Amino Acid Composition of Planktonic Foraminifera: A Paleobiochemical Approach to Evolution

Abstract. *A unique, species-specific amino acid composition is identified with each of 16 species of planktonic Foraminifera isolated from the tops of deep-sea sediment cores. This amino acid pattern appears to directly reflect the genotype. The total amino acid content ranges from 2.0 to 4.2 micromoles per gram of calcified tissue or 0.02 to 0.04 percent by weight. Analyses of two Early Miocene species indicate that characteristic compositional differences are sufficiently well preserved over geologic time to determine phylogenetic affinities among extinct species living at least 18 million years ago.*

Planktonic Foraminifera provide an ideal fossil record for the study of evolution. These amoeboid, calcareous protozoans occur in great abundance and diversity in deep-sea sediments to form a relatively complete, continuous stratigraphic record extending back to Early Cretaceous time (about 130 million years ago). They have evolved rapidly, with morphological diversity expressed in more than 40 genera and nearly 1000 species, most of which are extinct.

Although the sequence of species in many evolutionary lineages has been determined for biostratigraphic purposes, the phylogeny of planktonic Foraminifera has not been firmly established. This has largely been due to the necessary reliance on shell morphology as the sole taxonomic character. Only the shell is preserved as fossils, and the living species have a low grade of organization that precludes effective comparative anatomy of the soft parts. As a result, a number of taxonomic schemes (*1*) have been proposed, each emphasizing different "diagnostic" morphological features and each reflecting true phylogenetic relationships to an unknown degree.

This study was undertaken to investigate the possibility of using a quantitative biochemical character, amino acid composition of the shell, to supplement descriptive morphology in determining phylogenetic relationships in living and fossil planktonic Foraminifera. The amino acid patterns of mollusks, another major group of calcareous invertebrates, parallel morphology and shell structure in living species (*2*).

The 16 Recent species analyzed represent one-half of the planktonic foraminiferal species now living in the world oceans (*3*). The calcareous tests (shells) of the individual species were isolated from the tops of deep-sea sediment cores (*4*). To minimize the possible effect of environmental factors, the 16 species were sampled from as few core locations as possible (*5*); 11 species were separated from one equatorial Atlantic core (V25-59 TW) with an especially abundant and diverse fauna. Samples were dissolved and hydrolyzed (*6*) prior to analysis by an ultrasensitive ion-exchange chromatographic system (*7*).

The most striking result of the amino acid analyses is that each species has a unique pattern that differs, to a greater or lesser degree, from the compositions of all the other species (*5*). In terms of the average composition of all 16 species, the eight most abundant amino acid residues are Asp (20 percent), Gly (13 percent), Glu (11 percent), Ala (9 percent), Ser (8 percent), Thr (8 percent), Val (6 percent), and Pro (5 percent), representing a total of 80 percent of all residues (*8*). Aspartic acid shows the widest spread (maximum to minimum) between species (112 residues per 1000), followed by Gly (60 residues per 1000) and Ala (53 residues per 1000). The total amino acid concentration in the shell material ranges between 2.0 and 4.2 μmole/g (*5*). Only four species, however, have total contents greater than 3.0 μmole/g.

The amino acid data were subjected to a Q-mode factor analysis (*9*) to determine whether the 16 species could be statistically grouped into a few assemblages for comparison with the taxonomic classification. The first analysis was conducted with a 16 by 17 data matrix with the 17 amino acids expressed in residues per 1000; it resulted in a two-factor solution. A plot of the species in relation to the two independent factor axes (*5*) showed a generally smooth succession through the four major groups of a current taxonomic classification based on morphology (*3, 10*).

