A minimally invasive approach for the identification of proteinaceous binders in works of art by mass spectrometry

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Introduction

A medium also known as "binder" is a material in which the pigments are suspended to obtain a coloured paint adhering onto an artwork surface. The identification of the employed binders may contribute to the conservation and the restauration treatments [1]. Animal protein-based media (*e.g.*, animal glues, caseins and whole egg or white and yolk) are widespread binders [2]; proteinaceous binders are usually characterized by removing small amounts of specimen and analyzing them through a bottom-up proteomic approach [3]. Since the non-invasiveness represents the main target to investigate delicate, precious and unique samples, there has been extensive research regarding the development of non-invasive techniques [4,5].

Materials and Method

Here we introduce a quasi-non-invasive protocol to investigate proteinaceous binders in artworks based on a small hydrogel (< 10 mm^2) made of poly(2-hydroxyethyl methacrylate)/poly(vinylpyrrolidone) (pHEMA/PVP) previously loaded with trypsin and applied onto the objects' surface for the in-situ digestion of proteins (Fig. 1). The released peptides from the pictorial contact area were recovered into the gel by soaking the hydrogel in an acidified acetonitrile solution in an ultrasonic bath. Peptides were subsequently identified by either matrix-assisted laser desorption/ionization (MALDI) time of flight (ToF) MS or reversed-phase liquid chromatography (RPLC) coupled to an electrospray ionization (ESI) interface and a linear ion trap (LIT) MS [6,7].

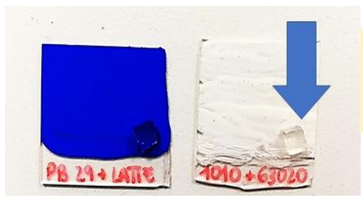


Figure 1. Pictures of gel applied to some paint replicas.

Results and Discussion

Preliminarily, the trypsin's activity into the hydrogel was assessed by comparing *the in-situ* digestion of a model standard protein (*i.e.*, bovine serum albumin, BSA) with the conventional overnight digestion in solution and in both cases BSA was appropriately recognized. The efficiency of the loaded hydrogel was corroborated by acquiring peptide mass fingerprinting (PMF) of paint models composed of chicken egg yolk, collagen, and caseins from cow milk mixed with various pigments (Fig. 2). Database search allowed us to assign the main peptides of paint replicas to all these binders. To ensure a reliable sequence assignment, the same tryptic digests were analyzed by RPLC-ESI-MS and some selected peptides were subjected to tandem MS (MS/MS) experiments by collision induced dissociation (CID). LC-MS/MS experiments lead to a confident identification of the proteinaceous because isobaric peptides were chromatographically separated. Finally, the same protocol was successfully applied to a painting on wood mockup, a liturgical scroll *Benedictio ignis et fontis* (Benedizionale) of the Museo Diocesano of Bari (Fig. 3) and a statue exposed in San Lorenzo church (Bisceglie, Bari, Apulia, Fig. 4). The acquired MALDI mass spectra are shown in Figure 5 respectively for scroll (A), statue (B) and painting (C). The result interpretation was performed by comparing spectra with those of the painting tests along with PMF in the Mascot database search [8].

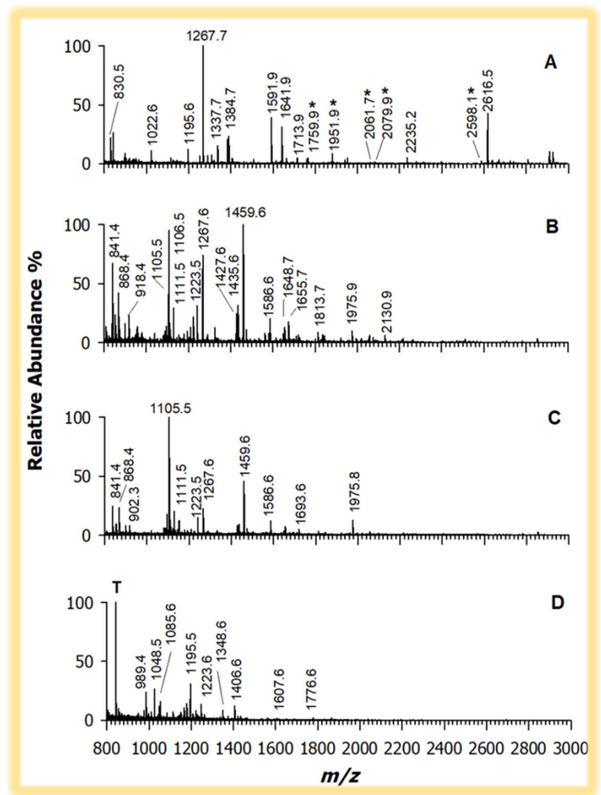
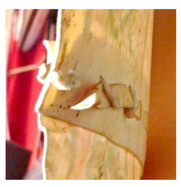


