Biogeosciences

Diagenetic alterations of amino acids and organic matter in the upper Pearl River Estuary surface sediments

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Abstract. The objective of this study was to investigate the diagenetic alteration of sediment organic matter (OM) in the upper Pearl River Estuary. Sediment analyses were conducted for three size fractions of OM, including coarse particulate OM (CPOM), fine particulate OM (FPOM), and ultrafiltered dissolved OM (UDOM). Results showed that the highest and lowest carbon (C): nitrogen (N) ratios were in CPOM and UDOM, respectively, indicating that CPOM was relatively enriched in organic C. The highest average total N content in the FPOM fraction showed that FPOM was enriched in N-containing molecules. Our study showed that the "size-reactivity continuum" model was applicable to sediment particulate and dissolved OM. Distributions of amino acids and their D-isomers among the sediment fractions indicated that the amino acid-based diagenetic index, C:N ratio, and percentage of total N represented by total hydrolysable amino acids could be used as diagenetic indicators. Furthermore, the diagenetic state of sediment OM could also be characterized by C- and N-normalized yields of total D-amino acids, and C- and N-normalized yields of D-alanine, D-glutamic acid, and D-serine.

1 Introduction

Bio-molecules undergo major transformations in the natural environment following the disintegration of bacterial cell materials through death (Tremblay and Benner, 2009), viral lysis (Riemann and Middelboe, 2002), and protist grazing of source organisms (Nagata and Kirchman, 2000). Heterotrophic microorganisms are the primary agent of decomposition, and exhibit a significant impact on preserved organic matter (OM) abundance and biochemical composition (Tremblay and Benner, 2009). Over half of the nonliving (or detrital) OM in aquatic environments still remains unidentified at the molecular level (Hedges et al., 2000), although extensive effort has been taken to determine its origins, productions, and diagenetic alterations.

Several studies have confirmed the occurrence and origin of bacterial biomarkers in OM from water columns and sediments (Jørgensen and Middelboe, 2006; Lomstein et al., 2006; Lomstein et al., 2009; Pedersen et al., 2001; Tremblay and Benner, 2009). Among these biomarkers are amino acids and the respective D-enantiomers. Amino acid-based parameters have been shown to be useful as source indicators of particulate OM through the comparison of the yields and compositions of amino acids and the associated D-enantiomers between OM samples and source materials (Cowie and Hedges, 1992; Lomstein et al., 2006; Pedersen et al., 2001).

The yields and compositions of total hydrolysable amino acids (THAA) pool have been used successfully as diagenetic indicators because they are selectively decomposed or preferentially preserved during diagenesis, and seem uncompromised to source variations (Amon et al., 2001; Dauwe and Middelburg, 1998; Keil et al., 2000; Vandewiele et al., 2009). Based on microbial decomposition of dissolved OM in a marine water column on a short time scale, Davis et al. (2009) reported that carbon (C)-normalized amino acid yields were the most effective indicator for early dissolved OM diagenesis, relative abundances of amino acid yields for intermediate stages of diagenesis, and the mole percent composition of the non-protein amino acid (γ-aminobutyric acid) for advanced diagenesis. Dauwe et al. (1999) revealed systematically compositional changes upon progressive particulate OM degradation and derived a quantitative degradation index based on amino acid compositions of particulate OM samples from various marine environments. Also, the amino acid-based degradation index has been successfully used to characterize the diagenetic status of OM in marine and coastal sediments (Dauwe and Middelburg, 1998; Lomstein et al., 2006). However, there is little information on the diagenetic alteration and degradation status of OM with
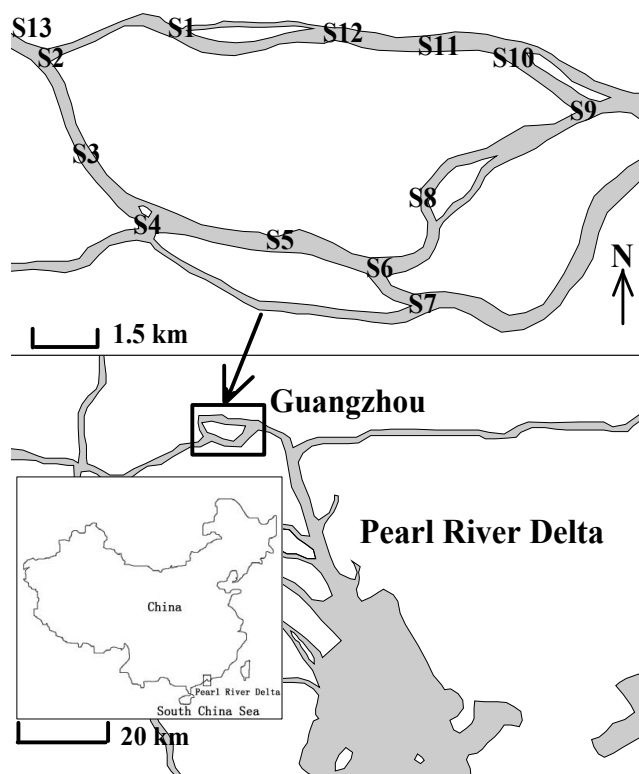
Fig. 1. Sampling stations along the Guangzhou reaches of the Pearl River Estuary, South China: S1 (Ersha Island), S2 (Baietan), S3 (Hedong Bridge), S4 (Yuxi Community), S5 (Luoxi Bridge), S6 (Xiaozhou Village), S7 (Nansha Express), S8 (Luntouhai), S9 (Emei Zhou), S10 (Xinchong Kou), S11 (Linjiang Road), S12 (Liede Village), S13 (Huadi Chong).

