Comparison of Gold–PAMAM and Gold–PPI Dendrimer Nanocomposites for Antioxidant Action

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Abstract

Gold–dendrimer nanocomposites were prepared in the presence of poly(amidoamine) (PAMAM) dendrimer (generation 3, 3.5, 5, and 5.5) or poly(propyleneimine) (PPI) dendrimer (generation 3 and 4) via reduction of HAuCl₄ with sodium borohydride. The average particle sizes of the gold nanoparticles were independent of the dendrimer concentration as well as the dendrimer generation, ranging between 3.0 and 4.3 nm in diameter for PAMAM dendrimers and between 2.1 and 2.3 nm in diameter for PPI dendrimers. The catalytic activities of the gold-dendrimer nanocomposites upon elimination of hydroxyl radicals formed in an H₂O₂/FeSO₄ system was examined using a spin–trapping method. The catalytic activity of gold–PPI dendrimer nanocomposites was slightly lower than that of gold–PAMAM dendrimer nanocomposites. In addition, the gold-dendrimer nanocomposites exhibited high catalytic activities which were hardly affected by the concentration as well as the generation of the dendrimer except PAMAM dendrimer 3.5. The highest activity for the gold–PAMAM dendrimer G 3.5 nanocomposites was 85 times that of ascorbic acid.

Key-words: Gold–dendrimer nanocomposites, Poly(amidoamine) dendrimer, Poly(propyleneimine) dendrimer, Hydroxyl radical, Spin–trapping method.

1. Introduction

All living organisms suffer from the damage caused by free–radical oxygen species which attack unsaturated acids in the cell membrane. Fortunately, a protective enzyme, superoxide dismutase, completely converts these free–radical oxygen species into two water molecules and oxygen, but this action decreases with aging. Thus, the anti–oxidants play a pivotal role in the inhibition of oxidative damage of DNA, proteins, and lipids by scavenging radicals before they attack the substrates. So, it is a challenging task to develop new catalysts for the elimination of active radical species.

Very recently, we have demonstrated that the gold–chitosan nanocomposites exhibit an extraordinary activity upon elimination of hydroxyl radicals formed in an H₂O₂/FeSO₄ system and their activity is 80 times higher than that of ascorbic acid which is a typical antioxidant. It is also found that stable gold–chitosan nanocomposites having diameter of 6–16 nm are obtained by chitosan molecules adsorbing on the gold nanoparticles where chitosan operates as a protective colloid.

As one class of polymers, dendrimers have been paid attention because they consist of regularly branched structure which can be controlled by chemical structure, molecular weight, and its distribution as well as molecular size and molecular shape. Accordingly, dendrimers can provide a dimensional functionality which is different from conventional linear polymers. Recently, metal–dendrimer nanocomposites have been synthesized and characterized. Among various dendrimers, poly(amidoamine) (PAMAM) dendrimers are the most frequently studied. It has been found so far that we can control the size of metal nanoparticles by changing various factors such as PAMAM generation and the concentration ratio of PAMAM to metal ions. Further, metal–dendrimer nanocomposites have many applicable potentials including nanocapsules, gene vectors, catalysis, resonance imaging agents, and photon transduction. On the other hand, there are...
very few studies of metal-dendrimer nanocomposites using poly(propyleneimine) (PPI) dendrimers, although they are available commercially. Like the PAMAM dendrimers, the PPI dendrimers have interior tertiary amine groups, but they do not contain amide groups. Further, the diameter of PPI dendrimers with surface amino groups is smaller than that of PAMAM dendrimers with surface amino group at the same generation. These different properties will affect the formation of metal nanoparticles as well as the stabilization of metal nanoparticles. Actually, in the reduction reaction of 4-nitrophenol using gold-dendrimer nanocomposites, it is found that the rate constants for the PAMAM dendrimers are higher than those for the PPI dendrimers at the same generation. Very recently, we have found that gold-PAMAM dendrimer nanocomposites exhibit a high antioxidant activity against hydroxyl radicals. So, it is still interesting to compare the catalytic activity against antioxidant action for the gold-PAMAM and gold-PPI dendrimer nanocomposites.

