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Case study

Application of peptide mass mapping on proteins in historical mortars

Stepanka Kuckova [a,*,b,c], Radovan Hynek [c], Milan Kodicek [c]

[a] Department of Chemistry and Chemical Education, Charles University, M.D. Rettigové 4, 116 39 Prague 1, Czech Republic
[b] Institute of Theoretical and Applied Mechanics ASCR, Prosecká 76, 190 00 Prague 9, Czech Republic
[c] Department of Biochemistry and Microbiology, Institute of Chemical Technology, Technická 3, 166 28 Prague 6, Czech Republic

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Abstract

The reliable identification of proteinaceous binders in historical mortars and plasters represents a complicated analytical problem. In this paper the possibility of peptide mass mapping (PMM) in connection with the mass spectrometry is demonstrated. The presence of milk and collagen proteins was trustworthy proved in the samples of mortars taken from the Romanesque rotunda of Saint Catherine in Znojmo (Czech Republic).

Keywords: Peptide mass mapping; MALDI-TOF MS; Historical mortars; Proteinaceous additives; Rotunda

1. Research aims

We tested the method of the peptide mass mapping (PMM), which was recently developed in our laboratory, on protein identification in the historical mortars. In this type of the samples the proteinacous additives are present in extremely low concentrations that can be further decreased by the activity of microorganisms and aggressive processes should affect the protein chemical structure during the hardening; that is why the identification of proteinaceous binders in the mortars represents a complicated analytical problem. In this paper the procedure of identification of protein additives in mortars is applied to samples from 12th century originated from the rotunda of Saint Catherine in Znojmo (Moravia, Czech Republic). It is one of the oldest and most significant churches in this country with well-preserved frescoes, which are believed to be the portraits of Czech princesses of Premyslid dynasty.

2. Experimental section

2.1. Introduction

The organic binders have been added into the plasters and mortars for different purposes since the ancient times, because their addition improved properties of these materials (Table 1) [1]. The wide range of plant and animal materials were used:

- eggs (whole egg or egg white and yolk individually);
- bovine milk or its constituents (casein, curd or whey);
- bovine blood;
- animal glues;
- gelatin;
- beer;
- malt;
- sugar;
- fruit syrup;
- treacle;
- grease;
- oils, etc. [1,2].

Among other characteristics, the additives change the water distribution in recently prepared mortars and accelerate their drying. Within the drying, the additives, namely the proteinaceous ones, initiate crystallization and thus change properties of the hardened mortar.

The proteinaceous materials were probably the most common additives in the mortars. Thus its identification could help to explain many of the mortar unusual properties such as high firmness, hydrophobic surface and, for frescoes, their high stability [3]. As there is usually extremely low content of the proteinaceous additives in mortars, the reliable identification of them represents a complicated analytical problem. In most

* Corresponding author. Tel.: +420 221 900 125; fax: +420 220 445 167.
E-mail addresses: rubisco@volny.cz (S. Kuckova), rh@biocentrum.dtu.dk (R. Hynek), Milan.Kodicek@vscht.cz (M. Kodicek).

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Table 1
Summary of protein additives in mortars and their classification by their effects on fresh and hardened mortars.

Effect	Protein additives
Accelerator of hardening	Egg white, blood, curd
Retarder of hardening	Egg white, blood
Plasticizer	Milk, egg white
Adhesive	Casein, animal glues, gelatin
Firmer	Milk, egg white, casein, cheese, blood

Some of the protein additives are matched into more, sometimes even contradicting, categories, due to their different effect on fresh and moderately hardened mortars.

of common analytical methods the identification of proteins is based on monitoring of the ratios of certain amino acids [4–8]; these parameters are not specific enough for individual proteins. Moreover, the protein decomposition is time consuming and the determination of amino acids ratios is not sufficiently reliable because of their modification and losses under the hydrolysis conditions. The identification of protein mixtures like the binders is essentially ruled out for these methods. Although some works deal with the identification of proteins in pictorial coating mainly by gas chromatography (GC) and pyrolysis–GC (Py–GC) [9,10].

In method PMM, which was developed at the end of last century by biochemists for proteomic purposes, the studied proteins contained in the sample are specifically enzymatically cleaved to peptides, whose composition is characteristic for each protein. Then, the mass spectrum of the peptides is measured. Each protein gives a set of peptide molecular masses (fingerprint) by which it can be safely characterized. The detection limits of proteins and the sample consumption are, in comparison with other analytical methods, very low [11]. Moreover, not only individual proteins, but also its characteristic mixtures (egg, animal glue, milk proteins, etc.) can be identified.

2.2. Material and methodologies

We received seven samples from the Romanesque rotunda of Saint Catherine in Znojmo without the preliminary knowledge of their sampling (Fig. 1) and description (Table 2); these

Table 2
The description of analyzed mortar samples.

