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Identification of collagen-based materials in cultural heritage

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All stakeholders in cultural heritage share an interest in fabrication methods and material technology. Until now methods for analysis of organic materials, particularly proteins, have not been widely available to researchers at cultural institutions. This paper will describe an analytical method for the identification of collagen-based materials from soft tissue sources and show examples of its application to diverse museum objects. The method, peptide mass fingerprinting (PMF), uses enzymatic digestion of extracted proteins to produce a mixture of peptides. The mass spectrum of the mixture contains characteristic marker ions—a peptide mass fingerprint—which are compared to species-specific markers from references as the basis of identification. Preliminary results indicate that analysis of materials from aged samples, several different tissue types, and tanned or untanned materials yields comparable PMF results. Significantly, PMF is simple, rapid, sensitive and specific, has been implemented in a museum laboratory, and is being practiced successfully by non-specialists.

Introduction

Collagen-based material (skin, intestine, esophagus and tendon) is used pervasively in a wide variety of applications including clothing, religious objects, watercraft, tools, toys and models. In the context of cultural heritage, species identification of these materials has historically relied on oral tradition and visual (macroscopic and microscopic) and tactile examination. Such methods may be limited due to availability of adequate references, sample condition, or the expertise of the researcher.[1] This paper will describe an alternative approach, the use of peptide mass fingerprinting (PMF), to identify collagen-based materials in objects of cultural heritage. Results will be shown to demonstrate that the described method can be implemented in a museum laboratory, provide material information to a previously unobtainable level of accuracy and specificity, and be practiced successfully by non-specialists.

Background

A wide variety of analytical techniques is in place for the analysis of materials found in artworks and objects of cultural heritage,[2] and mass spectrometric techniques are especially well represented. For example, gas chromatography-mass spectrometry (GC-MS), pyrolysis gas chromatography-mass spectrometry (Py-GC-MS), and liquid chromatography-mass spectrometry (LC-MS) are used to characterize art and archaeological materials including proteins, waxes, lipids, varnishes, resins, synthetic polymers, pigments and dyes. Colombini and Modugno[3] provide an extensive collection of reviews covering these areas.

Proteins are present in artworks and historic artifacts in many forms—as paint binders, adhesives and coatings—to name a few, and their identification is important for knowledge of artists' techniques and choices, and to guide conservation treatments. Currently, quantitative amino acid analysis (AAA) by GC-MS is the technique most often used for protein analysis, and it can detect and broadly classify proteins originating from egg, collagen and casein (Colombini and Modugno,[3] Chapter 9, for example) but cannot differentiate among sources. The objective of the work presented here is to add to the established capabilities for protein analysis by providing a method that can be used in a museum laboratory for indentifying the source of one class of proteins, collagen, with the expectation that it will in due course be extended to others.

In the last few decades there has been increased migration of analytical techniques and applications from traditional laboratory settings to use in cultural institutions, particularly to supplement or improve established methods for analyzing proteins and other organic materials. For example, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and database searching, originally developed for proteomics research,[4] has recently been applied to the analysis of a variety of artworks including paintings, gilt work, paint binders and

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protein remains in archaeological pottery.[5–9] Immunological techniques are being developed to identify proteins and gums in artworks;[10] and laser desorption mass spectrometry (LDMS) is being used to complement traditional methods for identifying organic dyes and pigments in paintings and sculpture.[11–16] The increasing availability of analytical tools and techniques provides an exceptional opportunity for conservators to acquire technical information about art and cultural heritage heretofore unobtainable. It is in this vein that we have adapted PMF as a routine means of identifying collagen-based materials in a museum laboratory.

Proteomics in cultural heritage research

Proteomics is an especially powerful tool for the study of cultural heritage as it can provide not only a sensitive means of characterizing the protein composition of a given sample,[17] but also the added possibility of species identification of the proteins themselves and, thus, the material used to create the object. The importance of such information is illustrated by recent studies where soft-ionization mass spectrometry, a mainstay in biotechnology, was applied to cultural materials and provided valuable new information and insights. In one study, Hollemeyer *et al.*[18] determined that the keratinous component of the clothing on the 5300 year-old Tyrolean mummy called iceman or Oetzi, "…was mainly consistent with what would have been expected to be available for use in the everyday life of a herdsman following sheep, cattle and perhaps goats…" and not a hunter-gatherer, who would likely have used hides from wild game. In another study, Solazzo *et al.*[19] used a proteomics approach to determine species origin of hair used to weave Coast Salish blankets, long thought to be composed of only dog hair. That study corroborated the use of dog hair, but surprisingly, discovered for the first time the importance of sheep wool in the Salish weaving tradition. Following from the above studies of keratin-based materials, our work utilizes a similar approach to identify collagen-based materials for which little or no published sequence data are available. The work presented here relies on species-specific peptide markers and spectra derived from reference materials for material identification.

Soft tissues in cultural heritage

Distinct from the keratin-based objects studied in the above applications, soft tissue derived from hide, gut and other connective tissues is composed primarily of collagen and is used pervasively in native cultures. Many cultural institutions steward extensive collections of skin, leather, and other collagen-based objects originating from nearly every geographic region of the world. Florian[20] provides a very comprehensive treatment of the use of collagen and other fibrous proteins found in cultural heritage. The present study deals with collagen originating from soft tissues; similar studies of collagen from archaeological bone have been published.[21–23]

Soft tissue material identification in current museum and library practice depends on visual examination, oral traditions and published literature, or with reference to availability and geographic ranges of mammalian populations.[24] Each of these approaches is limited and subject to difficulty. Because there are large collections that could benefit from an improved and readily available means of materials' identification, we pursued the goal of developing a simple, sensitive, accurate and specific method that could be implemented in a museum and practiced routinely by non-specialists. As has been shown by several academic groups, PMF has the necessary micro sample requirements, sensitivity, and specificity to be used successfully for the identification of proteins in artworks[25] and historic artifacts.[26] For these reasons, in addition to its lower cost and ease of implementation, we chose to use PMF *in lieu* of other techniques, such as LC-MS/MS, for protein analysis in the museum laboratory.

