage of 20 kV. Transmission electron microscopy (TEM) was carried out with a Jeol-2000EX TEM operating at 160 kV. Samples for inspection by TEM were prepared by slowly evaporating one drop of prepared gold nanoparticles in solution at room temperature on a 400 mesh copper grid, which was covered by a carbon support film. All the specimens were conserved at room temperature before inspection by TEM. Energy dispersive X-ray (EDX) elemental line profiles were collected in the scanning TEM mode. The binding energy of dry gold powder placed on PET film as the substrate were measured by X-ray photoelectron spectroscopy (XPS), MKII (VG Co., UK), operated with an ALKα X-ray source (1486.6 eV) at 220 W (11 kV × 20 mA) and analyzed with a CAE model.

RESULTS AND DISCUSSION

Peptide MS14 adherence to gold

Following Frens's method, colloidal gold particles with average diameter of 10 nm were prepared by mixing sodium citrate solution and tetrachloroauric acid while boiling. When MS14 was placed into colloidal gold solution, the gold nanoparticles congregated into large-scale particles without typical configuration after being incubated for 24 h at room temperature. The color of the mixture gradually turned from orange into grey. On the TEM micrograph (Fig. 1) we could see well dispersed spherical nanoparticles (average diameter of 10 nm) congregated into larger particles (approximate size 100–500 nm). The corresponding electron diffraction (ED) pattern reveals that the larger particle is an aggregate of single crystals. In control experiments we replaced MS14 by a number of other peptides but found no such aggregation behavior. Combining the observed results and relevant documentations about gold crystallization by peptide[18,18] we assume that the peptide–Au(0) interaction occurred under ambient environment.

In order to confirm the specific binding feature of MS14 and explore feasible methods for biomimetic synthesis of gold nanoparticles, we designed a simple assembly. A surface-positively charged PET film prepared by UV aminolysis and subsequent protonation, according to Yang *et al*.,[17] was soaked in the solution at pH 9 containing MS14 peptide for 1 h. The MS14 peptide, pI 8.52, in the solution at pH 9 could be regarded as an anionic polyelectrolyte. MS14 peptide was adsorbed on the surface of the film through electrostatic interaction to form the MS14-PET complex, followed by stringent washing with double-distilled water to remove the unadsorbed MS14 peptide. The control was with a blank PET film treated with the same procedures as mentioned above, except for MS14 peptide treatment.

MS14-PET film was soaked in 2.0 mM l^{-1} tetrachloroauric(III) acid ($HAuCl_4$) stock solution at room temperature and at atmospheric pressure for 6 h and then was put into a desiccator for drying. The dried sample was observed using SEM.

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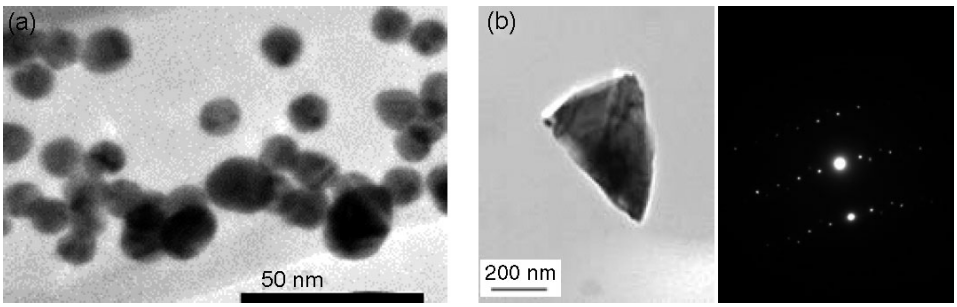

Figure 1. (a) TEM micrograph of the gold particles produced by Frens's method. (b) TEM micrograph and ED pattern of the composite produced by reaction of colloidal gold and MS14 for 24 h.

