NEW ZEALAND
ARCHAEOLOGICAL
ASSOCIATION

ARCHAEOLOGY IN NEW ZEALAND

NEW ZEALAND
ARCHAEOLOGICAL ASSOCIATION
ARCHAEOLOGY IN NEW ZEALAND

THE POTENTIAL FOR AMINO ACID RACEMIZATION DATING IN NEW ZEALAND ARCHAEOLOGY

Judith Robins[1], Martin Jones[1]
and Elizabeth Matisoo-Smith[2]

[1] Centre for Archaeological Research, The University of Auckland.
[2] Anthropology Department and Centre for Archaeological Research, The University of Auckland.

Introduction

Amino acid racemization (AAR) is used to determine the relative age of biological material such as bone, shell and teeth, and has been used in an archaeological context for over 30 years. During this time a number of significant results have been generated. However, many of these have been questioned and the technique remains controversial. In spite of this, the possibility of reliable AAR dating is attractive. The technique potentially serves as an independent method for dating faunal material, which is useful in the context of providing support for chronometric information produced via other methods. Further to this, AAR allows the possibility of being able to provide information on palaeotemperature histories through analysis of cross-dated specimens. The purpose of this paper is to introduce AAR and provide some assessment of the potential for AAR analysis in New Zealand archaeology. This is a summary document of an assessment of AAR run by the Centre for Archaeological Research (Robins 1999). A much more detailed discussion is presented on the CAR website and interested readers are referred to that document (Robins *et al.* 2001) for further detail.

Amino Acid Racemization Dating

Amino acids are the building blocks of protein and most can exist in two different forms, levorotatory (L) and dextrorotatory (D), known as enantiomers. In living organisms the amino acids occur almost exclusively as L enantiomers.

However, in most cases after the death of the organism amino acids spontaneously convert between the L and D forms through a process known as racemization. As a result, the proportional ratio of D and L enantiomers (the D/L ratio) shifts through time from zero towards one. Thus it is possible to estimate how long racemization has been taking place by measuring the relative proportions of amino acid enantiomers in organic material. This is the basis of AAR dating. The idea is that by establishing the rate at which racemization occurs and measuring the D/L ratio, we can estimate how long the racemization process has been taking place. While simple in theory, in practice the estimation of racemization rates is complex. The analysis is further confounded by the extent to which the observed D/L ratio is a function of racemization alone, given that processes other than racemization can influence this measure. In order to fully evaluate the potential for AAR as a dating tool that can be applied in New Zealand archaeology it is necessary to understand amino acids and the processes that affect racemization.

Amino Acids

There are approximately twenty different amino acids. With the exception of one (glycine) all occur as enantiomers and accordingly are candidates for AAR dating. However, each of the different amino acids racemize at different rates and exhibit different chemical characteristics and only a small number have been identified as potentially suitable for AAR dating. Isoleucine and aspartic acid are the most commonly used amino acids in AAR dating. Aspartic acid, like most amino acids, undergoes racemization as described above. The change from isoleucine to alloisoleucine is referred to as epimerisation. This is a process similar to racemisation, only slightly more complex, due to the more complex molecular structure of isoleucine. Isoleucine changes slowly and is suited to studies where the age of the artefact is expected to be 20,000 to 200,000 years old at temperatures of 18-24°C (Brooks *et al.* 1990). Where ambient temperatures are lower, the range shifts and isoleucine is suitable for even older samples. Aspartic acid racemizes quickly and is the amino acid most researchers have used for younger samples ranging from a few thousand to 80,000 years old depending on temperatures (Bada 1985a). Goodfriend and colleagues (Goodfriend *et al.* 1992, Goodfriend 1992) show that it can be suitable for samples as recent as 300 years old. However, due to the chemical complexities of the breakdown processes in aspartic acid, there is still considerable debate as to its reliability for dating (Bada 1990, Collins *et al.* 1999, Marshall 1990).

Processes affecting racemization

To apply AAR it is necessary to be able to accurately measure the D/L ratio, model the racemization rate, and account for factors that may confound the

observed D/L ratio. Precise techniques for amino acid analysis exist and the most critical issues in AAR dating relate to modelling the racemization rate and accounting for confounding factors that affect the rate. These issues are largely a function of the specific material analysed (e.g. bone, shell, teeth etc.), as the nature of the proteins and the potential reactions of the amino acids can vary with material type. Racemization rate is also greatly influenced by environmental conditions such as temperature, pH and moisture.