Peptide mass fingerprinting

PMF uses enzymatic digestion to cleave proteins at specific amino acid sites forming a peptide mixture. Each protein sequence is unique, thus the mixture of peptides is unique. The mixture is analyzed by MALDI-ToF-MS[27] resulting in a spectrum containing characteristic marker peptides—a "peptide mass fingerprint." Database searching of collagen-based fingerprints is not suitable for identifying proteins to the species level since very few mammalian collagen sequences have been published. Thus, species identification by PMF requires the generation of a reference database. Recently, Buckley *et al.*[21–23] demonstrated that PMF could be used to determine mammalian sources of archaeological bones based on species-specific sequence variations in collagen, and they developed a set of peptide markers as the basis of an identification scheme. In this work we use new sea mammal markers along with previously published markers[21,22] and visual comparison of unknown and reference spectra as the basis of soft tissue material identification.

Experimental

Reagents and materials

Trypsin (E. C. 3.4.21.4), sequencing grade, modified, Promega, Madison, WI. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), Pierce Chemical, Rockford, IL. Iodoacetamide (IAA), ammonium bicarbonate (AMBI), trifluoroacetic acid (TFA), acetonitrile (ACN), and alpha-cyano-4-hydroxycinnamic acid (αCHCA), Sigma-Aldrich Corp., St. Louis, MO, were analytical grade and used without further purification. C18 ZipTip® Pipette Tips, Millipore, Billerica, MA. Calibration standards were from AB SCIEX, Framingham, MA and included: des-Arg^1-Bradykinin (904.4681$^+$), Angiotensin I (1296.6853$^+$), Glu^1-Fibrinopeptide B (1570.6774$^+$), Neurotensin (1672.9175$^+$), ACTH (clip 1–17) (2093.0867$^+$), ACTH (clip 18–39) (2465.1989$^+$) and ACTH (clip 7–38) (3657.9294$^+$).

Extraction and tryptic digestion

Samples are removed from objects using tweezers or a sharp scalpel and are placed in 600 μL Eppendorf tubes. All subsequent operations take place in the same tube to minimize contamination and sample loss. Samples weights are not measured but are estimated to be no more than a few 10's of micrograms. Generally, as small a sample as can be handled

with a fine tweezers and observed at 30× magnification is sufficient to produce satisfactory PMF results. Samples from kayaks, hide garments, and gut objects are taken from tears or abrasions when possible and may consist of only a few thin fibers. Analyses are generally run in batches of 10–20 samples plus positive and negative controls. Total analysis time is one and a half days from extraction through MALDI analysis.

Sixty μL of 50 mM AMBI is added to samples in Eppendorf tubes and heated to 80 °C for 1 hour with intermittent agitation and visual observation to assure that the sample remains in the liquid. Samples are cooled to RT and placed in an ultrasonic bath for 10 min then heated again to 80 °C for an additional 30–60 min. Samples are cooled to RT, and 3 μL 20 mM TCEP in 50 mM AMBI is added followed by incubation at 37 °C for 20 min. Samples are cooled to RT, and 3 μL 40 mM IAA in 50 mM AMBI is added followed by incubation at RT in the dark for 30 min. Trypsin (8 μL, 0.02 μg μL^{-1} in 50 mM AMBI) is added, and the samples are incubated at 37 °C overnight. Digestion is terminated by the addition of 1 μL 10% (v/v) TFA, and samples are analyzed directly without further purification except as noted below in ZipTip® fractionation.

Procedures for the analysis of collagen from bones have been published previously.[21]

Zip tip fractionation

In some cases ZipTip® fractionation is used to help differentiate collagen species, especially to observe D and G peptides clearly, according to Buckley *et al.*[23] Digest samples (1–10 μL) are loaded onto a ZipTip® which has been conditioned with 80% ACN (v/v) 0.1% TFA followed by 0.1% TFA. Loaded samples are washed with 0.1% TFA and eluted first with 10% (v/v) ACN/0.1% TFA (Fraction 1), then 50% (v/v) ACN/0.1% TFA (Fraction 2) and analyzed separately by MALDI.

Table 1 Peptide markers used to identify mammalian materials. Family: (a) Canidae; (b) Ursidae; (c) Felidae; (d) Odobenidae; (e) Otariidae; (f) Phocidae; (g) Phocidae, Phocini tribe; (h) Bovidae; (i) Cervidae; (j) Equidae; (k) Delphinidae; (l) Phocoenidae; (m) Monodontidae; (n) Physeteridae; (o) Balaenopteridae; (p) Escgrichtiidae; (q) Balaenidae. Numbers in the column headings indicate the peptide position in the protein chain. P1 and P2 are recent additions to the original scheme of markers[21] and are useful for separating cetaceans

