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Enzymatically hydrolyzable amino acids in North Sea sediments and their possible implication for sediment nutritional values

by B. Dauwe¹, J. J. Middelburg¹, P. Van Rijswijk¹, J. Sinke¹,
P. M. J. Herman¹ and C. H. R. Heip¹

ABSTRACT

The nutritional value of peptidic material in five North Sea sediments that differ in organic matter quality has been investigated with an enzymatic approach measuring the digestibility (rate constant k of proteolysis by protease) and the proportion of bioavailable fraction of amino acids (%EHAA-i:THAA). The bioavailable fraction in sediments varied between 14–50% EHAA-i:THAA and was generally lower than in potential source organisms such as algae (40–43%), plankton (~80%) and bacteria (57–72%). The rate of proteolysis of the amino acids varied among stations ($k = 0.3$ – 3.1 h^{-1}) with a systematic decrease when going from labile to more refractory organic matter. The concentration of both the total and the enzymatically hydrolyzable amino acid pool increased toward the north where fine-grained sediments accumulate whereas the relative contribution of the labile fraction (% EHAA-i:THAA) decreased. Differences were found between the amino acid composition of the EHAA and the THAA pool in sedimentary organic matter, but these differences were minor in source organisms. Reasons for the selective liberation behavior of protease- k toward peptidic material in sediments include (A) specific cleavage pattern of protease- k preferring certain bounds (B) selection against amino acids protected by structural matrixes (holds for glycine and methionine) (C) protection by adsorptive binding to sediment particles (basic amino acids arginine, histidine, methionine). It is argued that arginine and histidine play an important role in deposit feeder nutrition since they are deficient in the sedimentary food source and therefore may be limiting growth. Their strong adsorption affinity, low susceptibility toward cleavage and occurrence in structural organic matter make them poorly available to deposit feeders. The relative content of basic amino acids also co-varies systematically with increasing nutritional quality.

1. Introduction

In coastal systems, a substantial fraction of the organic material produced in the euphotic zone reaches the sediment in a more or less degraded state as sinking detritus. Some of these particles are incorporated in the sediment quickly by burial and bioturbation. Most benthic deposit feeders rely on this external food input for their energy and nitrogen requirements (Christensen and Kannevorff, 1985; Graf, 1992). Especially, organic nitrogen is a highly desired and limiting nutrient in deposit feeder diets (e.g., Tenore, 1981;

1. Netherlands Institute of Ecology, Centre for Estuarine and Coastal Ecology (NIOO-CEMO), P.O. Box 140, 4400 AC Yerseke, The Netherlands. *email: dauwe@cemo.nioo.knaw.nl*

1988). It has been established that conventional bulk measurements of total nitrogen (TN) or protein are not suitable as indicators of the nutritional value of detritus and sedimentary organic matter due to the abundance of indigestible nitrogenous compounds (Rice, 1982).

Although amino acids usually do not comprise the major fraction of total sedimentary organic nitrogen (Cowie and Hedges, 1994; Wakeham *et al.*, 1997; Dauwe and Middelburg, 1998) they most likely form the principal source of nitrogen for benthic heterotrophs. Conventional methods, using strong acids to hydrolyze peptides (THAA), liberate most of the particle bound amino acids regardless of their degree of polymerization and adsorption to the sediment matrix. Animals do not use strong acids to liberate amino acids since their gut pH values are very similar to the surrounding sediment (Plante and Jumars, 1992). Instead, they rely primarily on enzymatic attack to solubilize amino acids from ingested food (Cowie and Hedges, 1996). Protein digestibility of potential food sources for marine animals has been estimated with multiple enzyme techniques and with gut extracts (Lan and Pan, 1993). Recently, Mayer *et al.* (1995, 1986) introduced a method using nonspecific proteolytic enzymes (protease-k) to distinguish between large polypeptides and smaller oligo- and monomers that are within reach of the organisms.

In this paper we report on the applicability of this method to investigate the enzymatic degradability of organic matter in five North Sea sediments covering a broad range of organic matter decompositional states (Dauwe and Middelburg, 1998) and different macrofaunal community structures (Dauwe *et al.*, 1998). We will compare the enzymatically available amino acids among stations, between sediments and potential sources of organic matter (bacteria and algae), and within sediment profiles. The rates of proteolysis and amino acid liberation patterns by protease-k are discussed in relation to deposit feeder diets and distribution.

2. Material and methods

a. Sediment sampling stations

Five stations in the eastern part of the North Sea were selected (Fig. 1). The most southern stations are the very shallow "Brouwershavensche gat A" (BG-A), then the 20–40 m deep "Broad Fourteens" (BF), "Frisian Front" (FF) and "German Bight" (GB) and finally the 280 m deep "Skagerrak" (SK). Details of the physical and chemical characteristics of the study stations have been summarized by Dauwe and Middelburg (1998). The water columns at the shallow coastal stations were completely mixed, but the 280 m deep Skagerrak was temperature stratified, with a minimum water temperature of 6°C at the bottom. The station GB and BG-A have somewhat lower salinities compared to the other stations due to the influence of river runoff.

The sediment granulometry reflects the hydrodynamic regime of the North Sea to a large extent (Otto *et al.*, 1990; Wiesner *et al.*, 1990). The southern part, with the highest current velocities, is largely dominated by sandy sediments (BG-A, BF), mixed with varying amounts of mud. Temporary deposition areas (FF, GB), where slow currents allow particles to stay a few weeks or longer, consist of fine sands mixed with clay. Silty sediments are

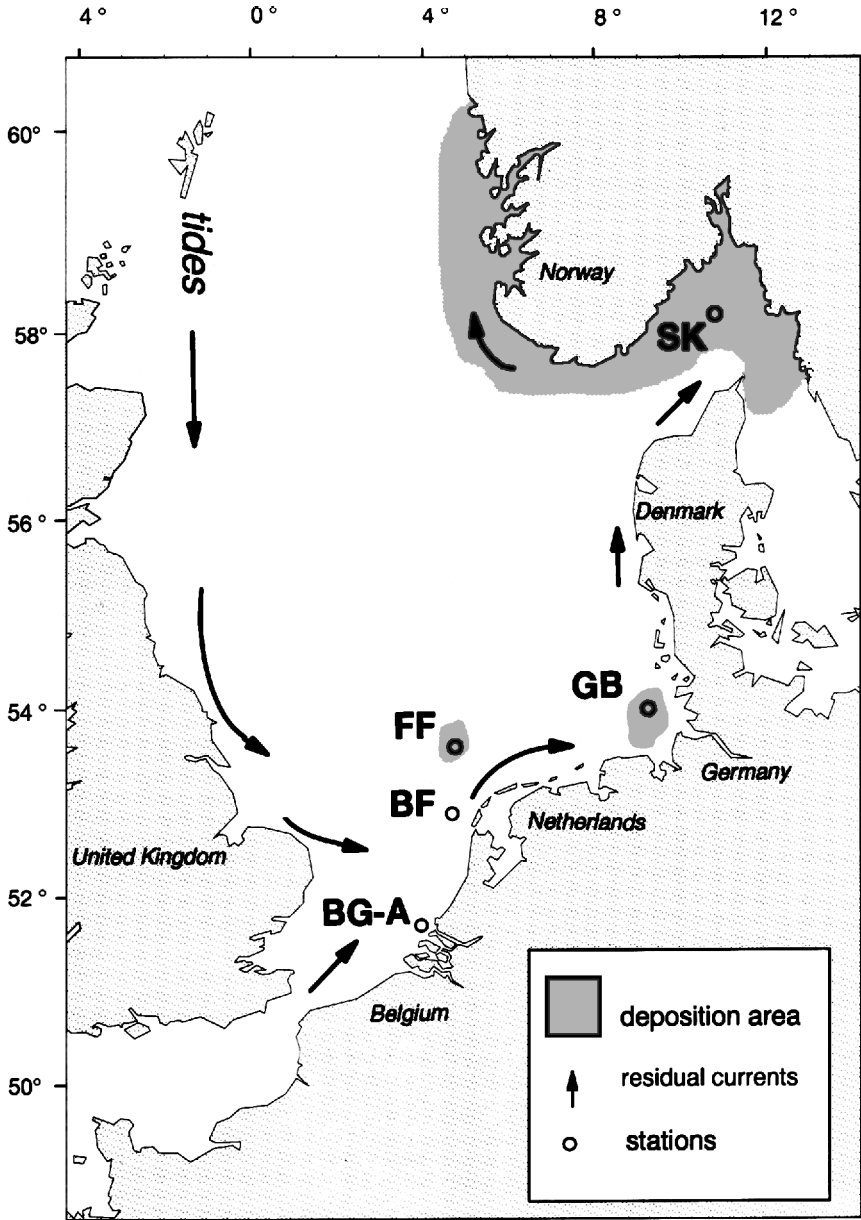


Figure 1. Sample stations in the North Sea. Arrows indicate the anticlockwise direction of the residual tidal currents.

