Amino acid racemisation dating

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The potential of the time-dependent amino acid racemisation reaction as a method of age assessment was first reported by Hare and Abelson (1968). They noted that in specimens of the bivalve mollusc *Mercenaria* sp., greater concentrations of amino acids in the D-configuration with increasing fossil age. Hare and Abelson (1968) also reported negligible racemisation in a modern specimen of *Mecanaria* sp. On this basis they suggested that the extent of amino acid racemisation (epimerisation in the case of isoleucine) may be used to assess the age of materials within and beyond the range of radiocarbon dating. For the past thirty years amino acid racemisation has been extensively applied in Quaternary research as a method of relative and numeric dating, and a particularly large literature has emerged on the subject (see summaries in Miller and Brigham-Grette, 1989; Murray-Wallace, 1993; Rutter and Blackwell, 1995).

The racemisation (epimerisation) reaction

During diagenesis amino acids and their precusor peptides may undergo numerous reactions that include oxidation, decarboxylation, deamination, hydrolysis and racemisation (epimerisation). In the protein of living organisms, amino acids are bound in peptides as left-handed molecules (L-amino acid: *levorotatory*), a phenomenon that has been related to enzymic reactions (Williams and Smith, 1977). Following the death of an organism, the enzymic reactions that formerly maintained the disequilibrium state cease (i.e. exclusively L-amino acids) and amino acids then slowly and progressively interconvert from a left-handed to a right-handed counterpart (D-amino acid: *dextrorotatory*). In this context, the event dated by the racemisation reaction is the time of death. Exceptions to this, however, relate to teeth and eye lenses, matrices in which racemisation is known to occur in during life. This process is termed amino acid racemisation for amino acids with only one chiral carbon centre (*i.e.* enantiomers such as aspartic acid) and epimerisation for amino acids with two carbon centres (*e.g.* isoleucine). The interconversion of L- to D-amino acids continues until equilibrium (i.e. D/L=1 for enantiomers and approximately 1.3 for diastereoisomers such as isoleucine).

Parameters that influence racemisation in natural systems

As the amino acid racemisation reaction is senstive to a variety of environmental parameters, considerable caution should be exercised when collecting samples for analysis. Broadly, in order of significance the major environmental parameters which may impinge on the utility of the method for a particular field setting include: (1) diagenetic temperature history; (2) fossil genus and matrix; (3) moisture regime; (4) hydrolysis state of peptides; and (5) pH and clay mineral catalysis.

Diagenetic temperature history

The most important parameter influencing amino acid racemisation in natural systems is the diagenetic temperature history. This refers to the integral of all temperatures a sample has experienced from the onset of racemisation to the time of sampling. Most significantly, temperature changes have an exponential influence on racemisation rate, such that for every 1^{o}C

increase in temperature, the racemisation will increase by approximately 20%. Accordingly, racemisation rates double for every 5°C increase in temperature, a phenomenon predicted by the Arrhenius equation (Williams and Smith, 1977). This relationship is also apparent in fossil marine molluscs of Last Interglacial age (*ca.* 125 ka; Oxygen Isotope Substage 5e; Murray-Wallace, 1995). The temperature dependence of racemisation reactions is also illustrated by the fact that aspartic acid will undergo ten times the extent of racemisation in 1000 years at 25°C than in 2000 years at 12.5°C (Davies and Treloar, 1977).
As temperature is such a significant consideration for amino acid racemisation dating, it is imperative that samples be obtained from well-buried situations (i.e. > 1 m). This will reduce the influence of diurnal and seasonal temperature extremes, such that the temperature changes which will have influenced racemisation in the materials selected for dating relate to longer-term climatic change.

Other considerations that relate to temperature centre on whether the fossils have been subaerially exposed for a lengthy part of their diagenetic history. In such situations, high summer temperatures will have disproportionately influenced racemisation rate. In some circumstances, however, this may be useful as it provides an additional means for assessing whether the materials have been exhumed and then re-buried. From an archaeological perspective, there is the further question of whether the materials have been burnt. Here, issues such as the intensity and duration of the heating event become important. For example, a short-term heating event from a low intensity fire might have a negligible influence on the extent of racemisation for archaeological materials that are several thousand years old. Evidence for heating events are more likely to be identified in younger materials and include: (1) unusually high extents of racemisation with respect to age, and (2) rapid loss of unstable amino acids such as threonine and serine.

