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Size-dependent flocculation behavior of colloidal Au nanoparticles modified with various biomolecules

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ABSTRACT

Au nanoparticles with different sizes were prepared and modified with various biomolecules including amino acids (arginine, lysine and cysteine), glutathione (GSH), oligopeptides, and proteins (bovine serum albumin (BSA), human serum albumin (HSA) and mouse IgG). The flocculation behaviors of the modified Au nanoparticles were investigated by observing their colorimetric and morphological changes with UV–vis spectrophotometer and transmission electron microscope (TEM), respectively. Consequently, we found that the flocculation behavior of the modified Au nanoparticles was quite different depending on both the size of the Au nanoparticles and the modified biomolecules. When modified with an amino acid, small-sized Au nanoparticles flocculated more easily than large ones while the modification with oligopeptides resulted in the flocculations of all tested Au nanoparticles due to the large number of hydrogen bonding between adjacent particles. The small Au nanoparticles were very effectively stabilized by protein capping, while the stabilization effect was not so good with large ones. The possible explanations for these size-dependent flocculation behaviors were discussed. This study would widen the understanding for the interaction involved in the Au nanoparticles modified with biomolecules.

1. Introduction

The synthesis and physical characterization of metal nanoparticles are currently very active fields of research due to their unique electronic, optical and catalytic properties [1–4] originating from their quantum-scale dimensions [5]. A number of outstanding studies already dealt with this rapidly increasing research area [6] highlighting their promising application in materials science and molecular biotechnology [7]. Especially, biomolecules conjugated with Au nanoparticles have received the most attention because of their high stability, good biocompatibility and high affinity for biomolecules [8,9].

Major strategy for the conjugation of biomolecules to Au nanoparticles was generally based on the direct interaction between some functional group within the biomolecule and the metal surface [10]. Typically the Au nanoparticles can be readily modified with thiol-containing biomolecules because the thiol group forms a strong interaction on the metal surface [11–13]. This thiol-induced modification of metal nanoparticles has been extensively employed in the relevant research field. For example, Mirkin group [12] exploited the colorimetric detection technology of DNA utilizing the aggregation of the oligonucleotide-modified Au nanoparticles which were prepared through the thiol interaction. Proteins or small biomulecules containing the thiol group were also widely employed to modify the Au nanoparticles [13,14]. Several researches were also performed to investigate Au nanoparticles modified with biomolecules which do not contain a thiol group [10].

In this work, we prepared Au nanoparticles with several different sizes, modified them with various biomolecules, and investigated their flocculating behavior depending on the size of the Au nanoparticles and the biomolecules. As a modifying biomolecule, we mainly studied amino acids, oligopeptides, and proteins without a thiol group although thiol-containing molecules like cysteine (Cys) and glutathione (GSH) were also covered.

2. Experiment

2.1. Materials

Hydrogen tetrachloroaurate ($HAuCl_4$), trisodium citrate, lysine (Lys), arginine (Arg), Cys, GSH, bovine serum albumin (BSA, MW = 66 kDa), human serum albumin (HSA, MW. 67 kDa) and mouse IgG (MW = 130 kDa) were purchased from Sigma-Aldrich Co. Sodium chloride was purchased from Junsei Co. The following

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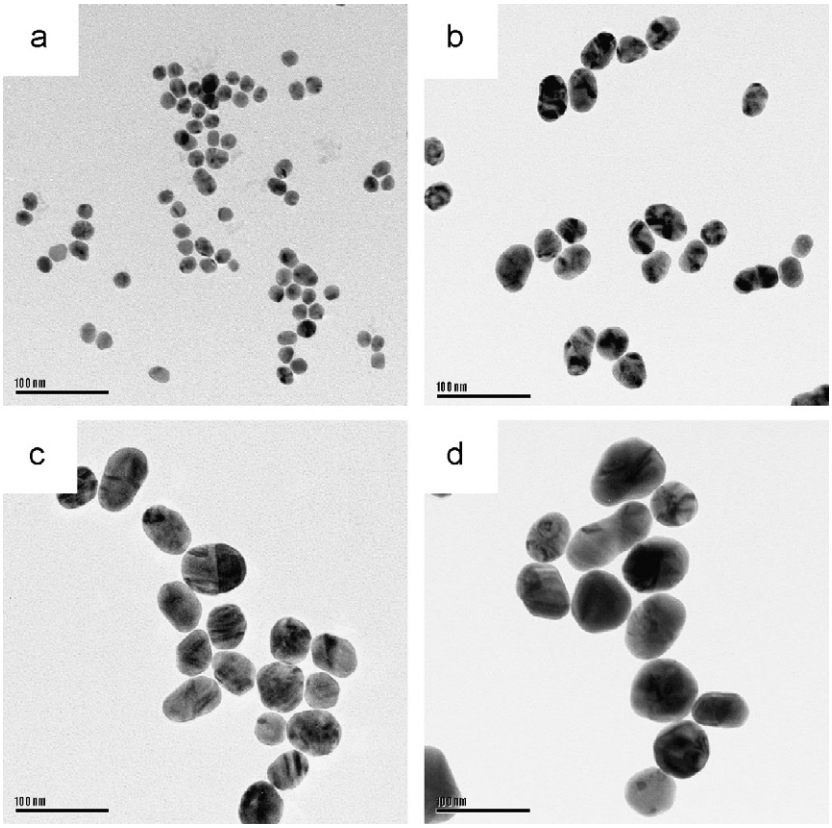