In this study, we have studied the catalytic activity of gold-dendrimer nanocomposites upon the elimination of hydroxyl radicals using an electron spin resonance (ESR) trapping method. Here, PAMAM dendrimers with amino terminal groups (generation of 3 and 5) or carboxyl groups (generation 3.5 and 5.5) are used. PPI dendrimers with amino terminal groups (generation of 3 and 4) are also used.

2. Materials and methods

2.1 Materials

PAMAM dendrimers were synthesized according to the literature. Their purity was confirmed by 1H- and 13C-NMR. PPI dendrimers were obtained from Aldrich Chemical Co. Tetrachloroauric acid tetrahydrate (HAuCl₄·4H₂O) was kindly supplied by Tanaka Kikinzoku Kogyo K. K. Milli-Q water was used in this study. All other chemicals were of reagent grade. The chemical structures of dendrimers (PAMAM G3 and PPI G3) are given in Scheme 1.

2.2 Methods

The preparation of gold-dendrimer nanocomposites in aqueous solution was conducted by the chemical reduction of HAuCl₄-dendrimer mixtures with sodium borohydride. For a typical experiment, 1 cm³ of freshly prepared 2 mmol dm⁻³ HAuCl₄ aqueous solution was added to 8.6 cm³ of dendrimer of various concentrations, and the solutions were stirred for 30 min. Then, 0.4 cm³ of 50 mmol dm⁻³ freshly prepared ice-cold sodium borohydride was quickly added to the solutions under stirring and left stirring for 30 min.

The gold-dendrimer nanocomposites obtained were analyzed by transmission electron microscopy (TEM) and UV-visible absorption spectroscopy. TEM observation was performed for the samples dried on carbon-coated copper grids. A Hitachi H-9000 NAR transmission electron microscope was operated at an accelerating voltage of 200 kV and direct magnification of 200,000 x. The size distribution of the gold nanoparticles was determined from about 200 particles.

The catalytic activity of the gold-dendrimer nanocomposites upon the elimination of hydroxyl radicals was estimated by using a spin trapping technique. In a typical experiment, solutions containing hydrogen peroxide, ferric sulfate, DMPO (5,5-dimethyl-1-pyrroline N-oxide), and the gold-dendrimer nanocomposites were measured with ESR: stable

Scheme 1 (a) PAMAM G3 (b) PPI G3
DMPO/OH adduct can be detected by a competitive reaction of OH radicals with DMPO or the gold-dendrimer nanocomposites. The measurements were carried out at 1 min after mixing of the solutions at room temperature. The rate constant (Kₙ) of the catalytic activity of the gold-dendrimer nanocomposites can be evaluated by following equations:

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\begin{align*}
F (1 - F) &= R \times [S] / [\text{DMPO}] \\
R &= Kₙ / Kᵢ \cdot F \cdot [\text{DMPO}] / (1 - F) \cdot [S]
\end{align*}
\]

where \( R \) is the degree of reaction, \( F \) the degree of decrease of ESR signal intensity, \( Kᵢ \) and \( Kₙ \) the rate constants of DMPO and the gold-dendrimer nanocomposites, and \([\text{DMPO}]\) and \([S]\) the concentrations of DMPO and the gold-dendrimer nanocomposites. Here, the value for \( Kᵢ \) by DMPO is taken as \( 3.4 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \). The concentration of the dendrimers referred to that of the terminal groups. The catalytic activity of ascorbic acid was also evaluated using solutions containing hydrogen peroxide, ferric sulfate, DMPO, and ascorbic acid.

3. Results and discussion

In the synthesis of the gold-dendrimer nanocomposites in the presence of PAMAM dendrimer, two types of PAMAM dendrimers are used: one is carboxyl group as terminal one (G 3.5 and G 5.5) and the other is amino group (G 3 and G 5). On the other hand, PPI dendrimers having amino terminal groups (G 3 and G 4) are used.

