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Original article

Application of redox proteomics to the study of oxidative degradation products in archaeological wool

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ABSTRACT

Most archaeological and historical textiles (clothing, tapestries, blankets, carpets, etc.) present traces of UV-induced damage when exposed to light during their lifetime. Yellowing of the fibres, fading of the dyes and loss of physical properties, such as tensile strength are the typical indicators of photodegradation. Natural fibres made of proteins, such as wool and silk are particularly sensitive to UV damage. Photo-oxidative damage is caused by the accumulation of chemical modification at the amino acid residue level that lead to a range of oxidation products, including chromophores responsible for changes in coloration, as well as to the breaking of peptide bonds in the protein backbone. Amino acid residues with aromatic side-chain groups are particularly sensitive to photo-oxidation and breakthroughs have been made in recent years in the field of protein science to identify the photoproducts and locate them within proteins. This study explores new methodologies using redox proteomics-based strategies to assess the extent of photodamage in ancient wool textiles, by identifying modifications occurring at the molecular level. Using a scoring system to determine the level of oxidation in amino acids with aromatic side-chains (tryptophan, tyrosine, histidine and phenylalanine), we compare the effects of dyes and mordants on fibres after UV ageing, and assess the extent of oxidation on the different proteins composing the wool fibres. We determine that dyes and mordants have the capability of slowing down photo-oxidation during ageing. We also assess the effect of UV irradiation on deamidation, a modification targeting glutamine and asparagine, as it is a common marker of ageing in ancient proteins.

1. Research aims

Important breakthroughs using redox proteomics have been made in recent years to identify the coloured photoproducts formed in proteins exposed to UV light (chromophores) [1]. A redox proteomics approach is based around characterisation of the complex cascade of oxidation and reduction events occurring at the protein primary level [2]. Proteomics is underpinned by mass spectrometry, with electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) the predominant modes used [3]. A redox proteomic approach consisting of:

- digestion with a proteolytic enzyme to produce peptides;
- separation of the peptides with appropriate liquid chromatography;
- tandem mass spectrometric peptide fragmentation;
- targeted bioinformatic evaluation for key redox products can provide detailed identification and location of modifications throughout the wool proteome.

In this study, these relatively new tools are applied to investigate changes occurring at the amino acid level in keratins when wool has

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been exposed to UV light. Changes in wool after exposure to UV light for up to 48 h were evaluated by mass spectrometry. Chemical changes (oxidation and deamidation) were observed in both undyed and dyed samples. Fabrics buried for more than eight years in North-European experimental burial sites [4] offered a basis for evaluating possible increases in oxidation in wool by simulating archaeological biodegradation. Finally, we tracked these modifications in actual archaeological finds from medieval sites in the UK and Iceland to determine whether it was possible to identify known products typically associated with photo-oxidation in archaeological samples after hundreds of years of burial.

2. Introduction

Exposure to natural light is one of the major factors that contribute to fibres' fragility and loss of coloration in ancient textiles. Photodegradation of wool by UV light is associated with yellowing [5,6], fading of the dyes [7–9] and loss of physical properties such as tensile strength [10]. Not only the length and intensity of exposure to UV light produce long-term damage, the treatment of fibres with dyes and mordants can also influence photodegradation positively or negatively [5,11], either by improving the photostability of the wool or by increasing the level of phototendering resulting in loss of strength and flexibility [12]. The presence of trace metals (for example iron and copper) that increase the production of hydroxyl radicals also influence wool photostability and accelerate photoyellowing [13,14].

Photoyellowing results from exposure to high-energy UV light in the 320–400 nm (UVA) and 280–320 nm (UVB) range, while filtering of UVA and UVB can lead to the competing process of photobleaching dominating (blue light 400–460 nm) [5]. Photooxidation is initiated via radical species that react with atmospheric oxygen and produce peroxide radicals. Reactive oxygen species (ROS) attack both amino acid residue side-chains and the protein backbone itself. When dyed, photochemical reactions are also transferred to the dyes as UV radiation is absorbed by the dye molecules. Dye chromophores are destroyed, resulting in dye fading (photofading). Dyes can be affected by UV light or by photoproducts of the substrate itself that react with the dyes. In addition, photodegradation leads to peptide chain scission, as well as cleavage of disulphide bridges, while it might inversely contribute to the formation of cross-links that will increase tensile strength but may also result in increased brittleness.

Redox proteomic-based evaluation of wool has found that oxidation products of phenylalanine, tryptophan and tyrosine are mainly responsible for the discoloration of wool due to the susceptibility of aromatic side-chains to oxidation [3,15–17]. The level of oxidation was calculated according to a classification system of the products resulting from the oxidation of the aromatic amino acids, while deamidation was also calculated in key peptides (see below). Supplementary Table S1 summarises the samples and the associated experiments.

