

Local Structure of sulfur in organic molecules immobilized on ferrite nanoparticles

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Introduction

Ferrite nanoparticles now have most extensively studied due to their magnetic and biocompatible properties resulted from their size [1]. Functional ferrite particle which fixed selective biomolecules on the surface is desired for biomedical application such as contrast medium of MRI and affinity separation of proteins which separates and refines only specific protein from a cytoplasmic extract [1].

We have studied chemical interactions between biomolecules and ferrite nanoparticles. Recently, we developed the novel method for the synthesis of ferrite nanoparticles in the presence of protein and amino acids [2]. Since this methodology simultaneously can perform both a ferrite synthesis and an immobilization of biomolecules on the surface of ferrite under low temperature and neutral conditions, biomolecules are directly bound to ferrites without an activation of biomolecules. Therefore, we synthesized ferrite particles in the presence of a cytoplasmic extract prepared from HeLa cells. The results showed that acidic amino acids may contribute to their selective proteins binding to ferrite particles. To understand the determinants for this selective protein binding, we synthesized ferrite particles in the presence of 19 L-amino acids (except for Trp). Consequently, cysteine, aspartic acid and glutamic acid had high affinity for ferrite nanoparticles [3]. In particular, a characteristic of cysteine has a thiol group in the chemical structure, indicating that chemical state of sulfur atom contribute to binding to ferrite surface. To discuss chemical binding of cysteine to ferrite, the information on local structure of sulfur atom is indispensable.

Among the various analytical method for direct determination of the chemical state of elements, XAFS spectroscopy is one of the most powerful methods focusing on local structure of a specific element. We have applied XANES to characterize the chemical state of sulfur atom of cysteine immobilized on the ferrite nanoparticles.

Experimental

Ferrite particles were synthesized from an aqueous solution at 4°C as previously described by Nishimura et al. [2]. The reaction solution (25 ml) was prepared by adding FeCl₂ (2.5 mM) and FeCl₃ (1.25 mM) solutions to

degassed distilled water. Subsequently, the resulting solution was added at 4°C, along with a NH₄OH solution (4.8% aqueous solution), to a 1 M NH₄Cl buffer (pH 8.4) that contained the cysteine. A proportion of the Fe²⁺ ions gradually oxidized into Fe³⁺ ions and these could be precipitated as ferrite particles (around 12 nm in size) in an alkaline solution. After this reaction, the ferrite particles were washed several times by magnetic separation and dialyzed in 500 ml of distilled water for 24h.

The XAFS measurement was carried out using synchrotron radiation ring at BL-9A, Photon Factory KEK, Japan. Si(111) double-crystal monochromator and high order rejection mirror was used. The sulfur K-edge XANES spectra of the samples were measured in fluorescence mode using a Lytle-type detector modified for soft X-ray region analysis. To detect the fluorescence X-ray, the sample chamber of the detector was displaced to Helium atmosphere.

Results and Discussion

Sulfur K-edge XANES spectrum of the cysteine immobilized ferrite was measured and analyzed. As a control, ferrite particle and cysteine were measured. The position of absorption edge for the different size of ferrite particles is almost same. The remarkable differences in a spectrum of the luminescence were found between cysteine and cysteine-immobilized ferrite. The component of sulfur in the cysteine immobilized ferrite sample was estimated to be several oxidized states because peak of absorption edge shifted to higher energy. This result indicates that sulfur atom is interacted with ferrite by fixation. Furthermore, small peaks were detected near the absorption edge. The oxidation of sulfur atom is ascribed to formation of chemical bond between cysteine and ferrite particles. Data processing and refinement are in progress.

References

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