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Reworking of amino acid in marine sediments: Stable carbon isotopic composition of amino acids in sediments along the Washington coast

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Abstract

The stable carbon isotopic composition of nine individual amino acids were investigated in phytoplankton and zooplankton, estuarine plankton, terrigenous material, marine fecal material, and clay mineral isolates collected along the Washington coast. The clay fraction was isolated from suspended sediments of the Columbia River (the mineral source) and three shelf and slope stations (mineral deposition sites). In the sediments, terrigenous amino acids were replaced by those of marine origin, and microbial reworking of the amino acids further influenced their $\delta^{13}C$ compositions. Based on changes in isotopic composition, individual amino acids could be roughly divided into three groups. (1) Leucine and proline had isotopic shifts similar to bulk organic matter. On average, 80% of these river-delivered amino acids were replaced by marine-derived material. (2) Alanine, isoleucine, glutamic acid, aspartic acid, and phenylalanine had intermediate isotopic shifts. These isotopic compositions are consistent with both the expected isotopic fractionation associated with microbial resynthesis of amino acids using marine substrates and/or preservation of ~50% of the terrigenous component in the marine environment. (3) Glycine and valine exhibited isotopic values outside the range of our sampled end members. Their unusual isotopic compositions are attributed to reworking of their isotopic signal during diagenesis. Microbial resynthesis of amino acids during growth on mixed substrates may account for nearly all the observed variation in amino acid isotopic composition. Similarity between the amino acid isotopic composition of the fecal material and that of the clay isolates suggests that alterations of the isotopic composition of the amino acids might occur while the amino acids are in distinct organic-rich debris, prior to long-term association with the sediment.

One common assumption regarding stable carbon isotopes is that there is little alteration of the bulk organic carbon isotopic composition during early diagenesis (Fogel and Cifuentes 1993; Hayes 1993; McArthur et al. 1992). Although it is well established that the fractionation of carbon isotopes during respiration is minimal (McArthur et al. 1992), diagenesis also entails synthesis of organic matter as microbial biomass. This process, microbial reworking of organic matter, can potentially alter the stable carbon isotopic composition of individual organic molecules that are produced by multiple sources (Macko and Estep 1984). During the growth of heterotrophic organisms, amino acids can be respired, assimilated, and incorporated directly into biomass or resynthesized from other assimilated compounds (sugars, nucleic acids, etc.). Determining the sources of amino acids used by microorganisms for growth, and by extension investigating the diagenesis of source materials, thus potentially requires knowledge of the isotopic composition of all the source materials (amino acids, sugars, nucleic acids, etc.) that are commonly used for production of microbial biomass.

The use of compound-specific isotope analyses allows the fate of individual compounds with multiple sources to be investigated for the first time (Prahl et al. 1992; Hayes 1993; Fogel et al. 1997). By monitoring the concentration and isotopic composition of an individual compound class and its source materials, it is possible to assess whether the isotopic signature obtained for a sedimentary sample represents dilution by material from a different source, diagenetic reworking of the initial material, or a combination of factors. Using this approach, Prahl et al. (1992) identified soil and riverine sources of diploptene (a pentacyclic triterpene) and showed that only the soil-derived biomarker was preserved in coastal sediments. The objective of the present study was to assess the factors that influence the isotopic composition of amino acids and proteins during the transport and deposition of sediment grains from a river to a coastal zone. Although amino acids are ubiquitous and abundant in both terrigenous and marine POM, there have been no previous attempts to determine the relative fates of terrigenous and marine-derived amino acids in the marine environment.

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Materials and methods

Location and sample collection—The continental margin along the Washington coast has been widely studied from biological, geological, and geochemical perspectives (Landry and Hickey 1989). The margin has a 25- to 60-km wide shelf that is intersected by a series of marine canyons and a steep shelf break at ~175 m water depth. The dominant sediment source is the Columbia River, which provides >90% of the mineral deposited along the Washington margin (Sternberg 1986).

Surface sediments (0–5 cm) from three locations were collected as described by Keil et al. (1994). Samples were from the sedimentary plume of the Columbia River approximately 30 km from the river mouth at a depth of 103 m (hereafter referred to as plume), the northern end of the mid-shelf silt deposit (MSSD) at a depth of 133 m, and the continental slope west of Grays and Willapa Canyons at a depth of 1,835 m (slope). The slope station contained small amounts of distinct fecal matter with pellets having an approximate size of 150–500 μm. This material was hand picked and analyzed separately. It is not clear whether this fecal matter is derived from benthic macrofauna or from plankton. Suspended particulate material (SPM) from the Columbia River estuary was collected during both maximal flood and ebb tide and combined after processing to isolate only mineral particles (river; Keil et al. 1994; Prahl et al. 1997).

Marine planktonic source materials were collected from vertical net tows taken approximately midway between the plume and the MSSD stations and were provided courtesy of Dr. Fred Prahl (Oregon State University). Material was fractionated into greater than and less than 64-μm material in order to coarsely separate macrozooplankton from phytoplankton. Samples were then manually sorted into phytoplankton (largely miscellaneous diatom species) and zooplankton samples (mostly small copepods). Terrestrial plant debris was handpicked from the >64-μm suspended sediment samples obtained in the estuary and was a mixture of pine needles, tree bark of unspecified origin, and deciduous leaf matter. Diatoms isolated from the estuary were of the genus *Skeletonema.*

Mineral isolation and analysis—Clays were isolated from the bulk sediment in order to assure that the comparison of amino acid content and composition was independent of mineral grain effects (e.g., Keil et al. 1998). A split-flow lateral transport thin separation cell (SPLITT) was used to isolate the clay-sized fraction (<3 μm) from bulk sediment (Keil et al. 1994). After isolation, density fractionation was used to separate clays from the small quantities of biogenic opal and quartz mineral present in the clay-sized sample. Fractionations were conducted using a density gradient of sodium metatungstate (Johnson Matthey) from 1.8 to 2.6 g cm^{-3} adjusted to a pH of 7.5 with HCl. The density gradient was formed by layering distilled water over the sodium metatungstate solution (2.6 g cm^{-3}) and allowing the gradient to form diffusively over a 18–36 h period. Samples were gently introduced to the top of the tube and then the tube was centrifuged (10 min at 5,000 × g). After centrifugation, the tubes were frozen in liquid nitrogen and thin slices were cut from the frozen block. The fraction richest in expandable clay (as determined by X-ray diffraction) was isolated from slightly above the bottom of the centrifuge tube (i.e., $\rho <$ 2.6). This material contained <10% identifiable quartz and opal and represented roughly 30–50% of the original sediment mass in the <3-μm fractions.