In the following sections we give an overview of the types of material that have been used in AAR studies previously and discuss some of the specific problems encountered. The prospects for AAR as a chronometric tool in New Zealand Archaeology are then assessed.

AAR and Bone

Though some would argue that in specific contexts reliable AAR dates can be obtained from bone (Csapó *et al.* 1998), the consensus opinion now seems to be that in most cases bone is an extremely unreliable material for AAR dating:

> "Now, we know better than to attempt to date bones and teeth with amino acids, but the exercise taught us much about the geochemistry of bones, teeth and proteins" (Rutter and Blackwell 1995, p. 147).

However, many of the early AAR efforts did focus on dating bone, and much of the initial controversy over AAR resulted from estimations of the age of human skeletal remains in North America. A detailed review and analysis of the evidence can be found in Pollard and Heron (1996, Chapter 5). However in summary, initial AAR results (Bada and Helfman 1975) indicated the possibility of human presence in America as early as 70,000 years B.P.: some 30-40,000 years earlier than had been previously proposed. Over the decade following these results it was demonstrated that the AAR age estimates were in error by as much as 60,000 years. These AAR estimates were later revised on the basis of further work (Bada 1985b). All fell within the Holocene but had much larger error estimates than those of the corresponding AMS radio carbon values. Although Bada claimed consistency between AAR and AMS dates, others (Pollard and Heron 1996, p. 228) argue that the dates only appear to be consistent with one another because of the unacceptably large error range associated with the AAR dates. The use of AAR for the dating of bone continues to be controversial.

Most of the experimental work on AAR in bone is conducted in laboratories, at a high temperature, and essentially within a closed system (Johnson and Miller 1997). In contrast when AAR takes place in an archaeological specimen it occurs in the field; an environment that is anything but a closed system. Given that the bone matrix is porous amino acids can be leached out and lost, and contaminants can be introduced from the general environment. In most cases the researcher has no control or record of these events. However, some workers consider that if bones are well preserved, have a known temperature history and have not been exposed to leaching they can be used for relative dating (Johnson and Miller 1997).

AAR and Teeth

Researchers have suggested that teeth may be a valuable material for AAR dating because tooth enamel provides some protection of amino acids from environmental contaminants (Bada 1984). However, unlike bone where racemization begins post mortem, racemization in teeth begins immediately after the tooth is fully formed and continues throughout the life of the host (Helfman and Bada 1976). Racemization continues after death, but at a reduced rate due to the fact that in most cases post mortem environmental temperature is lower than body temperature (Carolan *et al.* 1997). It is generally assumed that as long as burial temperature is low and time since death reasonably short, post mortem racemization will be negligible. Accordingly, AAR of aspartic acid in teeth has been used to estimate age at death of some mammals, for example humans (Gillard *et al.* 1990) and rats (Ohtani *et al.* 1995). Modern teeth of known host age, obtained from dentists, showed increased aspartic acid racemization in both dentine and enamel which was directly correlated with increasing host age (Bada 1984). However, when Gillard *et al.* (1991) used aspartic acid AAR in teeth to estimate age at death of archaeological human remains from 18th century vaults in England, they were unable to correctly assign age at death. AAR estimates could only provide, at 95% confidence level, ages of ± 24 years. This is compared to their estimates of ± 4 years for modern samples. A more recent analysis (Carolan *et al.* 1997) using calibration curves derived from modern tooth racemization data to estimate age at death in early 19th century dental remains, showed that the regression lines for ancient and modern samples were not parallel. There was a tendency for young individuals to be estimated as too old and old individuals to be estimated as too young. They suggest that this appears to reflect a fundamental difference in the racemization behaviour of dental proteins pre and post-mortem, the causes of which are currently not understood. In light of this Carolan *et al.* (1997) caution that use of AAR in teeth is currently of limited value for archaeology, but remains a useful tool for modern forensic research.