Sample	Description
1	Lean mortar taken from the surface of the stone construction of the vertex center
1A	Rich mortar taken from the surface of the stone construction of the vertex center
2	Mortar taken from the grout of the "rim" at ashlars masonry in the lantern opening
3	Mortar from the grout between the center (made up from the vertically laid stones) and the rest of the aisle construction
4	Lean mortar from the construction of the vertex center
8	Mortar from the grout between the vault-stone of the apse and rotunda aisle masonry
10	Mortar from the rotunda aisle

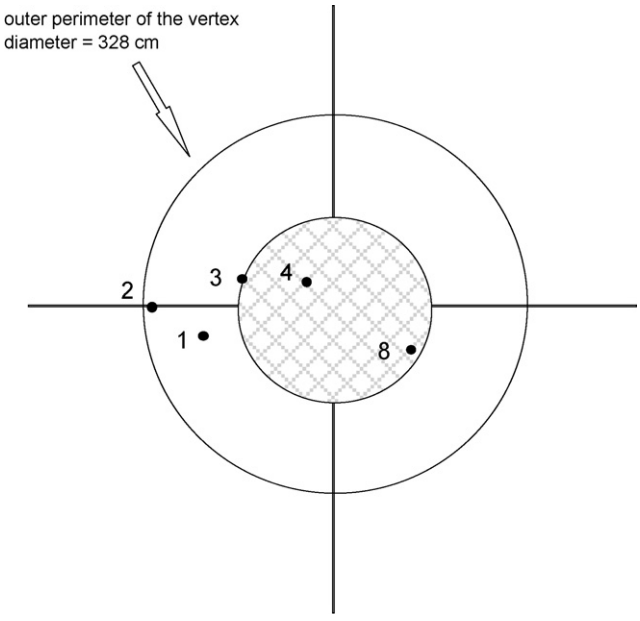

Fig. 1. The sketch of the back of vertex of the rotunda of Saint Catherine in Znojmo. The inner part is crosshatched and the places of sampling are labeled. Sample 1A is the second layer of the sample 1. The sample 10, coming from the rotunda aisle, is not shown on this sketch (author: Dr Jaromír Kovárník).

information we obtained after the analysis. From all samples the representative parts were taken; the weights of these parts were 0.1–0.4 g. These parts were digested approximately in 80 μl of 50 mM ammonium hydrogen carbonate containing approximately 10 μg/ml of trypsin, at room temperature for two hours. An aliquot of the obtained peptide solution (2 μl) was mixed with 4 μl of 2,5-dihydroxybenzoic acid (DHB) solution (15 mg of DHB in 1 ml of mixture of acetonitrile/0.1% trifluoracetic acid (1/2 [v/v]). The resulting mixture (1.5 μl) was spotted on the stainless steel MALDI target and dried on air. Mass spectra were acquired by Bruker-Daltonics Biflex IV MALDI-TOF mass spectrometer equipped with standard nitrogen laser (337 nm) in positive reflector mode; at least 200 laser shots were collected for each spectrum. The spectra were analyzed using the XMASS software (Bruker) and our database of reference proteinaceous binders [12].

2.3. Results and discussions

The results of the analyses are summarized in Table 3. The milk proteins, which were found in the rotunda samples, should

Table 3
The results of mortar samples.

Sample	Place of sampling (Fig. 1)	Proteinaceous additives
1	Outer part of vertex	Collagen materials
1A	Outer part of vertex	–
2	Outer part of vertex	Collagen materials
3	Inner part of vertex	Milk
4	Inner part of vertex	Milk
8	Inner part of vertex	Milk
10	Rotunda aisle	–

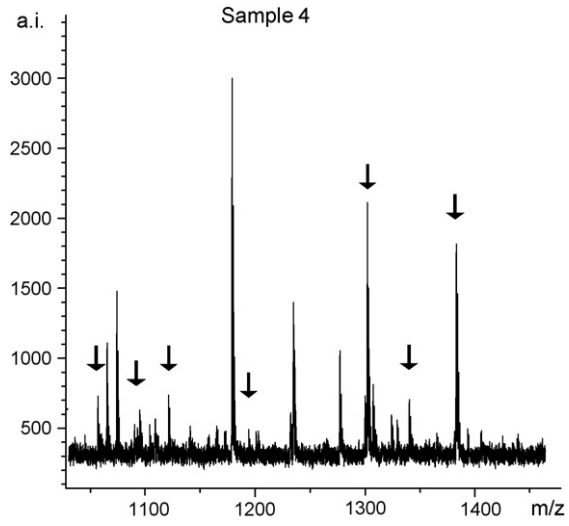

Fig. 2. The mass spectrum of sample 4. The peaks of milk proteins are displayed.

come from the curd or whey (Fig. 2). At this moment, we are not able to distinguish between these sources of proteins as none of them was, most probably, completely separated from the other one; it is also possible that middle-age bricklayers used the whole milk as the additive. Similarly, the applied method did not allow the reliable resolution of collagen-originated materials. In our case, most probably of bovine bone glue and gelatin (Fig. 3), because the amino acid sequences of the contained peptide chains are very similar; both materials are prepared by boiling of bones, leathers and other ligaments and they differ only in the time of boiling that influences the physical structure and consequently the properties of the binders.

