Chapter 27
Various Shapes of Gold Nanoparticles Synthesized by Glycolipids Extracted from *Lactobacillus casei*

Yugo Kato, Fumiya Kikuchi, Yuki Imura, Etsuro Yoshimura, and Michio Suzuki

Abstract Gold nanoparticles have particular properties distinct from bulk gold crystals. The gold nanoparticles are used in various applications in optics, catalysis, and drug delivery. Although many reports on microbial synthesis of gold nanoparticles have appeared, the molecular mechanism of gold nanoparticle synthesis in microorganisms is unclear. Previously we reported that the amounts of diglycosyl diacylglycerol (DGDG) and triglycosyldiacylglycerol (TGDG) bearing unsaturated fatty acids were much reduced after formation of gold nanoparticles. DGDG purified from *L. casei* induced the synthesis of gold nanoparticles in vitro. These results suggested that glycolipids, such as DGDG, play important roles in reducing Au(III) to Au(0). In this paper, we reported that the concentration change of DGDG induced various shapes of gold nanoparticles in vitro. Our work will lead to the development of novel and efficient methods to synthesize metal nanoparticles using microorganisms.

Keywords Gold nanoparticle · *Lactobacillus casei* · Glycolipid

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27.1 Introduction

Gold nanoparticles (containing a few tens of gold atoms) have various unique properties. A gold nanoparticle solution is wine-red in color, because of surface plasmon resonance (SPR) (Jin 2010; Zhang et al. 2010). Over the past two decades, such nanoparticles have found novel applications in general industry, chemistry, biology, and medicine. Antibody-bearing nanoparticles are useful labeling agents in electron microscopy and can reveal the detailed locations of organic molecules within cell organelles (Bendayan and Garzon 1988). Gold nanoparticles attached to DNA fragments can detect DNA-DNA interactions (which trigger color changes) (Storhoff et al. 2000). This technology is used to diagnose viral infections (Wang et al. 2001; Cao et al. 2002). When synthesizing gold nanoparticles, the appropriate selections of reducing agents active on gold ions, and dispersing agents that hold the particle size in the nanometer range, are important. Industrially, large amounts of gold nanoparticles are synthesized in the reaction of gold with citric acid under conditions of high temperature and pressure (Frens 1973). Novel methods using macromolecular polymers or biomacromolecules, such as proteins and DNA, have been used to develop more functional gold nanoparticles (controlled in terms of shape) at low financial and energy costs, in the absence of unwanted by-products (Selvakannan et al. 2004; Xie et al. 2009; Liu et al. 2011). Recently, many methods using microorganisms to synthesize metallic nanoparticles have been reported. For example, some previous works about synthesize gold nanoparticles using by *Rhodobacter capsulatus* (Feng et al. 2008), silver nanoparticles using by *Fusarium oxysporum* (Ahmad et al. 2003), CdS quantum dots using by *Escherichia coli* (Sweeney et al. 2004) were reported. In such processes, the microorganisms are cultured at normal temperature under normal pressure and do not produce any toxic by-product. Such benefits suggest that the use of microorganisms to synthesize gold nanoparticles will be important in the future. To improve the efficiency of such synthesis, it is essential to clarify the molecular mechanisms involved. Recently, we reported that diglycosyl diacylglycerol (DGDG) plays important roles in reducing Au(III) to Au(0) by *Lactobacillus casei* (Kikuchi et al. 2016). This report shows the function of DGDG for the synthesis of gold nanoparticles.

27.2 Materials and Methods

We extracted lipid from *L. casei* (strain JCM1134, purchased from RIKEN Microbe Division) according to the Bligh and Dyer method (Bligh and Dyer 1959). We extracted thin layer chromatography (TLC) analysis. After spotting of samples in the origin point, plates were transferred to TLC chambers saturated with the chromatographic solvent (chloroform/methanol/acetic acid, 65:25:10). DGDG was extracted from TLC plate and dissolved in ethanol. To confirm DGDG, we measured mass spectra on a matrix-assisted laser-desorption ionization–time-of-flight

mass spectrometer (ultraflex MALDI-TOF/TOF, Bruker). The sample solution was mixed with 500 mM 2, 5-dihydroxybenzoic acid in chloroform/methanol (1:1) solution as the matrix and dried on the plate.

