Journal of Archaeological Science 49 (2014) 524–535

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Species identification by peptide mass fingerprinting (PMF) in fibre products preserved by association with copper-alloy artefacts

Caroline Solazzo [a, b, *], Penelope Walton Rogers [c], Leslie Weber [b], Harriet F. Beaubien [b], Julie Wilson [d, e], Matthew Collins [a]

[a] BioArCh, Department of Archaeology, Wentworth Way, University of York, York YO10 5DD, UK
[b] Smithsonian's Museum Conservation Institute, 4210 Silver Hill Road, Suitland, MD 20746, USA
[c] Anglo-Saxon Laboratory, Bootham House, 61 Bootham, York YO30 7BT, UK
[d] Department of Mathematics, University of York, York YO10 5YW, UK
[e] Department of Chemistry, University of York, York YO10 5YW, UK

ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form
12 June 2014
Accepted 18 June 2014
Available online 27 June 2014

Keywords:
Textiles
Wool
Pelts
Mineral-preserved
Mineralisation
Copper alloys
Pseudomorph
Peptide mass
fingerprint (PMF)
Deamidation

ABSTRACT

Fibre products, such as textiles and animal pelts, are often recovered in the corrosion crust of archaeological metal artefacts. Because clothed burials are an important resource for the study of past societies, accurate fibre identification is important. However, extreme mineralisation of animal fibres can render microscopic visualisation difficult for species identification. Peptide mass fingerprinting (PMF) has been successfully used to identify the species origin in both collagen and keratin-made archaeological artefacts. The approach requires little material but the state of degradation (protein hydrolysis) is a limiting factor as it might impact on the identification of key markers. In this study we analysed pelt and textile fragments found in association with copper-alloy objects with different degrees of mineralisation; samples were obtained from a Viking-Age (10th c.) grave in Britain and from a burial in Mongolia (3rd c. BC to 2nd c. AD). Species identification was possible in all but one sample, revealing PMF can be applied to corrosion products, thereby further expanding the value of these objects for textile research.

1. Introduction

Textiles, animal pelts and other fibre products are frequently recovered, well preserved and in large quantities, in waterlogged soils, permafrost or arid conditions. Their preservation in graves in temperate climates, however, is mostly poor. Graves are hardly ever dug into waterlogged ground and most organic materials will decay rapidly once they have been buried in free-draining soils and subjected to fluctuating temperatures and rainfall (Jakes and Sibley, 1983; Sibley and Jakes, 1984). On the other hand, where the arrangement of metal garment accessories and beads in the grave indicates that the body must have been once clothed, or where the cremated remains associated with metalwork have been wrapped, patches of poorly preserved textile are often found in the corrosion crust on the metal (Janaway and Scott, 1989; Walton Rogers, 2007, 49–110). Since clothed bodies in inhumations provide highly significant evidence for the social organisation of past societies, the accurate identification of these small remains is of considerable importance to archaeologists.

1.1. The mineral preservation of fibres

The complex mechanisms by which fibre products are preserved in association with metalwork in inhumations have been studied by several authors, some of whom have worked at the theoretical and experimental level (Cooke, 1990; Gillard et al., 1994; Jakes and Sibley, 1984; 1983; Janaway, 1983a,, 1989; 1985) and some of whom have collected data from excavations (Janaway, 1983b; Walton Rogers, 2007, 50–60). Significant factors have proved to be the soil pH, the amount of air and moisture in the soil, the

* Corresponding author. Present address: Smithsonian's Museum Conservation Institute, 4210 Silver Hill Road, Suitland, MD 20746, USA. Tel.: +1 301 238 1284.
E-mail address: solazzo.c@gmail.com (C. Solazzo).

http://dx.doi.org/10.1016/j.jas.2014.06.009
0305-4403/

composition of the associated metalwork, and the nature of the raw textile material (proteinaceous animal fibre or cellulosic plant fibre).

Wool and other animal fibres can suffer physical degradation, especially through alkaline hydrolysis (Sibley and Jakes, 1984, 21–3, Timár-Balázsy, 1989), although even where soil conditions are basic, the mildly acidic environment produced by the decaying human body in the critical early stages of burial may have a protective influence (Walton Rogers, 2007, 59). They will also be susceptible to attack from aerobic bacteria, unlike plant fibres, which are more at risk from oxidation and fungal damage in the same conditions (Cooke, 1990, 9, Jakes and Sibley, 1983, 36–7, Sibley and Jakes, 1984, 25). Finally, they will be affected by metal salts from the corroding metalwork, which can, on the one hand, inhibit microbiological attack (where the metal is an alloy of copper), and, on the other, replace the organic material with minerals (Janaway, 1989). The mineralisation process can result in fibres which are wholly or partially replaced by the mineral, or a hollow cast of the fibre can form as the fibre decays. These are sometimes termed 'positive' and 'negative' casts respectively, and the fully mineralised fibres are called 'pseudomorphs' (Gillard et al., 1994, 133). Which of the two develops depends on the relative rates of fibre decay and corrosion deposition, although casts tend to be more common in association with iron (Janaway, 1989, 21, 24). In practice, mineralised textiles can retain intact fibres, identifiable by microscopy, in the core of the yarns (Walton Rogers personal observation). Organic material can also prove to be detectable by analysis in positive casts and Gillard et al. (Gillard et al., 1994) have argued that full mineralisation is probably relatively rare.

1.2. Analytical strategies

A variety of analytical techniques have been developed to determine the origins of fibre degradation products. X-ray diffraction (XRD) and Fourier transform infrared (FTIR) microspectroscopy have been used to identify the broad fibre type (Chen et al., 1996a, Chen, et al., 1996b; Gillard and Hardman, 1996; Jakes and Howard, 1986). Anheuser and Roumeliotou (2003) applied chemical staining to highly mineralised fibres from archaeological iron- and copper-alloy objects and determined that proteinaceous fibres (wool and silk) tested positive to naphtol blue black. In well-preserved specimens, the preferred method for animal fibres is transmitted-light microscopy, using cross-sections and whole mounts. This allows external diagnostic features such as the cuticular scale pattern, internal features and pigmentation to be viewed simultaneously (Appleyard, 1978; Textile Institute, 1975; Wildman, 1954). Scanning electron microscopy (SEM) is conventionally used where transmitted light will not penetrate the pseudomorph (Janaway, 1983a; Walton Rogers, 2007, 60–1). It is most useful in this context because it can produce clear images of the cuticular scale pattern, but where the fibre has suffered physical deterioration prior to mineralisation even SEM produces few diagnostic images. The most diagnostic technique to determine the source of proteinaceous fibres is by obtaining their amino acids composition: wool (keratin) is rich in cysteine, while silk (fibroin and sericin) is predominantly composed of serine, glycine and alanine. Amino acid analysis was successfully used in identifying silk and wool in pseudomorph-type textiles from Mongolian tombs (Vanden Berghe and Wouters, 2005). The technique, however, would not allow species identification as differences due to the degradation of proteins would be more significant than differences between species.

In this study, we approach the feasibility of identifying intact protein markers in mineralised archaeological samples using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF-MS has recently been successfully used to identify the animal fibre source of the Ice Man's clothing (Hollemeyer et al., 2012, Hollemeyer et al., 2008), and of the Coast Salish blankets (Solazzo et al., 2011). The approach relies on the identification of specific peptides obtained from enzymatic digestion of the keratin proteins that make up the intermediate filaments, the core structure of animal fibres. We have recently identified and sequenced some key markers for animal fibre identification in mammalian keratinaceous materials; these markers allow identification up to the genus level and are summarised in Table 1 (for full list, see (Solazzo et al., 2013a)).

1.3. Sampling of fibres preserved with copper-alloy artefacts

Two series of fibre products found in conjunction with copper-alloy artefacts were analysed, the first from a grave in Cumbria, UK, and the second from a burial in the Gol Mod 2 cemetery in Mongolia.