found in the GB and the deep final deposition area SK. The major source of organic material in the North Sea is local primary production as indicated by the stable carbon isotope composition ($\delta^{13}\text{C}$) of about -21% to -22% at all stations (Dauwe and Middelburg, 1998), matching the value typically found in North Sea marine plankton (Dauby *et al.*, 1994). Most of the primary production takes place in the shallow, unstratified southern part of the North Sea (BG-A, BF, FF). After the spring bloom, the fresh material sinks to the bottom, ages and is transported by bed-load transport successively to the north, with SK as the final deposition area (Van Weering *et al.*, 1993; Eisma and Kalf, 1987a,b). The stations have been chosen on the basis of these contrasting features to make sure that a broad quality range would be covered. The quality of sedimentary organic matter as revealed by the amino acid and hexosamine composition (Dauwe and Middelburg, 1998) is highest at station BF that receives fresh material and lowest at stations with more refractory organic matter (e.g. SK).

b. Preparation of sediment samples

Stations Broad Fourteens (BF), Frisian Front (FF), German Bight (GB) and Skagerrak (SK) were visited with the R. V. *Pelagia* in August 1994, and the most southern station Brouwerhavenschegat A (BG-A) with the R. V. *Luctor* in May 1996 (Fig. 1). At each station four box cores (diameter 50 cm) were recovered and each was subsampled immediately with plastic liners having a diameter of 3.3 cm and a length of 50 cm. These subcores were successively sliced in vertical horizons and samples from each horizon were pooled after sectioning to prevent selective sampling. The cores were sliced at 0.25 cm resolution in the top cm, at 0.5 cm resolution down to 3 cm, at 1 cm resolution down to 7 cm and 1.5 cm thick slices down to 15 cm depth. The sediment was immediately frozen at -40°C on board and then freeze-dried.

c. Organic carbon total nitrogen and total hydrolyzable amino acid fraction (THAA)

Total organic carbon (TOC) and total nitrogen (TN) were determined on freeze-dried samples that had been finely powdered and homogenized. A 20–50 mg split was combusted at 1010°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). Reproducibility is about 2% for both TOC and TN.

THAA were analyzed from freeze dried materials that were hydrolyzed as described in Dauwe and Middelburg (1998). THAA-samples were analyzed in duplicate and the reproducibility of the method was 2–5%. An 0.1–0.2 ml aliquot of the hydrolyzate was added to 2 ml of a pH 10 potassium borate buffer and neutralized with 0.1 ml of 6 N NaOH. The solution was vortexed, incubated for one hour at 20°C and vortexed again to set free any ammonium present in the mixture. 0.2–0.4 ml of this solution was added to 2 ml phosphate buffer in a cuvette and vortexed. An equal amount of the o-phthaldaldehyde (OPA) reagent (0.2–0.4 ml) was added and the fluorescence was measured after two minutes at excitation-emission wavelengths of 340/455 nm in a spectrofluorometer. Stan-

standard curves are made from an amino acid mixture (Sigma). An average molecular weight of 120 g mole⁻¹ was assumed for calculations on a weight basis. Individual amino acids and hexosamines were determined by reverse phase HPLC after Lindroth and Mopper (1979) as described in Dauwe and Middelburg (1998).

d. Enzymatically degradable amino acid fraction (EHAA)

The enzymatically hydrolyzable amino acids (EHAA) were determined with the method described by Mayer *et al.* (1995) in duplicate samples. The reproducibility was 2–5%. Briefly, digestion in the animal gut is mimicked by adding a nonspecific proteolytic enzyme (“Proteinase-k” Sigma No. P8044) to freeze dried sediment which generates polymers of varying complexity. 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 protease were incubated 6 hours on an orbit shaker (300 rpm) at 20°C. To exclude the higher molecular weight peptides and enzymes from the supernatant, and to stop the reaction, 75 µl of 100% trichloroacetic acid (TCA) was added, it was vortexed and then refrigerated for 30 minutes and centrifuged again for 10 minutes. Precipitation with TCA yields the “bioavailable” fraction containing oligo-peptides with chains containing <7–15 amino acids and monomers (Mayer *et al.*, 1995). This low molecular fraction was then hydrolyzed and measured in the same way as described for THAA.

Blank-assays containing solely freeze dried sediment and buffer without added protease-k represent the concentration of free oligo- and monomeric amino acids already present in the sediment (or released by freeze-drying) that are most readily available (“soluble” amino acids = SAA). SAA were subtracted from the experimentals to yield EHAA, the pool liberated by protease-k. The EHAA-i fraction refers to EHAA including SAA, indicating the total bioavailable pool.

e. Kinematic series of EHAA

Incubation slurries of the surface (0–0.25 cm), subsurface (2–2.5 cm) and deep (10–11 cm) layers were made in 4.5 ml polypropylene microcentrifuge tubes, with three replicates for each time point and one blank following the above described procedure. At various time points (after 0.1, 0.5, 1, 2, 3, 5, 10 and 25 hours, respectively), the incubation tubes were centrifuged and the incubation was stopped by adding TCA to the slurries. The first obtainable time point after addition of the protease-k was after 10 minutes ($t = 10$ min), due to handling and centrifugation of the sample. After addition of protease-k a direct release of EHAA was measured at $t = 10$ min, followed by a slower release of EHAA that continued up to six hours (Mayer *et al.*, 1995). The sum of the amino acids liberated by the fast reaction till $t = 10$ min and the SAA already present in the sediment can be considered to be an immediately available pool. Using nonlinear regression, curves were fitted to Eq. (1): $C_t = C_{10} + C_{\text{final}} (1 - \exp^{-kt})$, with C_t being the EHAA concentration at time t , C_{10} being the EHAA concentration released by the fast reaction after 10 minutes and C_{final} being the asymptotically approached EHAA concentration released during slow hydrolysis

and k being the first order rate constant describing the slower hydrolysis. Curves were not fitted through $t = 0$ because liberation rates of the fast pool could not be determined due to the poor time resolution.

f. Bacterial- and algal cultures

Potential source organisms of protein were examined for their EHAA and THAA content in fresh and freeze dried samples of bacteria, freeze dried samples of algae (*Phaeocystis* sp., *Thalassiosira pseudonana*) and a plankton sample from the Wadden Sea. A bacterial culture was started by inoculating an agar dish with a drop of coastal water (Eastern Scheldt). One of the colonies was isolated and grown in 1.75 l filtered seawater (0.2 μm) enriched with 100 mg yeast extract. The bacteria were harvested in the stationary phase and then isolated by repetitive centrifugation at $25000 \times g$ and washing in filtered sea water. The pellet was transferred into 25 ml of isotonic solution, resuspended, divided over 24 vials each containing 1 ml of bacterial suspension and then centrifuged again, discarding the supernatant. Nine freeze-dried pellets (Bact IA) and another nine fresh pellets (Bact IB) were treated in the following manner separately: For the EHAA determination: three blanks containing 1 ml inhibitor and 0.1 ml phosphate buffer, three experimentals containing 1 ml inhibitor and 0.1 ml protease-k, three controls containing inhibitor and 50 μl amino acid standard 43.75 $\mu\text{mol/ml}$ to check for the function of the inhibitor on amino acid uptake by bacteria. Three pellets were hydrolyzed for determination of THAA. This procedure was repeated to yield a second bacterial culture (Bact II) that was freeze dried.

