Direct Detection of Redox Reactions of Sulfur-containing Compounds on Ferrite Nanoparticle (FP) Surface

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Direct observation of sulfur sites for sulfur-containing compounds (cysteine and its oxidative derivatives) on ferrite nanoparticles (FP) was performed by X-ray adsorption near edge structure (XANES) measurements. XANES spectra of cysteine derivatives conjugated with FP indicated that redox reactions occurred on the FP surface. Cysteine was completely oxidized to cystine and cysteinesulfinic acid and cysteic acid were partially reduced. These studies revealed that the FP surface should possess both an oxidation and reduction reactive center for sulfur-containing functional groups.

Recently, molecules possessing multiple anionic groups such as citric acid, cysteine, and dimercaptosuccinic acid have been noticed because of their strong coordination to iron ion on FP.[1–3] These molecules could be adsorbed onto FP by simply mixing an aqueous solution without any complicated process. This easy adsorption process is quite important for various biotechnological applications such as bioseparation, magnetic resonance imaging (MRI), and hyperthermia.[4] If there are functional groups that do not participate in immobilization to FP, they are then exposed on the FP surface. As a result, the compound-conjugated FP possesses high dispersibility in liquid media. Thus, they work as a dispersant and it is expected that they decorate the FP surface. However, insufficient understanding and lack of information about the binding configuration of molecules and surface adsorption reactions on FP have prevented research and development toward various biological applications. There are two major reasons that make their analyses hard. First is an extremely trace amount of these adsorbed molecules on FP relative to the iron atoms in FP. Second is complicated reactivity of iron ions on FP relative to another molecule. Borghi et al. reported a surface reductive dissolution of ferrite by mercaptocarboxylic acid.[5] In adsorbing various molecules, an oxidation/reduction reaction occurred between a ferrite and the absorbing molecules making analysis of the reaction mechanism especially complicated. To analyze a reaction mechanism on ferrite, understanding of the binding configuration of these molecules on ferrite is very important.

In our previous study, we found that cysteine, cystine, and cysteic acid were efficiently immobilized onto FP.[6] Our result indicated that there was a selective adsorption of sulfur atom onto FP and/or a strong interaction between sulfur and iron on the FP surface. Therefore, we focused on uncovering the surface reaction between a sulfur functional group and an iron ion on FP. We describe herein a chemical state of the sulfur atoms of cysteine and its analogs (cystine, cysteinesulfinic acid, and cysteic acid) adsorbed onto FP surface measured by XANES spectra.

We synthesized FP through conventional chemical coprecipitation. All procedures in preparation of samples were carried out in glove box under N_2 to prevent oxidation of FP. A 25 mL portion of 0.1 M aqueous iron solution ($FeCl_2/FeCl_3 = 1/2$) was added into 50 mL of 0.2 M aqueous NaOH to give FP suspension (Produced FP is a mixed solid solution between Fe_3O_4 and γ-Fe_2O_3 and its size was around 8 nm.). Cysteine (75 μmol) or its derivatives (cystine, cysteinesulfinic acid, and cysteic acid) was dissolved in 0.2 M aqueous NaOH. Then, 1 mL of alkaline soution of cysteine or its derivatives was added to the obtained FP suspension. After vigorous stirring for 1 h at room temperature, cysteine-conjugated FP was collected magnetically and washed with MilliQ water. The conjugated FP was dried under vacuum to give a black plate. After grinding of the plate-like FP under N_2, the FP powder was put on Kapton tape to measure XANES spectra in fluorescence detection mode at KEK-PF (Tsukuba, Japan) using beamline-9A with Si(111) double crystal monochromator under ring conditions of 2.5 GeV and 348.5–347.3 mA. The entire path of the X-ray beam was in He atmosphere. The monochromator was tuned to 65% intensity, and rhodium-coated mirror was used both for suppressing higher harmonics. As standard sulfur K-edge XANES spectrum, $Na_2S_2O_3 \cdot 5H_2O$ was measured (2472.0 eV).

The XANES spectra of cysteine and its derivative-FP are shown in Figure 1, and the observed peak energies of sulfur functional group are summarized in Table 1. The spectrum of cysteine–FP gave two strong peaks (2472.7 and 2474.2 eV), which corresponded to each reference peak of cystine completely (2472.7 and 2474.2 eV, respectively).[7] These spectra indicate that a thiol group in cysteine was transformed into a disulfide by oxidation to produce cystine on FP. This oxidation reaction (disulfide formation) was not observed in cysteine without FP. XANES spectrum of cysteine–FP also provided significant information on the binding configuration of cysteine oxidized on FP. If there is any chemical bond between a sulfur atom and an iron atom on the FP surface, there must be an energy shift of sulfur K-edge in the XANES spectrum. Our XANES measurement of cysteine–FP exhibited no energy shift. Therefore, it is