2.1. Calculation of the oxidation score

MS/MS data were obtained by nanoLC-ESI-MS/MS to locate the induced oxidative modifications in the aged, buried modern and archaeological fibres. Based on reported photomodifications to aromatic amino acid residues [3,15,17], single and double oxidation on aromatic residues (tyrosine, tryptophan, histidine and phenylalanine), quinone and hydroxyquinone (oxidized tyrosine) and kynurenine and hydroxykynurenine (oxidized tryptophan) were chosen as variable modifications (Fig. 1). The degree of oxidative degradation for each modified peptide has been evaluated by assigning a score to each individual observed oxidative modification within the peptide based on the relative level of the modification within this oxidative cascade. Scores were assigned as 1 for those modifications classified as single oxidation, 2 for double oxidation, 3 for quinone and kynurenine formation and 4 for hydroxyquinone and hydroxykynurenine formation. The score given to each modification reflects the relative level of modification with respect to the native residue; with initial oxidation products being further modified themselves in a cascade of degradation (Fig. 1). Quinone, hydroxyquinone, kynurenine and

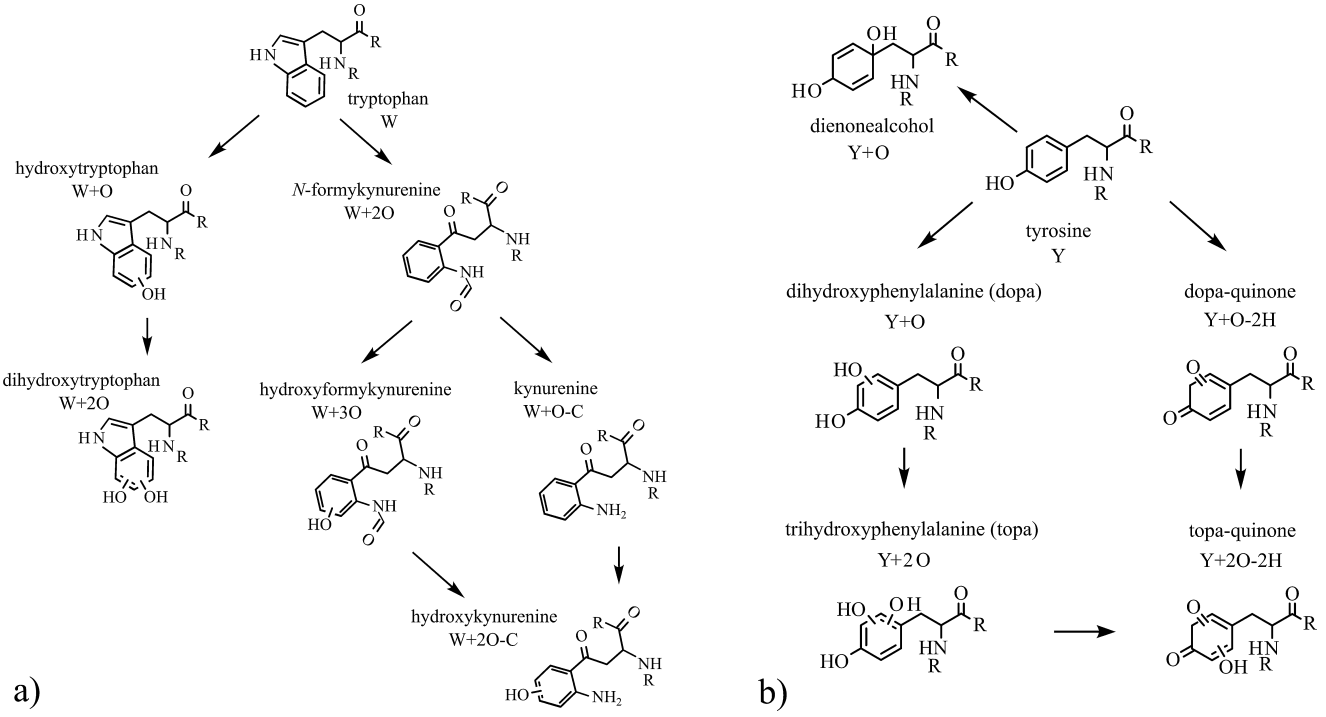

Fig. 1. Pathways of oxidation showing the resulting products of oxidation of (a) tryptophan, and (b) tyrosine.

Table 1
Deamidation markers identified by MALDI-TOF-MS: sequence, theoretical $[M+H]^+$, modifications, and deamidation sites [18].

Sequence	[M+H]+	Modifications	Deamidation sites
QNQEYQVLLDVR	1487.74	Gln->pyro-Glu N-term Q or Glu->pyro-Glu N-term Q	Q1-N2-Q3-Q6
QNQEYQVLLDVR	1504.77		Q1-N2-Q3-Q6
LNVEVDAAPTVDLNR	1625.84		N2-N14
TVNALEVELQAQHNLR	1834.97		N3-Q10-Q12-N14

hydroxykynurenine are the most important modifications as they are the chromophores primarily responsible for yellowing.

2.2. Calculation of deamidation

Deamidation rates were calculated for selected peptides commonly observed by peptide mass fingerprinting and previously reported (Table 1) [18]. Peptide mass fingerprints were obtained by MALDI-TOF-MS. 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 [19]. We refer to the percentage of non-deamidated peptide as %Gln–Asn. The level of deamidation was calculated on peptides previously characterised and described in Solazzo et al. [18].

3. Experimental

3.1. Dyeing and mordanting

Raw wool from Icelandic sheep was obtained from the Honorwood flocks (Cefn Llanfair Llanfair Road, Llandysul Ceredigion SA44 4RB). Alum ($AlK(SO_4)_2{\cdot}12H_2O$), madder and weld were obtained from the mulberry dyer shop (http://www.mulberrydyer.co.uk/) and mordanting and dyeing was done according to traditional dyeing recipes using natural dyes [20].

