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XANES measurements of cysteine and its oxidation products at the S-K absorption edge and their application to the oxidative state in human hair using soft X-ray contact microscopy

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Introduction

The effectiveness of XANES profile for mapping a specific chemical bond or molecules containing the bond has been demonstrated for biological specimens such as DNA distribution in a chromosome[1] and in a sperm[2] at the C-K absorption edge. We have measured XANES profiles of sulfur containing biomolecules at the S-K absorption edge and found that a peak of cysteine having S-C bond and that of cystine, a oxidative form of cysteine, having S-S bond is separated each other[3].

In the present study we have extended our XANES measurement to further oxidation product of cystine, cysteic acid, and tried to apply the measurement to oxidative state in human hair specimen mainly composed of sulfur rich proteins. The results showed that the oxidation treatment to the human hair reflected the increase of the peak intensity unique to cysteic acid.

Materials and Methods

For XANES measurement we used sulfur containing amino acid cysteine, its oxidative form cystine, and cysteic acid, an oxidation product by oxygen addition. A droplet of water solutions of these molecules was placed on a collodion membrane supported by an EM-grid and then dried to form a thin film. The transmittance was measured by detecting transmitted X-rays by a silicon photodiode. For contact X-ray microscopy with an electronic zooming tube human hair was cut into small portions with approximate 20 μm thickness, and then placed on a SiN membrane. The opposite side of the membrane was coated with Au for a photocathode of the zooming tube. The oxidation treatment of hair was carried out to soak in the mixture of 3% H_2O_2 and 1.2% ammonia.

Results and Discussion

Fig. 1 shows XANES of cysteine, cystine and cysteic acid at the S-K edge. Arrows indicate the characteristic peaks for these compounds. In accord with our previous observation, the peak of cystine was located at the lower energy than that of cysteine. The peak of cysteic acid was found to be significantly shifted to the higher energy. The contact image of hair was presented in Fig. 2. XANES of a small area in cortex and medulla region (5.4x5.4 μm for cortex and 1.3x1.3 μm for medulla) was measured. The cystine peak observed in the spectrum of the cortex indicated that cystine was a major component, which is consistent with the present knowledge on the hair composition, while the higher peak intensity for cysteine in the medulla spectrum suggests that cysteine was rather rich in this region. XANES of cortex region in the oxidation treatment hair were also inserted in Fig. 1. The significant increase of the peak intensity assigned to cysteic acid was observed. The mapping of oxidative area in the treated hair is in progress.

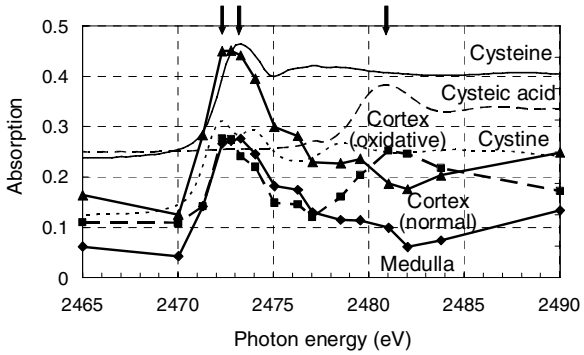

Fig. 1. XANES of thin film of sulfur containing amino acids (cysteine, cystine, cysteic acid), and XANES of intracellular areas of human hair with experimental points.

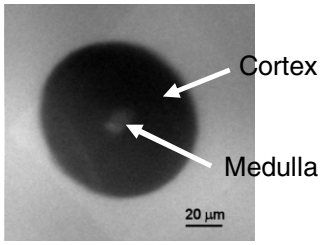

Fig. 2. Cross-sectional image of normal human hair by X-ray contact microscopy at 2465 eV.

References

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