A *Phaeocystis* sp. culture was grown in a 2.5 l PEP medium (enriched sea water with 1 mmol NaNO_3 , 6.3 μmol $\text{NaH}_2\text{PO}_4 \times 2\text{H}_2\text{O}$, 44 μmol $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, trace elements and vitamins following Kester *et al.*, 1967) and harvested after three weeks at a cell density of 8×10^5 cells/ml and then freeze-dried (Phaeo). A *Thalassiosira pseudonana* culture was grown in 5 l F2 medium (Guillard and Ryther, 1962) at 16°C for 2 weeks. The algae were harvested after repetitive rinsing and centrifugation $10000 \times g$ and then freeze-dried (Thala). A plankton sample from a *Phaeocystis* sp. bloom in the Dutch Wadden Sea was retrieved with a plankton net and freeze dried (Plank-W). All samples were further treated for EHAA and THAA analysis as described above.

3. Results

a. The effect of freeze-drying on total yield and relative contribution

Freeze-drying enlarged the amount of THAA in algae (*Phaeocystis* sp.) by $21 \pm 7\%$ and bacteria by $21 \pm 2\%$. Also the amount of EHAA was $15 \pm 7\%$ (*Phaeocystis* sp.) and $26 \pm 4\%$ (bacteria) higher in freeze dried material than in fresh materials. Upon freeze drying soluble amino acids (SAA) increased $13 \pm 5\%$ in *Phaeocystis* sp., but decreased by $41 \pm 10\%$ in bacteria (Table 1). For consistency, we only used freeze-dried samples.

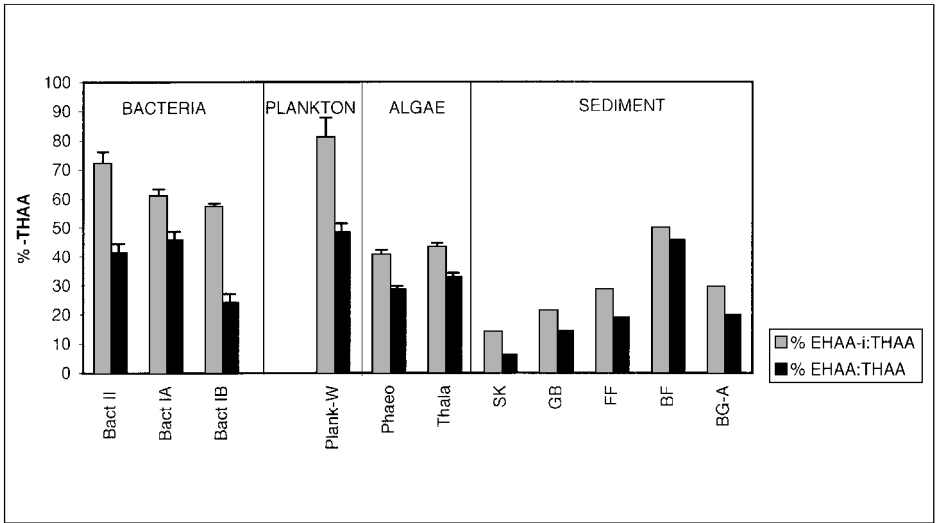


Figure 2. Contribution of enzymatically hydrolyzable amino acids to total hydrolyzable amino acids in source material (bacteria, plankton, algae) and the sediments (0–10 cm depth-averaged values). %EHAAs:THAA = blanks without enzyme subtracted, %EHAAs-i:THAA = inclusive blanks.

b. EHAAs in source organisms

The fraction of enzymatically available amino acids in source organisms was higher than that in sediments (Fig. 2). In bacteria between 57%–72% of the THAA were degradable by protease-k. The plankton sample contained the largest fraction of degradable peptidic material (~80% EHAAs-i:THAA). Algae generally contained a lower fraction of protease-k degradable amino acids of (41–43%). In source materials, the contribution of the pool of soluble amino acids % SAA:THAA is higher (13–42%), and also more variable compared to that in sediments (5–10%).

c. Depth-averaged contents of EHAAs in sediments

The sediments with the highest organic carbon (TOC) content were SK ($\pm 23 \text{ mg} \cdot \text{g}^{-1}$) and GB ($\pm 11 \text{ mg} \cdot \text{g}^{-1}$). Sediments at FF and BG-A were intermediate and sediments at BF contained the least ($\pm 0.5 \text{ mg} \cdot \text{g}^{-1}$ TOC) (Table 1). The large variations of the TOC content among stations were reflected by their TN (total nitrogen) contents.

The sizes of the different amino acid pools THAA (up to $7.6 \text{ mg} \cdot \text{g}^{-1}$), EHAAs-i (up to $1 \text{ mg} \cdot \text{g}^{-1}$) and SAA (up to $0.6 \text{ mg} \cdot \text{g}^{-1}$) were all positively correlated to TN content of the sediments. The relative contribution of the acid hydrolyzable fraction % THAA-N:TN varied between 34–51% of the nitrogen at all stations (Table 1). The enzymatically hydrolyzable nitrogen fraction varied much stronger among the North Sea sediments (2–15% EHAAs-N:TN) and showed a negative correlation with the TN content, with lowest values at high nitrogen sediments (Table 1).

The variation of the % EHAA:THAA concentration among the investigated sediments was remarkable, increasing with a factor 8 from the most degraded material at SK ($\pm 6\%$ EHAA:THAA) to the most labile organic matter at BF ($\pm 46\%$ EHAA:THAA) (Fig. 2). Intermediate EHAA contributions to THAA were found at BG-A ($\pm 20\%$) and FF ($\pm 19\%$) and GB ($\pm 14\%$). Also the contribution of the bioavailable fraction of the amino acids % EHAA:THAA was inversely correlated with total nitrogen content of the sediments. Since the contribution of % SAA:THAA showed little variation among the sediments (between 5–10% at all stations), this inverse correlation with TN holds also for total available fraction % EHAA-i:THAA that varied between 14–50%.

d. Vertical profiles of THAA and EHAA

Depth profiles of total nitrogen were matched by those of THAA and are, therefore, not given in Figure 3. A homogeneous depth profile of all the nitrogen containing organic matter fractions was found at station SK at all depths up to 20 cm. At station GB, surface concentrations of THAA and EHAA-i were in the same range as those at station SK, but they sharply declined with increasing depth. The FF sediment showed a homogenous profile of THAA and EHAA-i with a slight increase toward the surface in the top centimeters. BG-A has a distinctive subsurface peak in all the three nitrogen-fractions at the 10–11 cm (“deep”) section and somewhat elevated concentrations toward the surface. At station BF, the THAA and EHAA-i concentrations were extremely low and profiles are rather homogeneous, showing only variation within the confidence limit of the measurements. The SAA-pool at station BF was very small and sometimes below the limit of detection ($\sim 0.1 \mu\text{mole/g}$).

e. Time course of SAA and EHAA release

The bulk of the SAA was released immediately after addition of the buffer and remained constant during the entire 28 hours time course of the incubation (Fig. 4). Therefore the mean value for each sediment section was used to correct the EHAA-i for this soluble fraction to yield the amino acids liberated enzymatically during the time course (EHAA). After 10 minutes SAA usually accounted for the major fraction of the EHAA (up to 100%) %SAA:EHAA at the beginning of the time course (t_{10}), but their relative contribution decreased to 20–60% at t_{final} (Table 2).

