Original Paper

Reaction of L-cysteine on Rh(PVP) nanoparticle surface by NEXAFS

S. Gohda[1], T. Ashida[1], S. Yagi[1], H. Namatame[2], M. Taniguchi[2]
[1]*Department of Quantum Engineering, School of Engineering, Nagoya University; Furo-cho, Chikusa-ku, Nagoya, 464-8603 Japan*
[2]*Synchrotron Radiation Center, Hiroshima University; 2-313 Kagamiyama, Higashi-Hiroshima, 739-8526 Japan*
h076409m@mbox.nagoya-u.ac.jp

(Received: November 28, 2007; Accepted: April 04, 2008)

We have studied the reaction of L-cysteine [$HSCH_2CH(NH_2)COOH$] on the Rh(PVP) nanoparticle surface in the colloidal solution by the atmospheric pressure X-ray absorption fine structure (XAFS) measurement system. The measurement sample is prepared by dissolving L-cysteine into the Rh(PVP) colloidal solution and being left at the room temperature. The sulfur K-edge NEXAFS spectra of the mixed solutions show four chemical states which are atomic sulfur, L-cysteine thiolate, L-cysteine and cystine. As a result, it is considered that L-cysteine dissolved into the Rh(PVP) colloidal solution dissociates to L-cysteine thiolate or atomic sulfur on the Rh nanoparticle surface and L-cysteine thiolates lead to the formation of cystine molecules [$(SCH_2CH(NH_2)COOH)_2$] by the S-S bond. We have concluded that the cystine molecules are synthesized gradually in the Rh nanocolloidal solution at room temperature.

1. Introduction

Recently the transition metal nanocolloids, which have a highly effectiveness for removing an active oxygen, have been paid attention on the medicine and pharmaceutical fields. However, there is few report about an influence of nanoparticles in vivo. Most of the studies which have ever been reported about the nanoparticle and the biological molecule have been researched for the bioelectronic applications so that they have not denoted the details of the nanoparticle-biomolecule reaction [1, 2]. Therefore, it is a great important thing on the above fields to reveal the mechanism of reaction between the metal nanoparticle surface and the biomolecule in vivo.

Many researchers have investigated the reaction of biomolecule on Au surface and shown that the sulfur containing molecules decompose to thiolate on Au substrate [3-6]. Besides, T. Nomoto has presented that the dimethyl sulphide dissociates to the methanethiolate on the surface of Rh [7, 8], which is one of the platinum family elements including Au. We have succeeded in synthesis of the Rh(PVP) nanoparticle controlled its diameter and elucidation of the properties [9]. The Rh(PVP) nanoparticles are synthesized by reducing Rh ion in ethanol-water solvent with water-soluble polymers of PVP (polyvinylpyrrolidone). To clarify the reaction between the metal nanoparticles and biomolecule in vivo, we thought that there is a need to measure the reaction under the solution environment. Since the nanoparticles exist inside a water pool in the solution, we can imitate the reaction between the metal nanoparticles and biomolecule in vivo by dissolving an organic molecule into the solution. L-cysteine, which is one of the amino acids, has a thiol group that indicates a high reactivity on the metal surface so that the studies about the adsorption behavior of L-cysteine on various kinds of metal surfaces have been reported [10-12]. We have focused on the reaction between L-cysteine and nanoparticle surface. The purpose of this study is to reveal the reaction of L-cysteine on the Rh(PVP) nanoparticle surface in the colloidal solution by near edge XAFS (NEXAFS).

2. Experimental

In this study, we have prepared the Rh(PVP) nanocolloidal solution by the reduction method [9]. Rhodium

chloride tri-hydrate ($RhCl_3(3H_2O)$: Rh content is 39 wt%) and PVP(K-15) are purchased from KISHIDA CHEMICAL Co., Ltd. and Mitsuwa Chemicals Co., Ltd., respectively. First, 0.05 mmol $RhCl_3(3H_2O)$ and 0.5 mmol PVP were dissolved into a mixed solvent of distilled water and ethanol (the water ratio to the solvent: 10 Vol%). Next, the Rh(PVP) nanocolloidal solution was produced by the reflux-flow system at 353 K for 5 hours. The formation of the nanoparticles was judged from the change of the solution color from rose pink to dark brown. Furthermore, 0.01 mmol L-cysteine obtained from Sigma Aldrich Japan Co., Ltd was dissolved in 2 ml Rh(PVP) colloidal solution. We have left the mixed solution for the appropriate time at room temperature to stimulate the reaction. The mixed solutions that passed each time after dissolving L-cysteine powder (5, 24, 48 hours and 32 days) were put into the NEXAFS measurement cell for the liquid specimens, which made of a polyethylene film.

