Superoxide Scavenging Activity of Gold, Silver, and Platinum Nanoparticles Capped with Sugar-based Nonionic Surfactants

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Abstract: Metal nanoparticles have the ability to remove superoxide via changes in the surface electronic states at the large surface area. Gold, silver, and platinum nanoparticles were prepared in the presence of three sugar-based nonionic surfactants using NaBH\textsubscript{4} as a reducing agent. The surfactants (glycosyloxyethyl methacrylate: xGEMA) contain sugar oligomers of various lengths (x), are biodegradable, and act as protecting groups for the nanoparticles. Three types of xGEMA were used: dodecyl and hexadecyl chains containing amphiphilic oligomers (C\textsubscript{12}-3.0GEMA and C\textsubscript{16}-3.2GEMA) and multi-dodecyl chain with multiple sugar side chains (1.8C\textsubscript{12}-4.7GEMA). We found that the type of nonionic surfactant affected the size of the nanoparticles. The average size of the gold, silver, and platinum nanoparticles ranged from 1.9 to 6.6 nm depending on the surfactant. The trend in the size of gold nanoparticles in relation to the chosen surfactants was different from that for the silver and platinum nanoparticles. Moreover, the gold nanoparticles did not show effective antioxidant activity for superoxide, whereas the silver and platinum nanoparticles removed superoxide to a certain extent. The general order for superoxide scavenging activity increased in the following order: gold \textless platinum \textless silver. In particular, the largest size of silver nanoparticles capped with C\textsubscript{16}-3.2GEMA had a similar ability for the removal of superoxide as superoxide dismutase (ca. 3999 unit/mg) on the basis of the mass concentration.

Key words: nanoparticle, anti-oxidative activity, SOD-like activity, nonionic surfactant

1 Introduction

Metal nanoparticles have distinct properties compared to small molecules and bulk materials, including their melting point, magnetic susceptibility, and light absorption\textsuperscript{1,2}. In addition, metal nanoparticles have a large specific surface area, which can result in high catalytic activity via the changes in the electronic states of the surface atoms\textsuperscript{3-4}. This catalytic activity has potential applications in cosmetics and medicine\textsuperscript{5,6}. In particular, nanoparticles can show an antioxidant effect similar to that of superoxide dismutase (SOD), which removes reactive oxygen species in biological systems\textsuperscript{7}. Reactive oxygen species are related to the onset of various diseases\textsuperscript{8}. Therefore, metal nanoparticles have been modified to increase their biocompatibility\textsuperscript{8,9,10}. In addition, gold nanoparticles have been studied for use in photothermal therapy and as radiosensitizers by exploiting their photoelectric properties\textsuperscript{11,12}. Silver and platinum nanoparticles have been used as antimicrobial agents and their toxicity for different applications has been evaluated\textsuperscript{13,14}.

In this study, we prepared gold, silver, and platinum nanoparticles using a reducing agent (NaBH\textsubscript{4}) in the presence of three types of sugar-based nonionic surfactant. The surfactant molecules are composed of single alkyl chain (C\textsubscript{12}-3.0GEMA and C\textsubscript{16}-3.2GEMA) or multiple alkyl chains (1.8C\textsubscript{12}-4.7GEMA) containing amphiphilic oligomers with multiple sugar side chains, as shown in Fig. 1\textsuperscript{15}. In general, sugars are abundant natural resources and are more biodegradable than ionic aliphatic surfactants. The bulkiness of surfactants may affect the formation of nanoparticles and control their size by acting as protective agents. The superoxide scavenging activity of metal nanoparticles was measured to estimate the function of percentage by mass. Our results will be useful in planning the design of next-genera-
2 Experimental Procedures

2.1 Materials

The preparation and purification of single-alkyl chain (C_{12}-3.0GEMA and C_{16}-3.2GEMA) and multi-alkyl chain (1.8C_{12}-4.7GEMA) containing amphiphilic oligomers with multiple sugar side chains have been reported previously. The molecular structures of the three kinds of sugar-based nonionic surfactant are shown in Fig. 1. Hydrogen tetrachloroaurate (III) tetrahydrate (HAuCl_{4}·4H_{2}O) and hydrogen hexachloroplatinate (IV) hexahydrate (H_{2}Cl_{6}Pt·6H_{2}O) were obtained from Tanaka Kikinzoku Kogyo K.K. Silver(I) nitrate and sodium tetrahydridoborate (NaBH_{4}) were purchased from Wako Pure Chemical Co. Superoxide dismutase (SOD) from lyophilized bovine erythrocytes powder (3999 units/mg solid) was obtained from Sigma Co. The commercial materials were guaranteed reagents and were used without further purification. Pure water was obtained by ion-exchange treatment with a Milli-Q Plus.