The immediate release of EHAA was followed by a slower release of EHAA that continued for hours (Fig. 5) and generally reached an asymptote within 6 hours. Table 2 gives the variables of the slow reaction for the North Sea sediments. The model generally offered a good fit to the liberation of EHAA ($R^2 > 0.9$), except for stations BF and BG-A with extremely labile organic material. The degradation rate constants varied between 0.14 and 3.1 h^{-1} among the three investigated depth strata and sample stations. Generally the rate constant increased with increasing bulk contribution of % EHAA:THAA in the sediments going from SK via GB and FF to BG-A and BF (Table 2). The degradation constants varied considerably among depth strata except at station SK where they remained

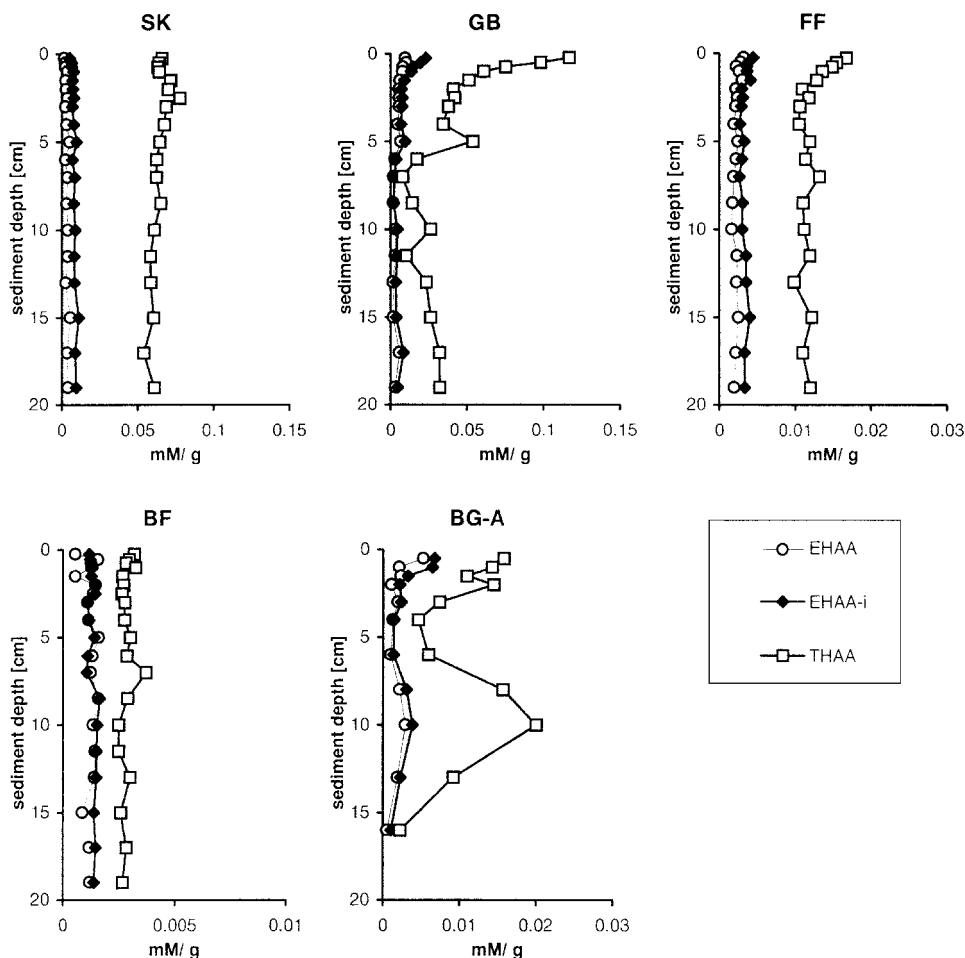


Figure 3. Depth profiles of EHAAs, EHAAs-i and THAA within the sediment.

constant ($k = \pm 0.3 \text{ h}^{-1}$). At the other stations the surface layer was more rapidly degradable compared to the subsurface layer, and in all cases 10–11 cm deep layers had distinctly higher degradation constants compared to the more shallow layers (Table 2).

f. Amino acid spectra

The EHAAs spectra of the sedimentary organic matter at stations SK, FF and GB were dominated by glycine, aspartic acid and alanine with each contributing ~ 10 – 20 mole % to the total pool. Glutamic acid, serine, valine, leucine, threonine, arginine and isoleucine accounted for 5–10 mole %. A minor group of phenylalanine, tyrosine, methionine, histidine, the non-protein amino acids γ -aminobutyric acid and β -alanine, and the amino sugars glucosamine and galactosamine contributed less than 5 mole %.

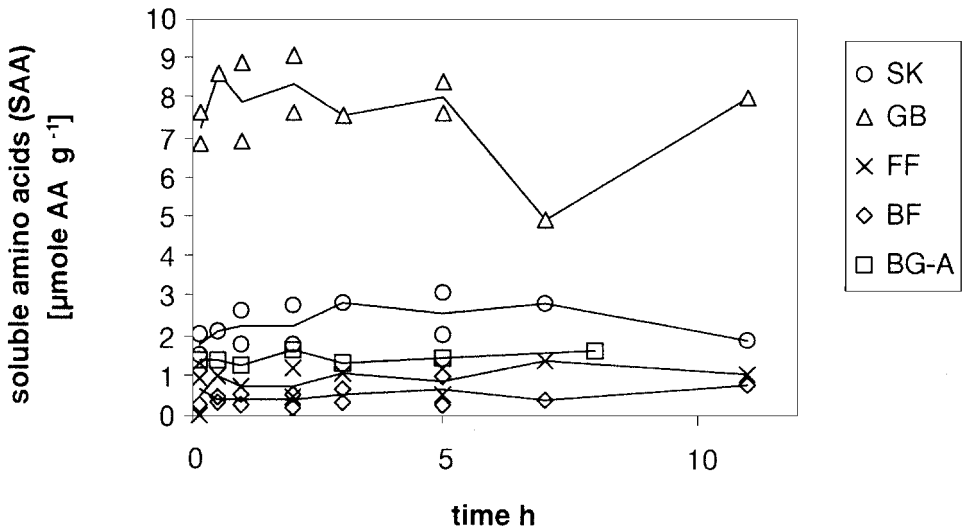


Figure 4. Time course of the soluble amino acids (SAA) in the 0–0.25 cm surface layer of the sediments.

However, there were major and consistent differences in the amino acid spectra between the total hydrolyzable and the enzymatic available fraction in sedimentary organic matter (Fig. 6A). In all sediments the acidic amino acids (glutamic- and aspartic acid) and the essential amino acids leucine, threonine, isoleucine and phenylalanine (only FF) and methionine (only GB) were enriched in the EHAA fraction with respect to their concentration in THAA. The amino acids alanine and serine and the essential amino acid valine were non-selectively liberated, resulting in about the same mole contributions in both, the THAA and the EHAA pool. However, the essential amino acids arginine and histidine were liberated much less efficient by protease-k reaching only 5–30% of their original concentration in the THAA pool. The amino acids glycine and tyrosine, the nonprotein amino acids β -alanine and γ -aminobutyric acid, and the amino sugars glucosamine and galactosamine were less effectively degraded by protease-k from sedimentary organic matter (generally <10% of the concentration in THAA).

In algae and plankton, the picture was more scattered, resembling the sediment in its deficiency of tyrosine, methionine, histidine, arginine and leucine (Fig. 6B). In bacteria the differences in liberation patterns between the two methods were minor compared to those in sedimentary organic matter and algae (Fig. 6C), except for methionine that was not liberated by protease-k and for glutamic acid and histidine that were preferentially liberated by protease-k.