Clay minerals were identified and quantified by X-ray diffraction with a precision of ±5–10%, similar to that reported for semiquantitative analysis of other size fractions from the Washington coast (Keil et al. 1994). Biogenic silica was determined by solublizing opaline silica in Na_2CO_3 and determining concentrations of dissolved silica as described by DeMaster (1981). Carbonate was determined by difference between acidified and nonacidified samples on a Carlo Erba model 1106 analyzer. Mineral surface area was determined by nitrogen adsorption, using the one-point Brunauer, Emmett, and Teller (BET) method on a Quantachrome Monosorb surface area analyzer fitted with a 0.3 mole fraction N_2 in He gas (Mayer 1994). Samples were run in triplicate with a standard error of <4%.

Organic matter characterization—Average weight percentages of organic carbon and total nitrogen were determined in duplicate after acidification using a Carlo Erba model 1106 analyzer with a sample mean deviation of <4% of the measured value for all reported analyses (Hedges and Stern 1984). Nineteen hydrolyzable amino acids (15 primary amines and four nonprotein amino acids) were extracted and quantified using the charge-matched recovery standard technique of Cowie and Hedges (1992*a*) except that a Gilson model 231 autosampler was used and 2-μL glacial acetic acid was used to stop derivatization and stabilize the products prior to injection (Keil and Kirchman 1991). The nonprotein amino acids α-aminoadipic acid (acidic), γ-methylleucine (neutral), 1-methylhistidine (intermediate), and δ-hydroxylysine (basic) were used as charge-matched internal recovery standards. Proline was quantified for this study after derivatization to its isopropyl-N-trifluoroacetate analog and after separation by GC and detection by FID (Silfer et al. 1991).

Protein contents were determined after hot water (95°C) extraction for 45 minutes. While still hot, the sediment was separated from the water by filtration (Gelman GF/G) and then, after cooling, the Coomassie Blue protein assay was applied as described by Mayer et al. (1986). The protein RuBP carboxylase was used as a standard and expressed in units of mg AA gdw^{-1}. The Coomassie Blue assay does not respond to peptide sequences smaller than approximately ~10–15 amino acids in length (Mayer et al. 1986).

Isotope measurements—Clay isolates were acidified to remove inorganic carbon and then combusted in sealed glass ampoules. The stable carbon isotope composition of total organic matter was determined using a Finnigan MAT 251. Data are reported as $\delta^{13}C$ values versus the PDB carbon standard. Stable nitrogen isotopes were analyzed on a Nier-Johnson type double-focusing isotope ratio monitoring mass spectrometer after Dumas combustion in the presence of Cu and CuO to N_2 gas and are reported as $\delta^{15}N$ relative to air.

The $\delta^{13}C$ ratios of individual amino acids were measured

with a gas chromatograph–combustion system coupled to a Finnigan 252 isotope ratio mass spectrometer as described by Silfer et al. (1991). Using an HP Ultra-1 column (Hewlett Packard), we were able to measure nine amino acids: alanine (Ala), glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid + asparagine (Asx), glutamic acid + glutamine (Glx), and phenlyalanine (Phe). Positive identification of amino acids was achieved prior to isotope composition measurement by GC separation coupled with quadrupole mass spectrometry. Prior to chromatographic separation and isotope ratio measurement, the amino acids were converted to their isopropyl-N-TFA derivatives. This procedure adds five to eight carbon atoms to individual amino acids and requires an empirical correction be made to the data (Silfer et al. 1991). This correction was made by measuring the isotopic composition of amino acid standards with and without the isopropyl-N-TFA derivative. First, individual amino acid standards were combusted to CO_2 in glass ampoules and then analyzed for their carbon isotopic composition. Then the amino acid standards were mixed together and treated as samples (i.e., derivatized, separated, and analyzed in one batch on the GC/C/IRMS). The differences between static combustion isotopic compositions and GC/C/IRMS values were calculated each day and used to correct values obtained for derivatized samples. Owing to sample size limitations, samples were hydrolyzed and derivatized in duplicate (except the MSSD station, which was analyzed in triplicate, and the phytoplankton sample for which only one hydrolysate was analyzed) and then multiple injections (2–4) were made of the derivatized sample. Using this method, our analyses have an overall standard deviation of approximately 1.5 ± 0.9‰ (*see results*). Standard deviations were calculated for all amino acids after unit normalizing each replicate around the mean for that station and then comparing the deviation about the mean. The standard deviation reported here accounts for all the variance in sample and standard preparation during the weeks that the samples were run and should be viewed as a representative estimate of the true deviation. Standard deviations of <0.4‰ are associated with the derivatization and analysis portions of the procedure, respectively.

Modeling loss and replacement—The extent to which terrigenous amino acids are lost from the clay isolates or replaced with marine material was estimated using a simple two end member mixing equation that takes both the concentrations and isotopic compositions of the amino acids into consideration. Use of the equation implicitly assumes that there are no diagenetic effects that alter the isotopic composition of the amino acids other than loss and replacement (this assumption is clearly violated; *see later discussion*). It also assumes that the amino acid concentration and isotopic composition of the material exiting the river represents one end member (i.e., estuarine suspended particulate matter is used as the integrated terrigenous signal) and uses the averaged isotopic composition of each amino acid in the zooplankton and phytoplanton net tow samples as the marine source. Finally, it is assumed that there is no seasonality in the isotopic composition of the end members. The relationship between the concentration and isotopic composition of the sample and the isotopic composition of the source was computed as

$$F_{\text{T-lost}} = 1 - f_{\text{terr}} \times f_{\Delta}, \quad (1)$$

where $f_{\text{T-lost}}$ is the fraction of river-borne (terrigenous) amino acid lost, f_{terr} is the fraction of amino acid of terrigenous origin, and f_{Δ} is the fractional change in amino acid concentration normalized to mass (mgAA/mgdw) between each marine station and the riverine station. f_{terr} was determined from the standard isotopic mixing equation

$$f_{\text{terr}} = \frac{\delta^{13}C_s - \delta^{13}C_m}{\delta^{13}C_{\text{terr}} - \delta^{13}C_m} \quad (2)$$

where $\delta^{13}C_s$ is the stable carbon isotopic composition of the sample, $\delta^{13}C_m$ is that of the marine end member, and $\delta^{13}C_{\text{terr}}$ is that of the terrigenous end member.

