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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]. Photo-oxidation 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

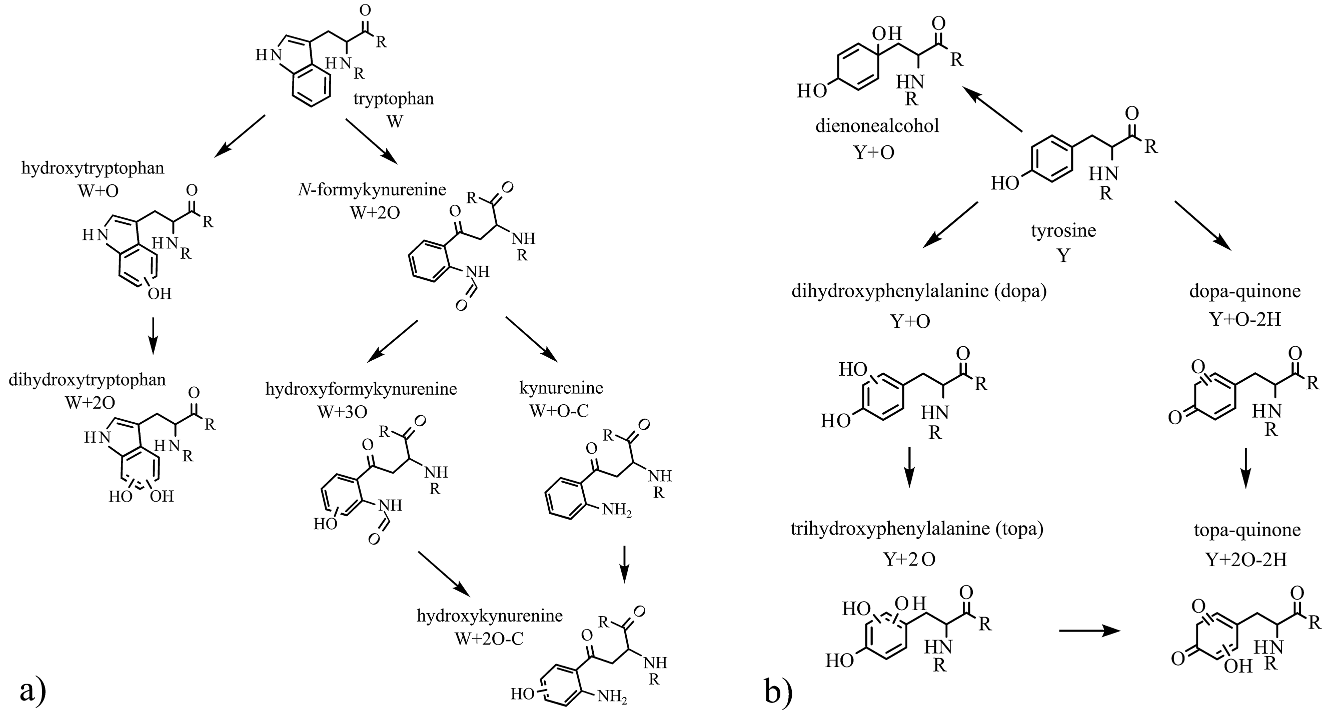

Fig. 1. Pathways of oxidation showing the resulting products of oxidation of (a) tryptophan, and (b) tyrosine.