Fig. 1. TEM images of Au nanoparticles: (a) 13 nm, (b) 35 nm, (c) 45 nm and (d) 68 nm.

oligopeptides were provided by Bachem.: H-Pro-Thr-His-Ile-Lys-Trp-Gly-Asp-OH (MW = 953.07), H-Lys-Arg-Pro-Ala-Gly-Phe-Ser-Pro-Phe-Arg-OH (MW = 1162.36).

2.2. Sample preparation

Au nanoparticles were obtained by conventional citrate reduction method [15]. A 0.01% $HAuCl_4$ solution was reduced by 1% sodium citrate with vigorous stirring at near boiling temperature. The size of the nanoparticles was controlled by adjusting the amounts of sodium citrate.

The modifications of the Au nanoparticles with three amino acids (Lys, Arg, and Cys), GSH, two oligopeptides, and three different proteins (BSA, HSA, and mouse IgG) were carried out as follows: typically, the prepared colloidal Au nanoparticle (90 ml) was added to 10 ml of the aqueous solution of biomolecule at various concentrations allowing the conjugation of the biomolecule to the Au nanoparticle surface.

2.3. Measurements

Absorption spectra of the prepared Au nanoparticles and their conjugates with biomolecules were recorded by UV–vis spectroscopy at a resolution of 1 nm on Varian CARY-100 Conc. All spectra were collected over the range of 200–800 nm with 2 nm resolution. Transmission electron microscopy (TEM) measurements were performed on a TECNI F20 model 1300 KX instrument operated at an accelerating voltage of 131 eV.

3. Results and discussion

We prepared Au nanoparticles with four different sizes of about 13, 35, 45 and 68 nm determined by TEM (Fig. 1). The λ_{max} values of the Au nanoparticles were about 519, 528, 538 and 545 nm, respectively. These different-sized Au nanoparticles were modified with various biomolecules and the flocculation behaviors of the modified Au nanoparticles were investigated depending on the sizes of the Au nanoparticles and the conjugated biomolecules, by monitoring changes in their optical properties.

3.1. Flocculation behavior of Au nanoparticles conjugated with amino acids and oligopeptides

First, we modified the Au nanoparticles with three amino acids (Arg, Lys, and Cys) and observed their flocculation behaviors. As shown in Table 1, the modification of Arg or Lys caused flocculation for the small 13 nm Au nanoparticles over some concentration while large-sized Au nanoparticles (35, 45 and 68 nm) were not flocculated upon their modification. The flocculation behavior was also observed by the absorption peak shift of their surface plasma resonance in UV–vis spectra. As shown in Fig. 2, the spectrum of the Au nanoparticles (13 nm) modified with 1.0×10^{-3} M Arg had λ_{max} around 520 nm (curve b), which is almost same as that of unmodified Au nanoparticle (519 nm, curve a). The conjugates had red-purple color indicating that they were well suspended. On the other hand, the λ_{max} was shifted to 650 and 670 nm (curves c and d) when the nanoparticles were modified with 5.0×10^{-3} and 1.0×10^{-2} M Arg, respectively. Their colors were changed to blue. With these samples, both the long-wavelength (~650 nm) and short-wavelength (~520 nm) peaks were observed and finally

Table 1
Flocculation induced on Au nanoparticles when conjugated with various biomolecules, as a function of the final concentration of biomolecule and the size of Au nanoparticles

