Pergamon

Geochimica et Cosmochimica Acta, Vol. 65, No. 22, pp. 4103–4114, 2001

0016-7037/01 $20.00 + .00

PII S0016-7037(01)00688-3

Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids

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(*Received December* 11, 2000; *accepted in revised form March* 30, 2001)

Abstract—The chemical structure of organic nitrogen and the mechanisms of its cycling in the oceans still remain elemental questions in contemporary marine sciences. The Arctic Ocean provides a model system for studying the fate of terrigenous compounds in the ocean. We chemically characterised and traced the discharge of dissolved organic nitrogen (DON) and its particulate counterpart (PON) from the Russian rivers into the central Arctic Ocean. We focused on the D- and L-enantiomers of amino acids, the principal organic nitrogen compounds of living biomass. Total dissolved and particulate hydrolysable amino acids (TDAA, PAA) exhibited highest concentrations in the rivers (TDAA: 3.2 μM; PAA: 5.0 μM on average), contributing ~40% to DON and ~60% to PON. In the Arctic Ocean, TDAA and PAA decreased to concentrations of <1 μM, accounting only for ~10% of DON but ~80% of PON. Dominant amino acids in TDAA were glycine and alanine (in the rivers, 35% of TDAA; in deepwater, 49%), followed by aspartic acid, glutamic acid, and serine. Threonine was also abundant in the rivers, and leucine in deep seawater. Microbial-derived D-enantiomers of aspartic acid, glutamic acid, serine, and alanine were found in significant amounts in all river and seawater samples, both dissolved and suspended. In riverine TDAA D-aspartic acid was most abundant (21% of total aspartic acid); in deep seawater D-alanine predominated (44% of total alanine). The proportions of all D-enantiomers were significantly higher in oceanic versus riverine TDAA and increased with depth in the Arctic Ocean. PAA exhibited much lower proportions of D-enantiomers than TDAA (generally <10% of the respective amino acid).

This first direct and complete quantification of D-amino acids dissolved and suspended in seawater provides molecular evidence for microbial contribution to marine organic nitrogen. Particulate D-amino acids, present even offshore in the euphotic zone, indicated microbial biomass and fast turnover of decaying phytoplankton. However, recognisable microbial-derived compounds contributed only a minor fraction to marine DON. The amino acid signature of DON can be explained largely by conservative mixing of recalcitrant compounds of terrestrial (soil) and marine origin. They behaved biogeochemically stable in the brackish mixing zone of the estuaries and in the Arctic Ocean over years to decades. The high amino acid content and the low D-enantiomer proportion of soil-derived DON indicate that terrestrial diagenesis is much more efficient than marine diagenesis in protecting amino acids from bacterial degradation. The huge amounts of dissolved organic nitrogen transported by Siberian rivers into the Arctic Ocean therefore do not substantially support the productivity of the Arctic Ocean. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

The availability of a few elements controls the productivity of the world's oceans. It is primarily nitrogen that limits phytoplankton growth in the euphotic zone. Besides atmospheric inputs and autochthonous fixation of atmospheric N_2, continent–ocean fluxes are the primary source for bioavailable nitrogen to the oceans. The major fraction of this nitrogen is bound in organic molecules and available for most primary producers only following bacterial mineralisation (Cornell et al., 1995, and references therein). The chemical identity of the organically bound nitrogen therefore determines the impact of the enormous continental fluxes on marine productivity. The chemical structure of terrigenous and marine-derived organic nitrogen and the mechanisms of its cycling in the oceans still remain elemental questions in contemporary marine sciences.

The Arctic Ocean provides a model system for studying the fate of terrigenous compounds in the ocean. The Arctic Ocean is, on a volume basis, the ocean with the highest terrestrial input in terms of freshwater and organic matter. Because of the large freshwater input, it is well stratified and characterised by a distinctive surface layer of reduced salinity. The transport of riverine freshwater to outer-shelf areas is slow and lasts several years. Advective mixing of surface waters with halocline water takes ~10 yr (Schlosser et al., 1995).

Despite great efforts in the last decades, it is not possible so far to isolate efficiently dissolved organic matter from a seawater matrix for extensive chemical characterisations. In recent years, ultrafiltration and solid phase extraction have been applied most frequently. However, these methods generally isolate only a minor fraction (<30%) of marine dissolved organic matter (Benner et al., 1997). The major fraction therefore awaits chemical identification. To improve our knowledge on the composition of bulk organic matter, we established an analytical method for the direct quantification of the principal organic nitrogen compounds of living biomass, D- and L-amino acid enantiomers, in seawater (Fitznar et al., 1999). Bacterial biomass is rich in D-amino acids, whereas phytoplankton and other primary producers contain almost exclusively L-enantiomers (e.g., Jørgensen et al., 1999). Abiotic racemisation and inorganic degradation also generate D-amino acids, but these processes are slow and mostly negligible in the presence of

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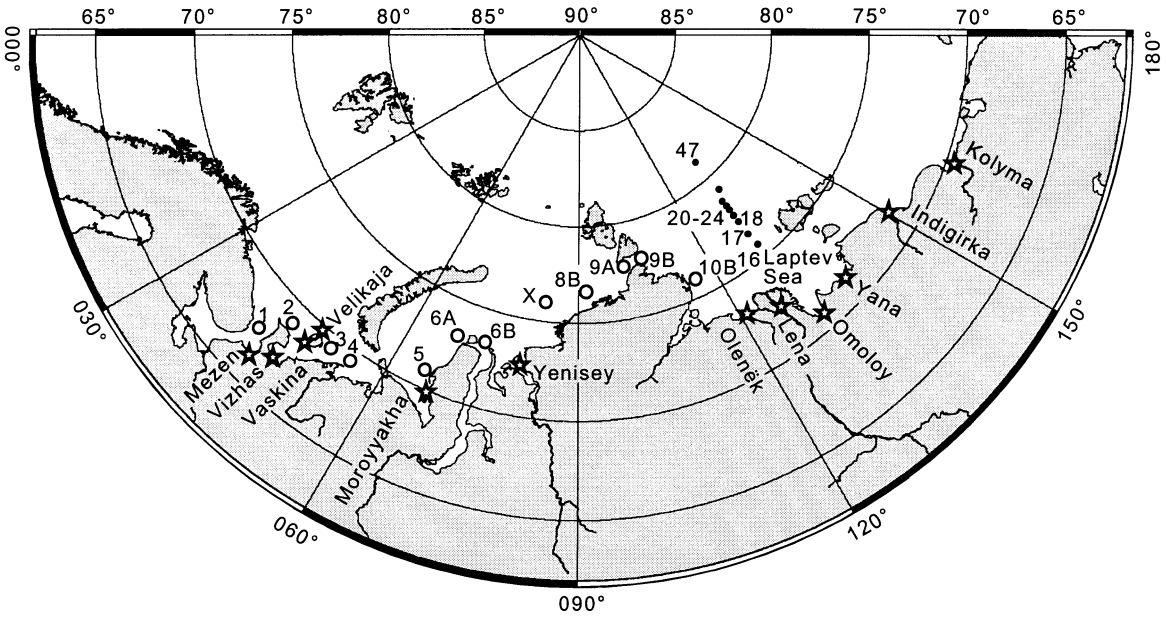

Fig. 1. Map of sampling site and stations. Station numbers are from the various expeditions. Stars denote river stations, open circles denote near-shore stations, and filled circles denote offshore stations.

active bacteria (Bada, 1972). Earlier studies on dissolved organic nitrogen fractions isolated from seawater by solid phase extraction (Cu-ligand exchange chromatography; Lee and Bada, 1977; Bada and Hoopes, 1979) and ultrafiltration (>1 kD; McCarthy et al., 1998) show that the D-enantiomers of some amino acids are present in significant concentrations in seawater. Alanine is the amino acid with highest D-enantiomer proportions of up to 50%. McCarthy et al. (1998) deduced a major bacterial contribution to marine DON. The D-enantiomers of alanine, aspartic acid, and glutamic acid are principal constituents of peptidoglycan, a structural biopolymer of bacterial membranes. Cell-wall constituents are presumably less accessible to biodegradation than bulk organic matter (Tanoue et al., 1995; Nagata et al. 1998a) so that the respective D-amino acids may therefore accumulate during diagenesis. The formation of submicron particles from bacterial degradation may protect even labile compounds (Borch and Kirchman, 1999). Bacteria may therefore play a central role for the generation of recalcitrant organic nitrogen in the ocean.

