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Evolved Gas Analysis-Mass Spectrometry to Identify the Earliest Organic Binder in Aegean Style Wall Paintings

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Abstract: *An organic binder was identified in the painted fragments from the Canaanite palace of Tel Kabri, Israel. Recently dated to the late 18th century B.C.E. by ^{14}C, Tel Kabri is the most ancient of the Eastern Mediterranean sites in which Aegean style paintings have been found. The application of pigments was suspected to be using an organic binding medium, particularly for the Egyptian Blue pigment. Samples of blue paint were examined using evolved gas analysis-mass spectrometry (EGA-MS) in order to overcome the analytical challenges imposed by highly degraded aged proteinaceous materials. Egg was identified as the binder based on the presence of hexadecanonitrile and octadecanonitrile, confirming the use of a secco painting technique. Lysozyme C from Gallus gallus was detected by proteomics analysis, confirming the presence of egg. To our knowledge, this is the earliest use of egg as a binder in Aegean style wall paintings.*

The discovery of Aegean-style paintings in Eastern Mediterranean archaeological sites is of great interest for its implications in terms of the cultural influence and contacts between Bronze Age societies.[1] The study of the materials used in these paintings was conducted in order to better understand how the Aegean societies produced such significant works of art during the Bronze Age. It also adds to our knowledge and understanding of trade, technological aspects, materials use and artistic transfer between the Cannanite coast and the Aegean region.

Until now, the morphological and inorganic composition of Aegean paintings, taken from different archaeological sites (Crete, the Cyclades and Greek mainland) including Eastern Mediterranean sites such as Tell el Dab'a and Tel Kabri, have been studied by means of SEM-EDS, XRD, XRF, LIBS and micro-Raman spectroscopies.[1–7]

However, the study of the binders of these paintings has been rarely attempted. It has been previously suggested that Aegean paintings were executed in *a fresco* technique, with pigments applied directly on lime-based plaster without any organic binder. Though a few scholars suggested that *a secco* technique was used, analytical confirmation has been lacking.[8–13] Investigation by Brecoulaki et al. on the organic binding media of Aegean wall-paintings from the Mycenaean "Palace of Nestor" in Pylos (ca. 1200 B.C.E.)[4,14] and the "West House" at Mycenae (ca. 1250 B.C.E.)[15] proved, for the first time, the use of organic binders in Aegean style wall-paintings.

Tel Kabri was the capital of a Canaanite Kingdom during the Middle Bronze Age (ca. 1850–1650 B.C.E.). In a palatial complex, approximately 2000 painted plaster fragments of Aegean style painting were found. Based on recent ^{14}C analysis, the end of phase DW IV, to which the wall paintings belong, is dated to the end of the 18th century B.C.E.[16] Consequently, these paintings are the earliest known among the four sites with Aegean-style wall paintings in the East Mediterranean—Tel Kabri, Qatna, Tell el Dab'a and Alalakh.[17] Most interestingly, in a larger context, paintings from Tel Kabri were painted possibly before the appearance of comparable paintings in the Aegean area in the Late Minoan IA or Late Cycladic I contexts in Crete and the Cyclades.[18]

Following the discovery of new fragments in the 2008–2011 excavation seasons, the polychromy of painted fragments from Tel Kabri was investigated.[6] The blue color, identified as Egyptian Blue, was the most common color in the palette (Figure 1 and Supporting Information), in agreement with other studies on Aegean wall paintings.[3,4,19] The powdery appearance of the blue paint layers (Figure 2a), the poor adherences of the color to the support, the distinctive flat interface between the paint layer and its support, as well as the clear presence of a preparation layer (Figure 2b), suggested that an organic binding medium might have been used to paint the blue decorations. As a result, blue samples became first candidates for the study of the binding media.[6]

The fragments analysed (K1, K7, K20) belong to the defined group A by Linn et al.[6] The fragments in this group

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Figure 1. One of the fragments with blue design that was uncovered during the 2008–2011 excavation seasons, showing probably a part of a wing (Photo: R. Linn).