2.2 Synthesis of gold, silver, and platinum nanoparticles capped with sugar-based nonionic surfactants (C_{12}-3.0GEMA, C_{16}-3.2GEMA, and 1.8C_{12}-4.7GEMA)

The gold nanoparticles were synthesized by the chemical reduction of HAuCl_{4}·4H_{2}O in three kinds of sugar-based nonionic surfactants by NaBH_{4}. In a typical reaction, 1 mL of HAuCl_{4} aqueous solution (2 mmol L^{-1}) was added to 8.6 mL of sugar-based nonionic surfactant (0.05 mg mL^{-1} of C_{12}-3.0GEMA, C_{16}-3.2GEMA, or 1.8C_{12}-4.7GEMA). The mixed solution was stirred for 30 min at room temperature. Then, 0.4 mL of sodium borohydride (50 mmol dm^{-3}) in ice was poured into the solution. The total volume of the solution was 10 mL, and the solution was stirred for 30 min. Therefore, the final concentration of gold was calculated to be 0.2 mmol L^{-1}(0.0394 mg mL^{-1}), and the concentration of the surfactants used as a protective agent was 0.043 mg mL^{-1}. The syntheses of silver and platinum nanoparticles were similarity performed by replacing the HAuCl_{4}·4H_{2}O starting material with AgNO_{3} and H_{2}Cl_{6}Pt·6H_{2}O, respectively. The nanoparticles were used without further purifications. The combination of nanoparticle precursors and three kinds of surfactant solutions yielded nine nanoparticle samples, as shown in Table 1.

2.3 Measurements

2.3.1 Ultraviolet and visible (UV-vis) absorption spectrum of nanoparticles

The absorbance of nanoparticles in the surfactant solutions were measured by UV-vis spectrophotometry (Shimazu, UV-2500PC).

2.3.2 Transmission electron microscopy (TEM)

A drop of a sample solution was dried on a carbon-coated copper grid for one day in a desiccator. The observation of the nanoparticles on the copper grid were performed using a JEOL-1011 TEM with acceleration voltages of 100–300 kV. The average size of the nanoparticles was determined from ca. 200 nanoparticles using image processing analysis (Image J).

2.3.3 Superoxide scavenging activity

The assay for superoxide scavenging activity was an SOD Assay Kit – WST (Dojindo Molecular Technologies, Inc.). The superoxide was generated by mixing xanthine and xanthine oxidase. The superoxide scavenging activity in the system was determined by measuring the absorbance of generated formazan (pigment) at 450 nm. Formazan is the final product in the oxidation reaction of the tetrazolium salts in the system. If oxidation to the water soluble

Fig. 1 Molecular structure of single- and multi-chain sugar-based nonionic surfactants (C_{12}-3.0GEMA, C_{16}-3.2GEMA, and 1.8C_{12}-4.7GEMA).
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tetrazolium salt is fully inhibited by the addition of the scavenger (nanoparticles or SOD), the tetrazolium salt (pigment material) is not oxidized to formazan, i.e., the absorbance of the solution in the visible light region is almost zero. The ratio of inhibition for each nanoparticle sample was evaluated using equation (1) based on experiments varying the concentration of nanoparticles.

\[
\text{Inhibition ratio}(\%) = \frac{[A_{\text{blank1}} - A_{\text{blank2}}] - [A_{\text{sample}} - A_{\text{blank1}}]}{[A_{\text{blank1}} - A_{\text{blank1}}]} \times 100
\]

Here, \(A_{\text{blank1}}\) denotes the total absorbance without inhibition at 450 nm, and \(A_{\text{blank2}}\) and \(A_{\text{blank1}}\) refer to the absorbances from the sample and reagent solutions without xanthine oxidase, respectively. \(A_{\text{blank1}}\) is the absorbance of the sample solution with reagent and xanthine oxidase. Next, the actual procedure is described. 1. An aqueous sample solution (120 \(\mu\)L) was added to a mixture of xanthine and tetrazolium salt (400 \(\mu\)L) in a plastic cell. 2. Xanthine oxidase solution (40 \(\mu\)L) was poured into the above cell. 3. The solution was incubated in thermostat with a shaker function at 310 K. 4. The absorbance of the solution was measured at 450 nm. The measurements were performed three times and the average values were calculated.