	13 nm (λ_{max} = 519 nm)	35 nm (λ_{max} = 528 nm)	45 nm (λ_{max} = 533 nm)	68 nm (λ_{max} = 545 nm)
Arg (M)				
1.0×10^{-2}				
5.0×10^{-3}				
1.0×10^{-3}				
Lys (M)				
1.0×10^{-2}				
5.0×10^{-3}				
1.0×10^{-3}				
Cys (M)				
1.0×10^{-2}				
5.0×10^{-3}				
1.0×10^{-3}				
GSH (M)				
1.0×10^{-2}				
5.0×10^{-3}				
1.0×10^{-3}				
Oligopeptide (MW. 953) (M)				
1.0×10^{-5}				
5.0×10^{-6}				
1.0×10^{-6}				
Oligopeptide (MW. 1162) (M)				
1.0×10^{-5}				
5.0×10^{-6}				
1.0×10^{-6}				
BSA[a] (M)				
1.0×10^{-4}				
5.0×10^{-5}				
1.0×10^{-5}				
HSA[a] (M)				
1.0×10^{-4}				
5.0×10^{-5}				
1.0×10^{-5}				
Mouse IgG[a] (g/L)				
1.0×10^{-3}				
5.0×10^{-4}				
1.0×10^{-4}				

The "blue box" indicates flocculation and the "violet box" indicates mid-flocculation of the Au nanoparticles, while "Solid line" means no flocculation of the Au nanoparticles.

[a] 0.1 ml of 10% NaCl solution was added to 1 ml of protein-capped Au colloidal solution.

the short-wavelength peak disappeared as the flocculation became dominant. The almost same tendency was also observed with the Au nanoparticles modified with Lys.

The flocculation of Au nanoparticles modified with amine-rich amino acid like Arg and Lys is considered to be induced by the interaction among amino acids attached on the nanoparticle surface. Generally, the amino group in a side chain binds to the Au nanoparticle surface while the terminal amino group forms hydrogen bonding with the carboxyl group of another amino acid on an adjacent nanoparticle (Fig. 3a) [10]. In contrast, there was no flocculation observed when large Au nanoparticles were modified with the Arg or Lys (Table 1). We suppose that the hydrogen bonding interaction between the modified Au nanoparticles is not strong enough to attract the large nanoparticles together leading to their flocculation in these cases [16].

Upon the addition of Cys, immediate flocculation was observed with the Au nanoparticles of all sizes tested in this study (Table 1). With Cys, the thiol group can form a strong chemisorption on the Au surface instead of the side amino group in Arg or Lys and the terminal amino group can form a hydrogen bonding with the terminal carboxyl group of another Cys on the adjacent Au nanoparticle in the same manner with the case of Arg or Lys (Fig. 3b) [17–19]. Being distinct from the case with Arg or Lys, the interaction by thiol group is supposed to be strong enough to lead to the flocculation via the subsequent hydrogen bonding even with the large Au nanoparticles. GSH, tripeptide molecule showed almost same flocculation behavior with Cys due to the presence of the thiol group within the molecule (Table 1).

Fig. 4 shows TEM images of 13 and 35 nm Au nanoparticles after conjugation with Arg and Cys, respectively. Interestingly, in

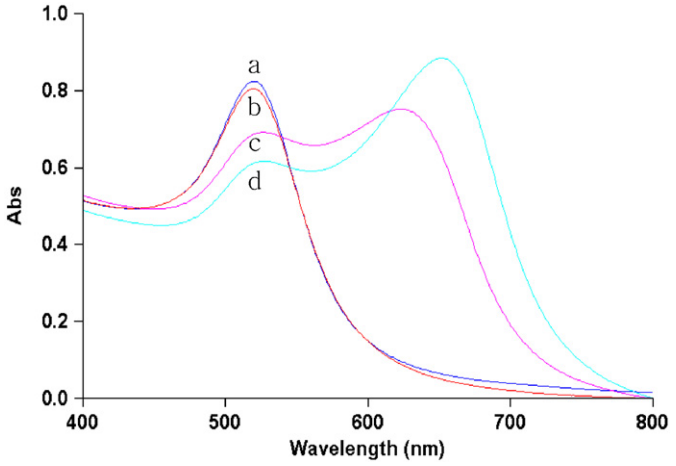

Fig. 2. UV–vis spectra of 13 nm Au nanoparticles conjugated with different concentrations of Arg: (a) unmodified Au nanoparticles, (b) Au nanoparticles conjugated with 1.0×10^{-3} M Arg, (c) Au nanoparticles conjugated with 5.0×10^{-3} M Arg and (d) Au nanoparticles conjugated with 1.0×10^{-2} M Arg.

