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# 12. Data Report: D/L Ratios and Concentrations of Selected Amino Acids in Interstitial Waters, Equatorial Pacific and Peru Margin, ODP Leg 201[1]

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## INTRODUCTION

Proteins and their amino acid building blocks form a major group of biomolecules in all organisms. In the sedimentary environment, proteins and amino acids have two sources: (1) soft tissues and detritus and (2) biotic skeletal structures, dominantly from calcium carbonate–secreting organisms.

As organisms die, their soft tissues are metabolized by other organisms and converted into insoluble fecal material or dissolved molecules. Eventually, some fraction of the remains settles through the water column or is adsorbed to clay particles to be deposited in sediments. Active microbial activity in surface sediments and within the sediment column further affects the composition of sedimentary organic matter by adding and removing biomolecules (see Burdige, 2002, for a recent review). In addition, the concentration of biomolecules such as amino acids should decrease with increasing sediment depth as a result of condensation reactions that incorporate amino acids into humic substances and kerogen (Amon and Benner, 1994).

Protein within biotic carbonates is largely protected from the usual metabolic and diagenetic reactions mediated by microorganisms. In carbonate sediments, proteins and amino acids undergo a series of predictable chemical reactions that include hydrolysis, decarboxylation, deamination, and racemization (Mitterer, 1993). Because of these reac-

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tions, the general trend in carbonate sediments with increasing age is a decrease in overall concentration of amino acids and an increase in the extent of amino acid racemization (increasing D/L ratios) (Mitterer, 1993). In agreement with this trend, Mitterer et al. (2001) showed that amino acid concentrations generally decrease downcore in carbonate sediments from Ocean Drilling Program (ODP) Leg 182 (Great Australian Bight).

The focus of this report is on D/L ratios and concentrations of selected amino acids in interstitial waters collected during ODP Leg 201. The Peru margin sites are generally low in carbonates, whereas the open-ocean sites are more carbonate rich. Seifert et al. (1990) reported amino acid concentrations in interstitial waters from Site 681 (ODP Leg 112) comparable to Leg 201 Site 1229.

## METHODS/MATERIALS

Concentrations and D/L values were determined for selected amino acids in interstitial water samples extracted from whole-round core intervals that were placed in a titanium squeezer (as modified from Manheim and Sayles, 1974). Water samples for amino acid analyses were placed in baked (450°C) glass vials, acidified to pH 1, and stored at 4°C prior to transport for shore-based analyses. Samples were stored onshore at freezing temperatures until analysis.

Concentrations and D/L ratios of free plus combined amino acids were determined by reverse-phase high-pressure liquid chromatography (RPLC) at the Amino Acid Geochronology Laboratory at Northern Arizona University. An aliquot of 20 µL of interstitial water was evaporated to dryness and hydrolyzed in 7-N HCl at 110°C for 6 hr to release all peptide-bound amino acids to the free state. The hydrolyzate was evaporated to dryness, taken up in 5 µL of acid buffer, and applied to the RPLC column without removing sea salt (see Kaufmann and Manley, 1998, for analytical details). Because of low concentrations or interferences from unknown constituents during chromatography for most amino acids, only values for aspartic acid, glutamic acid, serine, and glycine are reported.

Some racemization occurs during acid hydrolysis of proteins, with aspartic and glutamic acids exhibiting the largest effects (~2%–3% D-enantiomers formed), whereas hydrolysis-induced racemization of serine is negligible (Kaiser and Benner, 2005). Consequently, concentrations of the D-enantiomers of aspartic and glutamic acids in samples will be overestimated; this effect will be significant only for low D/L values.

## DISCUSSION

Some of the D/L ratios in Table T1 are less than those expected if hydrolysis-induced racemization occurred during sample preparation. The complex nature of amino acid associations in marine sediments (i.e., free, peptide-bound, cellular, humic and fulvic acids, and kerogen) may limit the extent of this effect in the types of organic matter in interstitial water samples. Because of the uncertain, but very low, extent of racemization occurring during hydrolysis, the D/L values in Table T1 have not been corrected for this effect.

