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Proteomic evaluation of the biodegradation of wool fabrics in experimental burials

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ABSTRACT

Woollen textiles recovered from archaeological excavations are usually fragile, discoloured, mineralised, or highly biodeteriorated fragmentary remains. The nature and extent of preservation is highly dependent on the site of burial and factors such as soil composition, pH, temperature, oxygen content, and contact with a wood coffin or metals. Understanding the particular biodegradation in archaeological sites is important for biomolecular studies of textiles, and to assist in the conservation of these finds. Wool fabrics dyed and buried for up to 8 yr in bog-type soils in Denmark (Lejre) and Norway (Rørmyra), and in marine sediments in Sweden (Marstrand) were evaluated by proteomics analysis. Wool degradation was found to occur through a range of differing mechanisms, mainly due to the complex nature of wool itself with its many families of proteins (keratin and keratin-associated proteins) and structures. Microbial activity was a large contributory factor to the physical deterioration of the wool fabrics at Lejre and Marstrand, and might result in faster loss of keratin-associated proteins over keratins. Additional hydrolysis took place at Marstrand, influenced by the environmental conditions of the sediment, and in particular the alkaline pH, contributing to the degradation of keratins. However, cross-linking was associated with the long-term preservation of the fabrics at Rørmyra, where pH, temperature, and vegetative composition of the bog prevented microbial activity, and sphagnum moss might preserve wool by binding with keratins.

1. Introduction

Archaeological hair and wool are becoming of increasing interest in biomolecular studies. In recent years, DNA has been extracted from mammoth (Gilbert et al., 2007) and human hair (Rasmussen et al., 2010) that was preserved in permafrost for thousands of years. Isotopic analysis of human hair has been used to reveal the diet of ancient populations (Macko et al., 1999; Sharp et al., 2003; Wilson et al., 2007b). Similar studies on wool have recently been published to identify the source of textiles, through DNA analysis (Brandt et al., 2011), protein characterisation (Solazzo et al., 2011; Hollemeyer et al., 2008, 2012), and isotopic analysis of strontium and light isotopes (Hedges et al., 2005; Frei et al., 2009a,b, von Holstein, 2011). The protein composition of wool and silk has also been investigated to date textiles by means of amino acid racemisation and deamidation evaluation (Araki and Moini, 2011; Moini et al., 2011). These recent innovations are opening up exciting new areas within the field of ancient textile research. However, little is known with respect to fibre degradation at the molecular level that could potentially influence the feasibility and interpretation of such biomolecular studies, in particular for textiles excavated from archaeological sites.

Deterioration of archaeological textiles is dependent on burial conditions (soil composition, pH, temperature, oxygen content, contact with wood coffins, metals, etc.), and on how these environmental factors regulate microbial activity. In wool, a high cysteine content confers high resistance to chemical degradation by forming extensive cross-linking (disulphide bridges). Keratins are therefore primarily degraded in soil via specialised keratinolytic

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microorganisms, as they produce enzymes capable of degrading keratins. At least 300 reported fungi use keratins as a source of nutrients (C, N, and S) (Błyskal, 2009). Fibre degradation is initiated by denaturation of the constituent proteins and breaking of the disulphide bridges, necessary steps for proteolytic enzymes to access and attack keratin. Surface erosion and radial penetration are the two known mechanical modes for microorganisms to attack wool fibres (Filipello Marchisio, 2000; Korniłłowicz-Kowalska and Bohacz, 2011). The former results in progressive degradation from the cuticle to the cortex, while in the latter holes in the fibre are caused by perforating organs or hyphae. Wilson et al. (2007a) assessed microorganism activity on human hair buried in soil and observed fungi deterioration in the form of tunnelling. Cases of both surface erosion and radial penetration were observed, showing either fibrillation (loss of cuticle and separation of cuticle cells), or collapse of the fibre by loss of the cortex (degradation of the cortex with intact cuticle) (Wilson et al., 2010). Studies on human hair have also demonstrated that the structures that make up the cuticle and cortex are gradually degraded by fungi. In both cuticle and cortex, the cell membranes and cytoplasmic residues are attacked first. This is followed by invasion of the endocuticle, and of the intermacrofibrillar matrix and microfibrils in the cortex. The exocuticle, a-layer, and intermicrofibrillar matrix are the most resistant to enzymatic digestion (Filipello Marchisio, 2000).