different sizes. Amon and Benner (1996) proposed the "size-reactivity continuum" model for aquatic dissolved OM, in which OM reactivity decreases with declining size as diagenetic alteration progresses. Tremblay and Benner (2009) found a consistent trend of increasing proportions of DAAs among Amazon River detrital size fractions in water columns and suggested the portion of DAAs as a useful diagenetic indicator for water column particulate and dissolved OM. Nevertheless, until now no studies are available on the diagenetic state of sediment OM of different sizes, especially in estuarine sediments.

The yield and composition of THAA pool have been used to quantify bacterial contributions to OM (Lomstein et al., 2009). Pedersen et al. (2001) showed that the percentages of four DAAs in the THAA pool increased with the sediment depth and that bacterial peptidoglycan contributed to the pool of organic nitrogen (N). Measuring the DAA composition in THAA, Kaiser and Benner (2008) found that bacterial detritus was the major component of particulate OM and an important source of submicron size particles and colloids in the ocean water columns. These amino acid-based indicators are

affected by many factors, such as sediment depth (Pedersen et al., 2001), diagenetic status (Lomstein et al., 2009), and OM size (Kaiser and Benner, 2008).

Therefore, the aim of this study was to assess the diagenetic status of sediment OM of different size fractions through measurements of compositions and concentrations of sediment particulate and dissolved amino acids. Moreover, the applicability of the "size-reactivity continuum" model in sediments was also investigated.

2 Materials and methods

2.1 Site description

The Guangzhou reach of the Pearl River Estuary (GZPR) was selected as the study site (Fig. 1). The Pearl River crosses Guangdong Province, China in the subtropical region, and opens to the northern part of the South China Sea (Yin and Harrison, 2008). The GZPR is in the upper Pearl River estuary (PRE). The tidal range at the GZPR is nearly 1.2 m, affected by the Guangdong micro-tidal coast (irregular semidiurnal tide) and the inverted funnel-shape topography of the PRE (Harrison et al., 2008). The GZPR is quite shallow with an average water depth less than 10 m. The fresh water from the GZPR discharges into the PRE and adjacent South China Sea through the Humen Gate, locating at the northeast of the PRE with a yearly sediment load of 658×10^4 t (Dong et al., 2006). The yearly freshwater discharge from the Guangzhou channel is 3.8×10^{10} m^3. Suspended sediment in the GZPR is composed mainly of clay and silt with a high percentage of organic particles because of urban sewage (Dong et al., 2006). The average sediment accumulation rate in the GZPR was 1.17 cm yr^{-1} (Zhang et al., 2002). The GZPR system is strongly affected by the combination of seasonal monsoon winds, tidal dynamics, complex topography, and large seasonal variations in both the runoff and coastal circulation (Dong et al., 2006). The terrestrial organic C contribution to the surface sediments in the GZPR is ca. 78 % (He et al., 2010). However, evidence for bacterial contributions to Pearl River OM has been reported, including relatively lower C:N ratios than those in local plants and enriched concentrations of deoxy sugars in the surface sediments (fucose and rhamnose) (He et al., 2010).