The objective of our study was to trace the flux of dissolved organic nitrogen (DON) and its particulate counterpart (PON) from the Russian rivers into the Arctic Ocean. We sought to identify major sources of organic nitrogen to the Arctic Ocean and diagenetic alterations characteristic of different environmental conditions. We focused our attention primarily on amino acids, the principal organic nitrogen compounds of living biomass and also on the quantification of D-enantiomers. We highlight their use as tracers for bacterial impact on organic nitrogen in this first comprehensive study of hydrolysable D- and L-amino acids in total dissolved and suspended seawater organic matter.

2. MATERIAL AND METHODS

The 12 Russian rivers studied (Fig. 1) account for 43% of the riverine freshwater discharge to the Arctic Ocean (Arctic River Database, 1998) and for 38% of the dissolved organic carbon discharge (Lobbes et al., 2000). Siberian rivers become free of ice in early summer and discharge >90% of the annual delivery to the Arctic Ocean from May to July. We traced this summer discharge from the estuaries to the near-shore coastal zone and along a transect from the Laptev Sea into the Amundsen Basin. Below the halocline, a layer of Atlantic water is located. Deeper water layers (>500 m) are similar in terms of salinity and temperature and are referred here as "deepwater." Samples were collected from the upper part of the estuaries (salinity ~0) and along the coast (salinity 25–34) over an east–west distance of ~4000 km of coastline (Fig. 1). These samples were taken from surface water, except for the Yenisey (10 m), after the maximum water discharge in summer. Both dissolved and particulate samples were acquired from all rivers, except the Vaskina, Omoloy, and Yana (no dissolved samples) and Velikaja (no particulate sample). Additionally, dissolved samples were obtained at standard oceanographic (CTD) depths from the Laptev Sea along a 550-km transect. All samples were taken from Niskin bottles fitted to a CTD-probe rosette system.

Samples from the Lena, Yana, and Omoloy Rivers were taken during two expeditions with the research vessel (RV) *Prof. Makkaveev* by the Alfred Wegener Institute in co-operation with the Geographical Faculty of the Moscow State University in July 1994 (Lena) and August 1995 (Yana, Omoloy). Sampling at the other estuaries and along the coast was carried out during the SWEDARCTIC Tundra Ecology-94 expedition with the RV *Akademik Fedorov* in June–July 1994. For detailed information on the expedition see Lobbes et al. (2000) and references therein. The Laptev Sea was sampled during the RV *Polarstern* expedition ARK XI/1 from July to September 1995 (Rachor, 1997; Kattner et al., 1999).

Water samples were filtered onboard through precombusted (500°C, 5 h) Whatman GF/F filters (nominal pore size ~0.7 μm). Water samples from Lena, Yana, and Omoloy were poisoned with $HgCl_2$ to a sample concentration of 100 mg $\cdot$ L^{-1} (Kattner, 1999), stored in 1-L polyethylene bottles at 4°C and filtered in the home laboratory. The filters were kept frozen (−30°C) in precombusted sealed glass vials until determination of particulate organic carbon (POC), nitrogen (PON), and amino acids (PAA). Water aliquots for analysis of inorganic nutrients and dissolved organic nitrogen (DON) were poisoned with $HgCl_2$ and kept at 4°C in polyethylene bottles. Aliquots for determination of dissolved organic carbon (DOC) were adjusted after filtration to pH = 2 with 1 N HCl and stored in precombusted glass

ampoules at 4°C. Total dissolved amino acid (TDAA) samples were kept frozen (−30°C) in precombusted sealed glass ampoules.

Salinity was measured with a CTD-probe (Seabird SBE-11 plus; Laptev Sea) and a salinometer (WTW LF191; estuary and near-shore samples). Total suspended solids (TSS) were gravimetrically quantified on the dried GF/F filters (80°C, 12 h). POC, PON, and stable carbon isotope analyses were performed with a Europa Scientific ANCA SL 20–20 stable isotope analyser. Results were normalized to the PDB standard and expressed as $\delta^{13}C$ (Fry and Sherr, 1984). DOC was determined by high-temperature catalytic oxidation with a Shimadzu TOC 5000 or a Rosemount Dohrman TOC 190 analyser. Inorganic nutrients were determined with a Technicon Autoanalyser II system according to Kattner and Becker (1991). Dissolved organic nitrogen (DON) was measured by wet oxidation with potassium persulfate (Koroleff, 1983). The relative standard deviations for each method and each run were ≤3.5% (p = 0.05; German standard method; Funk, 1985). If a run exceeded this value or if the coefficient of variation between duplicates exceeded 5%, the determination was repeated.

The sum of dissolved combined and free amino acids (TDAA) was quantified because free amino acids are present in natural waters in negligible concentrations only; for example, in Greenland Sea and Lena River <2% of TDAA (Hubberten et al., 1994; Lara et al., 1998). For the determination of TDAA and PAA, samples were hydrolysed with HCl (16%) in the presence of ascorbic acid (55 μM) at 110°C (24 h) in precombusted sealed glass ampoules according to Fitznar et al. (1999). Glycine (Gly) and the D- and L-enantiomers of the individual amino acids aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), arginine (Arg), alanine (Ala), γ-amino butyric acid (GABA), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Iso), and leucine (Leu) were determined in the hydrolysates (adjusted to pH = 8.5) by high-performance liquid chromatography (HPLC)/fluorescence detection (Ex: 330 nm; Em: 445 nm) after precolumn derivatisation with *o*-phthaldialdehyde and *N*-isobutyrylcysteine (Fitznar et al., 1999). We used a Merck LaChrom HPLC system with intelligent autosampler for automatic derivatisation, reversed phase column (Superspher RP 18, 4-μm particle diameter, 125 mm length, 4 mm inner diameter) and a multistep gradient system. External standards of the amino acid enantiomers (Fluka, Switzerland; Aldrich, USA; Sigma, USA) were used for calibration. Each sample was analysed in duplicate with two derivatising agents (*N*-isobutyryl-L-cysteine and *N*-isobutyryl-D-cysteine). We only report amino acid concentrations that were reproducibly quantified after derivatisation with both reagents and only those D-enantiomers that were significantly different from the racemisation blank. The coefficients of variation between the duplicates were 1 to 8%; the relative standard deviation for the individual amino acids and each run was ≤3.5%.

The validity of the method was checked by standard addition to a subset of samples before and after hydrolysis. Systematic errors determined in this way were below 5% of sample concentration, including possible losses during hydrolysis. Chemical hydrolysis induces racemisation of amino acids. Therefore, the extent of racemisation during hydrolysis was determined by exposing a composite of L-amino acids to the hydrolysation procedure. The percentage of D-amino acids in the hydrolysates ranged from 0.5 to 5%. These racemisation blanks are not representative for environmental samples because racemisation is essentially dependent on the chemical environment and bonding of the amino acids (Manning, 1970). Because the true value for TDAA and PAA samples is not accessible, the results were not corrected for the racemisation blank. Because of characteristic and highly distinctive D-amino acid patterns of the different samples, we infer negligible racemisation during hydrolysis compared with naturally occurring D-amino acid contents.