If amino acids in the sedimentary environment were sourced solely by deposition of organic matter from the overlying water column, then

T1. Concentrations and D/L-amino acid ratios, p. 7.

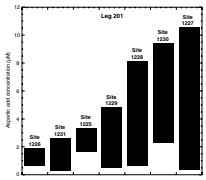
concentrations of amino acids should generally decrease with increasing depth below the seafloor due to various chemical reactions, including those that incorporate amino acids into humic substances and kerogen. More importantly, racemization of most amino acids (except glycine) should lead to a systematic increase in D/L ratios with depth (i.e., increasing age) within the sediment column if there are no additional inputs of amino acids during burial. Amino acids in Leg 201 cores exhibit neither of these trends. Concentrations of amino acids do not systematically decrease, nor do D/L ratios generally increase downcore. Instead, both parameters are variable as a function of sediment depth. The downcore variations in concentrations and D/L values exhibit no apparent relationship to other chemical parameters such as sulfate or dissolved inorganic carbon concentrations. These results agree with those of Seifert et al. (1990) on the lack of any systematic trends in amino acid concentrations with depth in interstitial waters from Site 681 (also Site 1229) (D/L amino acid ratios were not determined by Seifert et al.)

Despite the lack of any definitive trends in downcore concentrations of the individual amino acids, some overall patterns are apparent. Throughout the cores, of the four amino acids, glycine generally occurs in highest concentration and aspartic acid in lowest concentration. This relationship reflects, in part, the relative concentrations of these amino acids in proteins and, in part, the production of glycine as a diagenetic product of other amino acids (Mitterer, 1993). All four amino acids occur in highest concentrations overall in Site 1230 samples; this site also has the highest dissolved inorganic and organic carbon concentrations of the Leg 201 sites (**Smith,** this volume; Shipboard Scientific Party, 2003).

A significant difference between the open-ocean and Peru margin sites is the range of amino acid concentrations in each region. Figure **F1** illustrates the range of aspartic acid concentrations for each of the Leg 201 sites; concentration ranges for glutamic acid and serine display comparable patterns. As Figure **F1** demonstrates, the ranges of aspartic acid concentrations for the three open-ocean sites (1225, 1226, and 1231) are relatively narrow, with ranges of ~2 µM and maximum values up to 3 µM. In contrast, the four Peru margin sites have wider ranges in aspartic acid concentrations, from 4 to 10 µM, and maximum aspartic acid concentrations from ~5 to 11 µM. Despite the lower overall concentrations in the open-ocean sites, the aspartic acid concentrations overlap the lower end of the values for most of the Peru margin sites.

The variable concentrations and D/L ratios of these selected amino acids indicate that microorganisms are adding and removing amino acids to the reservoir of these compounds in the interstitial waters. Whereas this result is not surprising for the Peru margin sites, which display evidence of extensive microbial activity throughout the sediment column (Shipboard Scientific Party, 2003), the open-ocean sites also appear to have some limited microbial activity.

**F1.** Aspartic acid concentrations, p. 6




## SUMMARY AND CONCLUSIONS

Dissolved amino acid concentrations and especially D/L ratios do not exhibit any systematic trends in interstitial waters with increasing depth either in the Peru margin or open-ocean sites. Amino acid concentrations and D/L ratios have overlapping values in the interstitial

water samples from both regions, indicating that some microbial activity occurs in all these sites.

## ACKNOWLEDGMENTS

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**Figure F1.** Range of aspartic acid concentrations in interstitial waters from Leg 201 sites.

