Ancient Protein Sequencing Report

Proteomic analysis of one sample from an Etruscan ash urn (HIN 171) from Ny Carlsberg Glyptotek in Copenhagen, Denmark

Fabiana Di Gianvincenzo
fdigianvincenzo@palaeome.org

October 22nd, 2021

Materials

One sample from an Etruscan ash urn (HIN 171) was provided by the Ny Carlsberg Glyptotek (Copenhagen, Denmark) for proteomic analysis. The sample was collected from the yellow paint residues on the pillow of the sculpted urn.

Methods

The sample was processed, along with a protocol blank, following the protocol described in Mackie et al. (2018)[1]. Briefly, protein residues were extracted from the sample using a lysis buffer, and enzymatic digestion was performed with LysC and Trypsin. The resulting peptides were immobilised on C18 Stage-Tips and analysed by nano-liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS). The MS/MS spectra were identified with the MaxQuant software, matching them against a reference database containing all the publicly available sequences of proteins contained in the most common proteinaceous artistic materials: collagens, egg proteins, and milk proteins. In order to investigate the presence of protein residues originating from other sources, the spectra were then matched against a larger database (SwissProt, from UniProt), containing all publicly available and manually reviewed protein sequences. The matches were against fully tryptic peptide sequences, with no taxonomic restriction.

Proteins are considered confidently identified if at least two unique non-overlapping peptides are observed, unless otherwise specified. Peptides were considered species-diagnostic when, after BLAST search against the entire nrNCBI protein database, they were assigned to a single species, or to a limited number of species among which only one can be considered plausible, based on: (i) the nature of the samples, (ii) the geographic origin of the sample, and (iii) the dating of the sample. Peptides assigned to recurrent contaminant proteins were filtered out and not considered further. Contaminants include primate keratins (likely from the laboratory space or through human handling of samples), excess trypsin, and Bovine Serum Albumin (a common laboratory reagent).

Results

Collagen alpha-1(I) and collagen alpha-2(I) were confidently identified in the sample. More details on the proteins identified are provided in Table 1, and their diagnostic peptides in Table 2. All

[1] Mackie, Meaghan, Patrick Rüther, Diana Samodova, Fabiana Di Gianvincenzo, Clara Granzotto, David Lyon, David A. Peggie, et al. "Palaeoproteomic Profiling of Conservation Layers on a 14th Century Italian Wall Painting." *Angewandte Chemie International Edition 57*, no. 25 (2018): 7369-74.

reported proteins were identified when matching the spectra against a reference database containing all the publicly available sequences for likely paint binders (animal glue, egg, and milk), since the search against the SwissProt database did not lead to any other identification.

The identification of collagens in the sample indicates the use of animal glue, possibly as paint binder. The biological origin of the material could not be confidently determined, as none of the identified peptides is species-diagnostic. For collagen alpha-1(I) in particular, the origin could not be restricted further than Mammals, although two peptides not matching the corresponding human sequence confirmed that the presence of this protein is not due to human contamination (Table 2).

Two peptides, among the ones supporting the identification of collagen alpha-2(I), restrict the biological origin of this proteins to species belonging to the Pecora infraorder. The complete list of species matching the sequence of each peptide is reported in Table 2. Based on the historical and geographical context of production of the Etruscan urn, it is likely that the species of origin is either cow (*Bos taurus*) or red deer (*Cervus elaphus*). Nonetheless, the use of other species cannot be excluded based solely on this evidence.

Since all the identified peptides from collagen alpha-1(I) also match these species, both collagens can be assumed to come from the same species for the parsimony principle.

Conclusions

A sample from an Etruscan ash urn was provided for species identification via proteomic analysis. The results indicate the presence of animal glue in the sample. The biological origin of the glue could not be identified at the species level, but only at infraorder level (Pecora). However, cow (*Bos taurus*) and red deer (*Cervus elaphus*) seem to be the most likely sources out of the species matching the sequence of the identified peptides.

Table 1: Observed proteins and their taxonomic identifications. *The indicated accession nr corresponds to the protein from *Bos taurus*.

Protein accession nr	Protein name	Taxonomic identification	Total peptides	Unique peptides	Total sequence coverage (%)	Sequence length	Total MS/MS spectra
-	Collagen alpha-1(I)	Mammals	6	6	7.7	1461	8
P02465*	Collagen alpha-2(I)	Pecora	7	7	7.6	1364	9

Table 2: Observed diagnostic peptides. The underlined species are the ones considered most likely in the historical and geographical context of the production of the Etruscan ash urn.

Protein	Peptide sequence	Matching species (BLAST)	Length	Mass	MQ score	MS/MS spectra
Collagen alpha-1(I)	GEPGPTGIQGPPGPAGEEGK	Unspecific - but not matching *Homo sapiens*	20	1830.87	134.9	2
	GETGPAGPAGPIGPVGAR	Unspecific - but not matching *Homo sapiens*	18	1559.81	228.3	1
Collagen alpha-2(I)	GEPGPAGAVGPAGAVGPR	Pecora (*Bison bison bison, Bos indicus, Bos indicus x Bos taurus, Bos mutus, <u>Bos taurus</u>, Bubalus bubalis, Cervus canadensis, <u>Cervus elaphus</u>, Cervus elaphus hippelaphus, Muntiacus muntjak, Muntiacus reevesi, Odocoileus virginianus texanus*)	18	1515.78	66.5	1
	IGQPGAVGPAGIR	Pecora (*Bison bison bison, Bos indicus, Bos indicus x Bos taurus, Bos mutus, <u>Bos taurus</u>, Bubalus bubalis, Capra hircus, Cervus canadensis, <u>Cervus elaphus</u>, Muntiacus muntjak, Muntiacus reevesi, Odocoileus virginianus texanus, Oryx dammah, Ovis aries, Sus scrofa, Sus scrofa domesticus*)	13	1191.67	94.7	2