1.3.1. Cumwhitton animal pelts

The remains of two animal pelts were found in association with metalwork in Grave 27 at Cumwhitton Viking-Age cemetery, Cumbria (Walton Rogers forthcoming). One was sandwiched between the plates of a buckle and the other lay between the back of a strap end and a thick layer of wood, probably the base of the coffin (Fig. 1). These objects were close together in the burial, in the region of what must have been the right hip or waist. Despite their proximity, from visual inspection, it was obvious that the two pelts were different. It was concluded that the buckle originally fastened a skin garment, while the strap end had been in contact with a pelt on the floor of the grave. The fibres of the buckle-fastened garment had mineral salts deposited on the surface but were still transparent and could be examined by transmitted-light microscopy. The fibres of the pelt with the strap end were much more degraded, had lost most of their cuticular scale pattern and were partially mineralised.

The fibres in the buckle plates were short and straight, and dominated by coarse fibres, 75–200 microns diameter, interspersed with finer fibres of around 25 microns (Fig. 2A–B). The cross-sections were flat or elliptical. Medullas were rare and narrow, and there was medium and light pigmentation, arranged in large globules which were close to the core in the lighter fibres. In the limited areas where the scales were visible, the pattern was irregular waved mosaic with smooth close margins with some areas that were petal-like (Fig. 2B–D). These features indicated the coat of seal (Appleyard, 1978). The second pelt, beneath the strap end, had longer fibres (full length difficult to determine), 20–80 microns in diameter; cross-sections were oval, medullas were rare, narrow and sometimes fragmented, and pigmentation was absent. The scale pattern was poorly preserved in the sample examined, and, although sheepskin seemed a likely identification, it was far from certain.

Since sealskin had not been identified in a Viking-Age burial from Britain before this time and confirmation would be desirable, and since the second pelt had not been identified with confidence, both samples were selected for proteomics analysis.

1.3.2. Gol Mod 2 cemetery textiles

Textile remains were found associated with copper-alloy artefacts found in burial#21 in the Tomb 1 complex of the Gol Mod 2 cemetery. At an altitude of 1600 m in central–north Mongolia (Allard et al., 2002, Miller et al., 2006, Miller et al., 2008), the necropolis yielded over 400 burials (Brosseder, 2009) associated with the Xiongnu (3rd c. BC and 2nd c. AD). The disk shaped object and the small hemisphere (leaded bronze with 5 and 8% Sn and 10 and

Table 1
Main markers for identification observed by PMFs (see (Solazzo et al., 2013a) for details).

m/z[a]	Peptide sequence	*Ovis*			*Capra*						*Bos*						
		Urial	Mouflon	Domestic sheep	Ibex	Markhor	Domestic goat	Chamois	Tahr	Muskox	Cow	Yak	Deer	Reindeer	Camel	Horse	Harbour seal
952.50	LQFFQNR	+	+	+	+	+	+		+				+				
968.49	LQFYQNR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1041.49	WQFYQNR															+	+
1169.55	WQFYQNQR	+	+	+	+	+	+	+	+	+	+	+			+	+	+
1051.48	DVEEWFAR[3]														+		
1109.53	DVEEWYIR	+	+	+	+	+	+	+	+	+	+	+		+			+
1896.86	DVEEWFTTQTEELNR															+	
1543.73	SFNFCLPNLSFR + acetyl N term	+	+	+	+	+	+	+	+	+							
1545.68	SYSCCLPNLSFR + acetyl N term										+	+					
1545.68	SYSCFLPNLSCR + acetyl N term															+	
1559.73	SYNFCLPNLSFR + acetyl N term	+	+	+	+	+	+	+	+	+	+	+	+	+			
1834.98	TVNALEVELQAQHNLR	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
1848.99	TVNALEIELQAQHNLR															+	+
2063.03	SDLEANSEALIQEIDFLR/SDLEANVEALIQETDFLR		+	+			+	+	+	+	+	?		+			
2075.06	SDLEANVEALIQEIDFLR										+	+					+
2164.11	Sequence not known																+
2191.12	KSDLEANSEALIQEIDFLR/KSDLEANVEALIQETDFLR		+	+				+		+	+	?		+			
2203.16	KSDLEANVEALIQEIDFLR										+	+					+
2563.34	YSSQLSQVQGLITNVESQLAEIR															+	
2576.36	Sequence not known																+
2576.33	YSCQLAQVQGLIGNVESQLAEIR										+	+	+	+			
2583.35	YGSQLSQVQGLITNVEHQLAEIR[3]														+		
2637.37	YSCQLSQVQSLIVSVESQLAEIR	+	+	+	+		+		+	+							
2662.30	Sequence not known																+
2664.38	YSCQLSQVQSLIVNVESQLAEIR/YSCQLNQVQSLIVSVESQLAEIR	+	+	+	+				+	+							
2679.35	YSCQLNQVQSLISNVESQLAEIR	+	+	+	+	+	+	+	+								+
2691.39	YSCQLNQVQSLIVNVESQLAEIR				+	+	+	+	+	+							

[a] *m/z* values calculated as $[M + H]^+$ with carbamydomethylation modification on cysteine (+57 da), or observed *m/z* for unknown peaks.

Fig. 1. Copper-alloy objects with associated animal pelts from a Viking-Age grave at Cumwhitton, Cumbria, pre-conservation. Above, the buckle fragment with the pelt between the plates marked by a black arrow (see also Fig. 2A); below, the back view of the strap end, showing remains of a different animal pelt adhering to the surface. Scale in mm. © The Anglo-Saxon Laboratory.

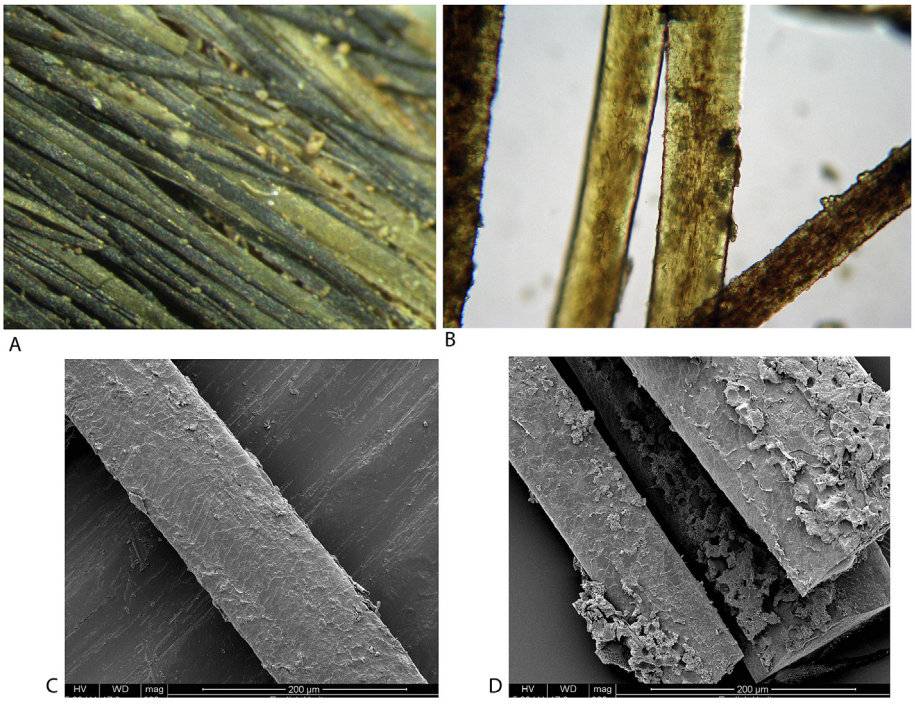

Fig. 2. Micrographs of the seal fibres taken from between the plates of the buckle from Cumwhitton. A: close-up of the fibres; B: fibres viewed by transmitted light; C: scanning-electron micrograph of coarse fibre, mid-shaft; D: scanning-electron micrograph of coarse fibre, proximal end. Images A–B © The Anglo-Saxon Laboratory; images C–D Crown copyright.

