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Preservation of amino acids from in situ–produced bacterial cell wall peptidoglycans in northeastern Atlantic continental margin sediments

Abstract—In this study we present the results of total hydrolysable amino acids (THAA) and amino acid D/L-enantiomers in northeastern Atlantic continental margin sediments. There is increasing evidence that intrinsically labile amino acids are present in old marine sediments as part of a refractory network of peptide-like material. We used amino acid enantiomers to identify the contribution of amino acids from bacterial cell walls to THAA in organic matter ranging from relatively young to 18,000 yr old. The ratio of D/L-amino acids increased with depth in the sediment mixed layer. Application of a transport-racemization-degradation model excludes a significant production of D-amino acids by racemization and implies in situ bacterial production as the main source. Amino acids associated with a refractory pool of bacterial cell walls could account for approximately one third of the THAA deeper in the sediments. We propose that in situ bacterial production and the primary flux of labile organic matter from the water column result in a small but highly reactive pool of amino acids in the surface mixed sediment only, whereas amino acids associated with refractory cell walls persist in marine sediments.

The contribution of amino acid-nitrogen to total nitrogen (%AA-N) as well as the spectrum of individual amino acids have been used as indicators of organic matter (OM) reactivity in marine sediments (Cowie and Hedges 1992; Grutters et al. 2001). Preferential enzymatic degradation of amino acids relative to bulk OM (Harvey et al. 1995) causes the %AA-N to decline from 75% to 90% in fresh plankton to 40% to 50% in OM in sinking aggregates and further to 10%–30% in sedimentary OM (Lee 1988). However, the sorption of OM to mineral surfaces is assumed to result in the preservation of these intrinsically labile compounds (Keil et al. 1994). Amino acids are known to be a major constituent of peptidoglycans, the main structural components of bacterial cell walls (Schleifer and Kandler 1972). It has been reported that peptidoglycans contribute significantly to dissolved OM in the deep ocean (McCarthy et al. 1998). In addition, Parkes et al. (1993) proposed that with increasing depth in the sediments up to 16% of the total organic carbon (TOC) that cannot be accounted for by amino acids and carbohydrates (uncharacterized TOC) comes from dead bacterial biomass. They suggested that this bacterial ''necromass'' is relatively recalcitrant and perhaps contributes to OM preservation. Therefore, we hypothesize that the in situ production of bacterial cell walls contributes to the preservation of amino acids in marine sediments. In addition, benthic production of peptidoglycan may be a source to suspended particulate organic matter in the lower water column of the ocean (Bauer and Druffel 1998).

Materials and methods—Particulate matter was collected from sediment traps (Antia et al. 1999), situated 400 m above bottom, at water depths of 1,445 and 3,650 m across the Goban Spur northeastern Atlantic continental slope (49°24.89′N, 11°31.42′W to 49°05.30′N, 13°26.18′W). Sediment samples were taken from multicores and piston cores (representing ages of 7, 10, 18 kyr) at water depths of 651, 1,296, and 3,650 m (Lohse et al. 1998). Sediment trap samples (pooled averages from individual cups covering a time span of 14 months) as well as sediment samples were analyzed for total hydrolysable amino acids (THAA) and D/L enantiomers of aspartic acid, glutamic acid, serine, and alanine, the major peptidoglycan amino acids.

THAA were measured by reverse-phase high-performance liquid chromatography (HPLC) analysis, after liquid-phase hydrolysis (110°C for 24 h) and precolumn derivation with *o*-phtaldialdehyde (OPA) (Grutters et al. 2001). Amino acid enantiomers were analyzed by HPLC after vapor-phase hydrolysis at 150°C for 3 h and precolumn derivation with OPA/*N*-acetyl-L-cysteine and ultraviolet-fluorescence detection (Glavin et al. 1999). Prior to derivatization, hydrolysates were desalted by use of cation exchange resin (AG50W-X8, Bio-Rad) and the desalted extracts were stored in borate buffer (pH 9.4). Concentrations of the THAA and enantiomers were calculated by comparison of the amino acid peak areas to those of a standard run in parallel. Very low levels of amino acids were detected in a blank carried through the same processing procedure as the sediment samples and were subtracted from the measured peak areas of the samples. The precision of the THAA analysis, expressed as the