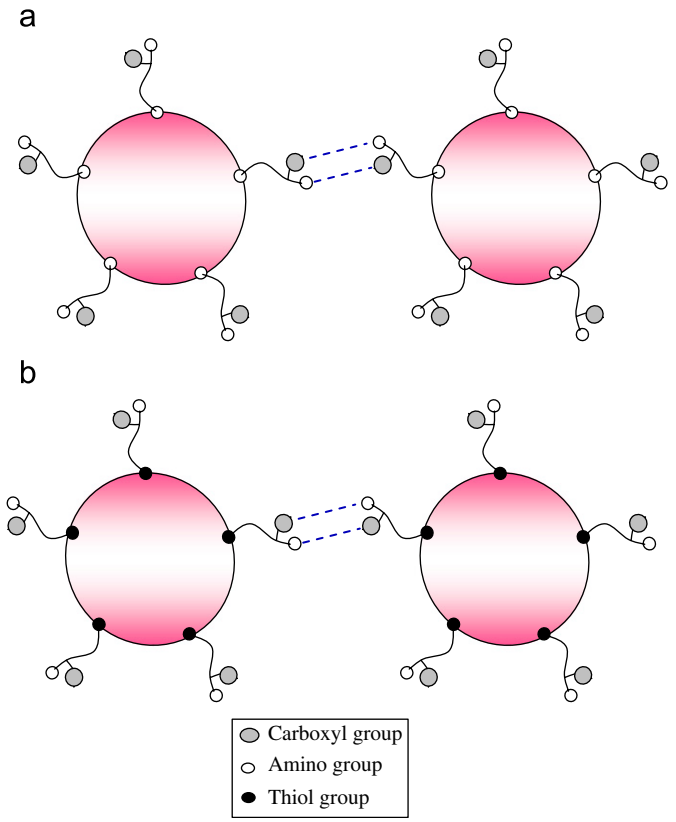

Fig. 3. Illustration of the assembly of Arg-modified Au nanoparticles (a) and Cys-modified Au nanoparticles (b) induced by hydrogen bonding between amino acid molecules in adjacent Au nanoparticles.

contrast to the largely isotropic character for unmodified Au nanoparticles, the flocculated Au particles show a dominant anisotropic pattern indicating the presence of chain-like or band-like elongation [20,21].

Next, we investigated the flocculation behavior of Au nanoparticles modified with oligopeptides (Table 1). Two oligopeptides consisting of 8 and 10 amino acids were studied in this work. Like the thiol-containing Cys and GSH, the modification with the oligopeptides resulted in the flocculations of all sized Au nanoparticles even though much lower concentrations were used. This strong capability to induce the flocculation could be explained by the fact that the oligopeptides have a large number of functional groups (amine, carboxyl, etc.) to form many hydrogen bondings, which can contribute to the flocculation of the modified Au nanoparticles.

3.2. *Flocculation behavior of Au nanoparticles capped with proteins*

Being distinct from the cases with amino acids and oligopeptides, modifications with proteins rarely cause the Au nanoparticle to flocculate. On the contrary, the modification with protein is known to stabilize the Au colloidal solution generating an additional repulsion energy term [22,23].

Therefore, in this case, we added NaCl solution to the protein-modified Au nanoparticles and studied their flocculation behavior. As shown in Fig. 5b, unmodified 13 nm Au nanoparticles rapidly precipitated upon the addition of 10% NaCl. Because of the screening effect of NaCl, the electrostatic repulsion of the Au nanoparticles is minimized to cause flocculation, which accompanied the plasmon resonance band shift from 524 to 691 nm. However, the addition of NaCl no longer caused the aggregation of the 13 nm Au nanoparticles when capped with BSA, indicating that the absorbed BSA molecules on the nanoparticle surface prevented them from flocculating (Fig. 5c).

