analytical chemistry

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Modeling Deamidation in Sheep α-Keratin Peptides and Application to Archeological Wool Textiles

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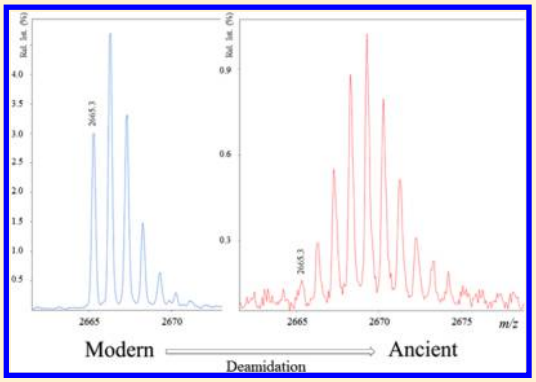
Supporting Information

ABSTRACT: Deamidation of glutamine (Q) and asparagine (N) has been recognized as a marker of degradation and aging in ancient proteins. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to study deamidation in wool textiles, we identified eight peptides from α-keratin proteins in sheep wool that could potentially be used to assess the level of degradation. For each chosen peptide, the extent of deamidation was determined by comparing the calculated theoretical distribution with the measured distribution using a genetic algorithm that gives the best fit to the measured distribution. Variations in the levels of deamidation were observed between peptides and in modern wool samples buried for up to 8 years in which deamidation levels were relatively low under short-term burial. In contrast, deamidation was higher in archeological textile fragments from medieval sites ranging from the 9th to 13th century in York (United Kingdom) and Newcastle (United Kingdom) and from the 13th to 16th century in Reykholt (Iceland). Major differences were observed between the British and the Icelandic samples, showing a negative correlation between age of samples and levels of deamidation, but highlighting the effect of local environment. In addition, nanoscale liquid chromatography−electrospray ionization tandem mass spectrometry (nanoLC−ESI-MS/MS) data indicated that deamidation in wool's α-keratin was influenced by primary and higher-order structures. Predominance of deamidation on glutamine rather than asparagine in the archeological samples was attributed to a higher abundance of Q in the α-helical core domain of keratins, neighboring residues and steric hindrance preventing deamidation of N.



The importance of wool as a major source of fiber and economic trade in Europe throughout the medieval times has long been established.[1] Archeological textiles recovered from excavations, although fragmentary and fragile, are valuable finds for the study of textile production in the past (e.g., weaving and dyeing techniques, patterns, and styles), its trade (e.g., textile provenance), and the development of sheep breeding (e.g., fineness of wool, type, origin[2,3]). In an earlier publication,[4] we highlighted predominant degradation routes for modern wool fabrics buried in different soil contexts, combining the effects of dyes and burial environment to assess degradation at the protein level of wool fiber. Fibers were either degraded through hydrolytic processes or preserved through protein cross-linking, depending on the soil conditions.

Degradation is also visible at the amino acid residue level: protein deamidation, the process by which glutamine (Gln/Q) and asparagine (Asn/N) are converted into glutamic (Glu/E) and aspartic (Asp/D) acids (resulting in a mass shift of +0.984 Da), has been recently identified as an important biomolecular marker of the deterioration and natural aging of proteins in artistic and archeological materials.[5−10] In vitro and in vivo

Received: August 19, 2013
Accepted: December 3, 2013
Published: December 3, 2013

   dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

Figure 1. Type I and type II proteins with α-helical chains 1A, 1B, 2A, and 2B (red), linkers L1, L12, and L2, and C- and N-termini (black). N and Q are in bold, and peptides mentioned in the text are underlined.

studies have moreover established that nonenzymatic deamidation of asparaginyl and glutaminyl residues acts as a molecular clock to time biological processes such as the regulation of protein turnover and tissue aging.[11−17] The modification was found, for instance, to accumulate in human eye lenses' crystallins with age; the aggregation of modified proteins leads to stiffness of the lens and to age-related issues such as presbyopia and cataract.[16,18,19] Likewise, deamidation is expected to introduce irreparable damage to ancient proteins from postmortem tissues.

Wool's fibers are made principally of α-keratins packed into intermediate filaments, and these proteins are rich in Asn and Gln (Figure 1). An important introduction of negative charges will create extensive disruptions on the three-dimensional structure,[16] resulting in denaturation of the α-helices. Deamidation occurs either by direct hydrolysis or via cyclic intermediates that are also disruptive for the protein structure by causing racemization and isomerization (Asp only), as well as peptide bond cleavage on the carboxyl terminus of the Asn and Gln residues.[15,16] These mechanisms are susceptible to increase the solubility of the protein chains, and to contribute to the degradation of fibers, eventually affecting the strength and aesthetic aspect of textiles.

