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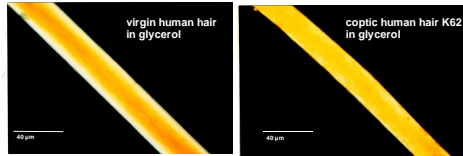
Latent Damage in Keratin Fibres

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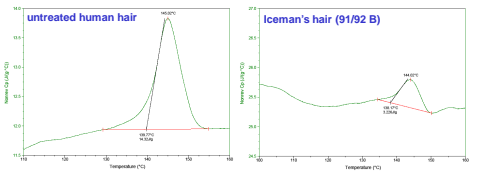
The investigation of archaeological keratin fibres by means of polarization microscopy shows a discrepancy in the results for the degree of damage when applied in non-swelling and swelling agents. On the basis of these observations, the concept of latent damage was developed. Latent damage is specified as an underlying but hidden damage in the protein framework, which cannot or only to a limited extent be assessed by non-destructive analytical tools (e.g. WAXS). This type of damage is located in the α -helical material of the intermediate filaments (IF).



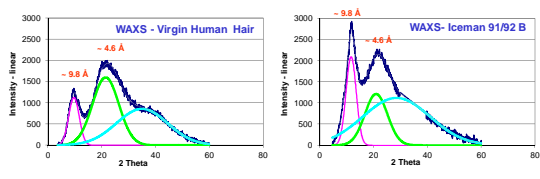
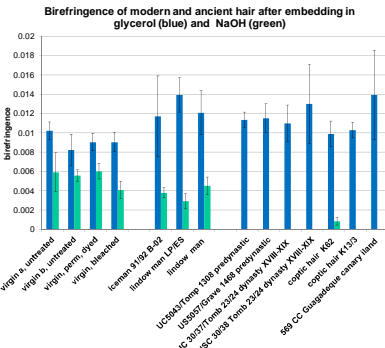
Polarising microscopical image of modern and ancient human hair

Latent damage in archaeological keratin fibres

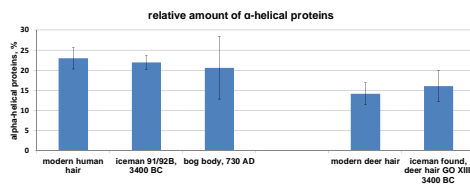
Using non-denaturing (polarising microscopy in glycerol, WAXS) and denaturing analysing tools (polarising microscopy in NaOH, DSC) show differences in the degree of structural damage of α -helical IF proteins. Protein denaturing methods as well as swelling agents can show a pronounced disturbance in the arrangement of the seemingly intact helical protein fragments for archaeological fibres.



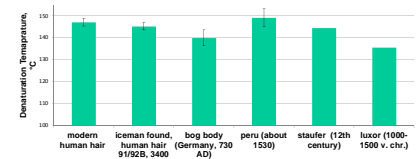
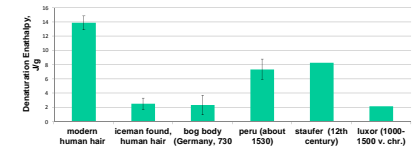
DSC of modern and ancient human hair in water



Equatorial scan of x-ray diffraction pattern



Relative α -helix content of keratin fibres in comparison to modern hair



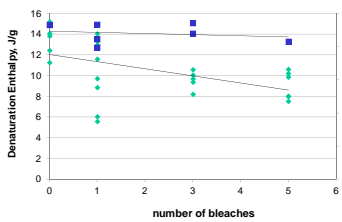
Denaturation Enthalpy ΔH_0 and Denaturation Temperature T_0 in archaeological hair

Keratin fibres show birefringence due to the semi-crystalline, α -helical intermediate filament proteins (IF). Embedding archaeological keratin fibres in glycerol (non-swelling agent) these fibres show birefringence similar to modern hairs. After embedding in 0.1 N NaOH fibres swell. In consequence birefringence decreases. Ancient fibres show a more significant swelling in NaOH than native, undamaged keratin. The increased swelling and decreased birefringence, respectively, is caused by oxidized disulphide bonds in the matrix and peptide bond breakage in the IFs. In some cases (e.g. hairs from Egyptian mummies) no birefringence can be detected after treatment with NaOH.