For any given amino acid at any station other than the river station, there are multiple combinations of changes in amino acid concentrations and $\delta^{13}C$ isotopic compositions, each with distinct potential interpretations. (1) If there is no change in either the isotopic composition or quantity of amino acid, then there has been no loss of terrestrial material and no addition of marine material. (2) If the isotopic composition does not change but the quantity of amino acid decreases, then there has been loss of terrestrial material without replacement by marine material. (3) If the isotopic composition of the sample becomes more enriched and there is an increase in the concentration of the amino acid, then dilution of the terrigenous isotopic signal due to addition of marine material is dominating. (4) If the isotopic signals are more enriched than can be accounted for by simple addition of marine material, then loss of terrigenous material must occur as well. (5) Finally, if the calculation cannot be balanced, then either the sample contains an unaccounted end member or diagenetic reworking has changed the isotopic composition of the amino acid.

Results

Organic matter composition: sources—Bulk organic characteristics of source materials (woody debris, estuarine diatoms, phytoplankton and zooplankton, fecal matter) are typical of organisms from the region (Table 1; Prahl et al. 1992, 1994, 1997). Stable carbon isotope compositions of the bulk organic matter in the terrestrial and marine source materials differ by approximately 6.5‰, with the marine material being more enriched in ^{13}C. Nitrogen isotopic compositions are also variable. Although there is no strong ^{15}N signal to distinguish the terrigenous and marine sources, there may be a trophic level signal, as the autotrophs all have ^{15}N isotopic ratios that are between 3.4 and 5.7, whereas the zooplankton and fecal material have enriched isotopic values of 10.7 and 9.3, respectively (Table 1). Similar shifts along trophic levels have been observed in other marine systems (Fogel and Cifuentes 1993).

The amino acid compositions of the autotrophic end members resemble those of most autotrophic organisms and show little contribution of nonprotein amino acids (Table 2; Cowie and Hedges 1992*b*, 1994; Cowie et al. 1992). Variation in

Table 1. Mineralogical compositions of clay isolates (wt %), bulk organic matter content (wt %), and stable isotope compositions (‰) of sediment fractions and source materials (‰). SA = nitrogen-specific mineral surface area; OC : SA = organic carbon to mineral surface area ratio in units of mg OC m^{-2}; C/N_a = atomic ratio of organic carbon to total nitrogen.

Sample	%Clay	%Opal	SA	%OC	OC : SA	$C : N_a$	$\delta^{13}C$	$\delta^{15}N$
Terrestrial plant debris				41		47.8	−26.6	3.4
River diatoms				33		7.5	−26.3	5.7
Fecal matter				43		8.1	−21.4	10.7
Phytoplankton (<63 μm net)				36		7.2	−20.2	4.0
Zooplankton (>63 μm net)				40		6.6	−20.8	9.3
River	90	<5	68.2	3.08	0.45	10.0	−26.4	5.4
Plume	90	<5	63.4	3.48	0.55	9.4	−22.9	6.4
MSSD	85	<5	73.5	3.99	0.54	9.9	−23.6	6.5
Slope	90	<5	69.7	3.21	0.46	9.9	−22.1	7.7

mole percentages between zooplankton and phytoplankton were minimal and indicate no discernable change in bulk amino acid composition between autotrophs and heterotrophs.

The stable carbon isotopic compositions of the nine measured amino acids range from −5‰ to −25‰ for the marine sources and −10‰ to −30‰ for the terrigenous and riverine sources (Table 3, Fig. 1). These values are in good general agreement with other studies (Abelson and Hoering 1961; Macko et al. 1987; Fogel and Tuross 1999). The large range in stable carbon isotope compositions for different amino acids within a single sample (e.g., the 20‰ range in the marine phytoplankton sample) reflects, in part, differences in the biochemical pathways used to synthesize specific amino acids (Abelson and Hoering 1961; Macko and Estep 1984; Fogel and Cifuentes 1993). As with bulk organic carbon, the marine-derived amino acids are all isotopically enriched relative to the terrestrial or riverine values (Table 3, Fig. 1). The magnitude of the offset in $\delta^{13}C$ between terrigenous and marine amino acids is not uniform; the isotopic difference between terrigenous and marine for Ala is 14‰, whereas it is only 3‰ for Gly (Fig. 1).

Least-squares correlation of all the amino acids in the individual samples provides a means of evaluating similarity among samples. Although individual amino acids appear to be isotopically distinct between the two plankton samples (e.g., Gly, Leu, Phe; Table 3), the overall isotopic compositions of amino acids in the phytoplankton and zooplankton samples are identical within the ability to make the measurements; the correlation has a slope of 1 ± 0.2 and an intercept of 0 ± 1‰. The estuarine diatoms and hand-picked terrigenous debris are also similar in isotopic composition (Table 3). Only Glx and Leu are significantly different between these two samples (Table 3). It is fortuitous, but unclear, why these two samples should be so similar in composition. Although both plants synthesize amino acids using common pathways, we expected the estuarine diatom population to show isotopic shifts relative to higher plants due to growth within an aqueous system.

Amino acids from the fecal matter have a unique carbon isotopic composition. In addition to the bulk organic carbon isotopic composition (−21.4‰), only three amino acids (Gly, Pro, Glx) have values similar to the marine plankton samples (Fig. 1). Three amino acids (Val, Phe, and Leu) have isotopic compositions resembling the terrigenous end members, and three (Ala, Asx, and Ile) have values somewhat in-between the marine and terrigenous end members (Fig. 1).