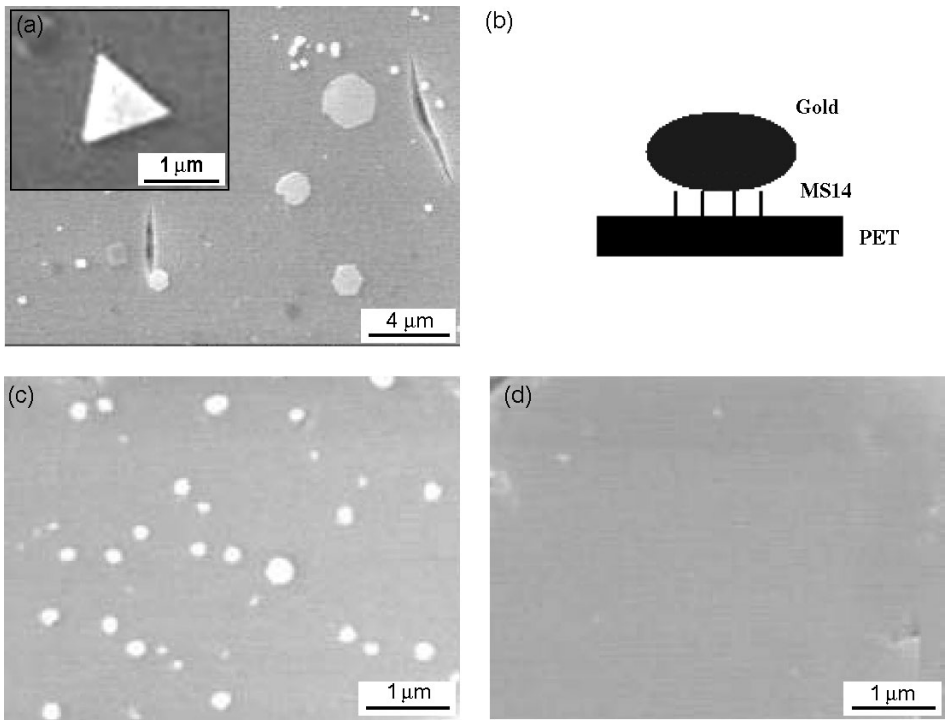

Figure 2. SEM image and schematic illustration. (a) Various (including triangular, hexagonal and cubic lattice) single crystallites formed on PET and not moved away with ddH_2O. (b) Schematic illustration of (a). (c) Spherical particles deposited on blank PET film. (d) Control: there were no particles on the blank PET film after washing with ddH_2O.

Gold single-crystallite formatted on the surface of MS14-PET film exhibited hexagonal, triangular and quadrangular thin microparticle morphology, 1–3 μm in size, and small spherical particles, approximately 200 nm in size [Fig. 2(a)]. These crystallite could not be removed easily with ddH_2O. In contrast, only spherical particles with size of 200 nm deposited on the blank PET film surface [Fig. 2(c)], but all were removed after the film surface was washed with ddH_2O [Fig. 2(d)].

The above results verified the adherence of MS14 to gold and the feasibility of forming crystallite, although the particles formed were limited in number and their size and morphology lacked uniformity.

Gold crystals formed by single peptides MS14

Gold crystal formation in a biomimetic environment is the main problem considered in this work. In previous studies, biological substances, such as bacteria,[19] peptides[12,20] and biomass,[21] have been utilized to produce metallic particles. Thus, the gold-binding peptide MS14 could also be an appropriate reagent to produce gold particles.