Whether deamidation could be used as a dating technique in postmortem tissues is, however, open to discussion. In the most recent example, deamidation levels in collagen peptides from a 700 000 year old horse specimen buried in permafrost were found to be lower than 11−19 000 years old mammoths from temperate regions.[20] The problem of burial environment can to some extent be overcome by adjusting the chronological ages of samples to their thermal ages (expressed in years equal to exposure at 10 °C,[21] www.thermal-age.eu). This has allowed reasonable comparisons between bone samples from different areas and periods in Europe.[8] A pilot study on wool (but on a limited data set) suggested it was possible to estimate the age of museum textiles using deamidation, as long as they had been maintained in constant and supposedly comparable environmental conditions.[5] The provenance of artifacts in museums is not always clear and to guarantee that such factors (i.e., humidity, temperature, light, and pollutants) have been stable over hundreds of years of storage can only be hypothetical: efficient climate control only started to be implemented in museums in the 1950s,[22] and appropriate guidelines are still being debated.[23] Deamidation is influenced by pH and temperature,[13,24,25] but other parameters such as humidity and the structural integrity of the protein will also play a role. Assessing deamidation in wool is therefore critical for (1) evaluating deamidation as a good marker of molecular damage in wool textiles, (2) identifying reliable peptides for studying deamidation, and (3) validating deamidation as a time-dependent modification.

In this study, we adapted a previously published methodology[10] to resolve deamidation in the isotopic distributions of key peptides identified by peptide mass fingerprinting (PMF) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Using the peptide profile of sheep wool,[26] α-keratin markers that contain between two and five deamidation sites (no peptide could be found on the MS profiles with only one site) were identified, and deamidation was evaluated in aged, buried, and archeological samples.

α-Keratins are made of a rod domain of left-handed coiled-coil segments (secondary structure) and of nonhelical end and tail domains. The helical chains 1A, 1B, 2A, and 2B are separated by flexible linker regions L1, L12, and L2[27] (Figure 1). Keratins are furthermore assembled in heterodimers (tertiary structure) of a type I (acidic pI) and a type II (basic to neutral pI) protein, themselves assembled into tetramers (quaternary structure). All selected markers for deamidation are part of the rod domain, which contains most of the basic residues targeted by trypsin, the enzyme used for digestion, and all but two are from type I keratins (Table 1). In studies of

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

Table 1. Deamidation Markers: Sequence, Type (Acidic I or Basic II), and Chain Domains (α-Helical 1A, 1B, 2A, and 2B, or Linker L1, L12, and L2), Theoretical $[M + H]^+$, Modifications (Carboxymethylation of Cysteine Adds 58 Da), Deamidation Sites, First-Order Deamidation Half-Times in Days at pH 7.4, 37.0 °C, 0.15 M Tris−HCl,[a] and the First Residue To Be Deamidated in the Archaeological Samples As Observed after ESI-MS/MS Analysis

sequence	type: domain	$[M + H]^+$	modifications	deamidation sites	first-order deamidation half-times in days[a]	residue primarily deamidated first
QNQEYQVLLDVR	I: 2B	1487.74	Gln → pyro-Glu N-term Q or Glu → pyro-Glu N-term Q	Q1-N2-Q3-Q6	N1, N2, Q3 = NA; Q6 = 8000	not conclusive
QNQEYQVLLDVR	I: 2B	1504.77		Q1-N2-Q3-Q6	N1, N2, Q3 = NA; Q6 = 8000	Q1 > N2
LNVEVDAAPTVDLNR	I: L12−2A	1625.84		N2-N14	N2 = 294 ;N14 = 62	N2
TVNALEVELQAQHNLR	I: 2B	1834.97		N3-Q10-Q12-N14	N3 = 27; N14 = 116; Q10 = 6000; Q12 = 5500	N3 (group B) but Q10 (group A)
(a) SDLEANVEALIQETDFLR	II: 1B	2063.02		N6-Q12	(a) N6 = 254; Q12 = 7700	(a) Q12
(b) SDLEANSEALIQEIDFLR	II: 1B				(b) N6 = 15; Q12 = 7700	(b) Q12
LEAAVTQAEQQGEVALNDAR	II: 2B	2113.04		Q7-Q10-Q11-N17	N17 = 32; Q7 = 4300; Q10, Q11 = NA	Q10
SQQQEPLVCPNYQSYFR	I: L1−1B	2144.96	carboxymethylation C	Q2-Q3-Q4-N11-Q13	N11 = 114; Q13 = 6400; Q2, Q3, Q4 = NA	Q3/Q4 (1 sample)
(a) YSCQLNQVQSLIVSVESQLAEIR	I: 2B	2665.30	carboxymethylation C	Q4-N6-Q7-Q9-Q18	(a) N6 = 57; Q4 = 4800; Q9 = 4400; Q18 = 6100; Q7 = NA	(a) Q9
(b) YSCQLSQVQSLIVNVESQLAEIR	I: 2B				(b) N14 = 291; Q4 = 4800; Q9 = 4400; Q18 = 6100; Q7 = NA	(b) N14 > Q9

[a]Ref 31.

bone collagen, deamidation rates were found to be faster on Asn than on Gln,[8,20] consistent with half-times predicted from synthetic short peptides[15,28−31] that show deamidation of Asn is generally faster based on the primary structure alone.[32] The rates of deamidation are, however, also influenced by higher-order structures of proteins, and by their local environment.[16,17,33] The effect of primary and secondary structures was qualitatively assessed after nanoscale liquid chromatography−tandem mass spectrometry (nanoLC−MS/MS) analysis, and considerations could be formed about the prevalent sites of deamidation, emphasizing the importance of glutamine deamidation in archeological wool.