Organic matter composition: clay isolates—All four clay isolates have organic matter contents between 3 and 4% organic carbon (OC) (Table 1). Normalized to mineral surface area, the carbon content of the isolates is 0.49 ± 0.04 mgC m^{-2}. These values are at the low end of the range (0.5–1.1 mgC m^{-2}) commonly measured for river suspended materials (Keil et al. 1998) and continental margin sediments (Keil et al. 1994; Mayer 1994). Bulk organic matter in the clay isolates have $\delta^{13}C$ values that range between −26.1 to −22.1‰ and generally become more enriched with increasing distance from the river mouth (Table 1). Clay isolate bulk $\delta^{13}C$ values are similar to those of the total unfractionated

Table 2. Concentration (AA; mg gdw^{-1}) and mole percent composition of individual amino acids hydrolyzed for all samples.

	AA	Asp	Glx	Ser	Gly	Thr	Ala	Tyr	Met	Val	Phe	Ile	Leu	Pro	His	Arg	Lys	βala	γaba	αaba
Terrestrial	49	12.5	10.7	4.7	10.1	5.8	10.4	2.7	3.3	7.3	2.7	5.8	8.8	4.5	2.4	6.9	5.5	0	0	0
River diatoms	294	10.5	11.1	5.7	11.8	5.2	9.7	2.8	2.2	5.9	4.3	5.3	8.1	4.8	2.1	4.3	9.3	0.04	0.15	0
Fecal matter	210	9.8	9.3	6.1	12.2	5.0	10.2	2.6	2.1	5.8	5	5.8	9.3	5.5	2.0	4.0	9.7	0.1	0.7	0.11
Phytoplankton	346	11	11.9	5.9	10.5	4.9	9.8	3	2.5	5.9	4.6	5.1	8.1	6.0	1.8	4.2	8.9	0.05	0.2	0.03
Zooplankton	355	10.2	10.9	5.5	12.4	5.1	9.8	2.7	2.1	5.9	4.1	5.5	7.8	5.2	2.2	4.1	9.9	0.06	0.4	0.1
River	14.6	11.9	12.94	6.3	11.4	3.81	12.9	2.1	1.7	4.8	3.6	4.5	4.3	4.8	3.7	4.9	3.6	4.34	0.12	2.78
Plume	18.6	11.7	9.05	6.1	8.2	3.18	10.1	4.9	0.5	9.0	3.5	5.9	7.0	4.9	1.9	5.1	3.0	6.91	0.57	1.64
MSSD	17.6	11.5	7.7	7.2	13.4	4.7	11.0	0.7	1.8	5.3	2.6	3.7	4.8	4.4	4.4	4.9	5.5	4	1.66	3.87
Slope	15.6	10.4	8.6	7.1	9.8	2.8	10.0	5.3	0.6	8.5	3.1	5.4	7.5	6.0	1.8	4.9	4.2	5.59	1.42	1.02

Table 3. Stable carbon isotopic composition and standard deviations of individual amino acids for all samples. OC is the isotopic composition of the bulk organic matter in the sample.

	Terrigenous debris		Estuarine diatoms		Columbia River SPM		Plume		MSSD		Slope		Fecal debris		Phytoplankton		Zooplankton	
	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std	$\delta^{13}C$	std
Gly	−8.7	1.1	−11.5	2.1	−16.1	1.1	−1.0	0.9	−14.4	1.9	5.8	0.8	−5.6	1.3	−5.4	1.0	−8.6	0.7
Ala	−23.5	1.7	−22.6	3.6	−22.0	2.6	−20.5	1.9	−13.6	2.7	−20.4	1.0	−19.9	1.7	−9.5	0.8	−6.1	1.3
Asx	−30.7	1.5	−27.1	1.4	−21.5	1.1	−18.4	2.1	−15.7	2.1	−18.2	1.6	−18.9	1.2	−11.3	0.5	−10.3	0.1
Ileu	−28.0	1.3	−29.5	2.7	−24.3	1.2	−24.8	3.6	−28.2	0.1	−22.3	2.4	−20.8	0.3	−15.0	0.9	−14.8	1.9
Pro	−29.8	0.9	−28.1	0.1	−26.4	1.3	−18.2	0.7	−13.3	3.4	−14.4	1.7	−15.1	1.8	−15.0	1.7	−13.8	0.9
Glx	−26.0	2.5	−19.5	2.0	−20.7	0.3	−18.4	3.5	−19.3	1.6	−23.6	1.2	−13.5	3.2	−16.1	1.1	−14.3	0.3
Val	−26.6	2.3	−28.7	1.2	−27.6	3.4	−22.1	0.5	−30.0	3.2	−20.1	2.2	−28.6	1.1	−16.3	0.1	−19.9	0.3
Phe	−26.4	1.7	−28.1	1.1	−29.0	0.5	−24.5	1.0	−25.6	3.4	−26.7	3.2	−25.7	0.7	−18.7	3.2	−24.4	0.4
Leu	−26.3	1.8	−30.6	1.1	−28.8	1.8	−26.1	0.4	−21.7	1.6	−25.0	2.1	−27.0	0.3	−24.9	1.8	−20.9	0.8
OC	−26.6	0.1	−26.3	0.1	−26.1	0.2	−23.3	0.2	−22.9	0.2	−22.1	0.2	−21.4	0.2	−20.2	0.1	−20.8	0.2

Fig. 1. Average (± std) stable carbon isotopic composition of individual amino acids from the two marine end members (net tow samples) and of the two terrigenous end members (terrestrial plant debris and estuarine diatoms). The marine fecal debris and Columbia river SPM samples are also plotted to illustrate similarities between the source materials and these two samples.

sediments (Keil et al. 1994). Nitrogen isotopes range from 5.4 to 7.7‰ and increase with distance from the river mouth (Table 1).

Total yields of hydrolyzable amino acids in the clay isolates range from 14.6 to 18.6 mg gdw^{-1} (Table 2), at the high end of the values commonly observed in modern coastal marine sediments (Henrichs 1987; Burdige and Martens 1988; Cowie and Hedges 1992*b*; Cowie et al. 1992; Whelan and Emeis 1992). Relatively high amino acid yields have also been observed in other clay-sized isolates (Tanoue and Handa 1979; Keil et al. 1998). The clay isolates contain roughly 18–22% of their organic carbon and 49% of their nitrogen in amino acids. Amino acid compositions are typical of these coastal marine sediments (Keil et al. 1998). Relative to source materials, the clay isolates are slightly depleted in concentrations of Glx, Thr, Met, Leu, and Lys and are enriched in the nonprotein amino acids β-alanine, γ-aminobutyric acid, and α-aminobutyric acid (Table 2). These distributions are common in many bulk marine sediments (Whelan and Emeis 1992) and are indicative of sediments that have undergone appreciable but not extreme diagenesis (Cowie and Hedges 1994; Dauwe et al. 1999).