The essential amino acids made up between 30–45 mole % of the THAA pool in all investigated materials (Fig. 7) and were generally more concentrated in source materials compared to sedimentary organic matter. The EHAA pool in all sediments was enriched with essential amino acids compared to the THAA pool. Among the sediments, the relative

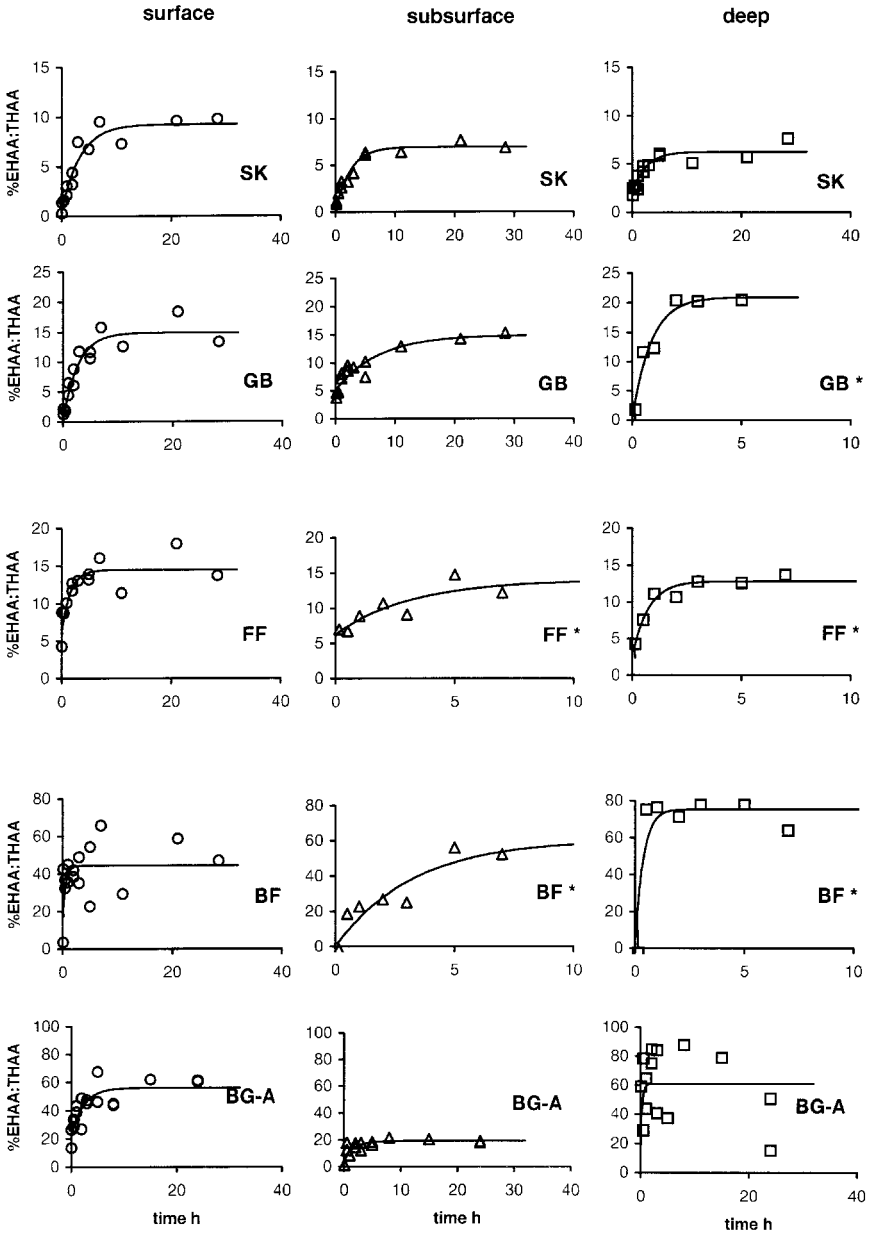


Figure 5. Degradation kinetics at three depth strata: surface: 0–0.25 cm, subsurface: 2–2.5 cm, deep: 10–11 cm. The curves were fitted to equation (1) and regression results are summarized in Table 2. *indicate a different scale on the x-axis due to shorter time series.

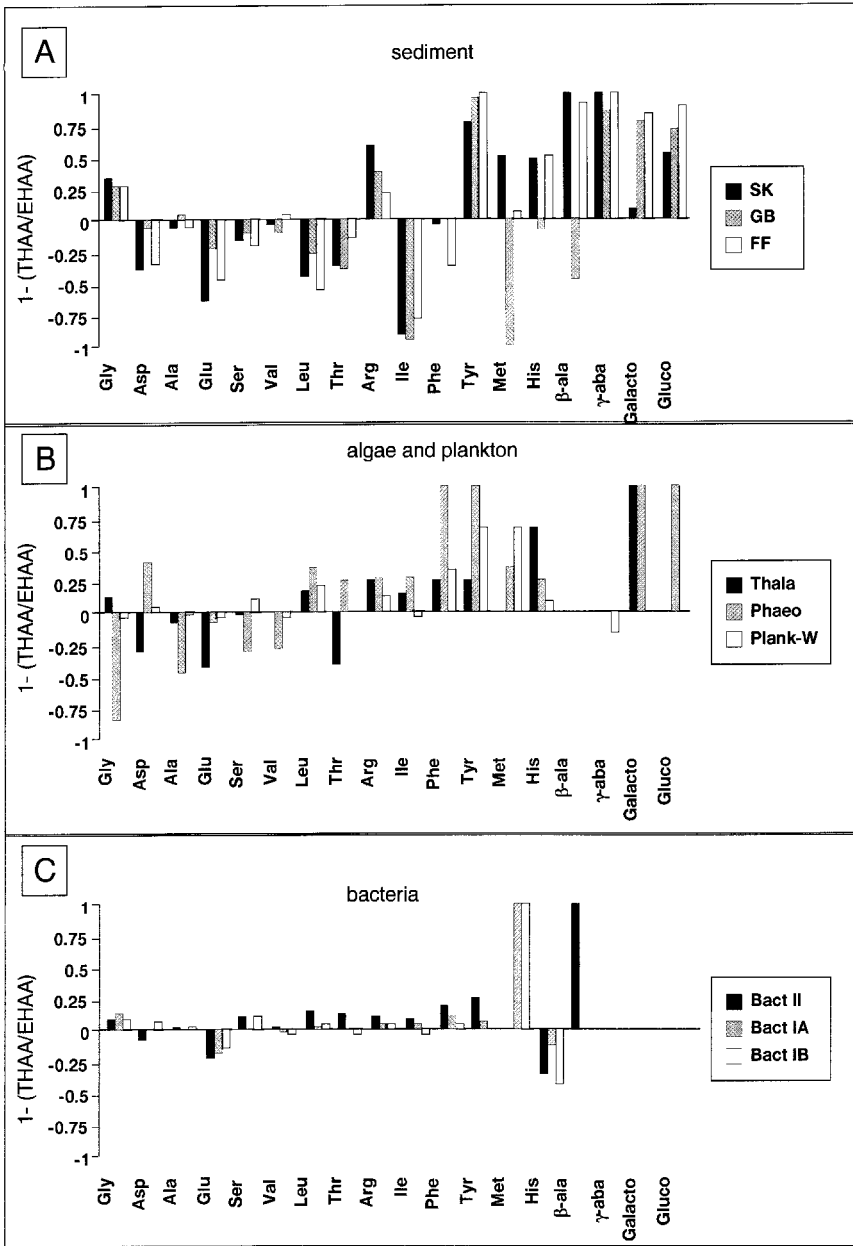


Figure 6. Deficiency and accumulation of analyzed amino acids in the EHAA-fraction versus the THAA fraction in North Sea sediments (SK, GB, FF) based on their mole % concentration (A), algae (Thala, Phaeo) and plankton (Plank-W) (B) and bacterial cultures (Bact II, Bact IA, IB) (C). Zero indicates an equal distribution in both fractions, positive values indicate a relative depletion of the component in the EHAA fraction compared to its concentration in the THAA pool. A negative value indicates an enrichment.

