2/ Understanding Medieval Textile Production and Provenience in the Darkhad Valley Through Biomolecular Analyses

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Abstract

In the spring of 2019, Dr. Bayarsaikhan Jamsranjav from the National Museum of Mongolia brought textile fragments (11th to 14th c.) recovered from recently looted graves to MCI, among them some unique silk pieces with gold thread. The gold threads of Mongolia belong to a particular type of metal thread that are made by coating an organic substrate (paper or animal skin) with a protein adhesive and a layer of gold or gilt silver. The corpus of samples from Mongolia is very diverse and contains a large variety of elaborately dyed silk woven fabrics as well as fragments of garments made with animal fibers and leather. Woolen fabrics, fur, and leather will be analyzed by ZooMS (zooarchaeology by mass spectrometry), a method using peptide mass fingerprinting to identify collagen proteins in leather and keratin proteins in animal fiber to species. Proteomics analysis will be performed: (1) on the silk fibers themselves to inform on the type of silk (wild or domesticate) and degradation of the textiles, and (2) on the protein layers of the metal threads to determine the type of proteins and the species used, thus revealing the technologies of fabrication and possibly origin of the threads. Biomolecular analysis of the corpus from the Darkhad Valley will shed a new light on herding and trade networks of the people living in northern Mongolia in medieval times.

Introduction

Not much is known about the archaeology of the Darkhad Valley, with the notable exception of the Mongolian Deer Stone Project, initiated in 2002 by the Smithsonian's Arctic Studies Center. This project has primarily focused on the investigation of deer stones— megalithic standing stones dating from the Late Bronze Age (1400-700 BCE), with carved decorations that would have played an important part in forming a ritual landscape. The project has also afforded the opportunity for researchers to engage in ethnographic studies of the local populations, khirigusuur monuments in the valley, climatic and floral analysis, and more work highlighting the ethnographic and archaeological nature of the Darkhad Valley and the larger Hovsgol aimag and Northern Mongolia region. This work, however, has been primarily focused on the Bronze Age, with the exception of the ethnographic work done on local and surrounding populations. The discovery in 2017 of medieval cemeteries at Khorig and Nomt Mountain and their subsequent collection and analysis is the first work done on a medieval site in the Darkhad Valley.

Dr. Bayarsaikhan Jamsranjav and his team salvaged textile fragments and other artifacts by sieving the spoil piles of 29 graves. The collection ranges from small fragments to nearly complete garments, many of which retain their original strength, weaving characteristics, and

decorative patterns. Among the materials salvaged is the most important archaeological collection of medieval silk garments ever recovered from the Mongol Empire period (12-15th C.), and because of their provenance from a single period and geographic region, potentially one of the most important collection of silk in the world. Some silk pieces have gold threads of a particular type that are made by coating an organic substrate (paper or animal skin) with a protein adhesive and a layer of gold or gilt silver. Dr. Bayarsaikhan Jamsranjav donated samples of silk and other clothing elements (leather, fur) to MCI for scientific analyses. In particular, proteomics analysis will be performed: (1) on woolen fabrics, fur, and leather to identify collagen proteins in leather and keratin proteins in animal fiber to species, (2) on the silk fibers themselves to inform on the type of silk (wild or domesticate) and degradation of the textiles, and (3) on the protein layers of the metal threads to determine the type of proteins and the species used, thus revealing the technologies of fabrication and possibly origin of the threads.

Tissue and species identification by proteomics

Proteomics is a suite of techniques based on mass spectrometric analysis that studies the protein composition of tissues or other biological systems. The protein sequence is obtained from genes and the total of all proteins composing a sample or tissue is called a proteome. Proteins are made of long chains of amino acids (called residues when they are linked by peptidic bonds in the protein chain), forming polypeptides. They are macromolecules of high molecular weight, ranging from a few dozen residues to thousands of residues for the largest proteins. Structural proteins, such as collagen in skin (e.g. leather), keratin in hair and wool, and fibroin in silk, are fibrous proteins that provide mechanical support, strength, and a protective framework. They have three-dimensional structures in the form of alpha-helix (alpha-keratin), beta-pleated sheets (heavy-chain fibroin), or triple helix (collagen) that make them more resistant to environmental degradation [1]. For archaeological and historical textiles and clothing, characterizing ancient proteins has relevance for questions such as techniques of fabrication, material availability and variety, trade of materials, domestication and breeding of animals.