Scoured fibres were mordanted by simmering for 1 h in a water bath with alum (10% in weight of the fibre weight, pH ~ 3), let to cool down in the bath overnight, removed and rinsed. The fibres were dried and stored in the dark and at room temperature. For dyeing with weld, the dyestuff (50% in weight of the fibre weight) was simmered in water for 45 min The solution was cooled and strained. Wetted mordanted fibres were dyed by simmering for 30 min in the dye bath (pH ~ 8) then cooled down overnight, removed from the bath and rinsed in warm water, dried and stored in the dark at room temperature. The fibres took a bright yellow colour. For dyeing with madder, the dyestuff was first washed in boiled water for 3 min and the solution discarded. Dyestuff (50% in weight of the fibre weight to use) and wetted fibres were added to the same bath (pH ~ 8) and heated up to about 40–50 °C for 1 h, then cooled down overnight, removed from the bath and rinsed in warm water, dried and stored in the dark at room temperature. Mordanted fibres took on a red colour while non-mordanted wool was dyed a dark orange tone.

3.2. UV ageing

Snippets of wool were spread across a glass plate (22 cm × 19 cm) as a thin open web and irradiated in a Luzchem UV chamber (LZC4-14, Luzchem, Ontario, Canada), using UVB (Luzchem LZC-UVB) narrow bandwidth lamps–spectral distribution 281–315 nm, dose 35.33 Wm^{-2}. Samples were turned regularly to allow even irradiation as much as possible and sampling was done at 0, 24, and 48 h, with additional samples taken at 3, 6, 12 and 18 h for the undyed samples. After irradiation the samples were stored in the dark at room temperature prior to further handling. Note for reference that 3 h of accelerated ageing utilised in this study is approximately equivalent to 1.5 years actual ageing in sunshine based on average UV exposure levels in the United Kingdom.

3.3. Experimental burial

Between 1998 and 2006, samples of fulled twill (vadmel) fabrics (undyed or dyed with madder and weld) were buried in bogs at "Land of Legends Lejre" (Denmark) and Rørmyra, Sør-Trondelag County (Norway), while a second series of burial experiments was initiated in 2002 in the harbour sediment at Marstrand on the coast of West Sweden [4]. For the bog study, samples were placed together with excavated soil into perforated PVC plastic pipes (16 mm diameter) and the modules buried in hand-drilled boreholes 0.5 to 1 m deep. For the Marstrand study, samples were placed together with sediment in perforated trays and buried at a depth of 0.5 m in the harbour bottom. Control samples and samples retrieved from burial were kept in darkness in climate-control stores at the NTNU Museum, Norwegian University of Science and Technology in Trondheim, Norway (Table S1). They were subsequently kept in drawers, away from sunlight until analysis.

3.4. Medieval samples

Nine samples (9–13th c.) are from the textiles finds from the excavations conducted at 16–22 Coppergate in York in 1979–1981 [21] and seven (10–11th c.) from the excavations conducted at 6–8 Pavement on the site of the Lloyds Bank in York in 1972–1973 [22]. One sample was obtained from a 13th c. site on Queen Street, Quayside in Newcastle upon Tyne [23]. The final eight samples (13–16th c.) come from an archaeological high status farm site at Reykholt, Borgarfjörður in Iceland [24,25].

3.5. Sample preparation for mass spectrometry

Samples of up to 10 mg (10 mg for the UV aged samples, 2 to 10 mg for the experimentally buried samples [4] and between 1–3 mg for the archaeological samples [18]) were ground in liquid nitrogen unless too small to be handled. The samples were solubilised by overnight shaking in a solution of 8 M urea, 50 mM Tris and 50 mM TCEP at pH 8.3. An aliquot of the supernatant was alkylated with 150 mM IAA and vortexed for 4 h in the dark. This was followed by 24 h dialysis with 100 mM AB on 3500 MWCO Slide A Lyzer® Mini Dialysis units from Thermo Scientific (two changes). About 25 μg of samples were digested with 0.5 μg of trypsin, overnight at 37 °C. All samples were then dried down and re-solubilised in 10 μL of 0.1% TFA. A 1 μL aliquot was used for MALDI-TOF-MS analysis and a diluted aliquot (1:40) used for nanoLC-ESI-MS/MS.

3.6. Peptide mass fingerprinting by MALDI-TOF-MS

A matrix solution was prepared by diluting 0.1 mg of CHCA (α-cyano-4-hydroxycinnamic acid) in 97/3 (acetone/0.1% TFA) and 1 μL was applied onto an AnchorChip™ target (Bruker) and allowed to dry. A 1 μL aliquot of analytical solution was applied and

