Assessment of the State of Preservation of Keratin Material found with the Iceman in Comparison to other Mummy Hairs

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Assessment of the State of Preservation of Keratin Material found with the Iceman in Comparison to other Mummy Hairs

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Abstract

Keratinous materials, such as human or animal hair and feathers, are very stable and resistant to environmental influences. Consequently they are often found together with historical and archaeological human or animal remains. Environmental conditions during preservation induce specific changes of keratin structure and amino acid composition. Humidity, temperature, pH and chemical influences change keratin proteins in different ways and can be explained through the course of the preservation process. Even if methods such as X-ray diffraction and polarization microscopy show intact fiber structures, protein denaturation techniques reveal damage at the protein level. Results of microscopic analyses, X-ray diffraction, and thermal and protein chemical investigations synergistically identify specific facets of changes to the molecular and morphological structures in keratin fibers for a number of pertinent cases.

Introduction

Keratinous materials, such as human or animal hair and feathers, are very stable and resistant to environmental influences. Consequently, they are often found together with human or animal remains. For example, in the case of the Iceman find, a glacier mummy found 1991 in the Alps, a wide variety of hair materials were retrieved, originating from the Iceman himself as well as from pieces of leather and fur clothes.

Different environmental conditions during preservation induce specific changes of keratin structure and amino acid composition. Results of microscopic analyses, X-ray diffraction and protein chemical analysis present specific components of these changes. The results are presented and discussed in comparison and contrast to keratinous materials from different archaeological contexts.

Results and Discussion

Scanning Electron Microscopy (SEM)

SEM examinations of mummy hair of different origins show substantial differences in the state of contamination and preservation. These differences are dependent upon the surroundings and the environmental conditions of preservation. Keratin fibers found together with the Iceman under the glacier, though partly incrusted with soil, show an extremely good condition of the hair surface (Fig. 1a). Even intact hair roots can be detected. SEM images of other archaeological keratin fiber demonstrate significantly worse preservation. The hair fibers have lost the outer cell layer, the cuticle; show holes on the surface and the fibers are broken or split (Fig. 1b).

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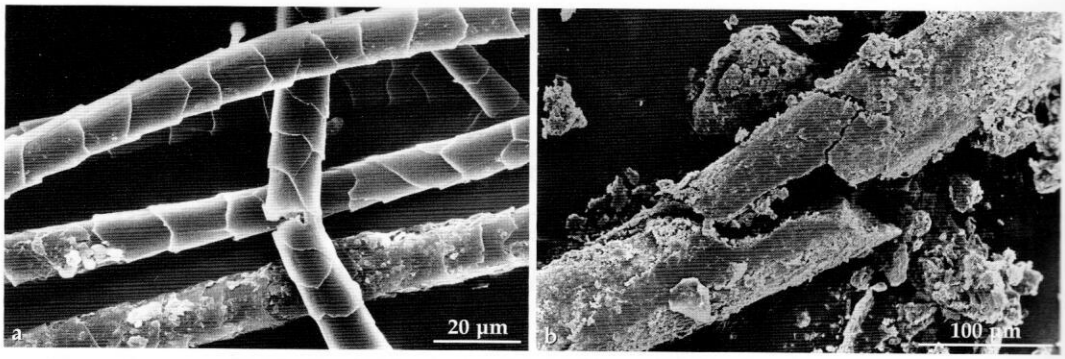

Fig. 1. **a,** Iceman 91/137, animal hair; **b,** animal hair, Vindolanda Textiles, about 200 AD.

Wide Angle X-Ray Diffraction

Wide Angle X-Ray Diffraction (WXRD) gives information about the structural state of the α-helical fraction of intermediate filament proteins (IF). The equatorial halo at 9.8 Å represents the distance between the centers of two α-helical protein chains; the meridian halo at 5.15 Å reflects the step height of the helices. A ring-shaped halo at 4.6 Å corresponds to β-random protein material in the fiber.

Changes in the degree of crystallinity of the α-helical proteins can be detected by the proportional change of halo intensities at 9.8 Å vs. 4.6 Å. X-Ray Diffraction of archaeological keratin fibers generally shows an increase of crystallinity in comparison to modern fibers (see Fig. 2). This suggests a well-preserved structure of the α-helical intermediate filament proteins in the fiber (e. g. Lubec et al. 1987, Bertrand et al. 2003).

