Linking diagenetic alteration of amino acids and bulk organic matter reactivity

Abstract—Examination of amino acids in particulate samples from a variety of marine environments (fresh phytoplankton to deep-sea sediments) revealed systematic compositional changes upon progressive degradation. These consistent trends have been used to derive a quantitative degradation index (DI) that is directly related to the reactivity of the organic material, as indicated by its lability to enzymatic decay and its first-order degradation rate constant. This direct link between molecular composition and degradation rate allows us to quantify the quality of organic matter based solely on its chemical composition.

Decomposition of particulate organic matter (POM) is responsible for oxygen consumption in the ocean and its sediments, for the recycling of essential nutrients, and for most early diagenetic processes. The heterogeneous composition of POM leads to selective preservation of more stable (or less available) molecular compounds and to the loss of labile compounds, resulting in a continuously altered biochemical composition of the material during diagenesis (Tegelaar et al. 1989; Cowie and Hedges 1994; Wakeham et al. 1997). These compositional changes in POM are probably the reason for a decreasing first-order degradation rate (Middelburg 1989) and a reduced nutritional value toward heterotrophic consumers (Tenore et al. 1984) as degradation proceeds. Although intrinsic differences in molecular structure (de Leeuw and Largeau 1993) and differences in physicochemical association with the sediment matrix (Keil et al. 1994; Mayer 1994) are documented factors acting on early diagenesis of POM, there are few studies that directly link the resulting shifts in biochemical composition to the degradation state (Cowie and Hedges 1994; Wakeham et al. 1997; Dauwe and Middelburg 1998). Moreover, compositional characteristics of organic matter have not yet been linked to its biological availability or its degradation dynamics.

A series of molecular diagenetic maturity indicators have been used to estimate the relative degradation state of the organic matter (Cowie and Hedges 1994; Wakeham et al. 1997), varying from short-term (e.g., chlorophyll) to longer term (e.g., nonprotein amino acids) indicators. Broadly applicable degradation state indicators should be based on major components that are widely distributed geographically and that are omnipresent in organisms so that variability in sources of organic matter is minimized. Moreover, they should ideally be sensitive to all stages of alteration. Proteins are ubiquitous components of all source organisms and degradation mixtures (Cowie and Hedges 1992). Although there is some dissimilarity in amino acid composition of the ultimate source organisms (e.g., diatoms, coccolithophorids, and bacteria) (Cowie and Hedges 1992), these differences are minor compared to the alteration of the spectra upon degradation (Dauwe and Middelburg 1998).
Table 1. Data matrix of the protein amino acid-based DI. (1–6) Source materials (Source): 1 microalgae (Brown 1991), 2 phytoplankton (Cowie and Hedges 1992), 3 bacteria (Cowie and Hedges 1992), 4 zooplankton (Cowie and Hedges 1992), and 5 and 6 sediment trap (T) material from Saanich Inlet (Saan) and Dabob Bay (Dab) (Cowie and Hedges 1992; Cowie et al. 1992). (7–17) Coastal sediments (Coastal): 7–13 North Sea (Dauwe and Middelburg 1998): SK, Skagerrak; GB1, GB2 = German Bight 1 and 2; FF, Frisian Front; BF, Broad Fourteens; BG-A, BG-B, Brouwershaven-gat A and B; 14, 15 surface sediment (S1); and 16, 17 subsurface sediment (S2) of Saanich Inlet (Cowie et al. 1992) and Dabob Bay (Cowie and Hedges 1992). (18–20) Eastern Mediterranean samples (34°52′30″N, 21°07′08″W, 2,539-m water depth): 18, 19 hemipelagic sediments (Med-1 and Med-2), and 20 sapropel (Sap), (21–28) Turbidite samples. Depth profile of the MAP turbidite in core 86P25 (30°44′22″N, 25°22′45″W, 5,400-m water depth) (de Lange et al. 1994): 21–24: Tur-ox, oxidized section, and 25–28: Tur-red, reduced section. The DI for samples outside our data set can be calculated on the basis of their protein amino acid spectra, and the factor coefficients based on the first axis of the PCA, listed according to the formula

$$\text{DI} = \sum_{i} \left[ \frac{\text{var}_i - \text{AVG var}_i}{\text{STD var}_i} \right] \cdot \text{fac coef}_i,$$