Fibrous proteins. Archaeological garments made of leather, animal fibers and silk can be identified to species by the proteomics analysis of their respective structural proteins. Collagen is the main component of hides and leather; the collagen fibrils consist of bound tropocollagen proteins, themselves assembled from three left-handed helical polypeptide chains. The three helical chains are twisted together and stabilized by hydrogen bonds to form a right-handed triple helix. Collagen has a very repetitive pattern with abundant proline (Pro) and hydroxyproline (Hyp), and one amino acid, glycine, occurring every three residues in a Gly-X-X pattern. Mammalian hair has three structural elements: the outer layer made of cuticle cells that overlap to create a scale pattern used for microscopic identification, the cortex made of macrofibrils and, for some species, a medulla at the core of the fiber. The macrofibrils are composed of

intermediate filaments (IFs) of trichocyte keratins arranged longitudinally, cross-linked to structurally irregular keratin-associated proteins (KAPs) that compose the matrix surrounding the IFs. The hair keratins have a high content of the sulfur-containing amino acid cysteine, forming abundant cross-links (disulfide bridges) between the keratins' head and tail domains and the KAPs. The KAPs fall into three main categories: high-sulfur (HSPs), ultra-high-sulfur (UHSPs) and high glycine-tyrosine (HGTPs) proteins. Silk is an extracellular proteinaceous fiber reeled from the cocoon of silkworms. It is primarily produced from the domesticated silkworm *Bombyx mori* but other species have been exploited and are referred to as wild silks (for example Tussah, Muga or Eri silk). The reeled fiber is made of two brins of highly crystalline and insoluble proteins, the fibroins. The fibroins are very distinct between the domesticated silk and the non-domesticated silk from Antheraea and Samia genera (most commonly employed in textile production). The differences are characterized by higher ratios of the amino acid alanine in wild silk leading to differences in secondary structure, the presence of a light-chain fibroin in the domesticated silk but not the wild silks, and differences in the fiber's secretion mechanisms.

Proteomics analysis. In proteomics analysis, proteins are solubilized and cleaved at specific residues through enzymatic digestion; the resulting fragments are called peptides that are characterized by mass spectrometry. In mass spectrometry, molecules are ionized and identified by means of their mass-to-charge ratios (*m/z*). The resulting mass spectra are plots of the relative abundance of ions as a function of their *m/z* values. In MS mode, peptides are identified by their mass only; in MS/MS mode, a peptide is selected, isolated and fragmented in a collision cell, and the resulting fragments are acquired by a mass analyzer to form a spectrum that will be read as the amino-acid sequence of the peptide. The mass spectra generated are compared with protein sequences in databases.

Collagen and keratin-based tissues are processed using a similar extraction protocol adapted to archaeological samples that is based on solubilization in a urea solution and digestion with trypsin [2]. Because silk is insoluble in water due to the compact beta-sheet arrangement of the heavy-chain fibroin and does not easily denature in any common protein extraction buffer [1], specific protocols were developed for solubilization and digestion of the fibroins using calcium salts and digestion with chymotrypsin. So far, seven different species of Bombyx, Antheraea, and Samia silks can be distinguished using the newly-developed proteomics methodologies [3].

All samples are analyzed on a Thermo Scientific Dionex Ultimate 3000 UHPLC system (for peptides separation) directly coupled to a Thermo Scientific LTQ Velos Dual Pressure Linear Ion Trap mass spectrometer. PEAKS 8.5 (Bioinformatics Solutions Inc.) is mainly used to search the RAW data for matches against publicly available sequences in imported UniProt (www.uniprot.org) and NCBI (https://www.ncbi.nlm.nih.gov/protein) databases.

The samples

During the 2018 season of the "Northern Mongolia" archaeological project, the project team recovered silk and other textile fragments from medieval grave sites on the adjacent mountains of Khorig and Nomt, in the Darkhad Valley. The two sites are close in proximity to one another, and the Nomt site is visible from Khorig. The graves and monument found consisted of circular stone mounds. The presence of these cemeteries had been well known by locals, but previously undisclosed to foreign archaeologists prior to the looting. About 40 samples were given to MCI from graves from Khorig, and one non-grave monument at Nomt (Table 1).

