Spectroscopic Study on the Reaction of L-Cysteine in Rh(PVP) Nanoparticle Colloidal Solution*

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We have investigated the reaction of L-cysteine in Rh(PVP) nanoparticle colloidal solution using S-K and Cl-K edges near edge X-ray absorption fine structure and circular dichroism measurements. In our previous study, it was shown using S-K edge NEXAFS technique that cystine molecules were synthesized by dissolving L-cysteine into the Rh(PVP) nanocolloidal solution. In this study, we have revealed the details of cystine synthesis reaction. There are four reactions, which are the adsorption of L-cysteine on Rh nanoparticle surface, the L-cysteine decomposition to cystine thiolate, the formation of the complex structure similar to $(\text{NH}_4)_3[\text{RhCl}_6]$ and the synthesis of cystine molecule on Rh nanoparticle surface. These reactions start immediately after dissolving L-cysteine into the nanocolloidal solution. [DOI: 10.1380/ejsnt.2009.314]

Keywords: Rhodium; Nanoparticle; L-cysteine; Cystine; Surface chemical reaction; NEXAFS; Circular dichroism

I. INTRODUCTION

The platinum group elements show a high reactivity for halogens and chalcogens. Many studies have been undertaken into the catalytic performance of platinum group metal halide and chalcogenide [1–7]. It is considered that the platinum group metal halide and chalcogenide have stable structures and we can use the platinum group metals as a remover for the halogen and chalcogen. Because the ratio of the surface atom to internal atom increases on a nanoparticle phase, the nanoparticle made of the platinum group metal is expected to be particularly efficient at removing halogens and chalcogens. Therefore, this nanoparticle has been used in the cosmetic and medical products in these years.

In our previous study, we have investigated the reaction of L-cysteine on Rh(PVP) nanoparticle surface in the colloidal solution by S K-edge near edge X-ray absorption fine structure (NEXAFS) [8]. This study indicated that L-cysteine molecules decompose to cystine thiolate, the thiolate adsorbs on Rh nanoparticle surface through the sulfur and cystine molecule is produced by making the S-S bond from two cysteine thiolates. However, it is not clear whether there exists a population of dipeptides in cystine state in the aqueous environment that is free of the Rh(PVP) nanoparticle. This paper focuses on circular dichroism (CD) and NEXAFS spectroscopy. CD was employed as it specifically reports the binding state of dissolved chiral molecules, whereas NEXAFS can measure the time-dependent Cl K-edge spectra after dissolving L-cysteine into the nanocolloidal solution; this is especially important where chlorine ions remain in the nanocolloidal solution may influence the reac-

FIG. 1: A photographic view of the NEXAFS measurement cell for liquid samples.

tion between L-cysteine and Rh(PVP) nanoparticle. In this work, we have clarified the reaction of L-cysteine in Rh(PVP) nanoparticle colloidal solution by S-K and Cl-K edges NEXAFS and CD measurements.

II. EXPERIMENTAL

We have prepared the nanocolloidal solution of Rh(PVP) by the chemical reduction method [9]. Rhodium chloride tri-hydrate ($\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$; Rh content is 39 wt%) and PVP (polyvinylpyrrolidone; molecular weight is 10,000) were purchased from KISHIDA CHEMICAL Co., Ltd. and Mitsuwa Chemicals Co., Ltd., respectively. $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ and PVP were used as a precursor for Rh nanoparticle and as a surfactant to protect aggregation of Rh nanoparticles, respectively. $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ (0.05 mmol) and PVP (0.5 mmol) were dissolved into a mixed solvent of distilled water and ethanol. The Rh(PVP) nanocolloidal solution was produced by

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FIG. 2: S K-edge NEXAFS spectra for L-cystine aqueous solution, L-cystine aqueous solution and the mixed samples that have been left for arbitrary time.

the reflux-flow system at 353 K for 5 hours. The average size of Rh(PVP) nanoparticle is estimated to be 3.2 ± 0.7 nm [9]. L-cysteine (0.01 mmol) obtained from Sigma Aldrich Japan Co., Ltd was dissolved into 2 ml Rh(PVP) nanocolloidal solution. We have left the mixed sample for the appropriate time at room temperature to stimulate the reaction.