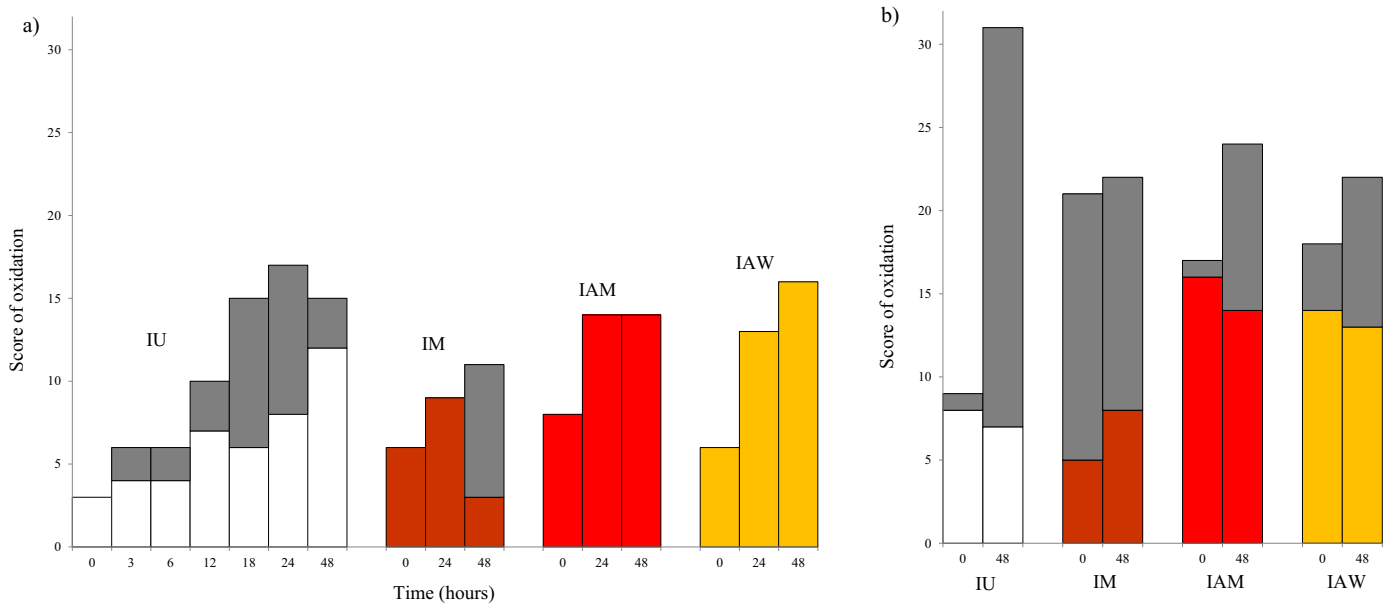

Fig. 2. Oxidation scores in UV aged samples in (a) single LC–MS/MS run and (b) by GPF or Gas Phase Fractionation. Bottom = keratins. Top (in grey) = keratin-associated proteins KAPs. IU = undyed (white); IM = Madder-dyed (orange); IAM = alum/Madder-dyed (red); IAW = alum/weld-dyed (yellow).

then removed after one minute and 1 μL of washing buffer (0.1% TFA) added. The residual droplet was removed and 1 μL of recrystallisation solution (0.1 mg of CHCA in 6/3/1 (ethanol/acetone/0.1% TFA)) applied. The plate was loaded in an UltraflexTM III mass spectrometer (Bruker), and analyses were carried out in positive reflector mode using a Nd:YAG laser operating at 355 nm. Spectra were acquired using flexControl 3.0 (Bruker) on a mass range of 700–4000 Da with an accumulation of 500 shots on the standards and 1000 shots on the samples. The calibration standard (Bruker) 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.

3.7. Protein analysis by nanoLC-ESI-MS/MS

Protein separation was carried out on an Ultimate nanoflow nanoLC equipped with Famos autosampler and Switchos column switching module (LC-Packings, The Netherlands). A 10 μL sample was loaded on a C18 precolumn (Varian Microsorb 300 μm ID, 5 μm particles, 300 Å pore size) at a flow rate of 8 μL/min. The precolumn was then switched in line with the analytical column (Microsorb C18, 20 cm, 75 μm ID, 5 μm particles, 300 Å pore size), and eluted at a flow rate of 150 nL/min, with a gradient from 2% to 55% B in 50 min. Solvent A was HPLC-grade H_2O (Fisher Scientific, USA) with 0.2% formic acid, solvent B was LC–MS grade ACN with 0.2% formic acid. Using a stainless steel nanospray needle (Proxeon, Denmark), the column outlet was directly connected to a Q-STAR Pulsar i mass spectrometer (Applied Biosystems, USA) which was programmed to acquire MS/MS traces of 1+, 2+, 3+, 4+ and 5+ peptides. MS data was acquired from *m/z* 350–1200 and MS/MS from 40–1600 *m/z* accumulating three cycles over 1.3 s duration each.

One run was conducted for all UV-irradiated samples from 0 to 48 h. In addition, the control samples (0 h) and the 48 h UV-irradiated samples were analysed by Gas Phase Fractionation (GPF); by defining smaller mass-to-charge ranges, this method allows multiple analyses to be performed and increases the number of lower abundance peptides identified. Four runs were performed between *m/z* 400–550, 550–680, 680–785 and 785–1200, then combined into a single file.

3.8. Bioinformatic analysis

Mascot Daemon (Matrix Science, UK) was used to extract peak lists from the LC–MS/MS data files. The peak lists from all *m/z* segments of each sample were concatenated and imported in Protein-Scape v2.1 (Bruker Daltonics). Subsequently, Mascot was used to search for matches with known *Ovis aries* sequences, using an in-house database compiled and curated by AgResearch, NZ. Parameters were set as followed: no enzyme, peptide mass tolerance (MS) of 150 ppm, fragment mass tolerance (MS/MS) of 0.4 Da, carboxymethylation of cysteine as a fixed modification and acetyl (*N*-term), carbamyl (*N*-term), deamidated (NQ), Gln- > pyro-Glu (*N*-term Q), methyl (DE) and oxidation (M) as variable modifications. For oxidative modifications, single and double oxidation of H, W, F and Y was allowed, as well as kynurenine and hydroxykynurenine, quinone and hydroxyquinone. The peptide score cut-off was set at 30.