Table 1: Short sample description of the Khorig and Nomt samples received by MCI

Material	Site Name	ID Number	Material	Site Name	ID Number
Silk + gold	Khorig	L-1-T1	**Silk**	Nomt	NU-T1a
Leather	Khorig	L-12-LTHR1	**Fur**	Nomt	NU-T1b
Silk	Khorig	L-12-T12	**Wool**	Nomt	NU-T2
Silk + gold	Khorig	L-12-T2	**Silk**	Nomt	NU-T3a
Silk + gold	Khorig	L-12-T3	**Silk**	Nomt	NU-T3b
Silk + gold	Khorig	L-12-T4	**Silk**	Nomt	NU-T3c
Silk + gold	Khorig	L-14-T1	**Silk**	Nomt	NU-T4a
Leather	Khorig	L-14-LTHR1	**Silk**	Nomt	NU-T4b
Leather	Khorig	L-23-LTHR1	**Silk**	Nomt	NU-T4c
Silk	Khorig	L-20-T1	**Silk**	Nomt	NU-T5a
Leather	Khorig-2	L2-497-LTHR1	**Silk**	Nomt	NU-T5b
Silk + gold	Khorig-2	L2-498-T1	**Silk**	Nomt	NU-T5c
Leather	Khorig-2	L2-504-LTHR1	**Fur**	Nomt	NU-T5d
Leather	Khorig-2	L2-504-LTHR2	**Silk**	Nomt	NU-T6a
Silk	Khorig-2	L2-504-T1a	**Silk**	Nomt	NU-T6a
Silk + gold	Khorig-2	L2-504-T1b	**Silk**	Nomt	NU-T7
Leather	Khorig-2	L2-516-LTHR1	**Silk**	Nomt	NU-T8
Leather	Khorig-2	L2-516-LTHR2	**Silk**	Nomt	NU-T9
Leather	Khorig-2	L2-530-LTHR1	**Silk**	Nomt	NU-T10a

Silk	Nomt	NU-T10b

Preliminary results

Fur. Furs are an important element of Mongolian clothing. Furs were acquired from animals such as sable, ermine, squirrel, rabbit, fox or even snow leopard and lynx. Two fur samples were obtained from the Nomt site radiocarbon dated to the 11th c.-mid 12th c. The fur comes from inside of the silk textiles, and the two fur samples have been very well preserved. Proteomics identification indicates that both samples are from a Mustelidae species. **Table 2** shows in details the main keratin proteins identified in NU-T1b: type I keratins Ha1 to Ha8 (acidic type) and type II keratins Hb1 to Hb6 (basic type). Because *Mustela putorius furo* (ferret) and *Enhydra lutris kenyoni* (sea otter) are the only two species represented in the public database of keratin sequences available to date, our set of proteins match either one of these two species. However, other Mustelidae species in Mongolia include the ermine (Mustela sp.), sable (Martes sp.) wolverine (Gulo sp.) and European otter (Lutra sp.). Further identification will require a robust database of keratin sequences from reference specimens.

Table 2: Main protein matches in sample **NU-T1b**, showing protein accession number in NCBI, protein name, and identified species (*Mustela putorius furo:* ferret; *Enhydra lutris kenyoni:* sea otter). The protein score (-10lgP), protein percentage coverage (%) and number of peptides (#) identified are obtained from PEAKS PTM after searching against an NCBI database containing all keratin sequences for mammals. Searches were carried out using trypsin as enzyme, one allowed non-trypsin cleavage at any end, one missed cleavage, peptide mass tolerance (PMS) of 10 ppm, fragment mass error tolerance (MS/MS) of 0.02 Da, carbamidomethylation as a fixed modification, and deamidated (NQ), and oxidation (M) as variable modifications. A maximum of three PTMs (Post-Translational Modification) were allowed.

