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Microspectroscopic soft X-ray analysis of keratin based biofibers

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ABSTRACT

Scanning soft X-ray transmission microspectroscopy (STXM) and transmission electron microscopy (TEM) have been employed for a high-resolution morphological and chemical analysis of hair fibers from human, sheep and alpaca. STXM allows optimum contrast imaging of the main hair building blocks due to tuneable photon energy. Chemical similarities and deviations for the human hair building blocks as well as for the three investigated species are discussed on the basis of the local near-edge X-ray absorption fine structure (NEXAFS). The spectra of melanosomes corroborate the state-of-the-art model for the chemical structure of eumelanin. Complementary TEM micrographs reveal the occurrence of cortex sectioning in alpaca hair to some extent. A spectroscopic analysis for human hair cortex indicates low mass loss upon soft X-ray irradiation, but transformation of chemical species with decreasing amount of peptide bonds and increasing NEXAFS signal for unsaturated carbon–carbon bonds.

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1. Introduction

Biofibers have gained specific scientific interest during the last years due to their remarkable physical properties (e.g. tensile strength, Young's modulus, low weight), comparably low costs, biodegradability, and variable applications in composite materials (Saheb and Jog, 1999; Mohanty et al., 2000; Hearle, 2007; Eichhorn et al., 2010; Faruk et al., 2012). The properties of biofibers are fundamentally influenced by their nanoscopic morphology and internal molecular interactions. Soft X-ray microspectroscopy provides an excellent tool to investigate such materials with high resolution, high contrast without staining due to resonant imaging, orientation dependent dicroism and relatively low irradiation dose (Ade and Hitchcock, 2008). Scanning transmission soft X-ray microspectroscopy (STXM) has been successfully used to gain insight into the chemical morphology and molecular orientation within various natural fibers with spatial resolutions below 100 nm (Hernández Cruz et al., 2006; Rousseau et al., 2007). Taking advantage of modern Fresnel zone plate technology, the resolution of STXM can reach even below 20 nm (Vila-Comamala et al., 2009).

A typical example for physically and chemically stable keratin based biofibers is mammalian hair. Although significant morphological variations are found for hair specimens of various mammals, the overall properties and basic building blocks are very similar (Menkart et al., 1966). The main building block of hair is called cortex. Most hair fibers contain more than one type of fibrillar cortical cells that can be differentiated by their size and shape (Mercer, 1953; Randebrock, 1964). An asymmetric distribution of these cell types into distinct para- and orthocorteces is usually the natural origin of hair curvature (Orwin et al., 1984; Thibaut et al., 2007). The cortex cells consist of an amorphous lightly cross-linked matrix and highly ordered spindle-shaped macrofibrils that are built up by filamentous polypeptides (Popescu and Höcker, 2007; Robbins, 2012). The cortex is surrounded by a varying number of overlapping non-crystalline scales that form the cuticle (Orfanos and Ruska, 1968). The cuticle is chemically very resistant and serves as protection layer (Geiger, 1944). With increasing thickness a porous medulla can arise in the center of mammalian hair. This structure can occur in very different morphologies according to age, origin and species of the investigated mammal and can therefore contribute to forensic hair identification (Robbins, 2012; Clement et al., 1981). The hair building blocks are internally and externally linked by specific lipid bilayers that are in total referred to as cell membrane complex (CMC) (Robbins, 2009). The natural dye of mammalian hair is melanin that is usually accumulated in oval granules (melanosomes). Melanin exists in hair as black eumelanin and/or reddish pheomelanin. Although the detailed molecular composition of these dyes is still discussed, the combination of various characterization methods and simulations strongly hints on covalently bound oligomers of dihydroxyindole derivatives that

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aggregate to larger particles by π,π-stacking and hydrogen bonds (Watt et al., 2009; Chen et al., 2013).

The physical and chemical properties of (human) hair as well as the effect of several treatments like bleaching and dyeing have been studied in detail by various mechanical characterization methods and electron microscopy (Robbins, 2012). X-ray micro-diffraction studies allow an in situ observation of the keratinization process in the hair follicle (Baltenneck et al., 2000). Also the influence of (cosmetic) hair treatments (Bhushan, 2008), intoxications (Xing et al., 2013) and even severe diseases like breast cancer (James et al., 1999) on the hair microstructure prove the demand for detailed microspectroscopic knowledge about the underlying mechanisms, there are just very few X-ray microscopic studies of this material so far. Hard X-ray Zernike phase contrast imaging was employed for a basic microscopic characterization of unsliced hair shafts with 100 nm resolution (Youn and Shin, 2007), while X-ray tomography showed decomposition effects in the cortex and medulla of bleached and permed hair (Takehara et al., 2010). Furthermore, the content of various amino acids within the keratin structure of the hair is different within its morphological components and influenced by genetics, weathering, diet or cosmetic treatments (Robbins, 2012). X-ray microspectroscopy has high potential to contribute to a detailed in situ chemical analysis with high spatial resolution.

Here we present a detailed microspectroscopic characterization of black human and alpaca hair and white sheep hair by means of transmission electron microscopy (TEM) and STXM including a dose dependent quantitative analysis of X-ray induced radiation damage within the keratin structure. The radiation induced decomposition mechanisms and chemical differences of the various specimens are discussed on the basis of near-edge X-ray absorption fine structure (NEXAFS) spectroscopy.

