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Protein Oxidation of a Hair Sample Kept in Alaskan Ice for 800–1000 Years

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Ancient finds of organic matter are not only of the highest value for palaeochemists and palaeobiologists but can be used to determine basic chemical reactions, such as protein oxidation, over long time periods. We studied oxidation of human hair protein about one thousand years old of an Alaskan child buried in ice, ten hair samples of copts of comparable age buried in graves of hot dry sand and compared the results to ten recent hair samples. Protein oxidation parameters o-tyrosine and cysteic acid of the Alaskan child were comparable to recent samples whereas they were higher in the coptic specimen. N-epsilon-carboxymethyllysine, a parameter for glycoxidation, however, was as high in coptic specimen. We conclude that ice in contrast to soil prevented protein oxidation but failed to inhibit glycoxidation, a reaction initiated by autooxidation of glucose. This study therefore has implications for the interpretation of oxidation and glycoxidation as well as preservation mechanisms of proteins.

Keywords: Protein oxidation, glycoxidation, hair, Alaskan mummy, o-tyrosine, di-tyrosine, amino acids, carboxymethyllysine, cysteic acid, copts

INTRODUCTION

Ancient finds of organic matter are of the highest value to the biochemist and biologist. Not only can we draw conclusions about origins of life from ancient DNA, we can learn about environmental influences on bio (macro-) molecules over long periods of time. Furthermore, we can learn about preservation or decay of biological matter. The find of an Alaskan mummy prompted us to study the mummy's hair protein biochemistry. Hair is a most useful find as it is an incredibly stable structure. In a previous study we have shown that hair is structurally stable for thousands of years[1] and there is no enzyme known so far to cleave native hair keratin. Mitochondrial DNA in hair is well-protected in the hair shaft and this forms the basis for genetic investigations on hair, both for forensic and palaeogenetic purposes.[2] In a previous study

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we have tried to characterize changes as protein oxidation and glycoxidation in the Ice Man[3] but the findings of protein oxidation in this study were difficult to interpret as a copper axe was found at the same location and this transition metal could have been responsible for the high degree of protein oxidation. Another confounding factor was the high altitude of the deposit in the glacier and increased (cosmic) irradiation or ozone could have been responsible for the oxidative modifications. We therefore investigated a hair specimen found at sea level in the absence of apparent causes of factors for protein oxidation.

METHODS

A hair sample of the Alaskan child was obtained from principal investigators of the ongoing National Science foundation funded project "Archaeology of the North Alaska Coast: A settlement pattern study" (Gregory Reinhardt, University of Indianapolis, IN, Glenn Sheehan and Anne Jensen, Bryn Mawr College, Bryn Mawr, PA). The archaeological team excavated and conducted research on the frozen body of a young girl found at Ukkuqsi in the old whaling village of Utqiogvik, which is in the modern town of Barrow in the North Slope Borough of Alaska. The study on the body was done at the request of the Barrow Elders and with initial funding by the Commission on Inupiat History, Language and Culture and ultimate funding through a supplemental grant from the NSF Office of Polar programs. Analysis of the archaeological context and evaluation of radiocarbon dates suggest that this little girl was a member of the semi-nomadic Thule culture (c. AD 800–1000). She was buried in a meat cellar dug partly through an abandoned and even older house floor. The house gives us the first evidence for residential occupation by the Birnirk peoples at the Utqiagvik site. Artifacts on the house floor are Birnirk, which date to c. AD 400–800. This find ties in with Birnirk burials found in 1981 further up the Kugok ravine from Ukkuqsi, which at that time could not be related to any nearby occupation site.

The examination of the child's body was performed by one of the authors (M.Z.) in August 1994 at Providence Hospital in Anchorage. The body was that of a 6.025 kg female Eskimo child appearing approximately 5 to 8 years old. It was partially encased in an extremely wet birdskin parka, with the arms and knees drawn up to the chest. Full juvenile dentition showed moderate attrition.

X-ray examinations showed a number of growth arrest (Harris) lines in the distal tibiae and indicated an approximate age of 5–6 years. No fractures or other evidence of trauma was seen. Several small (0.5 cm and less) ovoid opaque bodies were noted in the lower abdominal cavity.

Examination of the body revealed the chest and abdomen to be intact and there was no gross evidence of trauma or obvious emaciation (which is difficult to evaluate in a partially desiccated body). Internal examination revealed marked osteoporosis/osteomalacia. The lungs were severely anthracotic, most probably due to due to deposition of pigment from smoke in the dwelling place, a common finding in Eskimo and other mummies. The left lung was collapsed and both lungs showed scarring and emphysema on histological examination of the rehydrated organ. A chemical test on lung fluid in the chest was positive for blood pigment. The liver showed scarring and accumulation of an abnormal pigment in the liver cells. The gastro-intestinal tract was found to be filled with animal hair, gravel, sand and pebbles (the opaque bodies seen on X-ray).

