Unleashing top-down mass spectrometry in study of proteinaceous materials in museum objects: Method development using paint models

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Introduction

Proteinaceous material has been extensively used by the art community for centuries as paint binders, adhesives, and varnishes. Study of these materials can give new insight into the artists' techniques (e.g. choice of materials, provenance)(1), as well as both guide future conservation efforts and demonstrate the current state of preservation. The most common proteomics approach to the identification of protein compounds has been bottom-up proteomics, a technique introduced in the cultural heritage field in the early 2000s (2), where proteins are extracted from a sample and are digested into peptides using enzymes. This digestion step can often lead to loss of labile protein modifications or induce others, which in turn limits the information that these modifications can provide.

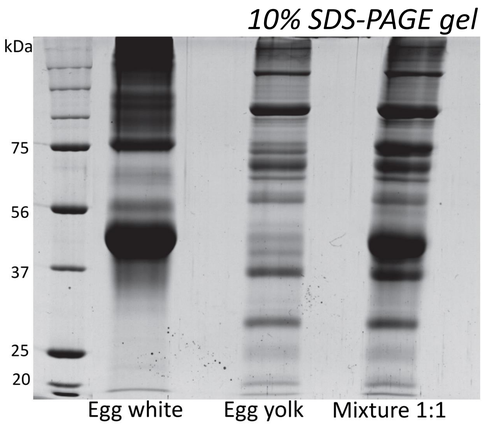
We present here the direct study of intact proteins using top-down analysis to accommodate the specific challenges of art samples. The method has been optimized using paint mock-ups based on egg protein binders. To achieve minimal invasiveness, the method had to be optimized for optimal sample extraction with minimal number of steps.

Workflow

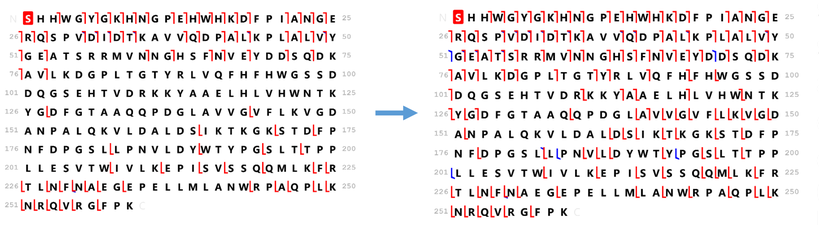
Egg-based models

Paint models were composed of egg white/yolk or single egg protein (lysozyme) mixed with pigment (lead white) and with/without linseed oil.
Here is shown an electrophoretic separation showing the separation of egg white and yolk proteins' extracts. Ovalbumin is the most intense band at 45 kDa (egg white and mixture).