Fig. 1(a) show the UV-visible spectra of H\text{AuCl}_4 aqueous solution in the presence of PAMAM G 3.5 (1 mmol dm⁻³) after addition of sodium borohydride. The spectrum exhibits an absorption band at around 510-520 nm which is a typical plasmon band, suggesting the formation of gold nanoparticles. In Fig. 1(b), the TEM image and particle size distribution using PAMAM G 3.5 are given. One can see that the average particle size and standard deviation of gold nanoparticles is 4.1 nm and 0.6 nm, suggesting that monodisperse gold nanoparticles are formed. In addition, the average particle sizes of gold nanoparticles obtained using PAMAM G 3.5 are almost independent.
of the dendrimer concentration. Using PAMAM G 5 and G 5.5 at 1 mmol dm$^{-3}$, monodispersed gold nanoparticles are also obtained (Fig. 1(c) and (d)). However, no stable gold nanoparticles are obtained at both 1 and 3 mmol dm$^{-3}$ of PAMAM G 3. The average particle sizes of gold nanoparticles using the other PAMAM dendrimers with amino terminal group or carboxyl one are very similar, ranging between 3.0 and 3.6 nm in diameter.

Similarly, gold–PPI dendrimer nanocomposites were prepared by reduction of HAuCl$_4$ with borohydride in the presence of PPI dendrimer. Spectral changes of gold–PPI dendrimer nanocomposites are very similar to those of gold–PAMAM dendrimer nanocomposites. In Fig. 2, typical TEM images of gold–PPI dendrimer (G 3 and G 4) nanocomposites are given, along with their size distributions. Although the nanocomposites are prepared by changing the concentration of PPI dendrimer, the average particle sizes are almost same, i.e., 2.3 nm for both G 3 and G 4. It is noteworthy that the average size of gold is smaller for gold–PPI dendrimer than that for gold–PAMAM dendrimer at the present experimental condition, suggesting that PPI dendrimers control more effectively the growth of gold nanoparticles than PAMAM dendrimers. The fact that PPI dendrimers are soluble in water, dimethylformamide, and dichloromethane, but PAMAM dendrimers are not soluble in dichloromethane indicates that the hydrophobicity of the PPI dendrimers is greater than that of the PAMAM dendrimers. Accordingly, there is a great possibility that the PPI dendrimers could preferentially adsorb on the gold nanoparticles compared to the PAMAM dendrimers, and prevent the growth of the gold nanoparticles, resulting in the smaller size of gold.

It is very important to characterize the structure of the gold–dendrimer nanocomposites. Because PAMAM dendrimers G 3 and G 5 are about 3.6 and 5.4 nm in diameter and PPI dendrimers G 3 and G 4 are about 2.4 and 2.8 nm in diameter$^{14}$ and these values are comparable to the sizes of gold nanoparticles obtained, it is reasonable to suggest that the gold–dendrimer nanocomposites are formed by dendrimer molecules being adsorbed on the gold nanoparticles rather than encapsulation of gold nanoparticles in the dendrimers. Furthermore, these structures are supported by FT–IR measurements$^{15}$. In addition, the interaction of gold nanoparticles with the dendrimer with amino terminal group seems to be much stronger compared to that with dendrimer with carboxyl terminal group because the absorption band of amino terminal groups with gold nanoparticles shifts, whereas no shift of the absorption band of carboxyl terminal group is observed in FT–IR spectra.