Her emphysema and liver pathology, diseases rare in children, might be a complication of a rare genetic disorder, alpha-1-antitrypsin deficiency.[4] Roughly 2 in 10.000 of the United States population suffer from this disorder. Isotope analysis on her hair, by Roy Crouse, PhD, Dpt of Physics, University of Calgary, Alberta, Canada, indicated that her diet was derived from marine food sources (R. Crouse, personal communication), similar to those of other ancient marine populations.

In conclusion, this 6 year old girl appears to have died of starvation about 800–1000 years ago. The foreign material found in the intestines indicate that normal food sources were unavailable and the Elders have told us that when they were children and hungry, their parents gave them animal skins to chew on. The terminal event was most probably pulmonary edema, secondary to the hypoproteinemia of starvation, with accumulation of bloody fluid in the chest and collapse of the left lung. A severe degree of emphysema, most probably secondary to a rare congenital disorder, alpha-1-antitrypsin deficiency, was a contributing cause to her death. This disease accounts for the multiple bouts of illness she undoubtedly suffered during her life, as evidenced by the numerous growth arrest lines seen in her long bones. Her state of chronic illness is probably related to her deliberate burial, a rare finding in ancient Eskimo populations. She was buried with a small sled, indicating that she was not ambulatory. It is clear that this chronically ill child was kept alive and treated with care in life and in death.

10 hair samples were from coptic graves, approx. 1000 years old, described and characterized in a previous publication.[1] Ten recent hair samples from a Caucasian/Austrian population, sex and age matched to the Alaskan girl were used as controls.

The hair samples were washed extensively in aequous (distilled water) and hydrophobic (acetone) phase to remove adherent debris.

Hair samples were then weighed and hydrolyzed by 6 N HCl at 105°C under nitrogen for a period of 16 hours. Samples were then evaporated to dryness and redissolved in the corresponding buffers used for HPLC systems. Samples were run in triplicate.

Determination of Protein Oxidation

Determination of o-Tyrosine (o-Tyr)

o-Tyr, the aromatic hydroxylation product generated by the hydroxyl radical from phenylalanine, was analyzed by a standard HPLC technique.[5] An HP 1050 high-performance liquid chromatograph with electrochemical detection HP 1049 A was used. The column used was Spherisorb ODS 2, 250 mm, the elution buffer was water:acetonitrile (99:1). The peak identity was verified by spiking samples with the o-tyrosine standard. The coefficient of variation was calculated as 6.7%.

Determination of Di-Tyrosine

An HPLC method was applied using o-phthalaldehyde precolumn automated derivatization. The column used was an Ultrashpere C 18 (Beckman). Solvent A was 12 mM Na-phosphate buffer pH 7.2, 2% acetonitrile, solvent B was 12 mM Na-phosphate buffer pH 7.2 plus 50% acetonitrile. Fluorescence was detected at an excitation wavelength of 230 nm and at the emission wavelength of 450 nm. The gradient formed is described in a previous paper.[3] The coefficient of variation was 5.2%.

Determination of Cysteic Acid (CA)

CA, the oxidation product of cysteine, was determined by a standard HPLC method for the detection of DABS—amino acids at picomole levels.[6]

Determination of N-Epsilon-Carboxymethyllysine (CML)

CML, a parameter for glycoxidation, was evaluated by a standard HPLC technique using a Shimadzu gradient system 6A and a Jasco fluorometer 820 FP, gain 10, excitation 340 nm, emission 450 nm with a sampler Shimadzu SCL 6B and a Shimadzu Integrator CR4AX. The column used for separation was an Astec C 18, 25 × 4.6 mm, 5 micrometer. The flow was 1 ml per min. Derivatization and the gradient are shown in a previous paper.[7] The coefficient of variance was estimated with 5.1%.

Amino Acid Analysis

A standard amino acid analysis HPLC method was used for the analysis of hair hydrolyzates to show the amino acid integrity or decomposition. The column used was a XL ODS reversed phase. 3 micrometers, 4 × 6 × 70 mm with guard column. Detection was by fluorescence at the excitation wavelength of 330 nm and the emission at 450 nm. The flow rate was 1.0 ml per min and the solvents used were eluant A (12.5 mM Na-phosphate pH 7.2 plus 2% acetonitrile plus 1% tetrahydrofurane) and solvent B (12.5 mM Na-phosphate buffer 7.2 plus 50% acetonitrile). The gradient is given in a previous publication.[3] The coefficient of variance was 7.4%.

No photodocumentation was provided as the Eskimos in Barrow requested that no picture of the body—out of respect to the deceased—should be published.

RESULTS

The ratio o-tyr/para tyrosine(p-tyr) was 0.043 ± 0.032 in recent hair samples, approx doubled in copts with a quotient o-tyr/p-tyr = 0.108 ± 0.034 and 0.038 in the Alaskan child.

Di-tyrosine was not detectable in recent hair samples, copts or in the Alaskan child.

Cysteic acid was 1.25 ± 0.23 micrograms/mg hair protein in recent hair samples, 2.16 ± 0.29 micrograms/mg hair protein in the coptic hair samples and 1.19 in the Alaskan child.