Polarization Microscopy

Polarization microscopy is another tool used to evaluate the degree of crystallinity of hair proteins by measuring the birefringence, which is caused by the intermediate filament proteins. By embedding hair fibers in 0.1 N NaOH the fibers swell, the helical structure is disrupted, and birefringence decreases.

According to the results of WXRD, the crystallinity of archaeological fibers seem to increase in comparison to modern hair after embedding in glycerol. After embedding the ancient fibers in NaOH, these fibers swell significantly more than native, undamaged hairs. This is attributed to oxidized disulphide bonds and peptide bond breakage. Birefringence for these fibers decreases significantly. Over the time of storage the protein stabilizing disulphide bonds are oxidized and peptide bonds are broken. This leads to a disruption of the crystalline, α-helical structure during the swelling process. In consequence the birefringence of archeological hair decreases significantly more than in undamaged hairs. In some cases (e. g. hairs from Egyptian mummies) no birefringence could be detected after treatment with NaOH (Fig. 3).

Electrophoretical Investigation

Electrophoretical fractionation of reductively extracted hair proteins according to their apparent molecular weight (SDS-Polyacrylamide Electrophoresis – SDS-PAGE) or to their charge (Isoelectric Focusing – IEF) leads to specific protein patterns. Protein bands in a SDS-PAGE pattern can be correlated to chemical-morphological components of the fiber cortex such as α-helical intermediate filament proteins, amorphous matrix as well as high-tyrosine-glycine proteins. Changes in proteins by environmental influences change their separation patterns in a typical way (Wilrich et al. 1994, Seiler et al. 1995, Mitu et al. 2003). SDS-PAGE only shows washed protein bands instead a of pronounced patterns because of the breakage of peptide bonds and the high degree of cystine oxidation to cysteic acid in archaeological hair. IEF-patterns show newly formed protein bands in the acid range (Fig. 4) due to the oxidation of cystine.

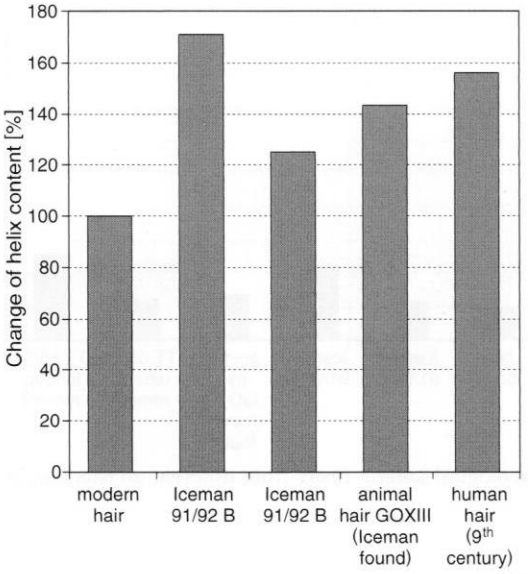

Fig. 2. Relative α-helix content of hair.

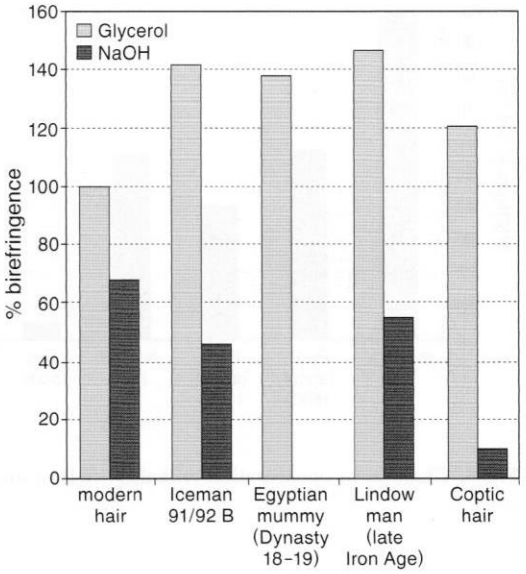

Fig. 3. Relative change in birefringence of modern and ancient hair after embedding in NaOH.