14% Pb respectively), possibly originally linked by a leather cordage, had each been wrapped in a wool fabric; on the hemisphere, the border of the textile is folded around the hemisphere's lower rim except for a small exposed section and tucked into the interior of the hollow dome, while on the disk, fragments remained on both sides wrapped around a portion of the edge (Fig. 3). The layering of the fabric around the objects and their positions in the grave seems to have influenced the extent of degradation of the fibres with at least four types of samples, ranging from physically robust to pseudomorph-type fibres. This range provided a unique opportunity to assess the protein content with different degrees of mineralisation.

Five samples were taken from the woven fabrics (Table 2) to test fibres at varying states of degradation, designated as types 1 and 2. On the disk, the two types of fabrics were evident on the B face of the disk (that was turned to the bottom in the grave). The type 1 textile (sample DB1-rb) was robust and soft and had a red–brown colour. The black type 2 textile overlapped the first fabric and was fragile and soiled. Sample DB2-bl was taken from some of the black textile and sample DB2-ps was from highly corroded material and had the appearance of a pseudomorph. Scanning electron microscopy of the DB2 fibres show damaged fibres with occasional transversal cracking (Fig. 4a). Distinct holes through the outer surface can be observed in some cases (see graphical abstract), revealing an insight into the interior of the fibre that might indicate microbial attack. The scales could not be made out and the average diameter of the fibres was 14 microns (Fig. 4b).

On the hemisphere the different areas are clearly delimited: a red–brown coloured area (again the most robust fibres), a fragile buff area (unstained) that is clearly degraded in some parts, and an area of very fragile, dark brown fibres similar to the pseudomorph-type fibres of the disk. Two samples were taken from the hemisphere, one from the buff area (HO1-bu) and one from the well-preserved red–brown area (HO1-rb). Evidence for slow corrosion is characterised by the absence of mineralisation in some parts of the textile, such as the buff area. The presence of an iron bridle in the vicinity of the hemisphere might also have played a role in the mineralisation of the textiles associated with the hemisphere, although it is not clear how the objects were positioned in relation to each other. A few fibres were observed with a degraded core (Fig. 4c) which would be consistent with iron mineralisation. But most fibres had a rough, eroded and soiled surface with no definite scales, similar to the fibres from the disk (Fig. 4d).

2. Materials and methods

2.1. Chemicals

Urea, tris(2-carboxyethyl)phosphine (TCEP), ammonium bicarbonate (AB), iodoacetamide (IAM), trifluoroacetic acid (TFA), trypsin and α-cyano-4-hydroxycinnamic acid (CHCA) were provided by Sigma-AldrichTM (USA), and acetonitrile (ACN) by Fisher Scientific (UK).

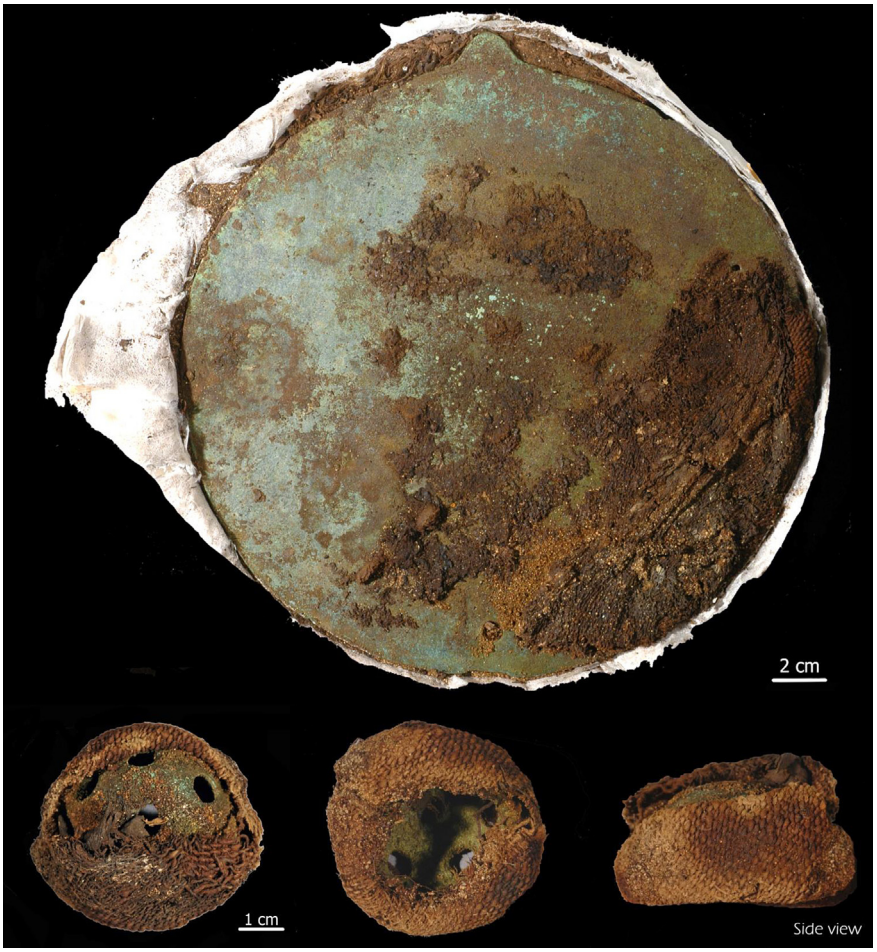

Fig. 3. Disk (side B) and hemisphere (top, bottom and side views) with wrapping textiles from the Gol Mod 2 burial#21, Tomb 1 complex. © Smithsonian's Museum Conservation Institute.

Table 2
Samples characteristics.

Name	Description	Origin	Period	Burial type	Mass (mg)[a]
DB1-rb	Disk red–brown fabric	Burial#21, Gol Mod 2 cemetery, Mongolia	3rd c. BC–2nd c. AD	Sandy soil, loose	1.24
DB2-bl	Disk black fibres	Burial#21, Gol Mod 2 cemetery, Mongolia	3rd c. BC–2nd c. AD	Sandy soil, loose	2.40
DB2-ps	Disk pseudomorph	Burial#21, Gol Mod 2 cemetery, Mongolia	3rd c. BC–2nd c. AD	Sandy soil, loose	3.90
HO1-bu	Hemisphere buff fabric	Burial#21, Gol Mod 2 cemetery, Mongolia	3rd c. BC–2nd c. AD	Sandy soil, loose	0.42
HO1-rb	Hemisphere red–brown fabric	Burial#21, Gol Mod 2 cemetery, Mongolia	3rd c. BC–2nd c. AD	Sandy soil, loose	0.25
G27-793	Fragments attached to a buckle	Cumwhitton, Cumbria	mid-10th century	Acidic soil	2.65
G27-795	Fragments found in grave	Cumwhitton, Cumbria	mid-10th century	Acidic soil	2.45
Seal hair	Modern hair	unknown	2009	NA	2.21
Merino wool	Modern wool	Wingham Wool Work, Rotherham, UK	2009	NA	5.00

[a] Mass of sample analysed/200 μl of analyte buffer.

