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PRECISION OF AMINO ACID ENANTIOMERIC DATA FROM FOSSILIFEROUS LATE QUATERNARY EOLIANITES SAN SALVADOR ISLAND, THE BAHAMAS

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ABSTRACT

Amino acid enantiomeric (D/L Leucine and Alloisoleucine/Isoleucine) values have been used to discriminate and correlate fossiliferous late Quaternary carbonate rock units in Bermuda, The Bahamas, and elsewhere. As a first approximation, higher Alloisoleucine /Isoleucine (A/I) values in mollusc fossils, or whole rock samples, represent greater age. However, previous work on San Salvador Island, The Bahamas has demonstrated that there are inconsistencies among the data that call the utility of amino acid data into question. In an attempt to better define aminostratigraphy in these carbonate sequences, a blind study using amino acid analyses of the land snail *Cerion* sp. was performed. The goals of this study were 1) to estimate the precision of *Cerion* shell amino acid data performed by several amino acid geochemists, and 2) to determine if aminostratigraphy can be used to understand the sea-level highstand data preserved on San Salvador Island.

Amino acid data presented here show good chromatographic precision. Multiple chromatograms from a single hydrolyzate (or shell) have coefficients of variation (%CV) of approximately 5 %. In addition, multiple chromatograms and samples of Interlaboratory Standard (ILC) mollusc shell powders analyzed during this investigation also have %CV of approximately 5%. However, variable A/I values are observed from fossil *Cerion* collected from a single lithologic unit at a single site. An analysis which considers the precision of analyses from several fossils from the same rock unit, and which provides a measure of the level of resolution that may be obtained from this amino acid data, was conducted. *Cerion* A/I values measured from single lithologic units on San Salvador Island show %CVs that range from 20-50%. At present, low sample precision precludes the use of aminostratigraphic methods to discriminate units deposited during different oxygen isotope substages (approximately $\pm$ 20,000 years. In addition, trends in A/I values are not always consistent with observed stratigraphic relationships, so that discrimination of units deposited during different oxygen isotope stages (approximately 100,000 years) may be performed only with difficulty.

Recently, whole-rock amino acid analyses have been used on Bermuda and San Salvador to refine lithostratigraphic interpretations. On San Salvador, such analyses have been utilized to identify previously unrecognized substage and stage-level temporal distinctions. *Cerion* shell A/I values presented here, from many of the same outcrops that have been characterized elsewhere by whole-rock A/I analyses, suggest that it is probable that the whole-rock results are substantially less reliable than those from *Cerion*. Radiocarbon analyses of an outcrop on San Salvador provides further support for this conclusion. Rocks collected from a unit reported to have been deposited during oxygen isotope substage 5a (circa 85,000 years ago) based upon whole-rock A/I ratios, yield radiocarbon ages of 3690 $\pm$ 70 and 4400 $\pm$ 70 yrBP. The radiocarbon ages are consistent with the stratigraphic interpretation of that outcrop as determined by the authors.

INTRODUCTION

Amino acid enantiomeric values have been used to correlate and discriminate late Quaternary carbonate rocks in The Bahamas (Carew, 1983a, b; Carew and others, 1984; Carew and Mylroie, 1985, 1987; Hearty, 1992; Hearty and Kindler, 1991, 1992; Kindler and Hearty, 1992) and Bermuda (Vacher and Hearty 1989; Hearty and others, 1992). The work

STANDARD	LABORATORY	A/I VALUE PEAK AREA	%CV	A/I VALUE PEAK HEIGHT	%CV	NO. OF CHROMATO-GRAMS
ILC-B	MSU (this work)	0.43 +/- 0.02	4.6	0.45 +/- 0.06	13	n=8
ILC-B	MSU (mean)	0.41 +/- 0.03	6.8	0.46 +/- 0.05	11	n=32
ILC-B	UD (1986-1988)	0.44 +/- 0.04	9.1	0.44 +/- 0.05	11	n=50

Table 1. Comparison of ILC mollusc shell standard data (Wehmiller, 1984b) run by the analyst (Mirecki). A/I values obtained during this project are compared with amino acid data obtained by Mirecki at the University of Delaware (UD) and at Memphis State University (MSU). The coefficient of variation (%CV) represents the percent variation by the standard deviation about each mean A/I value.