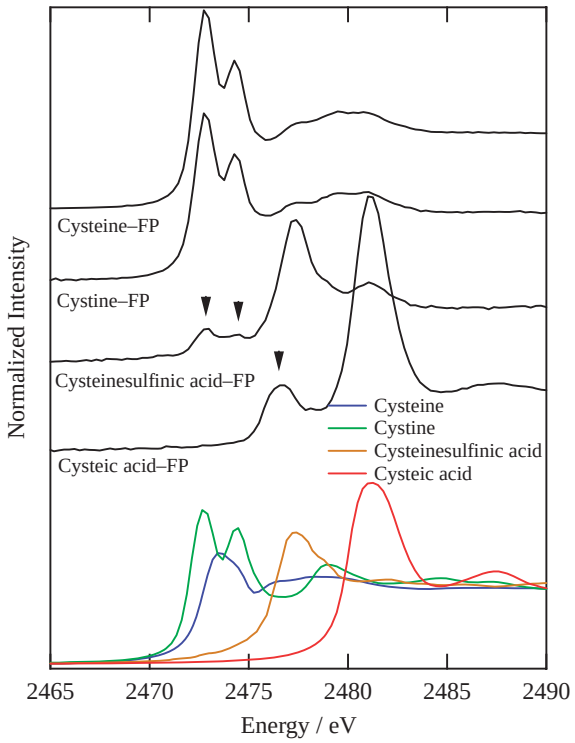

Figure 1. Sulfur K-edge XANES spectra of cysteine, cystine, cysteinesulfinic acid, and cysteic acid-conjugated FP, as well as reference spectra of the unconjugated parent compounds. The black triangles indicate pre-edge feature (peak) appeared in cysteinesulfinic acid– and cysteic acid–FP.

expected that oxidized cysteine (or cystine itself) molecule binds to FP through carboxyl and/or amino groups. This result is consistent with the reported reductive dissolution of iron oxide by thiol-containing molecules.[8] Since no characteristic change was observed in the XANES spectrum of cystine–FP, a redox reaction between cystine and FP did not occur.

The main XANES peaks in cysteinesulfinic acid–FP (2477.4 eV) and cysteic acid–FP (2481.3 eV) did not change compared to those of cysteinesulfinic acid and cysteic acid, respectively. Their sulfur functional groups did not react with FP. However, we observed an interesting weak pre-edge feature in cysteinesulfinic acid–FP and cysteic acid–FP. The pre-edge feature in XANES spectrum of cysteinesulfinic acid–FP appeared at 2472.7 and 2474.2 eV (Figure 1), which corresponds to the intense peaks of disulfide in cystine. Cysteic acid–FP showed an apparent pre-edge feature at 2476.7 eV (Figure 1), which was slightly lower than that of sulfinic acid. This peak did not correspond to any cysteine derivative (Table 1). This feature indicates the presence of reduced compounds derived from cysteic acid. Although we could not identify this species at present, this might be a deoxidized product with a sulfur functional group. From these results, we assumed that these peaks would indicate the existence of a reactive center on FP surface where sulfinic acid and/or sulfonic acid functional groups were deoxidized.

In summary, we directly observed an oxidative transformation of cysteine into cystine and a reduction of cysteinesulfinic acid to a disulfide compound, as well as cysteic acid to a reduced compound on FP surface in XANES. Disulfide did not undergo oxidation and/or reduction. Cysteine was oxidized to cystine completely on FP and cysteinsulfinic acid and cysteic acid were reduced partially on FP. These results would mean that a surface redox potential energy of FP is quite close to that of disulfide group. Currently, examination of the XANES spectra of other sulfur-containing compounds is underway in an effort to elucidate binding information between FP and sulfur-containing compounds.

Table 1. Observed peaks energies in cysteine and its derivatives conjugated to FP and observed sulfur K-edge energy peaks (reference peaks) in cysteine and its derivative

Sample	State	Peak energy/eV
Cysteine–FP		2472.7, 2474.2
Cystine–FP		2472.7, 2474.2
Cysteine sulfinic acid–FP		2472.7, 2474.2, 2477.4
Cysteic acid–FP		2476.7, 2481.3
Cysteine	–SH[a]	2473.4
Cystine	–S–S–[a]	2472.7, 2474.2
Cysteine sulfinic acid	$–SO_2H$[a]	2477.4
Cysteic acid	$–SO_3H$[a]	2481.3

[a]–SH, –S–S–, $–SO_2H$, and $–SO_3H$ are corresponding to cysteine, cystine, cysteinesulfinic acid, and cysteic acid, respectively. The sulfur K-edge XANES spectrum of $Na_2S_2O_3 \cdot 5H_2O$ was measured as standard (2472.0 eV).

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