The samples can be divided into three groups according to the obtained results. The first group (samples 3, 4, and 8) contains milk proteins. They were taken from top inner part of the rotunda vertex (Fig. 1); it can be deduced that higher amounts of milk proteins were added here in order to increase the firmness of the mortar. The second group (samples 1 and 2) contains

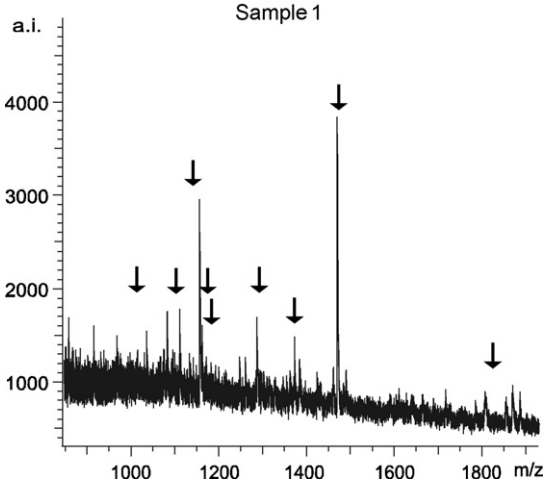

Fig. 3. The mass spectrum of the sample 1. The peaks of collagens are displayed.

predominantly collagens. They originated from the outer part of rotunda vertex. The animal glue (probably made from bovine materials) was added in to the mortar because the good adhesivity was required here. The mortar of the samples 1A and 10, which belong to the third group, does not contain proteinaceous binders. Sample 1A was the second layer found under the sample 1 and it represents so-called rich mortar that has hydrophobic protection properties probably caused by oil additive. In the sample 10, which comes from the rotunda aisle, the proteins were not expected because of the ordinary wall construction.

3. Conclusions

The PMM method combined with MALDI-TOF mass spectrometry opens the possibility to distinguish the main groups of proteinaceous additives (milk proteins, collagens, egg proteins, etc.) that are, usually in extremely low concentrations, often present in historical mortars. The two types of these additives were identified in the mortars in the rotunda of Saint Catherine in Znojmo (12th century).

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References

[1] P. Rovnaníková, Omítky. Chemické a technologické vlastnosti, STOP, Praha, 2002.
[2] J. Hošek, J. Muk, Omítky historických staveb, SPN, Praha, 1989.
[3] B. Slánský, Technika malby díl I., Malířský a konzervační materiál, SNKLHU, Praha, 1953.
[4] J. De la Cruz-Canizares, M.T. Doménech-Carbó, J.V. Gimeno-Adelantado, R. Mateo-Castro, F. Bosch-Reig, Suppression of pigment interference in the gas chromatographic analysis of proteinaceous binding media in paintings with EDTA, J. Chromatogr. A. 1025 (2004) 277–285.
[5] I. Bonaduce, M.P. Colombini, Gas chromatography/mass spectrometry for the characterization of organic materials in frescoes of the Monumental Cemetery of Pisa (Italy), Rapid Commun. Mass Spectrom. 17 (2003) 2523–2527.
[6] M.P. Colombini, F. Modugno, Characterisation of proteinaceous binders in artistic paintings by chromatographic techniques, J. Sep. Sci. 27 (2004) 147–160.
[7] G. Chiavari, N. Gandini, P. Russo, D. Fabbri, Characterisation of standard tempera painting layers containing proteinaceous binders by pyrolysis gas chromatography mass spectrometry, Chromatographia 47 (1998) 420–426.
[8] R. Mateo-Castro, J.V. Gimeno-Adelantado, F. Bosch-Reig, A. Doménech-Carbó, M.J. Casas-Catalán, L. Osete-Cortina, et al., Identification by GC-FID and GC-MS of amino acids, fatty and bile acids in binding media used in works of art, Fresenius J. Anal. Chem. 369 (2001) 642–646.
[9] F. Ospitali, A. Rattazzi, M.P. Colombini, A. Andreotti, G. Lonardo, XVI century wall paintings in the "Messer Filippo" cell of the tower of Spilamberto: microanalyses and monitoring, J. Cult. Herit. 8 (2007) 323–327.

[10] D. Bersani, P.P. Lottici, A. Casoli, D. Cauzzi, Pigments and binders in "Madonna col Bambino e S. Giovannino" by Botticelli investigated by micro-Raman and GC/MS, J. Cult. Herit. 9 (2008) 97–102.
[11] R. Hynek, S. Kuckova, J. Hradilova, M. Kodicek, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as a tool for fast identification of protein binders in color layers of paintings, Rapid Commun. Mass Spectrom. 18 (2004) 1–5.
[12] S. Kuckova, I. Nemec, R. Hynek, J. Hradilova, T. Grygar, Analysis of organic colouring and binding components in colour layer of art works, Anal. Bioanal. Chem. 382 (2005) 275–282.