MP 009 Collagen Remains in Palaeotherium Bone from the Isle of Wight (UK)

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Introduction

Analysis of original biomolecules in ancient bone continue to grow. A range of techniques are used including LC-MS/MS, microscopy, RAMAN and FTIR being prominent. However, one noticeable area that lacks data is the sedimentological aspect of the ancient bone specimens used, e.g. location, excavation details, geological sequence context, matrix context, and specimen preservation details. This study uses LC/MS following trypsin digestion to extract collagen remains in a sample of *Palaeotheriid* jawbone that was excavated from the Isle of Wight (UK), containing sedimentological data sets and 3D Scan/model of the fossil. LC/MS for collagen analysis in ancient bone, compared with optical techniques provides the ability to see trends in original biomolecule preservation when comparing geological context.

Methods

An exceptionally well preserved *Palaeotherium* jawbone (FOSS293) was excavated from the Bouldnor Formation, (Isle of Wight, UK) along with contextual data. The jawbone was reconstructed using standard palaeontological techniques. For visualisation, a 3-D scan was made of the entire jawbone. A 20mg sample was taken from the lower ramus of the jawbone, ground to a fine powder (< 50 microns), before trypsin digestion and analysis by LC-MS using a Waters ™ Xevo G2-XS QToF. Spectra were scanned for peptide matches using the MASCOT software search engine. ATR-FTIR was performed on a Bruker Vertex 70 © equipped with Deuterated Lanthanum α Alanine doped TriGlycine Sulphate (DLaTGS) detector. Spectra in the range 4,000 to 600 cm^{-1} were collected and analysed with OPUS software.

Preliminary Data

The FTIR spectra showed a peak for amide I group (carbonyl, C=O) absorption around 1650 cm^{-1}. One of the factors determining peak institutes in FTIR spectra is the concentration of the molecules in the sample. The peak ratio for carbonyl over phosphate ("CO/P") is indicative the presence of organics within the sample. Following trypsin digestion and analysis by LC-MS, the results from the *Palaeotherium* jawbone were compared with LC-MS/MS tests performed on modern turkey bone (*Meleagris gallopavo)*, dating from 2022 with high collagen content. Comparison of LC/MS results showed strong correlation between two samples, with 6 – 8 peaks in the mass range of 1084 – 1086 occurring at the same m/z value as those in modern bone. Relative heights of the corresponding peaks from both samples (*Palaeotherium and Meleagris gallopavo*) are also in a similar ratio, howbeit at a much reduced signal intensity. The MS peaks

from the modern turkey bone results correspond to type 1 collagen, strongly indicating the presence of collagen in the *Palaeotherium* jawbone sample also. Collagen is a principal component of animal and human bone, being the main structural protein in extracellular matrix. The presence of collagen in ancient bone (particularly fossil bone) is of particular current interest and has been both claimed and contested. This study provides additional evidence from ATR-FTIR and LC/MS following protein digestion of the presence of type 1 collagen in palaeontological bone. The apparent presence of collagen in the *Palaeotherium* jawbone makes this the first experimental observation of original biomolecules within fossil bone from the UK. The additional sedimentological data now allows for comparison with other results from ancient (fossil) bone from different geological sequences from the Cretaceous period.

Novel Aspect

- First original biomolecules from UK fossil bone
- Establishes new methodology for archaeological and palaeontological analysis
- Novel geological & geographical location

Conflict of Interest Disclosure

The authors declare no competing financial interest.