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Analytical pyrolysis of proteins in samples from artistic and archaeological objects

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ABSTRACT

The paper presents a study of proteins found in artistic and archaeological objects based on analytical pyrolysis. Proteins (mainly egg yolk and/or egg white, casein, animal glue and collagen) have been extensively used as paint binders, adhesives and varnishes in mural and easel paintings, and they can be found in archaeological findings, such as bones and skin tissues. In order to overcome limitations of wet chemical methods arising from the reduced solubility of aged proteins in samples of cultural heritage, a combination of analytical pyrolysis techniques was used to characterise reference materials, paint reconstructions and samples from different historical periods (2nd century BC-20th century AD) and geographical origins, which were collected from paintings and archaeological findings. In particular evolved gas analysis mass spectrometry (EGA/MS), pyrolysis coupled with gas chromatography/mass spectrometry (Py/GC/MS) and double shot pyrolysis/gas chromatography/mass spectrometry (DSP/GC/MS) were used. This analytical approach allowed us to characterise and differentiate the proteinaceous media, investigate their thermal behaviour and evidence changes occurring with ageing. Data clearly indicate that egg, casein and animal glue can be identified and distinguished in a sample of unknown composition using each of the analytical pyrolysis techniques used. With time though differences tend to disappear to the extent that extremely degraded samples present pyrolytic profiles extremely similar to each other, irrespective of the nature of the proteins present. The data also indicate that proteins tend to become more thermally stable with ageing, suggesting that extensive intramolecular and intermolecular aggregation, and/or covalent cross-linking occur with time.

1. Introduction

Identification of proteins in samples from cultural heritage is often a difficult task [1,2]. From a technical point of view, paintings, polychromies, archaeological and paleontological objects are complex systems that often consist of multiple heterogeneous layers, in which pigments, fillers and other inorganic matter are mixed with organic materials. Egg yolk and/or egg white, casein, gelatin, animal glue and collagen are among the most common proteinaceous materials that can be encountered in this type of objects [1,3]. Research has evidenced that, as an effect of the long term exposure to the changeable and sometimes harsh environments where the object is displayed or stored, degradation phenomena take place. These include deamidation, hydroxylation, oxidation and carbonylation, partial hydrolysis, aggregation, cross-linking, formation of complexes with other organic binders and inorganic pigments and fillers [4–10].

Many analytical methods for the identification and characterisation of proteins in samples from artistic, archaeological and paleontological objects have been presented [1,2,11], based on staining, immunological, spectroscopic, chromatographic, and proteomics techniques.

Staining techniques are usually performed on paint cross-sections. The localisation of the proteins by stains is based on the use of dyes that may react with primary amines, or interact with specific portions of the proteins, to produce coloured and fluorescent products [12–21]. The localisation and identification of proteins by immunological methods are based on the antigen/antibody interaction. The antibody often requires to interact with 6–10 amino acids of the antigen to form the complex [11–24]. The specificity and sensibility of the immune techniques depend on the antibody availability and on their multiple epitopes. Spectroscopic techniques used to identify and localise binders are based on Infrared and Raman spectroscopy. The simultaneous presence of other organic and inorganic materials can strongly compromise

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the success of these techniques [25–32]. Chromatographic techniques using high-performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC/MS) permit the identification of the material based on the determination of the amino acid composition evaluated after hydrolysis [3,33–38]. The thermal decomposition of the sample by pyrolysis coupled with GC/MS (Py/GC/MS) generates pyrolytic profiles which are useful for the identification of the materials [11,39]. Despite the potential of this technique, a relatively few examples in the literature discuss the identification of proteins in highly degraded samples, especially those from paintings and polychromies [39–45]. Proteomics techniques have been more recently adopted in the field of cultural heritage to identify proteinaceous materials by enzymatic digestion and peptides characterisation [46–54].

Each of these techniques has shown the potential of being able to successfully detect, and in some cases, identify, proteins in selected samples, but all of them present more or less drawbacks, which are seldom discussed and rarely linked to degradation phenomena.

The development of a robust and reliable analytical approach for the identification of proteinaceous materials in samples from artistic, archaeological and paleontological objects, as well as understanding its limitations, requires a better understanding of the physico-chemical changes undergone by proteins with time. Given the loss of solubility observed as a consequence of ageing [38,55–60], wet chemical methods may present insuperable difficulties, which may be, in part, overcome by analytical pyrolysis. In this paper, we present a study, based on analytical pyrolysis, aimed at characterising and better understanding the ageing of proteinaceous materials, and how this reflects on the identification of proteins in paintings and archaeological objects. Pure reference materials, paint reconstructions and a set of samples from paintings and archaeological objects spanning from the 20th century AD to the 2nd century BC were investigated using evolved gas analysis-mass spectrometry (EGA/MS), pyrolysis-gas chromatography-mass spectrometry (Py/GC/MS) and double shot pyrolysis-gas chromatography-mass spectrometry (DSP/GC/MS).