Amino acids in the riverine SPM clay isolate are isotopically equivalent to a mixture of ~50% river diatom and ~50% terrigenous debris ($r = 0.81$; e.g., Fig. 1). When correlated against the average of the terrigenous end members, neither the slope nor the intercept of the correlation is significantly different from one and zero, respectively. The only exceptions to this generalization are Gly, which is isotopically depleted relative to the terrigenous sources, and Pro, which is isotopically enriched (Fig. 1).

The marine sediments (plume, MSSD, and slope) best correlate with each other (average $r = 0.83$) and have isotopic compositions that are between the average marine end member and the average terrigenous end member (Table 3; Fig.

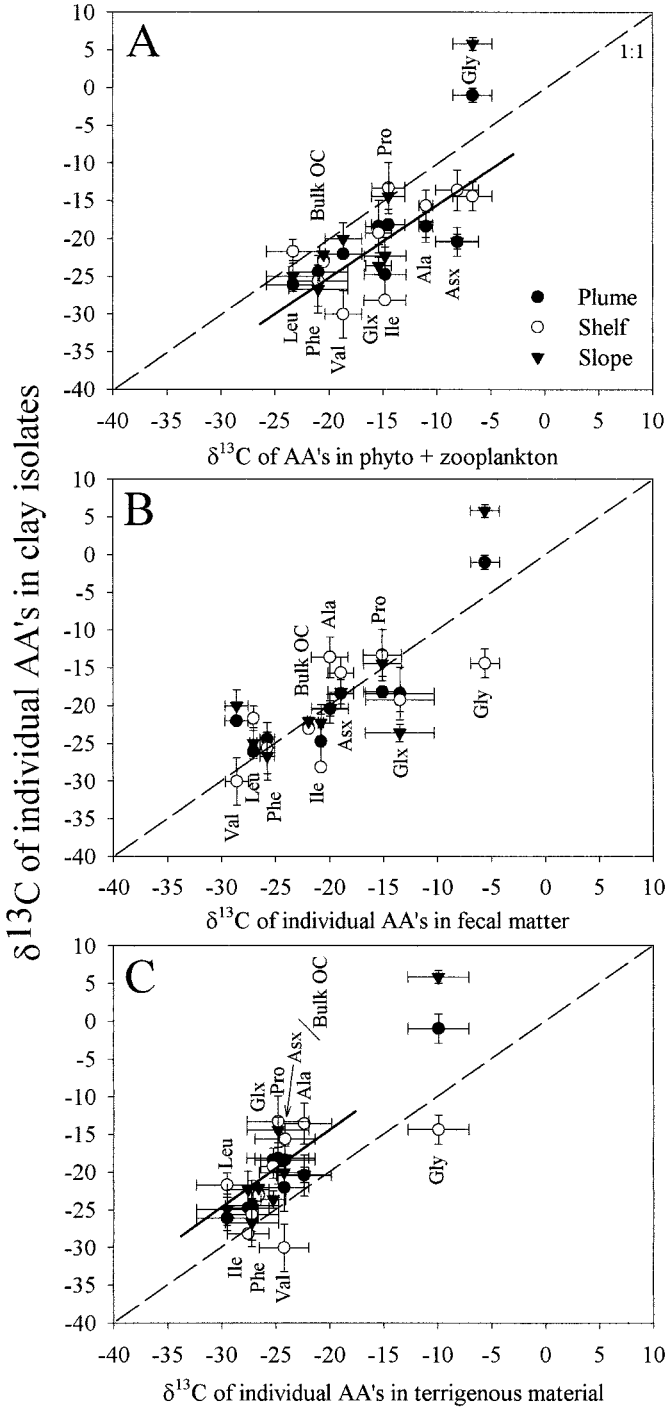

Fig. 2. Correlation between stable carbon isotopic compositions of clay isolate amino acids and potential source materials. (A) Relationship with marine plankton end members. The overall slope is 0.98 ± 0.2 and the intercept is −4.7 ± 2.8 (r = 0.71). (B) Correlation with fecal matter yields a slope of one and an intercept not significantly different from zero (r = 0.84). (C) Correlation with terrigenous end members yields a slope of 1.2 ± 0.2 and the intercept is +8.3 ± 3.8 (r = 0.82).

2). Relative to the marine plankton, the marine sediments are depleted in δ^{13}C by an average of 4.7 ± 2.8‰ (Fig. 2A). Other than self-correlation within the group of three marine sediment samples, these samples are most similar in composition to that of the fecal material (Fig. 2B). Only Glx is significantly different (depleted) in all three of the marine sediment samples relative to the fecal debris. Compared to the terrigenous material, the marine clay isolates are all isotopically enriched by an average of 6.3 ± 3.8‰ (Fig. 2C).

The observation that nearly all the amino acids in the marine sediment samples are isotopically between that of the terrigenous and marine end members suggests that their isotopic compositions may reflect mixing of the two sources. Comparison of the individual amino acids with the bulk organic matter can help evaluate this possibility. Bulk organic carbon isotopic compositions shift from a terrigenous to a marine composition (Fig. 3). Based on previous work (Hedg-