Fossil genus and matrix
Rates of racemisation vary according to fossil genus and the nature of the matrix host to the residual protein (*e.g.* molluscs, bone, teeth and wood). The genus-effect in marine molluscs relates specifically to the original make up of proteins and polypeptides. A strong correspondence between racemisation rates in different mollusc genera, and the classical taxonomic subdivision of the phylum mollusca has been documented (Wehmiller, 1984). Accordingly, amino acids that are bound in polypeptides with stable peptide-linkages will undergo slower rates of racemisation. This is because more time is required for hydrolysis reactions to break down polypeptides, such that amino acids located in the newly created terminal positions of peptides may undergo racemisation.

Moisture regime
The presence of water within deposits also influences racemisation rate as well as the measured extent of racemisation. The very low extent of racemisation in bones from the La Brea tar pits, (California), for example, has been attributed to the anhydrous nature of this environment (McMenamin *et al.*, 1982). Thus the presence of water has been frequently cited as an important determinant of racemisation. Samples that are permanently within the the water table and hence permanently saturated do not appear to present a major difficulty for racemisation studies. However, materials that experience significant changes in moisture regime, especially on a

diurnal basis may be difficult to date by amino acid racemisation. Materials from such contexts are often characterised by highly degraded surfaces that crumble readily and have lost a portion of their free amino acids as well as amino acids bound in lower molecular weight peptides. This is generally evident by the low concordance of results for the extent of amino acid racemisation for sample replicates from a single deposit. Thus, the moisture regime is significant for not only influencing the *rate* of racemisation, but also the measured *extent* of amino acid racemisation.

Hydrolysis state of peptide residues

Rates of racemisation also vary according to the location of amino acids within peptide chains (Mitterer and Kriausakul, 1984). Accordingly, the relative rates of racemisation follow the relation:
NH_2-terminal > diketopiperazine >> COOH-terminal ~= interior positions ~= free amino acids

pH and clay mineral catalysis

Although pH and clay mineral catalysis are known to influence racemisation, for many natural matrices such as aragonitic and calcitic fossil shells, the matrix protects the residual protein from these effects. The calcium carbonate matrix of shells, for example, prevents catalytic surface effects from clays such as montmorillonite. In addition, the range of pH typically experienced in archaeological and geological deposits (pH 5 to 9) appears not to influence racemisation (Bada, 1985).

Applications of amino acid racemisation

Amino acid racemisation has a variety of applications in Quaternary studies. The method has been used in relative and numeric age assessments of Quaternary fossils (Wehmiller, 1984; Murray-Wallace, 1995), idenification of reworked fossils (Murray-Wallace and Belperio, 1994), palaeotemperature calculations and for screening samples before analysis by uranium-series disequilibrium or radiocarbon dating and assessing the veracity of finite ages close to the practical limits of radiocarbon dating.

Sampling requirements

Samples for amino acid racemisation analysis should be from well-buried contexts (i.e. > 1 m). The materials should be well-preserved and not exhibit chalky surfaces or crumble readily. At least 1 g of matrix is required (*e.g.* shell calcium carbonate). Larger samples are preferred, however, as this permits a more rigorous pretreatment before isolation of the residual protein, as well as the opportunity to analyse free amino acids in addition to the total hydrolysate. Replicate samples should also be collected to assess within-deposit enantiomeric ratio variation between fossils. In the case of materials such as fossil molluscs, sample selection should involve single species. As with any dating method a very detailed site description is required so that the results may be evaluated.

Sample processing: Chemical and Physical

Several analytical approaches may be used for the separation and quantitation of amino acid isomers. The method adopted at Wollongong University, and widely used overseas, involves ion-exchange separation of the residual protein followed by gas chromatography of N-pentafluoropropionyl D, L-amino acid 2-propyl esters. Both the total acid hydrolysate and free

amino acid fractions are analysed. This analytical scheme permits the analysis of nearly all the twenty protein amino acids as well as their diagenetic break-down products. This analytical scheme is particularly useful in assessments of contamination, as unstable amino acids such as serine and threonine, as well as amino acids associated with bacterial cell walls such as diamino-pimelic acid may be identified.

Price, turn around times

A limited number of samples are processed for researchers outside the university on a fee for service basis. A standard analysis (total acid hydrolysate) is A$500. At present the turn around time is approximately three months.

Further inquiries may be directed to:

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