MS14 powder was dissolved in $HAuCl_4$ aqueous solution in an Eppendorf minicentrifuge tube and the mixture was incubated for 24 h. Spherical and polyhedral particles were observed on TEM micrograph [Fig. 3(a)]. In the corresponding EDX graph [Fig. 3(b)], atom quantity data

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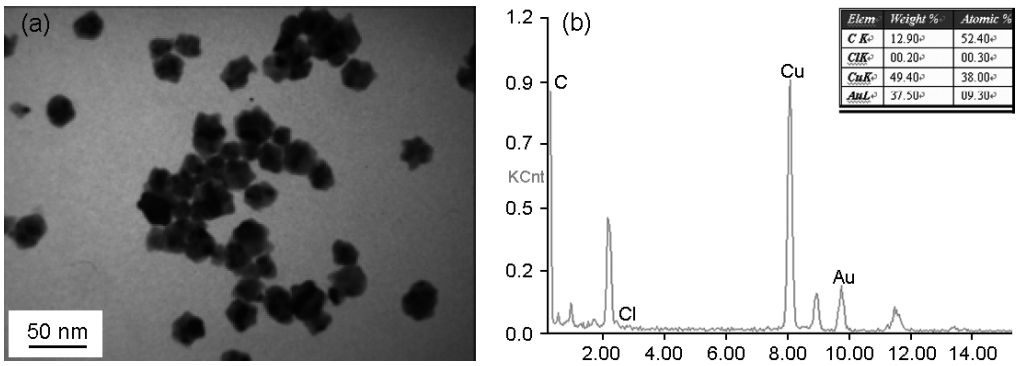

Figure 3. (a) TEM image of gold nanoparticles produced by MS14 at pH 7. (b) The corresponding EDX spectrum.

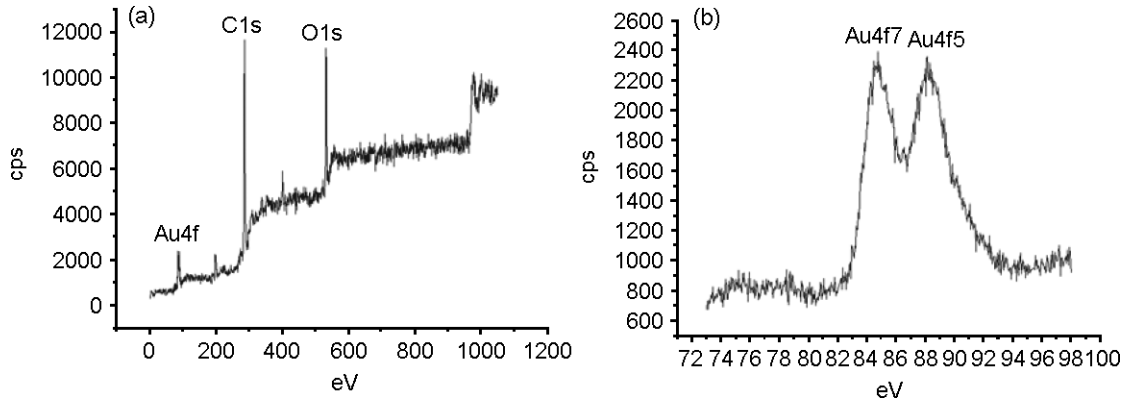

Figure 4. XPS graph of the gold sample gained by MS14 reduction. (a) The full spectrum. (b) Au4f spectrum.

of Au (9.30%) and Cl (0.30%) indicate that the amount of $HAuCl_4$ is limited. The detected Cl on the surface of the particles should come from the surrounding solution.

In the X-ray photoelectron spectroscopy (XPS) graph (Fig. 4), the measurement values were: C1s = 285.0, Au4f7/2 = 84.7, Au4f5/2 = 88.3. The standard correction factor of C1s was the chargeshift value 0.9eV, so the exact Au4f7/2 value was $84.7 - 0.9 = 83.8$ eV. No obvious Au(III)4f7/2 peak between 85 and 86 eV was observed. According to the standard binding energy (Au4f7/2 = 83.9 eV) and distance between the peaks Au4f5/2 and Au4f7/2 (3.6eV), the data confirms the reduced state of the gold nanoparticles.[22,23] Based on the observations and discussions above, we safely conclude that Au(III) in the form of $HAuCl_4$ was reduced to Au(0) to form nanoparticles.