2.2 Sediment sampling and sample processing

Thirteen sediment samples were collected at the study site in January of 2010. The sampling area and stations are shown in Fig. 1. Topographical characteristics of the sampling stations are summarized in Table 1. Sediment samples were collected using a piston lead core equipped with a PVC tube of inner diameter 6 cm and 47 cm long. The length of sediment cores collected was 35 cm. The cores were immediately stored in ice and transported to the laboratory. In the present study, we used the upper 8 cm of each sediment core, in which sediments were thoroughly mixed, for analyses.

Table 1. Topographical characteristics of the sampling stations.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Latitude (°) N	23°06′679″	23°06′323″	23°04′482″	23°03′052″	23°02′958″	23°02′366″	23°02′115″	23°04′061″	23°05′487″	23°06′129″	23°06′289″	23°06′585″	23°06′201″
Longitude (°) E	113°17′365″	113°14′588″	113°15′512″	113°16′556″	113°18′111″	113°21′224″	113°21′948″	113°21′947″	113°24′610″	113°23′710″	113°21′722″	113°21′022″	113°18′880″
H (m)	540	540	541	542	542	541	541	541	541	541	541	541	541
Water depth (m)	3.8	1.9	2.6	2.2	2.4	1.8	1.0	1.7	2.7	2.4	3.4	2.7	0.5

H, elevation.

Table 2. Average weight percents (%) of the three sediment fractions at the sampling stations with and without the treatment of freeze-drying and sonication.

Sediment fraction	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
					Treatments with freeze-drying and sonication								
CPOM (%)	75.9(0.17)	51.1(0.12)	9.96(0.09)	21.6(0.04)	7.28(0.11)	9.12(0.06)	54.1(0.77)	17.6(0.21)	26.1(0.08)	28.3(0.85)	72.8(0.09)	15.9(0.10)	13.3(0.10)
FPOM (%)	23.6(0.12)	48.3(0.09)	87.4(0.04)	72.8(0.06)	85.4(0.05)	87.9(0.08)	44.7(0.10)	79.3(0.69)	71.6(0.12)	64.4(1.01)	24.9(0.66)	70.7(0.09)	78.4(0.09)
UDOM (%)	0.500(0.03)	0.540(0.01)	2.64(0.08)	5.58(0.11)	7.34(0.11)	2.94(0.07)	1.26(0.08)	3.06(0.10)	2.22(0.09)	7.28(0.04)	2.34(0.10)	13.4(0.12)	8.32(0.12)
					Treatments without freeze-drying and sonication								
CPOM (%)	75.8(0.12)	51.1(0.11)	9.98(0.08)	21.5(0.12)	7.25(0.10)	9.18(0.04)	54.2(0.39)	17.9(0.07)	26.0(0.11)	29.6(0.50)	72.7(0.07)	15.9(0.10)	13.5(0.10)
FPOM (%)	23.7(0.05)	48.4(0.07)	87.3(0.12)	72.9(0.09)	85.3(0.08)	87.8(0.45)	44.5(0.29)	79.2(0.76)	71.6(0.11)	63.3(1.16)	24.9(0.75)	71.3(0.56)	78.3(0.56)
UDOM (%)	0.490(0.01)	0.520(0.01)	2.69(0.07)	5.67(0.11)	7.42(0.08)	3.05(0.08)	1.37(0.05)	2.95(0.10)	2.36(0.10)	7.13(0.16)	2.49(0.10)	12.8(0.69)	8.22(0.69)

CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; UDOM, ultrafiltered dissolved organic matter; standard deviation in the parenthesis.

Samples were freeze-dried, gently homogenized by grinding in an agate mortar, and stored in polyethylene vials prior to analyses.
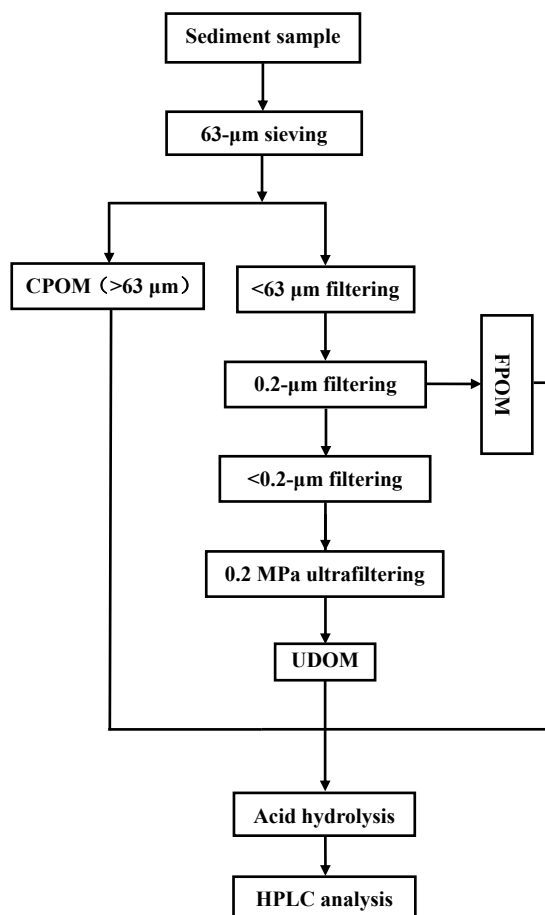
2.3 Sediment fractionation