Species	(P1)	α2(I) 988–1000 (A)	α2(I) 494–508 (B)	α2(I) 512–529 (C)	(P2)	α2(I) 803–826 (D)	α1(I) 602–634 (F)	α2(I) 767–799 (G)
Dog/wolf (a)	1105	1226	1453	1566		2131	2853	2999
Brown bear (b)	1105	1233	1453	1566		2163	2853	2957
Lion (c)	1105	1223	1453	1566		2147	2853	2999
Atlantic walrus (d)	1105	1221	1453	1566		2121	2853	3003
Pacific walrus (d)	1105	1221	1453	1566		2121	2853	3003
Northern fur seal (e)	1105	1221	1453	1566		2121	2853	2957
Steller sea lion (e)	1105	1221	1453	1566		2121	2853	2957
Bearded seal (f)	1121	1221	1453	1566		2171	2853	2957
Hooded seal (f)	1105	1221	1453	1566		2171	2853	2957
Ribbon seal (g)	1105	1221	1453	1566		2171	2869	2957
Spotted seal (g)	1105	1221	1453	1566		2171	2869	2957
Ringed seal (g)	1105	1221	1453	1566		2171	2869	2957
Grey seal (g)	1105	1221	1453	1566		2171	2869	2957
Common/harbor seal (g)	1105	1221	1453	1566		2171	2869	2957
Harp seal (g)	1105	1221	1453	1566		2171	2869	2957
Cow (h)	1105	1208	1427	1580		2131	2853	3033
Sheep (h)	1105	1196	1427	1580		2131	2883	3033
Goat (h)	1105	1196	1427	1580		2131	2883	3093
Musk ox (h)	1105	1208	1427	1580		2131	2883	3033
Elk/red deer (i)	1105	1196	1427	1550		2131	2883	3033
Caribou (i)	1105	1166	1427	1580		2131	2883	3093
Roe deer (i)	1105	1196	1427	1580		2131	2883	3059
Horse (j)	1105	1198	1427	1550	1682	2145	2883	2999
Bottlenose dolphin (k)	1079	1205	1453	1566	1638	2119	2883	3023
Euphrosyne dolphin (k)	1079	1205	1453	1566	1638	2119	2883	3023
Risso's dolphin (k)	1063	1205	1453	1566	1638	2119	2883	3023
Pilot whale (k)	1063	1205	1453	1566	1638	2119	2883	3023
Orca (k)	1079	1205	1453	1566	1652	2119	2883	3023
Dall porpoise (l)	1079	1205	1453	1550	1652	2119	2883	3023
Harbor porpoise (l)	1079	1205	1453	1550	1652	2119	2883	3023
Narwhal (m)	1079	1205	1443	1550	1652	2089	2883	3051
Beluga whale (m)	1079	1205	1443	1550	1652	2121	2883	3051
Sperm whale (n)	1079	1205	1453	1550	1652	2133	2883	3039
Minke whale (o)	1079	1205	1441	1566	1652	2135	2883	3023
Fin whale (o)	1079	1205	1453	1566	1652	2135	2883	3023
Humpback whale (o)	1079	1205	1453	1566	1652	2135	2869	3023
Gray whale (p)	1079	1205	1453	1566	1652	2135	2899	3023
Right whale (q)	1079	1205	1453	1566	1682	2135	2883	3023

MALDI analysis

αCHCA matrix is prepared as a saturated solution in 40% (v/v) ACN/0.1% TFA. Digest samples (2–4 μL) are mixed with 20 μL matrix in a separate tube and then spotted onto the MALDI sample plate. Six peptide standards from 904 Da to 3657 Da are used as external calibrants. A Waters microMX Laser Desorption-Time of Flight Mass Spectrometer operated in reflector mode was used to collect positive ion spectra from 800–3800 Da. Resolution was >8000 ($M/\Delta M$) measured at 2465 Da allowing baseline isotopic resolution over the mass range of interest (800–3800 Da) and monoisotopic mass accuracy of at least ±0.1 Da. Each spectrum is calibrated individually using the external standard mixture. In addition, spectra are further mass locked with the known collagen ion at 1105.5676 Da when that ion is observed. Once samples are dried onto the sample plate, they are stable indefinitely and, if necessary, can be transported to off-site MALDI instrumentation. Strict adherence to the above procedures is maintained to ensure the highest degree of repeatability.

Data analysis

Table 1 shows the peptide markers used for material identification. Some markers have been published previously,[21] whereas others, particularly additional whale, seal, dolphin and porpoise markers (Odobenidae, Otariidae, Phocidae, Delphinidae, Phocoenidae, Balaenopteridae, Eschrichtidae and Balaenidae) are new. As can be seen from Table 1, markers currently defined can readily differentiate among many mammalian families but do not always allow unique identification of each species within a family, for example among earless seals (Phocidae). Materials identified with markers may be visually compared with reference spectra, using mMass[28] in the examples below, to corroborate the identification based on similarities of the total spectra. Implicit in all the identifications using this method of data analysis is the fact that identities are based only on available reference markers and spectra, which may not necessarily include all species in a family, for example. This limitation will continue to diminish as we enlarge our databases. In many cases potential identifications can be eliminated with reference to either markers or reference spectra (negative comparison), and the number of possible materials reduced.

Results and discussion

Collagen as the basis of species identification

Collagen refers to a group of structurally distinctive triple-helical proteins that contains a high proportion (>30%) of glycine and (~20%) proline amino acids. It also contains the unusual amino acids hydroxyproline and hydroxylysine and is considered to be the most abundant protein in mammals. The high abundance of collagen in hide and connective tissues permits highly sensitive analyses. There are currently at least 28 types of collagen described, although over 90% of collagen in the body derives from Type I collagen. In some collagen types, including Type I, the three polypeptide chains are genetically distinct (heterotrimeric), and are numbered as alpha chains preceding the type they originate from (*e.g.*, Type I collagen is made up of two identical α1(I) chains and one distinct α2(I) chain). These triple-helical molecules aggregate to form much larger fibrils and fibers to give tissues structural support with great tensile strength.