2. Materials and method

2.1. Reference materials

Casein (CAS), egg white (EGW) and animal glue (GLU) were purchased from Bresciani srl (Milan, Italy) and were used as reference materials [4].

2.2. Paintings and archaeological samples

Easel and mural painting samples from artworks of different geographical origin and ages and one archaeological sample were investigated. Some of the samples are paint preparations which have been applied on a wooden support, and covered by paint layers. Others belong to mural paintings, which have, by definition, been more exposed to degrading environmental factors than easel paintings preparations. One sample is from an Egyptian mummy.

The sample code, the typology of the artwork and archaeological object, its age and geographical origin, as well as the nature of the proteinaceous materials present, as identified by GC–MS analysis [61] (see Supplementary Material; Table S.1) are reported in Table 1.

All samples were analysed as powders. Samples of bigger dimension, as those from reference materials, were homogenised with an agate mortar previous analysis. When paint samples were available as flakes, these were pulverised with the aid of a scalpel.

2.3. EGA/MS

The instrumentation consists of a micro-furnace Multi-Shot Pyrolyzer EGA/Py-3030D (Frontier Lab) coupled with a gas chromatograph 6890 Agilent Technologies (Palo Alto, USA) equipped with a deactivated and uncoated stainless steel transfer tube (UADTM-2.5N, 0.15 mm i.d. × 2.5 m length, Frontier Lab). The GC was coupled with a 5973 Agilent Mass Selective Detector (Palo Alto, USA) single quadrupole mass. A program temperature was chosen for the micro-furnace chamber: initial temperature 50 °C; 10 °C/min up to 700 °C. Analyses were performed under a helium flow (1 ml/min) with a split ratio 1:20. The micro-furnace interface temperature was kept at 100 °C higher than the furnace temperature until the maximum value of 300 °C. The inlet temperature was 280 °C. The chromatographic oven was kept at 300 °C. The mass spectrometer was operated in EI positive mode (70 eV, scanning m/z 50–600). The MS transfer line temperature was 300 °C. The MS ion source temperature was kept at 230 °C and the MS quadrupole temperature at 150 °C. Samples, ranging from 30 to 500 μg, were placed into a stainless steel cup and inserted into the micro-furnace. The amount of sample used depended on the sample nature: samples relatively rich in organic materials, such as reference materials, and easel paintings were smaller in size compared to those coming from mural paintings. The sample underwent a thermal decomposition in inert atmosphere (He) over the chosen heating range, and evolved gaseous compounds were transferred to the mass spectrometer and directly ionised and analysed as a function of time.

2.4. Py/GC/MS

The instrumentation consists of a micro-furnace Multi-Shot Pyrolyzer EGA/Py-3030D (Frontier Lab) coupled to a gas chromatograph 6890 Agilent Technologies (USA) equipped with an HP-5MS fused silica capillary column (stationary phase 5% diphenyl–95% dimethyl-polysiloxane, 30 m × 0.25 mm i.d., Hewlett Packard, USA) and with a deactivated silica pre-column (2 m × 0.32 mm i.d., Agilent J&W, USA). The GC was coupled with an Agilent 5973 Mass Selective Detector operating in electron impact mode (EI) at 70 eV. Samples (30 μg for reference materials, easel paintings and polychromies and 100 μg for mural paintings and archaeological residues) were placed into a stainless steel cup and inserted into the micro-furnace. Stainless steel cups, after use were emptied and flame cleaned. Each sample was placed in a clean sample cup, which was previously analysed without sample, in order to ensure the absence of contaminants. The pyrolysis temperature was set at 600 °C for 2 min and interface temperature was 180 °C. The split/splitless injector was used with at 1:10 split ratio and it was interfaced with a liquid nitrogen cryogenic trap (Micro Jet Cryo-Trap MJT-1035E, Frontier Lab). The use of the micro Jet cryo-trap was not necessary but improved the quality of the peaks in the first region of the chromatograms, where highly volatile molecules were eluted. Chromatographic conditions were as follows: initial temperature 40 °C, 2 min isothermal; 10 °C/min up to 140 °C; 6 °C/min up to 280 °C; 10 °C/min up to 300 °C, 30 min isothermal. Carrier gas: He (purity 99.995%), constant flow 1.2 ml/min.

A sample of GLU was analysed in triplicates by Py/GC/MS in order to estimate the reproducibility that can be expected when the same, homogenous, sample is analysed with the same technique. Extracted ion chromatograms were obtained for selected m/z: m/z 67–pyrrole, m/z 91-toluene m/z 107-phenol derivatives, m/z 117-indole and benzeneacetonitrile, m/z 131-methyl-indole, m/z 70 and 154-DKPs, m/z 186-diketodipyrrole (Fig. 4). Areas where integrated, and normalised for their sum. The relative standard deviations (RSD) of the normalised areas and retention times were calculated, and resulted below 20% and 1%, respectively. Detailed