Accession	Keratin	Species	Score	% cov.	# pep.
XP_004774748.1	Hb6	*Mustela putorius furo*	204.55	79	83
XP_004774745.1 **XP_022375067.1**	Hb5	*Mustela putorius furo* *Enhydra lutris kenyoni*	200.42	80	64
XP_022375216.1	Hb1	*Enhydra lutris kenyoni*	197.78	70	75
XP_022375220.1	Hb3	*Enhydra lutris kenyoni*	195.01	76	75

XP_022364865.1 **XP_004772812.1**	Ha3-II	*Enhydra lutris kenyoni* *Mustela putorius furo*	188.16 184.68	77 74	69 69
XP_012901344.1	Ha3-I	*Mustela putorius furo*	184.55	79	68
XP_022364879.1	Ha4	*Enhydra lutris kenyoni*	186.52	70	67
XP_022364864.1	Ha1	*Enhydra lutris kenyoni*	186.33	72	65
XP_004772830.1	Ha7	*Mustela putorius furo*	179.45	64	48
XP_004772809.1	Ha8	*Mustela putorius furo*	175.21	68	47
XP_022365048.1	Ha5	*Enhydra lutris kenyoni*	152.76	56	34
XP_022365105.1	Ha2	*Enhydra lutris kenyoni*	144.68	47	29
XP_004774805.1	Hb2	*Mustela putorius furo*	137.55	42	26

Golden threads. Out of the ten silk samples from Khorig (Table 1), seven were gilded with either gold leaf or powder (**Figure 1**) or had metal threads made with gold leaf applied to paper or skin strips (**Figure 2**), subsequently twisted or woven into the textile. Radiocarbon dates for Khorig were obtained from graves 1, 12, 19, 21 and 24. Only two of these graves (1 and 12) had textile fragments sent to MCI. However, the dates obtained, the late 13th c. to the late 14thc., correspond to the period of the Mongol Empire when textiles with gold threads were known to have been produced, thus confirming the medieval timeframe for at least six of the 19 samples from Khorig. Gold threads made with an organic substrate, such as the ones shown in figure 2, are indeed typical of this period and were produced in workshops in China and Persia. The threads covered with gold (figure 1), either painted or coated in gold powder or leaf is a very rare occurrence, rarely mentioned in texts in English [4,5].

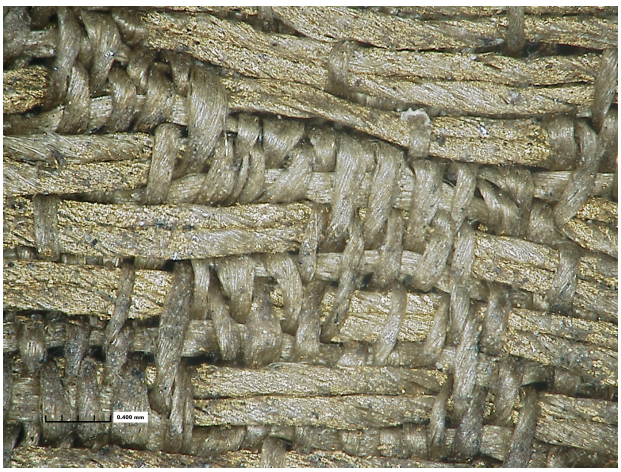

Figure 1. Left: L-12-T4 (100x magnification, scale 0.4 mm); **Right**: L-14-T1 (80x magnification, scale 0.4 mm). Both are likely examples of gold-coated threads with either gold leaf or gold powder. Images acquired by 3D digital light microscopy with HIROX KH-8700, by Jacob Kalodner, Museum Conservation Institution, Smithsonian Institution

