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LETTER

Aspartic acid racemization and protein diagenesis in corals over the last 350 years

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Abstract—D/L aspartic acid values from a 350-year time series of annual growth bands of a living colony of the coral *Porites australiensis* show a very regular pattern of increase with age. The initial rate of racemization is extraordinarily rapid (0.6% per year) but slows in older growth bands to 0.04% per year (4% per century). The skeletal proteins show progressive hydrolysis with increasing age, with free aspartic acid comprising 16% of the total aspartic acid in the 350-year-old band. The proteins are unusually rich in aspartic acid (nearly 50 mol%). The relative weakness of the peptide bonds formed by aspartic acid moieties is probably responsible for the rapid hydrolysis and consequent rapid racemization of aspartic acid. Racemization analysis provides a means for checking for sections of missing bands in corals and for screening of prospective samples for U-Th dating.

INTRODUCTION

RECENT STUDIES HAVE shown that aspartic acid has a particularly high rate of racemization in mollusk shells during the first few centuries following formation of the shell (GOODFRIEND et al., 1991; GOODFRIEND, 1992). The rate is much higher than in bones or teeth (at comparable temperatures) and opens the possibility of its use for dating of mollusk shells over this recent time period. Evaluation of the accuracy of the method, however, is difficult because independent means of determining the actual ages of samples in this time range are generally not available. Radiocarbon dating, for example, has limited usefulness for marine samples for this period because of (1) the uncertainties of the amount of the reservoir effect (the apparent age of marine bicarbonate, reflecting its average residence time in the oceans; STUIVER et al., 1986), and (2) fluctuations in atmospheric levels of ^{14}C as a result of natural variations in the ^{14}C production rate and anthropogenic effects (e.g., input of CO_2 from the burning of fossil fuels). Tropical corals produce distinct annual growth bands, which provide a time series of known-age samples. In some large coral heads, these bands provide a record stretching back hundreds of years. Such annual sample series have formed the basis for studies of trends of radiocarbon concentrations (DRUFFEL, 1982) and stable isotope variations (DUNBAR et al., 1991). In the present study, we use known-age samples of coral to evaluate aspartic acid racemization dating over the last few centuries. The results presented here are based on a 350-year time series of samples from a core cut through a large head of the coral *Porites australiensis* from the Great Barrier Reef, Queensland, Australia.

Racemization or epimerization of amino acids in mollusks is thought to be strongly dependent on hydolysis of shell proteins. According to the epimerization model of Mitterer and Kriausakul (KRIAUSAKUL and MITTERER, 1980; MITTERER and KRIAUSAKUL, 1984), progressive hydrolysis of proteins exposes formerly interior amino acids to terminal positions, where they epimerize rapidly, thus continuing to fuel the epimerization reaction. Progressive hydrolysis of shell proteins (as measured by the proportion of free amino acids, the final end product of protein hydrolysis) with increasing age and/or D/L amino acid values has been demonstrated in marine bivalves over time scales of millions of years (HARE and MITTERER, 1966; MILLER and BRIGHAM-GRETTE, 1989). In the present study, we determined the proportion of free aspartic acid in the coral sample time series in order to evaluate the relationship of protein hydrolysis to aspartic acid racemization.

Little work on racemization/epimerization in corals has been done previously. The comprehensive study of WEHMILLER et al. (1976) on mostly Pleistocene corals showed a rather wide scatter of D/L values in relation to U-Th ages and thus discouraged further work on corals. Possible problems of leaching of amino acids and contamination by non-indigenous amino acids were suggested as possible causes of the variability of D/L values.

MATERIALS AND METHODS

The 4.94-m core (ABR 1 C) was taken by Peter J. Isdale and colleagues (Australian Institute of Marine Science) in December, 1985. The coral colony was ca. 7 m in height and was located on the leeward side of Abraham Reef (22°06′S, 153°00′E) in southern Swains Reef, ca. 200 km off the Queensland coast. The core was cut with a tungsten cutting bit and stainless steel single-walled barrel. During drilling, the hole was flushed with seawater passing through a pump. In the laboratory, the core was slabbed, X-rayed, mapped, and cut using methods described in GRIFFIN and DRUFFEL (1985). For post-1948 bands, annual bands were cut; whereas for pre-1948 bands, two-year increments were cut. Samples were stored in the laboratory until they were analyzed.