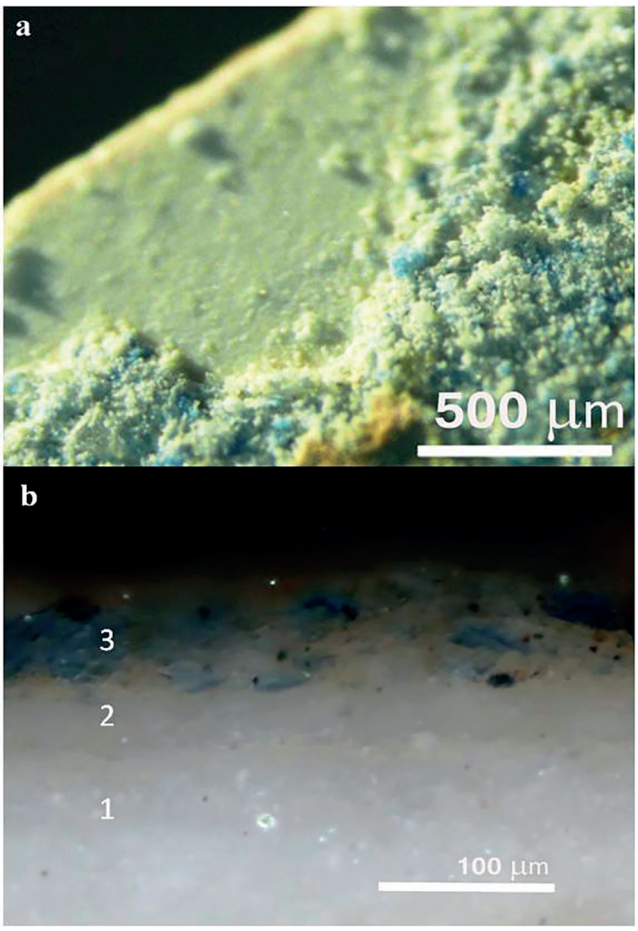

Figure 2. Sample K20 a) (top) A micrograph of the surface of the sample showing the powdery appearance of the paint layer made of Egyptian blue particles admixed with a white pigment (right). The distinctive flat smooth surface appears underneath on the left side. (Photo: R. Linn). b) (bottom) A micrograph of a cross section showing the blue paint layer (3) with Egyptian Blue particles on top of a distinctive white preparation layer (2) and a plaster layer (1) suggesting the use of *a secco* painting technique.(Photo: R. Linn).

(Figure 2b) show a thin paint layer ($\approx$10 µm), an even and flat surface, and interface between plaster and paint layer.[6] 80 % of the fragments studied from Tel Kabri were part of this group.

The study of ancient paintings is a significant challenge for the analytical chemist. It has been demonstrated that during aging, proteinaceous materials become highly thermally stable[20,21] and show very little solubility, challenging GC-MS and MS proteomics-based approaches that require an extraction of the proteinaceous material from the matrix.[22–24] Evolved gas analysis mass spectrometry (EGA-MS) instead is based on analytical pyrolysis and yields temperature-resolved mass spectrometric information on the gaseous products evolved from samples upon progressive heating. Though highly degraded samples yield pyrolytic profiles of proteinaceous materials without specificity, hindering the identification of the binder,[20] the technique has the advantage of giving rapid indications regarding the presence of organic media without the need for any sample work-up.[25]

A first sample (K1) was analysed by EGA-MS, sub-sampling the blue paint layer and the white preparation layer, separately. EGA profiles of the samples are shown in Figure 3. Data clearly show that both the blue paint layer of Egyptian blue as well as the preparation layer contain egg (which could be from either egg yolk or whole egg).

The Total Ion Thermogram (TIT) profile of both sub-samples (Figure 3a) shows a main degradation step (B) centered at 420 °C. The average mass spectra in the range 400–450 °C of both samples (Figure 3c) are dominated by fragment ions (*m*/*z* 67, 91, 107,117, 131) corresponding to nitrogen- and oxygen-containing aromatic compounds (i.e. pyrrole and alkylpyrrole, indole and alkylindole, phenol and alkylphenol, toluene, styrene and ethylcyanobenzene) associated with the thermal decomposition of the more thermally stable portion of proteins.[20]

The temperature range in which the evolution of aromatic compounds occurs, as well as the absence of fragmented ions ascribed to diketopiperazines, resulting from the cyclisation

