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Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter

Rainer M. W. Amon[1] *and Hans-Peter Fitznar*
Alfred Wegener Institute for Polar and Marine Research, Department of Biological Oceanography, Postfach 120161, D-27515 Bremerhaven, Germany

Ronald Benner
Department of Biological Sciences and Marine Science Program, University of South Carolina, Columbia, South Carolina 29208

Abstract

Bacterial growth and the chemical composition of dissolved organic matter (DOM) were followed during a 10-d decomposition experiment with fresh, algal-derived DOM from an Arctic ice floe. During the experiment ~30% of the dissolved organic carbon (DOC) was used by bacteria, indicating the highly reactive nature of this fresh DOM. Over half of the DOC consumption was accounted for as losses of combined neutral sugars and amino acids. The initial composition of the DOM was characterized by high neutral sugar (14% DOC) and amino acid (7.4% DOC) yields and the dominance of glucose (~75 mol%) and glutamic acid (~25 mol%). During microbial degradation the neutral sugar and amino acid yields decreased, and the molecular composition of the DOM became more uniform. The relatively constant abundance of D amino acids and the dramatic changes in the neutral sugar and amino acid compositions indicated that bacteria were important in shaping the chemical composition of marine DOM by selectively removing bioreactive components and by leaving behind biorefractory components. Based on principal component analysis and other parameters, neutral sugars and amino acids were found to be excellent indicators of the diagenetic state and bioavailability of marine DOM.

The interactions between bacteria and marine dissolved organic matter (DOM), one of the largest active reservoirs of organic carbon, play a major role in the global carbon cycle. Our understanding of the origin, composition, and reactivity of DOM in the ocean is still very limited. The majority of marine DOM has not been characterized on a molecular level, is very resistant to degradation, and appears to be of low molecular weight (Benner et al. 1992; Ogawa and Ogura 1992; Amon and Benner 1994). In order to account for the low molecular weight, low bioavailability, and uncharacterized nature of DOM, Amon and Benner (1996) proposed the size-reactivity continuum model. This conceptual model links the physical size of organic matter to its diagenetic state, suggesting a decrease in size with increasing diagenesis and chemical alteration. The model provides a framework for interpreting diagenetic alterations but it does not explain the mechanism for the production of biorefractory low molecular weight DOM. The concept of a diagenetic continuum (Cowie and Hedges 1994; Hedges and Oades 1997; Keil et al. in press) linking chemical composition to diagenetic trends has been proposed, but these studies are of a geochemical nature and only indirectly link observed diagenetic trends to specific processes, like microbial activity.

In the present study, changes in the chemical composition of algal-derived DOM were investigated during microbial decomposition under controlled conditions. An Arctic ice floe inhabited by algae was used as a source of relatively fresh and labile DOM. Previous studies (Thomas et al. 1995, in press) indicated high concentrations of DOC along with high bacterial activity in sea ice. Changes in concentrations and compositions of neutral sugar and amino acid were investigated because these two compound classes typically comprise a major fraction of cellular material. Amino acids and neutral sugars have been the focus of numerous studies, providing the opportunity for larger scale comparisons of diagenetic trends.

Material and methods

Sampling—During the research cruise ARK XIII/3 on the RV *Polarstern* in September 1997, we collected a piece of a multiyear ice floe (0.3 m^3), using a wire basket suspended from a crane. The ice was collected in the Fram Strait at 80°53.5′N and 2°34.2′W. After collection the ice was put into an acid-rinsed polyethylene tray, brought inside the laboratory, and thawed at room temperature. The melt water was filtered through a 0.6-μm pore size polycarbonate filter cartridge as it thawed. The ice was inhabited by ice algae, as indicated by a greenish-brown color.

[1] Corresponding author (ramon@awi-bremerhaven.de).

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The experiment was carried out in batch cultures. The filtered ice-floe water was placed into polycarbonate containers and sealed without a headspace using silicon stoppers. One sample and one reservoir bottle were connected by Teflon tubing and sampling was carried out following the protocol described by Amon and Benner (1996). The experiment was performed in triplicate with one treatment being amended with inorganic nutrients to reach a final concentration of 90 μM nitrate and 11 μM phosphate. The samples were incubated in the dark between 0 and 2°C for 10 d. Subsamples for a variety of measurements (dissolved oxygen, dissolved organic carbon, bacterial abundance, bacterial leucine incorporation, nutrients, neutral sugars, and D/L amino acids) were taken approximately every 48 h to follow bacterial growth and DOM decomposition.

Measurements—Bacterial abundance was determined by epifluorescence microscopy using DAPI as a stain (Porter and Feig 1980). Samples (4 ml) were preserved with formaldehyde (4% final concentration) and counted within 6 months of collection.

Bacterial growth was estimated from rates of radiolabeled leucine incorporation (Kirchman et al. 1985). Duplicate water samples (10 ml) and one killed control were incubated with 20 nM (final concentration) of [4, 5-^{3}H] leucine (specific activity of 153 Ci mmol^{-1}) for 1 h in the dark at 0–2°C. Incubated samples were filtered onto a 0.2-μm MF filter (Nuclepore), and filters were oxidized in a biological oxidizer (OX-500, Zinsser Analytic) and measured in a liquid scintillation counter (Packard 2550 TR/LL, Amon and Benner 1998).

Dissolved organic carbon (DOC) was measured using the high temperature combustion method and a Shimadzu TOC 5000 analyzer (Benner and Strom 1993). Samples for DOC determinations were stored in 125-ml Teflon-lined polyethylene flasks (FLPE, Nalgene) at −20°C until analysis at the home laboratory. Bacterial cells were removed by filtering the sample through an acid-rinsed polycarbonate filter (0.2-μm pore size).

Oxygen concentrations were determined by Winkler titration using an autotitration system (SIS GMBH) with photometric endpoint detection. The system consists of a Metrohm Dosimat 665 and a Metrohm 649 single beam photometer. Samples for oxygen determination were collected in acid-washed and rinsed quartz BOD bottles (115 ml). Respiration rates were calculated between the initial and the final time point using linear regression analysis.

