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Paper

Study on interaction between phosphatidylcholine(PC) liposome and gold nanoparticles by TEM observation

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The PC liposomes constructed with one lipid bilayer have been fabricated without using chloroform solution and phosphate-buffer saline. After reaction promotion, the Au NPs covered with PC molecules exist on the outside of the liposome membrane. The thickness of the liposome membrane is estimated to be approximately 5 nm.

1. Introduction

Gold nanoparticles (Au NPs) are expected to apply to Drug Delivery System in medical field. H. Takahashi *et al.* have reported that the Au nanorods surface modified by a biomolecule can possess a biocompatibility [1]. Since the activity of the Au NPs is high for the biomolecules, we think that the investigation of the biocompatibility for the NPs is important. Additionally, we focus on the cell membrane, because the Au NPs firstly touch at the cell membrane when the NPs are injected in our body.

In our previous study, we have reported that the adsorption morphology of the Au NPs interacted with a phosphatidylcholine (PC) liposome, which is cell membrane model, cannot clearly be understood [2]. That is, because the image observed by means of transmission electron microscope (TEM) has been a projection view for the prepared sample without slicing, we cannot decide the existence site of the Au NPs, which is the outside or the inside of the PC liposome membrane (Fig. 1). When we prepare the ultra-thin section of the TEM sample by slicing, we may be able to understand the existence site of the Au NPs.

On the other hand, many papers have been reported that the PC liposome is formed by through some procedures with some molecules of PC, chloroform solution and phosphate-buffer saline (PBS) [3,4]. However, we want to clarify the interaction between the liposome constructed by only the PC molecules and the Au NPs. Furthermore, we must prevent molecules other than the PC molecule. As a new protocol to fabricate the PC liposome, we introduce a simple procedure in this paper. The simple procedure elements are only the PC suspension and the phase transition temperature.

In this paper, we have investigated the existence site of the Au NPs for the PC liposome by TEM observation. The existence site means whether the NPs exist on the outside or the inside of the liposome membrane. In addition, we have studied the thickness of the PC liposome membrane fabricated by new protocol. Those points are shown in Fig.1.

2. Experimental

The PC liposome was fabricated by means of extrusion method [5] with the Mini-Extruder proposed from Avanti Polar Lipods, Inc. The PC powder (Egg, >99%)

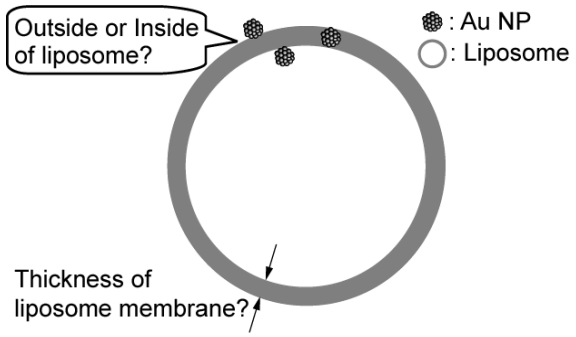

Fig. 1 Schematic view of our investigation points for the existence site of the Au NPs and the thickness of the liposome membrane.

of 5 mg purchased from Avanti Polar Lipods, Inc. was dissolved into the milli-Q water (≧18 MΩcm) of 1 mL and vortexed at room temperature. The PC suspension with white color was heated until 60 °C to exceed the phase-transition temperature of the used PC molecules, and went through a polycarbonate membrane with pore diameter of 400 nm to form the PC liposomes. The fabricated PC liposome aqueous solution was kept at room temperature.

The Au NPs colloidal solution was prepared by the solution plasma method [6, 7]. The Au rod (99.95%, 1 mm^{ϕ}) and the sodium chloride powder (NaCl: ≥99%) were purchased from Nilaco Co. and Wako Pure Chemical Industries Ltd., respectively. The distilled water was also provided. Two Au rods were faced each other with the distance of approximately 0.1 mm in the 10 mM NaCl aqueous solution of 180 mL. The pulse power source of AC-high voltage with both the frequency of 20 kHz and the voltage of 3.2 kV was produced by KURITA Seisakusho Co. Ltd. When the glow discharge occurred, the Au NPs were fabricated into the NaCl aqueous solution. The discharge time was for 5 minutes.

To reveal the details of the adsorption morphology between the PC liposome and the Au NPs, the PC liposome aqueous solution and the Au NPs colloidal solution were mixed and vortexed at room temperature. The mixed ratios for those solutions were (PC liposome aqueous solution : Au NPs colloidal solution) = (1 : 100) and (1 : 10). These samples were described in this paper as "PCA100" and "PCA10", respectively. The TEM observation samples for the PC liposome and the PCA100 were prepared by the negative staining method. The TEM sample for the PCA10 was prepared by the ultra-thin section method with freeze substitution [8].