Aliquots (20–25 mg) of thirty-three coral samples (spanning the period AD 1632–1985) were analyzed for D/L aspartic acid values by gas chromatographic separation of the N-trifluoroacetyl isopropyl ester derivatives of the hydrolysates on a Chirasil-val column. Details

of the hydrolysis and derivatization procedures are given in GOODFRIEND (1991). Analyses were carried out on an HP 5790 gas chromatograph. The D/L peak area ratios are reported. Replicate preparations and analyses were carried out for some of the samples (average of 1.4 analyses per sample, apart from the 1880–81 sample). From separate preparations and measurements (in different runs) of seven aliquots of the 1880–81 sample, the analytical error of a single analysis was determined to be ±0.00701 for a D/L value of 0.173 (4% error).

Amino acid analyses of both the total and the free amino acid fractions were carried out for selected samples. For analysis of total amino acids, samples were hydrolyzed in N_2-filled screw-top tubes at 150°C for 15 min and desalted with HF. The free fraction was prepared by adding the stoichiometric amount of cold 6N HCl to the powdered samples in 1.5-mL plastic microcentrifuge tubes in an ice bath. After no further reaction could be detected visually, the samples were briefly agitated on a vortex mixer. Buffer (pH 2) was added, the samples were centrifuged, and the solution was pipetted off and analyzed (without desalting). Analyses of both total and free amino acid fractions were carried out using post-column *o*-phthaldialdehyde/2-mercaptoethanol (OPA) derivatization with a fluorescence detector. Amounts of amino acids were determined by comparison of peak areas to a standard included in the same series of analyses; the error of measurement averages 5%. Amounts of proline were estimated from the gas chromatographic results.

PATTERN OF ASPARTIC ACID RACEMIZATION

A regular pattern of increasing D/L aspartic acid values with increasing age is seen (Fig. 1a). The trend consists of two segments: the most recent interval of the last ca. 60 y (1985 back to the 1920s), when the rate of racemization is extremely fast but decreases with increasing D/L value (convex upward trend), and an earlier interval (1920s to 1632) when the rate is slower and more or less constant. This earlier interval was evaluated by simple linear regression of the individual D/L measurements on age, which yields the equation

$$D/L = 3.966 \times 10^{-4} t + 0.1278, \quad (1)$$

where t is the age in years before 1985. The slope of this regression indicates a racemization rate of 0.040 (or 4%) per century for this interval. The correlation is very strong ($r = 0.99$). The average scatter about the trend line is given by the square root of the mean square error, 0.00725. This value is very close to the analytical error of the individual measurements (0.00701) determined from replicate preparations of the 1880–81 sample, which has a D/L value (0.173) that represents the median of the series. All the scatter observed about the trend line for this time interval can therefore be attributed to analytical error. The D/L aspartic acid values appear to be excellent predictors of age in this sample series, as far as can be determined within the analytical limits of the method.

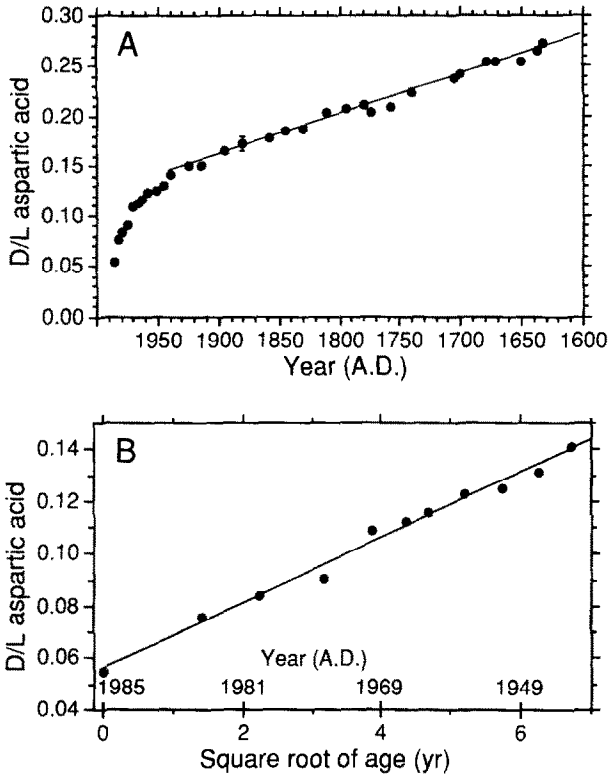

FIG. 1. Racemization (D/L values) of total aspartic acid (hydrolyzed samples) in relation to the age of annual bands in the coral *Porites* (a) racemization of samples from AD 1632–1985. A simple linear regression line for samples from 1632–1924 is shown. Where more than one analysis per sample was carried out, the mean of the analyses is plotted. Error bars (±1 S.D.) are shown for the 1880–81 sample; (b) parabolic kinetic plot (D/L vs. the square root of age) for samples from 1940–1985.