The concentration of individual neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose, and xylose) in hydrolyzed water samples was determined using a Dionex 500 anion-exchange chromatography system with pulsed amperometric detection (PAD) following the procedure described by Skoog and Benner (1997). Briefly, filtered (0.2 μm) water samples (9 ml) were brought to dryness in a SpeedVac system (Savant). One milliliter H_2SO_4 (72 w/w%) was added to the dry samples and mixed in an ultrasonic bath (15 min). After a total of 120 min, each sample solution received 9 ml of water (Milli-Q-UV Plus) and was transferred to a glass ampoule. Sealed ampoules were placed into a water bath (100°C), and samples were hydrolyzed for 3 h. Before analysis, hydrolyzed samples (10 ml) were neutralized in 1.44 g precombusted $CaCO_3$. The residual standard deviation including the sample preparation was between 5 and 30%. Recoveries for the individual sugars ranged between 70 and 90% (Skoog and Benner 1997).

The D and L enantiomers of individual amino acids (DL-aspartic acid, DL-glutamic acid, L-serine, L-threonine, glycine, L-arginine, DL-alanine, γ amino butyric acid, L-tyrosine, L-valine, L-phenylalanine, L-isoleucine, L-leucine) were determined by HPLC and precolumn derivatization with *o*-phthaldialdehyde and N-isobutyrylcystein after hydrolysis following the procedure described by Fitznar et al. (1999). In this study, we employed two derivatizing agents (n-isobutyryl-L-cysteine and n-isobutyryl-D-cysteine) to determine D and L amino acids. We only report amino acids that were reproducibly identified with both reagents, and only those D enantiomers that were significantly different from the racemization blank. The residual standard deviation for this analysis typically lies between 1 and 8% for the individual amino acids. Filtered (0.2 μm) water samples (10 ml) were mixed with 10 ml HCl (32%) and hydrolyzed in sealed glass ampoules for 24 h at 110°C. After hydrolysis the samples were neutralized with 8.9 ml borate buffer (0.5 M) and the pH was adjusted to 8.5 with 32% NaOH. In addition to the water samples, we obtained peptidoglycan (purified cell-wall preparation from *Staphylococcus aureus*: Fluka®) and eight bacterial cultures (courtesy of E. Helmke) isolated from the Arctic Ocean were also analyzed. The bacterial cell cultures were centrifuged, washed, pelleted, and dried. Dried bacterial cells and peptidoglycan were hydrolyzed in HCl (16%) and analyzed for D/L amino acids along with the time course samples.

Principal component analysis (PCA) was performed using the mol% compositions of individual neutral sugars and amino acids as the original data matrix and the Systat® statistical package. The main application of PCA is to reduce the number of variables and to detect structure in the relationships between variables. We used PCA in order to determine whether bacterial decomposition of DOM produces systematic differences in the molar composition of neutral sugars and amino acids. We expected PCA to order the time course samples according to their diagenetic state.

Results and discussion

The initial concentration of DOC in ice-floe water was high (112 μM), and relatively high concentrations of total hydrolyzable neutral sugars (THNS) and amino acids (THAA) were also measured (Table 1). The large contribution of neutral sugar and amino acid carbon to total DOC (represented as neutral sugar and amino acid yields) is indicative of the fresh nature of this DOM, which was most likely produced by ice algae. The predominantly algal origin of this DOM is supported by the high abundance of algae in the ice floe. The sea-ice community was dominated by the dinoflagellate *Echinum majus* and a mix of several diatoms (*Melosira sp.*, *Nitschia sp.*, J. Okolotkov pers. comm.). The initial DOC concentration (112 μM C, Table 1) is typical for water derived from Arctic sea ice inhabited by algae

Table 1. Initial composition of dissolved organic matter in sea ice melt water.

DOC (μM)	Total neutral sugars (nM)	Neutral sugar yield (% of DOC)	Total amino acids (nM)	Amino acid yield (% of DOC)	D amino acids (nM)	D amino acid yield (% of DOC)
112 ± 5 (SD)	2,620 ± 170 (SD)	14	1,831 ± 35 (SD)	7.4	68 ± 8 (SD)	0.25

(Bunch and Harland 1990; Thomas et al. 1995) but is also similar to DOC concentrations found in surface waters during phytoplankton blooms (Ittekkot 1982). Concentrations of THNS and THAA were 2,620 nM and 1,831 nM, respectively, contributing 21% to the DOC. These concentrations are higher than ambient concentrations of THNS (Rich et al. 1997; Amon and Benner in prep.) and THAA (Hubberten et al. 1995; Fitznar 1999) reported for Arctic Ocean surface waters. This also indicates that most of the sea-ice DOM was produced within the ice. The high neutral sugar yield (14% DOC) in the sea-ice DOM is comparable to neutral sugar yields in fresh phytoplankton-derived DOM reported by Biersmith and Benner (1998). Neutral sugar yield has been suggested as a robust indicator of the diagenetic state of organic matter, with relatively high yields representing fresh material (Cowie and Hedges 1994; Skoog and Benner 1997). The concentration of D amino acids was low (*see Table 1*), and the D/L ratios of alanine and glutamic acid were also low (*see Table 5*) and are indicative of fresh, algal-derived DOM (Jørgensen et al. 1999).

The concentration and chemical composition of sea-ice DOM indicated that it was freshly produced by marine algae (Ittekkot 1982; Biersmith and Benner 1998; Jørgensen et al. 1999). Therefore it appears that the sea-ice DOM collected for this study is representative of fresh, algal-derived, marine DOM.

Bacterial growth and bulk parameters during decomposition—In this experiment bacterial growth was characterized by an initial lag phase of about 50 h (Fig. 1), likely caused by the low initial cell concentration (7–9 $\times$ 10^4 cells ml^{-1}). After the lag phase bacterial growth and DOM decomposition increased rapidly as indicated by oxygen consumption, DOC consumption, bacterial leucine incorporation, and bacterial abundance (Fig. 1A–1D). There was no difference between treatments with and without nutrient additions, which indicates the high nutritional quality of the initial organic substrate (data not shown).

