MAPPING OF PIGMENT REMNANTS IN FOSSIL MATERIAL

B. van Dongen[1], P. Manning[1,2], N. Edwards[3], U. Bergmann[4], J. Anné[5], W. Sellers[1], A. van Veelen[6], D. Sokaras[4], V. Egerton[2], R. Alonso-Mori[7], K. Ignatyev[8], K. Wakamatsu[9], S. Ito[9], F. Knoll[1,10], and R. Wogelius[1].

[1]University of Manchester, UK; [2] College of Charleston, USA; [3]Stanford Synchrotron Radiation Lightsource, USA; [4]Stanford PULSE Institute, USA; [5]Children's Museum of Indianpolis, USA; [6]University of Southampton, UK; [7]Linac Coherent Light Source, USA; [8]Diamond Light Source, UK; [9]ujita Health University School of Health Sciences, Japan; [10]ARAID—Fundación Conjunto Paleontológico de Teruel-Dinópolis, Spain

Introduction

Determining the pigmentation (colour) of extinct organisms is one of the key challenges in paleontology. Advances in the analysis of soft tissue has recently provided great progress in this field. Novel state-of-the-art, non-destructive, chemical imaging and spectroscopy techniques, e.g. Synchrotron Rapid Scanning - X-Ray Fluorescence (SRS-XRF) imaging combined with X-ray absorption spectroscopy (XAS); has provided new information concerning the presence and distribution of eumelanin residue in fossil material over relatively large areas (dm^2), even after millions of years of potential degradation (Barden et al., 2015; Edwards et al., 2014; 2016; Manning et al., 2013; Wogelius et al., 2011). Eumelanin produces dark black/brown colours in vertebrates and invertebrates but is only one of the critical pigments governing color in ancient and modern lifeforms. Much less is known about the other melanin pigment, pheomelanin, responsible for the lighter reddish-brown colours. Until recently it was impossible to determine the presence and distribution of this pigment residue in fossil material.

Determining the presence of eumelanin in fossil material was possible since copper is the metal co-factor in the enzymatic process forming eumelanin (Hearing and Tsukamoto 1991), and elevated concentrations of organically bound copper can be correlated with eumelanin-rich fossil tissue (Gren et al 2017; Wogelius et al., 2011). Zinc, the second most abundant metal in mammal melanosomes, may correlate with the pheomelanin pigment. Pheomelanin synthesis requires the sulfur containing amino acid cysteine as a substrate, suggesting that there is sulfur contained within benzothiazole or benzothiazine units to which trace metals (zinc) could attach. This in contrast to eumelanin that has no sulfur groups that trace metals can bind to. Indeed, recent detailed SRS-XRF and XAS analyses of extant pheomelanin-rich feathers indicated a distinct chemical signature for zinc and organic sulfur, with a significant portion of the zinc inventory bound to sulfur (forming organosulfur-zinc complexes), almost certainly through the sulfur contained within the pheomelanin molecule (Edwards et al. 2016). In light of these new results, the aim of this study was to test whether, using similar SRS-XRF and XAS analyses, pheomelanin residue can now be resolved and mapped in extinct fossil material.

Results and discussion

Analyses of a range of well-preserved fossils indicate that the zinc coordination chemistry is comparable to zinc within modern pheomelanin-pigmented hair showing similar zinc/sulfur coordination chemistry and zinc-organosulfur compound distribution patterns. Sulfur spectroscopy of modern samples revealed a discrete signature caused by heterocyclic organic

sulfur compounds, such as benzothiazole, that can potentially be used as a biomarker for the presence of pheomelanin pigment in fossil material. However, analyses of fossil material indicate that the presence of other high-sulfur containing moieties, such as keratin (keratinous tissue) can complicate this signature. Breaking and oxidising of the disulfide bonds in keratin will result in an increased background in the sulfur K-edge spectra that could eventually obscure the weaker diagnostic (heterocyclic) sulfur signatures. This indicates that sulfur spectroscopy alone cannot be used to detect/map pheomelanin in fossil material. However, previous analyses of modern feathers indicate that zinc is not complexed with keratin but is almost entirely bound to the pheomelanin pigment by a zinc-sulfur bond (Edwards et al. 2006). Our analyses of a range of fossils suggest that this zinc-sulfur bond is more stable than the disulfide bond in keratin. Results show the presence of a significant amount of residual zinc-organosulfur compounds despite degradation of the surrounding keratin. By using a combination of organic sulfur XRF mapping and zinc spectroscopy we were able to resolve and map the distribution of zinc complexes in fossil material. Considering that these complexes are derived from pheomelanin residues, it was possible for the first time to resolve and map the presence and distribution of this pigment in fossil material.

Conclusions

Combined these analyses demonstrate that the spatial distribution of the different forms of melanin residues, eumelanin and pheomelanin, in extinct organisms may be resolved non-destructively over relatively large areas even after millions of years of degradation. This will ultimately help us to understand what these extinct organisms really looked like. Furthermore, it enhances our understanding of how these types of biomolecules have been preserved over time.

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