2.2. Sample preparation

Archaeological samples were washed in water, and gently broken down in solution by agitation, then let to dry overnight. The samples were solubilised in 200 μl of a solution of 8 M urea, 100 mM AB and 50 mM TCEP at pH 9.0 for three hours by shaking. The supernatant was alkylated for 45 min in the dark with 22 μl of 400 mM IAM for a final concentration of 40 mM IAM. The samples were then diluted with 50 mM AB, pH = 8.4 to 1 mL, and digestion was carried at 37 °C overnight with 1 μg of trypsin. Samples were then dried down and re-solubilised in 50 μL of 0.1% TFA. The samples were purified using 10 μL Millipore® ZipTips containing reversed-phase C18 resin. The pipette tips were washed with 100% ACN and equilibrated with 0.1% TFA. After loading the samples, the tips were washed with 0.1% TFA and the samples eluted with 0.1% TFA/50% ACN.

2.3. Peptide mass fingerprinting (PMF) by MALDI-TOF-MS

A matrix solution was prepared by diluting 10 mg of CHCA in 1 mL of 50% ACN/50% of 0.1% TFA, vortexing and diluting twice with the same solution. 1 μL was applied onto a MALDI target and followed by 1 μL aliquot of analytical solution. The plate was loaded in a Ultraflex™ III mass spectrometer (Bruker Daltonics GmbH, Germany), and analyses were carried out in positive reflector mode using a Nd: YAG laser operating at 337 nm. Spectra were acquired using flexControl 3.0 (Bruker Daltonics GmbH, Germany) on a mass range of 800–4000 Da with an accumulation of 500 shots on the standards and 1000 shots on the samples. The calibration standard (Bruker Daltonics) was prepared according to the manufacturer's instructions for instrument calibration and consisted of angiotensin I, ACTH clip(1–17), ACTH clip(18–39) and ACTH clip(7–38) peptides.

2.4. Protein analysis by maXis

Samples were loaded onto an Ultimate 3000 nanoLC system (Dionex) equipped with a PepMap100 5 μm C_{18} trap (300 μm × 5 mm, Dionex) and an Onyx monolithic C_{18} capillary column (100 μm × 300 mm, Phenomenex). The trap wash solvent was 0.05% (v/v) aqueous TFA. The separation used a gradient elution of two solvents (solvent A: 2% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid; solvent B: acetonitrile containing 0.1% (v/v) formic acid). Flow rates were 30 μL/min and 1.2 μL/min for the trap column and capillary column, respectively. Column temperature was 60 °C and the following gradient profile was as

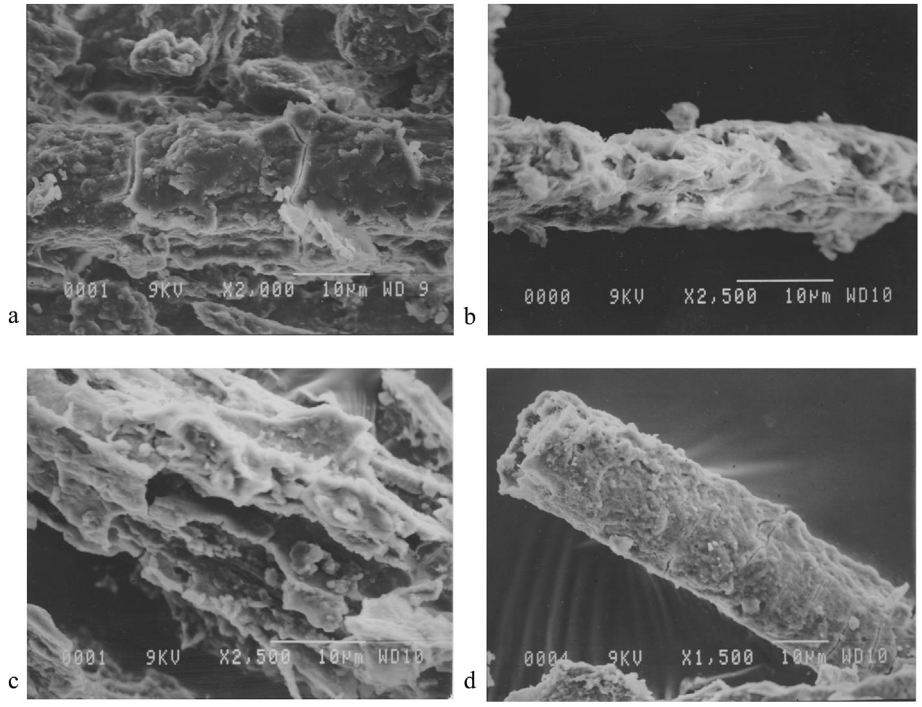

Fig. 4. (a) DB2 fibre with crack pattern; (b) DB2 fibre with surface erosion; (c) HO fibre with void visible inside the fibre and (d) HO fibre with no visible scales. © Smithsonian's Museum Conservation Institute.

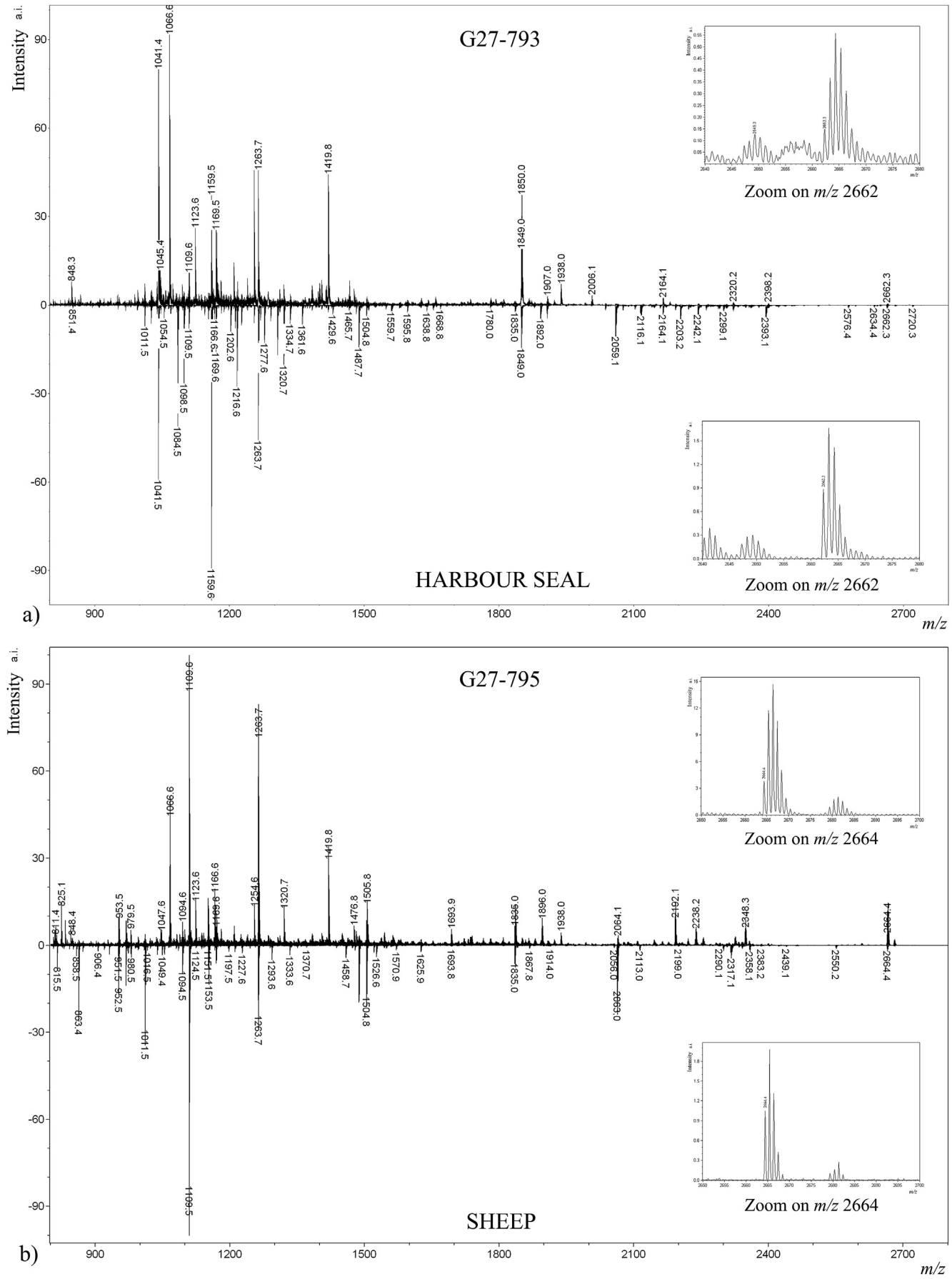

Fig. 5. (a) PMF of the animal pelt found with the buckle, compared to the PMF of modern Harbour seal hair; (b) PMF of the animal pelt found with the strap end, compared to the PMF of modern sheep wool.

follows: initial conditions 0% solvent B (5 min), followed by a linear gradient to 50% solvent B over 20 min, followed by a wash with 90% solvent B for 10 min. The column was returned to initial conditions and re-equilibrated for 15 min before subsequent injections.