Modulated High Pressure Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) of human hair in water (HPDSC) is a well-suited and readily-applied method for the investigation of changes of hair keratin caused by environmental influences (Wortmann & Deutz 1993). Modulated DSC gives more detailed information about the thermal properties of keratin fibers. Determination of the denaturation enthalpy (ΔH, J/g) gives a measure of the amount and state of the IFs, the structural backbone of keratin fibers. The denaturation temperature (ΔT) yields information about changes in the IFAPs. In comparison to virgin hair, the denaturation enthalpy ΔH in archaeological keratin decreases, which indicates a partial breakdown of the intermediate filaments (Fig. 5). This is attributed to the oxidation of cystine to cysteic acid and peptide bond breakage in the cortex proteins, inducing a destabilization of the α-helical material.

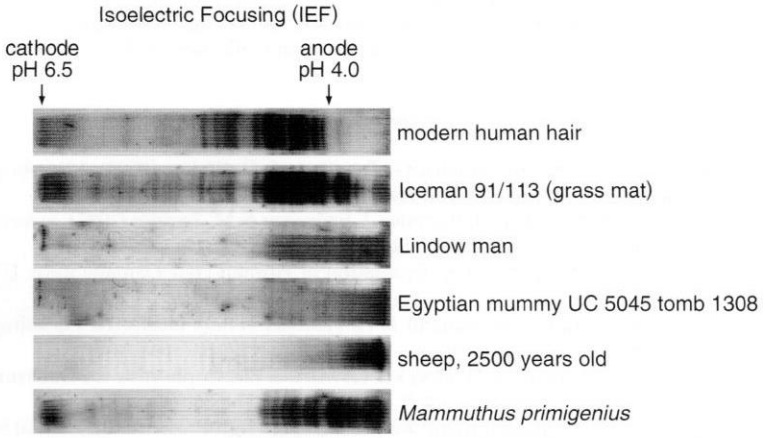

Fig. 4. Isoelectric focusing of extracted proteins of keratin fibers from different archaeological sites.

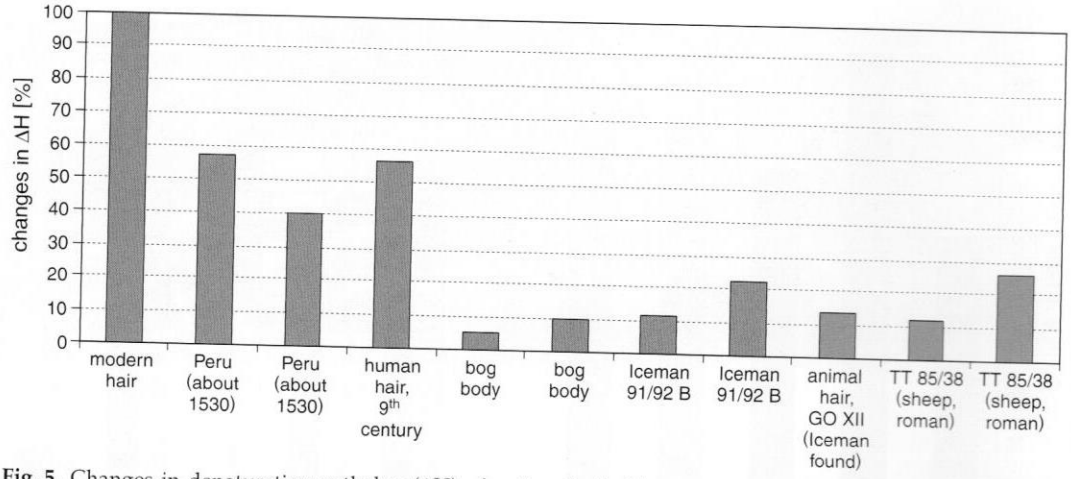

Fig. 5. Changes in denaturation enthalpy (ΔH) of archaeological keratin fibers from different archaeological sites.

Conclusions

Depending of the discovery situation and storage conditions, archaeological fibers are often in a very good external condition. Sometimes the microscopic images of these fibers can barely be distinguished from modern hair. X-ray diffraction and polarization microscopy show a high degree of crystallinity, which gives the impression of intact fiber structures. Nevertheless, time has left its mark. By protein denaturation techniques, it can be demonstrated that oxidation of cystine to cysteic acid and splitting of peptide bonds, proteins of archaeological fibers are degraded to different extents, depending on age and preservation conditions. Electrophoretical techniques show the extent of degradation of the α-helical proteins (intermediate filament proteins – IF) as well as of the amorphous matrix proteins (keratin-associated proteins – IFAPs) of the fiber cortex. MHPDSC revealed a decrease of up to 90 % of intact α-helical protein material in the fiber.

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