To reduce the overwhelming effect of aspartic acid in limiting the number of factors, the amino acid data were

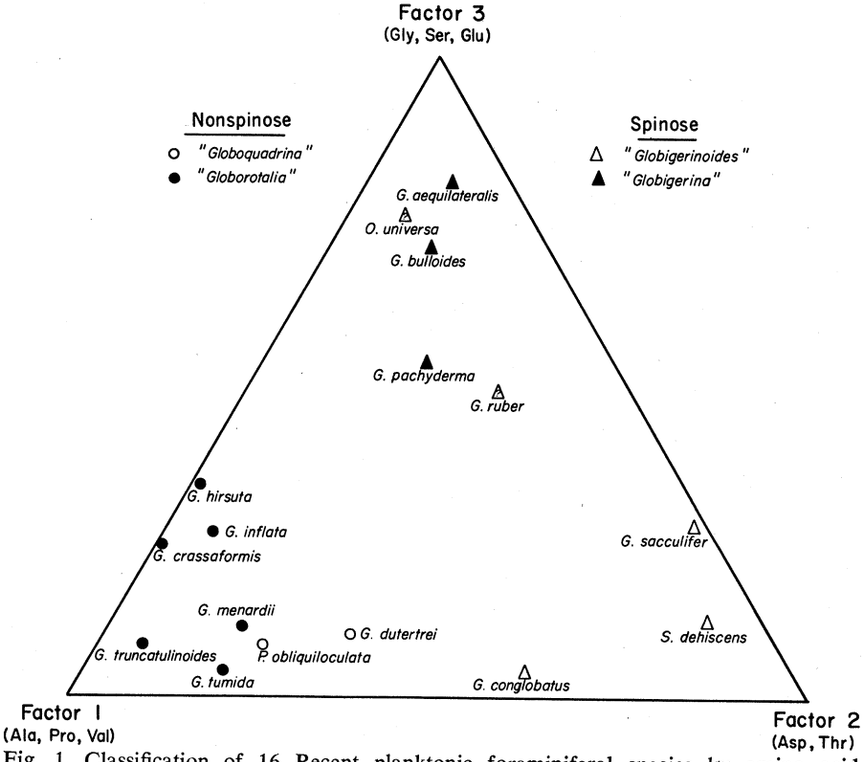

Fig. 1. Classification of 16 Recent planktonic foraminiferal species by amino acid composition by means of Q-mode factor analysis. The symbols represent groups of morphologically similar species in a current taxonomic scheme (*3, 10*).

Table 1. Comparison of amino acid concentrations in two extinct Early Miocene species (approximately 18 million years ago) and a Recent species; all three species are of the same genus, *Globoquadrina*.

Amino acid	Concentration (nanomoles per gram of shell material)		
	Recent	Early Miocene	
	G. dutertrei	*G. altispira*	*G. dehiscens*
Asp	467	398	273
Thr	172	11	11
Ser	166	15	18
Glu	252	193	168
Pro	139	118	115
Gly	273	164	155
Ala	215	265	230
Cys	25		
Val	136	129	99
Met	20		
Allo-Ile		56	46
Ile	81	43	35
Leu	95	65	49
Tyr	58	28	22
Phe	87	32	31
His	40	19	19
Lys	54	17	17
Arg	41		
Total	2321	1553	1288
Allo-Ile/Ile	0	1.30	1.31

transformed to percent of range and refactored. Also, the nine least abundant amino acid residues were deleted from the matrix, as they are characterized by either higher experimental error (Cys, Met, Tyr, Phe, His, Lys, and Arg) or minimal variability (Ile and Leu). The remaining eight amino acids account for about 80 percent of all amino acid residues in the 16 species and were measured with a reproducibility of ± 3 percent in duplicate analyses of 10 species. Analysis of this reduced, transformed data matrix yielded three important factors (Fig. 1). The composition of these factors, as indicated by the corresponding factor measurements matrix, shows that factor 1 is dominated by Ala, Pro, and Val; factor 2 by Asp and Thr; and factor 3 by Gly, Ser, and Glu.