The respiration was 0.15 μM O_2 h^{-1}, a rate similar to those measured in coastal waters of temperate regions (Biddanda et al. 1994) but much higher than respiration rates measured in Arctic Ocean surface waters (Cota et al. 1996; Amon unpubl. data). During the 220 h experiment, 34 μM or 30% of the initial DOC was degraded by bacteria, resulting in a final DOC concentration of ~75 μM (Fig. 1B). Bacterial abundance increased by an order of magnitude to 9 $\times$ 10^5 cells ml^{-1}. Bacterial leucine incorporation, a proxy for bacterial protein synthesis, increased from 0.01 nM leu-

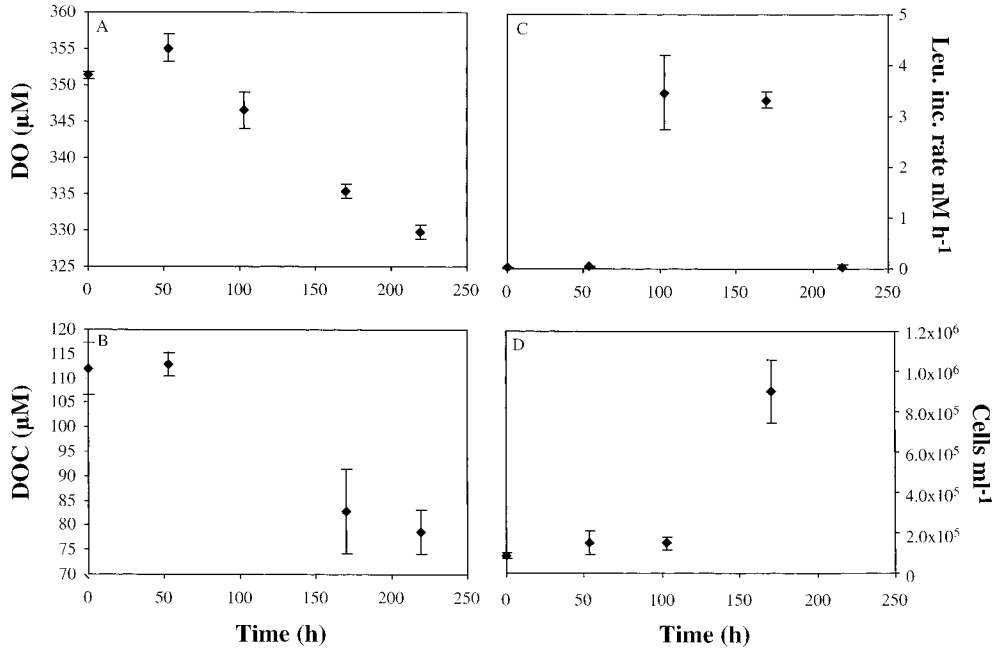

Fig. 1. (A) Dissolved oxygen, (B) dissolved organic carbon, (C) bacterial leucine incorporation, and (D) bacterial abundance during decomposition of sea-ice DOM. Each data point represents the mean of three replicates and error bars represent the respective standard deviation.

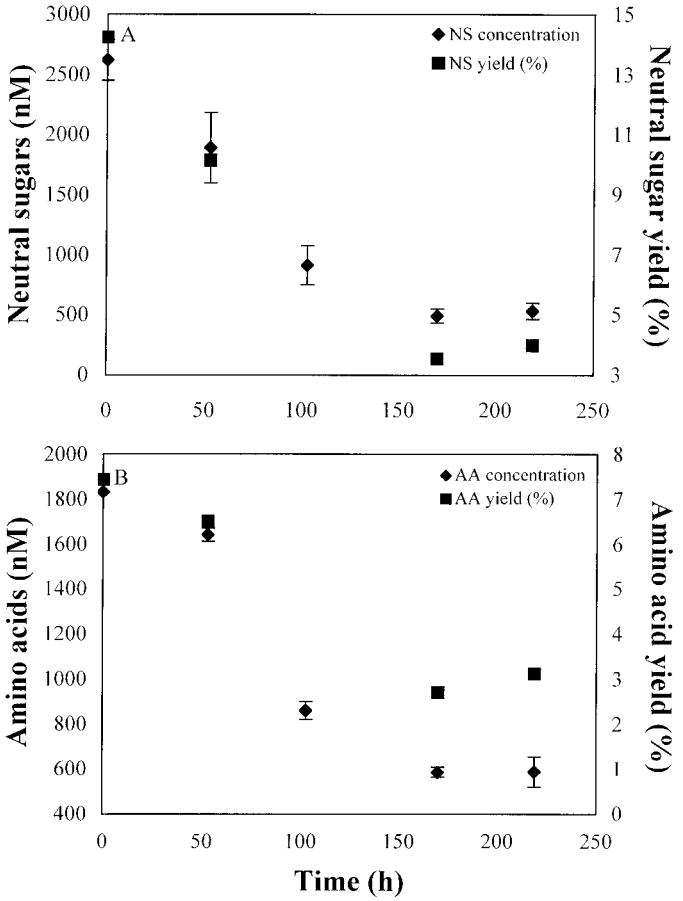

Fig. 2. (A) Concentration and yield of neutral sugars and (B) amino acids during decomposition of sea-ice derived DOM. Each data point represents the mean of three replicates and error bars represent the respective standard deviation.

cine h^{-1} during the lag phase to maximal values of 4.2 nM leucine h^{-1}. High bacterial production rates in sea ice have been reported by several investigators (Bunch and Harland 1990; Grossmann and Dieckmann 1994). These peak values during sea-ice DOM decomposition are as high as values usually measured in productive coastal waters (Chin-Leo and Benner 1992; Amon and Benner 1998). This experiment was conducted at 0–2°C, and the extremely low temperature observed in sea-ice brine pockets (down to −15°C, Weeks and Ackley 1982) could result in lower bacterial activity under natural conditions in sea ice. This, along with the extended light cycle during the Arctic summer (more time for photosynthesis), could explain the high concentration of DOM in sea ice. The decrease in bacterial activity after 170 h is likely due to the consumption of the bioreactive components of the DOM.