AAR and Molluscs

A considerable amount of research has focussed on AAR in mollusc shells. Leaching is less of a problem with calcium carbonate based shells than for bones (Miller and Hare 1980), and attempts are usually made to extract amino acids from internal portions of shell (Johnson and Miller 1997). However, it has been found that racemization rates vary among genera of molluscs (Lajoie *et al.* 1980) and have to be established for each organism of interest.

This has been achieved with varying degrees of success. For example Kimber and Griffin (1987) studied three molluscs, *Ostrea angasi*, *Anadara trapezia* and *Katelysia rhytiphora* and concluded that racemization varied among these, with *Katelysia rhytiphora* being the preferred species for dating. Another aspect of this is that the reliability of different racemization reactions may vary among organisms. Kimber and Griffin (1987) also showed that aspartic acid values should be used with caution, particularly for Ostrea shells. In contrast Goodfriend (1992) concluded that it was possible to date land snail shells less than 300 years old using aspartic acid.

In an earlier study of the racemization patterns of six amino acids Goodfriend (1991) concluded that isoleucine and glutamic acid racemization approximated first order kinetics and are useful for older samples. The faster racemizing amino acids, with the exception of aspartic acid, approximated parabolic kinetics i.e. when the D/L ratios were plotted against the square root of the radiocarbon age of the samples the relationship approximates a straight line. Goodfriend states that aspartic acid, which showed a very high initial rate of racemization, is particularly useful for young samples. However, little is known about the amino acid sequences of proteins in the molluscs (Collins *et al.* 1999) therefore, explanations of unusual kinetics are difficult. It is known that different genera have different proportions of the various amino acids in their proteins (Miller and Hare 1980), and it is assumed that this leads to differences in racemization rates (Johnson and Miller 1997). The kinetic model assumptions generally used are based on empirical relationships and not founded in theory (Goodfriend 1991).

AAR and Eggshells

Ratite eggshells appear to have excellent characteristics for amino acid racemization dating. They are essentially closed systems (Johnson *et al.* 1992) as cited by Johnson and Miller (1997) which are not susceptible to leaching. Therefore in the shell there is neither loss of protein hydrolysis products nor contamination from the environment. Brooks *et al.* (1990) showed that the epimerization of L-isoleucine in ostrich shell at high temperatures follows first

order reversible kinetics almost up to racemic equilibrium and used it to date sites beyond the range of radiocarbon dating. Similarly Miller *et al.* (1997) showed that isoleucine epimerization in emu eggshell is also reliable. Since ratite shell is commonly found in African and Australian archaeological sites there is considerable potential for dating in these regions.

This potential was demonstrated by Miller *et al.* (1992), who used isoleucine epimerization in ostrich eggshell to date strata at Border Cave, South Africa. Excavated ostrich eggshell fragments were analysed from each stratum of the site. The shell showed increasing alloisoleucine/isoleucine ratios with increasing stratigraphic age. The reaction was calibrated in the upper levels with radiocarbon dates on associated charcoal. One AMS date of eggshell confirmed the age association of the charcoal and the shell fragments. Anatomically modern human skeletal material from a well controlled stratigraphic context was recovered from the cave. Based on its association with dated eggshell, Miller *et al.* suggest occupation of the site by anatomically modern humans as early as 100,000 years BP.

Racemization rates in the egg shells of New Zealand ratites (moa and kiwi) have not been studied, but are likely to provide similar results to African and Australian ratites. While isoleucine epimerization in ratite eggshells appears to be a reliable dating method, it is of course limited to dating material in the 20,000 to 200,000 age range (Brooks 1990). Unfortunately this rate is too slow for applying to issues in New Zealand prehistory, and to date, little is known about the faster racemizing aspartic acid in ratite egg shell.

AAR and Palaeotemperature estimations

If the racemization rate for a particular amino acid system in any given material is known, then AAR can be used to estimate the thermal history of any cross-dated samples. However, good independent age controls and a reliable kinetic model for racemization rates are needed (Johnson and Miller 1997).

Although aspartic acid racemization has been used for palaeotemperature estimation (Shroeder and Bader 1973) such estimates from bone are now considered suspect (Rutter and Blackwell 1995). Miller *et al.* (1997), however, have successfully used isoleucine/alloisoleucine epimerization ratios in emu eggshell together with 14C dated eggshell to reconstruct temperature history for the 45,000 to 16,000 BP period in inland Australia. Until racemization rates of aspartic acid are studied in ratite egg shell AAR is of limited value for reconstructing temperature history of more recent periods.