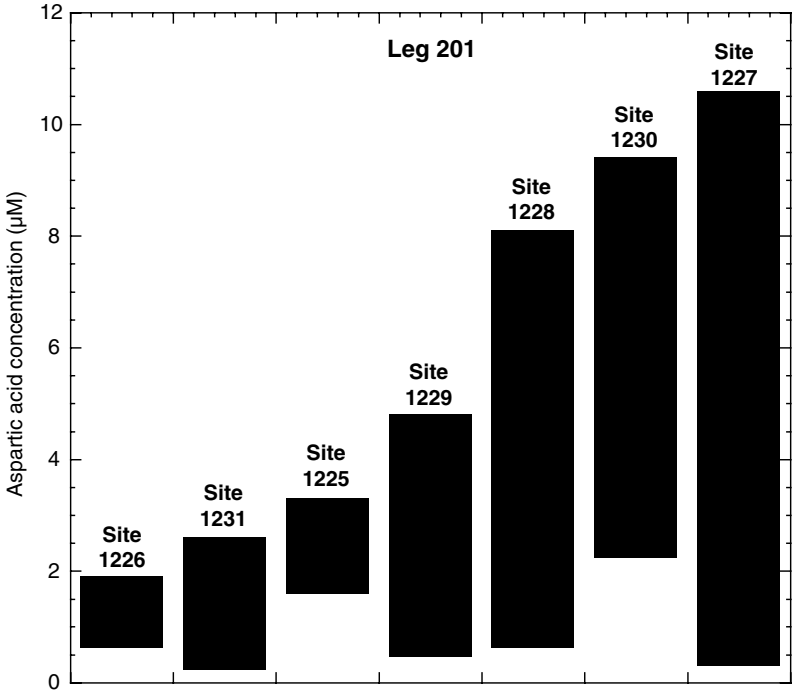

**Table T1.** Concentrations and D/L-amino acid ratios of selected amino acids in interstitial waters from Leg 201 sites.