Buckley *et al.*[21,23] noted that the α2(I) chain is much more genetically distinct than the α1(I) chains, the latter being much more restricted to the typical repeating Gly-Xaa-Yaa motif for which collagen is well-known. The majority of markers in Table 1 is derived from the α2(I) chains.

Data repeatability

Identification of species is made with reference to known mass markers or visual comparison with reference spectra or both. Thus, successful analysis relies on data reproducibility, and is the reason that the procedures described for sample preparation and analysis are followed exactly without deviation. Fig. 1 compares a portion of the mass fingerprints from the same

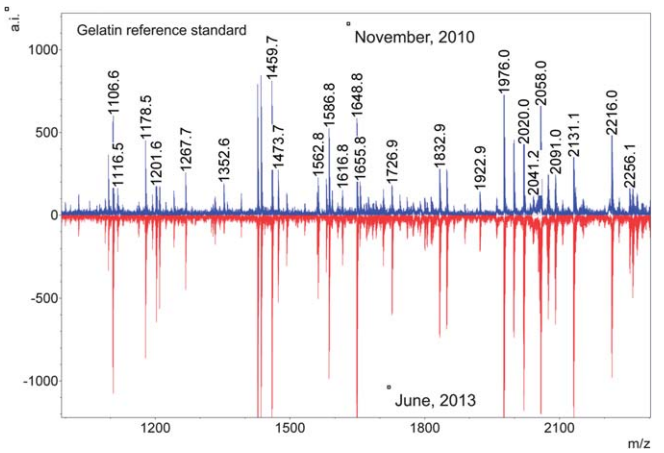

Fig. 1 Peptide mass fingerprints from the same gelatin reference sample made several years apart demonstrating the qualitative reproducibility of the described methods.

Fig. 2 9th c. Qur'an folio (Harvard Art Museums, Acc. # 1927.163) showing the sampling location along the left edge (inset). The parchment was identified as of sheep origin based on Table 1 markers.

gelatin reference sample produced almost 3 years apart, and is typical of the reproducibility we have observed with hundreds of samples and multiple non-specialist users. Since identification with marker ions is done without regard to intensity, as long as the ions are observed with signal to noise $(s/n) > 3$, the variations in intensity, such as are seen in these spectra, can be tolerated.

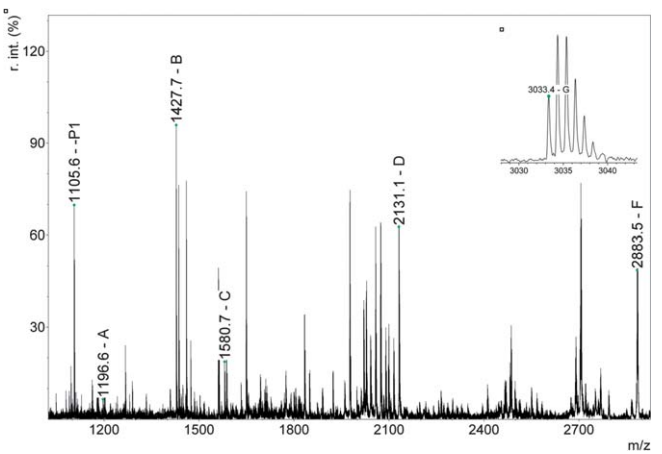

Fig. 3 Peptide mass fingerprint from the 9th c. Qur'an folio (Fig. 2) indicating marker ions (Table 1) used to identify the parchment material as sheep. The G-ion (inset) is from ZipTip® Fraction 2.

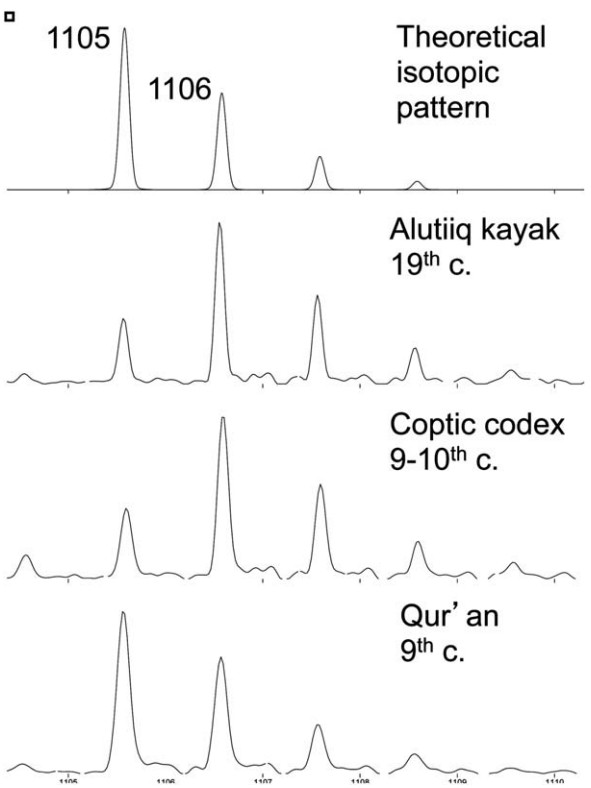

Fig. 4 Comparison of isotopic patterns for samples of different ages with the theoretical isotopic pattern for collagen α2(I), GVQGPP*GPAGPR, P* = hydroxyproline, 1105.57 Da, showing that deamidation resulting in a shift in the isotopic pattern does not necessarily track with sample age.