Possible Archaeological Applications in New Zealand

As part of a study of settlement in Aotearoa/New Zealand it was thought that AAR might be successfully used to date bones or teeth from the Pacific rat, *Rattus exulans*. Given the relatively short chronology of New Zealand prehistory (significantly less than 20,000 years), aspartic acid would be the only viable candidate for AAR dating. Given that Collins *et al.* (1999) present convincing evidence that racemization of aspartic acid in bones is inherently unpredictable as a dating method, pursuit of this technique is inappropriate.

It might be possible to develop AAR methods for New Zealand molluscs. However, given the precision with which it is possible to date mollusc shell using radiocarbon dating, there seems little benefit in developing AAR techniques.

Investment in AAR techniques might be justified in extremely specialised cases where material from an unknown radiocarbon reservoir needed to be dated e.g. fresh water molluscs, or possibly in experimental work to further refine marine calibrations. However, a large investment of both time and money would be required to develop this further for New Zealand.

The most promising material for AAR studies in New Zealand is ratite eggshell. If kiwi and moa eggshells behave similarly to those of emu and ostrich, they could provide a reliable source for both AAR dating and palaeotemperature reconstruction. However, as discussed earlier, to be of use in archaeological studies in New Zealand, we would first have to investigate the relationship in ratite eggshell between aspartic acid racemization and time. The measurement of racemization extent in samples of known or controlled age could be used to help build temperature histories for given locations or periods. This type of work would have to be based around measurement of racemization in aspartic acid within materials that have been demonstrated to give reliable results. Such research would involve a significant experimental program to enable a sound understanding of the relationship between racemization extent, racemization duration and environmental temperature regimes.

Conclusion

In light of the summary that has been presented above it would appear that AAR is of limited potential for chronometric control in New Zealand Archaeology. While some materials such as ratite shell or marine mollusc shell could possibly be dated via AAR it is already possible to provide reliable dates for this material through radiocarbon dating. Any future development of AAR in New Zealand for analytical purposes would require significant research investment.

Acknowledgements

This work was funded by a FoRST grant UOA-807 entitled Identifying and Dating Multiple Contacts and Colonizations of Aotearoa/New Zealand; joint use of chronometric and DNA techniques. Amino Racemization was one of the chronometric techniques investigated. We wish to thank Dr Christine Prior for valuable discussions on this topic.

References

Bada, J. L. 1984. *In vivo* racemization in mammalian proteins. *Methods in Enzymology* 106: 98-115.

Bada, J. L. 1985a. Amino acid racemization dating of fossil bones. *Annual Review of Earth and Planetary Sciences* 13: 241-268.

Bada, J. L. 1985b. Aspartic acid racemization ages of California Paleoindian skeletons. *American Antiquity* 50: 645-647.

Bada, J. L. 1990. Racemization dating. *Science* 248: 539-540.

Bada, J. L. and Helfman, P. M. 1975. Amino acid racemization dating of fossil bones. *World Archaeology* 7: 160-173.

Brooks, A. S., Hare, P. E., Kokis, J. E., Miller, G. H., Ernst, R. D. and Wendorf, F. 1990. Dating Pleistocene archeological sites by protein diagenesis in ostrich eggshell. *Science* 248: 60-64.

Carolan, V. A., Gardner, M. L. G., Lucy, D. and Pollard, A. M. 1997. Some considerations regarding the use of amino acid racemization in human dentine as an indicator of age at death. *Journal of Forensic Sciences* 42: 10-16.

Collins, M. J., Waite, E. R. and van Duin, A. C. T. 1999. Predicting protein decomposition: the case of aspartic-acid racemization kinetics. *Philosophical Transactions of the Royal Society London B* 354: 51-64.

Csapó, J., Csapó-Kiss, Z. and Csapó, J., Jr. 1998. Use of amino acids and their racemisation for age determination in archaeometry. *Trends in Analytical Chemistry* 17: 140-148.

