Biogeochemical data from well preserved 200 ka collagen and skeletal remains

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Abstract

Data presented herein form part of a pilot study to reconstruct palaeoenvironment using isotopic data from 200 ka collagen and skeletal remains. To date such a study has only been possible on materials of up to 120 ka, due to poor preservation, but amino acid compositional analyses of mammal molar collagen herein indicate excellent preservation of the 200 ka collagen. Samples studied were found in association with faunal evidence for a temperate climate, and oxygen and carbon isotope data support this evidence. Collagen δ^{15}N values, however, are akin to those typically found in collagen from modern semi-arid to arid environments. Similar data have been reported for the Eemian interglacial, and it is likely that these data reflect specific environmental adaptations, as opposed to aridity. This pilot study emphasises the need for complementary isotopic and stratigraphical analyses in palaeoenvironmental studies. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The isotopic composition of mammal skeletal and collagen remains has been used extensively to reconstruct palaeoenvironments and palaeodiet throughout the upper Pleistocene period (e.g. [1]). Such studies typically analyse carbon and nitrogen isotopes from collagen; and carbon and oxygen from skeletal material, i.e. teeth and bones. Carbon isotopes from collagen can be used to identify the animal’s environment as δ^{13}C/δ^{12}C ratios tend to be lower in closed-canopy forests than in open plant formations, such as steppes and grasslands. Nitrogen isotopes in collagen are used to infer aridity or water stress, and studies indicate that herbivores from arid, low rainfall environments exhibit higher N ratios than those from temperate environments [2,3]. In skeletal material, carbon isotopes largely reflect an animal’s diet, i.e. the difference between C_{3} and C_{4} plant intake, whereas oxygen largely reflects in...
gested water oxygen [4]. To date, palaeoenvironmental reconstruction using collagen and skeletal material has only been carried out on materials of up to 120 ka [1] because collagen is rarely well preserved beyond this timescale. Data presented herein form part of a pilot study on 200 ka collagen for palaeoenvironmental reconstruction.

Channel deposits at Stanton Harcourt in southern Britain are believed to correspond to marine oxygen isotope (MOI) Stage 7 (200 ka) [5], and floral and faunal remains are indicative of a fully interglacial temperate climate [6,7]. The site yields well preserved mammalian remains, including mammoth (Mammuthus sp.), straight tusked elephant (Palaeoloxodon antiquus), horse (Equus ferus), bison (Bison priscus), red deer (Cervus elaphus), brown bear (Ursus arctos), and lion (Panthera leo).

Mammuthus sp. remains far outnumber all other mammal remains at the site, which may indicate favourable conditions for this species. However, these mammoths had less evolved dentition and were about one third smaller in size than Mammuthus primigenius from cold stage deposits. Size reduction and ‘primitive dentition’ also characterise mammoths from other British sites attributed to MOI Stage 7 [8,9]. Reduction in mammalian body size has been attributed to insularity and/or an unfavourable environmental change [10,11].

Terrestrial biological data from MOI Stage 7 sites are rare; this site thus provides a unique opportunity to investigate terrestrial climatic conditions during this interglacial period. Herein we analyse oxygen, carbon and nitrogen isotopes extracted from collagen, carbonate and phosphate fractions of Mammuthus sp., P. antiquus, E. ferus and B. priscus molars. Other mammal samples had been chemically treated for preservation and were not suitable for analysis.

2. Materials and methods

2.1. Collagen extraction

Collagen from the dentine fraction was analysed following the method outlined in [12]. Collagen analyses were performed using a Carlo Erba automated carbon and nitrogen analyser coupled to a Europa Geo 20/20 isotope ratio-monitoring mass spectrometer. Typical replicate measurement errors are of the order of ±0.2‰ for both δ13C and δ15N, which are reported relative to PDB and AIR, respectively.

