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Recent advances in the characterization of hair of mummies from the Chilean Andean coast☆

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ABSTRACT

Two pre-Hispanic mummies from the Andean coast, belonging to a corpus of 16 mummies from the San Miguel de Azapa (Arica, Chile), were radiocarbon dated and analyzed in order to replace them in their historical context and to study the conservation state of the hair fibers and the heavy metal presence. The radiocarbon dating placed both mummies in the Formative period (1700 years BC to 500 years AD). Global and elemental analyses were performed using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy and using X-ray fluorescence spectroscopy. These combined techniques enabled to prove the good global conservation state of the mummies' hair and to detect iron, lead, bromide and also arsenic in some cases, in significant amounts inside the hair fibers. Fourier transformed infra-red spectroscopy seemed to prove the good conservation state of the hair surface at a structural level that is why the conservation of hair proteins at a molecular level will be investigated by a proteomics approach in future work.

1. Introduction

Among human remains from museum collections, mummies have intrigued researchers for a long time. They are subject to much research in order to understand the mummification processes, to improve conservation and restoration protocols, and to infer fascinating clues about their civilizations. Mummification rituals in the pre-Hispanic Andean culture started 7000 years ago with the Chinchorro civilization in the Camarones Valley in current Northern Chile, it spread to current Peru and to the south of the Atacama Desert in Chile and have lasted up to 6000 years [1,2]. Many mummies found in excavation sites during the last decades have not been dated yet and there is still a lot to learn about them and about their civilizations.

Studying human remains, like bones, teeth or hair, is of great importance in archaeology, since it enables a better understanding of the customs and traditions of ancient civilizations. Bone or tooth analysis is limited when it concerns the study of human remains from museum collections, whereas we can answer many questions thanks to hair analysis, without compromising the integrity of the remains. Hair is a very robust material that can be conserved throughout centuries and it is also a powerful biological indicator [3–5]. During its growth, hair integrates the different elements present in the blood, nutriments as well as toxic elements coming from the environment, the alimentation or some cosmetic treatments [6,7]. Along the hair fibers, we can thus follow the evolution of an individual's exposure to toxic compounds and update a lot of information about the environment of the peoples or about some of their customs, like their alimentation, their religious and cultural practices or their technical development [3,4,8–12]. Furthermore, the molecular fingerprint of the material have been modified by anthropic or natural alterations, before and during the funeral ritual or after the burial [13]. This complexity makes the analysis of archaeological remains a true analytical challenge.

Previous research on hair from mummies of the Arica and Camarones valley from Archaic to Late Intermediate periods (from 5000 years BC[1] to 1400 years AD) have shown high levels of heavy metals, such as arsenic and lead, using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [3,8]. This can be explained by the high concentrations in heavy metals in the rivers

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[1] BC: before Christ; AD: *Anno Domini*.

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and in the geological formations, due to intense volcanism of this area. The concentrations in arsenic have probably led to chronic poisoning and to a high infantile mortality in ancient pre-Hispanic populations [8,14,15]. To our knowledge, no thorough analysis was made in order to determine the conservation state of the hair components at structural and molecular levels. This problematic can however be essential in order to have a better view of the preservation state of the fibers and to see how the heavy metals interact with the hair molecules.

In the present work, we used a combination of optical and physical analysis techniques to have a global and structural overview of the conservation state of hair from two mummies from the Andean coast and belonging to a bigger corpus from Archaic to Inca periods (6500 years BC to 1500 years AD). The coupling with elemental analyses enabled to study the presence of heavy metals and non-common elements in the fibers. The optimization of the protocol for proteomics analyses is currently in progress to investigate the conservation of hair proteins at a molecular level and the first steps were applied to our archaeological samples.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and consumables

Sinapic acid (SA), ammonium bicarbonate (NH_4HCO_3) and sodium dodecyl sulfate (SDS) were purchased from Fluka (Buchs, Switzerland). Iodoacetamide (IAA), DL-dithiothreitol (DTT), sodium hydroxide (NaOH), Trizma® hydrochloride (Tris–HCl, buffer solution for proteins), thiourea, sodium deoxycholate (DCO), trichloroacetic acid (TCA), trifluoroacetic acid (TFA), formic acid (FA), dichloromethane, acetonitrile (ACN), acetone and silverwire for radiocarbon dating were from Sigma Aldrich (St. Louis, MO, USA). Urea was bought from Acros Organic (Geel, Belgium). Methanol (MeOH) and propan-2-ol (*i*PrOH) were supplied by Hipersolv CHROMANORM® (VWR Chemical Prolabo®, Radnor, Pa, USA). Ethanol was from AnalaR Normapur® (VWR Chemical Prolabo®, Radnor, PA, USA). Sequencing porcine grade trypsin was from Promega (Madison, WI, USA). Ultrapure water was produced with a water purification Purelab® UHQ system (ELGA LabWater VEOLIA Water, Anthony, France). Cupper oxide CuO was from VWR International (Radnor, Pa, USA), "Quartz" paper (glass filter prefilters, APFA02500) was from Millipore (Merck KGaA, Darmstadt, Germany) and dialysis cartridges (Slide-A-Lyzer 3.5 kDa, 3–12 mL) were from Thermo Scientific Pierce (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.1.2. Archaeological samples