EXPERIMENTAL SECTION

Control Samples. The fabric was a highly fulled twill (vadmel) woven in modern nonpigmented white wool fiber by Røros Tweed a/s (Røros, Norway) in 1997. A piece of the fabric was mordanted with alum ($AlK(SO_4)_2{\cdot}12H_2O$) and dyed red by the addition of dry madder (*Rubia tinctorum* L.) root while another piece of fabric was left undyed. The dyeing protocol followed procedures established at the Textile Workshop at "Land of Legends Lejre", Denmark, and replicated methods used in antiquity. Control samples were kept in darkness in climate-control stores at the NTNU Museum, Norwegian University of Science and Technology in Trondheim, Norway.

Hydrothermal Aging. Pieces from the control fabrics (15 mg) were weighed into sterile glass ampules and submerged in 900 μL of ultrapure water; each ampule was flame-sealed. Samples were placed in an oven maintained at a constant temperature of either 80 or 140 °C, for a specified time. Samples heated at 140 °C were removed from the oven after 1 and 6 h, while samples heated at 80 °C were removed after 120, 720, and 1440 h. The supernatant water was removed, and the sample was rinsed twice with ultrapure water and dried at <40 °C for a maximum of 12 h.[34]

Experimental Burial. Subsamples from the control fabrics were buried from 1998 to 2006 in two sites and retrieved after 1, 2, 4, and 8 years. They were placed together with excavated soil into perforated PVC plastic pipes (16 mm diameter), and the modules were buried in hand-drilled boreholes 1 m deep. The modules were buried in bogs at "Land of Legends Lejre" (Denmark) and Rørmyra, Sør-Trondelag County (Norway). The characteristics of each site and burial conditions have been described elsewhere.[4]

Medieval Samples. Nine samples (9−13th century) are from the textiles finds from the excavations conducted at 16-22 Coppergate in York in 1979−1981,[35] and seven are (10−11th century) from the excavations conducted at 6-8 Pavement on the site of the Lloyds Bank in York in 1972−1973.[36] One sample was obtained from a 13th century site on Queen Street, Quayside in Newcastle upon Tyne.[37] Finally eight samples (13−16th century) come from an archeological high-status farm site at Reykholt, Borgarfjördur in Iceland.[38,39] Details of the archeological samples are given in the Supporting Information, Table S1.

Sample Preparation for Mass Spectrometry. Samples between 1 and 10 mg (depending on availability of materials, see the Supporting Information, Table S4) were reduced to powder, either by using liquid nitrogen or, for degraded and archeological samples, by gentle agitation in deionized water to break down the samples. Samples were then left to dry and then solubilized by overnight shaking in a solution of 8 M urea,

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

50 mM Tris, and 50 mM TCEP at pH 8.3 (200 μL for the archeological samples, 500 μL for all others). A 200 μL aliquot of the supernatant was alkylated with 150 mM iodoacetic acid and vortexed for 4 h in the dark. This was followed by 24 h of dialysis with 100 mM ammonium bicarbonate (two changes) on 3500 MWCO dialysis Slide A Lyzer minidialysis units from Thermo Scientific (U.S.A.). The whole supernatant from archeological samples or approximately 25 μg from experimental samples (based on previous protein quantitation estimations[4]) was digested with 0.5 μg of trypsin, overnight at 37 °C. In addition, the insoluble fraction of the archeological samples was digested with 1.5 μg of trypsin in 50 mM ammonium bicarbonate at pH 8.3 for nanoLC−MS/MS analysis only. All samples were then dried down and resolubilized in 10 μL of 0.1% TFA.

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 and let to dry. A 1 μL aliquot of analytical solution was applied and removed after 1 min followed by 1 μL of washing buffer (0.1% TFA). The residual droplet was removed, and 1 μL of recrystallization solution [0.1 mg of CHCA in 6/3/1 (ethanol/acetone/0.1% TFA)] was applied. The plate was loaded in a Ultraflex 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.

Calculation of Deamidation. Deamidation rates were calculated for selected peptides commonly observed by PMF[26] and from known keratin sequences, containing one or more Gln or Asn residue (Table 1). Any peptides with isotope distributions that overlapped those of neighboring peptides were avoided as deconvolution of the various deamidation states would not be possible. The sequences of the selected peptides were checked against MS/MS data obtained by nanoscale liquid chromatography−electrospray ionization tandem mass spectrometry (nanoLC−ESI-MS/MS).