LOCATION	A/I (PEAK AREA)	%CV	A/I (PEAK HEIGHT)	%CV
Holocene North Point	0.37 +/- 0.03	8.1	0.07 +/- 0.02	28
Man Head Cay	0.63		0.69 +/- 0.02	2.9
Crab Cay	0.99 +/- 0.02	2.0	1.04 +/- 0.04	4.0
Crab Cay (rerun [same shell])	0.92 +/- 0.01	1.1	1.0	
Crab Cay (new *Cerion*)	0.66 +/- 0.03	4.5	0.63 +/- 0.13	21
Almgreen Cay	0.65 +/- 0.01	1.5	0.71 +/- 0.04	5.6
The Bluff	0.61 +/- 0.02	2.0	0.76 +/- 0.02	2.6
The Gulf (upper)	0.36		0.32 +/- 0.03	9.4
The Gulf (upper new *Cerion*)	0.41 +/- 0.03	7.3	0.46 +/- 0.04	8.7
The Gulf (sea level)	0.55 +/- 0.04	7.3	0.74 +/- 0.03	4.0
Upper Watlings Quarry	0.63 +/- 0.02	3.2	0.72 +/- 0.02	2.8
Sandy Pt/Conch Pt	0.46 +/- 0.01	2.2	0.52 +/- 0.03	5.8
Sandy Pt/Conch Pt (rerun)	0.42 +/- 0.01	2.4	0.49 +/- 0.02	4.1
Altar Road Cut	0.52 +/- 0.05	10	0.64 +/- 0.05	7.8
Owl's Hole (lower)	0.37 +/- 0.03	8.1	0.38 +/- 0.02	5.3
Owl's Hole (lower, new *Cerion*)	0.13 +/- 0.02	15	0.17 +/- 0.02	12

Table 2. Chromatographic precision of A/I values from San Salvador mollusc fossil analyses (*Cerion*). %CV is percent coefficient of variation.

LOCATION	A/I (PEAK AREA)	%CV	A/I (PEAK HEIGHT)	%CV	NO. OF FOSSILS
Crab Cay	0.86 +/- 0.14	16	0.82 +/- 0.20	24	2
The Gulf	0.44 +/- 0.08	18	0.51 +/- 0.17	34	3
Owl's Hole	0.25 +/- 0.12	47	0.28 +/- 0.11	38	2

Table 3. Geologic precision of A/I values from multiple analyses of fossil molluscs (*Cerion*) from San Salvador Island localities.

conducted on San Salvador Island, The Bahamas has produced some results that are inconsistent with interpreted stratigraphic position and radiocarbon ages (Carew and others, 1984; Carew and Mylroie, 1987; Mirecki and others, 1992). In an attempt to determine the utility of aminostratigraphy in these carbonate rocks, we undertook a blind study using the pulmonate gastropod *Cerion*. The goals of this study were to estimate the precision of *Cerion* shell amino acid data produced by different geochemists, and to determine if aminostratigraphy can be used reliably to understand the sea-level data preserved in the geologic record of San Salvador Island.

METHODS

Cerion shells were recovered from a variety of fossil and modern sites, including many that had previously been analyzed by another amino acid geochemist (Carew and others, 1984; Carew and Mylroie, 1987), and some from rocks which have recently been studied using whole-rock amino acid geochemistry (Hearty, 1992; Hearty and Kindler, 1991, 1992; Kindler and Hearty, 1992).