DGDG extraction from the TLC plate dissolved in 40 μL ethanol was applied to 960 μL of auric acid solution (final concentration of $K[AuCl_4]$: 250 μM). The mixture solution was incubated at 37 °C for 24 h. Forty microliters of ethanol without DGDG was mixed with 960 μL of auric acid solution (final concentration of $K[AuCl_4]$: 250 μM) as a negative control experiment. UV/VIS spectra of the supernatant were measured using UV/VIS spectroscopy photometer (V-550 spectrophotometer, JASCO). To examine the formation of gold nanoparticles, we used transmission electron microscopies (TEM) observation and energy dispersive X-ray spectrometry (EDS). TEM analyses were performed using a JEOL JEM-2000EX TEM operated at 200 kV, and EDS analyses were performed using a JEOL EX-24025JGT.

27.3 Results

27.3.1 Extraction of DGDG from L. casei

L. casei cells were suspended in chloroform/methanol solution to extract lipids, and these extracts were subjected to TLC. The extracted sample separated from the TLC was analyzed by MALDI-TOF MS. The spectrum of MALDI-TOF MS showed four major peaks at 939.6, 953.6, 967.6, and 981.6 (*m/z*) and certain isotope peaks (Fig. 27.1a). These peaks showed the different chain length of the unsaturated fatty acids in DGDG. The chemical structure of DGDG was shown in Fig. 27.1b. R_1 and R_2 mean the alkyl chains containing one double bond in each chain.

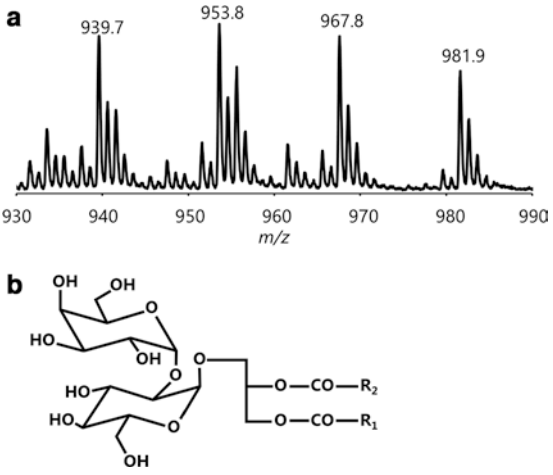

Fig. 27.1 (**a**) MALDI-TOF-MS spectrum of the extract from TLC. (**b**) The schematic structure of DGDG. R_1 and R_2 showed the alkyl chains of with one double bonding

