

Archaeology and Capillary Electrophoresis

A case of haemoglobin identification



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The Problem

The ability to perform DNA analysis on ancient organic material has provided archaeologists with a powerful tool to help understand the past. Unfortunately some remains do not contain sufficient DNA for analysis. Thus to prevent wasting time and money a preliminary test to indicate the possible survival of sufficient DNA was needed. Amino acid racemization has been proffered as this preliminary technique. However recent evidence indicates that this method is not as reliable as it first seemed (Collins et al. 1999). This study investigated the possible use of CE as a means of identifying proteins in ancient samples. For if proteins can be detected then DNA, in sufficient quantities for analysis, should also be present. However, this is not the only use of CE. This technique can be used to provide independent identification of residues and work as a complementary means of analysis to a number of existing techniques.

Amino Acid Racemization

All proteins are made from amino acids, the atoms of which are all in one conformation (D). Over time some of the atoms convert to an "L" form. When the number of D and L forms are equal the sample is said to be fully racemized. The rate of racemization is dependent upon many factors including temperature and moisture. Calculations of the rate at which hydrolysis takes place indicate that all proteins in a sample would be broken down into their amino acid components and diffuse out of the fossil by 100,000-1,000,000 years. Thus amino acid racemization has been used to estimate the degree of DNA preservation as a screening test prior to DNA analysis.

Samples



Alces alces, the modern descendent of *Alces latifrons*. Extracted residue from the right radius muscle, dated at 32,000BP. The sample is from the University of Alaska (Fairbanks) Museum.



Bison priscus. Extracted residue from the metatarsal muscle of the hind leg dated at 36,000BP. The sample is from the University of Alaska (Fairbanks) Museum.

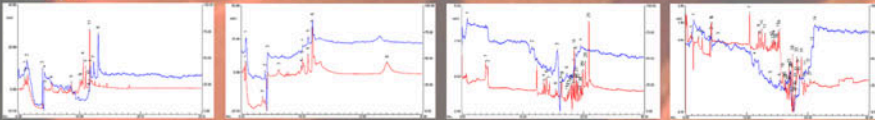


Diprotodon. Extracted residues from three bone samples dated at ca. 100,000yrs. The sample is from the Lake Eyre basin and is held at ANU



Residues extracted from a number of stone tools from British Columbia. Sites include Muncho Lake and Toad River Canyon.

Results



Alces latifrons sample. Red trace is at 280nm and blue trace is at 410nm. Nice clear peaks with reasonable alignment of peaks indicating presence of haemoglobin.

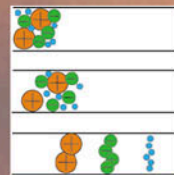
Bison priscus sample. Red trace is at 280nm and blue trace is at 410nm. Excellent alignment of peaks indicating the presence of haemoglobin.

Diprotodon scapula sample. Red trace is at 280nm and blue trace is at 410nm. Lots of proteins detected at 280nm with reasonable alignment indicating haemoglobin.

Diprotodon mandible sample. Red trace is at 280nm and blue trace is at 410nm. Again lots of proteins at 280nm and reasonable alignment indicating haemoglobin.

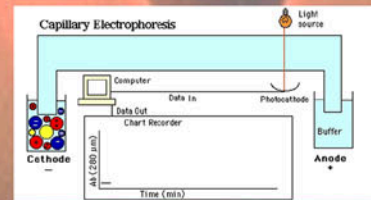
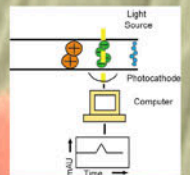
How CE Works

CE works by separating molecules based on their physical characteristics, usually charge to mass ratio.



When the electric current is applied the charged molecules move towards the appropriate electrode. As they move the molecules separate into discrete bands.

As the bands of molecules pass the detector variations in the UV absorbance is detected. A computer analyses the data to produce an electropherogram (EPG) showing the bands as peaks.



The sample is injected into the inlet end of the capillary, the capillary ends and the electrodes are then placed into vials of buffer. The buffer conducts the electricity through the capillary creating current which causes the molecules to move.

Modes of CE and their Archaeological Uses

Modes	Basis of Separation	Type of Molecules	Archaeological Use
Capillary Zone Electrophoresis (CZE)	Charge to mass ratio - charged molecules	Small ions, Small molecules, Peptides, and Protein	Analysis of lipids, proteins, fatty acids in plant and animal remains
Capillary Gel Electrophoresis (CGE)	Size exclusion - charged molecules	Peptides, Proteins, Oligo-nucleotides, DNA	Analyse denatured proteins, DNA visualisation, sexing and sequencing
Micellar Electrokinetic Capillary Chromatography (MECC)	Interaction of sample molecules with additives - neutral molecules	Small molecules, Peptides and Oligo-nucleotide.	Plant and animal material
Capillary Isoelectric focusing (CIEF)	Unique isoelectric point (The pH at which the molecule becomes neutral)	Peptides and proteins	Species identification based on the proteins isoelectric point.

Discussion

Using the isoelectric focusing capabilities of the capillary electrophoresis system, haemoglobin was detected in the tissue and bone extracts. The presence of haemoglobin as well as other proteins indicates that DNA should also be present in the samples. This is confirmed by previous analysis of these samples which detected and amplified DNA. Further work will need to be undertaken in order to establish this technique as a reliable indicator of the presence of DNA. Future research will also be needed to improve the results obtained from stone tools and extend the use of CE to other materials. The variety of modes available with CE means that a large variety of materials could be studied but each mode will need careful development. Capillary electrophoresis is a powerful method for analysing organic remains and warrants further study.

References

Collins et al. (1999) "Predicting protein decomposition: The case of aspartic-acid racemization kinetic" Philosophical Transactions of the Royal Society of London B 354:51-64

Acknowledgements

Dr Tom Loy, Carney Matheson, Ondrej Hlinka, Alison Crowther, Justine Eckersley and all the other members of Tom's Group.