Identification of the Sulfur-containing Pigment in the Biospecimen Studied by XAFS

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Melanin is a biologically important natural pigment found in living organisms. Melanin plays diverse functions such as protection against UV-induced damage, thermoregulation, immune response, and crypsis. It is primarily comprised of pheomelanin (PM) and eumelanin (EM), both of which are derived from oxidation and polymerization of tyrosine [1]. EM is a polymer of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxy-indole-2-carboxylic acid (DHICA) units that generate traits responsible for blackish colors. PM consists of sulfur(S)-containing benzothiazine and benzothiazole moieties. PM synthesis requires S-containing amino acid L-cysteine as a substrate, and PM leads to yellowish colors. Therefore, the PM/total ratio controls the coloration.

Direct chemical identification of melanin is complicated due to their low solubility, high thermostability, and complex polymer structure. When we observe the coloration of the biospecimens in detail, the color is different dependent on positions. Therefore, non-destructive new techniques, such as XAFS, and Raman, provide new insight into the characterization of melanin [2, 3]. In the present work, S K-edge XAFS was performed to characterize melanin in the biospecimen.

Hair samples of C57BL/6JHamSlc-ob/ob and A^y/+ mice (7 weeks old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). C57BL/6J ob/ob and A^y/+ mice possess uniformly black and yellow coats respectively. Both hairs contain EM and PM. Total melanin in black hair was higher than that in yellow, whereas the PM/total melanin ratio in black hair was lower than that in yellow [4]. Oxidized glutathione (GSSG), 2-Amino-6-chlorobenzothiazole (benzo-thiazole), 2H-1,4-Benzothiazine-3(4H)-one (benzo-thiazine) were used as standard samples.

The sample was fixed on a double-sided adhesive carbon tape attached to a sample plate and S K-edge XAFS spectra were taken with XAFS double crystal soft X-ray XAFS beamline (BL-13). The monochromator was operating with Si(111) crystals. The spectra were collected by partial fluorescence yield (PFY) mode with a silicon drift detector (SDD) and the total electron yield (TEY) mode by a sample current. The incident X-ray energy was calibrated with the first main peak of K_2SO_4 at 2481.7 eV [5].

Figure 1 shows S K-edge XAFS spectra of the hair of mice and standard compounds. The mice's hair spectra exhibit two clear peaks at around 2472 eV and 2473.2 eV. The hair consists of the structural protein (keratin) and melanin pigment. The peak features are in good agreement with that of the GSSG spectrum. In addition, mice's hair spectra clearly show a subtle small absorption between 2474.7 eV and 2476.7 eV. Both benzothiazole and benzothiazine exhibit a small absorption at ~2475 eV. Because benzothiazine is chemically transformed from benzothiazole by heat or light [4], the subtle small absorption is assigned to benzothiazole moieties. These results suggest that the absorption from the monomer of PM was detected by using S K-edge XAFS.

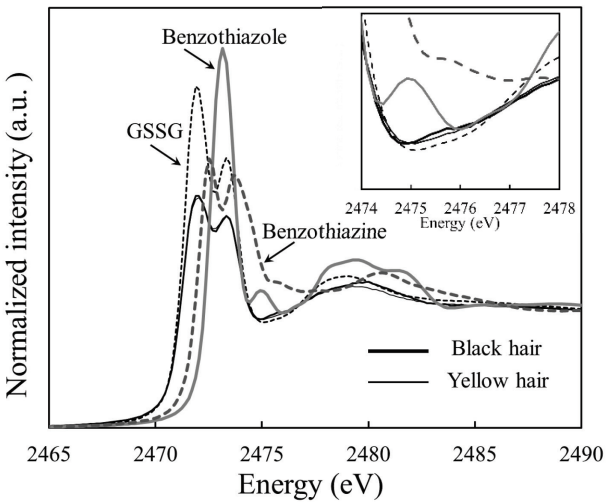

Fig. 1 S K-edge XAFS spectra of the hair of mice (PFY mode) and standard compounds (TEY mode).

References

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