The curve of inhibition yields the IC50 value, which is the nanoparticle concentration at 50% superoxide inhibition. The IC50 is important parameter for comparison with other antioxidant reagents. Moreover, the superoxide scavenging activity can be calculated in SOD units by comparison of the IC50 values of superoxide dismutase (3999 unit/mg) and a standard substance.

### 3 Results and Discussion

#### 3.1 Nanoparticle properties

Gold, silver, and platinum nanoparticles were synthesized using NaBH4 as a reducing agent in the presence of three kinds of sugar-based nonionic surfactants (C12-3.0GEMA, C16-3.2GEMA, and 1.8C12-4.7GEMA). The effects of the surfactants on the size and the shape of the nanoparticles and their protective ability in solution were investigated[17, 18]. In general, nanoparticles take on a core–shell structure capped with surfactants, and surfactant monomer exchange can occur in the bulk solution. Therefore, the surfactant does not bind to the surface of the nanoparticles with strong intermolecular forces. As shown in Fig. 1, the structures of the oligomeric surfactants are different from typical linear alkyl surfactants. In our previous report, we reported the critical micelle concentrations (CMC) of C12-3.0GEMA, C16-3.2GEMA, and 1.8C12-4.7GEMA to be 0.0258, 0.0106, and 0.043 mg mL\(^{-1}\) at 298.2 K[19]. The final concentrations of all nanoparticles and surfactants were fixed to 0.2 nmol L\(^{-1}\) and 0.043 mg mL\(^{-1}\), respectively. The surfactant concentrations are above the CMC which means that the nanoparticles are present with micelles.

The UV-vis absorbance spectra of aqueous solutions of the nanoparticles capped with sugar-based nonionic surfactant are shown in Fig. 2. Characteristic surface plasmon resonances at 520 and 410 nm were observed for the gold and silver solutions, respectively. In the case of the platinum nanoparticle solution, the peak from platinum ions at 260 nm disappeared and a broad peak appeared, which originated from the generated nanoparticles. Fig. 2 shows the spectra with depending on the kinds of surfactants from gold and silver particles. The small differences in the spectra of the nanoparticles formed in the presence of diff-

### Table 1 Properties of gold, silver, and platinum nanoparticles capped with sugar-based nonionic surfactants. Nanoparticle sizes were determined from TEM images. IC50 is the nanoparticle concentration at 50% inhibition ratio. Number of SOD units is calculated by comparing the IC50 between superoxide dismutase (3999 unit/mg) and nanoparticles.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Metal concentration (mmol L(^{-1}) (mg mL(^{-1}))</th>
<th>Particle size (nm)</th>
<th>IC50 (mg mL(^{-1}))</th>
<th>SOD unit (unit mg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au-C12-3.0GEMA</td>
<td>0.20 (0.039)</td>
<td>5.2 ± 2.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Au-C16-3.2GEMA</td>
<td>&quot;</td>
<td>2.5 ± 1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Au-1.8C12-4.7GEMA</td>
<td>&quot;</td>
<td>3.5 ± 1.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ag-C12-3.0GEMA</td>
<td>0.20 (0.022)</td>
<td>4.3 ± 3.2</td>
<td>0.0007</td>
<td>1739</td>
</tr>
<tr>
<td>Ag-C16-3.2GEMA</td>
<td>&quot;</td>
<td>6.6 ± 7.1</td>
<td>0.0004</td>
<td>2857</td>
</tr>
<tr>
<td>Ag-1.8C12-4.7GEMA</td>
<td>&quot;</td>
<td>4.9 ± 4.6</td>
<td>0.0013</td>
<td>928</td>
</tr>
<tr>
<td>Pt-C12-3.0GEMA</td>
<td>0.20 (0.039)</td>
<td>1.9 ± 1.2</td>
<td>0.0039</td>
<td>308</td>
</tr>
<tr>
<td>Pt-C16-3.2GEMA</td>
<td>&quot;</td>
<td>4.5 ± 3.8</td>
<td>0.0036</td>
<td>333</td>
</tr>
<tr>
<td>Pt-1.8C12-4.7GEMA</td>
<td>&quot;</td>
<td>2.6 ± 1.6</td>
<td>0.0093</td>
<td>129</td>
</tr>
</tbody>
</table>

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Fig. 2 UV-vis absorption spectra of aqueous solutions of gold and silver nanoparticles capped with three types of sugar-based nonionic surfactants. In the case of platinum nanoparticle, the typical spectrum of nanoparticle capped with C_{12}-3.0GEMA is shown in the figure.