About the negative staining method, the copper grid (G200HH, Nisshin EM Corporation) with carbon/support film was provided, and the surface of the grid with film was modified to the hydrophilic by soft plasma ion bombardment (PIB-10, Vacuum Device). The sample solutions for the PC liposome and the PCA100 were dropped in the grids, respectively. After 5 minutes, the grid was rinsed in negative staining solution with 2% uranyl acetate. The extra staining solution on the grid was removed by using filter paper, and the grid was dried in air.

About the ultra-thin section method with freeze substitution, the carbon/sapphire glass was provided and was modified to the hydrophilic by hard plasma ion bombardment. Additionally, the surface was altered by dropping Alcian Blue solution for 5 minutes, and rinsed with milli-Q water and dried in air. The sample solutions for the PC liposome aqueous solution and the PCA100 were dropped on the glass. The solutions on glass were frozen rapidly with a high-pressure freezing device (EMPACT-2, Leica, Wetzlar) and carried out the substitution (i.e. immersing frozen specimens in acetone containing 2% osmium tetroxide (OsO_4) at -80°C for 48 h) [8]. Subsequently, the sample was embedded in the epoxy resin (Poly/Bed 812, Polysciences Inc.) and the resin was sliced to sections with thickness of 50~70 nm by microtome. The sections were stained with uranyl acetate and lead citrate, and observed by a Transmission Electron Microscope (electron accelerating voltage: 100 kV, H-7600, Hitachi).

3. Results and discussion

3.1 Details of fabricated Au NPs

Figure 2 shows the size distribution of the fabricated Au NPs. This distribution is obtained by measuring the diameter of the Au NPs in TEM image. If the shape of the Au NPs is ellipsoid, we do not measure the short diameter but the long diameter. The numbers of the estimated Au NPs are 100. The average diameter with standard deviation is estimated to be 13.4±3.3 nm.

3.2 Fabricated PC liposome

Figure 3 shows the TEM image of the PC liposome prepared by the negative staining method. This PC liposome has been fabricated by use of the PC suspension

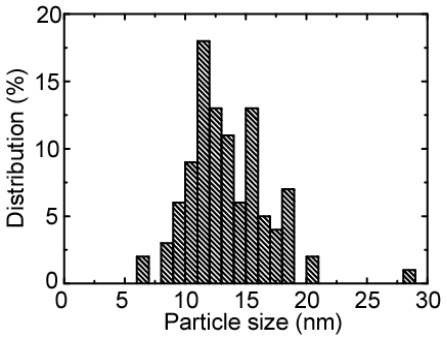

Fig. 2 Size distribution of the fabricated Au NPs.

only, although generally a liposome has been fabricated

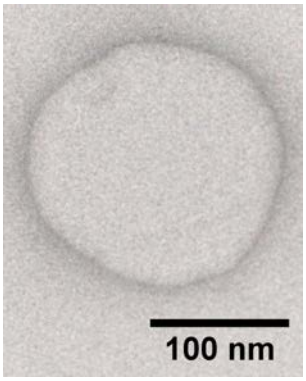

Fig. 3 TEM image of the PC liposome prepared by the negative staining method.

by use of the mixed solution, which is constructed from things such as lipid powder, chloroform solution and PBS [3]. Judging from the TEM image, it is revealed that we can fabricate the PC liposome by heating the PC suspension until the phase-transition temperature and going through filter.

3.3 PCA100 observed by negative staining

Figure 4 shows the TEM image for the PCA100 sample prepared by the negative staining method. This TEM image is the projection view. It seems that the Au NPs aggregate each other and touch the PC liposomes. In the enlarged view, a certain bright film is observed at around the aggregated Au NPs. The average thickness of the membrane with standard deviation is estimated to be 1.7±0.3 nm. Because the transmission efficiency of electron for carbon element is large, the hydrophobic group of the PC molecule has a low contrast. Moreover, since the average length of the PC molecule is presented approximately 2.0~2.5 nm [9,10], we think that the bright membrane is constructed with the PC molecules. In our

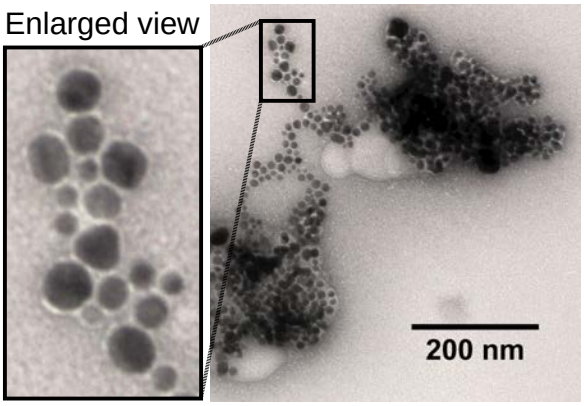

Fig. 4 TEM image for the PCA100 sample prepared by the negative staining method. The enlarged view is also shown.

previous study, it has been speculated that the PC molecule adsorbs on the Au sheet surface at the methyl group of N-CH_3, the phosphate group and the oxygen between hydrophilic and hydrophobic groups [2]. Thus, the Au NPs might aggregate through the hydrophobic group of the PC molecules adsorbed on the NPs. Considering to these results, we think that the aggregated Au NPs with the PC molecules touch the PC liposome.

