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Magnetic separation of amino acids by gold/iron-oxide composite nanoparticles synthesized by gamma-ray irradiation

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Abstract

Amounts of amino acids adsorbed onto the Au/γ-Fe_2O_3 composite nanoparticles synthesized by gamma-ray irradiation were measured using magnetic separation technique. Cystine and methionine, which are sulfur-containing amino acids, connected to Au by a Au–S bond could be selectively picked up by a magnet.

Keywords: Gold/iron-oxide; Composite nanoparticle; Magnetic separation; Amino acid; Irradiation; Gamma ray; Synthesis; Cystine; Methionine

1. Introduction

In recent years, much attention has been given to magnetic nanoparticles as magnetic carriers for biomedical applications such as drug delivery and targeting, MRI, DNA and RNA purification, immunoassays, cell separation and hyperthermia [1–5]. For these uses, the surface of the magnetic nanoparticle should be modified by an appropriate linker compound to bind functional biomolecules (i.e., DNA, RNA, antibody, protein, amino acid, etc.) onto them. On the other hand, gold nanoparticles are intensively studied for colorimetric DNA detection, because gold firmly combines with biomolecules possessing mercapto groups and the characteristic color of the gold colloidal solution may change from red to purple according to the agglomeration state of the nanoparticles owing to the DNA hybridization [6–9].

Recently, we have developed an original technique for immobilizing gold nanograins onto the surface of magnetic iron-oxide nanoparticles [10]. The target of these composite nanoparticles is to

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bind the functional biomolecules onto the gold grains via Au–S bonds and to manipulate them by an external magnetic field. The immobilization of gold on the magnetic iron-oxide particles enables the binding of functional biomolecules possessing mercapto groups without using a special linker molecule for each different application. We have successfully synthesized such composite nanoparticles in water irradiated with γ-rays. The adsorption affinity with mercapto groups was maintained in aqueous solution. We have confirmed that our particles adsorb mercapto groups and are attracted by a magnet by separation tests employing glutathione as a model compound [10,11]. In this paper, we report on the results of measurements performed to check whether sulfur-containing amino acids are adsorbed onto these composite nanoparticles. It was confirmed that only two sulfur-containing amino acids adsorbed onto our particles and were picked up by a magnet. Since proteins would have some sulfur contents, our technique will be an effective means for screening of proteins.

2. Experimental

The synthesis procedure of the present gold/iron-oxide composite nanoparticles was previously reported [10]. So it is just summarized here. γ-Fe_2O_3 nanoparticles (NanoTek®) with an average diameter of 26 nm were dispersed in an aqueous solution containing 0.5 mmol/l $HAuCl_4$, 0.125 mol/l 2-propanol and 10 g/l polyvinyl alcohol. The amount of γ-Fe_2O_3 in the dispersion was 1.0 or 0.1 g/l. (The initial composition of Au and γ-Fe_2O_3 in weight ratio was 1:10 and 1:1, respectively.) The dispersion was filled into a glass vial and irradiated with ^{60}Co gamma-rays at room temperature for 6 h. The obtained Au/γ-Fe_2O_3 composite nanoparticles were collected by a magnet and redispersed in water. The UV–vis absorption spectra of the dispersions were measured by a UV–vis spectrometer, Cary 50 (Varian). The composite nanoparticles were observed by a transmission electron microscope, H-8100 T (HITACHI) operated at 200 kV. The chemical composition of the composite nanoparticles was determined with an inductively coupling plasma (ICP) spectrometer, ICPS-7500 (SHIMAZDU).

We evaluated the amounts of amino acids adsorbed onto the Au/γ-Fe_2O_3 composite nanoparticles. A standard amino acid solution containing 17 α-amino acids of 200 nmol each was purchased from Wako Pure Chemical Industries Ltd. It does not contain asparagine, glutamine or tryptophan, but cystine, a disulfide of cysteine, instead of cysteine. The composite nanoparticles were washed twice with ultrapure water, separated, and dispersed into 0.02N HCl aqueous solution. After the particle dispersion was mixed with the amino acids and kept for two hours, it was divided into magnetic and nonmagnetic components by a magnetic separator, MidiMACS™ (Miltenyi Biotec GmbH). The amounts of amino acids remaining in the nonmagnetic solution were determined using an amino acid analyzer, L-8500A (HITACHI), by the ninhydrin method, measuring absorbances at 570 and 440 nm wavelength [12]. The amounts of the amino acids adsorbed onto the nanoparticles were thus indirectly evaluated as decrements from the initial concentration. We also directly evaluated the amounts of the sulfur-containing amino acids (cystine and methionine) adsorbed onto the nanoparticles by an elementary analysis of sulfur. The magnetic component, i.e., the nanoparticles with amino acids were dissolved by aqua regia, and sulfur contained in the solution was quantitatively determined by ICP spectrometry, measuring the emission intensity at 180.731 nm wavelength.