2.2. Amino acid analyses

The preservation of collagen can be tested through analysis of the amino acid profile [13]. For this purpose, the isolated collagen protein was dissolved in distilled water to create a solution containing approximately 6 μg of collagen in a 20 μl aliquot. In this study the collagen solutions were run on an ABI 420A derivatiser/analyser following hydrolysis for 24 h in 5.7 N hydrochloric acid at 110°C [14].

2.3. Enamel phosphate and carbonate analyses

Samples for phosphate δ18O analyses were prepared using the silver phosphate method [15], which has an analytical reproducibility of ±0.1–0.3‰ (1σ).

Samples for carbonate δ18O and δ13C analyses were pretreated with hydrogen peroxide before acidification with 100% H3PO4 at 90°C on an ISOCARB 2 preparation system. Analyses of the evolved CO2 were carried out on a VG PRISM mass spectrometer, with reproducibility of ±0.1‰ for δ13C and ±0.3‰ for δ18O.

3. Results

3.1. Collagen

Collagen δ13C and δ15N data from fossil samples SH6/152, SH6/244, SH1/353, SH4/45 and 339 are listed in Table 1. Four samples yielded C:N ratios of around 3.2, equivalent to that of fresh collagen [16], but samples SH7/126 and SH8/7 yielded insufficient collagen for analysis, and collagen from sample SH1/353 had a C:N value of 5, which may be suspect. Collagen δ13C data for the four good samples are in the region of −21.6 to
The N\textsubscript{15}N data are greater than 10\times for all samples, with a range of 10.7\textsuperscript{\textcircled{AIR}}\textsuperscript{13.2}\times.

3.2. Amino acid profiles

Amino acid profiles were analysed in mammoth and elephant samples – SH6/45, SH6/244 and SH6/152 – and compared to profiles in modern elephants from Amboseli National Park, Kenya. Similar quantities of amino acids were extracted. These data (Fig. 1) show the modern and ancient collagen profiles to be remarkably similar, which suggests that the 200 ka collagen is well preserved.

3.3. Enamel

Oxygen and carbon isotope data from molar enamel carbonate and phosphate are listed in Table 2. The mammalian skeleton consists of both phosphate and carbonate, and phosphate oxygen (\(\delta^{18}O_{\text{PO}_4}\)) is related to carbonate oxygen (\(\delta^{18}O_{\text{CO}_3}\)) by \(\Delta_{\text{CO}_3-\text{PO}_4} = 9.2\%\) [15]. From Table 2, we see that for samples SH6/152, SH1/353 and SH4/45 \(\Delta_{\text{CO}_3-\text{PO}_4}\) has a mean value of 9.3\%, consistent with modern values and therefore indicative of well preserved material. Carbonate \(\delta^{13}C\) ranges between 3\textsuperscript{12.1} and 3\textsuperscript{10.1}\times, and carbonate \(\delta^{18}O\) ranges between 3\textsuperscript{24.3} and 3\textsuperscript{26.5}\times.

Carbon data are reported relative to PDB and nitrogen to AIR. Analytical precision is ±0.2\% for C and N. \(n = 4\) for all samples, where \(n\) is the number of samples from each specimen. SH1/353 is shown in italics as the C:N ratio was outside the acceptable range [16].

![Amino acid data from modern elephant (Harriet, Omega and M178) and fossil elephant and mammoth (SH6/152, SH6/244, SH4/45) samples. Similar amino acid compositions were observed in both modern and fossil collagen. Abbreviations: aspartic acid – Asp, glutamic acid – Glu, hydroxyproline – OH-Pro, serine – Ser, glycine – Gly, histidine – His, arginine – Arg, threonine – Thr, alanine – Ala, proline – Pro, tyrosine – Tyr, valine – Val, methionine – Met, cysteine – Cys, isoleucine – Ile, leucine – Leu, phenylalanine – Phe, lysine – Lys.](image-url)
phosphate $\delta^{18}O$, for the three samples analysed, ranges between 15.0 and 17.1 $\%$.  