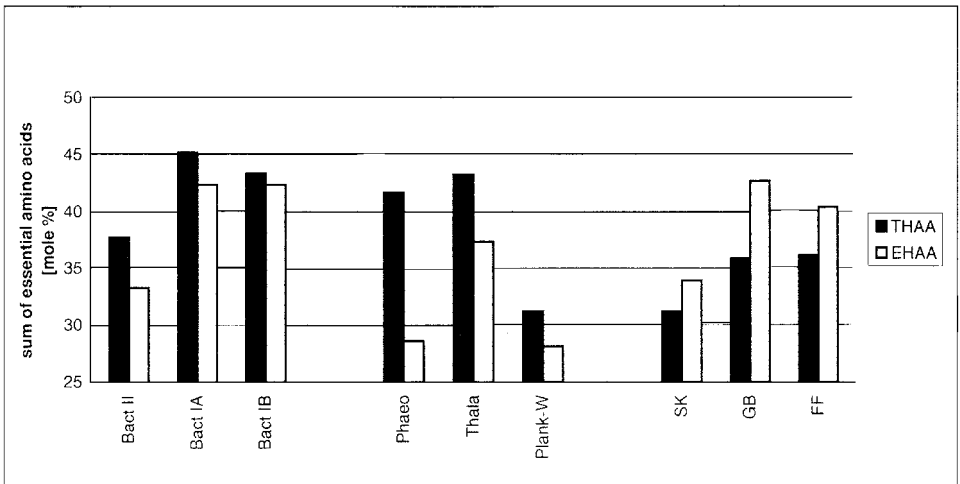


Figure 7. Concentration of the sum of essential amino acids [threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine (Phillips, 1984)] in the EHAA fraction and the THAA fraction in bacterial cultures (Bact II, Bact IA, IB), algae (Phaeo, Thata), plankton (Plank-W) and North sea sediments.

enrichment of essential amino acids in EHAA was maximal at station GB (20% higher mole % concentration in EHAA compared to THAA), followed by FF and the smallest enrichment (<8% higher mole % concentration) was found at SK. In source organisms, mole % concentrations of essential amino acids liberated by protease-k were either similar (Bact IA, Bact IB) or lower (Bact II and all algae samples) than those liberated by acid. The EHAA pool of *Phaeocystis* sp. was about 30% less concentrated in essential amino acids compared to the total amino acid pool.

4. Discussion

a. Effect of freeze-drying on protease activity

Our results show that freeze drying enhances the release of enzymatically (EHAA)- and acid hydrolyzable amino acids (THAA) from source materials such as bacteria and algae with ~20%. Compared to these source organisms, sediments showed a much higher increase of amino acid pools upon freeze-drying (~40%) (Mayer *et al.*, 1995). The positive effect of freeze drying on the EHAA yield in source organisms probably results from physical destruction of protective layers such as cell walls and cell membranes. The EHAA increase in sedimentary organic matter after freeze-drying has been attributed to the destruction of a “protective matrix” present in fresh material and not by enhanced release from living cells (Mayer *et al.*, 1995), since the extent of amino acid release was much too high to be accounted for by bacterial biomass alone (Mayer and Rice, 1992). Possibly, freeze-drying changes the molecular structure of the adsorbed peptides in sediments and makes them more exposed to disintegration. A similar increase of the lability of chlorophyll upon freezing of sediments has been found by Sun *et al.* (1991).

b. SAA in source organisms and sediments

THAA in marine organic matter comprises structural and storage amino acids as well as amino acids in an adsorbed/chelated state. In North Sea sediments, the fraction of soluble amino acids relative to THAA is usually small, with $\sim 5\text{--}10\%$ SAA:THAA (Table 2). The fraction of “free” amino acids is highest in fresh phytoplankton and decreases drastically as the material ages (Lee and Cronin, 1982). SAA comprise not only free amino acids, but also the water soluble fraction that is very loosely bound to the sediment matrix and may be desorbed during freeze-drying. The unassociated fraction is the most readily available amino acid pool and therefore probably of importance as food source for microorganisms. Adsorbed or dissolved monomeric amino acids are unlikely to provide a significant food source to deposit feeders because of their fast incorporation into bacterial biomass (Mayer, 1989). The concentration of SAA remained constant during the 28 hours time course of the incubations (Fig. 4). This indicates that this fraction is not liberated by sedimentary proteases, but was already present as a labile pool in the sediment and/or was released from biota by freeze drying. In fresh sediments, Mayer *et al.* (1995) observed a slow release of amino acids in blanks during the time course of the incubation, probably also due to release from biota (leaking of cells). The high content of SAA in the GB surface sediment compared to the deeper layer (Table 1) indicates an elevated nutritional quality. This agrees with the results based on a THAA-composition based quality index that allowed distinction between a labile upper layer and more refractory subsurface material at GB (Dauwe and Middelburg, 1988).

c. Digestibility

In contrast to the strong horizontal differences among the North Sea sites, there are minor variations in relative contribution of %EHAA:THAA within sediment profiles (deviation $<10\%$ of mean), probably due to extensive vertical mixing by bioturbation (Boon and Duineveld, 1998, Dauwe *et al.*, 1998). However, the specific degradability, as indicated by the rate constant k , shows considerable variation within the depth strata in all sediments, except at station SK, with the most refractory organic matter that has a rather homogeneous profile (Table 2). At all stations the surface layers are more easily degradable compared to the subsurface stratum, which can be explained by incorporation of recently deposited fresh material. At all stations the highest degradation constants were found in the deepest layer investigated (10–11 cm). These high rates of liberation ask for further study since deeper strata are generally considered to be more degraded compared to more shallow strata. At GB, the relatively large pool of readily available material at depth 10–11 cm may result from accumulation of fluff surface material in empty burrows (Aller and Aller, 1986) constructed by the abundant tubicolous polychaetes *Owenia fusiformis* (Dauwe *et al.*, 1998).

Among stations there is a positive relation between the relative contribution of EHAA to the THAA pool and the degradation rate constant of the peptidic material, with increasing values for both parameters when going from the refractory SK toward the more labile

stations (Table 2). The degradation rate constants within the North Sea sediments ($k = 0.3\text{--}3.1 \text{ h}^{-1}$) are higher and more variable than those for intertidal mudflats ($k = 0.15\text{--}0.52 \text{ h}^{-1}$; Mayer *et al.*, 1995) and those for estuarine seston ($k = 0.2\text{--}1.3 \text{ h}^{-1}$; Laursen *et al.*, 1996). Values of $k > 1.5$ have only been measured in the separated cytoplasmatic fraction of phytoplankton (Laursen *et al.*, 1996). The very high degradation constants cannot be quantified accurately, as indicated by the low R^2 values (Table 2). Also the high asymptotic standard error (A.S.E.) of the degradation rate constants at the stations FF, BF and BG-A (Table 2) indicates that the determination of k in the more coarse grained sediments with a low organic matter content is problematic. A better description of the rapid liberation of the EHAA would require more data in the beginning of the time course, but the method does not allow a higher time resolution and its applicability toward the quantification of extremely labile sources is therefore somewhat restricted. Since the degradation rate constant is largely determined by the % EHAA:THAA, we further consider the latter as an indication of nutritional quality.

d. Bioavailable fraction (EHAA)

The EHAA method is a relative measure of the bioavailable nitrogen fraction and does not give the absolute size of the available pool since it does not take into account (1) the combined action of the whole series of enzymes in animal guts (e.g., lysozyme to destroy bacterial cell membranes and get access to plasma proteins (McHenery and Birkbeck, 1982; Plante and Mayer, 1994; Lucas and Bertru, 1997), (2) the use of surfactants (Mayer *et al.*, 1997) and (3) particle selection by the organisms (Self and Jumars, 1988). The relative size of the available pool for deposit feeders as described by the % EHAA:THAA fraction is based on an incubation (i.e., gut residence) time of ~ 6 hours (Mayer *et al.*, 1995), even though small members of natural deposit feeder populations may have gut turnover times of only four minutes (Kofoed *et al.*, 1989) and may, therefore, not be able to digest the whole available fraction. As a consequence of the time dependence of digestion, the EHAA pool reflects a gross measure of the potentially available organic matter to the whole community. Nevertheless, the relative pool sizes (% EHAA) derived from freeze-dried samples can be used to compare among substrates.