2. Experimental

The human hair specimen was undyed adult hair donated by an Asian man. Alpaca wool was provided by Sun Star Alpacas (Trefling, Germany) and sheep wool originated from white-haired Anatolian sheep. While the human hair specimen was completely straight, the selected alpaca hair was wavy and the sheep wool curly. Sheep wool had been prepared for carpet production before and therefore the cuticle was reduced to one layer in most fibers. The various hair specimens were embedded in an epoxy resin derived from a 1:1 mixture of 4,4′-methylenebis(2-methylcyclohexylamine) and trimethylolpropane triglycidyl ether. After the resin was dried at 60 °C for 10 h the samples were microtomed to a slice thickness of 200 nm (according to interference color) and fixed on standard copper grids for TEM and STXM imaging. The samples were not stained.

TEM investigations were conducted with a Zeiss TEM 912 Omega at 80 kV accelerating voltage. STXM experiments were performed under vacuum conditions (10^{-5} mbar) at the PolLux endstation at the Swiss Light Source (SLS) in Villigen (Switzerland) (Raabe et al., 2008; Frommherz et al., 2010). The standard STXM setup uses high brilliance synchrotron radiation light that is focused on the specimen by a Fresnel zone plate. The sample is raster-scanned with interferometric control through the focal spot (diameter < 35 nm), while the transmitted photon intensity is recorded using a photo multiplier tube or photodiode. NEXAFS spectra were recorded by consecutive scanning of the investigated area with varying photon energies. All spectra were energy calibrated according to a polystyrene standard (Ade and Hitchcock, 2008; Morin et al., 2001). The absorption spectra were treated according to general procedures (Watts et al., 2006), i.e., normalization to the incident flux and subsequent constant background subtraction (resulting in negligible absorption in the pre-edge region). To obtain the normalized absorption spectra, the spectra were set to unity in the energy well above the spectral features (photon energy > 325 eV). Thus, a comparative analysis of spectral differences is facilitated.

3. Results and discussion

Fig. 1 presents a comparison of STXM and TEM micrographs of cross sections from black human hair. The images show the outer part of the cross section with protecting cuticle on the outside, cortex and melanosomes. A medulla structure was found for most individual hair fibers from this specimen, but is not depicted in these micrographs as it is located in the center of human hair. The specimen did not exhibit a sectioning of the cortex by ortho- and paracortical cells. Fig. 1A shows a STXM micrograph that was recorded at an illumination photon energy of 285.1 eV. This photon energy is perfectly suited to detect melanosomes as small round objects inside the cortex of the hair fiber. The CMC is also visible inside the cortex as vein pattern, but with moderate contrast. Furthermore the contrast of cuticle and cortex compared to the surrounding epoxy resin is rather low. This contrast can be significantly increased when the photon energy is changed to 288.1 eV (Fig. 1B). In this case cuticle and scales have a comparable high absorption and appear dark in the STXM image. On the other hand it is more difficult to differentiate melanosomes from intercellular cavities or holes within the thin cross section. Just larger melanosomes can be identified due to their regular structure. Optimum imaging of the CMC is achieved when the two previously described micrographs are divided by each other (Fig. 1C). The CMC appears as darker streaks inside the cortex and in between the several scale layers. The determination of melanosomes is again rather difficult. This comparison shows that the possibility of photon energy tuning in combination with image division allows generating high resolution STXM micrographs with optimum imaging contrast for all main building blocks of mammalian hair. Fig. 1D provides a TEM micrograph of a comparable sample position. Although the CMC shows lower contrast to the cortex than in Fig. 1, all the other features are clearly specifiable within this single micrograph.

However, STXM provides significant advantages compared to TEM. It has been proven in detail that the absorbed radiation dose and therefore also resulting beam damage is significantly lower in soft X-ray STXM than TEM imaging (Rightor et al., 1997; Hitchcock et al., 2008). This is especially important in the case of biological tissue specimens that are usually very prone to radiation induced decomposition (Meents et al., 2010; Späth et al., 2014). Furthermore, the chemical sensitivity of soft X-ray absorption directly allows a discussion of chemical composition from the above described micrographs. According to literature studies on pure amino acids (Zubavichus et al., 2005) a photon energy of 288.1 eV is expected to result in high absorption for peptide bond rich regions like the keratin based cortex and the cuticle, while the CMC (that consists to high portion of lipids) and melanosomes should appear bright. This is exactly what is found in Fig. 1B. Considering melanin as an indole derivative it is also reasonable that melanosomes show high absorption at 285.1 eV ($\pi^*_{C=C-C}$ resonance) (Zubavichus et al., 2005).

NEXAFS spectra of the various hair regions are presented in Fig. 2. It is clearly visible that cuticle, cortex and medulla exhibit the same main spectroscopic features that are very typical for amino acid based specimens and can be assigned in accordance to literature (Zubavichus et al., 2005) (Table 1).

The spectra are dominated by the π^* resonance of the peptide bond at 288.1 eV. A shoulder at 287.2 eV hints on a significant concentration of cystine and half-cystine that plays an important role in the formation of disulfide linkages inside the molecular

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