Figure 2. MALDI-ToF mass spectra of an in-situ trypsin-loaded hydrogel digestion of paint replicas from caseins (A), bovine collagen (B), rabbit collagen (C) and egg yolk (D). The asterisked peaks are due to phosphorylated peptides.

Figure 3. Detail of the *Benedictio ignis et fontis* on Folio n. 2 of the Benedizionale and zoom on the binding strip between Folio 1 and 2 (Museo Diocesano, Bari, Italy).



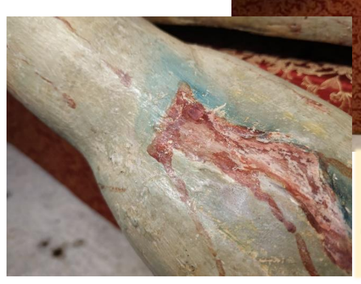


Figure 4. A fifteenth century dated wooden statue exposed in San Lorenzo church (Bisceglie, Bari, Apulia) attributed to Franciscan order. Two sampling points of the wooden statue exposed in San Lorenzo church (Bisceglie, Bari) are highlighted.

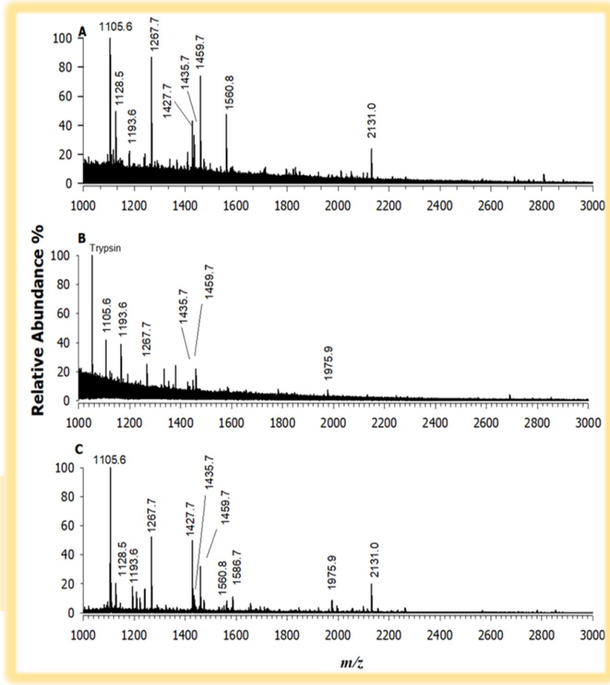


Figure 5. MALDI-ToF mass spectra of *in-situ* trypsin-loaded hydrogel digestion performed on 558 historical samples: (A) parchment scroll dated eleventh century, (B) wooden statue dated fifteenth century and (C) a 30 years aged paint mockup (collagen mixed with white gypsum area).

Conclusions

The proposed *in situ* sampling and digestion method lead to a sequence coverage comparable with the conventional digestion ones, but the whole protocol was reduced to less than one hour. The proteinaceous binder was successfully identified even in aged samples and works of art pieces as well [8]. The present protocol can be applied to ancient objects of cultural heritage for proteinaceous binder analysis, since it does not require sampling, or any specific technical expertise and the protein extraction leaves the surface unaltered.

References

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