

# Biomolecules in fossil remains

## *Multidisciplinary approach to endurance*

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Svante Pääbo, a leading pioneer in the study of ancient DNA, eloquently described the recovery of genetic information from the fossil record as a 21st Century form of genetic time travel<sup>1</sup>. The advent of PCR made possible the amplification of small amounts of DNA from fossil samples and allowed the direct study of phylogenetics from extinct organisms. Prior to this development, phylogenetic relationships determined by genetic variation relied mostly upon sequences from living organisms. The concept of time travel, via the analysis of ancient biomolecules, can be broadened to encompass numerous types of biomolecular information recovered from ancient bones. For example, palaeodiets and palaeoclimates can be reconstructed from stable isotopes of bone collagen, and estimations of age are obtained from amino acid racemization rates.

Despite notable successes in the retrieval of biomolecular information from ancient remains over the last 10 years, failure remains an occupational hazard. This is because of numerous complications inherent in the recovery of ancient biomolecules from the vertebrate fossil record. In spite of a rudimentary understanding of degradation reactions, problems of validity, contamination and degradation are compounded by the destruction of irreplaceable fossils, often without material benefit. In the case of DNA, some of these issues have been raised and recent reviews have called for more methodical and rigorous approaches to alleviate the present levels of scepticism<sup>2</sup>. However, a more encompassing, systematic approach to the problems associated with the recovery of biomolecular information from fossils is clearly required.

Identification of the mechanisms and controlling factors for biomolecular preservation in ancient material are the main objectives of the Ancient Biomolecules Group (ABG)

at the University of Newcastle upon Tyne. The ABG has an interdisciplinary and systematic approach to biomolecular preservation and works in close collaboration with colleagues studying ancient DNA (Dr Alan Cooper, University of Oxford), ancient proteins (Dr Peggy Ostrom, Michigan State University) and the application of synchrotron radiation to biological materials (Dr Tim Wess, University of Stirling). Research within the ABG encompasses both mineral and biomolecular degradation processes in ancient bone and relates these processes to the burial environment. Ultimately, the goal of this research is to address one of the most fundamental questions in bioarchaeology. To what extent are usable biomolecular signatures preserved into deep time?

### **Bone diagenesis**

The main stumbling block in the isolation of ancient biomolecules from fossil remains has always been the complicated issue of preservation. Understanding bone diagenesis, or

the post-mortem alterations in the physical and chemical composition of bone following its deposition in the geological environment, is critical to understanding biomolecular preservation. Studies focusing on archaeological material (<6000 years BP; Before Present) have suggested that biomolecular preservation is dependent upon the intimate relationship between protein (mainly collagen) and mineral (a carbonated form of hydroxyapatite) components, the integrity of which is maintained by key elements of the burial environment<sup>3</sup>. Subtle alterations measured in archaeological bone mineral structure have been related to degradation and loss of both osteocalcin and collagen<sup>3,4</sup>. In addition to the integrity of the mineral phase, bones yielding well preserved biomolecules show no evidence of microbial attack (as determined using current histological techniques)<sup>3</sup> – a result that echoes that of Cooper et al. for much older specimens<sup>5</sup>.

In its simplest form, bone diagenesis can be seen to proceed via three alternative pathways: chemical deterioration of the organic phase, chemical deterioration of the mineral phase, or biodegradation (utilization of the bone components as an energy source by micro-organisms) (Figure 1)<sup>3,6</sup>. These three pathways are by no means mutually exclusive, though it is not clear to what extent they can proceed in isolation. Which pathway predominates is dependent upon the burial environment. Studies on ancient samples have shown that once the stability of either the

mineral, or protein is compromised, the other becomes vulnerable to rapid deterioration<sup>3–6</sup>. Both dissolution of the mineral phase (pathway 2, figure 1), or microbial attack (pathway 3, Figure 1) are considered to be ‘fast’ pathways for biomolecular degradation<sup>3,6</sup>. If either of these pathways predominates, biomolecular survival is greatly compromised. In environments where the bone mineral phase is stable and where microbial attack is limited, the degradation of biomolecules is governed by ‘slow’ chemical processes (pathway 1, Figure 1), the rates of which are believed to be controlled by temperature and pH<sup>3,4,7</sup>. In these cases, the likelihood of finding fossil remains with intact biomolecules is considerably higher than in cases where pathways 2 and 3 predominate. The key to biomolecular survival, therefore, depends on which diagenetic pathway is followed. Fossils with diagenetic histories dominated by slow, chemical processes provide us with the best opportunity to explore fully the limits of biomolecular survival. These limits have been studied using the kinetics of chemical degradation for DNA, collagen and osteocalcin.

### Defining limits

Consistent with current thinking, success in sequencing DNA from

high latitude and permafrost fossils supports the contention that low temperatures enhance the preservation of biomolecules<sup>5,7,8</sup>. Using the kinetics of DNA depurination, members of the ABG have conducted a comprehensive estimate of the limit of DNA survival, which they believe to lie at 17500 years at a constant temperature of 10°C (Table 1)<sup>7</sup>. Using the kinetics of collagen and osteocalcin degradation, limits of survival have also been estimated for these biopolymers (Table 1). The predicted order of survival is DNA, collagen, osteocalcin. This order supports the contention that proteins offer a more stable substrate for ancient biomolecular studies<sup>4</sup>. These limits are appropriate at burial sites where the thermal history is the determining factor<sup>7</sup>, i.e. where diagenesis is governed by slow, chemical deterioration of the organic phase. This is because the key biomolecular degradation reactions (hydrolysis, gelatinization and browning), all have high activation energies and thus are likely to occur at very slow rates at low temperatures.