For each chosen peptide, the m/z at which it should be observed is calculated taking into account any modifications. Deamidation at a single site results in a mass shift of approximately +1 Da so that the first peak of the isotope distribution for the deamidated peptide coincides with the second peak of the isotope distribution for the nondeamidated peptide (at the resolution of our data). Figure 2a shows the overlapping distributions obtained for a peptide with 40% deamidation at a single site. The combined distribution that would be observed in this case is shown in Figure 2b. We denote the proportion of nondeamidated peptide by β_0 and the proportion of deamidated peptide by $\beta_1 = 1 - \beta_0$. Each additional deamidation results in a further 1 Da mass shift so that k possible deamidation sites lead to k overlapping isotope distributions, which must be deconvoluted in order to determine the proportion of the peptide in the various possible deamidation states. The proportions, β_j, with j sites deamidated (for $j = 0, ..., k$), are determined using a genetic algorithm to optimize the fit to the observed distribution as described in Wilson et al.[10] A description of the genetic algorithm is given in the Supporting Information. Each β_j shows the overall proportion with j sites deamidated and does not differentiate between sites, which could be either Gln or Asn. Here $\beta_0 + \beta_1 + ... + \beta_k = 1$ and the number of sites for which there is evidence of deamidation is j, where $\beta_j \neq 0$, but $\beta_i = 0$ for $i = j + 1, ..., k$. The percentage of nondeamidated peptide that still has either glutamine or asparagine residues, i.e., $100\beta_0$%, which we refer to as %Gln-Asn, has been shown by van Doorn and co-workers[8,10] to vary for glutamine residues in different peptides within the same PMF.

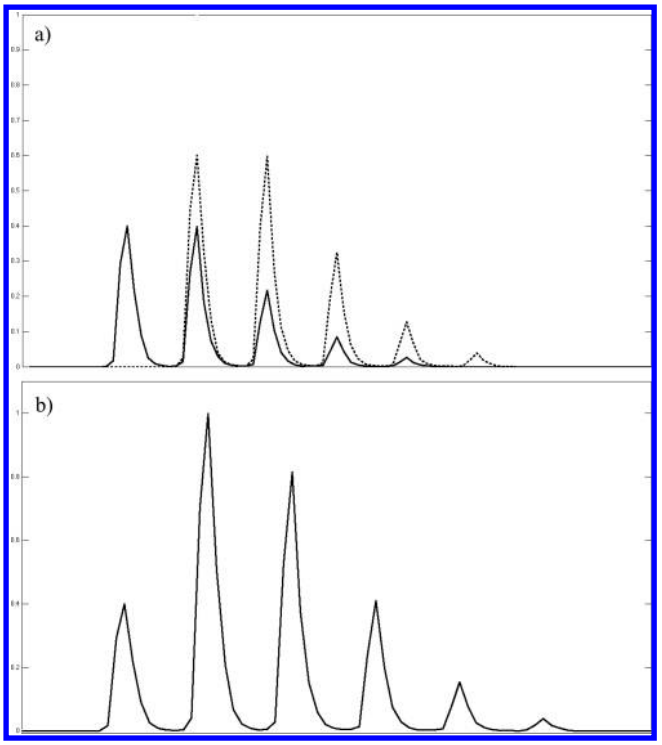


Figure 2. In panel a the overlapping isotope distributions for a peptide with 40% deamidation of one residue. The solid and dotted lines shows the isotope distributions corresponding to the undeamidated and deamidated peptide, respectively. The distribution in panel b corresponds to the combined distribution.

Protein Analysis by NanoLC−ESI-MS/MS and Mascot Search. Protein separation was carried out on an Ultimate nanoflow nanoLC equipped with Famos autosampler and Switchos column switching module (LC-Packings, The Netherlands) connected to a QSTAR Pulsar i mass spectrometer (Applied Biosystems, U.S.A.). Mascot Daemon (Matrix Science, U.K.) 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). The running and bioinformatics analysis parameters are described in the Supporting Information.

RESULTS

Calculation of Deamidation in Undyed and Madder-Dyed Wool. Deamidation was compared in undyed wool and wool dyed with madder, one of the most widely used dyes in the past. Levels of deamidation in controls, referred as %Gln-Asn (on a 0−100% scale, with 100% representing a peptide with no deamidation and 0% complete deamidation), are presented Figure 3 for the eight peptides described Table 1. Deamidation in undyed wool was observed only for peptides m/z 2063.02 and 2113.04, perhaps as a result of sample

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

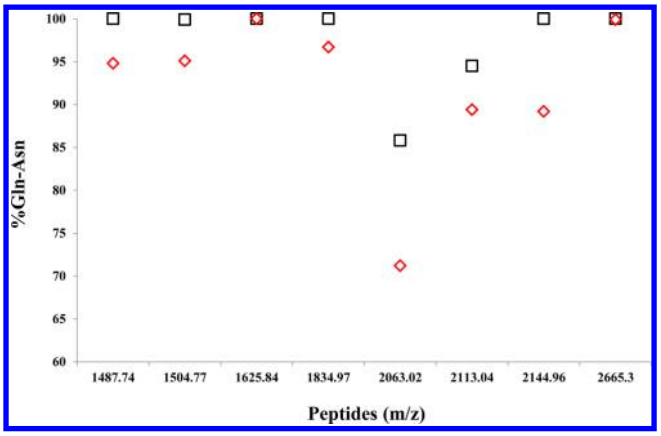


Figure 3. %Gln-Asn in undyed (square) and madder-dyed (diamond) wool controls for peptides m/z 1487.74, 1504.77, 1625.84, 1834.97, 2063.02, 2113.04, 2144.96, and 2665.30.

preparation. Artifactual deamidation during enzymatic digestion was found in previous studies to have little impact:[5,40] for instance, Li et al.[40] used $H_2{}^{18}O$ labeling and found no detectable Asn deamidation in a 4 h trypsin digestion of three rapidly deamidating peptides. The untreated control samples attest of the absence of artificial deamidation for a majority of the chosen markers. The peptides that are deamidated at $t = 0$ will consequently be considered unsuitable for studies on deamidation in wool, whether deamidation is a result of sample preparation, enzymatic digestion or natural. In contrast to undyed wool, madder-dyed wool was deamidated to various levels in all but m/z 1625.84 and 2665.30. The peptide least stable to deamidation was m/z 2063.02 with the highest levels of deamidation for both undyed and dyed samples.

