ANCIENT BIOMOLECULES INITIATIVE

Molecular Signatures from the Past

Proteins in dinosaur bones?

Can protein molecules survive inside dinosaur bones?

Michael Crichton's Jurassic Park excited the public imagination with a tale of the regeneration of dinosaurs from their DNA extracted from insects preserved in amber. The advances in modern DNA methods and computer modelling were the scientific basis for a really good yarn. But do organic molecules really survive well enough to enable scientists to do this?

DNA does not survive intact in dinosaur bones, but some amino acids - the building blocks of proteins - do. Maybe damaged protein molecules can survive over millions of years.

Dinosaur bones of 75.5 million years old have been reported still to contain an unusual amino acid known as Gla. We suspected that the Gla reported in dinosaur bones comes from osteocalcin, a small, very stable protein found in bones. Does this protein survive long enough to be found in the fossil record?

Our preliminary findings are of variable preservation, which can be excellent where the original microporosity of the bone has survived biodegradation

We took modern bone samples and artificially aged them to investigate the stability of osteocalcin. We used antibodies that recognise specific regions of the osteocalcin molecule to find out if the protein survives the ageing process.

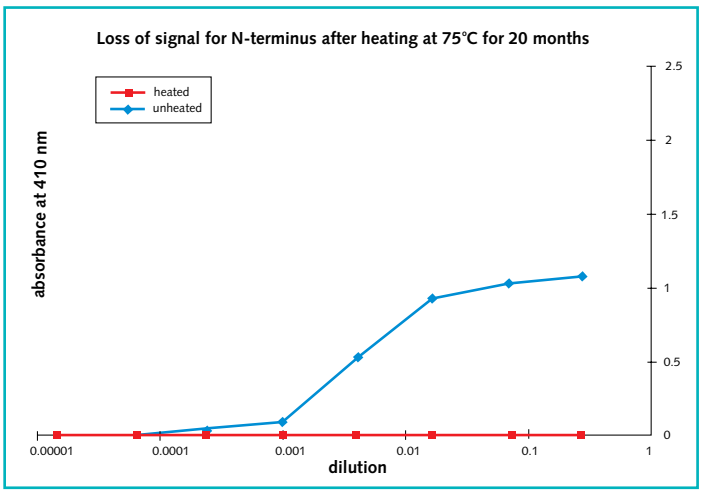
Our results show that some regions of the molecule survive better than others, and that the most robust parts may survive even in dinosaur bones.

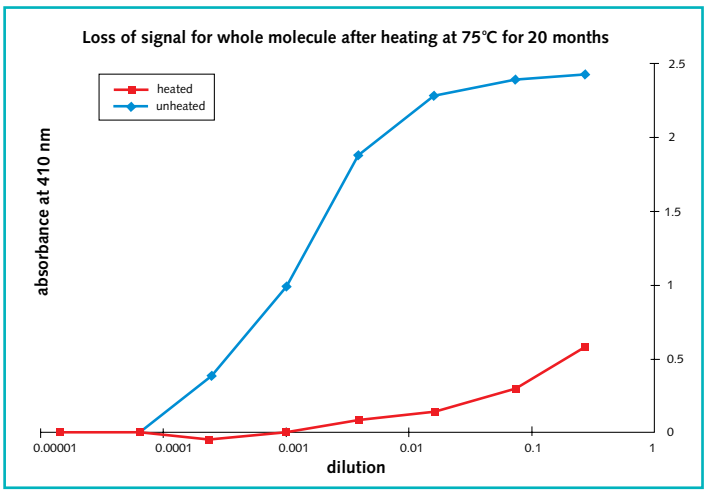
We used computer simulations to model the interaction of osteocalcin with calcium to investigate how the protein may be preserved. This work progresses; so far it has given us a valuable insight into the role of calcium in maintaining the protein integrity, and its stability with time.

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge.
The Initiative is funded by the Natural Environment Research Council.

Natural Environment Research Council

Proteins in dinosaur bones: the science in detail





Monoclonal antibodies that bind to the N-terminal end of unheated osteocalcin (unheated) can no longer bind to osteocalcin that has been heated at 75°C for 20 months (heated).

Fewer polyclonal antibodies bind to osteocalcin which has been artificially aged by heating at 75°C for 20 months (heated) than to intact unheated osteocalcin (unheated).