In the following experiment, we attempted to elucidate the influence factors and to explore the optimum conditions to produce ideal uniform nanoparticles by changing the pH and concentrations of the Au(III) in $HAuCl_4$–MS14 solution and by using extra reagent in the solution. Here, sodium citrate was utilized and its reducing ability and availability were highly regarded.

Factors influencing gold formation: pH and Au(III) concentration

Figure 5(a, b) shows TEM images of gold particles produced at different pH environments. Obviously, lower pH leads to more flat triangular and hexagonal single crystals with sizes between 100 nm and 1 μm, and the slightly alkaline conditions of the $HAuCl_4$–MS14 solution tends to result in the formation of smaller gold nanoparticles. Figure 5(c, d) shows the TEM images of gold particles with sizes of about 50 and 100 nm obtained at different Au(III) concentrations. At higher concentrations of $HAuCl_4$, it is easy to produce larger particles through aggregation of smaller ones. In general, most gold particles exhibit regular shape, such as spheres, triangles or hexagons. The tendencies of the gold size and morphology influenced by pH and Au(III) concentrations have been simplified and summarized in Fig. 6.

Function of sodium citrate in $HAuCl_4$–MS14 solution

Since sodium citrate was able to reduce Au(III) into Au(0) while being boiled by the chemical method, we attempted to use sodium citrate to help to produce gold nanoparticles from $HAuCl_4$ rapidly in an ambient environment.

In the repeating experiments carried out under the same procedure and conditions but with different concentrations

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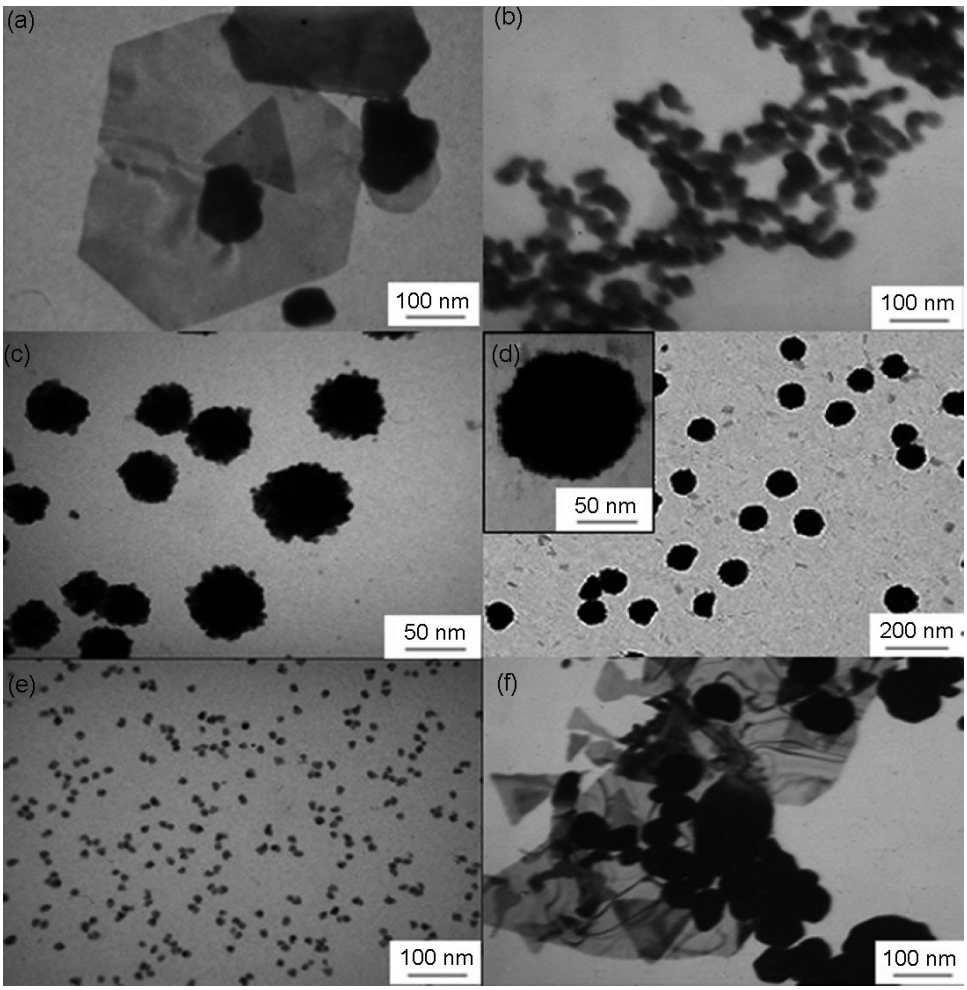