Use of THNS and THAA—Results of this experiment confirm the highly reactive nature of THNS. The concentration of THNS decreased by 80%, from 2,620 to 531 nM (Fig. 2A, Table 2). Neutral sugar yields decreased from ~14% DOC to below 5% DOC, which indicates the preferential use of neutral sugar carbon relative to bulk DOC (Fig. 2A). This is consistent with previous studies of neutral sugar yields in sediment trap samples, sediments, and DOM (Cowie and Hedges 1984, 1994; Hernes et al. 1996; Skoog and Benner 1997).

Like neutral sugars, THAA were rapidly used by the bacterial community with concentrations decreasing by 68%, from 1,830 nM to ~550 nM THAA (Fig. 2B). The preferential decomposition of THAA relative to bulk DOM is indicated by a decrease of the amino acid yield from 7.4% to ~3% DOC (Fig. 2B). This demonstrates that amino acid yield is also a good diagenetic indicator, analogous to neutral sugar yield. It is interesting to note that THNS were used at a higher rate (66 nM C h^{-1}) than THAA (40 nM C h^{-1}), which indicates the higher overall bioreactivity of neutral sugars relative to amino acids. This experiment demonstrates that microbial degradation of fresh, algal-derived DOM results in the preferential removal of combined neutral sugars and amino acids and leads to a decrease in neutral sugar and amino acid yields.

Changes in the molecular composition of DOM during bacterial degradation—Information on changes of the molecular composition of neutral sugars and amino acids during decomposition are primarily derived from geochemical studies of particulate and sedimentary organic matter diagenesis (Cowie and Hedges 1984, 1992, 1994; Dauwe and Middelburg 1998; Dauwe et al. 1999; Opsahl and Benner 1999; Keil et al. in press). Similar information on the molecular composition of neutral sugars and, especially, amino acids during natural DOM decomposition is sparse (Meon and Kirchman, pers. comm.).

Table 2. Concentrations (nM) of individual neutral sugars, sum of all neutral sugars (nM), and neutral sugar yield (% of DOC) during DOM decomposition. Fuc, fucose; Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; NS, neutral sugars.

Time (h)	Fuc	Rha	Ara	Gal	Glc	Man	Xyl	Sum of NS	NS yield*
0	32	31	61	275	1,938	177	105	2,620	14.25
53	32	29	37	111	1,549	110	53	1,890	10.15
103	32	31	28	80	575	118	64	911	nd
170	31	32	19	65	179	128	52	491	3.54
219	38	37	15	68	213	112	48	531	3.97
Avg. SD†	±2	±2	±2	±45	±84	±21	±24	NA	NA

* Neutral sugar yield = (neutral sugar C/total DOC) × 100.
† Average standard deviation for the individual neutral sugars.

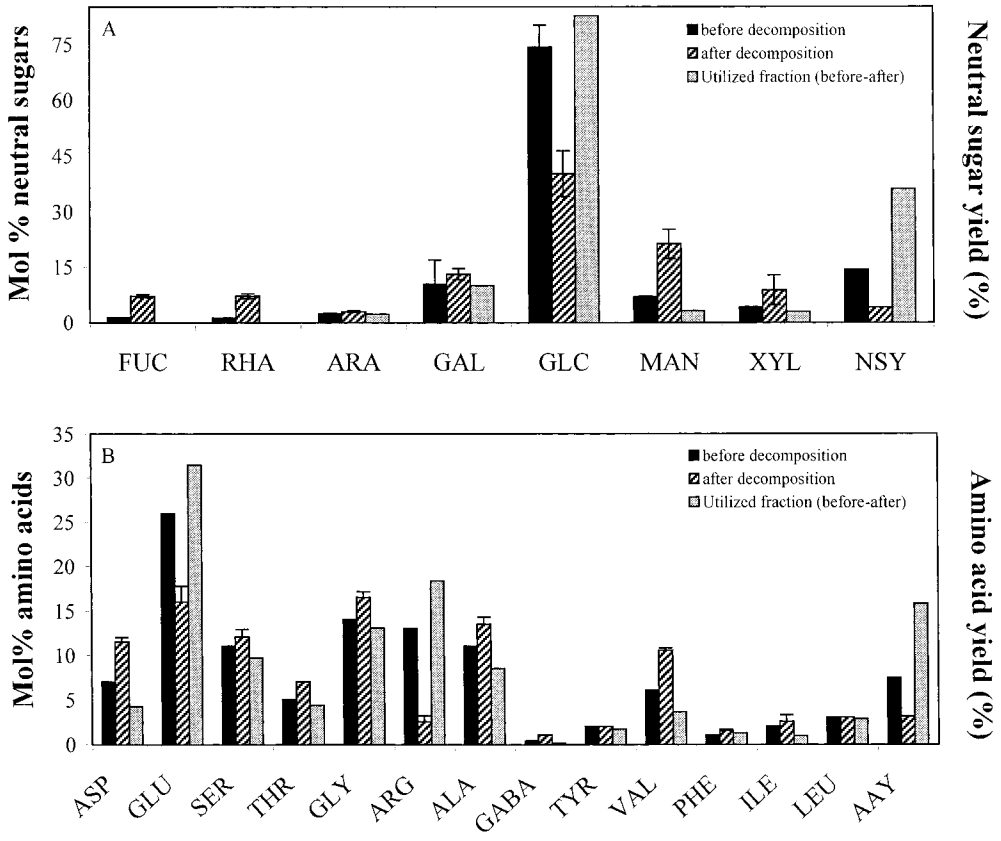

Fig. 3. (A) Changes in the mol% composition and yields of neutral sugars, and (B) amino acids during decomposition of sea-ice DOM. The used fraction was calculated by subtracting the neutral sugar and amino acid concentrations at the end of the experiment (220 h) from the concentrations at the beginning of the experiment (0 h). Abbreviations as in Tables 2 and 3 except neutral sugar yield (NSY) and amino acid yield (AAY).