The nanoLC system was interfaced with a maXis LC-MS/MS System (Bruker Daltonics) with a nano-electrospray source fitted with a distally coated silica emitter needle (360 μm O.D., 50 μm I.D., tapering to 30 μm I.D. at the tip, New Objective). Positive ESI-MS & MS/MS spectra were acquired using AutoMSMS mode. Instrument control, data acquisition and processing were performed using Compass 1.3 SP1 software (microTOF control, Hystar and DataAnalysis, Bruker Daltonics). Instrument settings were: ion spray voltage 1500 V, dry gas 6 L/min, dry gas temperature 150 °C and ion acquisition range *m/z* 50–3000. AutoMSn settings were: MS for 0.5 s (acquisition of survey spectrum); MS/MS (CID with N_2 as collision gas): ion acquisition range *m/z* 350–1600, 0.2 s acquisition for precursor intensities above 20000 counts, for signals of lower intensities down to 1000 counts acquisition time increased linear to 2 s, the collision energy and isolation width settings were automatically calculated using the AutoMSMS fragmentation table, 3 precursor ions, absolute threshold 1000 counts, preferred charge states 2–4, singly charged ions excluded, strict active exclusion. Two MS/MS spectra were acquired for each precursor and previously selected target ions were excluded for 60 s.

2.5. Bioinformatics analysis

Mascot Daemon (Matrix Science, UK) was used to extract peak lists from the LC–MS/MS data files. Subsequently, Mascot (Matrix Science, UK) was used to search for matches against the database of publicly available sequences NCBI (National Center for Biotechnology Information, US). Searches were carried out using semi-trypsin as the enzyme, two missed cleavages, peptide mass tolerance (MS) of 50 ppm and fragment mass tolerance (MS/MS) of 0.2 Da, carbamidomethylation as a fixed modification and acetyl (N-term), carbamyl (N-term), deamidated (NQ), Gln- > pyro-Glu (N-term Q), Glu- > pyro-Glu (N-term E) and oxidation (M) as variable modifications.

2.6. Calculation of deamidation

The deamidation of glutamine or asparagine results in a mass shift of +0.984 Da so that the isotope distributions for the deamidated and non-deamidated states of a peptide overlap. However, comparison with the theoretical distribution allows the percentage of each to be determined (Wilson et al., 2012). We refer to the percentage of undeamidated peptide as %Gln–Asn. The level of deamidation was calculated on peptides previously characterised and described in Solazzo et al. (Solazzo et al., 2014).

3. Results and discussion

3.1. Species identification

Fig. 5a shows the PMF of pelt sample G27-793 found on the buckle and its match against a modern sample of harbour seal hair (described Table 1). The sample can be formally identified thanks to peak *m/z* 1041 and 1849 which rule out the presence of bovidae, and more particularly the sample matches the *m/z* 2662, which sequence is unknown but is most likely an equivalent of type I

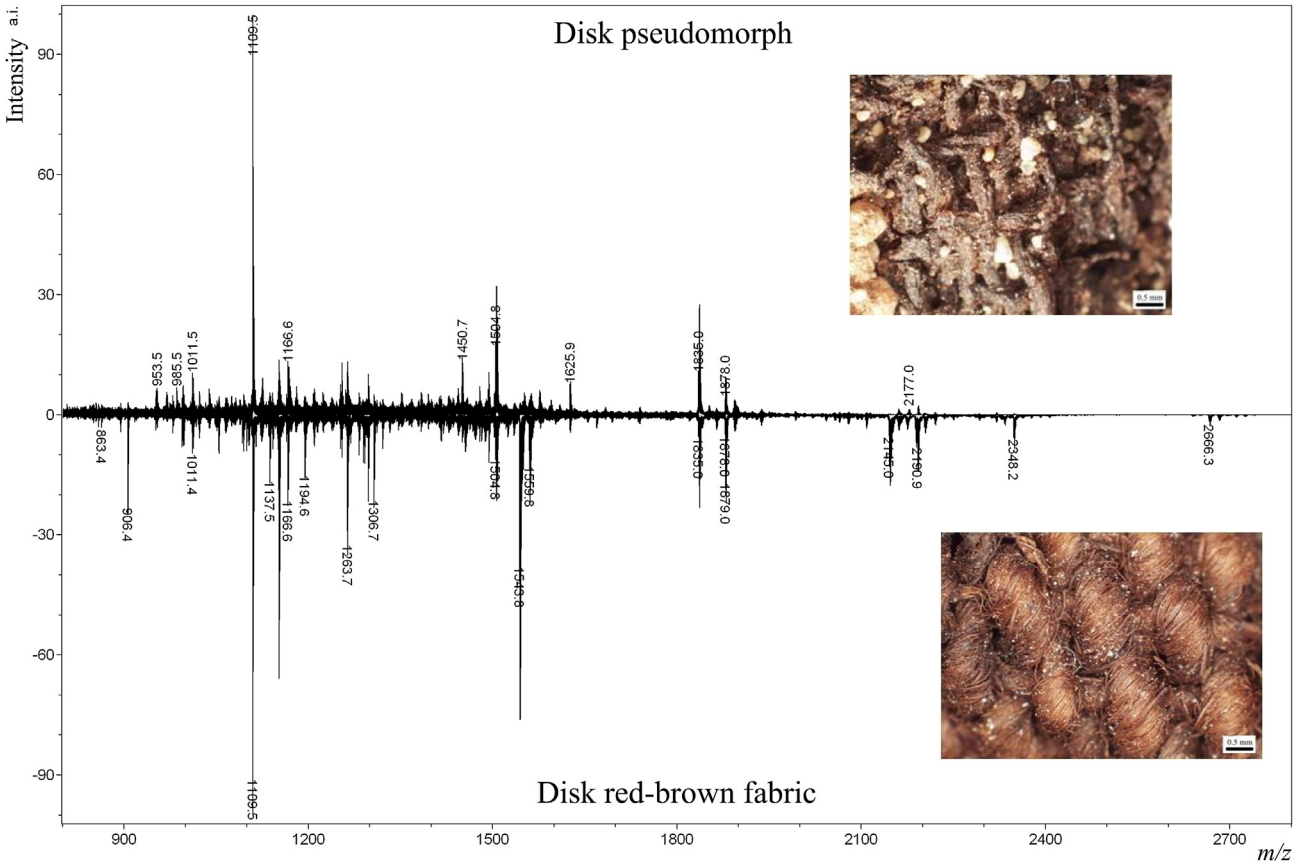

Fig. 6. PMF of DB2-ps (disk pseudomorph) compared to the PMF of DB1-rb (disk red–brown fabric). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