Dauwe and Middelburg (1998) introduced an empirical degradation index for protein amino acids on the base of a principal component analysis applied on a set of sedimentary amino acid data. The advantages of this degradation index are that the whole suite of protein amino acids is considered for the calculation and that different samples can be compared directly and quantitatively. The index characteristically reaches from +1 (phytoplankton, bacteria) to −1.5 (highly degraded oxic sediments). Recently, Amon et al. (2001) showed that this index is also meaningful for early diagenesis of marine dissolved organic matter. We applied this index on our dissolved and particulate samples of different origin and diagenetic state. The significance of this approach was tested by comparison with independent diagenetic indicators, for example, enantiomer ratios.

All correlation analyses performed in this study are based on Pearson's correlation coefficients, the significance of which was tested by the Student's *t*-test. Regression analyses were performed only if the values of one of the variables were known exactly without considerable errors (Webster, 1997).

3. RESULTS

3.1. General Parameters

The strong freshwater input of the Siberian rivers into the Arctic Ocean led to reduced salinities along the whole coastline and at the surface of the Laptev Sea, even at the most offshore stations. Despite the high variability between the individual rivers in terms of nutrient and organic matter concentration, their average pattern was significantly different from the oceanic stations that were pooled into near-shore and Laptev Sea categories. Furthermore, the Laptev Sea stations were grouped into surface (0–30 m), halocline (30–200 m), Atlantic water (200–500 m), and deepwater groups (>500 m). Average nutrient and organic matter concentrations for these subgroups are shown in Table 1.

In most of the rivers, silicate was the most abundant nutrient, with an average concentration of 15.9 μM. Silicate concentrations near-shore and in the Laptev Sea were lower at ~6 μM. In deepwater, silicate concentrations increased to 9.6 μM and dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium) was more abundant than silicate. The Arctic rivers were characterised by low concentrations of DIN and phosphate, exhibiting average values of 2.6 μM and 0.29 μM, respectively. The major fraction of the riverine nitrogen was bound in dissolved and particulate organic compounds (DON: 12.1 μM, PON: 11.4 μM). DIN and phosphate concentrations were low near-shore and at the surface of the Laptev Sea but increased considerably with depth to average concentrations in deepwater of 13.9 μM and 0.99 μM, respectively. DIN was composed principally of nitrate. Nitrite concentration was negligible (0.05 μM on average), and ammonium concentration was on average 0.7 μM, with no significant difference between the groups of stations. Contrary to DIN, DON decreased during the transition from the rivers to the ocean and continued decreasing with depth. In deepwater only a minor fraction of nitrogen was bound organically. DOC behaved similarly, but the decrease from river (624 μM) to near-shore (155 μM) was much more pronounced, leading to a strong decrease of the molar C/N ratio from an average of 51 in the rivers to 23 near-shore. PON and POC decreased near-shore to ~10% of the river concentration. The C/N ratio of particulate matter did not change significantly over this transition. Because of the strong near-shore decrease of PON, DON concentration greatly exceeded that of PON. The patterns of dissolved and particulate organic matter also changed during the transition from the rivers to the ocean. $\delta^{13}C$ of POC increased from −26.6‰ in the rivers to −25.2‰ near-shore. The organic carbon normalised molar yield of lignin phenol carbon (X_{lignin}) decreased from ~2‰ in both riverine particulate and dissolved organic matter to ~0.2‰ near-shore and in Laptev Sea surface water.

Table 1. Salinity, Nutrients, and Organic Bulk Parameters for Dissolved and Particulate Organic Matter.

			Laptev Sea			
	River	Near-Shore	Surface	Halocline	Atlantic Water	Deepwater
n (diss./part.)	9/11	12/12	16/4	21/4	15/4	19/5
Salinity	0.0 ± 0.0	31.4 ± 1.3	30.8 ± 0.6	34.1 ± 0.2	34.9 ± 0.0	34.9 ± 0.0
DIN (μM)	2.6 ± 2.0	2.9 ± 1.4	3.0 ± 0.8	8.1 ± 0.8	10.9 ± 2.2	13.9 ± 0.5
Silicate (μM)	15.9 ± 13.6	5.3 ± 3.2	6.4 ± 0.6	5.5 ± 0.9	5.7 ± 0.2	9.6 ± 0.9
Phosphate (μM)	0.29 ± 0.13	0.18 ± 0.12	0.51 ± 0.06	0.73 ± 0.04	0.87 ± 0.06	0.99 ± 0.04
DOC (μM)	624 ± 163	155 ± 26	125 ± 8	82 ± 8	62 ± 4	68 ± 5
DON (μM)	12.1 ± 2.2	7.4 ± 1.3	6.2 ± 0.4	4.5 ± 0.3	3.6 ± 0.4	3.4 ± 0.3
$C/N_{diss.}$	51 ± 9	23 ± 5	21 ± 1	19 ± 2	18 ± 3	20 ± 2
$X_{lignin,\ diss.}$ (‰)	2.1 ± 0.6	0.21 ± 0.08	0.27 ± 0.09	–	–	–
TDAA (μM)	3.2 ± 0.6	0.52 ± 0.08	0.52 ± 0.05	0.32 ± 0.02	0.27 ± 0.03	0.25 ± 0.03
TSS (mg L^{-1})	104 ± 47	28 ± 1	–	–	–	–
POC (μM)	115 ± 53	13 ± 5	–	–	–	–
PON (μM)	11.4 ± 5.9	1.2 ± 0.5	–	–	–	–
$C/N_{part.}$	11.0 ± 1.1	15.1 ± 5.4	–	–	–	–
$\delta^{13}C$ (‰)	−26.6 ± 0.3	−25.2 ± 0.8	–	–	–	–
$X_{lignin,\ part.}$ (‰)	2.5 ± 1.2	0.21 ± 0.12	–	–	–	–
PAA (μM)	5.0 ± 3.2	0.67 ± 0.35	0.17 ± 0.13	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02

Note: Average values, confidence intervals ($p < 0.05$) and number of samples (*n*) for the different areas of sampling. Our results were completed with nutrient, organic carbon and nitrogen data from Kattner et al. (1999; Laptev Sea) and Lobbes et al. (2000; Arctic Rivers) and lignin data from Lobbes (1998).

3.2. Amino Acids

Highest amino acid concentrations of 3.2 μM (TDAA) and 5.0 μM (PAA) on average were found in the rivers (Table 1). The organic nitrogen-normalised molar yield of amino acids was on average 38% for DON and 63% for PON (Fig. 2). Near-shore, the amino acid concentration decreased to 0.52 μM (TDAA) and 0.67 μM (PAA). Hydrolysable amino acids near-shore contributed only 10% to DON but made up 78% of PON. From surface to depth, TDAA concentration decreased parallel to DON to 0.25 μM in deepwater. Overall, amino acid yields remained almost constant at ~10% of DON.