Major decreases in the concentrations of arabinose, galactose, glucose, xylose, and mannose were observed during bacterial degradation, whereas the measured concentrations of deoxysugars (fucose and rhamnose) did not decrease (Table 2). The initial neutral sugar composition in the experiment was characterized by the dominance of glucose (>75 mol%) with minor contributions (<10 mol%) of the other neutral sugars (Fig. 3A). Glucose was the dominant neutral sugar in DOM harvested from a culture of the diatom, *Skeletonema sp.* (Biersmith and Benner 1998) and in DOM from phytoplankton blooms in the North Sea (Ittekkot 1982) and the Mississippi River plume (Benner and Opsahl in press). The two most striking changes in neutral sugar composition during decomposition were the rapid decrease in the mol% glucose, which results in a more heterogeneous neutral sugar composition and the increase in mol% deoxysugars (fucose and rhamnose) as decomposition progressed (Fig. 3A). A similar shift from glucose dominance of the neutral sugar composition to a more heterogeneous sugar composition was observed during the microbial degradation of vascular plant detritus (Opsahl and Benner 1999). A relative increase of deoxysugars during decomposition has been reported for several organic matter types and environments (Cowie and Hedges 1984; Frimmel 1998; Opsahl and Benner 1999), and it is often attributed to the abundance of fucose and rhamnose in bacteria.

The composition of the used (bioreactive) fraction of DOM can be calculated from the difference in neutral sugar concentrations at beginning and end of the experiment (Fig. 3A). The used or bioreactive DOM had a neutral sugar yield of 36%, indicating that more than one-third of the used DOC was neutral sugars. The neutral sugar composition of the bioreactive DOM was enriched in glucose and depleted in deoxysugars and mannose relative to the initial DOM (Fig. 3A). This indicates that individual neutral sugars are used to a different extent and on a different time scale and suggests that individual neutral sugars are associated with various biopolymers. Thus, the individual neutral sugars can originate from a number of macromolecules with varying reactivities (Cowie and Hedges 1984; Hernes et al. 1996; Boon et al. 1998).

The initial amino acid composition of sea-ice DOM was characterized by the dominance of glutamic acid (>25 mol%). Glycine, arginine, alanine, and serine contributed between 10 and 14 mol% each, and the other amino acids were typically less abundant (<7 mol%; Table 3, Fig. 3B). As with neutral sugars, the use of individual amino acids was not uniform. For example, 94%, 88%, and 81% of arginine,

Table 3. Concentrations (nM) of individual amino acids, sum of all amino acids (nM), and amino acid yield* (% of DOC) during DOM decomposition. Asp, aspartic acid; Glu, glutamic acid; Ser, serine; Thr, threonine; Gly, glycine; Arg, arginine; Ala, alanine; Gaba, γ-aminobutyric acid; Tyr, tyrosine; Val, valine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; AA, amino acid.

Time (h)	Asp	Glu	Ser	Thr	Gly	Arg	Ala	Gaba	Tyr	Val	Phe	Ile	Leu	Sum of AA	AA yield*
0	118	465	191	95	252	233	193	7	27	142	24	29	54	1,831	7.43
53	116	498	116	92	179	210	151	8	25	148	23	27	50	1,640	6.50
103	71	182	84	43	120	19	95	14	7	181	9	13	21	859	ND
170	58	110	59	36	88	13	76	6	6	96	8	13	17	586	2.70
219	64	87	66	39	89	15	79	6	6	96	8	14	18	586	3.12
Avg. SD†	±5	±17	±7	±3	±6	±4	±9	±2	±3	±3	±1	±3	±4	—	—

* AA yield = (amino acid carbon/total DOC) × 100.
† Average standard deviation for the individual amino acids.

tyrosine, and glutamic acid, respectively, were used. Most other amino acids decreased by ~65% during decomposition. Only aspartic acid and valine decreased by less than 50% (Table 3). The most pronounced changes in amino acid composition during DOM degradation were large decreases of arginine and glutamic acid and relative increases in aspartic acid, serine, threonine, glycine, alanine, and valine (Fig. 3B). In a recent review of the early diagenesis of particulate organic matter in the marine environment, Keil et al. (in press) summarized the most important patterns for amino acids. They stated that during the early stages of degradation organic matter becomes enriched in glycine, threonine, serine, and arginine. Except for arginine, this agrees well with the diagenetic trend we observed during DOM degradation. Colombo et al. (1998) and Keil et al. (in press) argue that glycine, threonine, and serine enrichment could be explained by their abundance in biogenic matrices (e.g., silicates, Hecky et al. 1973). Does the enrichment of glycine, serine, and threonine in degraded DOM indicate the existence of biorefractory dissolved organic-silicate complexes? This would have a major influence on our understanding of the oceanic carbon and silicate cycles and needs to be investigated further.

The amino acid composition of the bioreactive DOM was dominated by glutamic acid (32 mol%), arginine (18 mol%), glycine (13 mol%), and serine (10 mol%). All other amino acids were below 5 mol% (Fig. 3B). Amino acid carbon contributed ~16% to the DOC used during DOM decomposition.

Table 4. Concentration (nM) of individual D amino acids, sum of D amino acids (nM), relative (%) contribution of D amino acids to total amino acids, and D amino acid yields (% of DOC) during DOM decomposition.