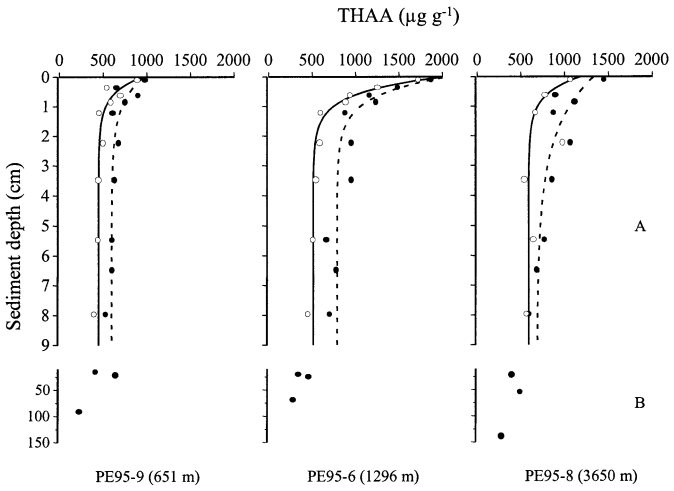

Fig. 1. THAA concentration profiles (μg g^{-1}) (solid circles) and profiles corrected for bacterial cell wall amino acids (AA_{CW}, open circles) in the (A) sediment mixed layer and (B) piston cores at 651-, 1,296-, and 3,650-m water depth across the Goban Spur continental slope. Piston core samples represented ages of 7, 10, and 18 kyr, determined from foraminiferal stratigraphy. THAA concentration profiles and the remaining profiles after correction in the sediment mixed layer were fitted by application of a ''diffusion-reaction'' model (dashed and solid lines, respectively). The area between the solid and dashed line denotes the contribution of AA_{CW} to THAA.

coefficient of variation, for duplicate samples varied between 5% and 15% for all amino acids except tyrosine (33%). The precision of the analysis of amino acid enantiomers was 3% for aspartic acid, 40% for glutamic acid, 21% for serine, and 15% for alanine. The reproducibility of the enantiomer analysis, determined from triplicate injections of several individual samples, was 1% for aspartic acid and alanine, 5% for serine, and 15% for glutamic acid.

Results and discussion—THAA concentrations decreased from 11 to 33 mg g^{-1} in the sediment trap samples to ~1–2 mg g^{-1} in the upper sediment layer (0–0.5cm). Concentrations further decreased from the sediment surface to the deeper layers with a strong gradient in the upper centimeters of the sediment, which indicates the degradation of amino acids during early diagenesis (Grutters et al. 2001) (Fig. 1). The concentration of D-amino acids (Fig. 2) increased in the upper centimeter of the sediment (glutamic acid and serine) or was constant with sediment depth (aspartic acid and alanine), whereas the concentration profiles of L-amino acids closely followed THAA concentration profiles. Concentrations of both D- and L-amino acids in the piston core samples were lower than in the sediment mixed layer. This could indicate the degradation of both enantiomers (O'Dowd and Hopkins 1998) at depth or their incorporation into refractory geopolymers that are resistant to HCl hydrolysis (Knicker et al. 2001). D/L amino acid ratios were low in sediment traps as well as in the upper sediment layer and increased strongly within the sediment mixed layer (Fig. 3). Apart from glutamic acid, D/L ratios in the piston cores fitted with those in the sediment mixed layer.

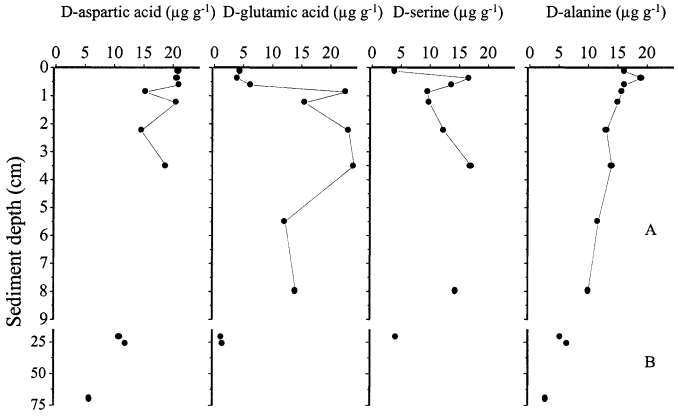

Fig. 2. Profiles of D-amino acids (μg g^{-1}) in the (A) sediment mixed layer and (B) piston core samples from station PE95-6 (1,296 m).