When the results are compared with a widely used classification based on morphology (*3*, *10*), this solution separates the nonspinose species (family Globorotaliidae) from the spinose species (family Globigerinidae). The two species in the nonspinose subgroup "Globoquadrina" (*Pulleniatina obliquiloculata* and *Globoquadrina dutertrei*) are adjacent to one another and are somewhat more strongly influenced by Asp and Thr than are the six members of the subgroup "Globorotalia" (genus *Globorotalia*).

The spinose species exhibit much greater variability in amino acid composition than the nonspinose forms, with the "Globigerinoides" subgroup showing the widest spread. *Sphaeroidinella dehiscens* and *Globigerinoides sacculifer* are close together, forming with *Globigerinoides conglobatus* a grouping of three species strongly dominated by Asp and Thr. The other species of "Globigerinoides" (*Globigerinoides ruber* and, particularly, *Orbulina universa*) are strongly influenced by Gly, Ser, and Glu, as are the members of the "Globigerina" subgroup (*Globigerina pachyderma, Globigerina bulloides*, and *Globigerinella aequilateralis*). Parker (*11*) has pointed out that (i) the genus *Globigerinoides* is undoubtedly polyphyletic, (ii) most of its included species should probably be transferred to genus *Globigerina*, and (iii) *G. sacculifer* should possibly be referred to genus *Sphaeroidinella*. These morphologically based conclusions are strikingly confirmed by the factored amino acid compositions.

To assess the feasibility of applying this technique at depth in cores to trace evolutionary lineages, we considered two questions about diagenetic changes in foraminiferal tests through geologic time: (i) Are amino acids preserved in sufficient amounts to quantify? (ii) Are the original species differences in amino acid composition maintained or are they destroyed, yielding a common residuum devoid of genetic information?

Two extinct species (*Globoquadrina altispira* and *Globoquadrina dehiscens*) of Early Miocene age (about 18 million years) were isolated from the same deep-sea core sample (V21-16) and were analyzed. The results are com-

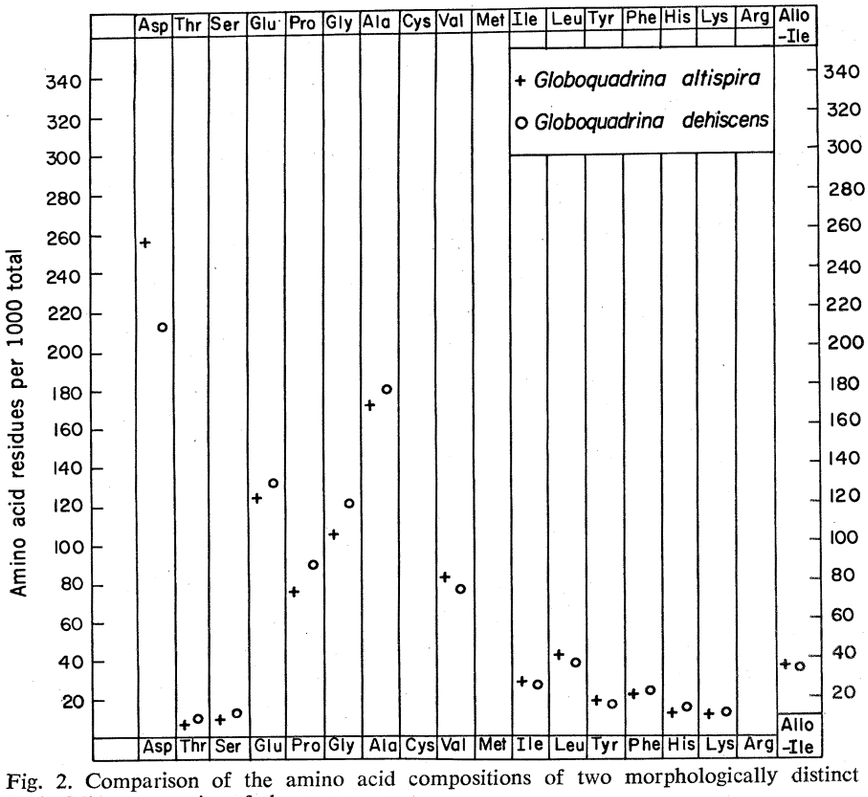

Fig. 2. Comparison of the amino acid compositions of two morphologically distinct Early Miocene species of the same genus.