4. Results and discussion

4.1. Oxidation and deamidation in UV-irradiated samples

After a short exposure to UV irradiation, undyed wool became yellow. This happened as quickly as 3 h after exposure. For dyed wool, discoloration was slower and irradiation was conducted for up to 48 h. The scores calculated for each sample are shown in Fig. 2 for the keratins and the keratin-associated proteins KAPs (in grey). Peptides observed with oxidative modifications are given Table S2. The oxidation scores, for both types of analysis conducted (single LC–MS/MS in Fig. 2a and Gas Phase Fractionation GPF in Fig. 2b) showed that the total oxidation score before irradiation was higher in the dyed samples than the undyed wool. Background oxidation has been observed in wool samples before irradiation [16] but comparison between undyed and dyed samples indicate that the dyeing and mordanting treatments induced an increase in damage prior to accelerated UV ageing. This is consistent with the higher level in 4-hydroxybenzoic, a product of the oxidation of amino acids with aromatic side-chains, observed in aged alum-mordanted wool compared to unmordanted wool [26]. Evaluation of

Table 2
Possible forms of oxidation taken by peptide DVEEWYIR, and presence in the UV-irradiated samples.

Peptide	Mr	Modifications	Name	IU	IM	IAM	IAW
R.DVEEWYIR.Q	1108.52	W	Tryptophan	0-3-6-12-18-24-48	0-24-48	0-24-48	0-48
R.DVEEWYIR.Q	1124.51	W + O	Hydroxytryptophan	3-6-12-18			
R.DVEEWYIR.Q	1140.51	W + 2O	Dihydroxytryptophan	3-6-12-18-24-48	48	0-24-48	24-48
R.DVEEWYIR.Q	1112.51	W + O–C	Kynurenine	12-24-48	24-48	24-48	24-48

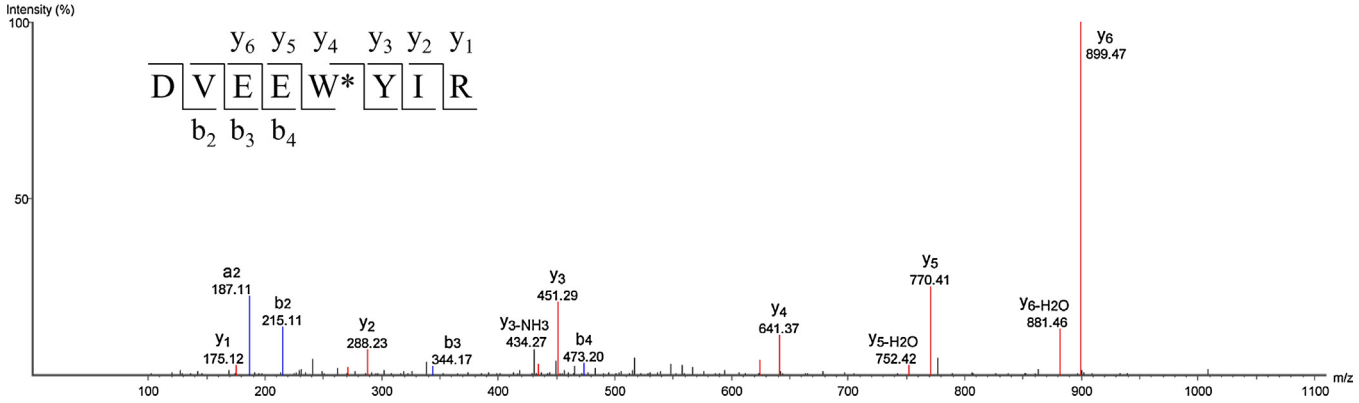

Fig. 3. Peptide DVEEWYIR with kynurenine modification in the undyed sample after 48 h of UV irradiation (IU48).

hydrothermal modification in wool has further revealed the formation of oxidative products of tyrosine and tryptophan, which were associated with the production of reactive oxygen species (ROS) [27]. The higher levels of oxidation in the dyed samples prior to irradiation can then be attributed to the in-solution heating of wool during the dyeing treatment rather than to the dyes or mordants themselves. Following irradiation, the analyses showed the highest increase in the oxidation score for the undyed sample (up to five times the initial score after 48 h). The increase was less significant for the dyed samples, consistent with lower oxidation