Fig. 3 Size distributions of nanoparticle samples by TEM. The samples of nanoparticle are indicated in the figures (a)–(i) with the corresponding abbreviations. The sample composition corresponds to the data in Table 1.
different surfactants originates from the size of the gold and silver nanoparticles, and the size distribution of the nanoparticles (Fig. 3) was determined by TEM observation (Fig. 4). The average sizes of nanoparticles are summarized in Table 1. The peaks of surface plasmon band for Au-C12-3.0GEMA and Au-1.8C12-4.7GEMA are red shift from those for Au-C16-3.2GEMA which may originate from their larger size of nanoparticles. In fact, the average sizes of Au-C12-3.0GEMA and Au-1.8C12-4.7GEMA are relative larger than Au-C16-3.2GEMA by TEM observation. As shown in Fig. 2, the red shift of silver nanoparticles was also almost consistent with the nanoparticle size dependency. However, it should be noted that the average size of the nanoparticles are estimated from the poly-dispersed distributions. As shown in Fig. 3, the size distributions follow neither the Gaussian nor Poisson distribution. The frequency decreases with increasing nanoparticle size in an exponential manner ($e^{-x}$). The one-sided distribution could be a result of coagulation during the process of nanoparticle generation. There was no particular trend in the size order between the different metals and surfactants. However, the silver nanoparticles have a relatively larger size than the gold and platinum nanoparticles (Table 1). The maximum and minimum size of nanoparticles were observed for Ag-C16-3.2GEMA (6.6 nm) and Pt-C12-3.0GEMA (1.9 nm), respectively. In general, the use of surfactants as protecting agents increased the size of the Ag and Pt nanoparticles in the following order C12-3.0GEMA ‘1.8C12-4.7GEMA ‘C16-3.2GEMA, and the order is corresponded to lowering CMC values$^{15}$. Yada et al. showed that the size of gold nanoparticles increased as the terminal group of polyoxyethylene chain changed from hydroxyl to methoxy, ethoxy, and trioxypropylene in dodecyl ether type of nonionic surfactants$^{17}$. The size of order for nanoparticles was also same as order of lowering CMC values. However, the order for gold nanoparticles in present study was showed reverse result for the above tendency. There is no doubt that the balance between the hydrophobicity and the hydrophilicity of the surfactant influences the growth of nanoparticles. Kazakova et al. also proposed an interaction model between gold nanoparticles and surfactants with two kinds of anionic and cationic surfactants that influenced the nanoparticle size$^{19}$. The properties of nanoparticles may be controlled by changing the kind of surfactant.
and the metal. However, in order to evaluate the influence of surfactants on the size of the nanoparticles, it is necessary to conduct studies with having simple structure of surfactant systems.

3.2 Superoxide scavenging activity by nanoparticles

Metal nanoparticles are catalytically active for various chemical reactions. The superoxide scavenging activity of nanoparticles has been studied experimentally and theoretically. In this study, the difference in the antioxidant activity of the nanoparticles was estimated by comparing their superoxide scavenging activity. Similar experiments were also performed for superoxide dismutase (SOD) and silver nitrate, which acted as a representative silver ion. The antioxidant ability of the nanoparticles is clear on comparison with the activity of the standard material. The inhibition ratio of superoxide with respect to the concentration of nanoparticles and the reference materials is also shown in Fig. 5. The IC_{50} is the nanoparticle concentration at a superoxide inhibition ratio of 50%, which was determined by drawing a line to the concentration axis from the 50% intersection.