Before analysis, a dental tool was used to abrade the inside and outside of the mollusc shells to remove extraneous calcareous material and the outer prismatic (pigmented) shell layer. Physical cleaning was followed by chemical cleaning using two successive washes of dilute HCl (ca. 1N), ultrapure water, then NH_4OH (ca. 7N). Samples were then dried in a vacuum oven (< 40° C), weighed, and dissolved in a stoichiometric volume of concentrated HCl, resulting in a 6N HCl hydrolyzate solution. These solutions were heated (22 hrs. at 110° C) to hydrolyze mollusc shell protein into individual amino acids for analyses by HPLC (high performance liquid chromatography) or GC (gas chromatography). Briefly, use of HPLC enables analysis of two amino acid diastereomers, alloisoleucine and isoleucene (see: Hare and others, 1985). The ratio of alloisoleucine to isoleucine (A/I value) increases with time since the death of the organism, at a rate that is temperature-dependent. GC analysis enables determination of D- and L-enantiomeric pairs of ten (10) amino acids; of those, the ratio between D-leucine and L-leucine (D/L leucine value) increases with time, and can be used as a stratigraphic tool (Wehmiller, 1984a; Miller and Brigham-Grette, 1989).

To ensure precision of fossil *Cerion* analyses, hydrolyzates of a standard mollusc shell powder (ILC-B; Wehmiller, 1984b) were run daily with the San Salvador samples. Mean A/I values for ILC-B hydrolyzate samples are tabulated for comparison (Table 1). Coefficients of variation (%CV) range between five (5) and ten (10) percent for peak area values, and from eleven (11) to thirteen (13) percent for peak height values.

RESULTS

The fundamental use of amino acid data is to characterize a fossiliferous unit with a statistically significant cluster of D/L leucine or A/I values. The hypothesis to be tested here is that superposed lithologic units deposited during sequential Pleistocene glacio-eustatic sea-level highstands can be differentiated using amino acid data. Amino acid enantiomeric data have been tested (and used successfully) elsewhere in a variety of clastic (Toscano and York, 1992) and carbonate environments. In order to objectively resolve fossiliferous units using amino acid data, any data set must fulfill the following criteria. First, amino acid values must increase from upper (younger) to lower (older) units. Second, each unit that is characterized by a cluster of amino acid values must show good precision. Precision can be quantified two ways. Chromatographic precision is calculated using several chromatograms (or "runs") of a single mollusc shell hydrolyzate, and typically ranges between 2-5% for HPLC and GC methods (Table 1; Wehmiller, 1984b). Sample precision is calculated from analyses of several mollusc fossils of the same genus from a single geologic unit defined in the field, and can range to 10% or higher. Lower precision (or higher %CV) limits aminostratigraphic resolution. If these apparently simple criteria are fulfilled, amino acid enantiomeric data can then be used objectively to differentiate superposed units, and to correlate these units within a limited geographic region.

CHROMATOGRAPHIC PRECISION

ILC-B standard mollusc shell powder analyses, and repeat runs (or multiple chromatograms) from single fossil shell hydrolyzates were used to estimate chromatographic precision. A/I values from ILC-B powders show good precision (4-10%, Table 1), and also good accuracy in that analyses run during this project are in agreement with analyses on HPLC instrumentation at the University of Delaware Amino Acid Laboratory. A/I values reported here were calculated from peak areas (areas under alloisoleucine and isoleucine peaks, measured by a Hewlett-Packard integrator) and peak heights (measured manually, in mm). Peak heights generally show slightly lower precision.

Replicate analyses of single fossil shell hydrolyzates also show reasonable precision, particularly if peak area measurements are used to calculate A/I values (Table 2). Chromatographic precision of A/I values from peak areas ranges from 1-10 %CV. As with the ILC-B powders, precision is lower using peak height values (1-28 %CV). The largest %CVs are encountered when measuring small alloisoleucine peaks with a ruler.

SAMPLE PRECISION

Precision problems are encountered when several molluscs from a single field-defined lithologic unit are considered. Multiple shell analyses from Crab Cay, The Gulf, and Sandy Point/Conch Point localities (see index map at front of volume) do not fulfill the criteria necessary for valid aminostratigraphic work . For example, A/I values (peak area) from Crab Cay and The Gulf localities show 18 %CV, which is too variable to define an aminostratigraphic unit in a small sample population (n =2 or 3) (Table 3). Similarly, A/I data from the Owl's Hole locality, and D/L leucine values from the Sandy Point/Conch Point locality both show 47-48 %CV (Tables 3 and 4).

