Spectroscopic and morphological studies on interaction between gold nanoparticle and liposome constructed with phosphatidylcholine

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Abstract. The gold nanoparticles (Au NPs) colloidal solution and the phosphatidylcholine (PC) liposome aqueous solution are fabricated by the solution plasma method and the extrusion procedure, respectively. When the Au NPs colloidal solution and the PC liposome aqueous solution are mixed, considering the TEM image, we think that the Au NPs firstly are covered with the PC molecules, which do not contribute to form the PC liposome, and subsequently the Au NPs covered with the PC adsorb on the PC liposome surface. We propose that the PC molecule adsorbs on the Au sheet surface at the methyl group of N-CH₃ and the oxygen atoms of P-O, P=O, C-O and C=O bonds, because each peak intensity of N, O and P K-edges NEXAFS spectra for the PC/Au sheet is reduced in comparison with that for the PC multilayer. Furthermore, the Au NPs covered with PC seem to be aggregated each other through the hydrophobic groups of PC on Au NPs.

1. Introduction

Gold nanoparticles (Au NPs) are paid attention to use of the hyperthermia for cancer cells [1] and the drug delivery system [2] in the medical field. However, since the activity of the Au NPs is high for the biomolecules, the investigation of biocompatibility for the NPs is important. About the biocompatibility of the NPs, many investigations have been carried out by observation for transformation of cells or animal tissues before/after providing with the NPs [3, 4]. On the other hand, the investigation by spectroscopic methods other than the elemental analysis has hardly been carried out, and the cause of the above transformation has not been revealed about the adsorption reaction between the biomolecules and the NPs. Thus, in our previous study, we have selected the reaction under water environment as one of the reaction conditions in our body (e.g. temperature, several molecules and pH), and elucidated the adsorption reaction between L-cysteine and the Au NPs under water environment by sulfur K-edge Near-Edge X-ray Absorption Fine Structure (NEXAFS) [5]. Generally, the NPs colloidal solution is fabricated by chemical reduction method with surfactant...
molecules. However, to clarify the adsorption reaction between the biomolecules and the NPs, we have fabricated the Au NPs colloidal solution by solution plasma method, which is a fabrication technique without using the surfactant molecules [5-7]. The L-cysteine has adsorbed on the Au NPs surface at thiol group and in addition those Au NPs have been aggregated by hydrogen bonding between the amino and carboxyl groups.

Subsequently, we focus on the influence of the Au NPs about cell membrane of our body, because we think the Au NPs interact with the cell membrane surface firstly when the NPs are injected into the body and reach at the target cell. The cell membrane is composed of mainly phospholipids and membrane proteins. The phospholipids construct the lipid bilayer by binding at the hydrophobic group each other, and form the spherical shape. Therefore, the liposome formed from the phospholipids is generally utilized as the cell membrane model [8]. Moreover, since it is reported that the most phospholipids of human cells are phosphatidylethanolamine (PC) [9], which structural formula is shown in figure 1, we have decided to reveal the adsorption reaction between the surfaces of the liposome formed from the PC molecules and the Au NPs. The liposome is called as “PC liposome” in this paper. However, in the case of carrying out the spectroscopic measurement for the above sample, it seems hard to obtain the signal with high Signal/Background ratio for the PC molecules which interact with the Au NPs. It is because the PC molecules exist not only in outer side of the lipid bilayer for the liposome but also in inner side. In addition, in the PC liposome aqueous solution, there is also a probability for the existence of the PC single molecules, which do not contribute to form the liposome and do not react with the Au NPs. The removal of those PC molecules is indeed difficult. Therefore, as an alternative to investigate the adsorption reaction between the PC liposome and the Au NPs, we have determined to elucidate the change of the chemical states for the “PC single molecule” before/after the adsorption reaction with the “Au sheet surface” under water environment. We think that it is easily possible to retain only the PC molecules chemisorbed on the Au surface by rinsing the Au sheet with the water after promotion of the adsorption reaction.

In addition, to apply the Au NPs as pharmaceutical products, we think that the Au NPs must indicate high biocompatibility. The Au NPs colloidal solution generally includes the surfactant molecules in the fabrication process. The molecules do not indicate biocompatibility. Although the surfactant molecules are used also to the fabrication of Au nanorod, H. Takahashi et al. have reported that the cell viability has increased when the surfactant molecules on Au nanorod surface have been replaced with PC molecules [10]. We have a question whether the molecular replacement has been carried out completely. Therefore, to prepare the Au NPs with high biocompatibility, we decide to use the Au NPs colloidal solution prepared by the solution plasma method and to cover the Au NPs surface with PC molecules. However, the behavior of Au NPs after covering with PC is unknown. Thus, the purposes in this study are three points. First one is to clarify the adsorption morphology between the PC liposome and the Au NPs by Transmission Electron Microscopy (TEM). Subsequently, we investigate the functional groups of the PC molecule contributing for the adsorption reaction between the PC molecule and the Au sheet surface by means of nitrogen (N), oxygen (O) and phosphorus (P) K-edges NEXAFS. Finally, we reveal the behavior of Au NPs after covering with PC molecules by means of TEM and propose the reaction model.