The sulfur K-edge NEXAFS measurements of the liquid samples were carried out by the yielding fluorescence X-ray using the atmospheric XAFS measurement system with a He gas at the beamline BL-3 on Hiroshima Synchrotron Radiation Center (HSRC) [13, 14]. The incident X-ray energy was calibrated on the assumption that the first peak of K_2SO_4 appears at 2481.70 eV. The fluorescence yield detection was employed using a gas-flow type proportional counter with P-10 gas (10 % CH_4 in Ar).

3. Results and discussion

Fig. 1 shows the sulfur K-edge NEXAFS spectra for L-cysteine powder, L-cysteine aqueous solution (A) and the mixed solutions. All spectra are normalized by the edge-jump. The peak of L-cysteine powder and L-cysteine aqueous solution are observed at 2472.8 eV and 2473.0 eV, respectively. These peaks are assigned to the transition from sulfur 1s to anti-bonding orbital of σ *(S-C). Since the peak positions have a slightly difference, this indicates that L-cysteine does not have a strong chemical bonding with water molecules. The peaks of the mixed solutions are located at 2472.6 eV, which is lower than that of L-cysteine aqueous solution. It shows that the peak of σ *(S-C) for L-cysteine is shifted by the interaction with Rh nanoparticle or PVP molecule. Those peaks of the mixed solutions become much broader than

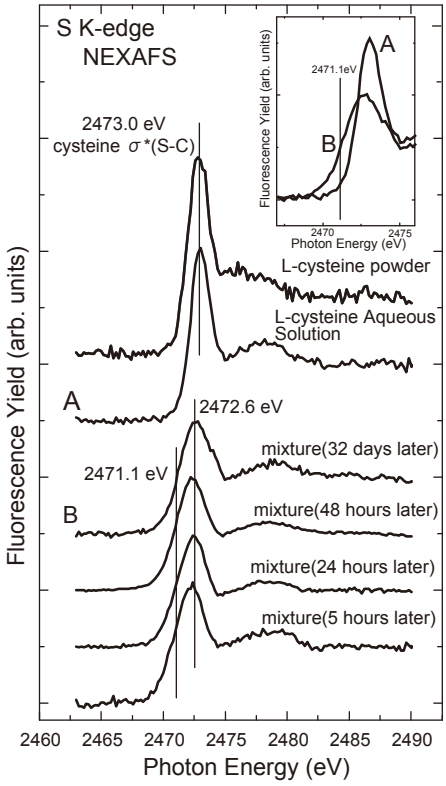

Fig. 1 Sulfur K-edge NEXAFS spectra for L-cysteine powder, L-cysteine aqueous solution and the mixed solutions that have left for arbitrary time. The inset shows the detailed NEXAFS spectra A and B.

that of L-cysteine powder and aqueous solution. It seems that a thiolate species originated from the L-cysteine exists on the Rh nanoparticle surface, because the peak at around 2472 eV has been reported as the thiolate adsorbate on the Rh surface [8]. Moreover, there is a small shoulder structure of the mixed solutions at 2471.1 eV. That can be assigned to atomic sulfur on rhodium surface, judging from the NEXAFS spectrum of atomic sulfur on Rh(100) [8]. It is considered that three chemical states, which are atomic sulfur, σ *(S-C) for L-cysteine thiolate and σ *(S-C) for L-cysteine, are found in the mixed solutions.

When we pay attention to the spectrum of the mixed solution after 32 days, the shoulder structure can be seen on higher energy side of the main peak. In summary, we know that there are four components originated from atomic sulfur, σ *(S-C) for L-cysteine thiolate, σ *(S-C) for L-cysteine and something. M. Ino has presented that L-cysteine is oxidized easily in vivo and converted L-cystine [15]. Additionally, C. Jing has documented that L-cysteine dissolved into the Ag nanoparticle colloidal