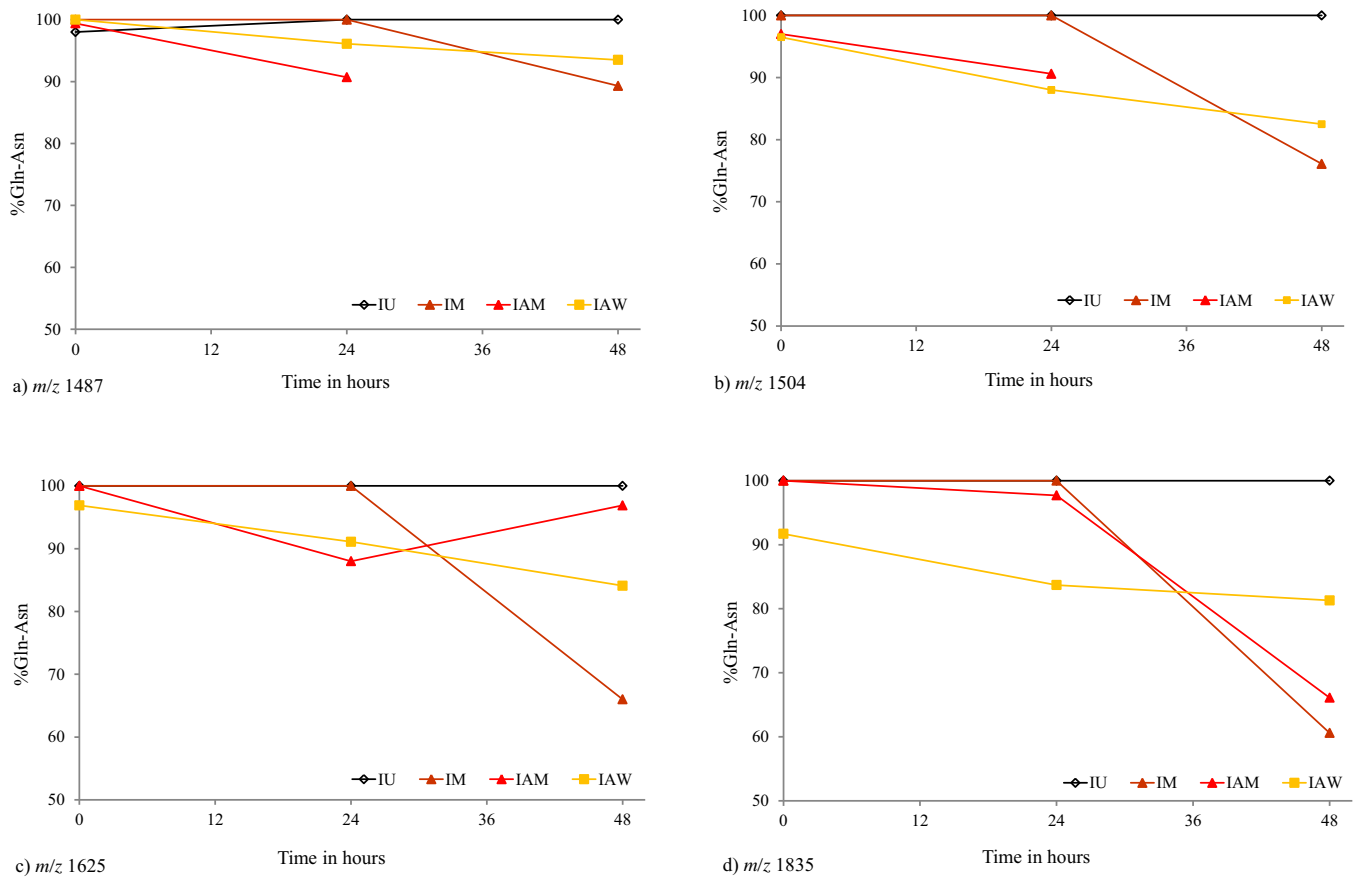

Fig. 4. Percentage Gln–Asn values over time for UV-irradiated wool (on a 0–100% scale, with 100% representing no deamidation and 0% complete deamidation); (a) QNQEYQVLLDVR, *m/z* 1487.74; (b) QNQEYQVLLDVR, *m/z* 1504.77; (c) LNVEVDAAPTVDLNR, *m/z* 1625.84; (d) TVNALEVELQAQHNLR, *m/z* 1834.97.