The potential contribution of D-amino acids by racemization, a process by which L-amino acids are converted into D-amino acids, was evaluated by application of a two-layer (0–8 and >8 cm) diagenetic model, including sedimentation, sediment mixing, degradation, and racemization. In both layers, a labile and a refractory fraction was taken into account (Fig. 3). The general solution for both enantiomers becomes

$$L(x) = 1/2(L_0 - D_0)e^{Ax} + 1/2(L_0 + D_0)e^{Bx} \quad (1)$$

$$D(x) = L - (L_0 - D_0)e^{Ax} \quad (2)$$

where the coefficients A and B stand for

$$A = \frac{\omega - \sqrt{[\omega^2 + (8r - 4m)D_b]}}{2D_b}$$

$$B = \frac{\omega - \sqrt{(\omega^2 + 4mD_b)}}{2D_b}$$

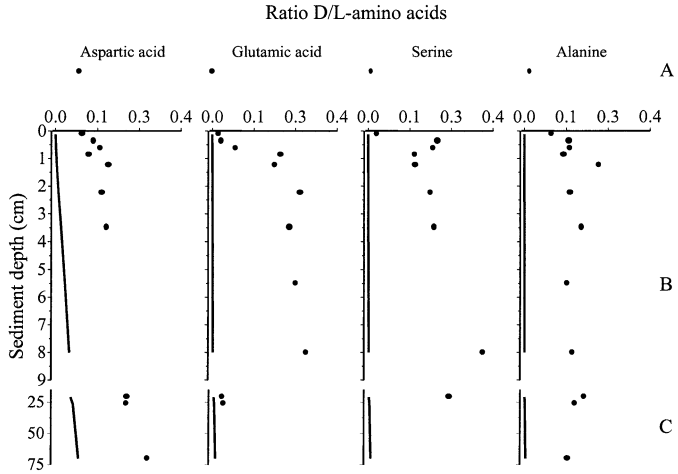

Fig. 3. D/L ratios (solid circles) of the enantiomers of aspartic acid, glutamic acid, serine, and alanine in samples from (A) sediment traps, (B) the sediment mixed layer, and (C) piston cores from station PE95-6 (1,296 m). Solid lines show D/L ratios calculated with the ''sedimentation-mixing-degradation-racemization'' model.

Coefficients r, m, ω, D_b, and z represent the first-order racemization rate constant (yr^{-1}), first-order degradation rate constant (yr^{-1}), sedimentation rate (cm yr^{-1}), sediment mixing coefficient (cm^2 yr^{-1}), and sediment depth (cm, positive downward), respectively. Sedimentation rates for the Goban Spur were taken from Van Weering et al. (1998). The sediment-mixing and amino acid degradation rates were taken from Grutters et al. (2001). Racemization rates were estimated by interpolating rates reported for free amino acids at 0°C and 25°C (Bada and Schroeder 1975) to the in situ temperature of 4°C by use of the Arrhenius equation. Equal rates for racemization as well as degradation were assumed for D- and L-enantiomers. It was taken into account that aspartic acid does not linearly racemize but rather has a ''fast'' and a ''slow'' component (Goodfriend 1991; Collins et al. 1999). Thus, the aspartic acid racemization rate was derived from running a model (Collins et al. 1999) under boundary conditions that mimic the environment in the sediments at the Goban Spur continental slope. The distribution of a labile and nondegradable fraction was determined from THAA concentration profiles (Fig. 1) as $(THAA_0 - THAA_\infty)/THAA_0 \times 100$. Below the sediment mixed layer, the concentration of enantiomers was calculated as a function of first-order degradation and racemization only, according to $L = 0.5(L_1 - D_1)\exp(-2rt - mt) + 0.5(L_1 + D_1)\exp(-mt)$ and $D = L - (L_1 - D_1)\exp(-2rt - mt)$. For t we used the age at the lower boundary of the sediment mixed layer (~3,000 yr) to 18,000 yr, the age of the oldest piston core samples. L_1 and D_1 refer to concentrations of L- and D-enantiomers at the lower boundary calculated from Eqs. 1 and 2.

The initial concentrations of L-amino acids at the sediment-water interface were calculated from measured concentrations in the sediment extrapolated to the sediment surface. The concentration of D-amino acids at the sediment-water interface was set to zero. This was supported by the low D/L ratios in the sediment traps (apart from aspartic acid), which indicated that settling organic matter is poor in D-amino acids. The model output from the lower boundary of the sediment mixed layer was used as input for the deeper zone. According to our model, the rapid increase in measured D/L ratios with sediment depth cannot be explained with racemization alone, which points at an additional source of D-amino acids in the sediment mixed layer.