Gillard, R. D., Hardman, S. M., Pollard, A. M., Sutton, P. A. and Whittaker, D. K. 1991. Determinations of age at death in archaeological populations using the D/L ratio of aspartic acid in dental collagen. *In* Pernicka, E. and Wagner, G. A. (Eds) *Archaeometry '90* Birkhäuser Verlag, Basel.

Gillard, R. D., Pollard, A. M., Sutton, P. A. and Whittaker, D. K. 1990. An improved method for age at death determination from the measurement of D-aspartic acid in dental collagen. *Archaeometry* 32: 61-70.

Goodfriend, G. A. 1991. Patterns of racemization and epimerization of amino acids in land snail shells over the course of the Holocene. *Geochimica et Cosmochimica Acta* 55: 293-302.

Goodfriend, G. A. 1992. Rapid racemization of aspartic acid in mollusc shells and potential for dating over recent centuries. *Nature* 357: 399-401.

Goodfriend, G. A., Hare, P. E. and Druffel, E. R. M. 1992. Aspartic acid racemization and protein diagenesis in corals over the last 350 years. *Geochimica et Cosmochimica Acta* 56: 3847-3850.

Helfman, P. M. and Bada, J. L. 1976. Aspartic acid racemization in dentine as a measure of aging. *Nature* 262: 279-281.

Johnson, B. J., Fogel, M. L., Miller, G. H. and Tuross, N. 1992. Isotopic and molecular characterization of fossil proteins in ostrich eggshell. *North American Palaeontol. Convention Abstracts and Program* 6: 151.

Johnson, B. J. and Miller, G. H. 1997. Archaeological applications of amino acid racemization. *Archaeometry* 39: 265-287.

Kimber, R. W. L. and Griffin, C. V. 1987. Further evidence of the complexity of the racemization process in fossil shells with implications for amino acid racemization dating. *Geochimica et Cosmochimica Acta* 51: 839-846.

Lajoie, K. R., Wehmiller, J. F. and Kennedy, G. L. 1980. Inter- and intrageneric trends in apparent racemization kinetics of amino acids in Quaternary mollusks. *In* Hare, P. E., Hoering, T. C. and King, K., Jr. (Eds) *Biogeochemistry of Amino Acids*. pp. 305-340. John Wiley and Sons, New York.

Marshall, E. 1990. Racemization dating: Great expectations. *Science* 247: 799.

Miller, G. H., Beaumont, P. B., Jull, A. J. T. and Johnson, B. 1992. Pleistocene geochronology and palaeothermometry from protein diagenesis in ostrich eggshells: implications for the evolution of modern humans. *Philosophical Transactions of The Royal Society of London Series B* 337: 149-157.

Miller, G. H. and Hare, P. E. 1980. Amino acid geochronology: Integrity of the carbonate matrix and potential of molluscan fossils. *In* Hare, P. E., Hoering, T. C. and King, K., Jr. (Eds) *Biogeochemistry of Amino Acids*. pp. 415-443. John Wiley and Sons, New York.

Miller, G. H., Magee, J. W. and Jull, A. J. T. 1997. Low-latitude glacial cooling in the Southern Hemisphere from amino acid racemization in emu eggshells. *Nature* 385: 241-244.

Ohtani, S., Matsushima, Y., Ohhira, H. and Watanabe, A. 1995. Age-related changes in D-aspartic acid of rat teeth. *Growth Development and Aging* 59: 55-61.

Pollard, A. M. and Heron, C. 1996. *Archaeological Chemistry.* The Royal Society of Chemistry.

Robins, J. H. 1999. Amino acid racemization: A relative dating technique. Presented at *New Zealand Archaeology Association Conference* Auckland, New Zealand.

Robins, J. H., Matisoo-Smith, E.A. and Jones, M.D. 2001. Amino acid racemization dating in New Zealand: An overview and bibliography. Report 1. www.car.auckland.ac.nz/publications

Rutter, N. W. and Blackwell, B. 1995. Amino acid racemization dating. *In* Rutter, N. W. and Catto, N. R. (Eds) *Dating Methods for Quaternary Deposits*. pp. 125-164. Geological Association of Canada.

Schroeder, R. A. and Bada, J. L. 1973. Glacial-Postglacial temperature difference deduced from aspartic acid racemization in fossil bones. *Science* 182: 479-482.