Paleoproteomics and Polychromy: The identification of peptides from paint binders from the Palace of Apries, Egypt

Luise Ørsted Brandt [1], *Cecilie Brøns* [2], *Jesper V. Olsen* [3], *Enrico Cappellini* [4]

[1] *Centre for Urban Network Evolutions, Aarhus University,* [2] *Ny Carlsberg Glyptotek, Denmark,* [3] *Centre for Protein Research,* [4] *Centre for GeoGenetics*

KEYWORDS

Proteomics, ancient polychromy, paint binders, collagen, ancient art

Fig. 1 Sampled fragment from the Palace of Apries (inv. no. ÆIN 1060), Memphis, Egypt, 26th Dynasty, 589–568 BCE, H 24.3 cm, W 14.6 cm, D 8.2 cm.

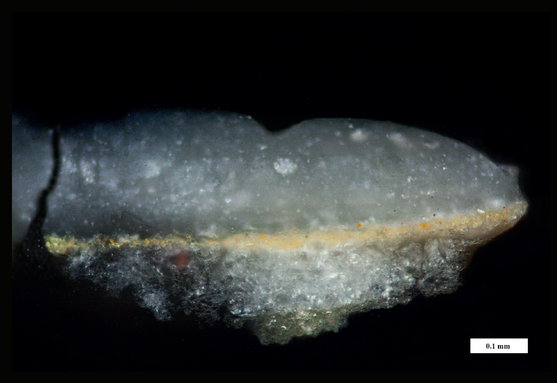

Fig. 2 Micrograph of cross-section of sample showing, from the bottom upwards: limestone, white preparatory layer, yellow paint layer, waxy surface coating. The yellow paint layer and the surface coating have been sampled separately and analysed using proteomics.

Fig. 3 Sampling of paint layers from the fragment from the Palace of Apries.

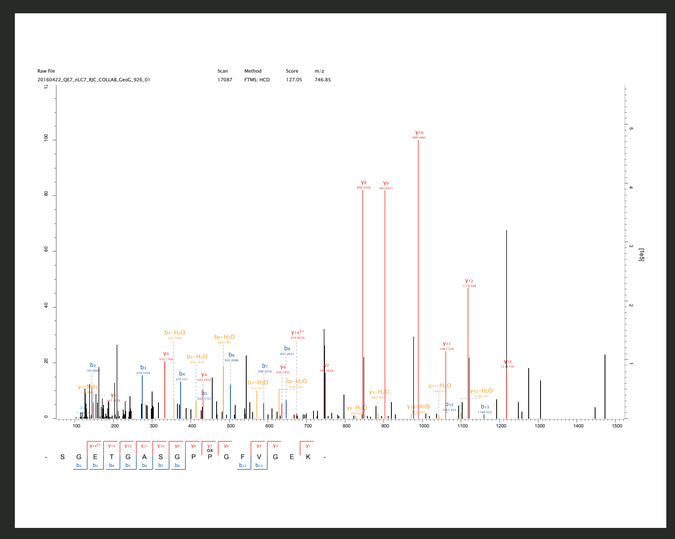

Fig. 4 Tandem MS spectra from the waxy surface layer supporting the identification of collagen alpha-2(I) diagnostic for *Bos Taurus*.

Proteins and peptides from the **waxy surface layer**

N.	Protein Name	Razor + unique peptides	Sequence coverage [%]	Matched spectra	Species Identification
1	Collagen alpha-1(I)	36	29,5	162	*Bos Taurus/Bubalus Bubalis*
2	Collagen alpha-2(I)	29	30,7	79	*Bos Taurus*

Proteins and peptides from the **yellow paint layer**

N.	Protein Name	Razor + unique peptides	Sequence coverage [%]	Matched spectra	Species Identification
1	Collagen alpha-1(I)	32	33,4	97	*Bos Taurus/Bubalus Bubalis*
2	Collagen alpha-2(I)	27	27,9	45	*Bos Taurus*

Fig. 5 Lists of proteins bearing observed species-diagnostic peptides.

ABSTRACT

Most of the cultural heritage objects produced using biogenic materials are rich in protein residues. This is also the case with paint binders that were used as media for pigments applied to sculptures and architecture in Antiquity.

So far, the research into ancient polychromy has, however, primarily focused on analyses and identification of pigments. The choice of the binding medium was, however, of crucial importance for the final polychrome appearance in terms of coverage, nuance, intensity, and gloss. The nature of the binding medium is therefore of paramount importance for our understanding of the original appearance of painted objects.

In this pilot study we identify collagen alpha-1(I) and collagen alpha-2(I) from *Bos Taurus* in a sample from a polychrome layer on an architectural element from the Palace of Apries, Egypt, (around 589–568 BCE) using mass spectrometry-based peptide sequencing.