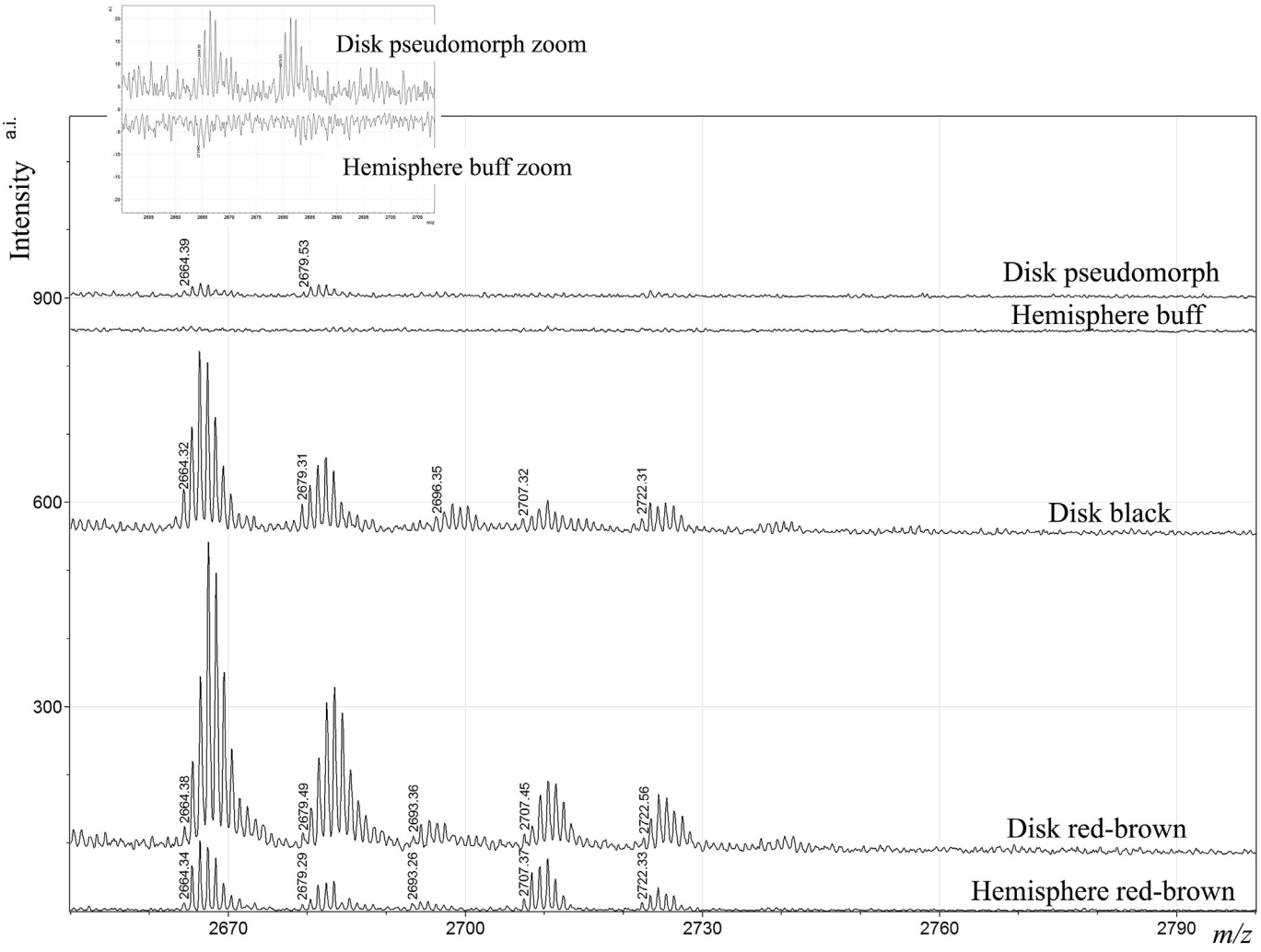

Fig. 7. Zoomed area *m/z* 2600–2800 from the PMFs of the textiles from Gol Mod 2. From top to bottom: DB2-ps, HO1-bu, DB2-bl, DB1-rb and HO1-rb.

peptide YSCQLSQVQSLIVNVESQLAEIR, the marker of the genus *Ovis* (Solazzo et al., 2013a). This marker is however identified in the second pelt sample, G27-795 (Fig. 5b) at *m/z* 2664, and indicates the presence of sheep (wild or domestic).

Fig. 6 shows the PMF of the pseudomorph-type sample compared to the red–brown sample from the disk (PMFs of all disk and hemisphere samples shown Figs. S1–5, Supplementary Information). Both samples show a high level of background noise, indicative of the deterioration of the samples with few diagnostic peaks visible on the pseudomorph-type sample. Both spectra are normalized to the peak at *m/z* 1109, which excludes camel as a candidate. The peak at *m/z* 1543 allows identification to the Caprinae family, while the peaks at *m/z* 2664 and *m/z* 2679 point out to the *Ovis* genus (zoomed area, Fig. 7). Note that the isotope clusters appear shifted due to high levels of deamidation (see below). The *m/z* 2664 and *m/z* 2679 peaks are followed by two clusters at +43 Da, representing a carbamyl adduct on both peptides. A small cluster appears at *m/z* 2693, but excludes goat which should appear at *m/z* 2691. Although the *Ovis* markers are present in low intensity compared to a modern sheep, it is possible to identify them in four out of five samples, including the pseudomorph-type sample. The sample where the markers appear the most deteriorated is the buff sample from the hemisphere.

3.2. Problems associated with degradation of archaeological artefacts

Protein degradation in archaeological samples eventually leads to the loss of important species markers, usually as a result of proteins breaking down (Solazzo et al., 2013b). With 112 distinct peptides identified (see nanoLC-ESI-MS/MS results Table 3 and Supporting Information, Tables S1 – S5), the red–brown samples DB1-rb and HO1-rb have the best protein coverage, followed by the black fabric DB2-bl with 74 distinct peptides. In comparison samples HO1-bu (buff sample) and DB2-ps (pseudomorph-type sample) yield very few peptides (49 and 38 respectively). The protein results recovered from the disk samples are consistent with the visual increase in deterioration (brittleness and shrinking) of the

Table 3
Peptide identification by nanoLC-ESI-MS/MS analysis by maXis in the Gol Mod 2 wool samples. The Keratins correspond to the α-helix proteins, while the KAPs stand for keratin associated proteins, the proteins that compose the matrix in which the keratins are embedded.

	DB1-rb	DB2-bl	DB2-ps	HO1-bu	HO1-rb
Total Keratins	107	69	37	47	109
Total KAPs	5	5	1	2	3
Total	**112**	**74**	**38**	**49**	**112**

Table 4
Levels of deamidation in archaeological samples for selected peptides in %: 100% represents no deamidation and 0% complete deamidation (at least one glutamine or one asparagine deamidated). – stands for no peak or peak below the signal-to-noise threshold. The peptides chosen for deamidation are as follow (available N and Q sites for deamidation are underlined): *m/z* 1487.74 = QNQEYQVLLDVR (with pyroglutamic acid N-term); *m/z* 1504.77 = QNQEYQVLLDVR; *m/z* 1625.84 = LNVEVDAAPTVDLNR; *m/z* 1834.97 = TVNALEVELQAQHNLR; *m/z* 1848.99 = TVNALEIELQAQHNLR; *m/z* 2664.38 = YSCQLNQVQSLIVSVESQLAEIR.

Provenance	Peptide *m/z*						
	1487.74	1504.77	1625.84	1834.97	1848.99	2662.30	2664.38
Harbour seal	100	87	NP		96	91	
G27-793, Cumwhitton	–	52	32		41	20	
Sheep	100	100	100	92			100
G27-795, Cumwhitton	–	40	–	38			32
Gol Mod 2_DB1-rb	–	25	87	15			0
Gol Mod 2_DB2-bl	58	28	95	23			16
Gol Mod 2_DB2-ps	–	33	79	30			44[a]
Gol Mod 2_HO1-bu	52	6	74	11			–
Gol Mod 2_HO1-rb	60	19	–	20			4
Average Gol Mod 2	57	22	84	20			16

[a] Value obtained at the signal-to-noise threshold limit.

fibres due to a combination of mineralisation and deterioration from the burial environment.