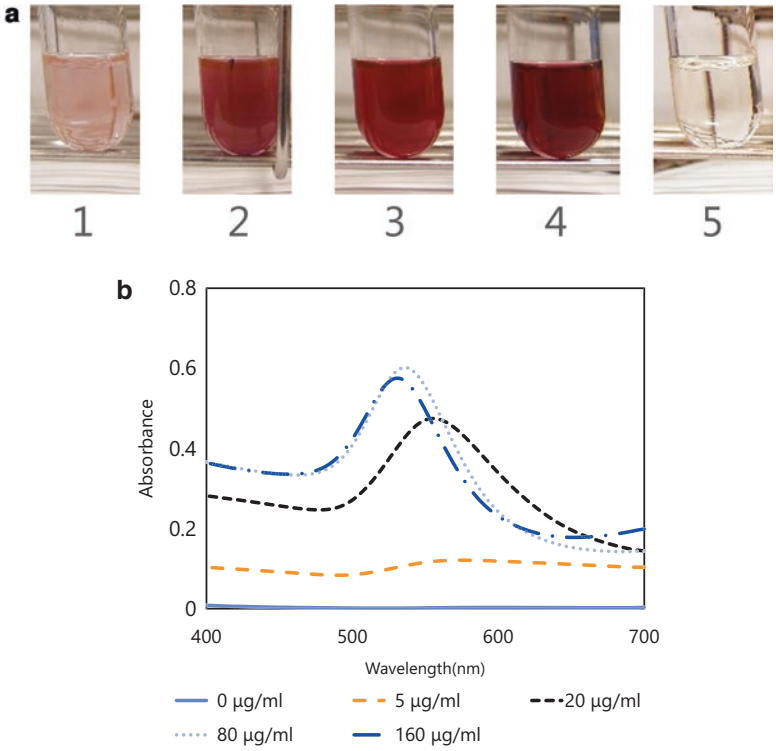

Fig. 27.2 (**a**) Auric acid solution (0.25 mM $K[AuCl_4]$) with DGDG purified from the TLC plate (1) 5.0 μg/mL, (2) 20 μg/mL, (3) 80 μg/mL, (4) 160 μg/mL, (5) 0 μg/mL), (**b**) UV/VIS spectra after 24 h. Light blue line, 0 μg/mL; orange break line, 5.0 μg/mL; black break line, 20 μg/mL; light blue dotted line, 80 μg/mL; dark blue dotted and break line, 160 μg/mL

27.3.2 Reaction of Auric Acid Solution with DGDG

Purified DGDG from *L. casei* was solubilized in ethanol to mix with auric acid. Then, the solution was incubated at 37 °C for 24 h. The colors of auric acid solution without DGDG kept the transparent yellow. On the other hand, the color of auric acid solution with DGDG became violet (Fig. 27.2a). The absorbance spectrum of each solution at wavelengths from 400 to 700 nm was measured (Fig. 27.2b). The intensity and wavelength of peak top varied by the DGDG concentration. The high concentration of DGDG showed the smaller wavelength indicating that the high concentration of DGDG synthesize the small nanoparticles. On the other hand, the low concentration of DGDG induced the bathochromic shift indicating that low concentration of DGDG induced the bigger nanoparticles.

27.3.3 Observation Nanoparticles by TEM

To investigate morphology of nanoparticles, the sample of each solution was subjected to TEM (Fig. 27.3a). TEM observations showed that black dots corresponding to gold nanoparticles were synthesized in all conditions. TEM observation of the condition with 5.0 μg/mL DGDG showed sphere shape of nanoparticles (Fig. 27.3a-1). 20 μg/mL DGDG synthesized small nanoparticles and rod shape of gold (Fig. 27.3a-2). 80 μg/mL DGDG induced the small and triangle shape of gold (Fig. 27.3a-3). 160 μg/mL DGDG synthesized the hexagonal shape of gold (Fig. 27.3a-4). EDS showed that the various shapes of nanoparticles were composed of Au (sphere shape (Fig. 27.3b-1), rod shape (Fig. 27.3b-2), triangle shape (Fig. 27.3b-3), and hexagonal shape (Fig. 27.3b-4).

27.4 Discussion

In the present study, incubation of DGDG with auric acid in vitro succeeded to produce gold nanoparticles, suggesting that DGDG has a function of both reducing and dispersing agents. The carboxylic groups of citric acid bind to the surface of Au(0) to create nanoparticles (Frens 1973). The degradation products of DGDG may interact with the Au(0) surface to stabilize nanoparticles. On the other hand, the size of particles synthesized by *L. casei* was 30 nm on average (Kikuchi et al. 2016). The gold nanoparticles synthesized by DGDG were slightly larger than those synthesized by *L. casei* cells, suggesting that the ability of dispersing capacity of DGDG is not enough to make the 30 nm nanoparticles. The high concentration of DGDG induced the various shape of gold crystals suggesting that DGDG also affected the crystal growth of gold. However, the other dispersing agents within *L. casei* cells may also play roles in inhibiting Au(0) aggregation and crystal growth. Further work is thus needed to reveal exactly how *L. casei* forms gold nanoparticles. Identification of the key organic molecules may allow modification of *L. casei* genes, permitting such recombinants to promote the synthesis of gold nanoparticles more effectively. In the future, the modified recombinant *L. casei* strains may be used for the applications in metal recycling and phytoremediation.