The gold nanoparticles did not show high antioxidant ability, even at the highest concentration (0.2 mmol L^{-1}). The maximum inhibition ratios for Au-C_{12}-3.0GEMA, Au-C_{16}-3.2GEMA, and Au-1.8C_{12}-4.7GEMA were 7.4%, 3.6%, and 10.5% respectively. The Au nanoparticles are not suitable for active oxygen removal. However, He et al. reported that relative smaller sized gold nanoparticles coated with polyvinylpyrrolidone or tannic acid could act in a similar way to SOD. The SOD-like activity of gold nanoparticles was highly dependent on their concentration. On the other hand, there are many reports that gold nanoparticles effectively remove hydrogen peroxide and hydroxyl radicals. Thus, gold nanoparticles are selective for the removal of free radical and reactive oxygen species. All samples of the silver nanoparticles showed effective antioxidant activity. As shown in Fig. 5, the IC_{50} of Ag-C_{16}-3.0GEMA approaches that of SOD (3999 units/mg solid). The antioxidant ability of Ag-C_{16}-3.2GEMA is almost equivalent to 2857 units/mg in SOD units by comparison of the IC_{50} values. The Ag-C_{16}-3.2GEMA nanoparticles have the largest size (ca. 6.6 nm) of the studied samples. The IC_{50} values for the silver nanoparticles decreased as follows: AgNO_{3} > Ag-1.8C_{12}-4.7GEMA > Ag-C_{12}-3.0GEMA > Ag-C_{16}-3.2GEMA (Fig. 6). The small IC_{50} value means that the nanoparticles can remove superoxide with only a low loading. The chemical reaction for the removal of active oxygen must occur at the surface of nanoparticles. The superoxide scavenging is mainly consist of protonation of O_{2}^{-}, adsorption, and rearrangement of HO_{2} on metal surfaces (O_{2}^{-} + H_{2}O = HO_{2} + OH^{-}) and then 2HO_{2} = O_{2} + H_{2}O_{2}^{2-}. According to Shen’s report, the adsorption energy of HO_{2} at the surface of Au, Pt, and Ag are estimated to -1.10, -1.31, and -1.52 eV (exothermic process), respectively. The lowering of adsorption energy of HO_{2} means free energy gap for generating O_{2} in a part of superoxide scavenging system which correspond to order of our
experimental result (Au<Pt<Ag). However, several factors affected on the process of superoxide scavenging. Our experimental results could show the superiority of each metal with regard to elimination of superoxide, but the detailed mechanism cannot be discussed at this point.

As shown in Fig. 6, the relatively larger size of Ag-C16-3.2GEMA resulted in good ability for the removal of superoxide compared to the smaller Ag-1.8C12-4.7GEMA and Ag-C12-3.0GEMA nanoparticles. Comparing Ag-1.8C12-4.7GEMA and Ag-C12-3.0GEMA, which have similar particle sizes, the former showed better removal of superoxide than the latter. The platinum nanoparticles also showed similar result for the removal of superoxide (IC50 values; Pt-1.8C12-4.7GEMA>Pt-C12-3.0GEMA>Pt-C12-3.2GEMA). The trend in IC50 values with type of capping surfactant is almost the same as those for the silver nanoparticle system.

In the case of using the same concentration for the preparation of nanoparticles, the increase in the size of the nanoparticles resulted in a numerical decrease in number of nanoparticles. In other words, the formation of larger nanoparticles means a lower solution particle concentration. However, the large nanoparticles show increased catalytic activity compared to those of the smaller particles, despite their lower concentration. Therefore, there must be an optimal particle size and protecting agent content when using nanoparticles as scavengers for superoxide.

The order of superoxide scavenging activity in relation to the metal increases as gold<platinum<silver nanoparticles. However, we must pay attention to the planned application, especially if the silver nanoparticles are used for medical or environmental applications.[27]

4 Conclusion

Gold, silver, and platinum nanoparticles capped with sugar-based nonionic surfactants were easily synthesized by chemical reduction with NaBH4. The three types of surfactants used as protective agents significantly affected the size of the nanoparticles. The trends in growth for the gold nanoparticles were different for the silver and platinum nanoparticles depending on the kind of surfactant used. The gold nanoparticles did not have a strong antioxidant effect for superoxide, whereas the silver and platinum nanoparticles showed good ability to remove superoxide. In particular, the silver nanoparticles (Ag-C16-3.2GEMA), which are relatively larger, showed good antioxidant action, similar to that of SOD. However, toxicity remains a problem if silver nanoparticles are used in large quantities for medical or environmental applications. Thus, it is desirable to improve the antioxidant action of gold and platinum nanoparticles.

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