The S-K and Cl-K edges NEXAFS measurements for the liquid samples were surveyed by measuring fluorescence X-ray yield using the atmospheric XAFS measurement system with He gas at the beamline BL-3 on Hiroshima Synchrotron Radiation Center (HSRC) [10, 11]. The photon energy was calibrated on the assumption that the energy of first peak in the K_2SO_4 spectrum occurs 2481.70 eV. The fluorescence yield detection was employed using a UHV-compatible gas-flow type proportional counter with P-10 gas (10% CH_4 in Ar). The samples were inserted into a specially designed measurement cell for the liquid specimens using a syringe. The cell is made of a polyethylene thin film (12 μm thickness) as shown in Fig. 1.

The CD spectra were measured with a J-720 spectropolarimeter (JASCO) at 298 K for 9-16 accumulations (1.0 mm path length, 1.0 nm bandwidth, 2 sec time constant, 100 nm/min scan speed). The energy calibration was assessed by monitoring the CD spectrum of ammonium (+)-camphor-10-sulfonate solution, which exhibited positive and negative peaks at 290.5 and 192 nm with a 1:2 intensity ratio, respectively [12].

FIG. 3: Relation between the reaction time and the relative peak intensity of $\sigma^*(S-C)$ for L-cystine.

FIG. 4: Cl K-edge NEXAFS spectra for Rh(PVP) nanocolloidal solution and the mixed samples after 3 hours and 180 days.

III. RESULTS AND DISCUSSIONS

A. S K-edge NEXAFS measurement

Figure 2 shows S K-edge NEXAFS spectra for L-cysteine aqueous solution, L-cystine aqueous solution and the mixed samples that have been left for different time durations (5 hours, 1, 30, 180 days). All spectra are normalized by the edge-jump. The spectra after 30 and 180 days have the shoulder structure at 2473.9 eV and this position is assigned to the peak position of $\sigma^*(S-C)$ for L-cystine. Figure 3 shows the relation between the reaction time and the relative peak intensity at 2473.9 eV. The relative peak intensities are estimated by subtracting the NEXAFS spectrum from the mixed sample after 5 hours. The amount of cystine produced gradually increases but then remains constant after 30 days. It is suggested that L-cysteine dissolved into the nanocolloidal solution is used

FIG. 5: Cl K-edge NEXAFS spectra for the mixed samples that have been left for arbitrary time.

up during the cystine synthesis reaction and the reaction is finished showing saturation levels of cystine after 30 days. However, it cannot be determined for S K-edge NEXAFS results as to whether the synthesized cystine molecules exist on the Rh nanoparticle surface or in the nanocolloidal solution.

B. Cl K-edge NEXAFS measurement

Figures 4 and 5 show Cl K-edge NEXAFS spectra for Rh(PVP) nanocolloidal solution and the mixed samples. All NEXAFS spectra are normalized by the edge-jump. In Fig. 4, two peaks of Rh(PVP) nanocolloidal solution are located at 2822.4 eV and 2828.8 eV. These peaks are attributed to a chlorine ion state and the bonding between chlorine and rhodium, respectively [9]. The main peak at 2828.8 eV shifts to 2827.4 eV after dissolving L-cysteine into the Rh(PVP) nanocolloidal solution and the peak intensity at 2827.4 eV increases with the reaction time. Rumpf *et al.* and Sugiura *et al.* have reported that the peak at around 2827.4 eV can be observed in the Cl K-edge NEXAFS spectra for the specimen of ammonium hexachlororhodate(III) complex $[(\text{NH}_4)_3[\text{RhCl}_6]]$ [13, 14]. Since this same complex structure cannot exist due to steric repulsions on the Rh nanoparticle surface, it is thought that similar structure is produced on the Rh nanoparticle surface in the mixed sample. In Fig. 5, it is observed that the peak intensity at 2827.4 eV increases

FIG. 6: CD spectra for (A) L-cystine aqueous solution and the mixed samples with Rh nanoparticle and (B) the mixed samples without the nanoparticle.

for the reaction time within 2 hours. The peak intensity of chlorine ion state observed at 2822.4 eV decreases with the reaction time as shown in Fig. 4. Therefore, it is proposed that a structure similar to $(\text{NH}_4)_3[\text{RhCl}_6]$ is being formed at surface Rh atoms on the Rh(PVP) nanoparticle, by reaction with chloride ion and L-cysteine in the nanocolloidal solution.