Pieces of the same fabrics were aged at two temperatures (short and long aging) in aqueous solution to evaluate deamidation as a function of time/temperature. Although the hydrothermally aged samples hydrolyzed rapidly (Supporting Information, Figure S1), peptides at m/z 1487.74, 1504.77, and 1834.97 were found in a large majority of samples (with the exception of madder-dyed samples during long aging at 80 °C, Supporting Information, Table S4). Figure 4 shows the %Gln-Asn values obtained during aging at 140 °C in the undyed (Figure 4a) and madder-dyed fabric (Figure 4b) from 0 to 6 h. The rates of deamidation and half-lives assessed from the exponential correlation (6.8 and 4.8 h in undyed and madder-dyed, respectively, at m/z 1487.74, 4.8 and 4.0 h at m/z 1504.77, and 4.6 and 3.7 h at m/z 1834.97) tend toward a faster rate of deamidation for the madder-dyed fabric, consistent with the control samples.

This experiment showed that (1) under constant temperature, a steady increase in deamidation with time of hydrothermal aging is observed, (2) deamidation is significantly accelerated with temperature, (3) peptides have different speeds of deamidation, and (4) during accelerated aging, the madder-dyed sample deamidates faster than undyed wool.

Calculation of Deamidation in Buried Fabrics. Fabrics evaluated after short-term burial showed no significant increase in deamidation in any of the experimental sites or between minimal and maximal burial times; therefore, values were averaged for all years by site of burial (Figure 5). Averaged values had small standard deviations for all peptides but m/z 2113.04 (Supporting Information, Table S4). In comparison, deamidation levels were systematically higher in the archeological samples that can be classified into two distinct groups: the Reykholt samples with high levels of deamidation in group A and the Newcastle–York (Coppergate and Pavement) samples with lower levels in group B (MS/MS data, see the Supporting Information, Tables S2 and S3, also indicated that peptides from the Reykholt samples were generally observed with all sites deamidated; this was not the case in the York and Newcastle samples).

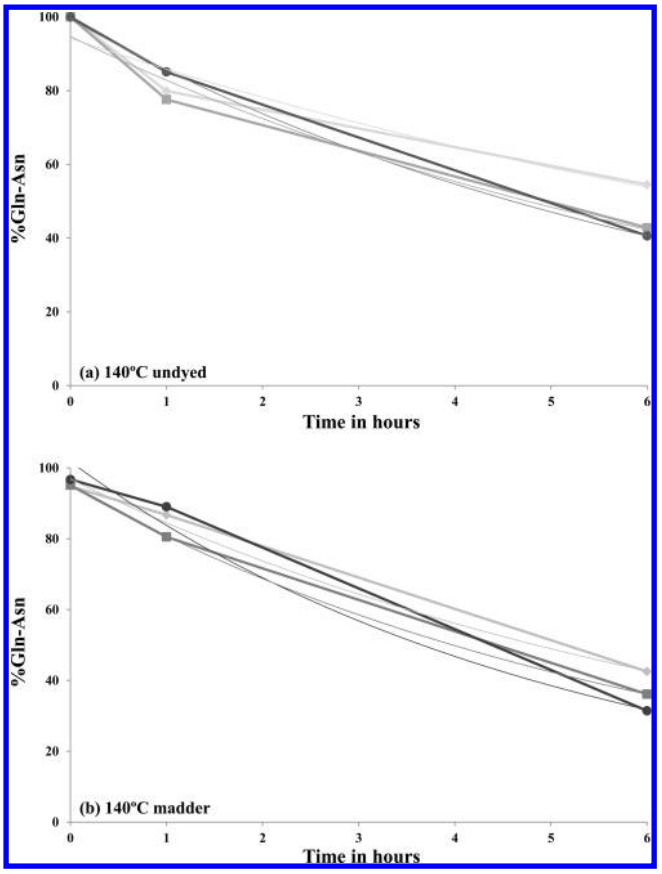


Figure 4. %Gln-Asn in thermally aged undyed (a) and madder-dyed (b) fabrics at 140 °C (0–6 h); m/z 1487.74 (diamond), 1504.77 (square), and 1834.97 (circle).

Both experimental sites Lejre and Rørmyra are acidic bogs; in addition the latter is located at higher latitude and is rich in sphagnum moss, which was found to inhibit bacterial degradation. Biodegradation was found to be faster at Lejre resulting in samples highly degraded and discolored after 8 years; in comparison samples at Rørmyra were well-preserved (with evidence of protein cross-linking) and had lost little dye.[4] As previously observed, the burial site had an impact on the data set of peptides obtained: frequently absent peptides in the experimental burial samples from Rørmyra (but not Lejre) were m/z 2063.02 and 2144.96. Both peptides, together with m/z 2113.04 were also regularly missing from the York assemblages (B), while at Reykholt (A), m/z 1625.84, 1834.97, and 2063.02 were primarily found, the latest two with very low %Gln-Asn. In the archeological samples and at Lejre, m/z 1625.84 had the lowest levels of deamidation (or high %Gln-Asn values), followed by m/z 2665.30 in group B, while at Rørmyra the lowest levels were found for m/z 1834.97, in contradiction with aging experiments.