The initial curved portion of the racemization pattern conforms very well to the parabolic kinetic model (Fig. 1b), proposed by MITTERER and KRIAUSAKUL (1989) to characterize the patterns of epimerization of mollusks and Foraminifera at higher D-alloisoleucine/L-isoleucine values. In this model, the D/L values are related to the square root of the age. Simple linear regression yields the equation

$$D/L = 0.01248\sqrt{t} + 0.0561. \quad (2)$$

The excellent fit of this model is indicated by the high correlation coefficient ($r = 0.99$). The rate of racemization at a given point in time can be determined from the first derivative of D/L with respect to age,

$$\partial(D/L)/\partial t = 0.00624 t^{-1/2} = 0.00624/\sqrt{t}. \quad (3)$$

From this equation, the initial rate of racemization (for the 1985 growth band) was determined to be 0.62% per year (equivalent to 62% per century). For the 1975 growth band (10 years of age), the rate has slowed to 0.20% per year.

The overall pattern of kinetics is similar to that observed in various time series of terrestrial mollusks (GOODFRIEND, 1992), in that there is a strong convex upward trend, with a very high initial racemization rate which rapidly slows down with increasing age. In order to compare the racemization rates in corals to those measured in mollusks, the different ambient temperatures of the samples must be taken into account, and the rates need to be compared over comparable ranges of D/L values, due to the change in rate with D/L. A mean annual sea-surface temperature of 24.3°C for the site was estimated from one year (1991) of weekly temperature measurements made by satellite. Aspartic acid in mollusk shells in the U.S. National Museum of Natural History has been shown to have a net racemization (measured D/L value minus D/L of zero-age material) of 5.8% in 110 years (GOODFRIEND, 1992). The coral samples, at presumably similar or slightly lower temperatures, took only 20 years to racemize to the same degree. Land snails buried 75 years ago

during construction of the Ottoman railroad in the Negev Desert, Israel, show a net racemization of 3.0%; the same amount of racemization occurred in the coral samples in 5.2 years. The mean annual temperature in the Negev (19.2°C at Beer Sheva; Israel Meteorological Service, 1983) is significantly lower than for the coral samples. The Arrhenius formula predicts a 2.3-fold difference in rate, assuming first-order kinetics and E_a = 28 kcal/mol for aspartic acid racemization (GOODFRIEND and MEYER, 1991). However, because of very high summer temperatures in the Negev (25.7°C mean monthly temperature for July and August), the effective temperature should be significantly higher than the mean annual temperature (WEHMILLER, 1977), since the racemization rate increases exponentially (not linearly) with temperature. Thus, the effective temperature in the Negev is probably close to that to which the coral was subjected. So in both cases, the corals show dramatically higher rates of aspartic acid racemization than occur in mollusks over similar time periods. These rates are the highest known for any amino acid in any biogenic carbonate.

PROTEIN HYDROLYSIS AND ITS RELATION TO RACEMIZATION

In the 1983 coral band, only a trace amount of free aspartic acid is present (Fig. 2b). The percentage of aspartic acid in the free form increases with increasing age, reaching 16% of the total aspartic acid in the 1632 band. The concentration of total aspartic acid present in the annual bands varies over time (Fig. 2a). Bands from the late 1600s and early to mid 1700s have lower amounts of aspartic acid than earlier or later samples, and concentrations in the samples from the mid 1600s are similar to those in the samples from 1800 to present. The trends in amino acid concentrations may relate to variations in some environmental factor. No notable differences in the relative amino acid compositions are seen for these different samples (Table 1). The absence of a general trend toward decreasing aspartic acid concentrations or changes in amino acid composition indicates that there was no measurable loss of either free amino acids or proteins from the coral skeleton over its 350-year history. However, the large amount of free aspartic acid in the older bands indicates that a considerable degree of hydrolysis of the coral skeletal proteins has occurred.