Identification of ancient paint binders will expand our knowledge of the painting techniques of Antiquity considerably. Moreover such identification will improve reconstructions of ancient polychrome artefacts in museum display.

INTRODUCTION

The study of the polychromy of ancient artefacts over the past decades has completely changed our perception of the ancient world and its aesthetics. Ancient art and architecture were very far from the pure white we usually see in museum displays. Rather they were covered in vibrant colours. It is now acknowledged that colour was an integral part of shape – its 'fourth dimension' – without which it cannot be understood.[1]

So far, the study of ancient polychromy has primarily focused on analyses and identification of pigments.[2] The choice of the binding medium was, however, of crucial importance for the original appearance of each painted object. The binders may be derived from proteinaceous materials such as egg or casein, vegetable gum, drying oil, or natural wax. Binders can be used alone or in mixtures containing binders from two or more sources.

Apart from considerably expanding our knowledge of the painting techniques of Antiquity, the paint binders have an enormous influence on the perception and final appearance of an object.
The interaction of paint layers with light is massively affected by the paint binders and has a crucial effect on the optical appearance of colour, including nuances, coverage, and intensity. The understanding of the composition and production of paint binders is therefore of paramount importance for our understanding of the original appearance of painted objects and will lead to 2D and 3D reconstructions of antique sculptures and architecture in museum collections.

So far, the most widespread analytical approach for identifying organic materials has been GasChromatography-Mass Spectrometry (GC/MS). However, when applied to proteinaceous binders, this solution only allows tentative indirect protein identification. This handicap was overcome with the recent introduction of MS-based methods for ancient protein sequencing.[3] This approach was applied to identify successfully protein binders in historical paintings,[4] as well as organic glues.[5] So far, however, the full potential of proteomic methods has never been applied to paint binders obtained from ancient art and architecture.

In this pilot project, we demonstrate how Mass Spectrometry-based ancient protein sequencing can be used to identify confidently the biological species of origin and the raw materials used as binders applied to architectural elements and sculptures from the collection at the Ny Carlsberg Glyptotek.

MATERIALS AND METHODS

Using LC-MS/MS proteomics we analysed two samples from an architectural fragment, which originally belonged to a large portal in a palace erected at Memphis by the Egyptian Pharaoh Apries (589–568 CE).[6] The limestone fragment depicts a fan, painted with green, yellow, and brown colours (Fig. 1). The paint binders have so far not been identified.

The yellow paint layer and a waxy surface coating were sampled for analysis (Fig. 2–3). The two samples were extracted using a guanidine hydrochloride extraction solution and enzymatically digested using Trypsin. The tryptic peptides were immobilised on C18 stage tips and sent to The Centre for Protein Research for sequencing. Peptide mixtures were analysed by online nanoflow reversed-phase C18 liquid chromatography tandem mass spectrometry (LC-MS/MS).[7] The LC-MS system consisted of an EASY-nLC™ 1000 system connected to a Q-Exactive HF (Thermo Scientific, Bremen, Germany) through a nano-electrospray ion source.

Raw files generated during spectra acquisition were searched on a workstation using the MaxQuant (MQ) algorithm, v. 1.5.5.30,[8] and the Andromeda peptide search engine.[9]

RESULTS

Two proteins, collagen alpha-1(I) and collagen alpha-2(I) were identified in both of the samples from the Palace of Apries.

In the sample from the waxy surface layer, collagen alpha-1(I) and collagen alpha-2(I) were assigned based on, respectively, 36 and 29 razor + unique peptides. In the sample from the yellow paint layer, collagen alpha-1(I) and collagen alpha-2(I) were assigned based on respectively 32 and 27 razor + unique peptides. Peptides diagnostic for *Bos Taurus* were found for both proteins in both samples (Fig. 4–5).

The results demonstrate that analytical approaches to protein sequencing can be applied with success even to very small samples of archaeological paint layers.

Collagen alpha-1 and Collagen alpha-2 are fibril-forming collagens found in most collagen-bearing tissues, such as bone, skin, and ligaments. Consequently, the collagen-based paint binders we detected in both the yellow paint layer and the surface coating, were most likely an animal glue produced from cattle skin or cattle bone.

CONCLUSION

We have demonstrated that LC-MS/MS-based ancient protein sequencing is a useful method for establishing the origin of paint binders from antique sculptures and architecture. In the case of the paint binder from the yellow paint layer and the waxy surface coating of Apries Palace, the paint binder was an animal glue most likely produced from either cattle skin or cattle bone.

ACKNOWLEDGEMENTS

We are grateful to the Ny Carlsberg Glyptotek for collaboration and provision of samples. We would also like to express our gratitude to Signe Skriver Hedegaard at Ny Carlsberg Glyptotek, Liam Lanigan, and Rosa Rakownikow Jersie-Christensen at the Centre for GeoGenetics and the Centre for Protein Research, respectively for their expertise and kind help with sample collection, sample preparation, and data generation.

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