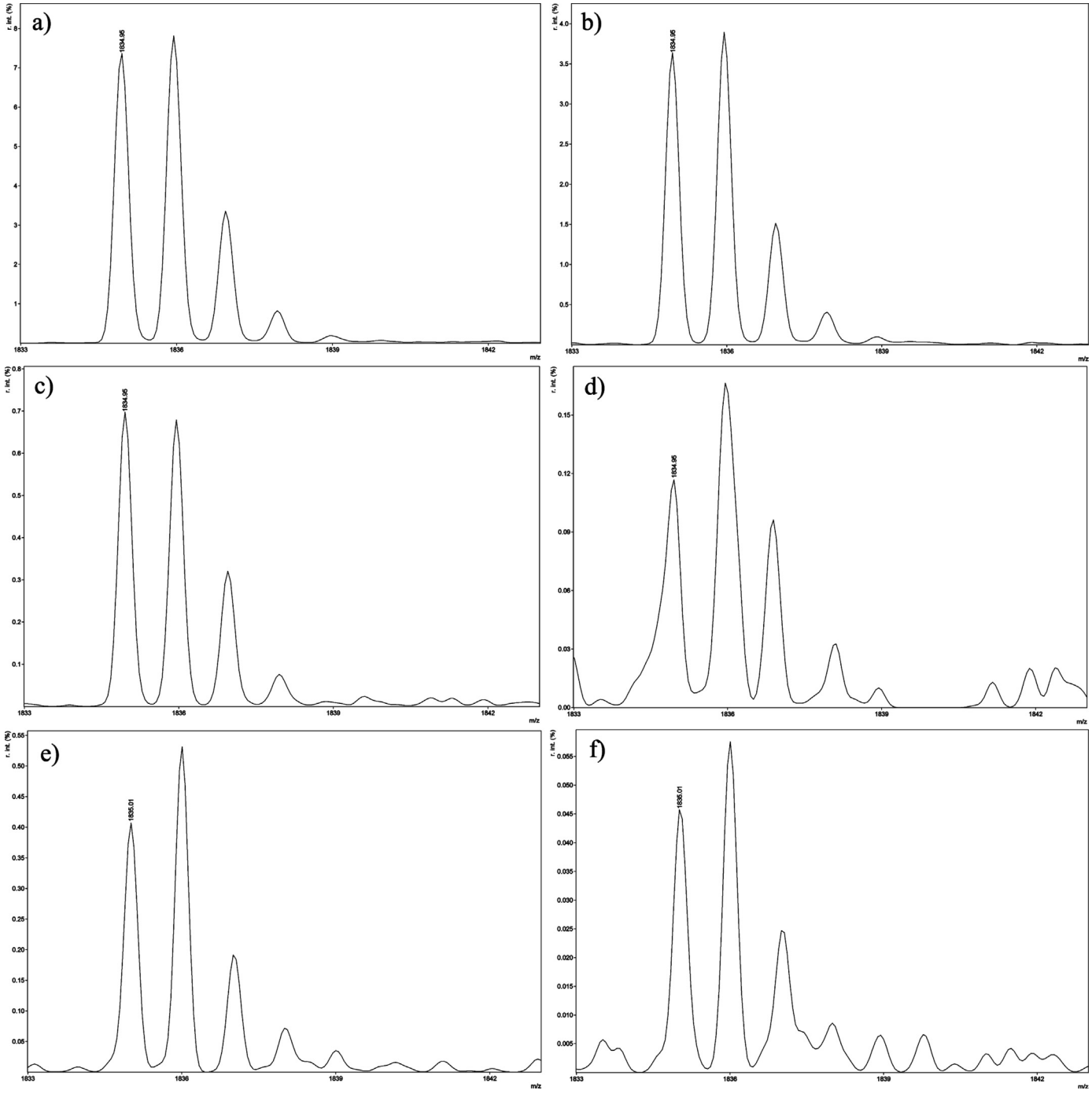

Fig. 5. Peptide *m/z* 1834.97 obtained by peptide mass fingerprinting and processed using mMass [28] (files for calculation of deamidation were processed using a specially-designed computed algorithm [19]) in undyed wool at (a) 0 h and (b) 48 h, in alum-mordanted madder-dyed wool at (c) 0 h and (d) 48 h, and in alum-mordanted weld-dyed wool at (e) 0 h and (f) 48 h.

Table 3
Oxidation scores in control (year 0) and experimentally buried samples (years 1, 2, 4, 7 or 8). U = Undyed; M = Madder-dyed; W = Weld-dyed. L = Lejre (Denmark); R = Rørmyra (Norway); Mu = Marstrand (Sweden) uncovered (sewn into an open mesh nylon envelope); Mc = Marstrand (Sweden) covered (sewn into an open mesh nylon envelope and enclosed in an additional envelope constructed of a non-woven geotextile fabric) [4].

Samples	U	M	W	LU				LM				LW				RU				RM				RW			
Year	0	0	0	1	2	4	8	1	2	4	8	1	2	4	8	1	2	4	8	1	2	4	8	1	2	4	8
Score	1	0	1	0	1	0	0	0	0	1	0	2	1	0	0	0	0	3	3	2	0	0	0	1	3	1	1

Samples	U	M	W	MuU				MuM				MuW				McU				McM				McW			
Year	0	0	0	1	2	3	7	1	2	3	7	1	2	3	7	1	2	3	8	1	2	7	8	1	2	3	7
Score	1	0	1	0	0	1	1	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	2	1	2	0

Table 4
Oxidation scores summarised for the archaeological samples.

	Coppergate (York)									Pavement (York)						
	3959	4060b	4066	4067	4070	4073	4076	4077	4078	4082	4085	4087a	4089	4091	4093	4094
Score W	0	0	4	0	6	7	0	0	0	7	9	5	2	3	9	7
Score Y	4	9	15	5	9	7	9	10	6	4	8	9	6	7	14	0
Score F	1	3	1	2	1	0	0	2	1	2	3	1	1	2	1	1
Score H	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0
Total score WYFH	5	12	22	7	16	14	10	12	7	13	20	15	9	12	24	8

	New	Reykholt (Iceland)						
	4544	2896	2897	2901	2902	2906	3962	3966	4120
Score W	9	0	0	4	8	4	2	2	2
Score Y	13	0	4	0	0	4	3	0	0
Score F	3	1	1	1	1	3	1	0	1
Score H	0	0	0	0	0	1	0	0	0
Total score WYFH	25	1	5	5	9	12	6	2	3

New: Newcastle.

levels observed in other studies by amino acid analysis [11] and suggesting a level of protection to photo-oxidation in dyed wool. Furthermore, oxidation in both mordanted weld and madder-dyed samples was primarily observed in the keratins while the unmordanted samples had a large contribution to oxidation from the KAPs.