During short-term burial at Rørmyra where samples remained colored, madder-dyed samples had lower %Gln-Asn

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567–575

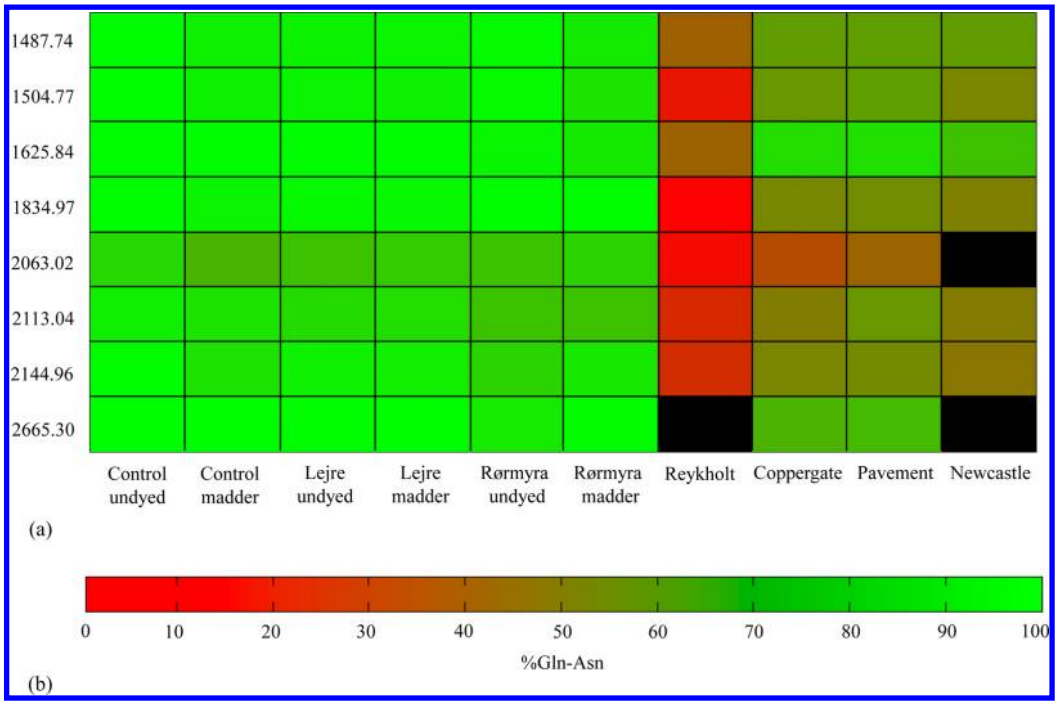


Figure 5. %Gln-Asn in the undyed and madder-dyed controls and short-term burial samples Lejre and Rørmyra (average values) and in the archeological samples (average values) for all peptides. In panel a the values are shown in a color-coded plot with the color key shown in panel b.

values for m/z 1834.97, 1625.84, 1504.77, and 1487.74 compared to undyed samples. However, at the Lejre site where the madder dye was washed away after 4 years of burial little difference was seen between undyed and dyed samples. Likewise, the few archeological samples with traces of madder dye had similar levels of deamidation than undyed samples from their respective archeological sites, which would indicate that the effect of dyes on deamidation is irrelevant under long-term burial, likely as an effect of dye deterioration.

DISCUSSION

Effect of Local Environment. Deamidation indicates that group A is chemically "older" than group B, although the time period determined from the archeological layers shows the samples from group A to be younger, even after adjusting to their thermal age (Supporting Information, Table S1). In Figure 6, %Gln-Asn is plotted against thermal age for peptides

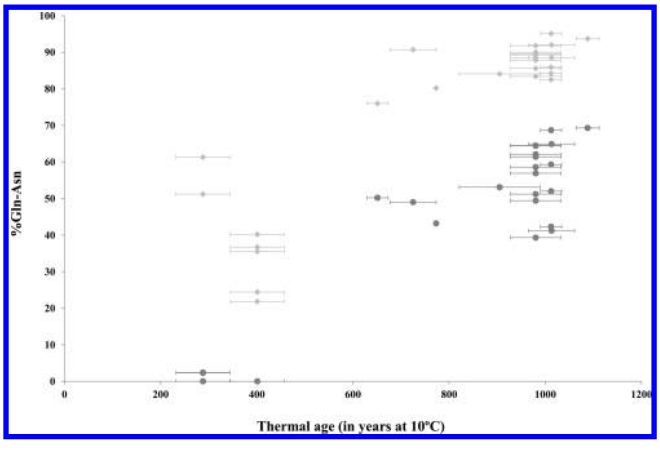


Figure 6. %Gln-Asn in the archeological samples adjusted to thermal age (range) for peptides m/z 1625.84 (diamond) and 1834.97 (circle).