Time (h)	D-Asp	D-Glu	D-Ala	Sum of D-AA	% D-AA	D-AA yield*
0	25	15	20	63	3.4	0.24
53	25	22	19	66	4.0	0.24
103	24	17	24	65	7.6	nd
170	23	16	18	57	9.7	0.26
219	25	15	21	61	10.4	0.32
Avg. SD†	±2	±2	±7	—	—	—

* D-AA yield = (D-AA carbon/total DOC) × 100.
† Average standard deviation of the individual D amino acids.

D amino acids, unlike L amino acids, are source specific and therefore have the potential to indicate organic matter origin as well as diagenetic state. Several studies (Lee and Bada 1977; McCarthy et al. 1998; Fitznar 1999) indicate that bacteria are an important source for D amino acids and DOM in the ocean. D amino acids are abundant in the cell wall of eubacteria (Schleifer and Kandler 1972; McCarthy et al. 1998), and they have also been detected in Archaea (Nagata et al. 1998, 1999). In this experiment the concentration of total D amino acids was 57–66 nM and did not show significant changes during decomposition (Table 4). Thus, D amino acids were present in the sea ice, and they were derived either from the seawater that formed the sea ice or from sea-ice bacteria. There was no net increase in D amino acid concentrations during the experiment, but the D amino acid yield increased during decomposition, which reflects the refractory nature of molecules containing D amino acids (Table 4). The D amino acid yield could therefore also be used as a diagenetic indicator, with higher yields in more diagenetically altered material. Ratios of amino acid enantiomers have recently been correlated with DOM bioavailability (Jørgensen et al. 1999), with low ratios indicating high DOM bioreactivity. Jørgensen et al. (1999) also reported increasing D/L amino acid ratios during incubation experiments, which is consistent with the results observed in the present study.

The three most abundant D amino acids detected during

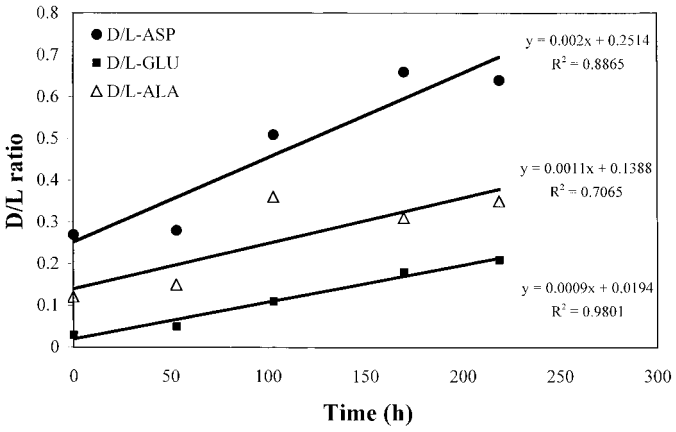

Fig. 4. D/L amino acid ratios for aspartic acid, glutamic acid, and alanine during decomposition of fresh DOM.

Table 5. D/L-amino acid ratios during a DOM degradation experiment, in DOM from surface waters of different oceanic regions, in cultured Arctic bacteria, cyanobacteria, and in commercially available peptidoglycan.

Sample	Asp	Ala	Glu	Reference
Fresh DOM	0.27	0.12	0.03	This study
After 53 h	0.28	0.15	0.05	This study
After 103 h	0.51	0.36	0.11	This study
After 170 h	0.66	0.31	0.18	This study
After 219 h	0.64	0.36	0.21	This study
Arctic Ocean	0.29	0.31	0.17	Fitznar (1999)
Baltic Sea	—	0.16–0.33	0.12–0.27	Jørgensen et al. (1999)
Gulf of Mexico	0.18	0.47	0.13	McCarthy et al. (1998)*
Central Pacific	0.39	0.52	0.23	McCarthy et al. (1998)*
North Sea	0.17	0.37	0.12	McCarthy et al. (1998)*
S. bacillaris	0.14	0.38	0.09	McCarthy et al. (1998)
Arctic bacteria	0.12	0.11	0.13	This study
Peptidoglycan	0.30	0.44	0.49	This study

* DOM samples were isolated using ultrafiltration and represent ~30% of the DOC in the seawater.

this experiment were D-aspartic acid (41%), D-alanine (34%), and D-glutamic acid (25%). Their relative concentrations (mol%) did not change during the experiment; however, the proportion of D amino acids relative to total amino acids changed dramatically during decomposition (Tables 4 and 5, Fig. 4) due to the rapid use of L amino acids. This indicates that L amino acids and D amino acids cycled on very different time scales in this experiment. A possible explanation for this observation is that most L amino acids are found in labile protein, whereas D amino acids are components of more refractory, bacterial cell-wall macromolecules, like peptidoglycan (Schleifer and Kandler 1972; Nagata 2000, and references therein). However, given the abundance of D amino acids in Archaea (Nagata et al. 1998, 1999), and other cellular components of bacteria, it appears likely that peptidoglycan is not the only important source of D amino acids in marine DOM.

Information on D amino acids in DOM is often presented as D/L amino acid ratios (Bada and Lee 1977; McCarthy et al. 1998; Jørgensen et al. 1999). We summarized some of the available data on D/L amino acid ratios to compare our results with DOM from different oceanic regions, bacteria (whole cells), and peptidoglycan (Table 5). Bacteria have relatively low D/L amino acid ratios, which reflects the dominance of protein-derived amino acids, whereas peptidoglycan has relatively high D/L amino acid ratios (Table 5). The D/L ratios of alanine, glutamic acid, and aspartic acid increased during the course of the experiment, which reflects the relative enrichment of D amino acids during DOM degradation (Table 5, Fig. 4). The shift of D/L amino acid ratios from low values in fresh material to higher values in diagenetically altered DOM (Fig. 4, Table 5) is consistent with the observed patterns of neutral sugar and amino acid composition during decomposition of fresh DOM. The D/L amino acid ratios of alanine and glutamic acid, the two most abundant D amino acids in bacterial peptidoglycan (Schleifer and Kandler 1972) become very similar to the respective D/L amino acid ratios found in marine DOM during the course of the experiment. However, D-aspartic acid is enriched twice as much as D-alanine and D-glutamic acid (*see the slopes of the regression lines in Fig. 4*) during the experiment. The D/L ratios for aspartic acid at the end of the experiment and in one DOM sample (Table 5) are higher than values expected for a typical peptidoglycan (Table 5, Schleifer and Kandler 1972). This could indicate that the different D amino acids (D-Asp, D-Glu, D-Ala) found in the ocean do not occur in the same molecular structure.