C. CD measurement

Figure 6 shows CD spectra for L-cystine aqueous solutions, (A) the mixed samples with Rh nanoparticle and (B) the mixed samples without the nanoparticle. In Fig. 6(A), the spectra for the mixed samples after 2, 20 and 30 days have a positive band at 279.7 nm and two negative bands at 244.5 and 316.0 nm. On the other hand, a small negative band at 248.4 nm is observed in Fig. 6(B). Since the intensity of the band at 248.4 nm in (B) is considerably smaller than that of the band at 244.5 nm in (A), the band at 248.4 nm in (B) has little contribution of the band intensity at 244.5 nm in (A). It is suggested that three bands observed in (A) are assigned to the polarization-sensitive material and the conformation, which are produced by the interaction between L-cysteine and Rh nanoparticle. The main band in the 235 to 260 nm wavelength range as shown in (A) is comprised of two spectral components, which are located at 244.5 and 255.8 nm, respectively. The negative band for L-cystine aqueous solution is observed around 255 nm and is attributed to S-S bond of cystine molecule [15, 16]. Therefore, the main band at 255.8 nm would correspond to S-S bond of cystine molecule under the aqueous solution environment. Thus, synthesized cystine molecules are dissolved into the nanocolloidal solution. Moreover, it has reported that the CD band intensity of S-S bond depends on the

FIG. 7: Proposed reaction mechanism of L-cysteine on Rh(PVP) nanoparticle surface.

chemical state of ionization of the amino group [17, 18]. The main band at 244.5 nm in (A) appears after 1 day and the intensity gradually increases. This indicates that the band at 244.5 nm can be assigned to S-S bond of cystine molecule which has an ionized amino groups near the S-S bond. This assignment agrees with the result of Cl K-edge NEXAFS which shows the existence of the complex structure similar to $(\text{NH}_4)_3[\text{RhCl}_6]$. The intensity of the band at 244.5 nm arises from the effect of ionized amino groups and from the increased concentration of cystine. In addition, this band becomes dominant in the spectrum after 2 days. We speculate that cystine molecules are synthesized during formation of the complex on Rh nanoparticle surface and the synthesis reaction of cystine gradually increases.

Though there is a slight difference between the spectra for the mixed sample after 20 and 30 days, it can be considered that those intensities are almost constant value. This result corresponds with the relation between the amount of cystine and the reaction time. Therefore, it is considered that the synthesis of cystine molecule gradually proceeds and is essentially complete after 20 days.

D. Considerable reaction mechanism of L-cysteine in Rh(PVP) nanoparticle colloidal solution

We can speculate that the reaction mechanism of L-cysteine in Rh(PVP) nanoparticle colloidal solution at

room temperature is as seen in Fig. 7. It seems that there are four reaction stages, which are (i) the adsorption of L-cysteine on Rh nanoparticle surface, (ii) the L-cysteine decomposition to cysteine thiolate, (iii) the bonding between chlorine ion and surface atom of Rh nanoparticle, the coordination bond between the ionized amino group of cysteine thiolate and $[\text{RhCl}_6]^{3-}$ and the formation of the complex structure similar to $(\text{NH}_4)_3[\text{RhCl}_6]$ and (iv) the synthesis of cystine on the Rh nanoparticle surface.

IV. CONCLUSION

We have investigated the reaction of L-cysteine in Rh(PVP) nanoparticle colloidal solution by the atmospheric pressure XAFS and CD measurements. L-cysteine dissolved into the Rh(PVP) nanocolloidal solution dissociates to cysteine thiolate. The complex structure similar to $(\text{NH}_4)_3[\text{RhCl}_6]$ is made from chlorine ion, Rh(PVP) nanoparticle surface atom and the cysteine thiolate-derived amino group. The cystine is synthesized by forming S-S bond and adsorbs on the nanoparticle surface of Rh(PVP) through the ionized amino group. Thus, the cystine molecule is produced from two cysteine thiolates on Rh nanoparticle surface. These reactions in the nanocolloidal solution start immediately after dissolving L-cysteine into the nanocolloidal solution and finish about 20 days later.

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