Peptides with tyrosine (Y) and phenylalanine (F) are particularly sensitive to degradation; *m/z* 952.5 LQFFQNR, *m/z* 968.5 LQFYQNR and *m/z* 1169.5 WQFYQNQR are for instance not observed in the hemisphere samples from Mongolia and only with low signal-to-noise in the disk samples (Fig. S6). In the Cumwhitton samples, all three peaks are observed in the sheep pelt and WQFYQNQR and *m/z* 1041.5 WQFYQNR are observed in the seal pelt with high intensity, showing the overall better preservation of the pelt samples. High-molecular mass peptides (*m/z* > 2000), which include the very specific markers between *m/z* 2500 and 2700, are also more susceptible to degradation. HO1-bu and DB2-ps, for instance, show the most important loss of peptides above *m/z* 2000 as a result of protein hydrolysis (Fig. S7).

In archaeological fibres, the formation of products due to degradation or ageing is usually observed. The most useful chemical modification to characterise the degradation of ancient proteins is the deamidation of glutamine (Gln) and asparagine (Asn) into glutamic acid and aspartic acid that results in a mass shift of +1 Da. Deamidation is a process that happens gradually with time if external conditions remain constant; peptides have different rates of deamidation that depend on the neighbouring amino acids and configuration of the proteins (Solazzo et al., 2014). Fig. 5a and b (zoomed areas) show isotopic clusters with high levels of deamidation in archaeological samples compared to the expected clusters from fresh hair. In ancient samples, the mass shift associated with deamidation gives a good indication that a marker originates from the sample and not from contamination, a potential problem when working with keratins (Oonk et al., 2012). However, it can cause problems in distinguishing between peptides with similar *m/z* values for example *m/z* 2662 in seal and *m/z* 2664 in sheep. The level of deamidation was calculated for five peptides (Table 4) as described previously (Solazzo et al., 2014, Wilson et al., 2012) and expressed as the percentage of peptide remaining in a non-deamidated form (denoted the %Gln–Asn value). The values are compared to those obtained for the modern reference (little or no deamidation) and for non-mineralised archaeological samples (Fig. 8). The values in the Cumwhitton and the Gol Mod 2 samples show a level of deamidation intermediate between medieval samples from York, UK (low deamidation) and from Reykholt, Iceland (high deamidation); in

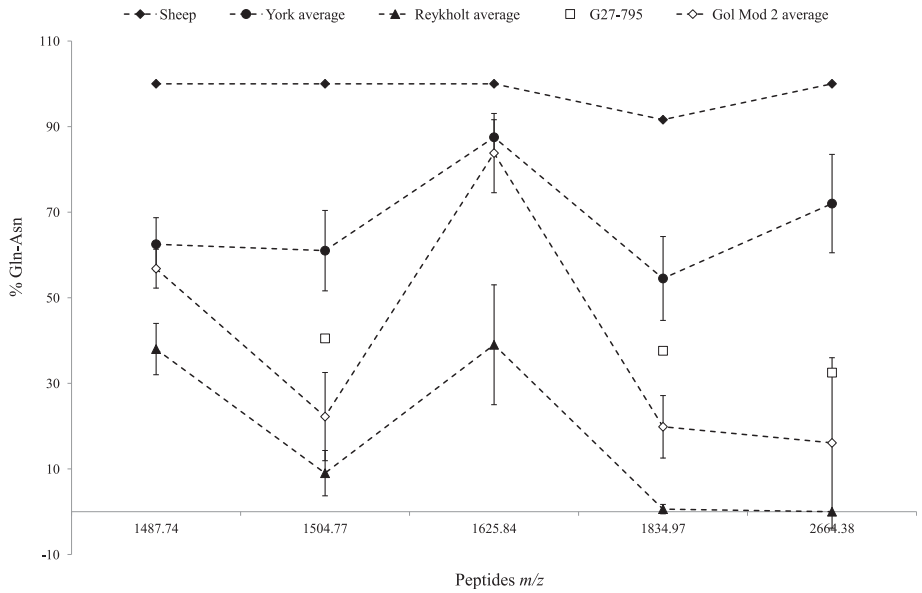

Fig. 8. Deamidation values in % Gln–Asn for peptides at *m/z* 1487, 1504, 1625, 1834 and 2664 for the sheep wool fabrics (Gol Mod 2 and G27-795) compared to modern sheep wool and archaeological samples from York (9th–13th c.) and from Reykholt (13th–16th c.).

archaeological samples, deamidation was found to depend on the burial conditions rather than simply be a function of age (Solazzo et al., 2014). The lowest % Gln–Asn values (i.e. the highest levels of deamidation) for all identifiable peptides are obtained for the buff sample from the hemisphere (Table 4). Curiously, the more mineralised the fabric is, the less deamidated it appears, indicating that the presence of copper corrosion products inhibit deamidation. In non-mineralized samples, deamidation proceeds at a rate imposed by the local environment; in the buff sample, the absence of a layer of copper corrosion products might also enhance biodeterioration.

4. Conclusion

Unlike bone, there is little protection from biodeterioration for buried textiles. It is well known that the presence of corroding metal helps preserve the physical traces of yarns by replacing the organic component with minerals, but we are now in a position to unpick the story of protein survival.

Our results are consistent with a positive cast, characteristic of textiles in contact with copper-alloy artefacts. While the cuticle of extensively mineralised artefacts might be difficult to interpret by microscopy the core of the fibre is sufficiently well preserved to be identified by mass spectrometry. Proteomic identification of a negative cast that results in partial or complete loss of the cortex might be more difficult, as the organic content would be mostly limited to the cuticle. In comparison to previous methods that can only determine the general class of the fibre, proteomics has proven more specific and we have been able to identify fibres to genus level, for both the Viking-Age and the Mongolian samples. In these cases, preservation was no doubt helped by the burial conditions: an acidic soil environment for the Cumwhitton site, characterised by the poor preservation of human remains, and the high-altitude sandy conditions of the Gol Mod 2 site (located in an area of low annual precipitations, low average annual temperature (Chuluunkhuyag, 2008), characterised by sporadic permafrost (Brown, 2001)).

Within a single context, the protein content (as analysed by MS and MS/MS) is highest in samples with initially extensive mineralisation. The concomitantly lower rates of deamidation indicate that chemical degradation tends to be slowed down by the mineralization of the fibres. However protein is inherently unstable and will continue to decay until only a pseudomorph (high mineral content and little organics left) remains. The corroding metal acts primarily as a biocide, and the presence of copper salts will also enhance preservation in other proteinaceous materials such as leather, horn and tortoiseshell.

Acknowledgements

This research was supported by a Smithsonian's Museum Conservation Institute post-doctoral grant (2008–2009) and by a Marie Curie International Outgoing Fellowship (FP7-PEOPLE-IOF-GA-2009-236425, THREADs) between the University of York, UK and AgResearch, NZ (2009–2012). The objects of the Gol Mod 2 cemetery were made available by Dr. Francis Allard (Khanuy Valley Project director), Dr. Diimaajav Erdenebaatar (Khanuy Valley Project co-director) and Bryan K. Miller (Khanuy Valley Project archaeologist) and we also thank Lynn Brostoff (MCI) for the XRF analysis. Proteomics analyses were carried at The Centre of Excellence in Mass Spectrometry, University of York, supported by Science City York and Yorkshire Forward, using funds from the Northern Way Initiative. We particularly thank Dr. David Ashford for carrying out analyses on the maXis instrument.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2014.06.009.