Glycoxidation as expressed by CML per lysine was 0.029 ± 0.013 in recent hair samples, 0.12 ± 0.043 in the coptic samples and 0.12 in the Alaskan child.

Amino acid analysis revealed that the most abundant hair amino acid, glutamic acid, indicated the absence of degradation as recent samples presented with 26.3 ± 3.9 micrograms per mg hair protein and the Alaskan child with 26.1 micrograms per mg hair protein. The lysine (p-tyrosine) content in recent hair was 8.1 ± 0.7 (6.8 ± 0.6) micrograms per mg hair protein, in coptic samples 7.9 ± 0.8 (6.9 ± 0.5) micrograms per mg hair protein and 8.2 (7.0) micrograms per mg hair protein in the Alaskan child. The ratios above were calculated from these data.

DISCUSSION

As indicated in the results we found no significant protein oxidation in terms of o-tyrosine, di-tyrosine or cysteic acid in hair protein of the Alaskan child. Ambient oxygen, ozone or irradiation did not obviously oxidize protein. Although we have no ambient temperature control we speculate that low temperature could be the underlying cause of unchanged protein oxidation. Another possibility, that hair was freeze dried and therefore no free water was available for the oxidizing reactions, is highly unlikely, as the coptic hair samples of about 1100 years of age and heat-dried in the hot dry sand of the graves in Egypt showed a high degree of protein oxidation. The anaerobic conditions in the latter case should not have favoured the oxidation process on the coptic hair, in contrast to the child's hair in ice, where oxygen, ozone and ionizing irradiation can easily diffuse or penetrate. Ionizing irradiation may not play an important role as the child's hair exposed to ionizing radiation showed lower protein oxidation than those protected against irradiation. The acid pH of the soil in which coptic hair was buried may have been a factor to be considered. The fact that low temperature inhibits protein oxidation (i.e. aromatic hydroxylation) by irradiation has been reported.[8] We cannot evaluate, however, whether oxidoreductases from microbes have been playing a role in protein oxidation or reduction processes.

An important protection factor against protein oxidation is the structure of the hair itself. It resembles the protection of molluscs and other fossils which are protected by shells or calcification. Hair structure is like reinforced concrete built from helical and nonhelical conformations, a dense scaffold

between water insoluble, non-biodegradable keratin[9] and the polyanionic acid glycosaminoglycans.[10] Insolubility may contribute to chemo protection as well as shielding against irradiation by the free radical scavenging carbohydrate moiety of the glycosaminoglycan part.

Sulfhydryls, under normal circumstances highly susceptible to oxidation to disulfide, cysteic acid, cysteine sulfinic acid, cysteine sulfone or sulfate were not significantly oxidized. The reason may well be that all cysteine (a major amino acid in hair protein responsible for cross linking, ref 10) of the hair protein is used for cross linking and helix formation by the formation of disulfide. Cysteine was not determined as it could have been easily oxidized and even in equilibrium with cystine, which makes the evaluation of cysteine highly unreliable. This is also the reason why modern literature rather reports the determination of e.g. homocyst(e)ine instead of homocystine and homocysteine.[11] We therefore decided to determine the stable oxidation product cysteic acid.

Another factor that may contribute to the understanding of our observation of unchanged sulfoxidation is that a pure thiol (homocysteine) solution seems to form a redox system: our own in vitro results administering different high doses of ionizing irradiation on a thiol only produced the disulfide but not higher oxidation products as shown by mass spectrometric investigations (unpublished). The disulfide therefore must be manyfold more resistant as the sulfhydryl residue and this seems to be the case in hair protein.

The exposure of the child's hair to smoke (indicated by the finding of smoke pigments in the child's chest) did not induce protein oxidation of the child's hair. Smoke could have been leading to the generation of the hydroxyl radical attack directly or by generation of active oxygen species from pyrolysis products.[12]

CML representing glycoxidation, however, was as high as found in coptic hair samples. Ahmed and coworkers and others observed that glycated proteins or amino acids, i.e. Amadori products, were oxidatively cleaved producing carboxymethyl amino acids as CML.[13,14] Also autooxidation of the carbohydrate moiety of hair glycoproteins may have led to glycoxidation according to the principle reactions described by Wolff.[15] This autooxidation and formation of nonenzymatic glycation (Maillard reaction) can be easily found in nutrients stored within months, even in the lyophilized state, when diffusion-controlled reactions are significantly reduced.[16]

The availability of trace metals/transition metal in soil for metal catalyzed generation[17] of active oxygen species along with higher temperatures could have been the most important factor explaining the high protein oxidation in coptic hair samples. Corrosive processes by metallic catalysts and acidity in the soil may have helped to attack coptic hair protein enabling oxidative processes secondarily, although no differences in the hair compositions between the Alaskan child and copts was found on amino acid analysis. We conclude, that no significant protein oxidation (as compared to recent hair specimens) was found in hair protein of an Alaskan child but significant glycoxidation occurred.

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