DISCUSSION

The low precision of amino acid enantiomeric data obtained by HPLC and GC methods indicates a limited ability to resolve lithologic units in this geologic setting. If each locality represents a single lithologic unit deposited during a single high sea-level episode, then it is unlikely that amino acid enantiomeric data can be used because the basic criteria for aminostratigraphy are not fulfilled. These two criteria are: 1) increasing D/L leucine or A/I values lower in the stratigraphic section (or in a calibrated sequence, with greater age); and 2) acceptable sample precision which enables discrete clusters of amino acid enantiomeric data to be recognized, with each cluster representing, in this instance, a sea-level highstand deposit.

The criterion defining the stratigraphic sequence of Quaternary units is not easily proven in the San Salvador eolian deposits. These deposits consist largely of a series of individual, overlapping, or stratigraphically stacked eolianites. As carbonate allochems can only be produced in a volume sufficient to yield the observed eolianites when the shallow platform is flooded by marine water; each eolianite "package" was deposited during a sea-level highstand. Discriminating upper/ younger from lower/older eolianite units can be difficult because both transgressive and regressive facies of each depositional package may exist, but they do not necessarily overlap or overstep one another. In addition, as each high sea-level episode reoccupies the platform at a similar position (sea level ± a few meters), allochems from preexisting units may be partially reworked into the younger units, and younger units may wholly or partially bury older ones. Although it is unlikely that *Cerion* shells are reworked in significant numbers, the reworking of sand-size allochems is a potential problem for whole-rock amino acid analyses. When intervening paleosols are exposed, the distinction between eolianites is usually clear; but that occurrence is more the exception than the rule, because exposures that cut deep enough to reveal paleosols between eolianites are relatively rare except on heavily developed islands. Therefore, inversions of D/L leucine or A/I values may be observed. In this San Salvador data set, one inversion in amino acid data is observed, at The Gulf locality. The criterion defining sample

LOCATION	D/L LEUCINE	%CV	NO. OF FOSSILS
Modern	0.02		1
Sandy Point/Conch Point	0.30 ± 0.14	47	3
Crab Cay	0.64		1
Almgreen Cay	0.64		1
Upper Watlings Quarry	0.72		1
Lighthouse Cave	0.51 ± 0.05	10	4

Table 4. Geologic precision of D/L leucine values from multiple analyses of fossil molluscs (*Cerion*) from San Salvador Island localities. Data from Carew et al., 1984; and Carew and Mylroie, 1987.