References

Allard, F., Erdenebaatar, D., Batbold, N., Miller, B., 2002. A xiongnu cemetery found in Mongolia. Antiquity 76, 637–638.
Anheuser, K., Roumeliotou, M., 2003. Characterisation of mineralised archaeological textile fibres through chemical staining. Conservator 27, 23–33.
Appleyard, H.M., 1978. Guide to the Identification of Animal Fibres. British Textile Technology Group, Leeds.
Brosseder, U., 2009. Xiongnu terrace tombs and their interpretation as elite burials. In: Bemmann, J., Parzinger, H., Pohl, E., Tseveendorzh, D. (Eds.), Current Archaeological Research in Mongolia. Papers from the First International Conference on "Archaeological Research in Mongolia", held in Ulaanbaatar, August 19th-23rd, 2007, Vor- und Frühgeschichtliche Archäologie. Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, pp. 247–280.
Brown, J., 2001. The News Bulletin of the International Permafrost Association. International Symposium on Mountain and Arid Land Permafrost and Field Excursions in Mongolia, FROZEN GROUND, vol. 25, pp. 7–11.
Chen, H.L., Jakes, K.A., Foreman, D.W., 1996a. SEM, EDS, and FTIR Examination of archaeological mineralized plant fibers. Text. Res. J. 66, 219–224.
Chen, H.L., Foreman, D.W., Jakes Kathryn, A., 1996b. X-ray Diffractometric Analyses of Microstructure of Mineralized Plant Fibers. Archaeological Chemistry, American Chemical Society, pp. 187–201.
Chuluunkhuyag, S., 2008. The Impact of Climate Change and Human Activity on Mongolian Water Resources. The 3rd WEPA International Forum on Water Environmental Governance in Asia, Putrajaya, Malaysia.
Cooke, W.D., 1990. Archaeological Textiles UKIC Occasional Papers. Fibre Damage in Archaeological Textiles, vol. 10, pp. 5–13.
Gillard, R.D., Hardman, S.M., 1996. Investigation of Fiber Mineralization Using Fourier Transform Infrared Microscopy. Archaeological Chemistry, American Chemical Society, pp. 173–186.
Gillard, R.D., Hardman, S.M., Thomas, R.G., Watkinson, D.E., 1994. The mineralization of fibres in burial environments. Stud. Conserv. 39, 132–140.
Hollemeyer, K., Altmeyer, W., Heinzle, E., Pitra, C., 2012. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry combined with multidimensional scaling, binary hierarchical cluster tree and selected diagnostic masses improves species identification of Neolithic keratin sequences from furs of the Tyrolean Iceman Oetzi. Rapid Commun. Mass Spectrom. 26, 1735–1745.
Hollemeyer, K., Altmeyer, W., Heinzle, E., Pitra, C., 2008. Species identification of Oetzi's clothing with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on peptide pattern similarities of hair digests. Rapid Commun. Mass Spectrom. 22, 2751–2767.
Jakes, K.A., Sibley, L.R., 1984. An Examination of the Phenomenon of Textile Fabric Pseudomorphism. Archaeological Chemistry-III, American Chemical Society, pp. 403–424.
Jakes, K.A., Howard, J.H., 1986. Replacement of Protein and Cellulosic Fibers by Copper Minerals and the Formation of Textile Pseudomorphs, Historic Textile and Paper Materials. American Chemical Society, pp. 277–287.
Jakes, K.A., Sibley, L.R., 1983. Survival of cellulosic fibres in the archaeological context. Sci. Archaeol. 25, 31–38.
Janaway, R., 1983a. Textile fibre characteristics preserved by metal corrosion: the potential of S.E.M. studies. Conservator 7, 48–52.
Janaway, R.C., 1983b. Catalogue and discussion of the graves and grave goods. In: Jarvis, K.S. (Ed.), Excavations Christchurch 1969–1980, Dorset Nat Hist and Archaeol Soc Monograph, pp. 112–136.
Janaway, R.C., 1989. Corrosion preserved textiles: Mechanisms, bias and interpretation. In: Janaway, R.C., Scott, B. (Eds.), Evidence Preserved In Corrosion Products: New Fields in Artifact Studies, United Kingdom Institute for Conservation (Occasional Paper 8), London, pp. 21–29.
Janaway, R.C., 1985. Dust to dust: the preservation of textile materials in metal artefact corrosion products with reference to inhumation graves. Sci. Archaeol. 27, 29–34.
Janaway, R.C., Scott, B., 1989. Evidence Preserved in Corrosion Products: New Fields in Artefact Studies. UKIC Occasional Papers.
Miller, B., Allard, F., Erdenebaatar, D., Lee, C., 2006. A xiongnu tomb complex: excavations at Gol Mod 2 cemetery, Mongolia (2002–2005). Mong. J. Anthropol. Archaeol. Ethnol. 2, 1–21.
Miller, B., Allard, F., Erdenebaatar, D., Lee, C., 2008. A xiongnu tomb complex: excavations at Gol Mod 2 Cemetery, Mongolia (2002–2005). Arkheologicheskie vesti. 15, 55–70.
Oonk, S., Cappellini, E., Collins, M.J., 2012. Soil proteomics: an assessment of its potential for archaeological site interpretation. Org. Geochem. 50, 57–67.
Sibley, L.R., Jakes, K.A., 1984. Survival of protein fibres in archaeological contexts. Sci. Archaeol. 26, 17–27.
Solazzo, C., Wadsley, M., Dyer, J.M., Clerens, S., Collins, M.J., Plowman, J., 2013a. Characterisation of novel α-keratin peptide markers for species identification in keratinous tissues using mass spectrometry. Rapid Commun. Mass Spectrom. 27, 2685–2698.

Solazzo, C., Wilson, J., Dyer, J.M., Clerens, S., Plowman, J.E., von Holstein, I., Walton Rogers, P., Peacock, E.E., Collins, M., 2014. Modeling deamidation in sheep α-keratin peptides and application to archaeological wool textiles. Anal. Chem. 86, 567–575.

Solazzo, C., Dyer, J.M., Clerens, S., Plowman, J.E., Peacock, E.E., Collins, M.J., 2013b. Proteomic evaluation of the biodegradation of wool fabrics in experimental burials. Int. Biodeterior. Biodegrad. 80, 48–59.

Solazzo, C., Heald, S., Ballard, M.W., Ashford, D.A., DePriest, P.T., Koestler, R.J., Collins, M.J., 2011. Proteomics and coast salish blankets: a tale of shaggy dogs? Antiquity 85, 1418–1432.

Textile Institute, 1975. Identification of Textile Materials. The Textile Institute, Manchester.

Timár-Balázsy, A., 1989. Deterioration process of fibers in different chemical actions. In: Járó, M. (Ed.), Conservation of Metals: Problems in the Treatment of Metal-Organic and Metal-Inorganic composite Objects (International Restorer Seminar, 1–10 July 1989), Veszprém (Hungary), pp. 77–82.

Vanden Berghe, I., Wouters, J., 2005. Identification and condition evaluation of deteriorated protein fibres at the sub-microgram level by calibrated amino acid analysis. In: Janaway, R., Wyeth, P. (Eds.), Scientific Analysis of Ancient and Historic Textiles: Informing Preservation, Display and Interpretation. AHRC Research Centre for Textile Conservation and Textile Studies, Winchester, UK, pp. 151–158.

Walton Rogers, P., 2007. Cloth and Clothing in Early Anglo-Saxon England (AD 450–700). CBA Research Report 145. CBA, York.

Wildman, A.B., 1954. The Microscopy of Animal Textile Fibres. WIRA, Leeds.

Wilson, J., van Doorn, N.L., Collins, M.J., 2012. Assessing the extent of bone degradation using glutamine deamidation in collagen. Anal. Chem. 84, 9041–9048.