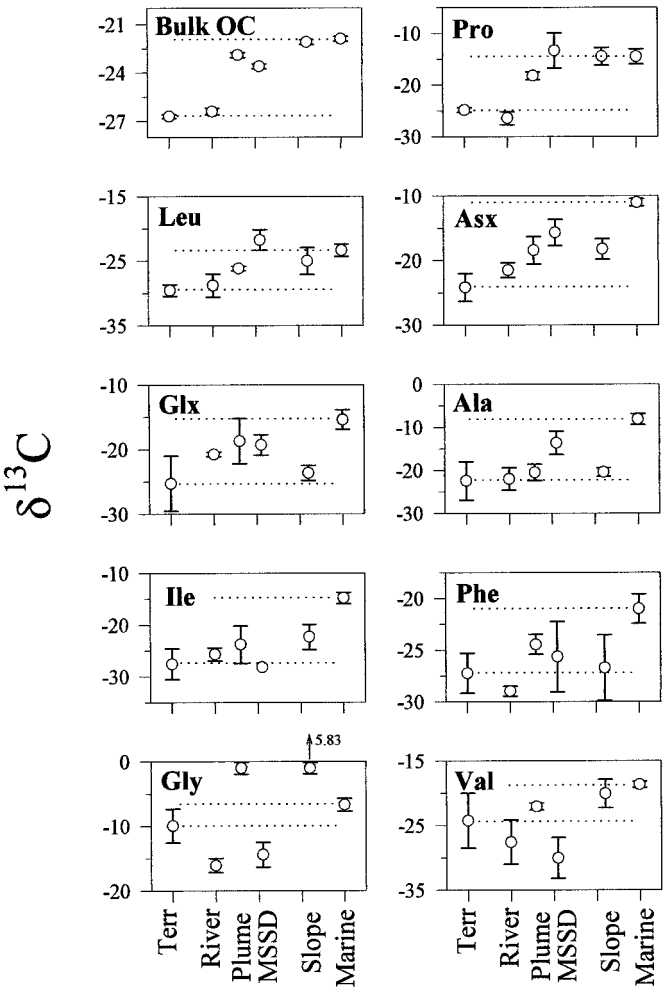

Fig. 3. Stable carbon isotopic composition of individual amino acids in the clay isolates as a function of sample location. Terr. and Marine denote the average composition of the terrigenous and marine end members, respectively (*see Fig. 1*). Average end member compositions are also marked with dashed lines. The relative spacing of the four clay isolates approximates their relative distance from the mouth of the Columbia river estuary.

Table 4. Results of the mixing model (*see Eq. 1*) for preservation of terrigenous amino acids in the three marine sediment samples. f_T lost = the fraction of original terrigenous material lost (missing) from the sample. std = one standard deviation. Dashes indicate that the sample data are not amenable to the model approach. Note that use of this equation assumes no reworking of amino acids in the sediment—*see text.*

	Plume		MSSD		Slope	
	f_T lost	std	f_T lost	std	f_T lost	std
Gly	—	—	—	—	—	—
Ala	0.18	0.22	0.62	0.33	0.14	0.15
Asx	0.39	0.21	0.55	0.45	0.43	0.15
Ileu	0.21	0.15	0.32	0.46	0.06	0.15
Pro	0.89	0.38	0.70	0.24	0.96	0.14
Glx	0.55	0.39	0.50	0.31	0.12	0.18
Val	0.33	0.45	—	—	0.58	0.29
Phe	0.51	0.28	0.33	0.15	0.14	0.27
Leu	0.52	0.45	1.12	0.28	1.01	0.58
OC	0.82	0.08	0.75	0.04	0.97	0.05

es and Mann 1979; Prahl et al. 1994), it is known that the MSSD station preferentially preserves lignin-rich woody debris and that the terrigenous contribution to total organic matter diminishes as a function of distance from the Columbia River. On average, 84 ± 9% of the bulk terrigenous organic matter is lost once it leaves the river mouth (avg. of OC data in Table 4). Individual amino acids behave differently from the bulk organic carbon (Fig. 3). The concentration of amino acids in the clay isolates rises slightly as particles are transported from the river to the shelf (Table 1) and the isotopic compositions sometimes change markedly. Pro and Leu have isotopic shifts similar to that of the bulk organic matter (Fig. 3) and ~75% of the terrigenous component of these amino acids is apparently lost (Table 4). Asx, Glx, Ala, Ile, and Phe have shifts in isotopic composition intermediate between the sources (Fig. 3). Less than 50% of the terrigenous source of these amino acids is apparently lost (Table 4). Gly and Val have isotopic compositions that are outside both boundaries of the measured end members. The isotopic composition of glycine in the clay isolates ranges from 0 to −15‰, with two measurements being more positive than the source materials and two values more negative. Of the four measurements of valine in the clay isolates, two are within the range defined by the sources and two values are more depleted (Table 3, Fig. 3).

Hot water extracted 81 ± 3% of the amino acids in the end members and 51 ± 3% of the total amino acids in the clay isolates (Table 5). Of the amino acids extracted from the end members, 85 ± 7% could be accounted for as protein detected by the Coomassie Blue assay. However, only 45 ± 4% of the amino acids extracted from the clay isolates responded to the protein assay (Table 5). The amino acid mole percent composition of the extracts and the whole samples were indistinguishable, and analyses of the extracted water for free amino acids (unhydrolyzed) yielded very low concentrations of free amino acids (data not shown).

Table 5. Amino acid and Coomassie protein content (mg gdw^{-1}) of clay isolates and water-extracted clay isolates. EE = extraction efficiency of amino acids using hot water; %Protein = the percent of extracted amino acids that respond to the protein assay.

Sample	AA Clay	AA H_2O extract	EE	Protein H_2O extract	% Protein
Terrestrial plant debris	49	40	82	32	79
River diatoms	294	241	82	227	94
Fecal matter	210	174	83	136	78
Phytoplankton (<63 μm)	346	259	75	215	83
Zooplankton (>63 μm)	355	295	83	277	94
Columbia estuary SPM	14.6	7.1	48	3.0	44
Plume	18.6	9.3	50	4.2	45
MSSD	17.6	9.7	55	3.8	40
Slope	15.6	7.9	51	4.1	51

Discussion

What happens to amino acids in sediments as sediments are delivered, transported, deposited, and degraded along continental margins? Are the dominant processes simply the mixing of marine material in with the terrigenous material and remineralization of both sources? Does microbial reworking of organic matter significantly influence amino acid isotopic signatures? For this discussion, we define microbial reworking as the synthesis of 'new' amino acids from other organic compounds during diagenesis and heterotrophic growth. This is a complication that does not routinely influence studies of sources and mixing because biomarkers are usually used to access sources and mixing (Hedges and Mann 1979). Along the Washington coast, specific terrigenous compounds (biomarkers) are transported and deposited in discernable patterns based on their hydrodynamic properties and their resistance to remineralization (Hedges and Mann 1979; Prahl 1985; Prahl et al. 1992). Since most biomarkers come from nonsedimentary sources, the impact of sedimentary diagenesis is only to lower their concentrations via degradation. However, for ubiquitous cellular components such as amino acids, a significant amount of the amino acids present in sediments could potentially be of bacterial origin. Is it possible to discern whether sedimentary amino acids have been significantly reworked (e.g., synthesized in situ) by microbial processes? Three lines of evidence suggest that microbial reworking of amino acids is an important component in amino acid cycling in sediments: the nonprotein amino acids found in sediments are indicative of diagenesis, shifts in the stable nitrogen isotopic composition are consistent with reworking, and the stable carbon isotopic composition of certain amino acids indicates resynthesis from other sources.