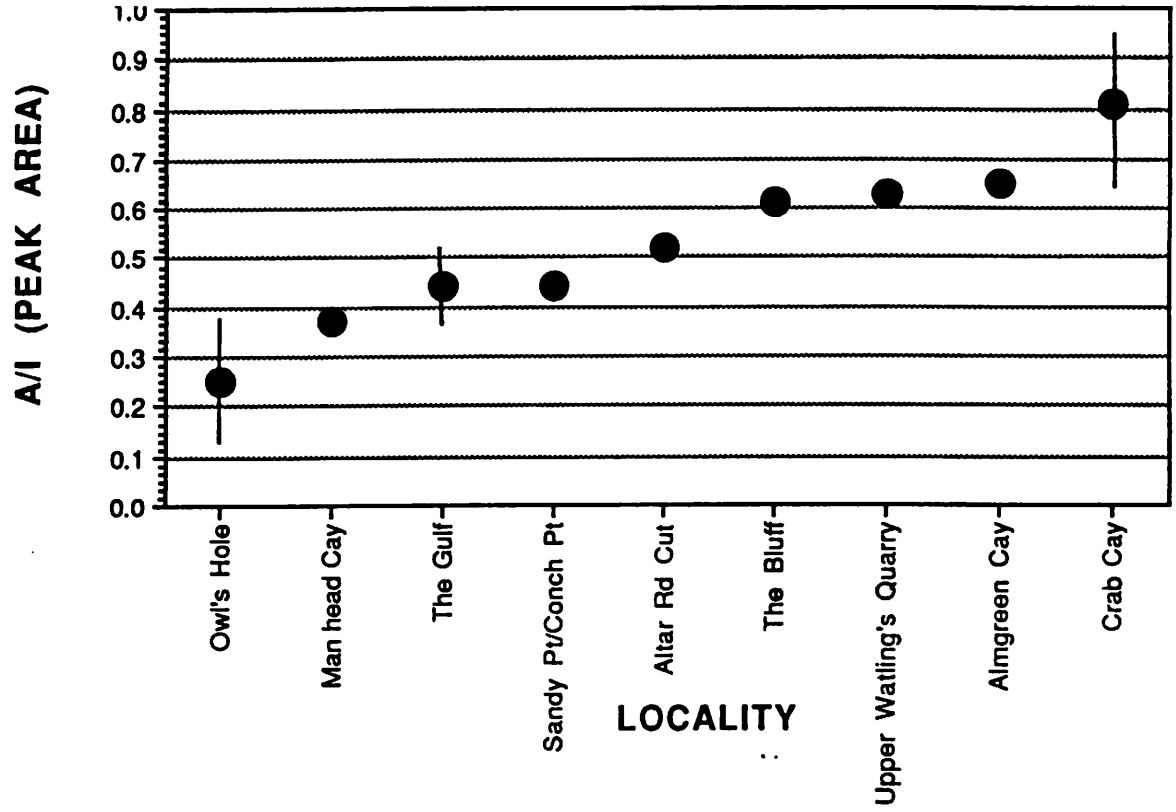

Figure 1. Mean A/I values from all fossil localities presented*in*this investigation. Lines represent standard deviation about mean A/I value, for localities where more than one fossil mollusc sample was analyzed. Localities arranged in order of increasing A /I value, and no stratigraphic significance is implied.

precision is easier to estimate from this data set. Although chromatographic precision is acceptable (i.e. HPLC and GC instrumentation performance is consistent), fossil mollusc shell precision (or sample precision) is frequently low. In fossils collected from a single unit, % CVs of up to 50% are reported, although not for both D/L leucine and A/I values. The lack of precision among multiple samples from a single locality seems to obscure the appearance of clusters of amino acid data, which could characterize each high sea-level deposit. A plot of mean A/I values from all sites reported in this investigation shows an apparent monotonic increase in A/I values, instead of clusters (Figure 1). Because this geologic setting is not characterized by continuous deposition, a steady increase in A/I values with time would not be expected.

From this investigation, amino acid enantiomeric data from *Cerion* collected from San Salvador do not show sufficient resolution to enable discrimination of deposits that represent deposition during individual Quaternary oxygen isotope sub-stages, or perhaps even stages. Diagenetic factors such as incorporation of ground-water-borne contamination into mollusc shell carbonate, or leaching may explain the poor precision of these data. In addition, reworking may also obscure the resolution of this stratigraphic record, but this would be expected to cause the greatest problems for whole-rock amino acid analyses.

Recently, it was suggested that based on whole-rock amino acid analyses the eolianites deposited during different oxygen isotope substages can be discriminated on San Salvador (Hearty and Kindler, 1991, 1992). The rocks at one locality (Three Dog Site) were assigned by those authors to oxygen isotope substage 5a (circa 85,000 years). However, based on field evidence we previously had identified those same rocks as Holocene (oxygen isotope stage 1). Radiocarbon analyses of those rocks obtained for this study yielded ages of 3690 ± 70 and 4400 ± 70 yrBP, which is consistent with the field data. Such a large discrepancy between the age assigned by whole-rock amino acid analysis and the radiocarbon age suggests that whole rock amino acid analyses may suffer from a similar, or even greater, lack of precision than that of analyses of individual mollusc shells.

The results of this study call in to question, until further research identifies the source of error, or a way to correct for it, the ages of Bahamian eolianites derived from amino acid analyses of *Cerion*. We intend to continue to investigate this problem with the goal of developing a methodology that is demonstrably reliable. At present, amino acid ages of Bahamian eolianites, where no clear stratigraphic relationships exist, should be considered suspect.

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