The composition of TDAA differed significantly from that of PAA, and riverine samples exhibited a different signature than oceanic samples (Fig. 3). The neutral amino acids glycine and alanine were the most abundant dissolved amino acids at all stations, followed by aspartic acid and glutamic acid (acidic amino acids) and serine and threonine (hydroxy amino acids). In deepwater the neutral amino acid leucine was also abundant. Glycine and alanine composed on average 35% of riverine TDAA and 49% of deepwater TDAA. Generally, marine samples showed a higher proportion of glycine and alanine than riverine samples, with this tendency increasing from surface to depth. The opposite trend was observed for threonine. At all stations the composition of PAA was much more uniform than for TDAA, and glycine and alanine did not predominate. Particulate glycine and threonine showed a similar trend from the rivers to deepwater as their dissolved counterparts. Alanine, serine, and leucine, however, in PAA and in TDAA behaved in opposite ways. The non-protein amino acid, γ-amino butyric acid, was typically close to the quantification limit and contributed weakly to PAA (0.2–0.5% on average at individual stations) and TDAA (0.5–1.3%).

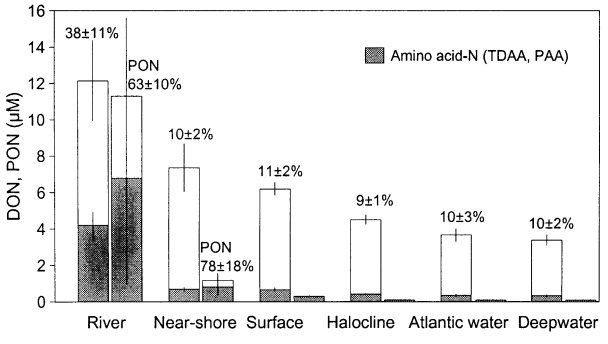

Fig. 2. Dissolved and particulate organic nitrogen (DON, PON) and amino acid nitrogen concentrations. Percentage contribution of amino acids to DON and PON. Average values and confidence intervals ($p < 0.05$) are for the different areas of sampling. Samples for PON determination were obtained only for the rivers and near-shore.

The D-enantiomers of aspartic acid, glutamic acid, serine,

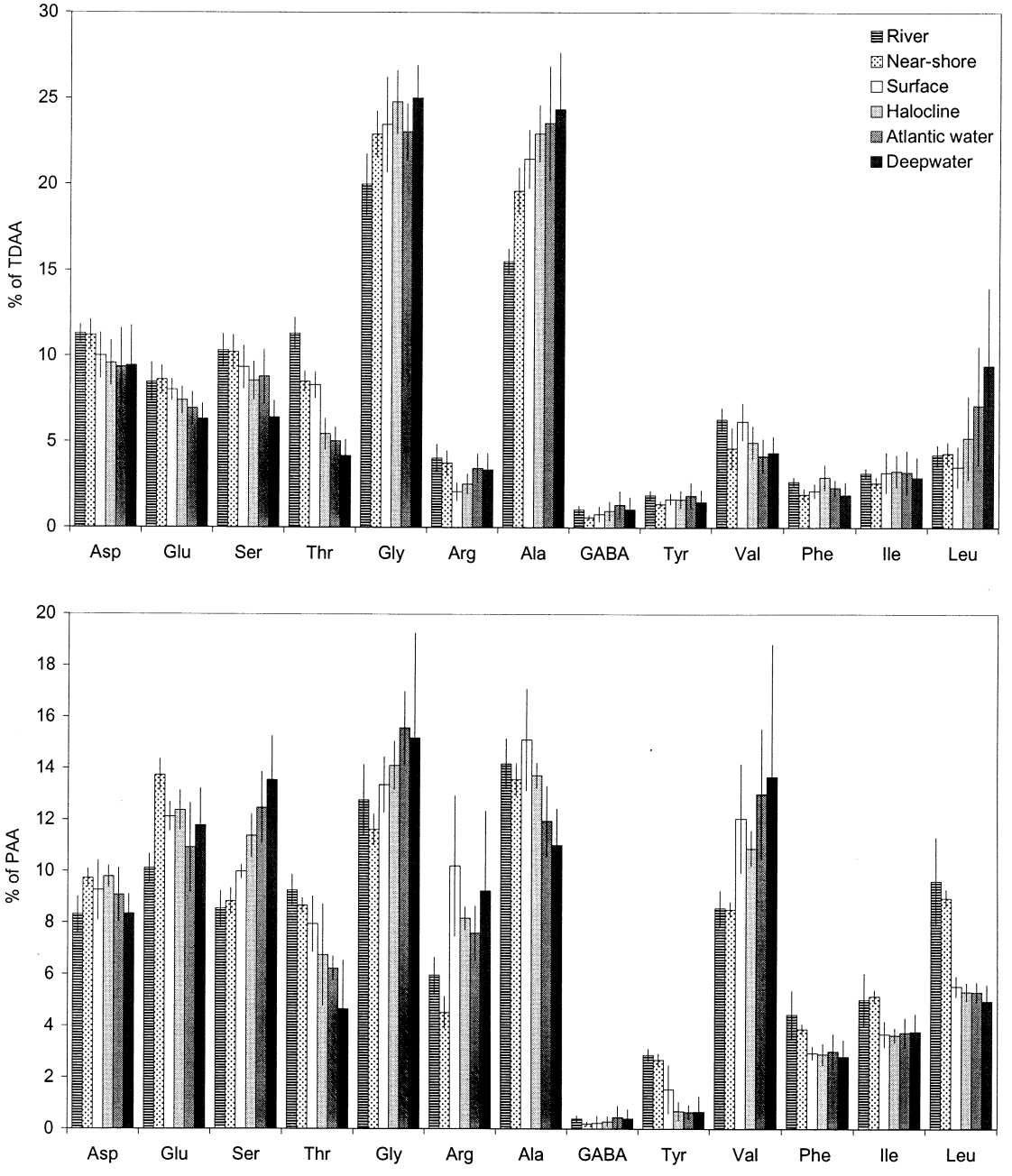

Fig. 3. Average mol percentages of individual total dissolved and particulate amino acids in the different sampling areas, with confidence intervals ($p < 0.05$).

and alanine were found in significant amounts in all dissolved and particulate samples (Fig. 5). Other D-amino acids were present only in negligible concentrations and could not reliably be quantified. The most abundant D-enantiomer in riverine TDAA was D-aspartic acid (21% of total aspartic acid) and in deepwater D-alanine (44% of total alanine). The proportions of all D-enantiomers were significantly higher in oceanic than in riverine TDAA and increased with depth in the Laptev Sea. PAA exhibited much lower proportions of D-enantiomers than TDAA. The most abundant particulate D-amino acid at all stations was D-aspartic acid with maximum percentages in Atlantic water (17% of total aspartic acid on average). Generally, particulate D-enantiomers contributed less than 10% to the corresponding amino acid concentration and less than 2% to serine. Particulate D-amino acids did not show a significant trend between the stations.