Bacteria produce nonprotein amino acids as a byproduct during degradation of protein amino acids and DNA. This leads to enrichment of nonprotein amino acids in sediments (Lee and Cronin 1982; Keil et al. 2000). All the clay isolates have nonprotein amino acid concentrations that are enriched by more than a factor of 20 over the source materials. This is indicative of diagenetic alteration of the material (Dauwe

et al. 1999). Stable nitrogen isotopes also become enriched in the clay isolates as distance from the river mouth increases (Table 1). Changes in $\delta^{15}N$ values are most likely due to increased importance of reprocessed material, as nitrogen isotopes are commonly enriched during degradation (Fogel and Cifuentes 1993). The marine fecal matter and zooplankton samples are both enriched in ^{15}N relative to the other samples, and the marine clay isolates are more enriched in ^{15}N than any of the autotrophic samples (Table 1). The fact that the sediments are not as enriched in ^{15}N as either the zooplankton or fecal material is consistent with the isotopic fractionations of nitrogen during microbial growth (Macko and Estep 1984; Hoch et al. 1996).

Our mixing equation can be used to both estimate whether preservation of terrigenous amino acids is occurring and to help gauge the potential importance of microbial resynthesis of amino acids from other source materials. If there is no resynthesis from other sources, then the amino acids found in the clay isolates can be proportioned into marine and terrigenous components. If synthesis of amino acids from other sources is a significant component of amino acid cycling in sediments, then it is expected that the amino acid isotopic compositions of certain amino acids might not fit within the constraints of the equation. Whereas samples that have isotopic values outside the range of the end members might clearly be ascribed to resynthesis, it is possible that resynthesis is also hidden within data that do fall within the boundaries of the mixing equation. After discussing the general results of our mixing calculations, we will then examine the importance of reworking.

Using the mixing model to estimate the percent terrigenous material lost from the clay isolates at each station, the bulk organic matter in the clay isolates at all the stations averages 84 $\pm$ 9% loss of terrigenous matter (Table 4). There is a statistically significant difference between the MSSD station and the other two stations that reflects changes in particle sorting (Prahl et al. 1992). Amino acids that show rapid loss of the terrigenous isotopic signal (similar to the pattern of the bulk organic matter) include Leu and Pro (Fig. 3, Table 3). Loss of river-delivered Leu is observed in all the stations and averages 70%. Replacement with significant quantities of marine-derived Leu occurs at the MSSD and slope stations. The carbon isotopic composition of Pro changes dramatically between the river and the MSSD stations, which suggests large losses (>70%) of the river-borne component. Since concentrations of Pro do not change markedly, there must be commensurate input of marine-derived Pro.

Amino acids that exhibit potential preservation of the terrigenous material include Glx, Asx, Ala, Ile, and Phe. Asx concentrations are higher at the marine stations than in the river, which indicates addition of marine-derived Asx. However, enrichment in the isotopic composition is insufficient given only addition of marine-derived Asx. At least 30–60% of the river-delivered Asx appears to have been lost (Fig. 3, Table 4). Isotopic changes for Glx cannot be explained by simple addition of marine material to the river-delivered Glx. Losses of terrigenous Glx are 20–55%. Except for the MSSD station, all changes in $\delta^{13}C$ for Ala can be explained by simple addition of marine material to the river-delivered Ala. At the MSSD station there must be significant loss (>60%) of the terrigenous Ala to balance the budget. Similarly, isotopic shifts for Ile can be explained by simple addition of marine material to the river-delivered Ile (Table 4; however, *see later discussion*). At the slope station, loss of up to 30% of the river-borne signal could occur. Phe compositions at the MSSD and slope stations can be explained by simple addition of a small amount of marine material, without loss of river-borne Phe. At the plume station as much as 60% of the terrestrially derived Phe could have been remineralized.

Only glycine and valine have isotopic compositions that cannot be successfully modeled using the mixing equation. As we will show, if reworking is a significant process affecting amino acids in sediments, these two amino acids are the most likely ones to exhibit significant variability in isotopic composition and are thus the most likely ones to pose significant difficulty when applying the mixing equation to the data. Thus, they are sensitive indicators of reworking.

Changes in the $\delta^{13}C$ composition of glycine for all four clay isolates are outside the range of the end members. The plume and slope samples are enriched, and the river and MSSD samples are depleted relative to either source (Fig. 3). Enrichment in the $\delta^{13}C$ of glycine could be achieved simply by synthesis from any other amino acid. Abelson and Hoering (1961) showed that the carboxyl carbon of amino acids is significantly enriched in ^{13}C relative to the other carbons within the molecule, often by as much as 10–20‰. The potential for resynthesis of glycine from other amino acids to result in highly enriched $\delta^{13}C$ values is highlighted by the fact that glycine is already enriched in $\delta^{13}C$ relative to all the other amino acids because it has only two carbons, whereas the others have three or more (Table 1; Fig. 1). Conversely, depletion in the $\delta^{13}C$ of glycine could be achieved through resynthesis if the source organic compounds were isotopically depleted relative to amino acids. Since many cellular components are isotopically depleted in ^{13}C relative to amino acids (Degens 1969), synthesis of glycine from other classes of organic compounds might lead to depleted isotopic values of glycine. Given that glycine is an intermediate in the fermentation of many organic compounds and can be easily shunted to anabolic pathways, it seems likely that the isotopic composition of glycine is influenced by its synthesis from other compounds. Similarly, consumption of glycine during biosynthesis of other compounds could lead to enrichment in the isotopic composition of the glycine that remains unused.