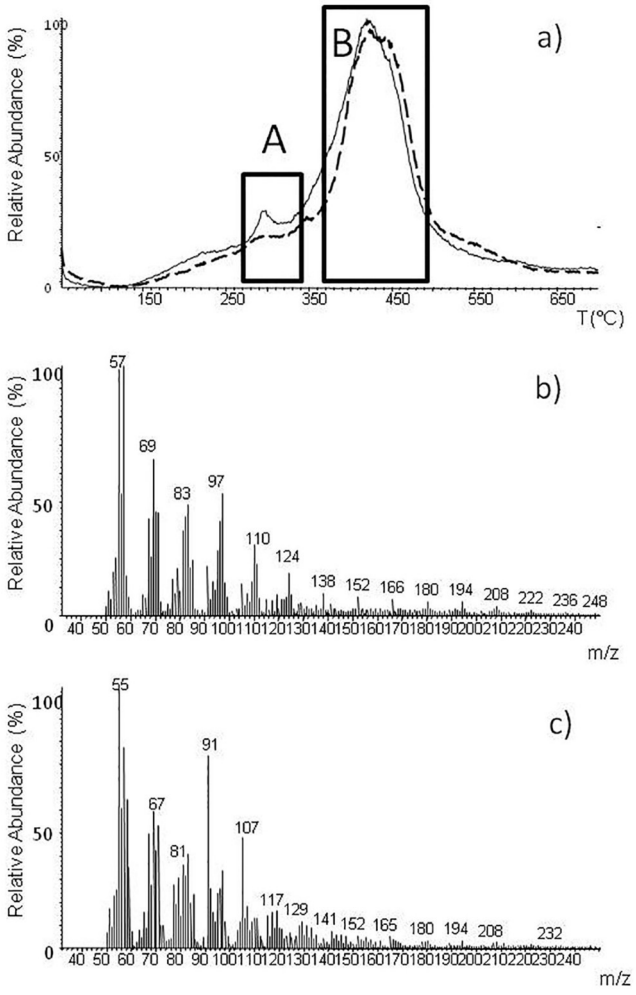

Figure 3. a) Total ion thermogram (TIT) of sample K1 showing Egyptian blue paint layer (straight line) and a preparation layer (dotted line). b) Average mass spectra of area A. c) Average mass spectra of area B.

of neighbouring amino acids in a polypeptide chain and specific for the different proteins, both indicate that the material is highly thermally stable and degraded.[21,22]

The TIT curve of the blue paint layer also shows a well-resolved peak (A) at around 300 °C (Figure 3 a). The average mass spectrum in this temperature range (290–310 °C) of both samples (Figure 3 b), shows the presence of fragmented ions ascribable to hexadecanenitrile and octadecanonitrile (*m*/*z* 194, 208, 222, 236).[26]

Hexadecanonitrile and octadecanonitrile are known markers of egg yolk[26] and have been identified in the pyrolitic profiles of degraded samples collected from artificially aged paint reconstructions[21] and in samples of polychromy on a clay sculpture from the 6th century C.E.[20] The formation of hexadecanenitrile and octadecanonitrile has been related to the pyrolysis of the products of a chemical interaction of carbonyl moieties of egg lipids and the primary amine group of Lys and Arg in egg proteins.[20] The absence of fragmented ions related to the thermal decomposition of the glycerolipid component and cholesterol confirms the observation that the organic material is degraded.[21]

EGA-MS has not been used yet to identify paint binders in samples from works of art and archaeological materials. To confirm these findings, two different blue samples (K7 and K20) were analysed by MS based proteomics, according to an analytical procedure implemented to increase sensitivity in detecting egg proteins in paint samples.[23]

Lysozyme C was unequivocally detected in both samples (9 and 11 peptides from *Gallus gallus* were identified in samples K7 and K20, respectively) confirming the identification of egg performed by EGA-MS. Details regarding the identification of peptides are given in the Supporting Information.

The data obtained proves that at least the blue paint layers from the Aegean-style wall paintings from Tel Kabri were executed in *a secco* technique using egg as binding medium. This result is in agreement with the study of Brecoulaki et al. (2012) on Mycenaean wall paintings from Pylos (ca. 1200 B.C.E.) confirming the practice of egg as a binder.[14] However, the current study allows us to extend the use of an organic binder in Aegean-style wall paintings back an additional 500 years and to a wider geographical area including the Eastern Mediterranean. As far as we are aware, this is the second oldest identification of an organic material used as binder in wall paintings, with the earliest use of egg as binder reported in the wall paintings from the Domus de Janas chamber tombs at Sardinia (3400–2700 B.C.E.).[27]

Since the Tel Kabri paintings are Aegean in their style and technique, this is indirect evidence that organic binding material and *a secco* painting technique were probably part of Aegean art from its early stages in the Middle Bronze Age II and III periods. The adoption of egg as paint binder in the Aegean and East Mediterranean regions allows us to hypothesize that it may have been used in Aegean style paintings more extensively than previously believed.

Given the discovery of an organic binder in these paintings, the conservation and possible future cleaning of the fragments should be carried out with particular care, given both the fragile adhesion of the large pigment particles and the potential loss of binding media.

Experimental Section

Samples (ca. 0.5 mg) for EGA-MS were placed into a stainless steel cup and inserted into the micro-furnace. They underwent a thermal decomposition in an inert atmosphere (He) over the chosen heating range and evolved gaseous compounds were transferred to the mass spectrometer, directly ionized and analysed as a function of time. The instrumentation used included: 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 single quadrupole mass selective detector (Palo Alto, USA). For proteomic analysis, 50 μL of AMBIC (ammonium bicarbonate) 50 mM containing 60 mU μL^{-1} of PNGaseF (Peptide:N-glycosidase F) solution was added to microsamples (ca. 0.6 mg) and incubated at 37 °C for 2 h. The reaction was stopped by boiling the sample for 2 min. The samples, incubated for 1 h in 20 μL in 6 M urea followed by sonication for 30 min at room temperature, were then 6-fold diluted with AMBIC 10 mM pH 7.5 and enzymatic digestion carried out by the addition of1 μg of trypsin at 37 °C for

16 hours. The supernatants were then recovered by centrifugation, filtered on 0.22 μm PVDF membrane (Millipore), concentrated and purified using a reverse phase C18 Zip Tip pipette tip (Millipore). Chromatographic, MS conditions and data handling are described in details in the Supporting Information.

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Conflict of interest

The authors declare no conflict of interest.

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