10% SDS-PAGE gel

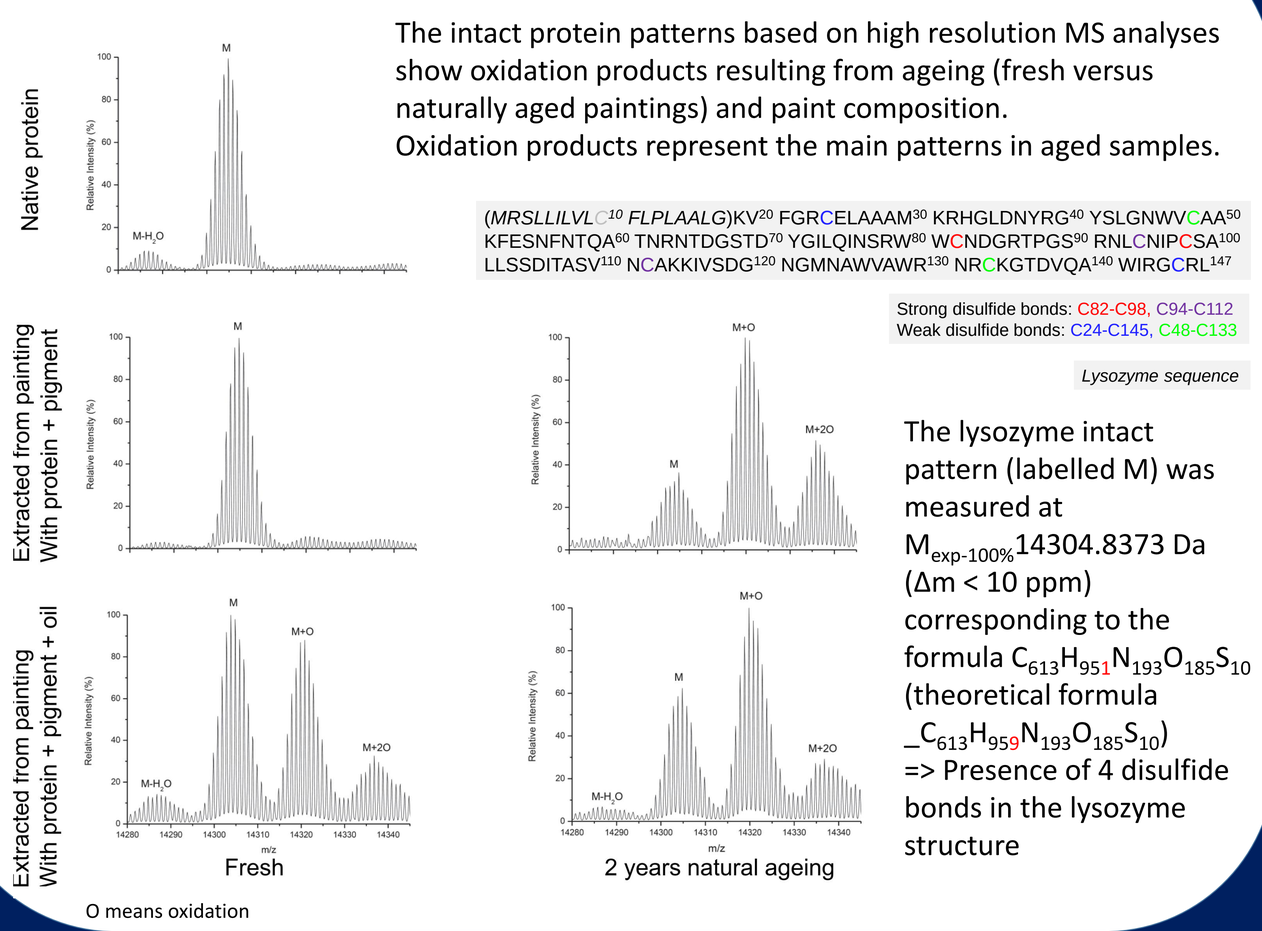


Method optimization



Separation and tandem mass spectrometry experiments settings had to be adapted to achieve improved sensitivity and optimal fragmentation in order to extract maximal information.
(here carbonic anhydrase used as model, red lines are marking fragment ions identified)

Results: focus on intact lysozyme protein



The intact protein patterns based on high resolution MS analyses show oxidation products resulting from ageing (fresh versus naturally aged paintings) and paint composition.
Oxidation products represent the main patterns in aged samples.

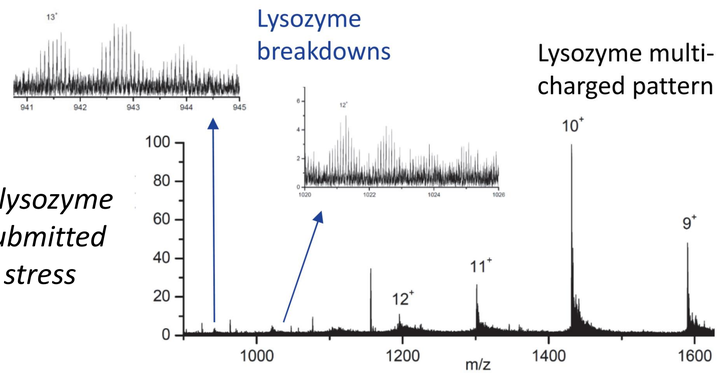
(*MRSLLILVLC[10] FLPLAALG*)KV[20] FGRCELAAAM[30] KRHGLDNYRG[40] YSLGNWVCAA[50] KFESNFNTQA[60] TNRNTDGSTD[70] YGILQINSRW[80] WCNDGRTPGS[90] RNLCNIPCSA[100] LLSSDITASV[110] NCAKKIVSDG[120] NGMNAWVAWR[130] NRCKGTDVQA[140] WIRGCRL[147]