where var$_i$ is the original (nonstandardized) mole percentage of amino acid $i$, AVG var, and STD var, are its mean and standard deviation in our data set, and fac coef$_i$, the factor coefficient for amino acid $i$.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Depth interval (cm)</th>
<th>DI</th>
<th>Amino acid</th>
<th>Factor coefficient (first axis)</th>
<th>AVG</th>
<th>STD</th>
<th>Factor coefficient (second axis)</th>
<th>Factor coefficient (third axis)</th>
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<tbody>
<tr>
<td>1</td>
<td>Phy-B</td>
<td>—</td>
<td>1.10</td>
<td>THR</td>
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<td>7.1</td>
<td>1.5</td>
<td>0.060</td>
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<td>ARG</td>
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<td>2.3</td>
<td>−0.206</td>
<td>0.059</td>
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<td>1.27</td>
<td>ASP</td>
<td>−0.102</td>
<td>13.4</td>
<td>2.7</td>
<td>−0.046</td>
<td>−0.247</td>
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<td>4</td>
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<td>GLY</td>
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<tr>
<td>5</td>
<td>Saan-T</td>
<td>—</td>
<td>1.25</td>
<td>VAL</td>
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<td>7.6</td>
<td>1.1</td>
<td>−0.219</td>
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<td>6</td>
<td>Dab-T</td>
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<td>11.8</td>
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<td>−0.148</td>
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Recently, the molecular composition of protein amino acids was used to derive a quantitative DI (Dauwe and Middelburg 1998). Here, we significantly extend this approach to a wider range of diagenetic conditions and present a more balanced data set, including older and calcareous sediments. The revised DI will be linked to the fraction of chemically hydrolyzable amino acids that is enzymatically available within 6 h and the first-order rate constant for mineralization of bulk organic carbon.

Materials—We have examined the protein amino acid composition (based on the 14 most common protein amino acids; Table 1) of marine particulate matter samples across a wide range of degradation states. The samples were comprised of unaltered source organisms (phytoplankton, bacteria, and zooplankton); partly degraded sediment-trap material from Dabob Bay (Cowie and Hedges 1992) and Saanich Inlet (Cowie et al. 1992); progressively degraded coastal sedimentary organic matter from the North Sea (Dau-
we and Middelburg 1998), Dabob Bay (Cowie and Hedges 1992), and Saanich Inlet (Cowie et al. 1992); and extensively degraded organic matter from hemipelagic and sapropelic sediments in the Eastern Mediterranean (de Lange et al. 1994). Eastern Mediterranean deep-sea sediments consist of interbedded organic carbon-poor and organic carbon-rich deposits. Sapropels, layers with >2 wt % of organic carbon, are formed during periods of enhanced carbon fluxes to the seafloor and/or increased preservation due to bottom-water oxygen depletion (Rossignol-Strick et al. 1982). We have also included a depth profile through the oxidized and reduced sections of an abyssal turbidite from the Madeira Abyssal Plain (MAP), eastern Atlantic (Prahl et al. 1989; de Lange et al. 1994). Turbidites are deposits originating from catastrophic deposition events that transport large masses of sediments from the continental slopes down to deep abyssal plains. A remarkable feature of the distal MAP turbidites is their homogeneity upon emplacement (Prahl et al. 1989). Postdepositional exposure to oxygen (and nitrate) under pelagic conditions for about 10–20 kyr caused carbon removal in the uppermost section due to a downward-progressing oxidation front (Wilson et al. 1986). The lowermost section of the initially homogeneous sediments has survived postoxic conditions without significant degradation for about 140 kyr (Weaver and Kuijpers 1983; Cowie et al. 1995; Prahl et al. 1997).

Analytical methods—Amino acid and hexosamine concentrations were determined on freeze-dried sediment after hydrolysis by reverse-phase high-pressure liquid chromatography (HPLC), as described by Dauwe and Middelburg (1998) or as taken from the literature. Literature data are also based on reverse-phase HPLC and precolumn derivatization with o-phthalaldehyde (OPA) (Lindroth and Mopper 1998) or as taken from the literature. Literature data are also based on reverse-phase HPLC and precolumn derivatization with o-phthalaldehyde (OPA) (Lindroth and Mopper 1979), and mole percentage values have been recalculated to a common set of amino acids.

