proteinspotlight
> ONE MONTH, ONE PROTEIN <

Issue 46, May 2004

www.proteinspotlight.org

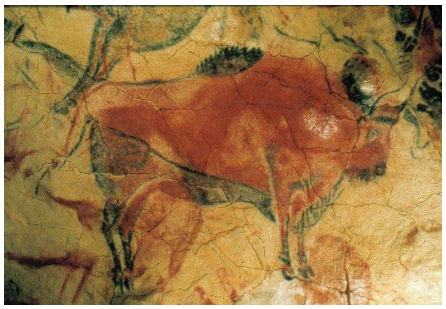
A small blast from the past

Vivienne Baillie Gerritsen

'What's a fossil Mum?' To which most Mums would answer, 'A fossil, sweetie, is bone which has become stone because it's been lying somewhere for a very long time.' The process of bone diagenesis is a complicated one but on the whole Mum's answer is not incorrect. However, before the bone becomes absolute stone and depending on the environment, some organic parts can survive for a long time. Even millions of years. And these are excellent candidates for study. Scientists have already managed to extract DNA from fossil bone – though in poor condition. Which is a pity, because DNA – though minute – can stash huge amounts of information. What is needed is something that not only lasts but is also informative from a biological point of view. A protein perhaps?

Yes. In the 1980s, a bone protein – osteocalcin – was detected in bovid bones which dated back 13 million years and rodent teeth that dated back 30 million years! A decade later, osteocalcin was found in 75 million-year-old fossils such as the duck-billed dinosaur. The tricky part was to extract the protein from the bone not only intact but also in sufficient quantities to be able to sequence it. This was only achieved a decade later in fossil bison bone. But what an achievement. And little did osteocalcin know that it would become such a famous molecule.

Bone is an exceptional organic tissue in that it is largely mineral (70-90%). The organic part is mainly collagen; non-collagenous matrix proteins make up the rest, of which osteocalcin represents just a tiny fraction. Osteocalcin is a small protein, barely 50 amino acids long, and is also known as the bone Gla-protein because it has a number of γ-carboxyglutamic acid (Gla) residues in its sequence. It is found exclusively in bone, where it is secreted by osteoblasts, and binds to the bone minerals. The binding of osteocalcin to the minerals is so strong that this is most probably what protects it from vanishing in the process of fossilisation. The protein is directly associated with bone formation and mineralisation – though in what way still remains unclear. What has been discovered is that impaired osteocalcin expression causes bone calcification to spread into nearby cartilaginous structures, so it must have a role in directing and controlling bone formation.



Bison from the Altamira Cave in Spain.

The osteocalcin that became a star was extracted from fossilised bison bone – *Bison priscus* – that was radiocarbon-dated back almost 60'000 years. *Bison priscus*, though now extinct and more commonly known as the steppe bison, remains familiar by way of prehistoric paintings in Paleolithic caves and fossils found in permafrost – in particular, a near intact carcass of an 8 to 9 year-old male found in 1979 in Alaska and known as 'Blue Babe'. 'Blue' because the specimen was almost entirely coated with vivianite – a blue iron-phosphate – and 'Babe' from the North American folk tales of Paul Bunyan, a lumberjack who once raised a blue ox…

Steppe bison stampeded the steppe-like grasslands of northern Eurasia and North America during the Pleistocene – 2 million to 10'000 years ago. The bone fossils, from which

was extracted osteocalcin, were found in Alaskan and Siberian permafrost.

Osteocalcin was successfully extracted from crushed bone fossil and fully sequenced – something that had met with hopeless failure until the year 2002. How was it done? Small amounts (20 mg) of osteocalcin were extracted thanks to techniques to which molecular geneticists are largely accustomed and are used for DNA purification – not protein purification. Until then, large amounts of protein had always been needed but were very difficult to come by. The novel techniques solved the 'quantity' problem. The sequencing was performed via matrix-assisted laser desorption ionisation mass spectrometry which – unlike sequencing by Edman degradation – is not hindered by amino-terminal blockage. This solved the 'sequencing' problem. As a result, the protein was fully sequenced and the ancient bison protein was found to match the modern osteocalcin bison protein. The sequence even predicted the single amino-acid substitution which makes the difference between cow and bison osteocalcin sequences.

What is the point of it all? Though DNA is far more informative from an evolutionary point of view than protein – and is also far easier to sequence – it is also far more subject to lab contamination. What is more, DNA does not survive as well as proteins do in the process of fossilisation. It has been estimated that protein matter could survive millions of years whilst the more fragile DNA seems to survive only thousands. This is an exciting prospect since proteins could take us right back to the beginnings of human evolution. The key now is to seek proteins which are more informative on the subject of evolution or even animal behaviour. Besides palaeontology, studies on the survivability of proteins – i.e. protein degradation – are also of great interest to those who work in the field of forensics. Furthermore, research on osteocalcin itself could inspire the design of drugs to treat bone diseases such as osteoporosis, in which osteocalcin may well have a role.

Cross-references to Swiss-Prot

Osteocalcin, *Bison priscus* (steppe bison) : P83489

References

1. Nielsen-Marsh C., Ostrom P.H., Gandhi H., Shapiro B., Cooper A., Hauschka P.V., Collins M.J.
   Sequence preservation of osteocalcin protein and mitochondrial DNA in bison bones older than 55 ka
   Geology 30:1099-1102(2002)

2. Nielsen-Marsh C.
   Biomolecules in fossil remains
   The Biochemist: 12-14(2002)

Protein Spotlight (ISSN 1424-4721), http://www.proteinspotlight.org, is published by the Swiss-Prot group at the Swiss Institute of Bioinformatics (SIB).