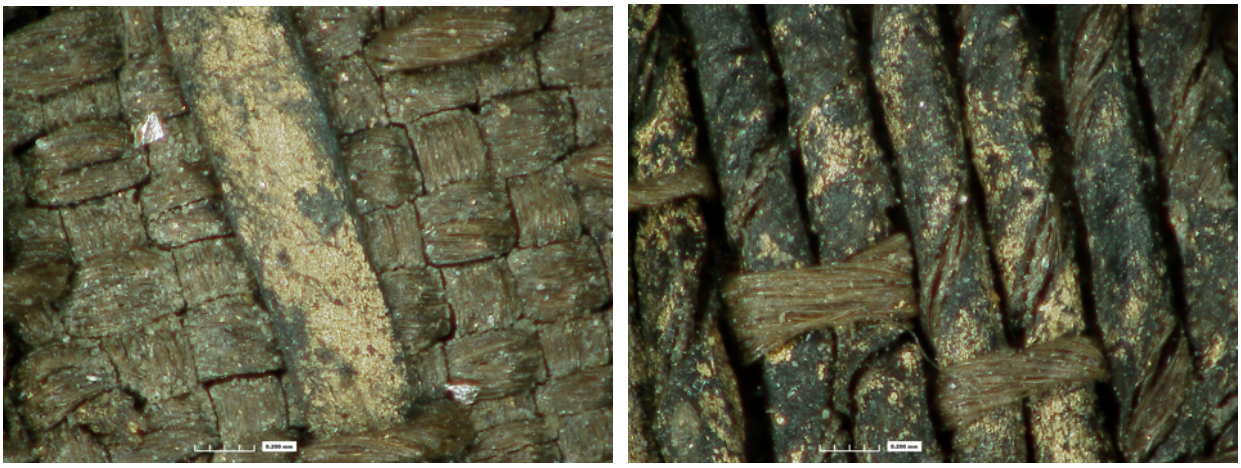

Figure 2. Left: L-1-T1 #1 gilded flat strip (150x magnification, scale 0.2 mm); **Right**: L1-T1 #2 wrapped threads (150x magnification, scale 0.2 mm). Images acquired by 3D digital light microscopy with HIROX KH-8700, by Jacob Kalodner, Museum Conservation Institution, Smithsonian Institution

With proteomics analysis and by using 1 mm of gilded thread or less, information is obtained on both the substrate, if made of a proteinaceous tissue, and on the adhesive if it contains a protein substance such as egg white or fish glue [6]. Analysis of the flat and wrapped threads in L-1-T1 revealed a skin-type of substrate made from sheep skin (**Table 3**) with an adhesive identified as a fish glue with a best match found for Amur sturgeon, consistent with other threads made in Central Asia [6]. Due to limitations in fish sequences available (only two species of sturgeon have collagen sequences in NCBI), the exact species identification is still under review.

Table 3: Proteomics identification of L1-T1 samples, showing the main collagen chains identified and the species with the best matches: Collagen Type I alpha-1 (COL1A1), Type I alpha-2 (COL1A2), and Type III alpha-1 (COL3A1). The protein score (-10lgP), protein percentage coverage (%) and number of peptides (#) identified are obtained from PEAKS PTM after searching against an NCBI database containing all collagen sequences for bovidae and fish. Searches were carried out using trypsin as enzyme, one allowed non-trypsin cleavage at any end, one missed cleavage, peptide mass tolerance (PMS) of 10 ppm, fragment mass error tolerance (MS/MS) of 0.02 Da, carbamidomethylation as a fixed modification, and deamidated (NQ), hydroxylation (P), and oxidation (M) as variable modifications. A maximum of six PTMs (Post-Translational Modification) were allowed.

Sample	Layer	Species Latin name	Species Common name	Protein	Score -10lgP	% cov.	# pep.
L1-T1 #1	Skin base identification	*Ovis aries*	Domestic sheep	COL1A1	253.94	66	189
				COL1A2	254.79	61	152
				COL3A1	198.89	49	92
	Adhesive identification	*Acipenser schrenckii*	Amur sturgeon	COL1A1	76.05	10	16
				COL1A2	65.71	11	10
L1-T1 #2	Skin base identification	*Ovis aries*	Domestic sheep	COL1A1	260.82	65	183
				COL1A2	251.43	64	151
				COL3A1	199.12	47	92
	Adhesive identification	*Acipenser schrenckii*	Amur sturgeon	COL1A1	80.12	16	24
				COL1A2	76.69	13	13

CONCLUSION

As this work is on-going, protein sequences database for the species of interest in Mongolia will be created and further analytical work on the fur, leather and golden threads will be conducted to better understand how the different elements of clothing were made and possibly give us clues on their provenance. Biomolecular analysis of the corpus from the Darkhad Valley will shed a new light on herding and trade networks of the people living in northern Mongolia in medieval times.

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