Enzymatically hydrolyzable amino acids (EHAA) and total hydrolyzable amino acids (THAA) were determined following the method introduced by Mayer et al. (1995), which has been described in detail by Dauwe et al. (1999). Briefly, sediment-buffer slurries with added bacterial inhibitor (0.1 M sodium arsenate and 0.1 mM pentachlorophenol in a pH 8 sodium phosphate buffer) and a nonspecific proteolytic enzyme (“Proteinase-k” by Sigma No. P8044) were incubated for 6 h. Trichloroacetic acid was added to stop the reaction and to precipitate the low-molecular-weight (LMW) bioavailable fraction. The LMW fraction and freeze-dried sediment were hydrolyzed, and the amino acid concentration was determined by fluorimetry following derivatization with OPA. The fraction EHAA:THAA will be used as a measure of bioavailability of the peptide pool. The reactivity of the organic material was calculated as a first-order decomposition rate constant (k yr$^{-1}$) (Hargrave and Phillips 1981) as $k = -(1/t_i)\ln(C_i/C_o)$ where k the first-order degradation constant, $t_i$ is the incubation period, $C_i$ is the organic carbon concentration at the beginning of the time course, and $C_o$ is the organic carbon concentration at time $t_i$ ($C_o - \Sigma CO_2$). The production of inorganic carbon was measured using sediment–water slurries during 3-week (North Sea samples) and 11-week (Eastern Mediterranean samples) incubations in the dark as described by Dauwe (1999).

Organic carbon was determined on freeze-dried samples that had been finely powdered and homogenized. A 20–50-mg split was combusted at 1,010°C in a Carlo Erba Elemental Analyzer NA-1500 after removal of carbonate by in situ acidification with 25% HCl within silver sample cups (Nieuwenhuize et al. 1994). The 0–15-cm depth-integrated values reported in Table 1 were derived from the analysis of multiple depth intervals within the sediment as described by Dauwe (1999) for the determination of the first-order degradation rate constant, by Dauwe et al. (1999) for the determination of %EHAA:THAA, and by Dauwe and Middelburg (1998) for the amino acid concentrations. The other samples have been analyzed only at the depth intervals indicated in Table 1.

Results and discussion—The protein amino acid spectra of these 28 samples were used in a variance-oriented method (Principal Component Analysis = PCA) to derive the principal components, which have the property that the maximum variance is found along the first axis, the maximum of the remainder along the second axis, etc. Factor coefficients (also known as unrotated loadings) give the relation between the PCA axis and the original variables (mole percentage of protein amino acids), while factor scores quantify relative positions of samples along the PCA axis.

The first axis of the PCA explains 36% of the total variation and has positive coefficients for tyrosine, leucine, histidine, isoleucine, phenylalanine, and methionine and negative coefficients for threonine, arginine, aspartic acid, and glycine (Table 1). The second axis of the PCA explains another 24% of the total variation and has positive coefficients for glycine and serine and negative coefficients for valine, arginine, alanine, isoleucine, leucine, and phenylalanine (Table 1). The third axis explains another 14% of the total variation, has positive loadings for valine, phenylalanine, isoleucine, glycine, and serine, and has negative loadings for glutamic acid, aspartic acid, alanine, and histidine (Table 1).

The behavior of most amino acids found in this comprehensive analysis agrees well with results of a PCA on a more limited data set, dominated by coastal sediments (Dauwe and Middelburg 1998). Because the first axis is interpreted to reflect organic matter degradation, scores on this axis can be considered as degradation state indicators (Fig. 1). The amino acids leucine, isoleucine, and phenylalanine decrease over the entire range of the DI (Fig. 1A–C), while the amino acids tyrosine, histidine, and methionine decrease rapidly during the initial stages of alteration (Fig. 1D–F).

The preferential accumulation of glycine and threonine is probably due to their concentration in cell walls (Hecky et al. 1973) that are preserved during sinking and decomposition (Siezen and Mague 1978; Lee and Cronin 1984; Müller et al. 1986), whereas amino acids that are concentrated in cell plasma (tyrosine, phenylalanine, and glutamic acid) (Hecky et al. 1973) tend to be depleted during degradation. Sediment-trap studies sampling POM at increasing depth in the water column confirm these compositional changes of amino acids during decay (Lee and Cronin 1984; Cowie and Hedges 1992; Cowie et al. 1992).
The amino acid-based DI varies from −2.2 to 1.5 (Fig. 1), with sources such as phytoplankton, bacteria, and sediment-trap matter having DIs between 1 and 1.5, which are higher than those of POM in coastal and ocean margin sediments (−0.3 to 1) and of refractory POM from pelagic deep-sea sediments (≤−1). Moreover, subsurface layers are generally more degraded than surface samples (Table 1), except for the Eastern Mediterranean sapropel that appears less degraded, perhaps due to bottom-water oxygen depletion (Rossignol-Strick et al. 1982). Coastal sediments are important sites of POM burial and degradation (Middelburg et al. 1997). The North Sea samples vary from labile (+1) to rather refractory (−0.3) due to extensive pre depositional degradation during repetitive deposition–erosion cycles (Dauwe and Middelburg 1998).