The minor variations of %THAA:TN contribution in the North Sea sediments (34–51%, Table 1), support our presumption that this chemically extractable fraction of peptidic material is not very sensitive to small changes in the food quality of sedimentary organic matter. In coastal sediments, THAA and TN usually comprise a large fraction of highly polymerized and relatively undegradable nitrogen and variations in contribution of %THAA:TN are mostly minor in sediments (e.g. Colombo *et al.*, 1996). The rather variable proportion of the enzymatically degradable fractions (2–15% EHAA-N:TN and 6–46% EHAA:THAA) reflects the large variations in the nutritional value of the organic matter in the investigated sediments experienced by benthic deposit feeders (Table 1). The depth-averaged concentrations of % (EHAA-N:TN in North Sea sediments (varying between 2–15%) are in the same range as those measured in coastal Maine sediments

(6–11% Mayer *et al.*, 1995). Laursen *et al.* (1996) reported higher contributions in suspended matter (37–63% (EHAA-N:TN), consistent with the large fractions of protease-k degradable peptidic material we measured in algae and plankton (Fig. 2). The large fraction of bioavailable amino acids in bacteria (± 60 –75% EHAA-i:THAA) and the algae *Phaeocystis* sp. and *Thalassiosira pseudonana* (43–50% EHAA-i:THAA) stresses the high potential food value of source organisms compared to the generally much lower content of degradable peptidic material present in sedimentary organic matter (~ 14 –50% EHAA-i:THAA) (Fig. 2). The broad range of the bioavailable pool demonstrates the contrasting food quality of sedimentary organic matter from the different deposits situated along the general transport route of organic matter in the North Sea. The largest proportion of enzymatically degradable amino acid was encountered at station BF, as demonstrated by its high EHAA-i- content (50%) approaching that of algae (43–50%) and bacteria (60–75%). The degradable fraction generally decreases when going north via stations BG-A \approx FF ($\sim 30\%$) and GB (21%) to station SK (14%) where the most degraded peptidic material is present in the sediment. The Skagerrak is supposed to be the final deposition area of organic material in the North Sea. It has been shown that more than 90% of the organic carbon buried in the deep SK region originates from sources outside this area, likely being transported by bed -and/or suspended load transport from the southern North Sea (de Haas and van Weering, 1997). Degradation during bed-load transport and during sinking in the 280 m deep water column, results in deposition and burial of rather degraded organic matter, as also has been inferred from the biochemical composition of the sediments (Anton *et al.*, 1993; Dauwe and Middelburg, 1998).

Besides the quality and quantity of the organic matter input, sedimentological properties also play an important role in the preservation of organic matter (Keil *et al.*, 1994a). The grain size of the sediments generally decreases along the array of the stations towards the north due to ongoing particle sorting (Dauwe and Middelburg 1998). With decreasing grain size, the surface area available for adsorption increases strongly (Mayer 1994). Consequently sorptive preservation of organic matter is very high in extremely fine grained deposits, such as those found in SK, compared to more sandy deposits. Indeed, the EHAA content of the fine-grained sediments at station SK is higher than that of sandy sediments (stations BF and BG-A).

Adsorption to sediment grains contributes to the conservation of intrinsically labile molecules such as amino acids in fine-grained deposits (Keil *et al.*, 1994b). The most fine-grained deposits contain the most refractory organic matter and the highest contents of all nitrogen pools (TN, THAA and EHAA) (Table 2). Although the TN content increases along the transport route BF < FF \approx BG-A < GB < SK, the share of the bioavailable pool becomes smaller when going north.

The inverse relation between relative quality in terms of % EHAA:THAA and the total amount of EHAA requires further consideration since it is not clear what is more relevant for deposit-feeder nutrition, the amount of available material or the relative size of the available fraction. Regarding the high sediment processing rates generally found in deposit

feeders, gut turnover time is likely to limit digestion (Kofoed *et al.*, 1989). Low quality sediments (e.g. SK) are dominated by small sized benthos (mean individual weight ± 0.55 mg AFDW \cdot organism⁻¹) whereas sediments containing more labile organic matter (e.g. FF) are inhabited by large sized animals (mean individual weight ± 3.9 mg AFDW \cdot organism⁻¹) (Dauwe *et al.*, 1998), with decreasing body size as the nutritional quality of the sedimentary organic matter decreases. Small sized species and individuals usually have higher size-specific ingestion rates and shorter gut turnover times compared to larger animals (Forbes, 1989; Cammen, 1989) and therefore probably need more easily digestible material. In North Sea sediments, however, the smallest organisms were found in the most refractory sediment at SK. In deep sea environments a comparable shift toward smaller species with increasing water depth was found (Thiel, 1975; Flach *et al.*, 1998).

In addition to ingestion rates, feeding types (selective versus non selective deposit feeders) are also likely to play a significant role. It is not clear yet whether adsorbed besides particular organic matter provides a significant nutritional pool. Auto-ecological studies of selective feeding by deposit feeders (e.g. Stamhuis *et al.*, 1998) have shown that specific gravity of particles is used to separate the light and more nutritious (Mayer *et al.*, 1993) particle fraction from the less nutritious fraction. Selective feeding on the basis of differences in specific gravity may be restricted to fine-sand habitats with a certain content of discrete low density particles (e.g. FF) and may be not effective in more homogeneous muddy sediments with mainly adsorbed organic matter (e.g. SK). Since most organisms at SK are deposit feeders, they would have to deal with the low % EHAA:THAA in their guts and digestion would be limited by the comparable low fluid/solid ratios in their guts compared to the EHAA-assay used in this study. This may be an explanation of the high contents of EHAA present in sediments with a low nutritional quality and low macrofauna biomass.

e. Molecular compounds of the diet

The diet of deposit feeders usually consists of a variety of organic particulates (diatoms, bacteria, detritus, encrusted mineral grains etc.). Budgetary calculations revealed that the nutritional needs of deposit feeders cannot be fulfilled by living organic matter alone and that the major fraction of the bioavailable pool is supplied by nonliving organic matter bound to mineral particles (Mayer, 1989). Even though the assimilation efficiency toward bacteria is much higher than that toward nonliving organic matter, bacteria are only a minor carbon source for deposit feeders (Cammen, 1980). Taghon (1989) stressed that not only the energy yield of the food is relevant for deposit-feeder nutrition, but also the content of essential nutrients (such as nitrogen, protein and essential amino acids). It has been hypothesized that deposit feeders obtain different components of the diet from different food sources; e.g., calories from sedimentary organic matter and protein from bacteria (Findlay and Tenore, 1982). Marsh *et al.* (1989) also emphasized the importance of micronutrients in deposit feeder nutrition and showed that the growth rate of the polychaete

Capitella sp.I fed on a variety of food types was positively correlated with the level of essential amino acids.

High liberation efficiencies of digestive enzymes (e.g. protease-k) toward essential amino acids may hint on a mechanism to fulfill the deposit feeder needs for specific nutrients, since this group of amino acids cannot be synthesized and consequently has to be taken up with the food. The highest liberation efficiencies (60–85% of the THAA) in sediments were reached by most of the essential amino acids (leucine, threonine, isoleucine and methionine (only GB)) and the acidic amino acids (glutamic- and aspartic acid) (Fig. 6), leading to an enrichment of essential amino acids (up to 8%) in the EHAA compared to THAA pool (Fig. 7). The enrichment of essential amino acids in the EHAA pool is more evident in the relatively labile organic matter at FF and GB compared to the refractory organic matter at SK (<2% more essential amino acids in EHAA). Protease-k can liberate the essential amino acids more effectively from sediments containing a large fraction of labile organic matter than from sediments containing highly degraded material. In the latter, the most easily accessible amino acids have already been removed by heterotrophs.