4. DISCUSSION

High amounts of reduced and oxidised nitrogen are transported by the Russian rivers into the Arctic Ocean. We estimated the riverine nitrogen discharge of the 12 rivers studied to

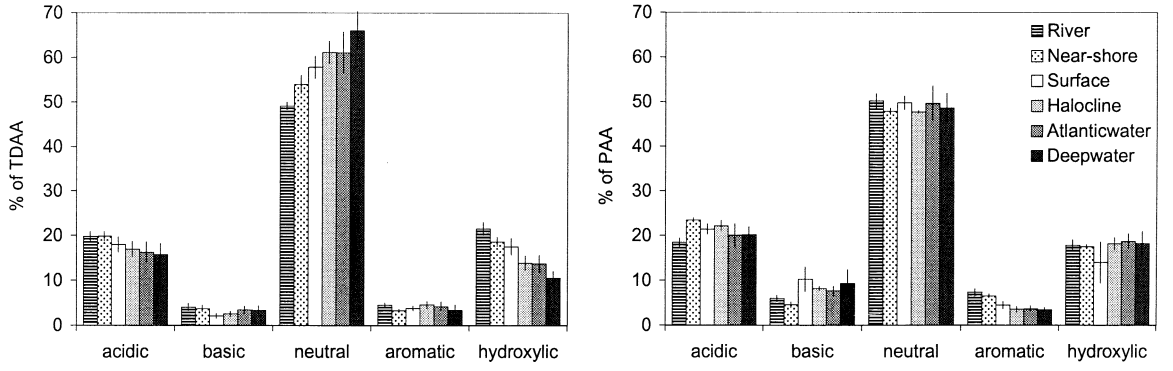

Fig. 4. Average mol percentages of grouped total dissolved and particulate amino acids in the different sampling areas, with confidence intervals ($p < 0.05$).

be $\sim 29 \cdot 10^9$ mol $\cdot$ yr^{-1}, using long-term means of annual water discharge (summarised by Lobbes et al., 2000 and references therein). The major nitrogen discharge is in the form of organic compounds (DON: $15 \cdot 10^9$ mol $\cdot$ yr^{-1}; PON: $9 \cdot 10^9$ mol $\cdot$ yr^{-1}), with an important contribution of amino acids (TDAA, PAA: $5 \cdot 10^9$ mol $\cdot$ yr^{-1} of each). The flux of DIN is relatively minor ($5 \cdot 10^9$ mol $\cdot$ yr^{-1}).

4.1. DON in the Arctic Rivers

Autochthonous production in Arctic rivers is low (Cauwet and Sidorov, 1996; Sorokin and Sorokin, 1996), and therefore a considerable production of DON from DIN is not expected. A common source and similar formation processes of TDAA and dissolved lignin in the rivers is indicated by their positive correlation (Fig. 6). Dissolved lignin is released during the degradation of debris in the soils of taiga and tundra, and the elevated acid to aldehyde ratio of the vanillyl phenol family is evidence for an advanced diagenetic state of lignin (Lobbes et al., 2000).

During decomposition of organic matter, the accumulation of cell wall constituents such as glycine and non-protein amino acids such as γ-amino butyric acid is commonly observed (Dauwe et al., 1999). Both amino acids were significantly enriched in riverine TDAA relative to freshly produced organic matter, indicating an advanced diagenesis of DON. This inference is supported by the degradation index for protein amino acids calculated after Dauwe et al. (1999); see Figure 7. Because the index for TDAA of Russian rivers is –1.0 on average (highly degraded), phytoplanktonic exudates are excluded as a significant source of TDAA in the rivers (Amon et al., 2001).

These findings are in accordance with considerable proportions of D-amino acids in the riverine TDAA. The primary source of D-amino acids is presumably bacterial peptidoglycan. The composition of peptidoglycan of gram-negative bacteria, predominant in aquatic systems, is relatively invariant. In accordance with its common architecture, total peptidoglycan-N can be estimated roughly as 5.7 $\times$ D-Ala-N (Rogers, 1983). Using this factor, peptidoglycan would account for 7 $\pm$ 2% of TDAA nitrogen in the Siberian rivers. However, the amino acid signature in the rivers may be influenced largely by diagenesis in the soils where gram-positive bacteria dominate (Schlegel, 1985). The proportion of D-alanine to peptidoglycan is more variable for gram-positive bacteria (Ghuysen and Shockman, 1973). Interpreting the estimates for the Siberian rivers, one also should keep in mind, that teichoic acids, which are rich in D-alanine, are major components of the cell wall membrane

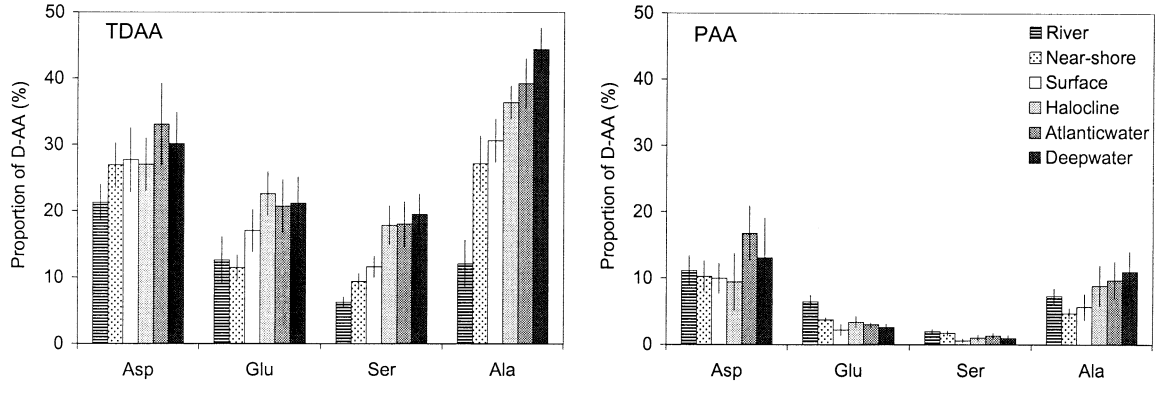

Fig. 5. Molar D-enantiomers fractions of the individual dissolved and particulate amino acids, calculated as D/D + L) amino acids.

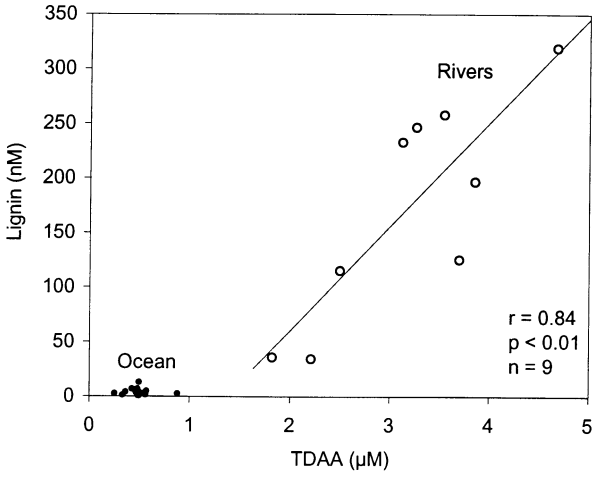

Fig. 6. Total dissolved amino acid concentrations versus dissolved lignin concentrations at the individual sampling stations. The correlation for the river samples indicates a common source.

complex in a large number of gram-positive bacteria (Perego et al. 1995, and references therein). Therefore, the calculated peptidoglycan value should be considered as a rough estimate.

Another possible source of D-amino acids are abiotic processes. However, stereochemical inversion at Arctic temperatures is probably too slow to account for appreciable racemisation over the residence time in the Siberian soils or the ocean (Bada, 1972). The radiocarbon age of POC in the Lena River is ~7000 yr (Kuptsov and Lisitsin, 1996), which is in the range of deep-sea DOC (Williams and Druffel, 1987). Abiotic degradation also may produce D-amino acids, such as the β-elimination of hydroxy amino acids (Bada et al., 1978; Bada and Hoopes, 1979). However, the absence of α-amino butyric acid, which would concomitantly have been built up during dehydration of serine, indicates a marginal production of D-enantiomers by abiotic reactions (Bada and Hoopes, 1979).

D-aspartic acid, which is characteristic of soil humic substances (Kimber et al., 1990), was the most abundant D-amino acid in the rivers. It even exceeded the concentration of D-alanine, which was most abundant at the near-shore and oceanic stations. Kimber et al. (1990) determined D-enantiomer proportions of 9% for aspartic acid, 8 to 15% for glutamic acid and 7 to 9% for alanine in particulate soil organic matter. Extracted humic substances exhibited higher proportions of up to 29% for aspartic acid, 20% for glutamic acid, and 16% for alanine, with a decreasing tendency of D-enantiomer proportions with increasing molecular weight. They found about double the D-enantiomer proportion of aspartic acid than of alanine in all humic acid extracts. This is in accordance with our findings in the Arctic rivers and is further evidence for soil-derived humic substances being the predominant origin of riverine DON. In contrast, bacterioplankton would produce higher D-alanine than D-aspartic acid proportions (McCarthy et al., 1998; Amon et al., 2001) and therefore probably play a subordinate role in the formation of riverine DON.