pared with a living species of the same genus (*Globoquadrina dutertrei*) in Table 1. Substantial levels of the more thermally stable amino acids are still present in the Miocene specimens. If it is assumed that the total concentration of amino acids in the Recent *G. dutertrei* approximates the original value of the Miocene species, about 67 percent of the original total content has been preserved in *G. altispira* and 56 percent in *G. dehiscens* (*12*). The less thermally stable amino acids are either absent (Cys, Met, and Arg residues) or present at greatly reduced levels (Thr and Ser residues).

The second question, regarding the persistence of original specific differences, was approached by studying the same two species (*G. altispira* and *G. dehiscens*) since they are morphologically distinct. Because of the parallelism in Recent specimens, one would predict from the morphologies that the amino acid patterns should be generally similar but should differ significantly in the more sensitive (variable) amino acids (such as Asp and Gly residues). A plot of the two compositions (Fig. 2) confirms this prediction. Thus, diagenesis does not obliterate significant differences in original amino acid compositions (*13*).

Contamination is always a potential problem in interpreting low-level occurrences of amino acids in geologic environments (*6*). However, several lines of evidence developed during the study strongly argue against the presence of amino acids from sources other than calcified proteins (*5*).

As the amino acid composition of the test apparently reflects the genotype of both living and fossil planktonic Foraminifera, this quantitative taxonomic character provides a new approach to constructing a "natural" classification based on phylogeny. Evolutionary lineages can now be traced paleontologically by studying biochemical, as well as morphological, variations through geologic time. The technique may also be useful in differentiating between species, subspecies, and phenotypic variants at a given horizon in time.