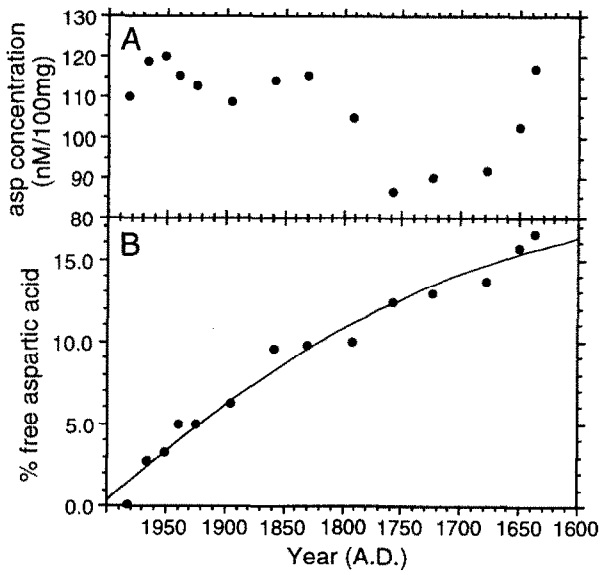

FIG. 2. Concentrations of free and total aspartic acid in relation to age. (a) concentration of total aspartic acid; (b) percent of the total aspartic acid that is free. A second-order trend line has been fitted to the data.

TABLE 1. Amino acid composition (in mole percent) of representative samples of annual bands of the coral *Porites*.

Amino acid	Year			
	1966	1858-59	1758-59	1678-79
asp	46.9	48.4	48.5	51.8
thr	3.9	3.1	3.0	2.8
ser	6.9	6.5	7.1	5.9
glu	10.8	11.3	11.5	10.5
pro	2.2	1.9	1.1	2.3
gly	11.3	12.0	12.3	10.8
ala	4.0	4.0	4.2	4.5
val	2.9	2.6	2.5	2.6
met	0.2	0.7	0.3	0.1
aile	0.04	0.06	0.10	0.12
ile	1.5	1.3	1.4	1.2
leu	2.9	2.7	2.9	2.6
tyr	1.0	0.9	1.0	0.8
phe	1.9	2.2	1.2	1.2
lys	2.0	1.9	1.8	2.0
arg	1.5	1.1	1.2	0.7

The coral skeletal proteins are unusually rich in aspartic acid, which makes up nearly 50 mol% of the amino acids (Table 1). High relative concentrations of acidic amino acids (aspartic acid and also glutamic acid) have been found also in other corals (YOUNG, 1971; MITTERER, 1978; CONSTANTZ and WEINER, 1988). Peptide bonds involving aspartic acid are relatively weak, so that hydrolysis of proteins occurs preferentially at such bonds (SCHULTZ, 1967; MARCUS, 1985). The very high aspartic acid content of the coral proteins is likely responsible for the rapid hydrolysis. This hydrolysis initially leads to exposure of internal aspartic acid residues to terminal positions, where they may be expected to racemize more rapidly, by analogy with isoleucine epimerization (MITTERER and KRIAUSAKUL, 1984). Thus, the high aspartic acid content of the coral skeletal proteins may be responsible for the unusually rapid rate of racemization observed. Note that isoleucine also shows a relatively high rate of epimerization (Table 1), attaining a D-alloisoleucine/L-isoleucine value of 0.097 in the 1678–79 sample. This is also likely related to the relative abundance of aspartic acid residues and consequent rapid hydrolysis of the matrix proteins.

Position effects probably also play an important role in controlling racemization rates. It is known from experimental studies of peptides that rates of epimerization and racemization depend on the identity of the neighbouring amino acid(s) (KRIAUSAKUL and MITTERER, 1980; SMITH and DE SOL, 1980; ENGEL, 1982; MITTERER and KRIAUSAKUL, 1984). In the eye lens protein α-crystallin, aspartic acid residues from various peptides show quite different rates of racemization (GROENEN et al., 1990).

DISCUSSION

The present study shows that over the time range of the last few centuries, racemization in corals shows ideal behavior. It remains to be seen how far back in time this pattern extends. Careful selection of samples, free from boring organisms and showing no recrystallization of aragonite to calcite, might extend the time scale over which racemization is a reliable predictor of ages in corals. On the other hand, the rapid hydrolysis of the skeletal proteins to free amino acids makes them particularly subject to leaching and thus may lead to poor preservation in older samples. This might explain the erratic patterns of epimerization found in older (mostly Pleistocene) corals by WEHMILLER et al. (1976).

The recent development of mass spectrometric methods for U-Th analysis (EDWARDS et al., 1987) now allows coral ages to be determined with high precision, with errors on the order of ±5 y for recent samples. Only for samples from the last few decades would aspartic acid racemization seem to offer higher precision, given the analytical techniques presently available. However, amino acid racemization analyses are far easier to carry out than mass spectrometric U-Th analyses and can be used to screen prospective samples for U-Th analysis. For annual series determined by counting of bands, racemization analysis can provide a check on the coral stratigraphy by determining whether certain sections have missing bands where the coral died but later overgrew.

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