Figure 5. TEM images of gold particles produced by HAuCl4-MS14 solution. (a) At pH 5.5. (b) At pH 8.5. (c) With high concentration (10 times normal) of Au(III) at pH 7. (d) With high concentration (100 times normal) of Au(III) at pH 7. (e) In the present of sodium citrate at pH 7. (f) In the present of sodium citrate at pH 5.5.

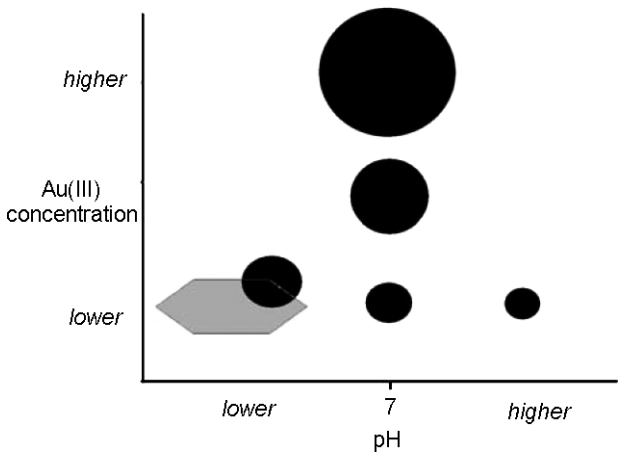

Figure 6. Illustration of the size and morphology variation controlled by Au(III) concentration and pH.

of sodium citrate, we found that the size of gold particles obtained decreased when sodium citrate and MS14 coexisted in the solution. Both the presence of sodium citrate and pH influenced the size and morphology of the particles obtained. Under neutral conditions, almost all gold nanoparticles exhibited nearly spherical shape, and the size decreased as the concentration of sodium citrate increased. Well dispersed spherical particles 10 nm in size were obtained when 50 μg ml^{-1} MS14 and 1 mM sodium citrate coexisted in 1 mM tetrachloroauric(III) acid ($HAuCl_4$) solution at pH 7 [Fig. 5(e)]. The size of particles increased remarkably and large thin flat crystals with triangular or irregular morphology appeared in an acidic environment [Fig. 5(f)]. The solution could show orange, purple or gray color as the size of gold nanoparticles varied, as expected.

Mechanism

According to related research on gold-binding peptides, it is believed that the formation of gold particles could

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be controlled by a combination of peptide–Au(0) and peptide–Au(III) interactions. Specifically, both the open, unfolded structure of the peptide, which is adhesive with forming Au clusters, and the presence of accessible proton donor/acceptor amino acids (Ser, Thr, Lys, Gln, His) in the peptide sequence, which may participate in the proposed acid-catalyzed or pH-mediated reaction, function in the formation of gold particles in solution.[15]

The size of the gold nanoparticles is decided by the balance of the amount of nuclei and the speed of aggregation. There have been enough experimental evidence and extensive studies to indicate that the large uniform particles consist of aggregates of small subunits. The mechanism by which the nanosize precursors irreversibly combine into colloids of various shapes has been explained and kinetic model has even been developed to determine the size of the aggregates.[4,24] Based upon these well developed studies and our experimental results, we will attempt to explain the mechanism in our system.