Sediment samples were dispersed by sonication. Coarse particulate organic matter (CPOM) in the sediment was separated by sieving onto a 63-µm Nitex screen and oven-dried at 40 °C. The size of CPOM is >63 µm. The sand-free suspension was then passed through a Nalgene filter with a 0.2 µm cutoff size, to get fine particulate organic matter (FPOM). The size range of FPOM is between 0.2 and 63 µm. The FPOM fraction was oven-dried at 40 °C. The ultra-dissolved organic matter (UDOM) fraction that permeated the 0.2 µm pore filter was recovered with MSC300 cup type ultra-filter system with a nominal cutoff size of 1000 atomic mass units (Daltons), corresponding roughly to 0.001 µm. The size range of UDOM is between 0.001 and 0.2 µm. The UDOM fraction was freeze-dried prior to analysis. The detailed sediment fractionation procedure is shown in Fig. 2. The weight percentages of the individual sediment fractions are shown in Table 2. To investigate the effects of freeze-drying and sonication on the yields of the three size fractions, sediment samples were fractionated according to the above procedure but without the steps of freeze-drying and sonication with three replicates. ANOVA analysis was used to test whether there were significant differences between the yields of the three size fractions with and without the treatment of freeze-drying and sonication using the statistical software SPSS (SPSS 16.0).

2.4 Elemental analysis

Total organic carbon (TOC) for CPOM and FPOM was determined using the wet oxidation method (Gaudette et al., 1974). About 0.2 g of CPOM and FPOM (dry weight) was used for TOC determination with a precision of ±0.7 %. Concentrations of total organic C and inorganic C for UDOM were measured with the high-temperature catalytic oxidation method using a Shimadzu TOC-V CPH TOC analyzer with precisions of ±0.9 % and ±0.8 %, respectively. About 20 mL of UDOM was used for total organic C and inorganic C analyses. Total N for CPOM and FPOM was determined using Kjeldahl digestion with a precision of ±0.8 % (Morgan et al., 1957). About 0.2 g of CPOM and FPOM was used for total N analysis. Total N for UDOM was determined using Persulfate digestion with a precision of ±0.6 % (D'Elia et al., 1977). About 20 mL of UDOM was used in determination of total N.

2.5 Amino acid analysis

Approximately 500 mg of dried CPOM or FPOM particles and 5 to 10 mg of UDOM powder were hydrolyzed with 6 M HCl at 110 °C for 24 h in a sealed screw capped glass vial (Labco Exetainer, UK) under N_2. The hydrolysate was filtered to remove mineral particles (Whatman GF/F), then the pH of the solution was adjusted with KOH to a range of 6.6 to 6.8. Thereafter, the hydrolysate was centrifuged and filtered before the chromatography analysis (Whatman GF/F). Derivatization with OPA and N-isobutyryl-L-cysteine (IBLC) was performed in a borate