The physical degradation of wool in different burial contexts has been reported from actual archaeological finds and experimentally buried fabrics and include loss of cuticular scale, fibrillation, pitting, discolouration, and staining (Peacock, 1996a,b). Wool, however, survives well in cold wet and hot arid environments, waterlogged soils, peat bogs and salt-saturated soils, when bacterial and fungal activity is reduced. We analysed wool fabrics from experimental burials to evaluate chemical deterioration and biodegradation in an archaeological context. The samples were derived from two separate experiments conducted between 1998 and 2009 (Table 1). The first experiment took place from 1998 to 2006, during which time wool fabrics were buried at Lejre in Denmark in a lowland bog, and at Rørmyra in Norway in a raised bog (Peacock, 2004; Turner-Walker and Peacock, 2008). The second, ongoing experiment began in 2002 and similar samples were buried in marine sediments in the harbour at Marstrand in Sweden for retrieval after periods of up to 48 yr. These samples are part of a project investigating reburial as a method for preserving excavated archaeological materials (Bergstrand et al., 2002; Bergstrand and Godfrey, 2007; Godfrey et al., 2009). In this experiment, one series of samples was exposed to the sediments, while a second series was enclosed in geotextile fabric. The fabrics were left undyed or dyed using madder, weld, and indigo. Samples included in this study were buried for up to 7 yr at Marstrand and 8 yr at Lejre and Rørmyra.

The physical deterioration of the samples was compared to the proteins identified after analysis by nanoLC-ESI-MS/MS. For each dye and burial site, we assessed the type and speed of protein degradation, compared to the controls kept in storage. The results indicating the occurrence of both hydrolysis and protein–protein cross-linking were highly variable with respect to dyeing treatment and site of burial.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Samples included in the study were of a burial-degraded modern textile fabric. The fabric was a highly fulled twill (vadmel) woven in modern natural pigmented white wool fibre by Røros Tweed a/s (Røros, Norway) in 1997. Pieces of the fabric were dyed red, yellow, and blue, respectively, at the Textile Workshop at "Land of Legends Lejre" (formerly Historical-Archaeological Research Centre Lejre, Denmark). A piece of

Table 1
Burial sites characteristics.

	Control (1998)	Lejre (1998–2006)	Marstrand (2002–2009)	Rørmyra (1998–2006)
Location	NTNU Museum, Trondheim, Norway	Land of Legends Lejre (formerly Lejre Historical Archaeological Research Centre), Denmark	Marstrand Harbour, Sweden	Rørmyra Nature Reserve, Bymarka, Sør-Trondelag County, Norway
Coordinates	NA	55° 36′ 30″ N; 11° 56′ 20″ E	57° 54′ 13″ N; 11° 32′ 43″ E	63° 21′ 30″ N; 10° 17′ 50″ E
Elevation	NA	58 m	0 m	175 m
Environment	Museum	Fenland bog, humid oceanic climate	Harbour	Raised bog, subarctic climate
Topography	NA	Low rolling hills	Sandy	Below wooded upland
Geology	NA	Clay	Marine sediments	Glacial till
Soil water pH	NA	5.6	7.2–7.5	5.0
Dissolved O_2	NA	0.5%	0%	1.6%
Annual air temperature (average)	18 °C, 50% RH	9.2 °C	7.4 °C	3.3 °C
Annual temperature 1 m (average)	NA	8.6 °C	NA	4.2 °C
Potential redox	NA	NA	−150 to −200 mV strongly reducing (sulphate reducing)	NA
Nature of the sediments	NA	"Sediments at Lejre were much darker and more silty, with less obvious organic matter and a strong smell of sulphur" "Closer to both urban areas and farmland" "the sub-soil to the bog is a silty clay and the lower levels of the peat contain considerable silt"	Fine sand. Analysis showed a near neutral pH, a reducing environment below 50 cm and low water content. Organic matter content 5–7%	"Almost pure sphagnum peat, with visibly well-preserved vegetable matter including small twigs and leaves" "Spectacular preserving qualities of sphagnum moss" due to sphagnan "Inhibits microbial action by deactivating proteolytic enzymes and binding free amino groups, thus denying micro-organisms access to nutrition"