The catalytic activity upon the elimination of hydroxyl radicals with the gold–dendrimer nanocomposites was estimated using a spin trapping technique. Fig. 3 shows a typical ESR signal change of DMPO/OH adduct in the presence of the gold–PAMAM dendrimer nanocomposites with various concentrations. A stable DMPO/OH adduct can be detected by a competitive reaction of OH radicals with DMPO or the gold–dendrimer nanocomposites. The intensity of the ESR signal decreases with the concentration of the gold–dendrimer nanocomposites, indicating that the gold–dendrimer nanocomposites have an ability to depress the activity of hydroxyl radicals. It should be mentioned that the action of the dendrimers itself is negligibly small compared to that by the gold–dendrimer nanocomposites. Using the equations described in the experimental section, the rate constants ($K_s$) for the gold–dendrimer nanocomposites were obtained. Fig. 4 shows plots of the rate constant versus the concentration of dendrimer added. The rate constants for all the gold–dendrimer systems range between $1.1 \times 10^{13}$ and $5.0 \times 10^{12}$ M$^{-1}$ s$^{-1}$ except the system for PAMAM G 3.5 (1 mmol dm$^{-3}$) whose rate constant is the highest (about $3.2 \times 10^{13}$ M$^{-1}$s$^{-1}$). This high activity may be attributed to the structure of the gold–dendrimer nanocomposites. PAMAM 3.5 interacts less strongly compared to those dendrimers with amino
terminal groups. Furthermore there is less adsorption of PAMAM G 3.5 on the gold nanoparticles resulting from the addition of a small concentration of PAMAM G 3.5, which means the gold-PAMAM dendrimer G 3.5 nanoparticles have more active sites compared to those for the other systems. As a result, hydroxyl radicals could interact with the active sites on the gold nanoparticles and would be effectively converted to water and oxygen. Another possibility is that the interaction of hydroxyl radicals with the gold surfaces being adsorbed by the dendrimer would be more hindered when the generation of dendrimer increases. However, no distinct differences in the rate constants are observed among the gold-PAMAM G 3, G 5, and G 5.5 nanocomposites. Accordingly, the coverage by the dendrimer on the gold nanoparticles would mainly control the reaction rate for the elimination of hydroxyl radicals. In the case of dendrimers with amino terminal groups, the rate constants for the gold-PAMAM dendrimer nanocomposites are very similar to those for the gold-PPI dendrimer nanocomposites. This may suggest that small molecules such as hydroxyl radicals can easily reach the gold nanoparticle surfaces having various coverages by the dendrimers, resulting in the similar rate constants. Thus, it is demonstrated that the antioxidant action for the gold-dendrimer nanocomposites is affected by the kind of the terminal functional groups of the dendrimers. On the other hand, a dependency of dendrimer concentrations on the rate constant for the reduction reaction of 4-nitrophenol by the metal-dendrimer nanocomposites has been observed. This difference in the change of the rate constant may be derived from different molecular sizes between hydroxyl radicals and 4-nitrophenol. Furthermore, it is interesting to compare the rate constant for the gold-dendrimer nanocomposites with that for ascorbic acid which is known as a typical antioxidant agent. Since the rate constant for ascorbic acid is obtained as $3.9 \times 10^{11}$ M$^{-1}$s$^{-1}$, the highest rate constant obtained in this study is about 85 times that for ascorbic acid. Also, the rate constants for the other gold-dendrimer nanocomposites are 12–30 times that for ascorbic acid. Thus, it is found that both the gold-PAMAM

![Fig. 3 Change of ESR spectra of DMPO-OH ESR signal with gold-dendrimer nanoparticles obtained in the presence of PAMAM G 3 (conc. 4 mmol dm$^{-3}$): [Au] = (a) 0; (b) 0.00313; (c) 0.025; (d) 0.05; (e) 0.2 mmol dm$^{-3}$.](image)

![Fig. 4 Plots of the rate constant for the gold-dendrimer nanocomposites vs. the concentration of dendrimer.](image)
dendrimer and -PPI dendrimer nanocomposites have a high activity upon the elimination of hydroxyl radicals.

4. Conclusion

In the present paper, gold-dendrimer nanocomposites are prepared using PAMAM and PPI dendrimers with different generations as well as different terminal groups. The average sizes of gold for the PAMAM dendrimers are 3-4 nm in diameter and those for the PPI dendrimers are about 2.3 nm which renders the particles insensitive to the concentration as well as the generation.

The catalytic activity by the gold-dendrimer nanocomposites upon the elimination of hydroxyl radicals formed in an \( \text{H}_2\text{O}_2 / \text{FeSO}_4 \) system is evaluated using a spin-trapping method. The catalytic activity by the gold-PAMAM dendrimer nanocomposites is very similar to that by the gold-PPI dendrimer nanocomposites which is not greatly influenced by the concentration as well as the generation of the dendrimer except PAMAM dendrimer G 3.5. In particular, the gold-PAMAM G 3.5 dendrimer nanocomposites exhibit the highest rate constant which is about 85 times that by ascorbic acid. Accordingly, the gold-dendrimer nanocomposites thus obtained have a great potential to be used for biological applications.

References