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2. P. E. Hare and P. H. Abelson, *Carnegie Inst. Washington Yearb.* **63**, 267 (1964); *ibid.* **64**, 223 (1965); E. T. Degens, D. W. Spencer, R. H. Parker, *Comp. Biochem. Physiol.* **20**, 553 (1967); M. T. Ghiselin, E. T. Degens, D. W. Spencer, R. H. Parker, *Breviora* **262**, 1 (1967).
3. A. W. H. Bé, in *Fiches Ident. Zooplancton*, No. 108 (1967).
4. The top 2.5 cm of each core was disaggregated ultrasonically in distilled water, wet-sieved, and air-dried to obtain the sediment coarse fraction (larger than 62 μm), which was predominantly foraminiferal tests. Individual specimens of each species were identified and separated from this fraction by the use of a binocular microscope and a vacuum picking device. Aggregate species samples were then cleaned ultrasonically in a series of sodium hexametaphosphate (1 percent) washes and distilled water rinses.
5. K. King and P. E. Hare, *Micropaleontology*, in press. A list is given of the four cores with their respective species, geographical location, and water depth. This reference also includes detailed analytical data and interpretive plots.
6. P. E. Hare, in *Organic Geochemistry, Methods and Results*, G. Eglinton and M. T. J. Murphy, Eds. (Springer-Verlag, New York, 1969), p. 438.
7. ———, *Carnegie Inst. Washington Yearb.* **70**, 268 (1971).
8. Standard abbreviations are used for the amino acids: Asp, aspartic acid; Gly, glycine; Glu, glutamic acid; Ala, alanine; Ser, serine; Thr, threonine; Val, valine; Pro, proline; Cys, cystine; Met, methionine; Tyr, tyrosine; Phe, phenylalanine; His, histidine; Lys, lysine; Arg, arginine; Ile, isoleucine; and Leu, leucine.
9. J. W. Harbaugh and D. F. Merriam, *Computer Applications in Stratigraphic Analysis* (Wiley, New York, 1968), p. 179. The analyses described in this report were performed on an IBM 1130 computer, with cosine-theta similarity coefficients and a varimax rotation of the factor axes.
10. F. L. Parker, *Micropaleontology* **8**, 219 (1962).
11. ———, *Bull. Amer. Paleontol.* **52**, 115 (1967).
12. The apparent increase in Ala concentration shown by the two Miocene samples is probably due to the generation of Ala in the diagenetic breakdown of Ser [see J. R. Vallentyne, *Geochim. Cosmochim. Acta* **25**, 157 (1964)].
13. The lower limit in fossil age for applying this method has not been determined but is currently being investigated through the analysis of successively older samples in the sediment column.
14. We are deeply grateful to J. D. Hays for his interest, encouragement, and suggestions throughout this investigation. Special thanks are due S. S. Streeter for his valuable help in conducting and interpreting the factor analyses. Gratitude is expressed to A. W. H. Bé and T. Saito for their valuable advice on questions of foraminiferal taxonomy and evolution. We thank P. H. Abelson, A. W. H. Bé, W. S. Broecker, T. C. Hoering, and T. Saito for reviewing the manuscript and making many helpful suggestions. J. E. Damuth brought core V25-59 TW to our attention. D. Breger drafted the figures and D. Ultsch prepared the manuscript. The deep-sea cores were collected on cruises of the Lamont-Doherty Geological Observatory research vessel, supported by ONR grant N00014-67-A-0108-0004 and NSF grant GA-29460. Work done at Lamont-Doherty Geological Observatory was supported by ONR grant N00014-67-A-0108-004, NSF grant GA-29460, and a predoctoral fellowship from the Geophysical Laboratory, Carnegie Institution of Washington. Contribution No. 1796 of the Lamont-Doherty Geological Observatory.

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Chemical Methods for Removing Radon and Radon Daughters from Air

Abstract. *Liquid bromine trifluoride and the solid complexes ClF_2SbF_6, BrF_2SbF_6, $BrF_4Sb_2F_{11}$, $IF_4(SbF_6)_3$, and BrF_2BiF_6 react spontaneously with radon and radon daughters at 25°C, converting the radioelements to nonvolatile ions and compounds. The reagents can be used in gas-scrubbing units to remove radon and radon daughters from air. The halogen fluoride–antimony pentafluoride complexes may be suitable for purifying air in uranium mines and analyzing radon in air, since they have low dissociation pressures at 25°C and are less hazardous to handle than liquid halogen fluorides.*

The chief radiation hazard to miners in underground uranium mines is that from radon gas, released by the decay of radium, and the short-lived radon daughters (^{218}Po, ^{214}Pb, ^{214}Bi, and ^{214}Po). Medical and epidemiological studies have shown that prolonged exposure to high concentrations of these radioelements, particularly the radon daughters, increases the incidence of lung cancer among miners (*1*). Since the discovery of this hazard, uranium mines have been force-ventilated to reduce the concentrations of the radioelements. In the United States, federal regulations now require mine atmospheres to be controlled so that a miner receives no more than four "working level months" of radiation exposure in any 12-month period, the "working level" being defined as the concentration of short-lived radon daughters in

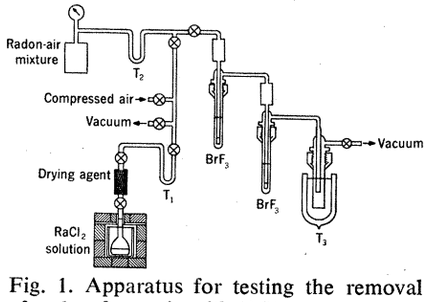

Fig. 1. Apparatus for testing the removal of radon from air with BrF_3.