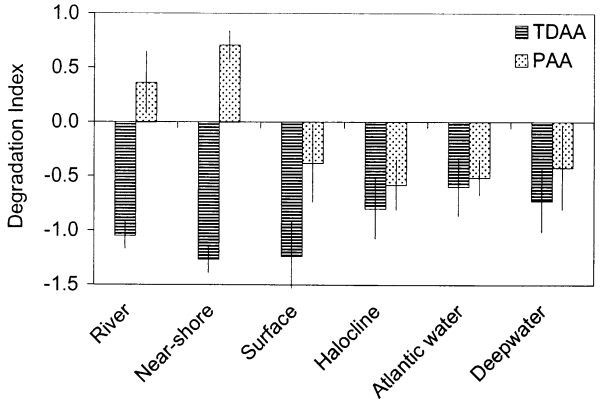

Fig. 7. Degradation indices of total dissolved and particulate amino acids. Average values and confidence intervals ($p < 0.05$) are for the different areas of sampling. Calculated after Dauwe et al., 1999. Low values are indicative of a high degree of degradation.

The composition of the precursor organic matter was directly reflected in the pattern of riverine lignin (Lobbes et al., 2000). However, the vegetation of the catchment area has no discernable influence on the amino acid signature of the rivers. No correlation was found between the proportion of taiga and tundra (estimated from the *Times Atlas of the World*,1997) and any of the dissolved or particulate amino acid parameters. This result shows that overall diagenetic processes in soils, which likely do not vary as much as vegetation between the different ecosystems, account for the formation of amino acid–containing humic compounds.

4.2. Fate of Terrestrial DON in the Ocean

Freshwater runoff is rapidly mixed in the coastal zone with saline waters. Within years to decades it mixes with outer shelf waters and surface and halocline waters of the Eurasian Arctic Ocean in the transpolar drift system (Schlosser et al., 1995). The primary source of freshwater to the Arctic Ocean is terrestrial runoff, whereas melting of sea ice plays a minor role (Bauch et al., 1995). Salinity therefore primarily traces river discharge. The simplest possible scenario for the dynamics of solutes in this system is conservative mixing of recalcitrant compounds of marine and terrestrial origin, without other sources or sinks. Deviations from conservative mixing would be evidence for additional processes. We modelled this scenario using average values of all rivers and of Laptev Sea deepwater as end members (Table 1). DOC concentration in the Laptev Sea closely followed the theoretical mixing curve. Such behaviour has already been described by other authors (Cauwet and Sidorov, 1996; Kattner et al., 1999) and is evidence for the biogeochemically stable character of DOC. Autochthonous production did not significantly contribute to the DOC pool of the Laptev Sea. Dissolved lignin concentrations reflect these features scattering around the theoretical mixing curve (Kattner et al., 1999). In contrast, DIN was low in all surface waters and increased strongly with depth. Assimilation by phytoplankton and remineralisation led to this pronounced deviation from conservative mixing. Dissolved phosphate behaved similarly, whereas the surface depletion of silicate was much less. DON correlated highly significantly with DOC in the Laptev Sea ($r = 0.83$; $p < 0.001$; $n = 94$) but also exhibited significant deviations from conservative mixing. To identify processes

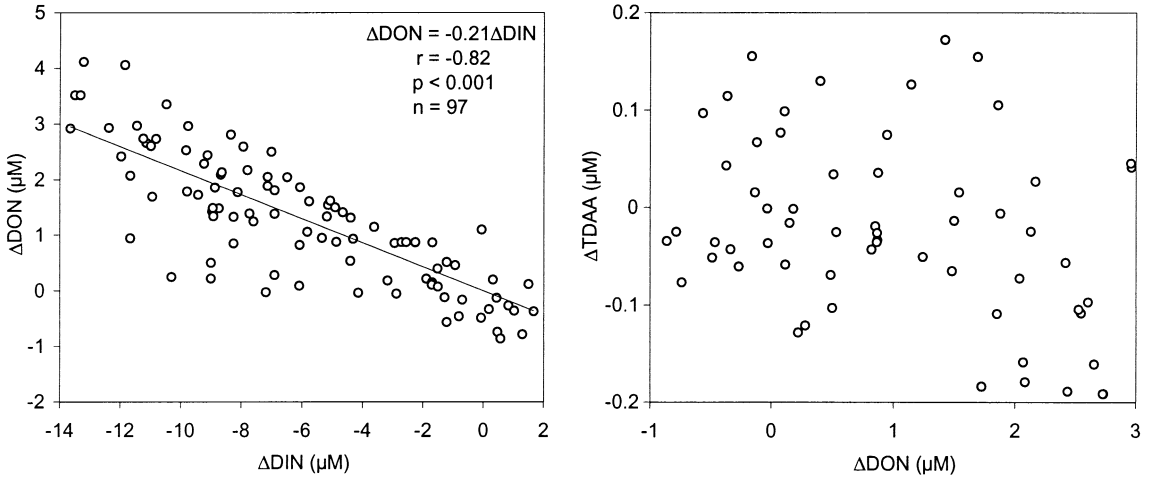

Fig. 8. ΔDIN versus ΔDON, and ΔDON versus ΔTDAA. Δ-values indicate deviations from conservative behaviour and are calculated as the difference between the theoretical concentrations of deep and river water mixing and the real concentrations.

causing these deviations, we calculated the differences (Δ) between the real concentrations and theoretical concentrations arising from mixing of river and deepwater. ΔDON correlated highly significantly with ΔDIN (Fig. 8). Per 5 mol DIN assimilated, ~1 mol DON was released. This ratio is similar to data from the Greenland Sea (Lara et al., 1993). DON in the Laptev Sea is therefore a mixture of biogeochemically stable, recalcitrant compounds (see river and deepwater end members, Table 1) and a labile, freshly produced fraction (ΔDON <4 μM as upper limit).

Freshly produced phytoplankton-derived DON is rich in amino acids (Cowie and Hedges, 1992). The Δ-values represent the autochthonous production of each parameter. A simultaneous increase of ΔTDAA with ΔDON might therefore be expected. However, ΔTDAA did not differ from zero on average, and the deviations of the individual samples were not coupled with ΔDON, scattering randomly around zero (Fig. 8). Rapid microbial metabolisation may explain very low yields of hydrolysable amino acids in fresh DON. Rich et al. (1997) found high bacterial production in the central Arctic Ocean, which at some stations was similar to primary production. Labile organic compounds were rapidly taken up and turnover rates were high. Amon et al. (2001) observed dramatic changes of TDAA yield and composition during early DON diagenesis. Within weeks its composition resembled that of ancient marine sediments. In the Arctic Ocean, phytoplankton-derived TDAA is probably rapidly incorporated into particulate biomass (bacteria) or in compounds, such as aminosugars, which are analytically not accessible via hydrolysis and amino acid detection. The observed amino acid signature and the proportion of D-amino acids support this inference. The concentrations of individual amino acids can be explained by conservative mixing of refractory terrigenous and marine substances; the deviations of individual samples were random and not coupled with ΔDON. On average, the concentrations of the D-amino acids also did not differ significantly from conservative mixing. The deviations of the individual samples, however, correlated with ΔDON ($r = 0.45–0.49$; $p < 0.001$; $n = 67$), with the exception of ΔD-serine. This observation also agrees with the presumption of fast microbial transformation of freshly produced phytoplanktonic TDAA. It can be assumed that TDAA in the Arctic Ocean off Siberia is primarily bound into biogeochemically stable compounds, the signatures of which can largely be explained by conservative mixing of terrestrial and marine components.