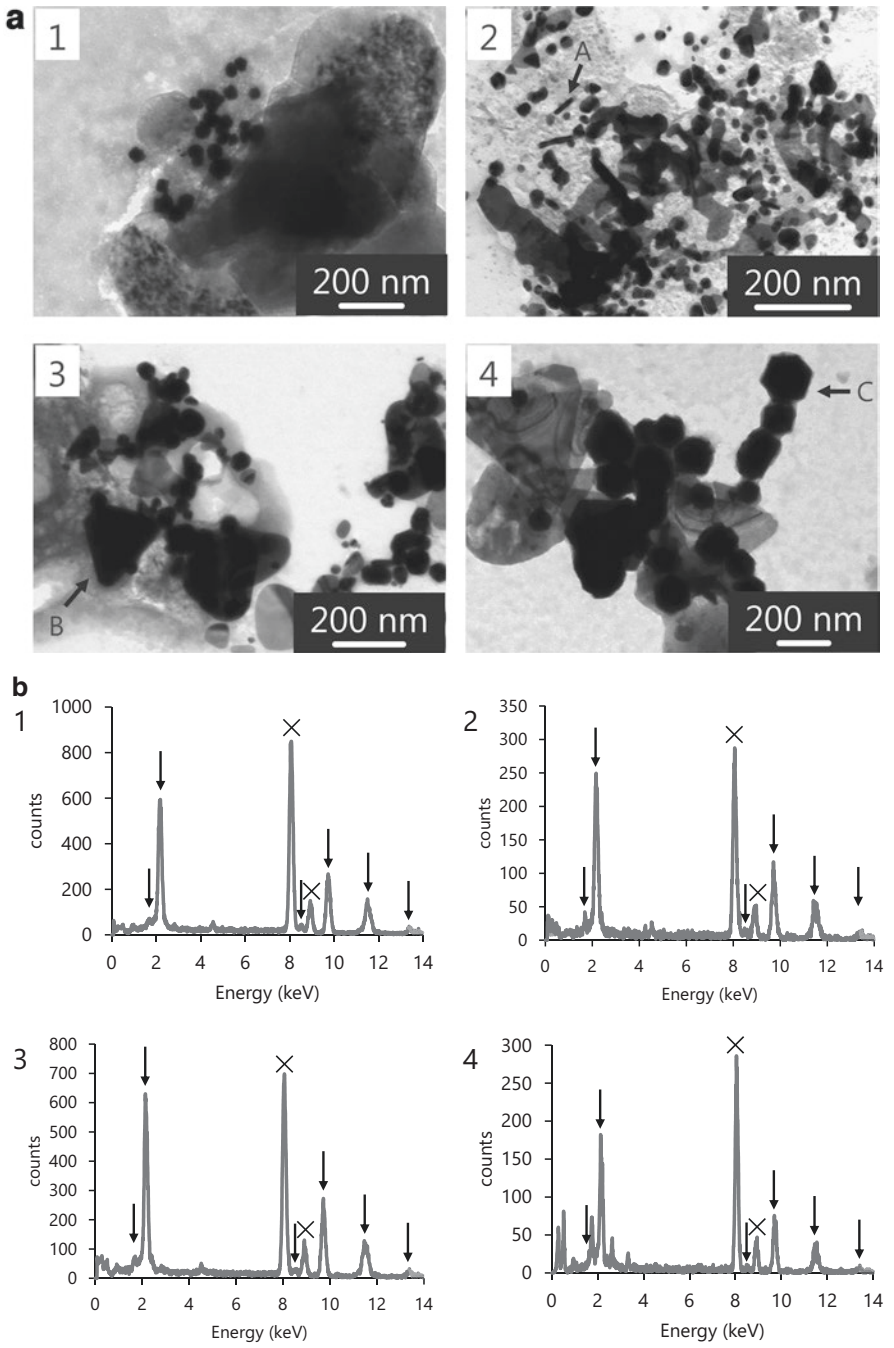

Fig. 27.3 (**a**) TEM image of gold nanoparticles synthesized by DGDG (*1*, 5.0 μg/mL; *2*, 20 μg/mL; *3*, 80 μg/mL; *4*, 160 μg/mL; DGDG). Arrow A indicates the rod shape of gold, arrow B indicates the triangle shape of gold, arrow C indicates the hexagonal shape of gold. (**b**) EDS spectra of nanoparticles of (**a**) (*1*, sphere shape; *2*, rod shape; *3*, triangle shape; *4*, hexagonal shape). The arrows showed the characteristic X-ray peaks of Au. X showed the peak of Cu from the copper grid

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