This scoring approach represents a relative evaluation of levels of oxidative damage rather than a quantitative measure of oxidation. However, specific markers of oxidation have previously been identified [16]. Peptide DVEEWYIR in particular is oxidised at several levels: single, double and kynurenine-type oxidation on the tryptophan [27,28]. Table 2 shows the products of oxidation created after UV irradiation in modern wool for peptide DVEEWYIR. In the undyed sample, single and double oxidations are detected after only 3 h. Single oxidation is found in the undyed sample only and up to 18 h, while the double oxidation is found up to 48 h in the undyed and dyed samples. The kynurenine modification (Fig. 3) is found after 12 h of UV exposure in the undyed samples and 24 h in the dyed samples. As would be expected, the cascade of oxidation is progressively observed in the undyed samples: the gradual evolution from single oxidation to kynurenine in undyed wool therefore represents a good basis to assess UV photodegradation in historical textiles exposed in the past or modern times to light.

In addition to oxidation, the mordanting and dyeing treatments have been shown to induce deamidation at specific residues (a post-translational modification that converts glutamine into glutamic acid, and asparagine into aspartic acid), adding a +1 Da to the mass of the peptide. Of the four selected peptides in Table 1, peptides at *m/z* 1834.97 and *m/z* 1625.84 were shown to have the fastest and the slowest rates of deamidation [18]. Fig. 4 shows the %Gln–Asn (on a 0–100% scale, with 100% representing no deamidation and 0% complete deamidation) for all four peptides and all irradiated samples. In contrast with the photo-oxidation scores, the undyed sample remained undeamidated for all peptides and up to 48 h of irradiation. The unmordanted madder-dyed sample also remained undeamidated for up to 24 h, but showed a large increase in deamidation for all peptides by the 48-h point. Alum-mordanted samples were deamidated as early as 24 h, and for the weld sample, deamidation was observed in the control sample for all but peptides *m/z* 1487.74. Fig. 5 shows the changes in the isotopic envelope due to deamidation for peptide *m/z* 1834.97 in madder and weld samples compared to the unchanged profile in the undyed sample.

4.2. Oxidation and deamidation in experimentally buried and archaeological samples

Since oxidation of the aromatic residues is likely primarily a pre-burial event triggered by the production of reactive oxidative species under UV irradiation, the amount of photo-oxidation calculated from the photoproducts detected, although not quantitative, can provide significant information on the use of a textile during its lifespan. To verify that the burial environment does not significantly influence oxidation, modern buried samples were analysed and the results given in Table 3 show that samples buried for up to 8 years have few or no oxidized peptides. The experimental burial control samples were kept in the dark in a controlled environment for 13 years before analysis and have oxidation scores of 0 for madder and 1 for undyed and weld. The buried samples generally display no observable oxidation either, with the maximum score of 3 found in some of the Rørmyra samples (undyed and weld).

Table 4 summarises the scores reported for each relevant amino acid (W, Y, F and H) and the total score in the archaeological samples. The observed peptides with oxidative modifications are given in Table S3. The scores are variable, with scores between 1 to 12 for the Reykholt samples, 5 to 22 for Coppergate, 8 to 24 for Pavement and 25 for the Newcastle sample. These scores mainly reflect the oxidation on the keratins rather than the KAPs, as KAPs are less-resistant amorphous proteins that are usually degraded first in buried samples. Less oxidation was generally observed in the Reykholt samples, potentially indicating that these textiles suffered less damage from photo-oxidation. In contrast, the highest levels of deamidation were reported for the Reykholt samples [18] (in Supplementary information Fig. S1), indicating that, in archaeologically buried samples, deamidation would be influenced more by the burial environment than pre-burial conditions. In the experimentally buried samples, the total time of burial (up to seven or eight years) was too short for any significant deamidation to be observed, although greater deamidation due to the dyeing treatment was evident in comparison to undyed wool.

5. Conclusions

Traditionally, the chemical and biodegradation of textiles has been investigated with techniques that focus on the overall changes of the material. For example, with FT–IR, changes in the bands' shape and intensity of functional groups either indicate damage or the creation of products of oxidation (for example, cysteic acid from cysteine). More specifically amino acids analysis can reveal patterns of oxidative degradation through the loss of certain amino acids, such as tyrosine and the formation of products, such as cysteic acid. But these methods are not precise enough to give details of the origin or location of the oxidative reactions. Methodologies based on mass spectrometric analysis of ancient proteins are complementary and have the potential to determine particular types of degradation pathways and the conditions that might be required to generate

them. We have demonstrated here how oxidative modifications at the amino acid residue level can be assessed qualitatively, offering a new analytical approach that should be considered when studying questions of fibre treatment and degradation in archaeological textiles. However using the scoring system alone, it is only possible to compare samples from similar contexts. Ancient samples, in particular from the ground, suffer protein damage, and possibly loss of oxidised peptides. KAPs are for instance easily lost in old wool. Other factors that could influence background oxidation is the type of wool, processing of the fibres from scouring to dyeing, type of dyes, or exposure to light before the product is finished, some of them difficult to assess in archaeological samples. Therefore, the oxidation scores could not be directly compared between modern and ancient samples. Specific markers of degradation have however been identified. As with deamidation [18], peptides are likely to have different rates of oxidation, which will have to be determined. This redox proteomics approach should be further developed by using quantitative methods (for example iTRAQ labelling, which allows peptides from different samples to be compared in the same analysis by binding them with specific isobaric mass tags). Such methods have been used to compare photo-oxidation in samples originating from similar contexts and comparable conditions [17] [29]. This approach would be particularly useful to the study of museum textiles, for which exposure to light and history are well documented.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.culher.2015.02.006.

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