The calculated contribution of D-amino acids by racemization based on our model was subtracted from the measured concentrations. The remaining concentration of D-amino acids is considered to represent the contribution of D-amino acids from bacterial peptidoglycan, derived from living bacteria and/or cell wall remnants. If we assume that the observed D-amino acid profiles (Fig. 2) reflect living bacteria only, published D/L ratios from bacterial cultures (Pedersen et al. 2001) (D/L_{LB}) can be used to estimate the contribution of amino acids from living bacteria (AA_{LB}) to the measured THAA as $\Sigma\ (D \times (1 + (D/L)_{LB}^{-1}))$, by taking into account that gram-negatives make up ~90% of the total bacterial community in aerobic surface sediments and ~70% in anaerobic, deeper sediment layers (Moriarty and Hayward 1982). The weighed average D/L ratio for aspartic acid, glutamic acid, serine, and alanine was, respectively 0.064, 0.103, 0.005, and 0.062. In this case, the total AA_{LB} would correspond to 11%–57% of the measured THAA in the sediment trap samples and in the upper sediment layer but would exceed the measured THAA concentration up to a factor of 5 in the sediment mixed layer. Furthermore, the bacterial abundance, as estimated from the calculated AA_{LB}, by use of empirical relationships between amino acid-nitrogen, bacterial cell-nitrogen, and cell abundance (Simon and Azam 1989), would exceed reported values of cell abundance for marine sediments (Boetius et al. 2000) up to a factor of 1,500. From these calculations, it is concluded that deeper in the sediment mixed-layer living bacteria cannot exclusively explain the observed profiles of D-amino acids. Thus, a significant contribution of D-amino acids has to be inferred from bacterial cell wall remnants.

The contributions of cell wall-associated D- and L-amino acids (AA_{CW}) to THAA in the sediment mixed layer were calculated by use of D/L ratios from peptidoglycan (D/L_{PEP}) as $1.2 \times \Sigma\ (D \times (1 + (D/L)_{PEP}^{-1}))$. D/L_{PEP} ratios were estimated from those types of peptidoglycan known to contain D- and L-aspartic acid, glutamic acid, serine, and alanine (Schleifer and Kandler 1972). The D/L ratio for aspartic acid, glutamic acid, serine, and alanine was, respectively, 3.3, 0.14, 0.25, and 0.63. The factor 1.2 corrects for the amino acids in peptidoglycans other than these four D- and L-amino acids (Schleifer and Kandler 1972). The contribution of AA_{CW} to THAA was only 1%–4% in the sediment trap samples and ranged between ~10% and 43% deeper in the sediment mixed layer (Fig. 1). The average contribution of AA_{CW} to THAA calculated with D:L ratios in peptidoglycans reported by Pedersen et al. (2001) and McCarthy et al. (1998) would range between 3% and 9% in the sediment traps and was ~5%–46% deeper in the sediment mixed layer. However, because the D/L values used in this study are on the low side relative to recently reported values for bacteria, cyanobacteria, and dissolved organic matter (Amon et al. 2001), these are conservative estimates only.

The estimated AA_{CW} were subtracted from the measured THAA concentrations to determine the pool of labile THAA. Profiles of labile THAA show a steeper concentration gradient in the upper layers of the sediment than those of uncorrected THAA (Fig. 1). THAA profiles were fitted with a ''diffusion-reaction'' model according to $C_{THAA}(z) = C_{L,0}\exp[-z\sqrt{(k/D_b)}] + C_{ND}$ in which C_{THAA} is the concentration of amino acids in the sediment. $C_{L,0}$ and C_{ND} are the labile and nondegradable fraction of the THAA (mg g^{-1}) at the sediment surface; z is the sediment depth (cm, positive downcore); D_b is the sediment mixing coefficient (cm^2 yr^{-1}) derived from modeling TOC concentration profiles (Grutters et al. 2001), and k is the first-order degradation rate constant of THAA (yr^{-1}). $C_{L,0}$, C_{ND}, and k were estimated by fitting the concentration profiles. The first-order degradation rate constants of labile THAA from the diagenetic model (Fig. 1) ranged between 1.4 and 8.4 yr^{-1} and exceeded values from uncorrected profiles (Grutters et al. 2001) by a factor of 2–10. They are consistent with amino acid degradation rate constants determined from laboratory experiments on amino acid degradation (Harvey et al. 1995). This small but reactive pool of amino acids in the upper sediment layers may originate from settling organic matter and in situ pro-

duction of cell plasma. We propose that the concomitant production of refractory bacterial cell walls, which explains at least one third of the amino acids in the sediment mixed layer may be the first step in the preservation of amino acids and, hence, initiates the preservation of organic matter.

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