4. Calculating palaeowater $\delta^{18}O$

There is a general relationship between precipitation $\delta^{18}O$ and ambient temperature [17-19], where lower water $\delta^{18}O$ values indicate lower ambient temperatures. This relationship can be complicated, however, by local environmental parameters. Precipitation in present-day southern Britain has a mean $\delta^{18}O$ value of around $3.7\%$. If similar environmental conditions prevailed at 200 ka, then similar precipitation $\delta^{18}O$ would be expected. A means of obtaining palaeoprecipitation $\delta^{18}O$ data is through interpretation of mammal phosphate and carbonate $\delta^{18}O$.

Mammal phosphate and carbonate $\delta^{18}O$ are directly influenced by body water $\delta^{18}O$, which is largely controlled by ingested (surface water) $\delta^{18}O$. This relationship, however, can also depend upon additional factors including animal species and dietary intake [20,21].

Different species can exhibit different relationships between phosphate (and carbonate) $\delta^{18}O_{PO_4}$ and ingested water $\delta^{18}O_{sw}$, i.e. the influence of ingested water on skeletal oxygen is variable due to variations in metabolism and water intake, but in general the relationship is approximately 0.75 [21]. Herein we analyse elephant, mammoth, horse and bison $\delta^{18}O$. The relevant relationships between $\delta^{18}O_{PO_4}$ and $\delta^{18}O_{sw}$ for these mammals are discussed below.

The investigation of [22] showed that the relationship between phosphate oxygen and ingested water oxygen ($\delta^{18}O_{PO_4}$-$\delta^{18}O_{sw}$) for elephants and mammoths had an unusually high slope. A more recent study [23] verified this relationship, on the basis of faunal and dietary data, to be:

$$\delta^{18}O_{PO_4} = 0.88 \times \delta^{18}O_{sw} + 23.9$$

(1)

The relationship between Bison sp. enamel and ingested water $\delta^{18}O$ has not been established. This can be developed, however, from data presented by [24]:

$$\delta^{18}O_{PO_4} = 0.77 \times \delta^{18}O_{sw} + 21.9$$

(2)

For Equus sp., $\delta^{18}O_{PO_4}$-$\delta^{18}O_{sw}$ has been extensively studied [25-27], and according to [27] it is:

$$\delta^{18}O_{PO_4} = 0.71 \times \delta^{18}O_{sw} + 22.6$$

(3)

Palaeowater $\delta^{18}O$ data for the Stanton Harcourt site, calculated using these species specific relationships, are listed in Table 2. Palaeowater $\delta^{18}O$ ranges from $-7.6\%$ to $-10.0\%$.

5. Discussion

The amino acid data show that the fossil and modern collagen profiles are very similar thus implying that the Stanton Harcourt collagen is well preserved, despite the antiquity of the samples. These are the first samples older than $\sim 120$ ka.

### Table 2

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sample</th>
<th>$\delta^{18}O_{CO_3}$</th>
<th>$\delta^{13}C_{CO_3}$</th>
<th>$\delta^{18}O_{PO_4}$</th>
<th>$\delta^{18}O_{water}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammuthus sp.</td>
<td>SH6/152</td>
<td>25.6</td>
<td>$-11.4$</td>
<td>16.2</td>
<td>$-8.7$</td>
</tr>
<tr>
<td>P. antiquus</td>
<td>SH4/45</td>
<td>24.3</td>
<td>$-11.3$</td>
<td>15.0</td>
<td>$-10.0$</td>
</tr>
<tr>
<td>Mammuthus sp.</td>
<td>SH1/353</td>
<td>26.5</td>
<td>$-11.3$</td>
<td>17.1</td>
<td>$-7.6$</td>
</tr>
<tr>
<td>P. antiquus</td>
<td>SH6/244</td>
<td>24.9</td>
<td>$-12.1$</td>
<td>$-$</td>
<td>$-9.3$</td>
</tr>
<tr>
<td>B. priscus</td>
<td>339</td>
<td>24.6</td>
<td>$-11.3$</td>
<td>$-$</td>
<td>$-8.5$</td>
</tr>
<tr>
<td>B. priscus</td>
<td>SH7/126</td>
<td>24.8</td>
<td>$-10.1$</td>
<td>$-$</td>
<td>$-8.1$</td>
</tr>
<tr>
<td>E. ferus</td>
<td>SH8/7</td>
<td>25.4</td>
<td>$-12.1$</td>
<td>$-$</td>
<td>$-9.0$</td>
</tr>
</tbody>
</table>