The variability we observe in the isotopic value of glycine suggests that its isotopic composition is sensitive to the concentrations and availability of amino acid and other types of organic matter during diagenesis. It is interesting to note that the two samples with depleted $\delta^{13}C$-glycine (river SPM and the MSSD) contain the highest relative abundances of terrigenous materials, whereas the sediments enriched in ^{13}C-glycine contain the largest relative abundances of marine-derived material (Fig. 3; *see also* Keil et al. 1998). Plankton are amino acid–rich relative to terrigenous plants, and terrigenous material is carbohydrate-rich relative to plankton (Cowie and Hedges 1992*b*; Cowie et al. 1992). We hypothesize that the enriched ^{13}C-glycine values found in the sediments containing the largest relative inputs of planktonic

materials reflect a greater degree of reworking of planktonic amino acids, whereas in the estuarine and MSSD samples, resynthesis of glycine is strongly influenced by reworking of a carbohydrate component.

The isotopic composition of valine in the river and MSSD samples is depleted relative to the sources (Fig. 3). This is consistent with de novo synthesis of this amino acid by bacteria. Plants and bacteria use different biosynthetic pathways to synthesize valine (Rawn 1989). Acetohydroxy acid synthetase, one of the bacterial enzymes responsible for synthesis of valine, fractionates to a greater degree than the enzyme used by plants, resulting in isotopic compositions of bacterially produced valine that are ^{13}C-depleted relative to the valine synthesized by plants. Thus, microbial synthesis of valine during early diagenesis should result in depleted ^{13}C-valine compositions relative to source materials. Our observations that the river and MMSD stations show the greatest depletions are consistent with the hypothesis that in these two samples, resynthesis of amino acids from carbohydrate-rich (terrigenous) materials dominates. The intermediate ^{13}C-valine values observed for the plume and slope sediment samples may reflect resynthesis of valine from other amino acids rather than simple mixing of marine and terrigenous source materials.

The idea that valine represents a sensitive marker for microbial reworking of source materials can be extended to include isoleucine. The synthesis of isoleucine in bacteria also involves use of acetohydroxy acid synthetase, and thus bacterially derived isoleucine is isotopically distinct from plant-derived isoleucine. Although our $\delta^{13}C$ values for isoleucine fall within those of the end members, we observe the same general pattern of depleted values in the river SPM and MSSD samples, and less depleted values in the plume and slope sediments. Thus, glycine, valine, and isoleucine isotopic compositions are all consistent with significant reworking of original organic materials. This conclusion is also consistent with the work of Fogel and Tuross (1999). They incubated different plant materials in marsh sediments and observed alteration in the stable carbon isotopic composition of several amino acids (including Gly, Val, and Ile) during diagenesis. Their results, as well as ours, suggest that heterotrophic reworking is a significant source of variability in amino acid isotopic compositions.

Is microbial reworking the dominant factor controlling $\delta^{13}C$ compositions of amino acids in our system? Macko and Estep (1984) and Macko et al. (1987) showed that when heterotrophic bacteria synthesize amino acids during growth on simple substrates, there is a fractionation of approximately −5‰ associated with growth. That is, bacterially produced amino acids are systematically depleted in ^{13}C by 5‰ relative to the substrate. Our data (Fig. 2) are consistent with this fractionation dominating the isotopic composition of the clay isolates. We see a systematic offset between the clay isolates and marine material of 4.7‰ (Fig. 2A). This brings up the possibility that the terrigenous amino acids are effectively remineralized and that the isotopic compositions we measure on the clay isolates generally represent complete reworking of marine material.

Different proteins from the same source can have amino acids with distinct isotopic compositions (Fogel and Tuross 1999). If different proteins have different fates during transport and deposition, then it is possible that selective diagenesis and reworking might account for some of the observed individuality in amino acid isotopic compositions. When we extracted the clay isolates with hot water and measured protein concentrations, we observed that only half the hydrolyzable amino acids responded to the assay. Since the Coomassie Blue assay is insensitive to small oligopeptides (Mayer et al. 1986), as much as half the amino acids present in the clay isolates could be present as low molecular weight peptides. The presence of low molecular weight peptides as a dominant component of the sedimentary amino acid pool has been observed in other studies (Mayer et al. 1986) and is consistent with immunological studies of degraded marsh plants (Fogel and Tuross 1999). If amino acids are being used as substrate by a marine microbial community, small peptides might be preferentially retained in the clay fraction depending on peptide sequence and solubility (Henrichs 1995) and these peptides could exhibit a carbon isotopic composition unique to the source protein or organism.

Regardless of which factors (loss and replacement, degradation or reworking) force changes in the stable carbon isotopic composition of the sediment-associated amino acids, it appears that changes are effected prior to burial of the organic matter and that the changes are imparted by both macrofauna and bacteria. The amino acid isotopic compositions of the three marine samples correlate best with the isotopic compositions of the fecal matter (Fig. 2). This suggests that the isotopic variations were introduced to the samples during the early stages of processing by heterotrophic communities (either benthic or planktonic). That is, diagenetic processing of organic matter while the material is being influenced by macrofauna might drive some of the observed changes in the stable carbon isotopic composition of the amino acids. Both clay minerals and organic matter reach marine sediments largely via packaging by zooplankton into materials such as fecal pellets, and within the upper layers of sediments macrofauna ingest large quantities of sedimentary material. Interactions between the clay and the organic matter while they are together, either while pellets fall through the water or while materials are in the gut of an organism, might be an important place where the clay gets its amino acid load and amino acid isotopic signature. Since we observe that the pellet and the clay have very similar amino acid isotopic compositions and that these compositions are very different from either the phytoplankton or the terrigenous material, we thus hypothesize that the reason the clay-bound amino acids have this unique isotopic composition is because they obtained it while they were being processed by macroheterotrophs. However, bacteria must also play a large role in altering the isotopic composition of the amino acids. Since valine is an essential amino acid to all eukaryotic heterotrophs (e.g., zooplankton and benthic macrofauna), it seems likely that it is microbial activity that alters the isotopic composition of valine and perhaps the other amino acids. Since all the marine sediments show signs of extensive reworking, we propose that many of the amino acids preserved in marine sediments may be derived from local bacterial populations.

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