Figure 1. Structural formula of PC molecule.
2. Experimental

2.1. TEM observations

2.1.1. Au NPs colloidal solution

The Au NPs colloidal solution was prepared by the solution plasma method [5-7]. The Au wire (99.95%, 1 mm $^{\phi}$) and the sodium chloride powder (NaCl: $\geq$99%) were purchased from Nilaco Co. and Wako Pure Chemical Industries Ltd., respectively. The milli-Q water ($\geq$18 M$\Omega$cm) was also provided. Two Au wires 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 region 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 10 minutes.

2.1.2. Mixing PC liposome and Au NPs colloidal solution

The PC liposome aqueous solution was fabricated by means of the extrusion procedure [11], and the Mini-Extruder proposed from Avanti Polar Lipods, Inc. was employed. The PC powder (Egg, $>99\%$) was purchased from Avanti Polar Lipods, Inc. The PC powder of 5 mg was dissolved into the milli-Q water of 1 mL and vortexed at room temperature. The PC suspension with white color was heated until 60 $^{\circ}$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 Au NPs colloidal solution and the PC liposome aqueous solution were mixed at volume ratio of 100:1 and vortexed at room temperature.

2.1.3. Au NPs covered with PC molecules

PC powder of 1 mg was dissolved into the milli-Q water of 2 mL. The PC suspension was kept at 4 $^{\circ}$C and centrifuged with 20000 rcf for 5 minutes at 4 $^{\circ}$C owing to the removal of the aggregated PC. The obtained PC supernatant solution was added to the Au NPs colloidal solution at 4 $^{\circ}$C, and the precipitate of Au NPs occurred after several hours. The Au NPs precipitate was picked up to other micro tube and rinsed several times with the milli-Q water.

2.1.4. Conditions for TEM observation

TEM images were observed by 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 state by soft plasma ion bombardment (PIB-10, Vacuum Device). Subsequently, the mixed solution of Au NPs and PC liposome or the precipitate of the Au NPs covered with PC molecules was dropped on the grid. After 5 minutes, the grid was rinsed in 2% uranyl acetate or in 2% phosphotungstic acid. In the case of use the phosphotungstic acid, the grid was subsequently rinsed in milli-Q water. The pH value of 2% phosphotungstic acid was calibrated to 7 by adding 2N NaOH. The extra staining solution on the grid was drawn off by using filter paper, and the grid was dried in air. The TEM observation under the electron accelerating voltage of 100 kV was carried out by H-7600 (Hitachi).

2.2. NEXAFS measurement

2.2.1. Sample preparations for NEXAFS measurements

The milli-Q water cooled at 4 $^{\circ}$C was provided. The PC powder of 1 mg was dissolved into the milli-Q water of 1 mL and vortexed. The Au sheet surface was polished with the alumina powder and cleaned in the ethanol solution by ultrasonic cleaning method. The Au sheet was dipped into the PC suspension, and the adsorption reaction was promoted for 36 hours at 4 $^{\circ}$C. The reaction temperature at 4 $^{\circ}$C has been selected to inhibit the formation of the PC liposome, because it has been generally
defined that the liposome is easy to form in the case of more than the phase-transition temperature. The reacted Au sheet was taken out from the PC suspension and dried in air after rinsing with the milli-Q water. This sample was called as “PC/Au sheet” in this paper. Furthermore, the PC multilayer sample, which did not react with the Au sheet surface, was prepared by dropping PC suspension on the Au sheet and drying in air. This sample was provided as a standard and was described as “PC multilayer”. As a standard for O K-edge NEXAFS spectrum, the FePO$_4$·$n$H$_2$O powder (Wako Pure Chemical Industries Ltd.) was rubbed on indium sheet.

2.2.2. Conditions for NEXAFS measurements
To investigate the adsorption chemical states for several functional groups of the PC molecules adsorbed on the Au sheet surface, the N K- and the O K-edges NEXAFS measurements using synchrotron radiation were carried out at the BL-2 of the SR center in Ritsumeikan University [12]. The NEXAFS spectra were obtained by total electron yield (TEY) method with the sample drain current under high vacuum condition. The incident X-ray energies for the N K- and the O K-edges NEXAFS measurements were calibrated with the first peak of the NEXAFS spectra for h-BN at 399.1 eV and α-Fe$_2$O$_3$ at 529.4 eV, respectively. The P K-edge NEXAFS measurements were carried out at the BL-6N1 in Aichi Synchrotron Radiation Center (AichiSR) and the BL-3 in Hiroshima Synchrotron Radiation Center (HiSOR) [13]. The P K-edge NEXAFS spectra were obtained by fluorescence X-ray yield (FY) method using the atmospheric XAFS measurement system with He-path [14]. The silicon drift detector (SDD) was used at AichiSR BL-6N1, on the other hand, the fluorescence yield detection for HiSOR BL-3 was employed using a gas-flow type proportional counter with P-10 gas (10% CH$_4$ in Ar). The incident X-ray energy was calibrated on the assumption that the first peak of FePO$_4$ appears at 2153.0 eV at both beamlines. In this paper, the shown spectra of the P K-edge NEXAFS are measured at AichiSR.