Wide angle x-ray diffraction provides information about the structural status of the α -helical fraction of intermediate filament proteins (IF). Wide Angle X-Ray Diffraction (WAXS) shows the distance between the centers of two α -helices (equatorial reflex, perpendicular to the fiber axis, 9.8 Å). At 4.6 Å a halo can be detected. After scanning the equatorial reflexes the relative amount of α -helical proteins can be calculated through peak deconvolution. X-ray diffraction of archaeological fibres generally shows still a high degree of crystallinity in comparison to modern hair.

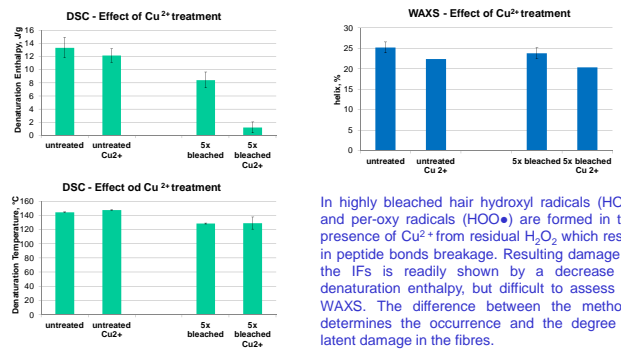
Differential Scanning Calorimetry (DSC) of human hair in water is a well suited and readily applied method for the investigation of changes of hair keratin caused by environmental influences. Denaturation enthalpy (ΔH_0) is a measure for the amount and state of Intermediate Filaments (IFs). Denaturation temperature (T_0) gives information about the changes in the amorphous IF Associated Proteins (IFAPs). In comparison to modern hair, ΔH_0 in archaeological keratin fibres decreases significantly. Denaturation temperature remains unchanged, so a decrease in ΔH_0 is assumed to be due to peptide bond breakage in IF proteins.

Latent damage in bleached human hair

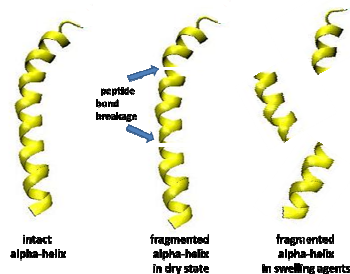


The underlying principles of latent damage were also applied to natural, recent hair for the case of multiple bleaching. WAXS only showed a moderate decrease in intact helical proteins at a very late stage of bleach repeats. By DSC for wet fibres a continuously increasing degree of damage of the IFs is observed with the number of bleaches.

Latent damage in Cu²⁺ treated human hair



In highly bleached hair hydroxyl radicals (HO•) and per-oxy radicals (HOO•) are formed in the presence of Cu²⁺ from residual H₂O₂ which result in peptide bonds breakage. Resulting damage of the IFs is readily shown by a decrease of denaturation enthalpy, but difficult to assess by WAXS. The difference between the methods determines the occurrence and the degree of latent damage in the fibres.



Principle of latent damage

Conclusion

When storing keratin fibres under environmental conditions with strong physical and chemical effects, strong bleaches or treating with Cu²⁺, both peptide bond breakage and oxidation and thus cleavage of cystine bonds occur.

If these effects are largely confined to the dry state, bond breakages would not necessarily affect the arrangement of helical proteins in the IFs. Helical protein fragments would still appear structurally undisturbed. Accordingly, clear damage to helical structures, e.g., by peptide bond breakage is thus expected to be detectable with some common analytical methods only in cases of severe damage.

In case of denaturing analytical tool or under swelling conditions helical fragments are too small to contribute to the measured α -helical value.

This damage of the hair fibre is referred to a 'latent'.

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