Water $\delta^{18}O$ data are calculated using animal specific relationships. Oxygen data are reported relative to SMOW, carbon to PDB. Analytical precision is $\pm 0.1\%$ for carbonate $\delta^{13}C$, $\pm 0.3\%$ for carbonate and phosphate $\delta^{18}O$. $n=4$ for all samples, where $n$ = number of samples from each specimen.

* Denotes values calculated from PO$_4$ or from CO$_3$ assuming $\Delta^{18}O_{PO_4-CO_3} = 9.2\%$.
to produce reliable collagen data, providing promise of further successful study of such ancient samples. That channel deposits have produced such high quality samples may be surprising, but similar collagen yields have previously been extracted from Seine River deposits [28].

Carbon isotope data obtained from both the carbonate and collagen fractions of the Stanton Harcourt mammal molars are consistent with an open environment consisting of C3 trees and plants [4], as expected from faunal and floral evidence. The oxygen isotope data from the carbonate and phosphate fractions can be used to infer that mean surface water δ18O at Stanton Harcourt was generally similar 200 ka to that of present-day Britain, thus suggesting that the Stanton Harcourt mammals experienced similar temperate conditions.

The collagen δ15N data, however, are higher than expected for a temperate climate. Animal collagen δ15N is influenced by dietary intake and because plants from arid water-stressed regions exhibit higher δ15N than normal, higher δ15N values are seen in animals consuming these plants [29,30]. Field data also show that mammalian δ15N may be further elevated relative to dietary δ15N under conditions of physiological stress, such as water stress associated with aridity [2,3].

Without baseline floral δ15N, it is impossible to interpret precisely the elevated δ15N measured in the Stanton Harcourt mammals, but it can be said that these values are similar to those of modern elephants from semi-arid environments (Fig. 2). The faunal and floral evidence from Stanton Harcourt, however, does not support a hypothesis of aridity, and as the empirical relationship between bison and mammoth collagen δ15N and precipitation has not been established, inferring aridity from these data may be premature. One may also expect δ18O values to be elevated if aridity was indeed a factor. It is of note that similar high N values have been recorded in Eemian herbivores from a forested environment [1], perhaps reflecting environmental parameters specific to certain parts of an interglacial period.

To date, collagen analyses of contemporaneous mammoth, horse and bison have shown mammoth δ15N to be notably higher than that of bison and horse [35–37], a feature that has been attributed to distinctive dietary adaptations [35]. However, at Stanton Harcourt the bison and elephant display similar δ15N values, which may be indicative of an environment that did not permit differentiation of feeding habitats. This hypothesis assumes, however, that these mammals would exhibit the same isotopic symptoms under the same environmental conditions. Such a scenario may have arisen from overcrowding or a reduction in food resources, but it has not been possible, as yet, to determine from stratigraphic data whether such conditions existed.

6. Conclusions

The collagen data are the first of this age to yield reliable amino acid profiles, indicative of unaltered collagen. The similarity in isotopic values between the mammoth, elephant and bison suggests that they shared a similar environment. However, the type of environment cannot be easily defined. The nitrogen data are similar to those from modern semi-arid environments but a hypothesis that such an environment existed at Stanton Harcourt does not tally with the faunal...
and floral nor the carbon and oxygen data. It is possible that certain parts of interglacials provide conditions that give rise to elevated $\delta^{15}N$ amidst temperate conditions but our current understanding of the factors affecting collagen $\delta^{15}N$ in large mammals cannot provide a definitive explanation.

This pilot study emphasises the need for the combination of several techniques for a more comprehensive understanding of palaeoenvironments. Work is underway to further investigate this site.

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