The DI also tracks the extent of degradation of the MAP turbidite as a result of post depositional exposure to bottom-water oxygen and nitrate (Fig. 2). The upper oxidized layer is depleted in organic carbon (0.2–0.4 wt % total organic carbon [TOC]) and has a lower DI (−2.2 to −1.4) than the reduced layer (1.1 wt % TOC with a DI = 0 to −0.3). The extent of degradation of the unoxidized layer is comparable to that of refractory North Sea sediments (−0.1 to −0.3), confirming the often-made assumption that this section has not been altered significantly since its export from the continental margin (Cowie et al. 1995; Prahl et al. 1997). Oxidation of the upper section has occurred in the absence of benthic animals, except for the top sample, which has the lowest index (−2.2), possibly due to mixing with more refractory pelagic material or stimulation of degradation by the activity of fauna (Kristensen and Blackburn 1987).

The correlation of the DI with the mole percentage of the nonprotein amino acids, γ-aminobutyric acid (GABA) and β-alanine (BALA) (Fig. 3A), and the hexosamines, glucosamine and galactosamine (Fig. 3B), demonstrates the consistency of the DI with well-established indicators of diageneric alteration. The microbial production of GABA and BALA from their protein precursors and their subsequent preservation in sedimentary deposits has been proposed as a degradation state indicator of marine sediments (Lee and Cronin 1984; Cowie and Hedges 1994). Similarly, hexosamines are generally incorporated into structural biopolymer matrices such as bacterial cell walls and chitinous material (de Leeuw and Largeau 1993) and are enriched in more refractory materials (Dauwe and Middelburg 1998). These
The DI can also be used to quantitatively link amino acid-based DI to other proxies for the alteration of organic matter. This biomimetic, kinetics-based approach (Mayer et al. 1995) is most sensitive during the initial to intermediate stages of alterations (DI > 0.5), similar to histidine, methionine and tyrosine (Fig. 1D–F). About 10% of the amino acids is always available from the pore waters or through desorption and does not require proteolytic enzymes (Dauwe et al. 1999). This relationship between amino acid composition characteristics and biologically available indicates that alteration results in compositional changes that lower the availability of amino acids to enzymes and hence, the nutritional value of the associated organic matter to organisms.

This applicability to a wide range of degradation states allows us to link the DI to the first-order rate constant (k) for POM degradation (Middelburg 1989). The logarithm of measured and model-based literature-derived first-order degradation rate constants for TOC is linearly correlated (r² = 0.47, n = 15, P < 0.005) with the DI based on protein amino acids (Fig. 3D). This is the first direct relationship between organic matter degradation state, amino acid compositional characteristics, and bulk organic carbon degradation kinetics. It appears that though they contribute only about 10–30% to the total carbon pool in sediments (i.e., Dauwe and Middelburg 1998), amino acids provide representative information on the bulk organic matter degradation state. Moreover, the logarithm of the first-order degradation rate constant is correlated linearly with the logarithm of degradation time (Middelburg 1989). Hence, through coupling of the DI–k relation and the power model (Middelburg 1989) time–k relation (Fig. 3D), there is in principle a coupling of degradation time with amino acid composition and amino acid compositional changes. Application of these relations to other sediments should make it possible to determine the lability, degradation history, and preservation potential of the associated organic matter. This tool will improve our capabilities of predicting the effect of diagenesis on carbon preservation and the formation of fossil fuel sources.

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Notes

nonprotein amino acids and hexosamines are sensitive indicators for intermediate to extensively degraded materials (DI < 0.5).

The results presented here confirm that the protein amino acid-based DI covers a very wide range of alteration stages, is uncompromised by source variations, and is robust with regard to differences in analytical methods (Fig. 2). It provides a solid frame to determine the relative reactivity, and hence applicability, of different biomarkers under various natural conditions, which is a prerequisite for reliable reconstruction of paleoenvironmental processes and environments. The DI can also be used to quantitatively link amino acid compositional characteristics of organic matter with dynamics of organic matter degradation and biological availability of organic matter.

The DI is correlated with the fraction of hydrolyzable amino acids (%EHAA : THAA) that is released after incubation with a proteolytic enzyme for 6 h (Fig. 3C). This biomimetic, kinetics-based approach (Mayer et al. 1995) is most sensitive during the initial to intermediate stages of alterations (DI > 0.5), similar to histidine, methionine and tyrosine (Fig. 1D–F). About 10% of the amino acids is always available from the pore waters or through desorption and does not require proteolytic enzymes (Dauwe et al. 1999). This relationship between amino acid composition characteristics and biologically available indicates that alteration results in compositional changes that lower the availability of amino acids to enzymes and hence, the nutritional value of the associated organic matter to organisms.

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