4.3. Terrigenous and Marine Recalcitrant DON

The samples from the Siberian rivers and the Laptev Sea deepwater reflect an integral signal of strongly contrasting primary organic matter sources and degradation processes. The conservative behaviour of the TDAA signature during the time scale of mixing, which lasts years to decades, indicates that TDAA in the Arctic Ocean off Siberia is a final product of diagenesis under marine and terrestrial conditions. Because the amino acid composition of proteinaceous sources is largely uniform (Cowie and Hedges, 1992), the different signatures of marine and terrigenous TDAA reflect fundamentally dissimilar pathways of degradation.

Terrigenous DON in the Siberian rivers was composed of hydrolysable amino acids to ~40%, comparable to the major world rivers (Ittekkot et al., 1983), and marine DON only to ~10%, which is similar to values from the Arctic Greenland Sea (Hubberten et al., 1994) and other ocean basins (e.g., Lee and Bada, 1977; McCarthy et al. 1997). Terrestrial diagenesis in the Siberian soils therefore includes processes that preserve hydrolysable amino acids much more efficiently than the processes involved in marine diagenesis. Terrestrial and marine samples exhibited significantly different amino acid patterns (Fig. 3). No direct hints about degradation, however, can be derived from these differences. Marine TDAA had higher proportions of glycine and alanine, which would be indicative of a higher degree of degradation (e.g., Dauwe et al., 1999). Threonine and leucine, however, point toward a lower diagenetic degree. Non-protein amino acids, such as γ-amino butyric acid, are preferentially preserved during diagenesis and should there-

fore correlate with the degradation index (Dauwe et al., 1999), just as D-amino acids. Such correlations were not found in our study. The degradation index indicates an apparently lower degree of alteration for deep-sea than for riverine samples (Fig. 7), despite much higher D-enantiomer proportions and γ-amino butyric acid yields in the deep-sea. In the case of TDAA, variations of the degradation index therefore reflect different pathways of degradation rather than different diagenetic states. In addition, amino acid partitioning between solid and liquid phases can produce compositional differences (Aufdenkampe et al., submitted), which may lead to inconsistent degradation indices under different environmental conditions.

Similarities of our data with amino acid signatures from the Amazon River system (Hedges et al., 1994, 2000a), other world rivers (Ittekkot et al., 1983), and the Greenland Sea (Hubberten et al., 1994; Lara et al., 1998) point to a dominant role of few universal diagenetic pathways for the formation of recalcitrant DON. Compared with the river samples, TDAA from the Greenland Sea exhibited markedly higher percentages of glycine and alanine and lower levels of threonine. These differences coincide with the general trend observed for TDAA from Siberian rivers towards the Laptev Sea.

The D-amino acid patterns provide new insights into the different diagenetic processes involved in the formation and preservation of marine and terrestrial TDAA. Deepwater samples exhibited highest D-enantiomer proportions, exceeding by far the values of bacterioplankton (McCarthy et al., 1998; Amon et al., 2001). The peptidoglycan contribution to TDAA nitrogen was estimated on the basis of D-alanine to be 53 ± 11%. For these estimates, we assumed a gram-negative bacterial community with invariant D-alanine proportion and the absence of other D-amino acid containing components. Recently, Karner et al. (2001) demonstrated archaeal dominance in the mesopelagic zone of the Pacific Ocean. Archaea do not contain peptidoglycan but soluble intracellular peptidyl D-amino acids (Nagata et al. 1998b). Our estimates are thus limited by the unknown abundance of specific microbial groups in the Arctic Ocean. It is therefore also matter of speculation whether the D-amino acids are incorporated into cell wall moieties. Nevertheless, our data strongly suggest a major microbial source of marine TDAA. D-amino acid containing compounds and non-protein amino acids are selectively enriched by passing repeatedly through the microbial loop.

These microbial-derived components can cover a wide range of molecular size classes. McCarthy et al. (1998) found a very similar D-enantiomer proportion in the high molecular weight fraction (>1 kD), which represents 20 to 30% of marine DON, compared with our study in which the complete DON pool is represented. Hydrolysable amino acids represent only ~10% of marine DON, both in the high molecular weight fraction and in bulk dissolved organic matter. A direct comparison of these results is limited because of differences in methodology and sampling sites. However, the remarkable similarities suggest a continuity of molecular subunits across all molecular sizes of marine DON.

Nuclear magnetic resonance spectroscopy revealed an almost invariant bulk composition of particulate organic matter and the dissolved high molecular weight fraction (>1 kD) in the course of diagenesis (McCarthy et al., 1997; Hedges et al. 2001). Amide bindings predominate in high molecular weight DON (McCarthy et al., 1997). This is surprising because the amount and composition of hydrolysable amino acids change dramatically from fresh biomass to marine DON. Bacterial membrane moieties may form heteropolycondensates and liposomes and thus protect labile amino acid compounds from biodegradation and analytical hydrolysis (Tanoue et al., 1995; Borch and Kirchman, 1999, Hedges et al., 2000b). The extrapolation of these findings and considerations from the high molecular weight fraction to bulk DON is problematic. Mopper et al. (1996) found evidence that organic matter concentrated by ultrafiltration is different from organic matter in unfractionated seawater in terms of chemical composition and spectroscopic properties. Amon et al. (1996) observed differences in the bioavailability of the two size fractions. High molecular weight organic matter was more easily accessible to biodegradation than the fraction <1 kD. Complexation or polymerisation of smaller "building blocks" probably does not occur during marine diagenesis (Amon et al., 1996), which would explain the presence of D-amino acid-rich subunits in all size classes. There is still no evidence for the overall chemical structure of marine DON. Hydrolysable amino acids are evidently microbial-derived, but the composition of TDAA, which comprises ~10% of DON, bears only weak information on bulk DON. It is therefore a matter of speculation to interpret the presence of D-enantiomers in TDAA as evidence for major bacterial contribution to marine DON, as it was recently done by McCarthy et al. (1998).

The influence of bacterial secondary production in the riverine samples was much less, as indicated by lower D-amino acid proportions. Peptidoglycan contributes <10% to TDAA. The difference between marine and riverine DON in terms of bacterial production becomes even more evident considering the high content of peptidoglycan in gram-positive bacteria that predominate in soils and contain about four times more peptidoglycan than gram-negative marine bacteria (Salton, 1994; Neidhardt, 1996). Amino acids in soil-derived organic matter were therefore protected from degradation in the aquatic environment not primarily by incorporation into bacterial structural polymers. Unselective abiotic mechanisms, such as the encapsulation of proteins by humic substances (Zang et al., 2000) and metabolisation by fungi, like protection by melanin (Parsons, 1981), probably play a more important role in the terrestrial than in the marine environment.