The differential enrichment of certain neutral sugars and amino acids, especially D amino acids during DOM decomposition, suggests the abundance of a variety of refractory cellular components with different origins. Potential sources include bacterial cell walls and bacterial capsular envelopes (Boon et al. 1998; Stoderegger and Herndl 1998).

Diagenetic trends in particulate and dissolved organic matter—The relative reactivities of amino acids and neutral sugars during organic matter degradation in sediments differs somewhat from the DOM decomposition results presented here. Although in sediments amino acids are usually more reactive than neutral sugars (Cowie et al. 1992, and references therein), neutral sugars were the more reactive component during the present experiment and in a recent study by Meon and Kirchman (pers. comm.). Amino acids are more abundant than neutral sugars in plankton, the ultimate source of most sedimentary organic matter, whereas neutral sugars are more abundant than amino acids in algal-derived DOM. Perhaps the relative reactivities of these biomolecules is related to their initial abundance. Regardless of this observed difference, there are several similarities in neutral sugar and amino acid diagenetic trends between particulate and dissolved organic matter. Diagenetic alterations of the neutral sugar composition in marine particulate organic matter (POM) are variable, but most available studies indicate relative decreases in glucose and increases in deoxysugars (fucose and rhamnose) during decomposition (Cowie and Hedges 1984; Cowie et al. 1995; Hernes et al. 1996; Opsahl and Benner 1999). The same general trend was observed during the short-term degradation of DOM; however, these trends will be dependent on the source composition, which can be quite variable (Biersmith and Benner 1998).

There has been a wealth of new information concerning diagenesis of amino acids in POM (Colombo et al. 1998; Dauwe and Middelburg 1998; Dauwe et al. 1999; Keil et al. in press). Despite the fact that geochemical studies on the early diagenesis of organic matter typically span annual and decadal time scales, general trends are very similar to what we observed during a short-term (220 h) decomposition experiment. The relative increases of serine, threonine, glycine, and the relative decrease in glutamic acid during DOM decomposition (Fig. 3B) was also reported in a recent review on early diagenesis of particulate amino acids (Keil et al. in press).

A comprehensive study linking amino acid composition to organic matter diagenesis was carried out by Dauwe et al. (1999), who used principal component analysis (PCA) to calculate a degradation index based on the mol% amino acid composition of several organisms and sediments. We used the same PCA (Dauwe et al. 1999) to directly compare our data from the decomposition experiment to their results. This was done using the mol% amino acid data from our experiment and the following formula:

$$\mathrm{DI} = \sum_i \left[\frac{\mathrm{var}_i - \mathrm{AVG\ var}_i}{\mathrm{STD\ var}_i} \right] \times \mathrm{fac.coef}_i$$

where DI is the degradation index, var_i is the individual amino acid (mol%), AVG var_i, STD var_i, and $\mathrm{fac.coef.}_i$ are the respective mean, standard deviation, and factor coefficient from table 1 in Dauwe et al. (1999). Using this approach, we calculated a degradation index for the different time points in our experiment. The calculated degradation indices reflect progressing diagenesis. More negative degradation indices represent a higher degree of decomposition (Fig. 5 inset). This shows that the general diagenetic pattern observed for particulate organic matter also applies to the diagenesis of DOM. To directly compare our DOM data to organic matter sources and organic matter in sediments, we summarized the data given in Dauwe et al. (1999) and Cowie and Hedges (1992) in Fig. 5. A diagenetic sequence going from source material (plankton) with a high degradation index and high percentage AA-N (amino acid nitrogen) to DOM with a low degradation index and low percentage AA-N is clearly indicated (Fig. 5). This pattern needs to be confirmed with a larger data set, but it is consistent with the ''size-reactivity continuum model'' (Amon and Benner 1996), which suggests a relationship between the physical size of organic matter and its diagenetic state. If this experiment is representative for a wider range of marine POM and DOM, the observed pattern also confirms the notion that DOM is a minor source for particle formation (aggregation) in the marine environment (Benner et al. 1997).

Neutral sugar and amino acid compositions as diagenetic indicators—The usefulness of amino acid composition to interpret organic matter diagenesis was demonstrated recently in sediment samples (Dauwe et al. 1998, 1999). We used the same statistical tool, principal component analysis, to determine the usefulness of neutral sugar and amino acid composition as diagenetic indicators for DOM. The calculated degradation index from PCA should reflect decomposition