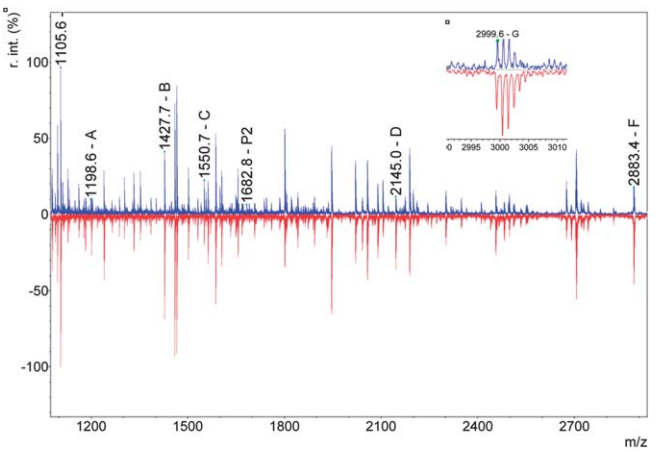

Fig. 5 Comparison of the peptide mass fingerprints from untanned horsehide (top, blue) and tanned horsehide (lower, red) with markers from Table 1 indicated. The G-ions (inset) are from ZipTip® Fraction 2.

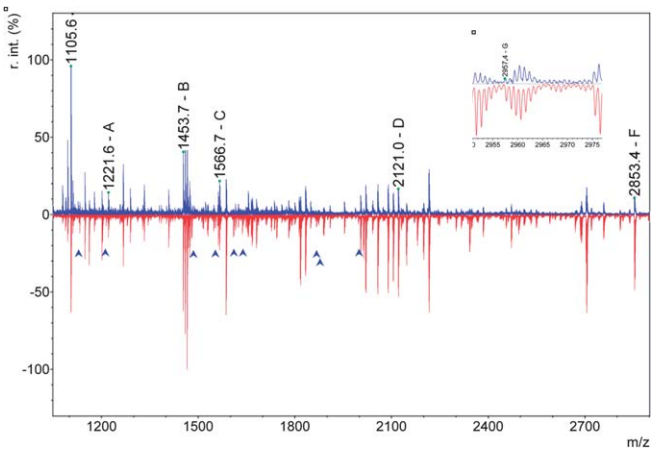

Fig. 6 Comparison of the peptide mass fingerprints from northern fur seal hide (blue, top) and esophagus (red, bottom) with markers from Table 1 indicated. Markers in the lower spectrum indicate additional ions observed in the esophagus sample. The G-ions (inset) are from the unfractionated sample.

Fig. 7 Yup'ik kayak (A) and Alutiiq kayak (B) from which samples of skin and stitching were taken for peptide mass fingerprinting. Multiple samples of stitching and covering were analyzed to verify that the same materials were used throughout.

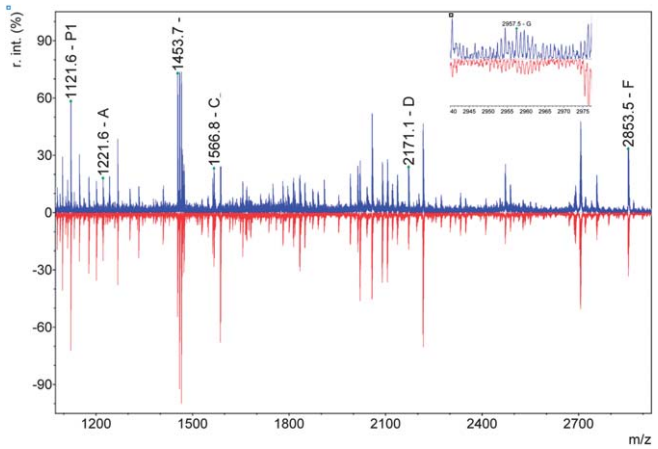

Fig. 8 Peptide mass fingerprints from the Yup'ik kayak deck strap and covering (blue, top) and bearded seal reference (red, bottom). Bearded seal markers from Table 1 are indicated in the Yup'ik kayak fingerprint. Bearded seal is unique among Phocidae with a 1121 Da A-ion and could be identified exactly among our references. G-ions (inset) are from the unfractionated samples.

Data analysis – parchment identification

A sample from a Qur'an folio dated to the 9th c. (Fig. 2) was analyzed to produce the peptide mass fingerprint in Fig. 3 and is shown here to illustrate the process of analyzing data using peptide markers. The sample was a small surface flake from a damaged area at the edge of the folio (Fig. 2 inset). The current practice for identifying parchment relies on visual and/or tactile examination of the material and usually depends on the observation of hair follicle patterns that can be compared to standards. In the case of this Qur'an folio, even though it was in very good condition, a clear follicle pattern could not be discerned, possibly the result of processing to prepare the surface for text application. The parchment was readily identified as of sheep origin based on the presence of the respective peptide markers (Table 1). Comparison with a standard fingerprint spectrum was unnecessary because other likely sources, such as cattle, goat and deer, could reliably be eliminated based on the markers alone.

Fig. 3 illustrates the data quality routinely obtained with samples from soft tissue-derived objects. Since soft tissue samples are nearly pure protein, abundant ions are produced and resolved with $s/n > 3$ to at least 3000 Da using the method described above. ZipTip® fractionation is used when necessary to clarify some marker ions (inset, Fig. 3).