3. Results and discussions

3.1. TEM observations

3.1.1. Mixing PC liposome and Au NPs colloidal solution
Figure 2 shows the TEM image of the projection view for the sample after mixing the Au NPs colloidal solution and the PC liposome aqueous solution. When we take notice of the enlarged view, it can be considered that the PC molecules adsorb on the Au NPs surface. It is because a certain bright film seems to be covered the NPs surface. The average thickness with standard deviation for the bright film is estimated to be 1.5±0.2 nm. The thickness is similar to the average length of PC molecule, which is defined as 2.0–2.5 nm [9,15]. H. Zhu et al. also have reported that the gold particles fabricated with phospholipids are coated with a phospholipid monolayer, because the average particle-to-particle distance is 4 nm [16] and the average distance means to be equal to the length of two phospholipids molecules. Thus, we think that the Au NPs firstly are covered with the PC molecules, which do not contribute to form the PC liposome, and subsequently the Au NPs covered with the PC adsorb on the PC liposome surface. Furthermore, considering to the results of section 3.1.2, it is speculated that the Au NPs surface is covered with two layers constructed with PC molecules as shown in figure 3 because the thickness of the certain bright film is same in comparison with the average distance between the NPs covered with PC. The PC molecules firstly adsorb on the Au NPs surface, and subsequently another PC molecule may interact with the PC molecule on the Au NPs through the hydrophobic groups.
3.1.2. Au NPs covered with PC molecules

Figure 4 shows the TEM image for the precipitate of the Au NPs covered with PC molecules. The Au NPs are constructed like 2-dimensional beads line. In the enlarged view, the NPs are covered with bright film and the average distance between NPs with standard deviation is estimated to be 1.5±0.4 nm. Considering the results of section 3.2, the PC molecules seem to adsorb on the Au NPs surface at methyl group of N-CH$_3$ and the oxygen atoms of P-O, P=O, C-O and C=O bonds. Therefore, the Au NPs probably interact through the hydrophobic group of the PC molecules on the NPs. Because the average distance between NPs is different compared with the length of two PC molecules connected linearly [9,15], we speculate the possibility of three causes. First one is that the PC adsorbs on the Au NPs surface with certain inclination. Second one is that the NPs approach each other when the water in the Au NPs precipitation is dried due to preparation of TEM sample. Third one is that the hydrophobic groups interact each other at molecular side, as shown in figure 5.
3.2. NEXAFS measurements

3.2.1. N K-edge NEXAFS

Figure 6 shows the N K-edge NEXAFS spectra for the PC multilayer as a standard and the PC/Au sheet. Those spectra are normalized by edge-jump. The obvious peaks for the PC multilayer and the PC/Au sheet are observed at 403.6 eV. We think that the peak position at 403.6 eV is assigned to $\sigma^*(N-C)$ orbital of the PC molecule. The peak intensity for the PC/Au sheet is reduced in comparison with that for the PC multilayer. S. Yagi et al. have reported that the peak intensity of the spectrum for L-cysteine on polycrystalline Cu surface is a little bit suppressed compared to the peak intensity for the L-cysteine bulk phase when the sulfur K-edge NEXAFS measurement has been carried out [17]. The suppression of the peak intensity has occurred by the charge transfer from the Cu surface to L-cysteine $\sigma^*(S-C)$ orbital. Therefore, we speculate that the reduction of the peak intensity at 403.6 eV occurs by the adsorption of the PC molecule at the methyl group of N-CH$_3$ and the charge transfer from the Au sheet surface to the $\sigma^*(N-C)$ orbital of the adsorbed PC molecule.
3.2.2. P K-edge NEXAFS