4.4. Particulate Organic Nitrogen

Particulate organic matter in the Siberian rivers is constituted primarily of vascular plant detritus from taiga and tundra (Lobbes et al., 2000). Phytoplankton and living bacterial biomass is negligible (Cauwet and Sidorov, 1996; Sorokin and Sorokin, 1996). C/N ratios and lignin yields (X_{lignin}) are low in the rivers compared with fresh vascular plant biomass but do resemble soil organic matter. The low acid to aldehyde ratios within the vanillyl phenol family indicate a lower diagenetic degree for particulate than for dissolved organic matter (Lobbes et al., 2000). Accordingly, the amino acid yield was higher in the particulate fraction, and the signature was much more homogenous. This results in positive degradation indices and the proportions of the D-enantiomers and the non-protein amino

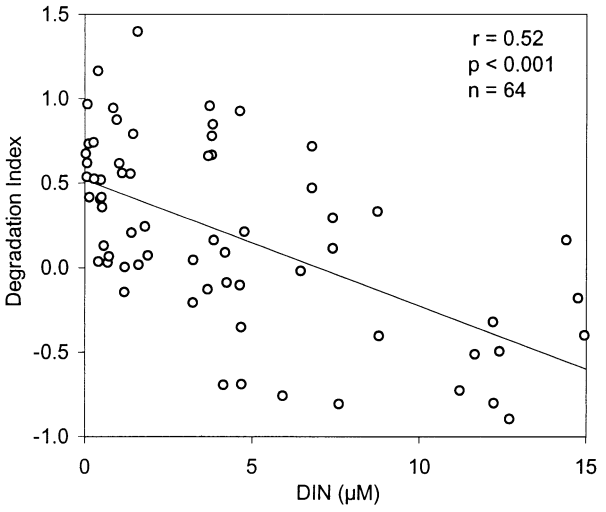

Fig. 9. Degradation indices of particulate amino acids, calculated after Dauwe et al. (1999), versus DIN concentrations at the individual sampling stations.

acid γ-amino butyric acid were much below the values of dissolved organic matter.

The near-shore decrease of TSS, POC, and PON corresponds to conservative mixing of river water with marine water in the brackish water zone. However, the source indicators X_{lignin} and $\delta^{13}C$ pointed to a changing composition of particulate organic matter toward lignin-poor and isotopically heavier phytoplankton-derived organic matter. This indicates that riverine particulate organic matter does not behave stable in the brackish mixing zones of the Siberian estuaries. With increasing distance from coast, suspended solids settle out and PAA concentration decreases relative to conservative mixing trends.

To differentiate diatomaceous from calcareous organic matter, the molar ratio of aspartic acid to glycine and the sum of serine + threonine proportions have been used (Ittekkot et al., 1984). No significant difference was found for these parameters between the marine stations. On average they exhibit Asp/Gly = 17.5 ±0.5 and Ser+Thr = 0.70 ± 0.03, which indicates a diatomaceous source for PAA in the ocean (e.g., Müller et al., 1986). For terrigenous organic matter, these parameters are not indicative of different autochthonous sources. In the Amazon, for instance, they would misleadingly indicate a diatomaceous source for soil-derived suspended PAA (Hedges et al., 1994). The variations between the different marine samples exceeded by far the differences between terrestrial and marine samples. The degradation index of Dauwe et al. (1999) reflected these source-independent patterns. Near-shore it was similar to the rivers, instead of falling below zero, which would be expected from mixing with marine PAA with negative degradation indices.

The degree of degradation apparently increased with increasing DIN concentration (Fig. 9). This is reasonable because high DIN concentrations reflect advanced mineralisation and diagenesis. Mixing of old and fresh sources and sedimentation of PAA may blur some of the relationships to the degradation index. However, it is remarkable that neither D-enantiomer proportions nor γ-amino butyric acid exhibited any correlation with the degradation index or with DIN. Most of the D-amino acids (except D-serine), however, correlated with γ-amino butyric acid ($p < 0.01$), indicating concomitant diagenetic dynamics. The reason for this asynchronous behaviour of the degradation index and the other diagenetic tracers might be different kinetics and sensitivities at different stages of degradation. The degradation index varied over a wide range between values of diagenetically fresh (river and near-shore waters) and old material (deepwater). In all samples, even offshore in the euphotic zone, the presence of D-amino acids indicated microbial biomass and fast turnover of decaying phytoplankton. For the first time, amino acid enantiomers were used as molecular biomarkers for microbial biomass in marine PON. No particulate D-amino acid concentrations in the ocean have been published before. The contribution of peptidoglycan to PAA nitrogen was estimated on the base of D-alanine. The rivers and Laptev Sea water below the euphotic zone (halocline, Atlantic water, and deepwater) exhibited the highest peptidoglycan proportion of 4.2 ± 0.6% and 4.8 ± 0.9%, respectively, and the D-enantiomer proportions were similar to fresh bacterial biomass (McCarthy et al., 1998; Amon et al., 2001). Near-shore and surface PAA was constituted of only 2.8 ± 0.4% peptidoglycan, which reflects the higher proportion of freshly produced phytoplankton-derived PAA in the euphotic zone. We infer that the degradation index for PAA is primarily sensitive on early stages of diagenesis. D-amino acids, however, are produced wherever bacteria metabolise organic matter.

5. CONCLUSIONS

We provide molecular evidence for bacterial contribution to marine organic nitrogen on the basis of the first direct and complete quantification of D-amino acids dissolved and suspended in seawater. We found D-amino acids to be powerful biomarkers to trace microbial-derived organic matter dissolved and suspended in the ocean. The dynamics of PAA, which always constitute the major fraction of PON, were uncoupled from the dissolved counterparts. Terrestrial PON was rapidly removed from the water column of the inner shelf. It is remarkable that D-amino acids were present even offshore in the euphotic zone, indicating bacterial biomass and fast turnover of decaying phytoplankton. Terrestrial PON was characterised by a lower diagenetic degree than DON and is probably more labile. Gradual release of nitrogen from decaying terrestrial biomass in the near-shore sediments may be a significant DIN source to the Arctic Ocean shelves.

Hydrolysable amino acids represent only ~10% of recalcitrant marine DON. The high D-amino acid proportion shows that these amino acids are microbial-derived and not released by phytoplankton and that TDAA repeatedly and more often than PAA has passed the microbial loop. TDAA exhibit a global similarity throughout the world oceans, in terms of its contribution to DON, amino acid composition and most probably also enantiomer ratio. This points to a dominant role of few universal diagenetic pathways for the formation of recalcitrant DON in the ocean. D-amino acids can be found in similar proportions in different molecular size classes, which indicates a continuity of molecular subunits across all molecular sizes of marine DON. However, even under consideration

of concomitant nitrogen-containing compounds of bacterial biomass, the major fraction of marine DON still awaits molecular identification, and hence source distinction. This is in clear contrast to terrigenous DON in the Siberian rivers. These recalcitrant compounds, which behave biogeochemically stable in the brackish mixing zone of the estuaries and in the Arctic Ocean over periods of at least years or decades, are constituted of ~40% hydrolysable amino acids. The influence of bacterial secondary production was low compared with the marine samples, as evident from lower D-amino acid proportions. The high amino acid content and the low D-enantiomer proportion of soil-derived DON indicate that terrestrial diagenesis is much more efficient in protecting amino acids from bacterial degradation than marine diagenesis. The huge amounts of dissolved organic nitrogen transported by the Siberian rivers into the Arctic Ocean therefore do not substantially support the productivity of the Arctic Ocean. It is of vital interest for our understanding of the global nitrogen cycle to identify the processes that lead to the immobilisation of nitrogen in the ocean and in the drainage areas of the rivers.

Acknowledgments—We thank V. Rachold for Lena and Yana samples and M. Wedborg for providing the sample material from the SWEDARCTIC Tundra Ecology-94 expedition. We thank C. Hartmann, M. Stürcken, and K.-U. Richter for technical support and J. Lobbes and R. Engbrodt for valuable discussions. We are also grateful to John Hedges, Wulf Amelung, and two anonymous reviewers for detailed and constructive comments on the manuscript.

Associate Editor: John I. Hedges

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