Sample age

Samples from objects as early as the 9–10th c. have been analyzed, and in all cases, age and sample condition have had no significant effect on the resulting spectra and subsequent identification. Deamidation, a chemical reaction in which the amide functionality in glutamine and asparagine is replaced by a hydroxyl group resulting in a 1 Da increase in mass, has been noted in deterioration/aging of proteinaceous binders in paintings.[29] We routinely monitor the highly conserved collagen ion (α2(I), GVQGPP*GPAGPR, P* = hydroxyproline) at 1105.57

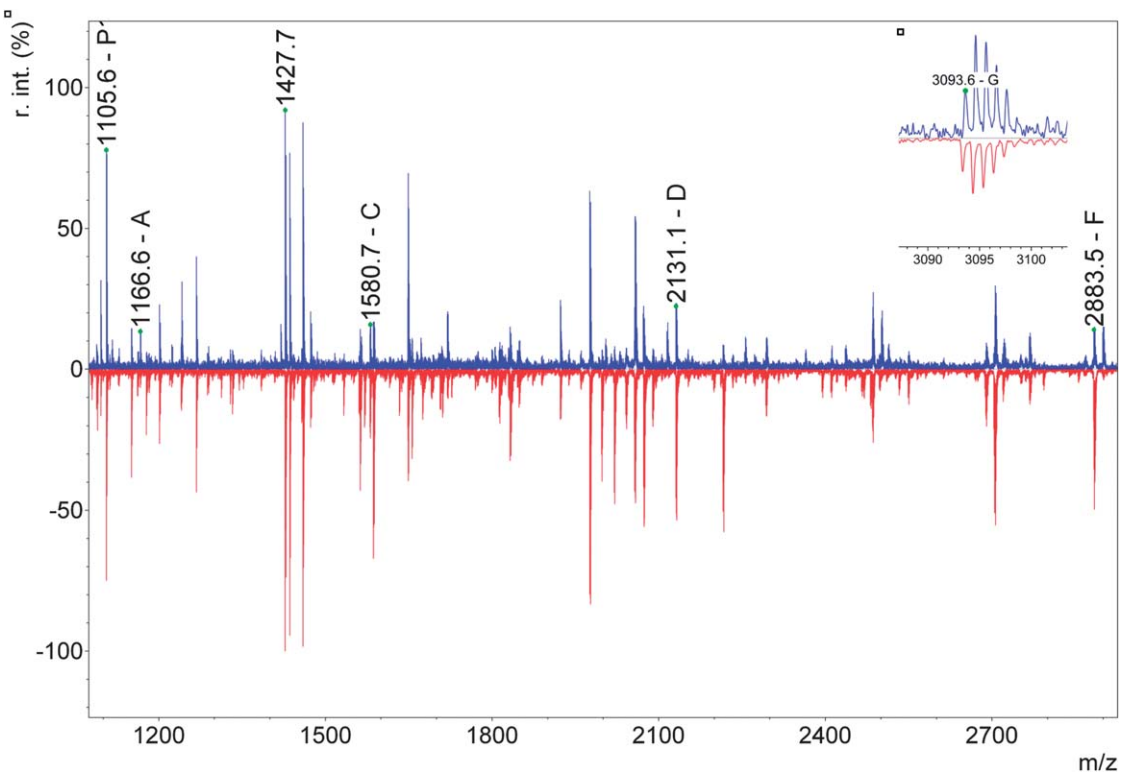

Fig. 9 Peptide mass fingerprints from the Yupi'k kayak stitching (top, blue) and caribou reference (bottom, red). Marker ions from the caribou reference in Table 1 are indicated in the kayak stitching spectrum. The G-ions (inset) are from ZipTip® Fraction 2.

Da as an indication of deamidation, and in our experience with a limited number of samples, deamidation has not correlated with age, but when observed is more likely related the production and/or use history of the object. For example, Fig. 4 compares the 1105 Da ion for the 9th c. Qur'an (Fig. 2), sheep parchment from a 9–10th c. Coptic codex, and seal skin from an approximately 200 year-old kayak, together with the theoretical isotopic pattern for the 1105 Da peptide. The kayak and codex spectra show deamidation, whereas the Qur'an spectrum does not, even though it is approximately as old as the codex. In the case of the kayak, environmental effects may be responsible for the observed deamidation. This peptide is observed in the majority of mammalian soft tissue samples and is used as an indication of deamidation to aid in reconciling mass shifts of other ions, particularly those greater than ~1700 Da, where the highest peak of the isotopic envelope is no longer the monoisotopic peak due to natural abundance of ^{13}C. In any case, deamidation does not interfere with data interpretation, and this may be an important consideration when PMF is extended to archaeological soft tissue samples.

Tanned *vs.* untanned

Hides and skin used in historical and contemporary clothing and other applications may be tanned, so we investigated whether the tanning process itself affects resultant fingerprints and the ability to identify species reliably. Fig. 5 compares fingerprints from tanned and untanned horsehide. A close visual comparison of the spectra shows only minor differences, mainly in relative intensities, consistent with spectral reproducibility discussed above. Considering peaks with $s/n > 3$, all ions observed in one are also observed in the other. Importantly, all marker ions are clearly and unambiguously observed in both spectra. Similar results were observed between tanned and untanned samples from rabbit and red fox. Thus, based on a limited set of observations, tanning does not significantly alter the resulting fingerprints.

Tissue types

Cultural objects can be composed of mammalian materials from a variety of tissues, such as skin/hide, intestine, esophagus, and tendon. It is important to know whether reference standards from one tissue type can be used as the basis for identification of other tissues from the same species. Fig. 6 compares fingerprints from northern fur seal hide and esophagus. The relative intensities vary, but, considering the presence or absence of ions with $s/n > 3$, the spectra are very similar. The main difference between the two spectra is the presence of additional ions in the esophagus sample. These ions have not been sequenced and their source is not known; they may originate from material processing. Differences noted are indicated (Fig. 6). Significantly, the differences are minor, especially considering the nature of the samples, and none of the marker peptides is affected. A similar comparison was made with harbor seal skin and untanned intestine with similar results.