Strong disulfide bonds: C82-C98, C94-C112
Weak disulfide bonds: C24-C145, C48-C133

Lysozyme sequence

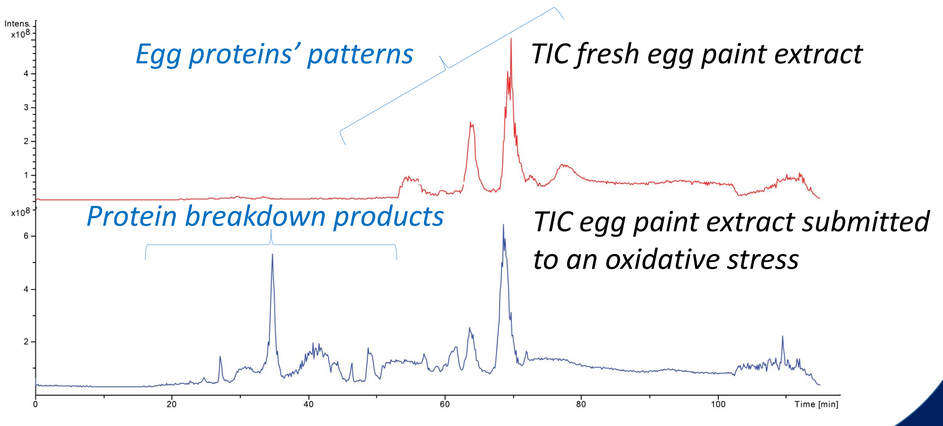
The lysozyme intact pattern (labelled M) was measured at $M_{exp\text{-}100\%}$14304.8373 Da ($\Delta m < 10$ ppm) corresponding to the formula $C_{613}H_{951}N_{193}O_{185}S_{10}$ (theoretical formula $C_{613}H_{959}N_{193}O_{185}S_{10}$) => Presence of 4 disulfide bonds in the lysozyme structure

Results: focus on breakdown products



MS analysis of lysozyme paint extract submitted to an oxidative stress

Paint exposition to an oxidative stress (UV irradiation) was used to optimize settings for the identification of protein breakdown products. An example of MS spectrum showing lysozyme breakdowns is shown above and total ionic current (TIC) of egg protein extract below.



Conclusion and Perspectives

The top down method appears as a novel powerful method to access new valuable information on the degradation mechanisms of the organic media (e.g. protein breakdown products) and protein chemical modifications. First results on egg-based model paints show informative spectra with good signal to noise, current work is focusing on the study of egg proteins of higher molecular weights and higher heterogeneity. The application of this method will provide new insights into the impact of restoration procedures and conservation conditions at molecular level.

References

1. Pozzi F. et al. Mixing, dipping, and fixing: the experimental drawing techniques of Thomas Gainsborough. Herit Sci. 2020 Aug 24;8, 85; **2.** Tokarski C. et al. Proteomics Approach for Binding Media Studies in Art Paintings. In Non-Destructive Testing and Microanalysis for the Diagnostics and Conservation of the Cultural and Environmental Heritage; Van Grieken, R., Van't dack, L., Meersman, G., Eds.; Antwerp, Belgium, 2002; Tokarski C. et al. Identification of Proteins in Renaissance Paintings by Proteomics. Anal. Chem. 2006, 78, 1494–1502; Dallongeville et al. Proteins in Art, Archaeology, and Paleontology: From Detection to Identification. Chem. Rev. 2016, 116, 2−79.