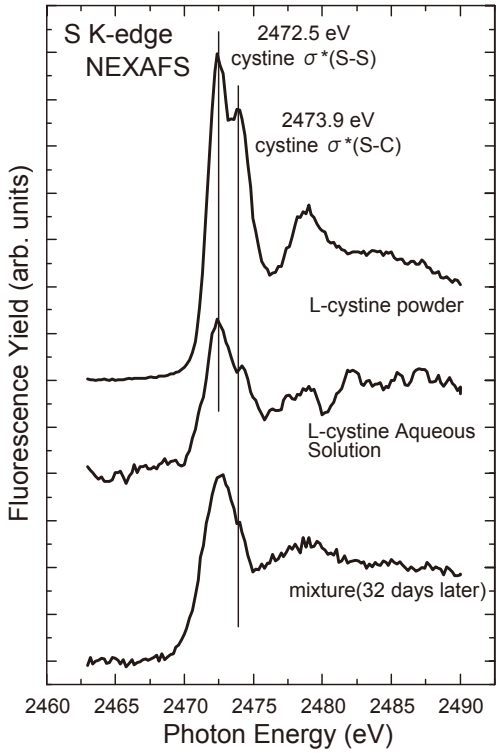

Fig. 2 Sulfur K-edge NEXAFS spectra for L-cystine powder, L-cystine aqueous solution and the mixed solution after 32 days.

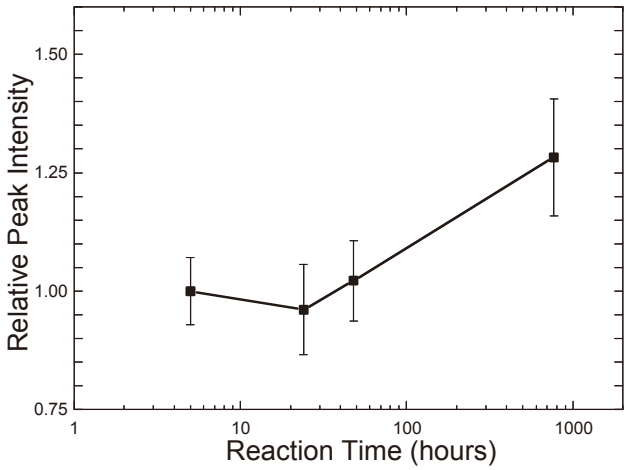

Fig. 3 Relation between the reaction time and the relative peak intensity at 2473.9 eV.

solution dissociates to L-cysteine thiolate on the Ag surface and L-cystine is synthesized [16]. Thus, we should compare the spectrum of L-cystine with the mixed solutions in the next section.

Fig. 2 shows the sulfur K-edge NEXAFS spectra for L-cystine powder, L-cystine aqueous solution and the mixed solution after 32 days. All NEXAFS spectra are normalized by the edge-jump. There is no change in the peak position between L-cystine powder and L-cystine aqueous solution. This suggests that the interaction between L-cystine and water molecule does not exist. Two peaks of L-cystine are located at 2472.5 eV and 2473.9 eV, which are attributed to the transition from sulfur 1s to anti-bonding orbital of σ*(S-S) and σ*(S-C), respectively. When one compares the spectrum of L-cystine aqueous solution with the mixed solution, the shoulder structure's position of the mixed solution corresponds to the peak position of σ*(S-C) for L-cystine. The peak position of the mixed solution at 2473.9 eV has 0.9 eV difference from that of σ*(S-C) for L-cysteine. Therefore, it is supposed that cystine is synthesized on the Rh nanoparticle surface in the mixed solution.

Fig. 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 solution after 5 hours. As seen in this figure, the amount of the compound cystine increases gradually in proportion to the reaction time. Thus, we conclude that the cystine molecules are synthesized by the catalytic reaction of the Rh nanoparticle at room temperature. It seems that there are four reactions, which are (i) the L-cysteine adsorption on the Rh nanoparticle surface, (ii) the decomposition of L-cysteine, (iii) the adsorption of L-cysteine thiolate on the Rh surface and (iv) the synthesis of cystine.

4. Conclusions

We have investigated the reaction of L-cysteine on the Rh(PVP) nanoparticle surface in the colloidal solution by the atmospheric pressure XAFS measurement system. Judging from the result of this study, L-cysteine dissolved into the Rh(PVP) colloidal solution dissociates to L-cysteine thiolate or atomic sulfur because of the interaction of the Rh(PVP) nanoparticle surface. Then, two L-cysteine thiolates form the S-S bond and one cystine molecule is produced. It is found that the cystine molecules are synthesized gradually in the colloidal solution of the Rh(PVP) nanoparticle at room temperature.

5. Acknowledgements

The authors are grateful for the financial supported of a Grant-in-Aid Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 15360358),

21-Century COE "Isotope Science and Engineering from Basics to Applications" and Innovation Hiroshima/Tokai of JST (Japan Science and Technology Agency). This work was performed under the approval HSRC Program Advisor Committee (Nos. 03-A-03, 05-A-15 and 05-A-16).

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