This stabilization effect of protein was found to depend on the size of the nanoparticles. As shown in Table 1, the small Au nanoparticles (13 or 35 nm) were so effectively stabilized by protein capping that no flocculation was observed upon the addition of NaCl while the large Au nanoparticles appeared not to be well stabilized by the protein. This difference could be explained by the DLVO theory describing the electrostatic stabilization of particles in a suspension [24]. The DLVO theory describes balanced forces between the electrostatic repulsion in particles and the attraction due to van der Waals attraction. Their combination supports the total interaction energy as a function of separation distance between two Au nanoparticles. The attractive force between two Au nanoparticles exists at primary minimum due to van der Waals interactions which cause aggregation in the absence of any other repulsive forces. At a relatively large distance of separation, there is a shallow minimum known as secondary minimum. The presence of a secondary minimum is due to the longer range of the attraction force compared to that of electrostatic repulsion. The depth of this minimum chiefly depends on the attractive force, which is proportional to the size of the particles [25]. In this reason, the large-sized Au nanoparticles are more prone to flocculation than those of small-sized ones. In addition, proteins may not entirely cover the surface of the large Au nanoparticles. Therefore, the protecting effects that generate the additional repulsion in small Au particles do not occur in large-sized ones.

When compared to oligopeptides, protein molecules are supposed to retain their inherent folded structures on the surface of the Au nanoparticles, consequently preventing them from forming hydrogen bonding with neighboring nanoparticles which is the common situation with oligopeptides.

4. Conclusion

In this study, we found that the Au nanoparticles flocculate when modified with amino acids or oligopeptides and the flocculating behavior is quite different depending on both the size of the Au nanoparticles and the used biomolecules. The stabilizing effect caused by protein capping is also dependent on the size of the Au nanoparticles. The results and scientific

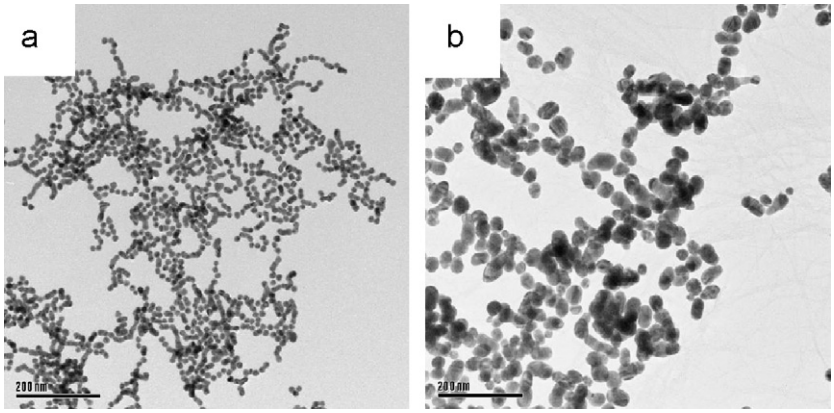

Fig. 4. TEM micrograph of the flocculated Au nanoparticles resulted from modification with amino acids: (a) 13 nm Au nanoparticles modified with 1.0×10^{-2} M Arg and (b) 35 nm Au nanoparticles conjugated with 1.0×10^{-3} M Cys.

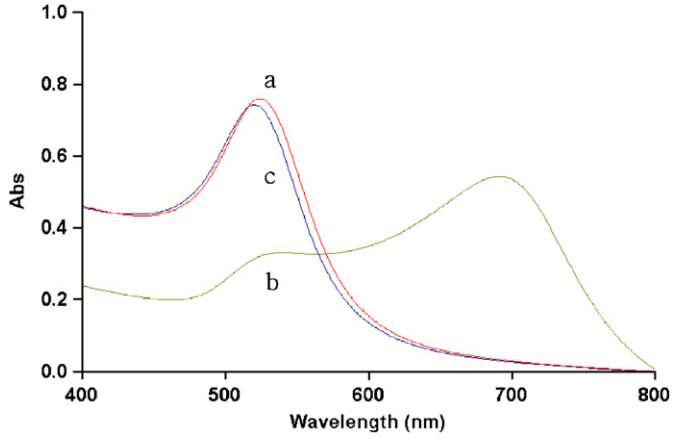

Fig. 5. UV–vis spectra of 13 nm Au nanoparticles capped with BSA: (a) unmodified Au nanoparticles, (b) unmodified Au nanoparticles after 10% NaCl addition and (c) BSA-capped Au nanoparticles after 10% NaCl addition.

interpretations made in this work would greatly help us to understand the interactions involved in the Au nanoparticles modified with biomolecules. The further characterization of interactions in biomolecule-capped Au nanoparticles and more precise explanation of the flocculation mechanism are underway to establish a comprehensive concept.

Acknowledgments

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