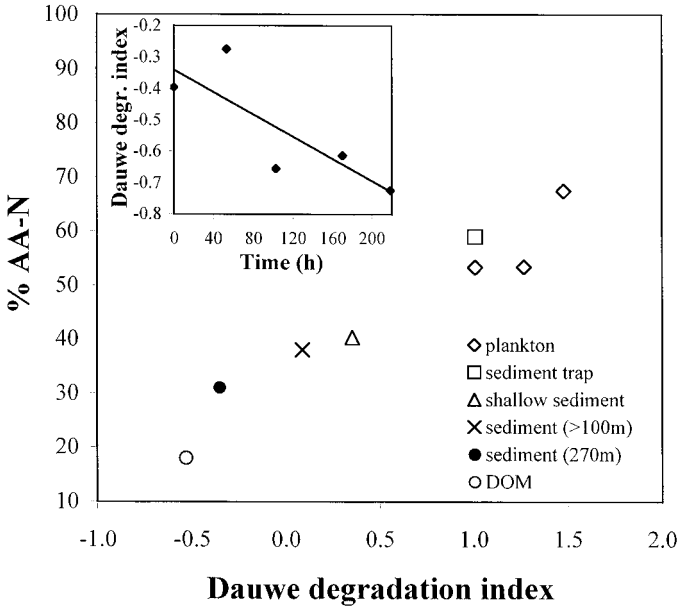

Fig. 5. Diagenetic trend indicating a sequence from fresh plankton, (phytoplankton, $n = 10$; zooplankton, $n = 4$; bacteria, $n = 3$) and sediment trap POM ($n = 27$) to POM from shallow sediments (<40 m water depth, $n = 5$), to POM from deeper sediments (>100 m water depth, $n = 2$), and finally to DOM ($n = 5$). The inset shows the decrease of the Dauwe degradation index with increasing time of DOM degradation. Data were taken from Cowie and Hedges (1992) and Dauwe and Middelburg (1998). Degradation indices for DOM were calculated based on data and the formula given by Dauwe et al. (1999). The Dauwe degradation index and the percentage AA-N represent two independent diagenetic indicators.

time and mirror other independent diagenetic indicators, such as neutral sugar yield. In order to compare neutral sugars and amino acids as diagenetic indicators, we did a separate PCA of both compound classes (Table 6). In each case the diagenetic changes in DOM were reflected by the derived degradation indices (Table 6). In the case of neutral sugars, 90% of the variability was explained by the first principal component axis, which was interpreted to represent organic matter degradation. For amino acids, 65% of the variability was explained by the first principal component axis. This indicates that neutral sugars and amino acids are useful indicators of the diagenetic state of DOM. In a comprehensive PCA including both neutral sugars and amino acids (Table 6), the first principal component axis explained 69% of the variability and the derived degradation indices agreed well with the neutral sugar yield (Table 2).

This simple DOM decomposition experiment demonstrated that bacteria play a crucial role in shaping both the concentration and composition of marine DOM. The increase in relative abundance of deoxysugars and D amino acids indicated that bacteria not only changed the composition of DOM supplied by phytoplankton, but they also produced DOM that is rather refractory in nature. D amino acids, which are considered to be of bacterial origin, were enriched in DOM during microbial decomposition. The importance of bacteria for the production of refractory DOM was also shown in a recent study of the molecular composition of

Table 6. Results of a principal component analysis (PCA) based on the relative concentrations (mol%) of individual neutral sugars (NS), amino acids (AA), and neutral sugars + D/L amino acids, respectively. The average (Avg) and standard deviation (STD) of individual monomers were calculated from the original data matrix. The factor score coefficients (FC) and the factor scores (degradation index, DI) of the first principal component were derived from the PCA. The first principal component axis explained 90% of the variability in the case of neutral sugars, 67% in the case of amino acids, and 69% for both compound classes. The first PCA axis was interpreted to present DOM degradation. DOM_{lab}, bioreactive DOM.

PCA—neutral sugars						PCA—amino acids						PCA—all compounds					
NS	Avg	Std	FC	Sample	DI	AA	Avg	Std	FC	Sample	DI	AA+NS	Avg	Std	FC	Sample	DI
Fuc	3.30	2.90	0.15	DOM_{lab}	0.93	Asp	8.20	2.70	0.12	DOM_{lab}	1.31	Asp	8.20	2.70	0.059	DOM_{lab}	1.28
Rha	3.30	2.90	0.15	0 h	0.53	Glu	24.30	6.30	−0.12	0 h	0.47	Glu	24.30	6.30	−0.059	0 h	0.48
Ara	2.70	0.70	0.14	53 h	0.98	Ser	10.30	1.70	0.07	53 h	0.81	Ser	10.30	1.70	0.037	53 h	0.77
Gal	10.30	2.80	0.13	103 h	0.04	Thr	5.80	1.00	0.10	103 h	−0.53	Thr	5.80	1.00	0.052	103 h	−0.34
Glc	63.20	20.50	−0.16	170 h	−1.27	Gly	14.40	1.90	0.11	170 h	−0.97	Gly	14.40	1.90	0.054	170 h	−1.09
Man	12.40	9.30	0.16	219 h	−1.12	Arg	8.50	7.00	−0.12	219 h	−1.09	Arg	8.50	7.00	−0.058	219 h	−1.10
Xyl	5.00	2.50	0.15			Ala	11.30	2.40	0.16			Ala	11.30	2.40	0.059		
						Gaba	0.80	0.80	0.09			Gaba	0.80	0.80	0.040		
						Tyr	1.90	0.70	−0.01			Tyr	1.90	0.70	0.001		
						Val	8.30	3.50	0.11			Val	8.30	3.50	0.051		
						Phe	1.20	0.30	0.10			Phe	1.20	0.30	0.051		
						Ile	2.00	0.50	0.10			Ile	2.00	0.50	0.052		
						Leu	2.90	0.20	−0.03			Leu	2.90	0.20	0.010		
												D-Asp	1.00	0.70	0.032		
												D-Glu	1.10	0.70	0.049		
												D-Ala	0.90	0.50	0.051		
												Fuc	3.30	2.90	0.059		
												Rha	3.30	2.90	0.059		
												Ara	2.70	0.70	0.054		
												Gal	10.30	2.80	0.042		
												Glc	63.20	20.50	−0.058		
												Man	12.40	9.30	0.058		
												Xyl	5.00	2.50	0.056		

colloidal DOM using direct temperature-resolved ammonia chemical ionization mass spectrometry (Boon et al. 1998).

Additional studies are needed to better resolve temporal scales of diagenetic alterations of DOM. The present study demonstrated that major bacterial alterations of DOM composition occur on time scales of days to weeks. Several studies (Benner et al. 1992; McCarthy et al. 1996, 1997; Skoog and Benner 1997; Boon et al. 1998; Clark et al. 1998) have noted similarities in the chemical composition of DOM collected from diverse geographic regions. This is somewhat surprising given the compositional heterogeneity of DOM sources. It appears that microbial decomposition processes could be responsible for much of the observed compositional similarity observed in marine DOM from different regions.

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