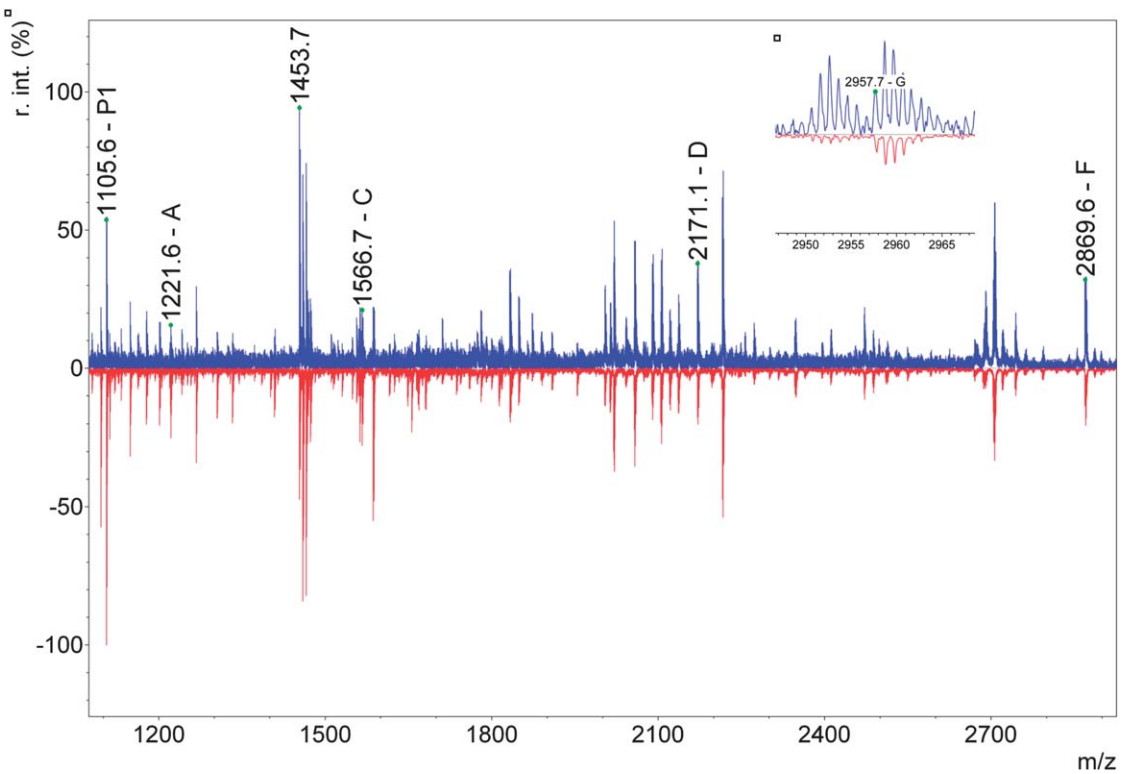

Fig. 10 Peptide mass fingerprints from the Alutiiq kayak covering and deck strap (top, blue) and harbor seal reference (bottom, red), a representative member of Phocidae, Phocini tribe. Marker ions from the harbor seal reference in Table 1 are indicated in the kayak spectrum. Phocini are differentiated from other Phocidae by the presence of the 2869 Da F-ion, but cannot be further separated with current references. G-ions (inset) are from ZipTip® Fraction 2.

Thus, as with the comparison of tanned and untanned materials and with a limited set of samples, tissue source does not significantly alter the resulting fingerprints where Type I collagen is the dominant protein.

Historic Alaskan kayaks

The following examples illustrate the use of PMF with historic objects in a museum setting. The impetus to study and conserve coastal Alaska Native objects in the collection of the Peabody Museum of Archaeology and Ethnology at Harvard University was the result of collaboration with members of the Alutiiq community in Kodiak, AK. This initiative began with the examination of two historic kayaks: a Yup'ik vessel and a single-hatch Alutiiq vessel. The Yup'ik kayak (Fig. 7A) is from Nunivak Island and dated to the late 19th c.; the Alutiiq vessel (Fig. 7B) is a single-man kayak from Kodiak Island and dated to approximately the mid 19th c. The current practice for identifying collagen-based cultural materials relies on visual examination, geographical provenance, and consultations with experts to narrow the range of possibilities. A precise identification is often impossible because of the absence of distinctive visual characteristics, particularly with internal tissues, such as the sinew used as stitching on the kayaks.

Samples of stitching and skin were collected from damaged or degraded areas of each kayak and analyzed by PMF. Based on the presence of characteristic peptide markers, the skin of the Yup'ik kayak was identified as a type of earless seal (Phocidae) and, more specifically, bearded seal, based on the presence of a unique marker ion (1121 Da, Table 1). Comparison of the kayak skin spectrum with the bearded seal reference spectrum corroborated this finding (Fig. 8). The Yup'ik stitching was identified as caribou by the presence of marker ions and was also corroborated by comparison with the standard peptide mass fingerprint (Fig. 9). Skin covering from the Alutiiq kayak was identified as a type of earless seal, and more specifically as the subset with a 2869 Da peptide F^{21} ion characteristic of the Phocini Tribe of Phocidae (Fig. 10). Comparison of available reference spectra did not show differences among the members of this group that could reliably allow differentiating them further. Stitching from the Alutiiq kayak was identified as whale, specifically humpback whale, based on marker ions (Fig. 11).