Figure 7 shows the P K-edge NEXAFS spectra for the PC multilayer as a standard and the "PC/Au sheet - (Au sheet)". The "PC/Au sheet - (Au sheet)" spectrum is obtained by subtracting the spectrum of the Au sheet from that of PC/Au sheet. The smoothed spectrum for the "PC/Au sheet - (Au sheet)" is also shown. The spectrum for the PC multilayer possesses a peak at 2151.8 eV, which is observed the enlargement of the full width at half maximum (FWHM) toward high energy side. Generally, if there is the double bond in a molecule and the NEXAFS measurement for the constituent element of the double bond is carried out, the $\pi^*$ peak appears at lower energy side in comparison with that of $\sigma^*$. Thus, we think that the energy position at 2151.8 eV indicates the transition from P1s to $\pi^*(P=O)$ molecular orbital. In addition, since the PC molecule possesses the bonds of both P=O and P-O, it is speculated that the enlargement of the FWHM toward high energy side shows the transitions to $\sigma^*(P-O)$ and $\sigma^*(P=O)$ orbitals. The peak and the shoulder structure for the "PC/Au sheet - (Au sheet)" are obtained at 2152.6 eV and 2151.8 eV, respectively. Because the energy position of the shoulder structure is equivalent to that of the peak for the PC multilayer and the peak intensity for the "PC/Au sheet - (Au sheet)" is reduced in comparison with that for the PC multilayer, we think that the PC molecule adsorbs on the Au sheet surface at the oxygen atom of P=O bond of PO$_4$ part. Since the intensity for energy region of $\sigma^*$ is also reduced, it is also possible that the PC molecule adsorbs on the Au sheet surface at the oxygen atom of P-O bond. The spectra of P K-edge NEXAFS measured at HiSOR BL-3, not shown in here, show the same results.

3.2.3. O K-edge NEXAFS

Figure 8 shows the O K-edge NEXAFS spectra for the PC multilayer as a standard and the "PC/Au sheet - (Au sheet)". The smoothed spectrum for the "PC/Au sheet - (Au sheet)" is also shown.

![Figure 7. P K-edge NEXAFS spectra for the PC multilayer as a standard and the "PC/Au sheet - (Au sheet)". The smoothed spectrum for the "PC/Au sheet - (Au sheet)" is also shown.](image)

The PC molecule has several kinds of O-P and O-C bonds. About the spectrum of the PC multilayer, the peak and shoulder structures are observed at 531.5 eV, 534.4 eV, 536.4 eV and around 545 eV. When we pay attention to the unoccupied orbital of O-C bond, the energy positions are derived from...
O1s(O=C)→π*(O=C) [18], O1s(O-C)→π*(O=C) [18], O1s→σ*(O-CH$_2$) [18] and O1s→σ*(O=C) [18], respectively. Subsequently, about the spectrum for the FePO$_4$·nH$_2$O powder, the peaks and the shoulder structures are observed at 530.2 eV, 531.5 eV, 537.3 eV and 544.6 eV. We speculate that those energy positions mean the transitions of O1s(O=P)→π*(O=P), O1s(O-P)→π*(O=P), O1s→σ*(O-P) and O1s→σ*(O=P), respectively. Other materials with O-P bonds also possess the π* peak at region of 530–532 eV and σ* peak at region of 535–538 eV [19]. Therefore, we think that the spectrum of the PC multilayer is constructed with the convolution of the peaks for both O-C and O-P bonds. Although the peak at 538.3 eV for the PC multilayer is also observed, the obvious assignation of the peak has not been defined. Because the peak position at 540.0 eV shows the transition of O1s→σ*(O-C) orbital [18] and the transition of σ*(O-P) attributed to PO$_4$ part has also been defined at 535–538 eV [19], we think that the peak at 538.3 eV is constructed with the convolution of those peaks.

Each peak intensity of the above unoccupied orbitals is reduced when the adsorption reaction between the PC molecule and the Au sheet surface is promoted. Thus, we propose that the PC molecule adsorbs on the Au sheet surface at the oxygen atoms of O-C and O-P bonds. Especially, considering the double bonds of the PC molecule, there is possible that the oxygen atoms of O=C and O=P for the PC molecule interacts to the Au sheet surface and the double bond is dissociated. It is because the peak of π* after adsorption reaction decreases and the shoulder structures of both π* and σ* disappear. This results correspond with the results for P K-edge NEXAFS.

![Figure 8](image.png)

**Figure 8.** O K-edge NEXAFS spectra for the PC multilayer as a standard and the PC/Au sheet. The spectrum of the FePO$_4$·nH$_2$O powder is also shown as a standard.
4. Conclusions
The Au NPs have been prepared by the solution plasma method. We think that the Au NPs firstly are covered with the PC molecules and subsequently the Au NPs covered with the PC adsorb on the PC liposome surface. Because each peak intensity of N, O and P K-edges NEXAFS spectra for the PC/Au sheet is reduced in comparison with that for the PC multilayer, the PC molecule seems to adsorb on the Au sheet surface at the methyl group of N-CH$_3$ and the oxygen atoms of P-O, P=O, C-O and C=O bonds. The Au NPs covered with PC seem to be aggregated through the hydrophobic groups of PC on Au NPs and are also constructed like 2-dimensional beads line.

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