Our project investigated the stability of the bone protein, osteocalcin, during artificial ageing. The reports of γ–carboxyglutamic acid (Gla) in dinosaur bones and the demonstration of osteocalcin in archaeological bones (7.5 million years old) where collagen has not survived well, has led to the suggestion that it is the Gla residues found in the helical region of osteocalcin that promote its preservation. We have done artificial ageing studies using bone powders (hydrated and dried) followed by immunochemical detection and chromatography to look at the preservation potential of osteocalcin in bone. We found that some regions of the osteocalcin molecule are much more stable than others.

The investigation

Organic materials, whether protein, nucleic acid or lipid, can be broken down both chemically and by microbes. The degree of degradation will depend upon the accessibility of the molecule and, in bone, the amount of protection afforded by the presence of the mineral matrix.

We studied the artificial ageing of osteocalcin, which is thought to be the best surviving protein in bone and which might be useful for the radiocarbon dating of archaeological bone in which collagen does not survive. Osteocalcin is a small bone protein (see diagram) made up of a chain of 49 amino acids, three of which are Gla, a highly acidic amino acid. In the presence of calcium, the Gla region of the molecule coils into a helix so that the acidic side chains of the Gla residues are aligned. On one end of the helix, the protein forms a loop; the two ends of the protein are tucked inside the helix and loop regions. The molecule is thought to be protected from degradation because of the close interaction of the Gla side chains with calcium hydroxyapatite, the mineral phase of bone.

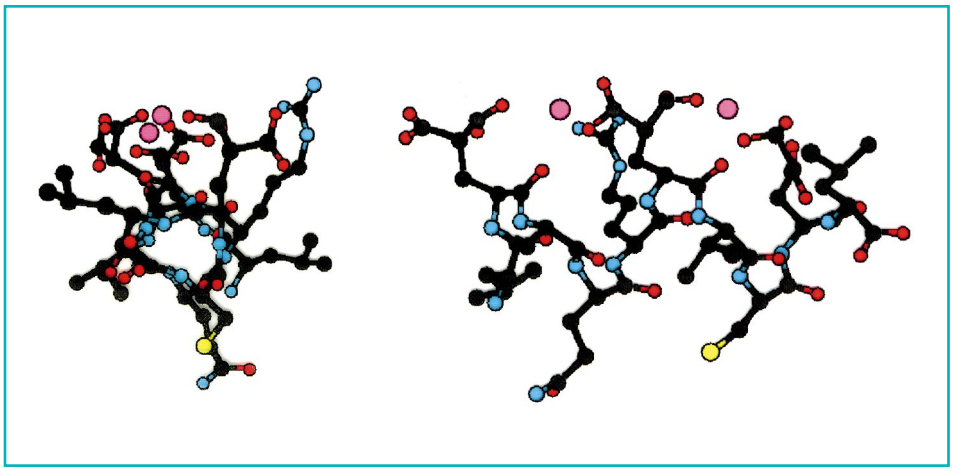
We chose antibodies that could bind to different regions of the osteocalcin molecule, so that we could tell which regions remained intact and which were destroyed. As we expected, heating decreases the ability of the antibodies to bind to osteocalcin. Some of the polyclonal antibodies (which bind to several different sites) still recognised osteocalcin after it had been heated for 20 months, showing that parts of the molecule still retain their original shape (see right-hand graph).

But the monoclonal antibody which binds only to the N-terminal region no longer recognised heated osteocalcin (see left graph), indicating that this part of the molecule has been lost, despite being 'tucked' inside the helix and loop regions.

It is possible that the polyclonal antibodies, although raised to several sites on the osteocalcin molecule, have one (or two) major sites of recognition. We are still finding out where these sites are, using fractions where known regions have been lost.

Methods

We artificially aged modern powdered bovine bone (hydrated at 95% relative humidity or freeze dried) by sealing it in tubes under argon, and heating it at 75°C, 85°C and 95°C for up to 20 months – a standard method for studying the effects of long-term chemical processes. We assessed the effects of this treatment by measuring how well certain antibodies bound to the osteocalcin before and after heating. If the antibodies no longer bind to the molecule, it shows that it has changed its shape or has started to break down.



End on and side view of a predicted conformation for the region of osteocalcin believed to bind to the bone mineral. The pink calcium ions are seen to be strongly held by acidic γ–carboxy glutamyl residues of the protein. It is this region of the molecule which we think is best preserved in ancient bones.

We have also performed computer simulations of the effects of heating on the structure of the Gla-helix region of the osteocalcin molecule in the presence and absence of calcium. These studies continue, but are giving us a valuable insight into the role of calcium in the maintenance of the helical structure; the partial preservation of the helix when one or more Gla residues decarboxylate to become glutamic acid (a phenomenon which is believed to occur during ageing) and hence the potential of the molecule to survive.

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