The strong tetrachloroauric(III) acid ($HAuCl_4$), the most accessible source of Au(III), totally dissociates in aqueous solutions generating $AuCl_4^-$ complex ions of square planar geometry. In neutral or slightly acidic solution, MS14 peptide, pI 8.52, carries an overall positive charge due to functional groups present in the sequence; thereby the negatively charged $AuCl_4^-$ ions are able to easily approach the binding sites due to $(AuCl_4)^-$/MS14 electrostatic interactions.[4,21] Since MS14 is both adhesive to original nuclei and the free $AuCl_4^-$, the process of enlargement of the nuclei accelerates and ultimately aggregates of nanoparticles are formed. By contrast, at higher pH, the accretion is difficult due to the repulsive $(AuCl_4)^-$/MS14 electrostatic interactions, and ultimately the nuclei only evolves into small aggregates. Moreover, higher Au(III) concentration also enhances the opportunity for nuclei to absorb free Au(III) ions in the solution, resulting in the formation of large aggregates. The addition of sodium citrate might reduce Au(III) more strongly than MS14 only and make it easy to form more original nuclei. However, the aggregation process is prevented by the electrostatic forces and better dispersed particles with smaller size are produced in the presence of extra reducing agent.

The large faces on the triangular and hexagonal crystals observed in Figs 2(a) and 5(a, b) are {111}, verified by electron diffraction (data not shown). A free crystal is inclined to adopt the equilibrium shape, which minimizes the total surface energy for a fixed volume. The crystal structure of gold is close-packed, face-centered cubic and the {111} faces possess the fewest number of broken bonds per atom and the lowest surface energy.[25] More energy is released by adding a gold atom to faces other than {111}. Crystal growth can be accelerated by biasing accretion onto a face other than {111}, thus increasing the area of the {111} faces. However, if accretion on all eight {111} faces were equally biased by the peptide, the process would be self-limiting as the {111} faces expand to form an octahedron. When accretion on only two of the {111} faces was affected by the peptide, the crystals could continue to expand by atoms accreting onto more energetically favorable faces. This would result in the formation of thin plates observed.[18]

It has been reported that similar large, thin gold crystals can also be produced by reducing Au(III) with boiling citric acid.[26] Also, in our experiment such flat crystals are only formed at pH lower than 7; we favor the explanation that the peptide emulates acidic conditions by regulation of proton concentration. Such a chemical environment in the vicinity acts to bias accretion on the thin edge of a flat crystal and thus a large-scale plate is formed. In comparison, the neutral and slightly alkaline environment does not favor such crystallization and only spherical colloidal particles are formed. Overall, control of local pH near the surface of the solid substrate partially by peptide is the possible mechanism of the crystal morphology.

Although significant advances have been made in the biosynthesis of nanogold particles using peptide, the exact impact of all possible parameters in the process, including temperature, reaction time and stir rate, needs to be further studied and a mathematical model should be established. It is believed that more complex multifunctional materials should be produced by utilizing the adhesion ability of inorganic-binding peptide.

CONCLUSIONS

The engineering peptide MS14 (MHGKTQATSGTIQS) can be utilized to catalyze and regulate nanogold crystallization. In aqueous solution the self-aggregation of gold nanoparticles into bigger crystals and the self-assembly of gold crystallites onto designed MS14-PET film complex proved the specific gold binding of the peptide MS14 *in vitro*. Moreover, pH of the solution, concentration of Au(III) and additional reducing agent sodium citrate influenced the size and morphology of the gold crystals obtained. In our experiments, uniformly dispersed spherical particles at approximate 10, 50 and 100 nm sizes were obtained in $HAuCl_4$ solution. We assume that the peptide–Au(0) interactions and peptide–Au(III) interactions make it possible to form gold particles whose size is decided by Au(III) concentration available in the solution and the presence of sodium citrate. The local pH partially controlled by the peptide results in the formation of flat crystals.

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