at m/z 1625.84 and 1834.97: while samples from group A have an increase in deamidation with increased thermal age, this is not the case for samples from group B. For instance, m/z 1834.97 is completely deamidated in group A, and further levels of deamidation (calculated between zero and two or more deamidated sites, Supporting Information, Figure S3) show that, with the exception of just one sample, the highest deamidation values in group A correspond to older samples (400 years). The high levels in deamidation observed in the Reykholt samples might be due to the actual temperature of the site; the samples were excavated from a former farm connected to a hot spring by stone-built conduits that allowed water and steam to circulate beneath the farm buildings.[38] This might have resulted in a higher ground temperature (in which case the calculated thermal age of the samples would be incorrect), and accelerated deamidation.

Effect of Primary and Higher-Order Structure. MS/MS data often indicated Gln as the prevalent site for deamidation in the archeological samples, in contradiction with the first-order deamidation half-times (given in days at pH 7.4, 37.0 °C, 0.15 M Tris−HCl) taken from Robinson and Robinson tables[31] (Table 1) that give shorter half-lives for the Asn residues based on the primary structure only. In peptide $\underline{Q_1}\underline{N_2}\underline{Q_3}EY\underline{Q_6}VLL$-DVR, for instance, m/z 1504.77 was more deamidated than m/z 1487.74 where residue Q1 is modified in pyroglutamic acid. Differences in levels of deamidation are evidence of some deamidation occurring on the Q_1 residue: using Mascot's site analysis, MS/MS analysis confirmed Q1 to be the primarily deamidated residue followed by N2 in m/z 1504.77. Mascot's site analysis of MS/MS data, using the Mascot Δ score (quantifying the probability of a modification happening on a specific site and previously described for the localization of phosphorylation sites[41]), is given for all peptides with two or more sites of deamidation in the Supporting Information. Figure 7 shows the MS/MS spectra of peptide SDLEA$\underline{N_6}$V-EALI$\underline{Q_{12}}$ETDFLR (m/z 2063.02) in an archeological sample

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

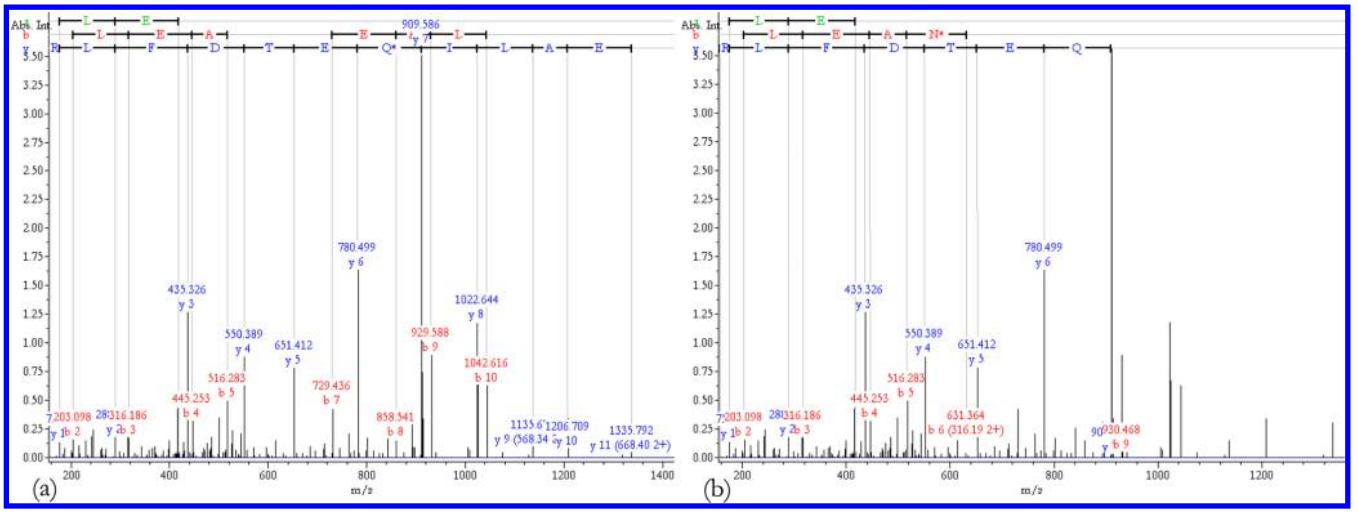


Figure 7. NanoLC−MS/MS spectra of triply charged peptide SDLEANVEALIQETDFLR (m/z 688.737) in the Reykholt sample 2906 with (a) deamidation of Q (Mascot score of 104) or (b) deamidation of N (Mascot score of 20).

showing a better match with deamidation on Q12 rather than N6. Preference for glutamine deamidation in these markers can be attributed to the presence of aspartic acid residues E directly preceding (m/z 2113.04), or following (m/z 1487.74/1504.77, 2063.02, and 2144.96) the glutamine residues.