If thermal history is the controlling factor, the kinetic models suggest that proteins may afford us the opportunity to recover genetic information from warmer environments, where attempts to recover ancient DNA are less sure of success<sup>2,7</sup>. In more temperate burial environ-

ments, osteocalcin has a predicted survival limit of 580 000 years at 20°C and 7 500 000 years at 10°C (Table 1).

Although the kinetic models predict a much longer propensity for the survival of proteins over DNA, they have yet to be tested with fossil material. If proteins do survive over great periods of time, they represent a source of genetic information that, although not as informative, can extend the molecular palaeontological record beyond that of DNA. Thus, building on foundations laid by Ostrom et al., the author has become involved in recent efforts to sequence ancient proteins using a mass spectrometric method; matrix assisted laser–desorption ionisation mass spectroscopy (MALDI–MS)<sup>9</sup>. Success with MALDI–MS would provide molecular palaeontologists with a new tool with which to recover genetic information from ancient bone.

### Beyond DNA?

Typically, organic material from fossils only survives in very small quantities and often in degraded and contaminated forms. With these limitations to consider, recent technical advances in biochemistry are particularly appropriate for exploitation by molecular palaeontologists for the analysis of ancient organic material. Both MALDI–MS and liquid

**Table 1.**

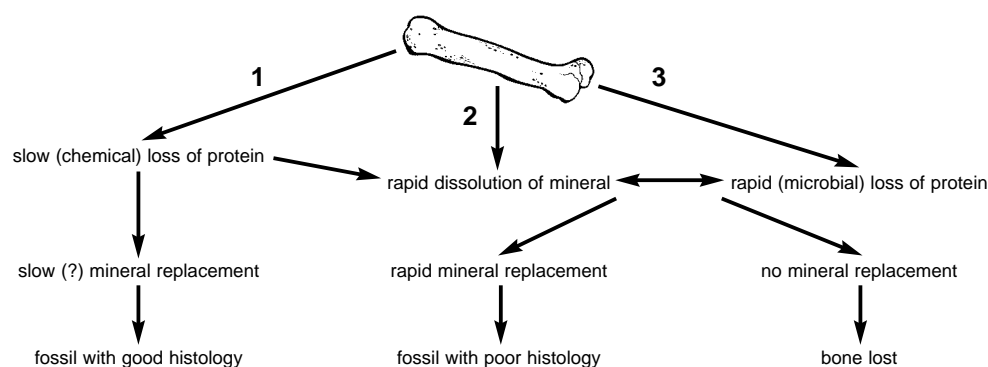
Expected persistence of biomolecules at temperatures of 0°C, 10°C and 20°C

	Concentration in bone (by weight)	Method	$E_a$ (kJ·mol <sup>-1</sup> )	Detection limit (years×10 <sup>3</sup> BP)		
				0°C	10°C	20°C
$t_{1/2}$ DNA	0.001%	Estimated based upon limit of amplification using $E_a$ for DNA depurination in solution <sup>7</sup>	127	125	17.5	2.5
$t_{1/2}$ Collagen	22%	Estimated based upon laboratory measured rates of gelatinization (M. Collins et al., unpublished work)	173	2700	180	15
$t_{1/2}$ Osteocalcin	0.2%	Estimated based upon laboratory measured rates loss of epitope for the Gla-rich mid-region (M. Collins et al., unpublished work)	175	110000	7500	580

chromatography–electrospray ionization–MS (LC–ESI–MS) are powerful tools to apply to the sequencing of ancient proteins. MALDI–MS requires only picomoles of sample and does not show interference from most salts and solvents used for protein purification<sup>10</sup>. LC–ESI–MS has a subpicomolar sensitivity for proteins and peptides and, therefore, may lower the detection limits that are achievable with MALDI–MS. Using minimal quantities of sample, both MALDI–MS and LC–ESI–MS have the potential to expand the field of ancient protein research in a manner analogous to the impact that PCR had on the analysis of ancient DNA, but if the estimations are correct, they will allow analysis of much older samples. Collaborations between the ABG and Michigan State University suggest that the isolation and sequencing of intact osteocalcin from fossil samples is imminent. If we can sequence ancient proteins in addition to DNA from ancient bone, we will augment our ability to derive phylogenetic information from fossil material and we will be much better equipped for time travel.

## Future directions

This article outlines current efforts from a multidisciplinary study that attacks the problems of biomolecular preservation on numerous fronts. Integrated studies involving multiple lines of investigation offer a new approach to the problems associated with recovering biomolecules from the fossil record. This author believes that our questions concerning biomolecular endurance will only be resolved through such encompassing research. A fundamental aspect of this approach is the constant search for more powerful analytical techniques that also limit



**Figure 1.** Alternative pathways of survival for bone in the burial environment. (1) chemical deterioration of protein; (2) chemical deterioration of mineral; (3) biodegradation of protein.

the destruction to valuable and irreplaceable fossils. One of the key issues to success in this research is a better understanding of bone diagenesis, and the relationship between the protein and mineral components. Just as exploitation of advances in proteomics represents a revolutionary direction for ancient protein sequences<sup>9</sup>, small angle X-ray scattering (SAXS) generated from synchrotron radiation, could revolutionize our understanding of the organic–mineral interactions in bone. Using SAXS, the structure of molecules and the architecture of tissues in the native state can be determined. By providing high-resolution details of bone mineral alteration, SAXS has the potential to further elucidate the nature of the relationship between biomolecules and the mineral matrix and to greatly enhance our understanding of diagenetic processes<sup>11</sup>.

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