The results from the kayak materials largely corroborated information provided by representatives from the Alutiiq community with one notable exception: the skin covering on the Alutiiq kayak was initially thought to be Steller sea lion, an eared seal (Otariidae). However, marker ions can clearly

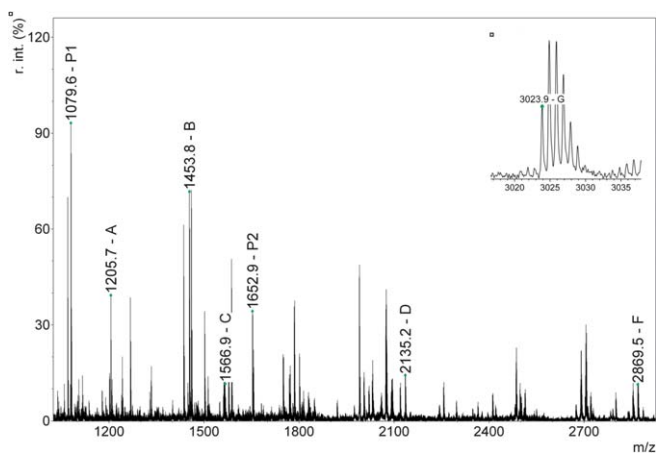

Fig. 11 Peptide mass fingerprint from the Alutiiq kayak stitching identified as humpback whale based on marker ions in Table 1. Humpback whale can be differentiated from the other baleen whales in Table 1 by the 2869 Da F-ion. The G-ion is from ZipTip® Fraction 2.

Table 2 A selection of the Alaskan Native objects analyzed and identified through PMF covering a wide variety of object types and materials. Numbers in parentheses are Peabody Museum of Archaeology and Ethnology accession numbers

Object	Sampling location	Museum ID	PMF ID
Gutskin bag (2103)	Gut	Seal	Eared seal
Gutskin bag (2103)	Painted stripe	Seal	Seal (Phocidae/ phocini)
Gutskin bag (2103)	Sinew	Unknown	Caribou
Gutskin bag (48414)	Gut	Walrus or sea lion	Eared seal
Gutskin bag (48414)	Black border	Walrus or sea lion	Bear
Gutskin bag (48414)	Sinew	Unknown	Caribou
Gutskin bag (76018)	Red painted stripe	Bear	Eared seal
Gutskin bag (76018)	Gut	Bear	Eared seal
Gutskin bag (76018)	Border sinew	Unknown	Eared seal
Gutskin bag (76018)	Embroidery sinew	Unknown	Dog/wolf
Gutskin bag (48414.1)	Gut	Unknown	Bear
Gutskin bag (48414.1)	Red and green border	Unknown	Bear
Gutskin bag (48414.1)	Sinew	Unknown	Caribou
Gutskin cap (48415)	Gut	Seal	Eared seal
Gutskin cap (48415)	Painted border	Unknown	Seal (Phocidae/ phocini)
Gutskin cap (48415)	Sinew	Unknown	Caribou
Spear with pouch and sinew (1620)	Pouch	Sea lion	Eared seal
Spear with pouch and sinew (1620)	Sinew	Whale	Caribou
Gutskin coat (56749)	Gut	Seal	Eared seal
Gutskin coat (56749)	Black overlay	Unknown	Eared seal
Gutskin coat (56749)	Sinew	Unknown	Eared seal
Kayak model (1203)	Skin	Unknown	Seal (Phocidae/ phocini)
Kayak model (1203)	Sinew	Unknown	Right whale
Kayak model (1204)	Skin	Sea lion	Eared seal
Kayak model (1204)	Sinew	Unknown	Right whale
Kayak model (11255)	Skin	Unknown	Eared seal
Kayak model (11255)	Gut	Unknown	Eared seal
Kayak model (11255)	Sinew	Unknown	Fin whale
Kayak model (11256)	Skin	Unknown	Humpback whale
Kayak model (11256)	Gut	Unknown	Eared seal

differentiate Otariidae and Phocidae (2121 Da *vs.* 2171 Da, Table 1), and within Phocidae to Tribe Phocini, which includes ribbon, spotted, ringed, gray, harp and common seals. This new information adds to the historical understanding of the kayaks and will positively impact the living art of kayak making on Kodiak Island.

Gut skin objects, kayak models and other objects

In addition to the kayaks, nearly 100 samples from 30 Alaskan Native objects were analyzed by PMF. Table 2 lists a selection representative of the entire list to illustrate the range of materials that have been encountered and successfully identified by a non-specialist in a relatively short period of time. The statistics clearly demonstrate the benefits of routine, sensitive and accurate materials identification in a museum setting: 63% previously "unknown" were identified to the species level in some cases and to the family level in others, 13% erroneously identified were corrected, and 13% were more accurately described.

Conclusions

The case studies presented in this paper show the results of analyses of cultural materials using PMF, as well as the benefits of employing this technique in a museum laboratory. Our goal of providing a simple, sensitive, specific and routine method for use by non-specialists for the identification of collagen-based materials has been demonstrated.

Using an extensive set of marker ions, some samples could be identified to a species level; all samples could be identified to a family level. The data in Table 1 allows specific identification of materials from 17 families ((a)–(q), Table 1) as well as to subfamily groups, such as cow, sheep, goat and musk ox (Bovidae), elk/red deer, caribou and roe deer (Cervidae) and, as has been pointed out above, within Phocidae to the Phocini tribe. In addition to the markers in Table 1, many other potentially diagnostic ions appear in all spectra, and these may in the future provide a means to differentiate further within families.

The PMF technique has allowed a considerable amount of data about diverse materials to be collected within a relatively brief time frame. Confirmed species identifications will allow researchers to better understand the availability of specific materials in a given region and, in some cases, help in sourcing an object of unknown provenance. Artisans and cultural groups can also use the information in their efforts to better understand and sustain their heritage.

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