3. Results and discussion

The chemical compositions of the Au/γ-Fe_2O_3 composite nanoparticles are tabulated in Table 1. In Fig. 1, the UV–vis absorption spectra of the dispersion of the present Au/γ-Fe_2O_3 composite nanoparticles are shown together with that of the monolithic Au nanoparticles synthesized by gamma-ray irradiation. The Au/γ-Fe_2O_3 spectrum involves a Au plasmon absorption peak located at around 520 nm as shown in the monolithic Au spectrum. The result indicates that the Au particles in the Au/γ-Fe_2O_3 sample particles are nanosized.

Table 1
Initial concentrations of source material and relative compositions of the present Au/γ-Fe_2O_3 composite nanoparticles

	Initial concentration		Results of chemical analysis	
	Au (g/l)	γ-Fe_2O_3 (g/l)	Au (wt%)	γ-Fe_2O_3 (wt%)
(a)	0.1	1.0	10	90
(b)	0.1	0.1	44	56

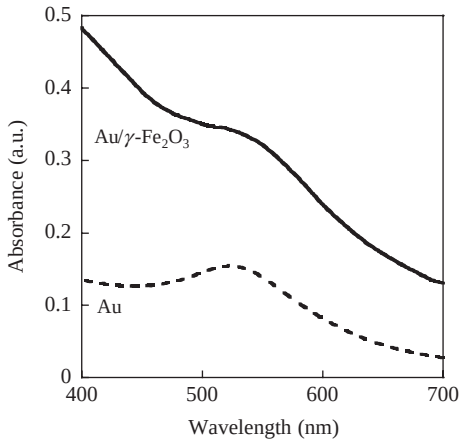

Fig. 1. UV–vis absorption spectra of the aqueous dispersion of (solid line) the present Au/γ-Fe_2O_3 composite nanoparticles (Au:γ-Fe_2O_3 = 44:56 (wt)) and (dashed line) the monolithic Au nanoparticles.

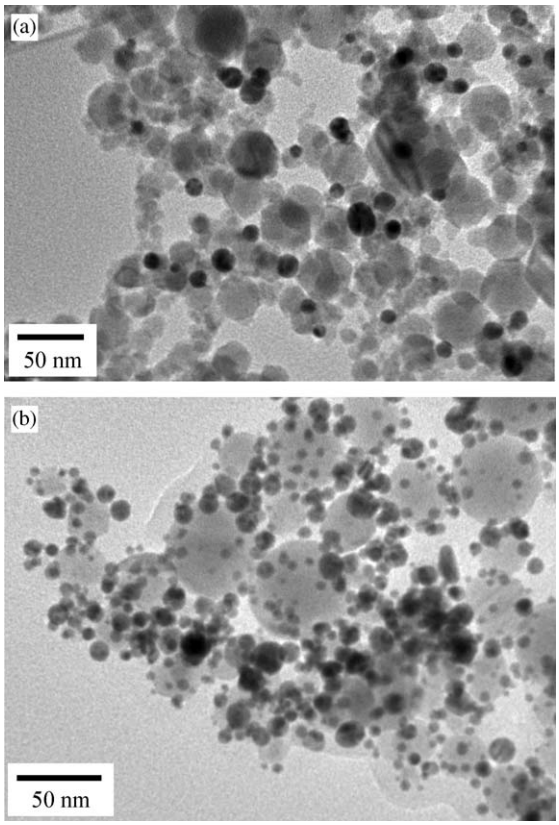

Fig. 2. TEM images of Au/γ-Fe_2O_3 composite nanoparticles synthesized by gamma-ray irradiation (Au:γ-Fe_2O_3 = (a) 10:90, (b) 44:56 (wt)).

Fig. 2 shows typical TEM images of the present Au/γ-Fe_2O_3 composite nanoparticles. The weight ratios of Au:γ-Fe_2O_3 were (a) 10:90 and (b) 44:56, respectively. Both images show that about 5 nm diameter Au particles with a stronger contrast are supported on the surface of the γ-Fe_2O_3 particles with a weaker contrast. It is noticed that much more Au nanoparticles are supported on γ-Fe_2O_3 in Fig. 2(b) than in Fig. 2(a), which is ascribed to the difference in the ratio of Au and γ-Fe_2O_3.