| Core, section, interval (cm) | Depth (mbsf) | Amino acid (μM) | | | | D/L | D/L | D/L |
|---|---|---|---|---|---|---|---|---|
| | | Asp | Glu | Ser | Gly | Asp | Glu | Ser |
| 201- | | | | | | | | |
| 1225C-1H-1-37 | 0.37 | 2.4 | 6.4 | 8.3 | 3.2 | 0.05 | 0.02 | 0.01 |
| 1225C-1H-3-65 | 3.65 | 1.8 | 3.1 | 4.6 | 2.7 | 0.08 | 0.03 | 0.02 |
| 1225C-2H-2, 135 | 11.65 | 1.7 | 1.9 | 1.4 | 7.2 | 0.37 | 0.19 | 0.00 |
| 1225C-3H-3, 135 | 21.15 | 3.3 | 5.1 | 6.0 | 9.6 | 0.18 | 0.08 | 0.08 |
| 1225C-4H-2, 37 | 30.65 | 2.9 | 4.6 | 7.7 | 6.1 | 0.08 | 0.03 | 0.02 |
| 1225A-10H-3, 0 | 83.3 | 1.6 | 3.9 | 1.2 | 1.1 | 0.08 | 0.04 | 0.11 |
| 1226B-1H-1, 130 | 1.3 | 1.9 | 2.1 | 1.9 | 8.7 | 0.36 | 0.20 | 0.24 |
| 1226B-3H-2, 130 | 21.2 | 1.5 | 1.8 | 2.0 | 7.0 | 0.36 | 0.18 | 0.25 |
| 1226B-4H-5, 130 | 30.7 | 1.4 | 2.2 | 2.5 | 1.8 | 0.09 | 0.04 | 0.02 |
| 1226B-5H-5, 130 | 40.2 | 0.9 | 1.4 | 1.9 | 1.3 | 0.13 | 0.05 | 0.03 |
| 1226B-20H-5, 130 | 182.7 | 1.1 | 1.3 | 1.6 | 1.2 | 0.07 | 0.08 | 0.05 |
| 1227D-1H-1-1 | 1 | 1.6 | 2.3 | 2.6 | 4.5 | 0.18 | 0.06 | 0.10 |
| 1227D-1H-2, 30 | 2.1 | 1.3 | 1.7 | 2.1 | 3.2 | 0.15 | 0.05 | 0.08 |
| 1227A-2H-3, 135 | 9.95 | 10.6 | 19.4 | 30.0 | 20.1 | 0.10 | 0.04 | 0.03 |
| 1227A-2H-6, 135 | 14.45 | 0.8 | 1.2 | 1.5 | 3.3 | 0.13 | 0.07 | 0.08 |
| 1227A-3H-3, 135 | 19.45 | 4.6 | 9.2 | 9.9 | 8.1 | 0.08 | 0.03 | 0.04 |
| 1227A-3H-6, 135 | 23.95 | 3.0 | 5.2 | 6.3 | 8.4 | 0.11 | 0.04 | 0.05 |
| 1227D-4H-2, 135 | 29.35 | 3.5 | 5.5 | 6.2 | 9.3 | 0.19 | 0.07 | 0.08 |
| 1227D-4H-5, 135 | 33.9 | 2.6 | 2.4 | 2.4 | 8.3 | 0.09 | 0.15 | 0.14 |
| 1227A-5H-1, 135 | 35.45 | 2.0 | 2.1 | 2.0 | 8.3 | 0.38 | 0.19 | 0.28 |
| 1227A-5H-3, 135 | 38.45 | 2.0 | 3.2 | 4.6 | 5.0 | 0.14 | 0.06 | 0.04 |
| 1227D-5H-2, 135 | 38.9 | 0.4 | 0.5 | 0.6 | 4.2 | 0.40 | 0.28 | 0.25 |
| 1227A-18H-2, 135 | 144.3 | 1.6 | 3.1 | 2.4 | 2.7 | 0.09 | 0.08 | 0.03 |
| 1228C-1H-1, 30 | 0.3 | 2.3 | 2.8 | 3.1 | 8.4 | 0.26 | 0.13 | 0.18 |
| 1228C-1H-1, 45 | 0.45 | 8.1 | 16.6 | 25.6 | 10.2 | 0.04 | 0.02 | 0.02 |
| 1228C-1H-1, 60 | 0.6 | 0.8 | 0.9 | 1.1 | 3.5 | 0.41 | 0.21 | 0.26 |
| 1228C-1H-1, 90 | 0.9 | 2.7 | 5.4 | 5.4 | 12.5 | 0.22 | 0.10 | 0.09 |
| 1228C-1H-1, 15 | 1.05 | 4.9 | 7.9 | 9.4 | 10.7 | 0.15 | 0.06 | 0.05 |
| 1228C-1H-1, 135 | 1.35 | 3.2 | 4.4 | 5.2 | 10.3 | 0.19 | 0.10 | 0.10 |
| 1228E-1H-3, 0 | 3.5 | 1.5 | 1.4 | 1.2 | 3.9 | 0.14 | 0.18 | 0.25 |