The presence of charged residues (particularly in C-terminus), such as the basic residue histidine, have been shown to accelerate deamidation[18,33] (TV$\underline{N_3}$ALEVEL$\underline{Q_{10}}$A-$\underline{Q_{12}}$H$\underline{N_{14}}$LR at m/z 1834.97, for instance, has the highest rate of deamidation). In contrast, the peptides with the lowest rates (Figure 4) are m/z 2665.30 (YSCQL(N/S)QVQSLIV(S/N)VESQLAEIR), where charged residues are not in direct vicinity, and m/z 1625.84, L$\underline{N_2}$VEVDAAPTVDL$\underline{N_{14}}$R, that contains no glutamines. On the basis of primary structure only that predicts rapid deamidation of the Asn residues (Table 1), this peptide should deamidate quickly. But in spite of N_{14} being next to a basic residue, it is still the peptide with the lowest rate of deamidation in controls and archeological samples. It has been shown that the α-helical structure poses conformational constraints to the deamidation of Asn residues since the preferred mechanism is through succinimide intermediates.[42] According to the α-helical assembly established in human hair keratins,[43] m/z 1625.84 is located partially on linker L12 and partially on the coiled-coil chain 2A (Figure 1). Segment 2A is the least flexible of all helical domains.[43] It is then not surprising that the residue to be deamidated first is N_2 (located in L12) and not N_{14} (located in 2A), in spite of its longer half-life (Table 1).

There are about 25% more Gln than Asn in the rod domain of keratins, while the end and tail domains have generally more Asn. A comparison with peptides from these areas would better highlight the effect of higher-order structure in nonhelical versus helical conformations. However, these domains contain few basic residues for trypsin digestion and tend to degrade quickly in archeological (buried) samples. Only type I peptide SF/Y$\underline{N}$FCLP$\underline{N}$LSFR (N-terminus) was observed (Supporting Information, Table S2): of all the peptides observed with two Asn, it was the only one found with the maximum number of deamidation (2) in the "slow" group B, consistent with easier deamidation of Asn in this area.

α-Helical structure also affects rates of deamidation by bringing residues together in close proximity, with a maximum found in the regions of three intervening residues (corresponding to a turn of the helix).[33] An example is peptide LES$\underline{E}$I$\underline{N}$TYR and its equivalents LEA$\underline{E}$I$\underline{N}$TYR and LEC$\underline{E}$I$\underline{N}$-TYR (two intervening residues): only peptide with S was found to be deamidated in group B, a possible consequence of the catalytic effect of the hydroxyl group in serine's side chain,[33] although the effect of higher order (neighboring proteins) could be considered too. In group A, however, all peptides were found with deamidation, indicative of the more advanced disruption of the secondary structure in the Reykholt samples. Other examples demonstrating the effect of the primary and higher-order structure are described in the Supporting Information.

■ CONCLUSIONS

Using the peptide mass fingerprint of sheep wool, we identified eight markers with two to five sites of deamidation. Five (m/z 1487.74, 1504.77, 1625.84, 1834.97, and 2665.30) are sufficiently stable (no deamidation in control wool) and reliable for extensive studies of deamidation in wool. Their different rates of deamidation make them good markers to track samples with a wide range of deamidation levels. The three remaining peptides are either readily lost in archeological samples, deamidate too quickly, or are too sensitive to sample preparation or chemical processing of the fibers.

All peptides are part of the rod domain rich in α-helix: the primary structure and the constraints imposed by the higher-order structure in keratins are conducive to the rapid deamidation of glutamine, in spite of the slow rates of deamidation observed by Robinson et al.[31] In addition direct hydrolysis is favored at low pH (hydrolysis via acid catalysis) relative to deamidation via the succinimide intermediate.[44] In archeological samples where acidic soils favor wool preservation (the pH of the archeological soils varies here from acidic at Reykholt (pH range of 5.25−6.00[38]) to slightly acidic to neutral at York[45,46]), direct hydrolytic deamidation of glutamine is facilitated.

Levels of deamidation in aged wool exposed to constant temperature confirmed that it is time-dependent in a controlled environment, thus validating that the genetic algorithm for calculating deamidation is well-adapted to keratin peptides with multiple sites of deamidation. In archeological wool, however,

dx.doi.org/10.1021/ac4026362 | Anal. Chem. 2014, 86, 567−575

deamidation correlated poorly with age of samples. Deamidation is a useful marker of degradation and should now be investigated in above-ground fabrics, in particular to understand the effects of a range of environmental factors (temperature, light, humidity), as well as wool treatments (dyeing, mordanting, wrapping in metal threads). Preliminary data have indeed indicated that treatment of wool (with dyes and mordants) increases deamidation under short-term UV accelerated aging (unpublished data). Recently, Kirby et al.[47] observed parchments of similar periods with different deamidation profiles, suggesting the level of deamidation was dependent on the process of fabrication of the samples rather than their age. Understanding how wool fibers are affected by deamidation is essential for the long-term conservation of archeological and historical textiles and could be a useful parameter to monitor aging of ancient textiles in museum environments.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ACKNOWLEDGMENTS

This research was supported by a Marie Curie International Outgoing Fellowship (FP7-PEOPLE-IOF-GA-2009-236425, THREADs) between the University of York, U.K. and AgResearch, New Zealand. All experiments were conducted in the proteomics facilities at the AgResearch Lincoln Research Centre.

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