3.4 Ultra-thin sections for PCA10

To reveal the existence site of the Au NPs for the PC liposome, the numbers of the adsorbed Au NPs must be reduced. Therefore, we have adjusted to the amount of one-tenth of the PCA100 sample; this sample is called as PCA10. The TEM image for the ultra-thin section of the PCA10 sample is shown in Fig. 5. We think that the bright area means the inside part of the liposome. Thus, we clarify the Au NPs exist on the outside surface of the PC liposome membrane. Subsequently, we focus on the thickness of the gray band between the Au NPs and the edge of the bright area. The gray band indicates the sum of thicknesses of both the liposome membrane and the PC molecule around Au NPs. The average thickness of the gray band with standard deviation is estimated to be 7.1±1.6 nm. The value is close to length of three PC molecules, because the average length of the PC molecule is presented approximately 2.0~2.5 nm [9,10]. Considering the result of section 3.3, since the Au NPs possess the bright film equivalent to one layer of PC molecules, it seems that the residual thickness equivalent to two layers indicates the liposome membrane. In general, the membrane of liposome is constructed with lipid bi-

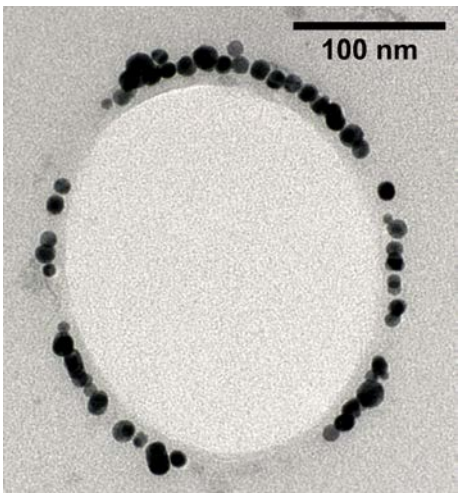

Fig. 5 TEM image for the PCA10 sample by the ultra-thin section method with freeze substitution.

layer. In addition, when the PC liposome is fabricated in aqueous solution, the hydrophilic group of the PC molecule appears the outside of the lipid bilayer. Therefore, we think that the PC liposome fabricated by new protocol is constructed with one lipid bilayer, and the outside of the lipid bilayer is appeared the hydrophilic group. Moreover, the thickness of the PC liposome membrane is estimated to be approximately 5 nm because the thickness is equal to the length of two PC molecules. However, one question occurs on this discussion. It is that the hydrophobic group of the PC molecules on Au NP touches the hydrophilic group of the PC liposome although the hydrophobic group generally cannot interact with the hydrophilic group. The structural model for this question is shown in Fig. 6. In this paper, we cannot clear the question, unfortunately. However, the question might be revealed by carrying out the small-angle X-ray scattering measurement in near future.

4. Conclusion

The PC liposomes constructed with one lipid bilayer have been fabricated, when the PC suspension at 60 °C goes through filter with 400 nm by the extrusion method. The thickness of the PC liposome membrane is estimated to be approximately 5 nm. The Au NPs are covered with the PC molecules and might aggregate each other through the hydrophobic group of the PC molecules adsorbed on the NPs. When the PC liposome aqueous solution and the Au NPs colloidal solution are mixed at room temperature, the NPs covered with the PC molecules exist on the outside surface of the liposome membrane.

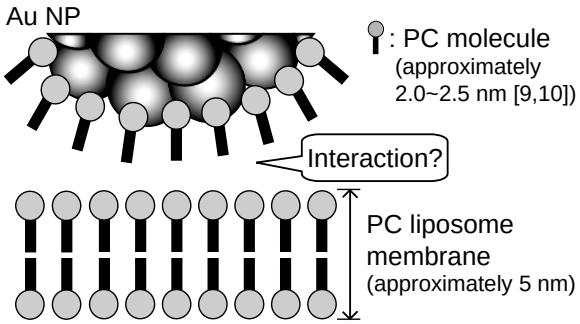

Fig. 6. Structural model for the interaction between the Au NP covered with PC molecules and the PC liposome.

In this paper, we cannot reveal the detail structural model between the Au NP covered with PC molecules and the PC liposome.

5. Acknowledgements

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