| 1228A-1H-3, 135 | 4.26 | 1.4 | 1.5 | 1.4 | 6.5 | 0.39 | 0.21 | 0.26 |
| 1228A-2H-1, 135 | 6.25 | 2.4 | 2.5 | 2.3 | 11.0 | 0.42 | 0.21 | 0.29 |
| 1228E-1H-4, 135 | 6.4 | 1.4 | 1.8 | 2.2 | 3.9 | 0.17 | 0.08 | 0.16 |
| 1228A-3H-5, 135 | 21.75 | 1.7 | 1.8 | 1.6 | 7.6 | 0.41 | 0.22 | 0.32 |
| 1228A-4H-1, 135 | 25.25 | 1.6 | 1.8 | 1.8 | 6.5 | 0.34 | 0.17 | 0.19 |
| 1228A-5H-1, 135 | 34.75 | 3.1 | 4.8 | 4.9 | 10.0 | 0.18 | 0.08 | 0.09 |
| 1228A-6H-1, 135 | 44.25 | 2.6 | 5.0 | 5.2 | 6.0 | 0.14 | 0.06 | 0.05 |
| 1228A-9H-1, 135 | 72.8 | 3.7 | 2.8 | 1.4 | 1.1 | 0.07 | 0.71 | 0.04 |
| 1228A-10H-1, 135 | 82.3 | 0.6 | 0.8 | 0.8 | 0.9 | 0.09 | 0.08 | 0.06 |
| 1229A-1H-3, 135 | 4.35 | 1.3 | 2.1 | 2.6 | 3.5 | 0.16 | 0.07 | 0.06 |
| 1229A-2H-3, 135 | 9.25 | 2.1 | 2.3 | 2.0 | 9.1 | 0.41 | 0.20 | 0.23 |
| 1229A-3H-3, 135 | 18.75 | 0.6 | 0.8 | 1.2 | 3.8 | 0.19 | 0.11 | 0.06 |
| 1229A-4H-3, 135 | 28.25 | 2.8 | 3.7 | 4.7 | 11.5 | 0.25 | 0.11 | 0.09 |
| 1229A-6H-3, 0 | 42.9 | 4.8 | 6.1 | 5.4 | 11.4 | 0.18 | 0.08 | 0.08 |
| 1229A-8H-3, 135 | 63.3 | 0.5 | 0.8 | 0.9 | 1.1 | 0.11 | 0.07 | 0.03 |
| 1229A-10H-2, 135 | 82.3 | 0.3 | 0.4 | 0.5 | 0.7 | 0.18 | 0.02 | 0.11 |
| 1229A-18H-3, 135 | 159.8 | 1.1 | 1.9 | 2.1 | 1.4 | 0.07 | 0.08 | 0.04 |
| 1230A-1H-2, 135 | 2.85 | 2.6 | 2.5 | 3.1 | 1.6 | 0.38 | 0.18 | 0.25 |
| 1230A-2H-2, 135 | 7.65 | 2.3 | 2.5 | 2.3 | 9.7 | 0.38 | 0.18 | 0.19 |
| 1230A-4H-3, 135 | 28.15 | 9.4 | 10.6 | 8.7 | 36.7 | 0.46 | 0.21 | 0.20 |
| 1230A-5H-2, 135 | 36.15 | 2.7 | 3.1 | 3.2 | 11.2 | 0.36 | 0.16 | 0.18 |
| 1230A-6H-2, 135 | 45.65 | 3.0 | 3.4 | 2.9 | 15.4 | 0.44 | 0.23 | 0.28 |
| 1230A-10H-1, 135 | 71.7 | 6.9 | 6.2 | 3.8 | 38.7 | 0.66 | 0.43 | 0.33 |
| 1230A-14H-5, 135 | 115.7 | 6.7 | 6.0 | 4.0 | 44.2 | 0.52 | 0.41 | 0.32 |
| 1230A-21H-4, 69 | 162.5 | 4.8 | 4.3 | 2.4 | 30.0 | 0.54 | 0.43 | 0.17 |
| 1231B-1H-1, 135 | 1.35 | 0.9 | 1.5 | 2.0 | 3.7 | 0.11 | 0.06 | 0.09 |
| 1231B-3H-3, 135 | 17.25 | 2.1 | 2.4 | 2.3 | 8.4 | 0.40 | 0.18 | 0.18 |
| 1231B-4H-3, 135 | 26.75 | 1.9 | 2.7 | 3.1 | 5.9 | 0.21 | 0.10 | 0.09 |
| 1231B-6H-3, 135 | 45.75 | 2.2 | 2.4 | 2.4 | 8.8 | 0.36 | 0.18 | 0.18 |
| 1231B-7H-3, 135 | 52.3 | 2.6 | 3.7 | 3.5 | 2.7 | 0.04 | 0.08 | 0.04 |
| 1231B-7H-3, 136 | 55.3 | 0.2 | 0.3 | 0.4 | 0.2 | 0.09 | 0.12 | 0.08 |
| 1231B-7H-3, 137 | 58.3 | 0.2 | 0.4 | 0.4 | 0.3 | 0.09 | 0.08 | 0.04 |

Notes: Asp = aspartic acid, Glu = glutamic acid, Ser = serine, Gly = glycine.