Fig. 3 shows the amounts of amino acids adsorbed and magnetically picked up by the present composite nanoparticles with the composition of Au 10 wt% and 44 wt%. For comparison, we used the monolithic γ-Fe_2O_3 nanoparticles. The data are presented as the amount for 5 mg γ-Fe_2O_3 contained in the composites. It was found that cystine (Cys_2) and methionine (Met), which are sulfur-containing amino acids, were preferably adsorbed on the Au/γ-Fe_2O_3 composite nanoparticles compared to the other amino acids without sulfur. The results indicated that cystine and methionine were certainly connected to Au by a Au–S bond. In contrast, the monolithic γ-Fe_2O_3 nanoparticles adsorbed almost evenly all kinds of the 17 amino acids. It is consistent with the report that the carboxylated species chemically adsorb onto the γ-Fe_2O_3 surface [13,14]. Especially aspartic acid (Asp) and glutamic acid (Glu) were relatively well adsorbed, which is probably due to the presence of carboxyl groups found in these molecules.

It should be pointed out that the amounts of the adsorbed sulfur-containing amino acids were

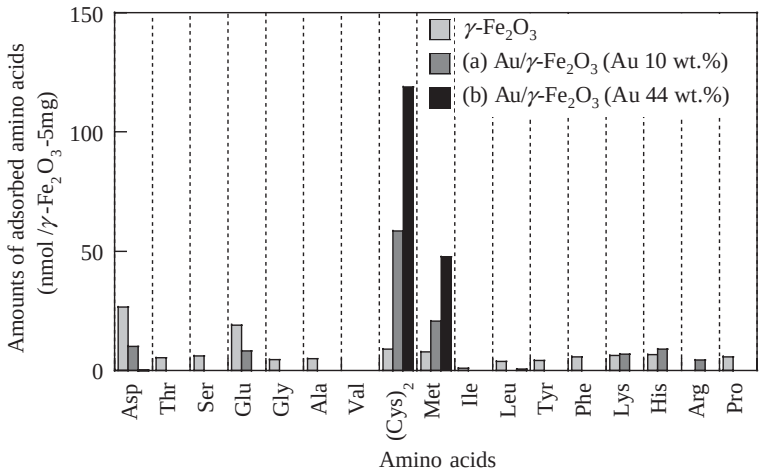

Fig. 3. Amounts of the 17 kinds of amino acids adsorbed onto the present nanoparticles.

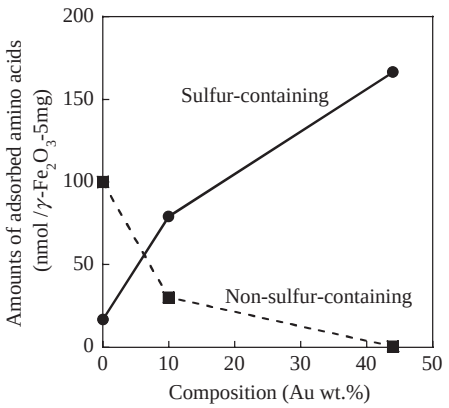

Fig. 4. Amounts of adsorbed sulfur-containing amino acids (●) and non-sulfur-containing (■) amino acids onto the nanoparticles against the Au weight ratio in the particles.

increased with the Au wt% in the particles, while those of the other amino acids were decreased. This fact clearly reflects the effect of coating with Au onto the γ-Fe_2O_3 surface. In Fig. 4, the adsorbed amounts of sulfur-containing amino acids and non-sulfur-containing amino acids are plotted against the Au weight ratio in the nanoparticles. As described above, this figure also shows that the enhanced loading Au ratio suppresses the non-specific adsorption of amino acids via carboxyl group and physical reactions, and promotes the effect of specific adsorption via Au–S bonds.

We tried to directly evaluate the amount of the sulfur-containing amino acids, namely cystine and methionine, adsorbed onto the Au/γ-Fe_2O_3 composite nanoparticles with Au 44 wt% (b) by the elementary analysis of sulfur. The result shows that 2.8×10^2 nmol of sulfur were adsorbed onto the composite nanoparticles containing 5 mg of γ-Fe_2O_3 (this weight is equal to that shown in Figs. 3 and 4). This agrees with 2.9×10^2 nmol determined from indirect evaluation shown in Fig. 3, since there are two sulfur in a cystine molecule and one in a methionine. These results indicate that the sulfur-containing amino acids connected to Au via a Au–S bond were successfully picked up by a magnet without significant loss.

4. Conclusion

We investigated the amounts of amino acids adsorbed onto the Au/γ-Fe_2O_3 composite nanoparticles synthesized using γ-rays. Only two sulfur-containing amino acids were adsorbed onto our particles and picked up by a magnet, which indicates that these amino acids were connected to Au contained in the composite nanoparticles by a Au–S bond. The present results show that our composite nanoparticles are very promising for screening of biomolecules such as proteins,

because of their ability to bind to biomolecules via Au–S bonds without any linker compounds.

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