The following factors play a role in the obvious differences between THAA and EHAA spectra of marine sedimentary organic matter (Fig. 6). Firstly, the cleavage pattern of protease-k. Proteolytic enzymes are rather specific in terms of the type of bonds they can break and in the geometric conditions necessary for them to act on the substrate (Mayer, 1989). Consequently, the liberation pattern of a protease is determined by its composition. Trypsin-type compounds cut mainly at the peptide bond adjacent to basic amino acids (which are all essential), and chymotrypsin cleaves the aromatic ends (phenylalanine is essential, tyrosine not). Lan and Pan (1993) found that protease isolated from the midgut gland of the shrimp *Penaeus* sp. contained a mixture of carboxypeptidases, aminopeptidases and trypsin-type enzymes. Mayer *et al.* (1995) showed that the cleavage pattern of protease-k resembles that of a deposit-feeding sea cucumber and is therefore probably representative for marine benthos. The protease-k approach measures only unprotected amino acids that are not surrounded by membranes, because this enzyme is incapable of cleaving the rigid skeletons of eubacterial cell walls. This results in the protection of amino acids that are incorporated in structural matrixes. This may explain the low availability of glycine, that is very common in cell walls and its association with lipids makes it difficult to digest. Glycine also accumulates in the THAA pool during long-term degradation (Dauwe and Middelburg 1998). The amino sugars are building parts of bacterial cell wall polymers (murein) and the chitinous exoskeleton of zooplankton. The common resistance of mureins towards peptidases, explains why no glucosamine has been found in the EHAA pool (Fig. 6). Living heterotrophic organisms generally use specialized enzymes such as lysozyme to enzymatically destroy microbial cell membranes to make the cytoplasm available for assimilation (Plante and Mayer, 1994). Non-protein compounds such as β -alanine and γ -aminobutyric acid in bacteria and sediment are also not degraded by proteinase (Fig. 6). Finally, protection of amino acids by adsorption to minerals or

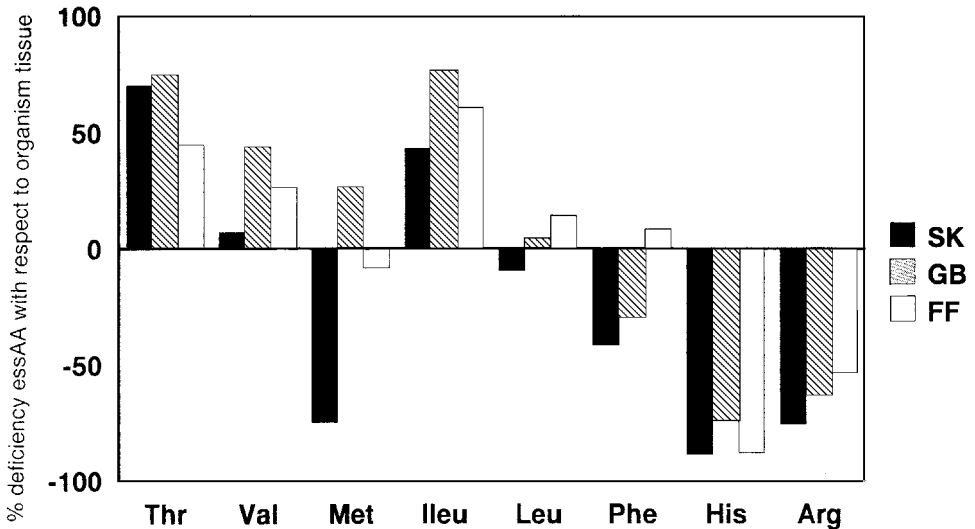


Figure 8. Percent deficiency of essential amino acids in the EHAA-fraction in North Sea sediments with respect to the composition of “marine organism tissue” (Phillips, 1984).

sedimentary organic matter may also affect EHAA spectra. Basic amino acids (histidine and arginine) are adsorbed much stronger to sedimentary materials than acidic and neutral amino acids (Henrichs and Sugai, 1993; Sugai and Henrichs, 1992). It seems that these functional group effects influence the digestibility toward protease in sediments, since acidic amino acids (glutamic- and aspartic acid) are liberated very easily by protease-k and basic amino acids are liberated poorly from sedimentary organic matter.

Essential amino acids are not necessarily limiting deposit feeder growth, because only those that occur in smaller proportions in the food (e.g. sedimentary organic matter) compared to animal body tissue are deficient and have to be taken up preferentially by the animals (Phillips, 1984). In North Sea sediments, arginine and histidine are most deficient with respect to a composite “marine organism tissue” (Phillips, 1984) (Fig. 8). Phillips (1984) also found these essential amino acids to be deficient with respect to other potential food sources such as microalgae, fungi, detritus and suspended particulate material. Mayer *et al.* (1995) reported that glycine, tyrosine, arginine and methionine were deficient in the sedimentary EHAA pool with respect to the tissue composition of a deposit feeding sea cucumber (*P. californicus*). It is possible that deficiencies of particular amino acids can be compensated by an increased enzymatic degradation efficiency and a higher absorption efficiency of the gut wall, as has been suggested for methionine (Mayer *et al.*, 1995). Furthermore, these authors argued that tissue concentration should be corrected for different amino acid residence times; e.g., glycine is a structural component of fibrous proteins with long residence times. The most deficient essential amino acids in marine sediments have in common that they are basic amino acids. Lan and Pan (1993) found that the sum of basic amino acids and the sum of aromatic amino acids had a positive

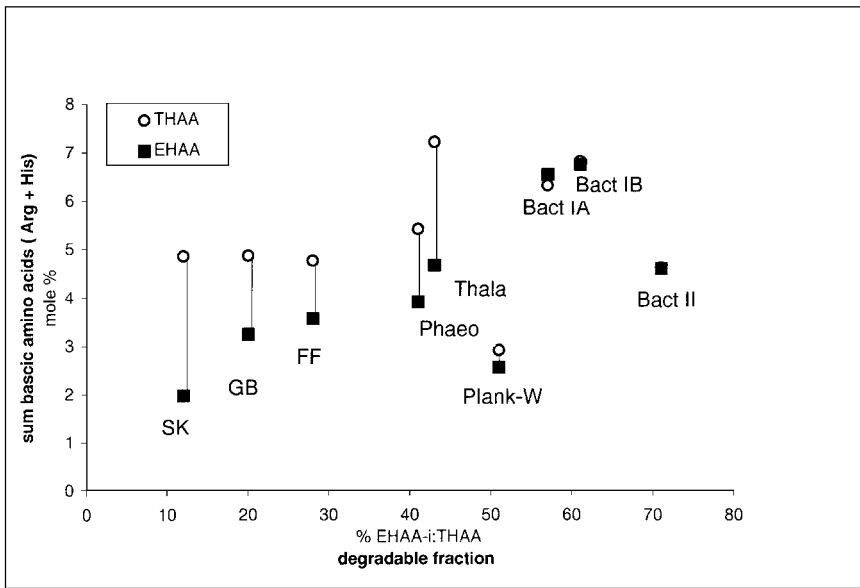


Figure 9. Sum of the basic amino acids (arginine and histidine) versus the relative size of the enzymatically degradable pool in the EHAA-fraction (solid rectangles) and in the THAA fraction (open circles).

correlation with the *in vitro* digestibility of the investigated protein food sources for shrimp. Marsh *et al.* (1989) found the highest significant correlation of growth rates with histidine (basic) and phenylalanine (aromatic) levels in their diets. Plotting the sum of the identified basic amino acids (% arginine and histidine) in the EHAA fraction against the food quality estimated by %EHAA:THAA over the range of sediments, bacteria and algae, we also found a general positive correlation between food quality and the sum of basic amino acids (Fig. 9). However, this relation was not clear in the acid hydrolyzable fraction because of rather constant values in the sediment and algae group. Accordingly, the basic amino acids histidine and arginine may be limiting components in sedimentary organic matter for deposit feeder growth. The basic amino acids probably have to be supplied by other food sources, such as bacteria and algae that can be assimilated very efficiently by deposit feeders (Bianchi and Levinton, 1984). The histidine concentration is elevated three times in EHAA from bacteria compared to algae and sediment, and also the arginine concentration is higher. Carnivory may be a solution in areas with poor food quality of the sedimentary organic matter. The North sea sediments containing low quality organic matter (SK), indeed showed the highest numerical and biomass contribution of predators (~20% of total macrofauna numbers and ~10% of total macrofauna biomass) compared to the other stations (Dauwe *et al.*, 1998). Féral (1989) also found that live food was most readily digestible (*Artemia* sp.), whereas protein sources with rigid cell walls showed lowest digestibility. He also found that the capacity of digestive enzymes is different for different

diets, leading to a clearly more efficient digestion in carnivores compared to deposit- or suspension feeders. The specificity of digestive enzymes toward essential micronutrients is a necessary co-evolution of the deposit-feeder digestive enzymes and the composition of their food items.

Finally, it should be kept in mind that “bioavailability” is not an intrinsic chemical characteristic of the food source alone, but also depends on the specific nutritional needs of deposit feeders and their ingestive and digestive capabilities. Future approaches estimating the nutritional value of organic matter to benthos should account for the energetic - as well as for the micronutrient requirements of organisms.

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