We focused here on two hair samples belonging to a bigger corpus studied in the frame of a project in collaboration between the Laboratory of Archaeometric Analyses and Research from the Department of Anthropology of the Tarapacá University (Arica, Chile) and the Research and Restoration Center for French Museums (C2RMF, Paris, France). The mummies of the corpus were discovered in excavations led in the 1960s and 1970s in the north of the Atacama Desert, on the coast of Arica and Parinacota region in Chile. Mummy bundles were found sitting or with hyper-bended legs, wrapped in textile mantles and they were deposited in circular pits dug in the sand, which walls were covered with plant mats. In general, different kind of offerings as ceramics, artifacts of everyday life and some plants were disposed near the mummies [16]. After the excavation, the mummies were brought to the San Miguel de Azapa Museum (Arica, Chile). For their study, mummy bundles were open and the heads were separated from the rest of the body. More particularly, the two heads of mummies which were studied in this work, PLM7_Cr3c and PLM7_T305 (Fig. 1), were found in the archaeological site Playa Miller 7 (Arica valley) by Guillermo Focacci in 1974 [16]. According to previous dates and observations from the same site, archaeologists postulate that this site belonged to the Formative period (1700 years BC–500 years AD). The first challenge of this study was thus to replace the two mummies in their archaeological context.

2.1.3. Modern samples

Modern hair samples were used as standards for different analyses. They were taken from one European woman, who had been exposed to chemical hair bleaching, for the physical and chemical analyses, and from one European man, who had not been exposed to any cosmetic or chemical treatment, in order to take the inter-individual variability into account.

2.2. Hair treatments

2.2.1. Pre-wash cleaning

After a manual selection under the binocular microscope to remove all solid contaminants, between 15 and 30 mg of hair were

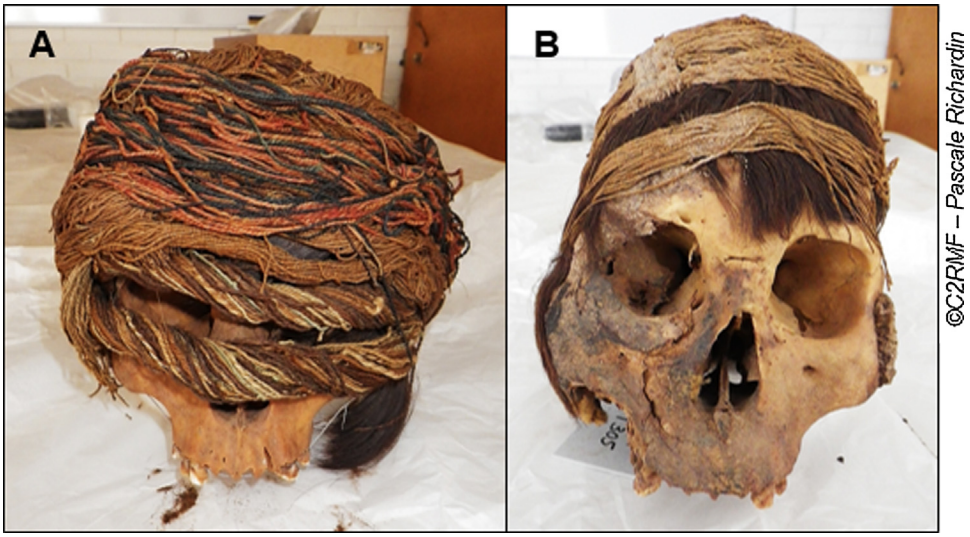

Fig. 1. Photographs of heads of mummies PLM7_Cr3c (A) and PLM7_T305 (B) from the excavation site Playa Miller 7 in the Atacama Desert, in Arica and Parinacota region (Chile).