Fig. 2. Schematic graph for sediment fractionation procedure, in which CPOM is for coarse particulate organic matter, FPOM for fine particulate organic matter, and UDOM for ultrafiltered dissolved organic matter.
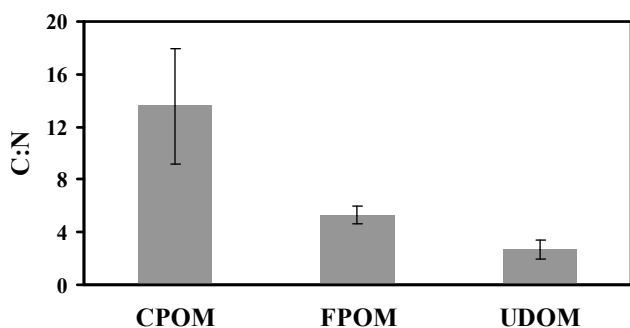
Fig. 3. Spatial-averaged mole C:N ratios for the coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and ultra-filtered dissolved organic matter (UDOM) size fractions based on values at the 13 sampling stations. Bars represent standard deviations.
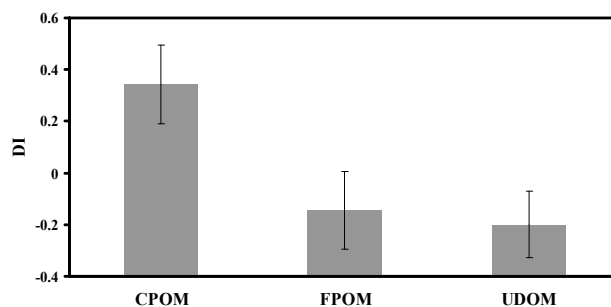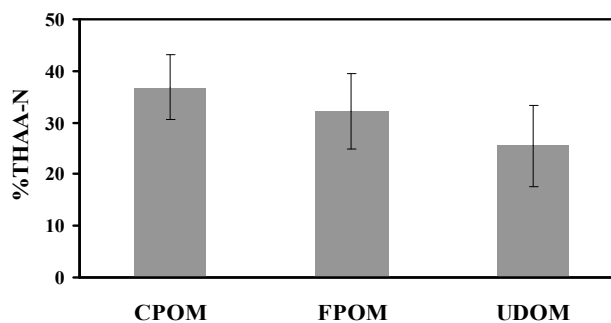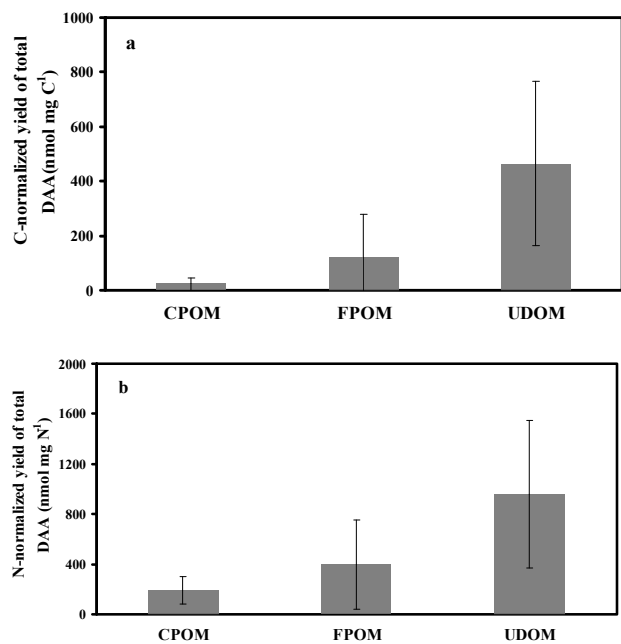

Fig. 4. Diagenetic index (DI) for the coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. Bars represent standard deviations.

Fig. 5. Spatial-averaged percentages of total nitrogen (N) as total hydrolysable amino acids (THAA) for the coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions based on values at the 13 sampling stations. Bars represent standard deviations.

buffer (pH 9.5) (Kaiser and Benner, 2005). Separation and detection of D-enantiomers were performed on an Agilent 1100 system with a Agilent Zorbax Eclipse XDB-C18 reverse phase column (150 mm × 4.6 mm, 5 μm) and a guard column (4 × 4 mm, 5 mL). The flow rate was 0.8 mL min^{-1} and the elution temperature was 20 °C. A mobile phase linear gradient was applied, from 100 % 23 mmol L^{-1} Sodium Acetate (pH 5.95–6.0) to 53.5 % methanol/acetonitrile (600:50 v/v) after 75 min and 80 % methanol/acetonitrile (600:50 v/v) after 85 min. The excitation wavelength was set at 230 nm (band width 25 nm). The emission length was set at 445 nm (cutoff filter 280 nm and band width 50 nm). DAA was quantified using four external DAA standard solutions containing a mix of D-serine (D-Ser), D-glutamic acid (D-Glu), D-alanine (D-Ala), and D-aspartic acid (D-Asp). The DAA measurements were corrected for chemical racemization occurring during hydrolysis. The correction was based on the average of measured racemization rates of free and protein amino acids (Kaiser and Benner, 2005). Percentages of D-enantiomers produced during acid hydrolysis of L-enantiomers for Asp, Glu, Ser, and Ala were 4.4 %, 2.0 %,
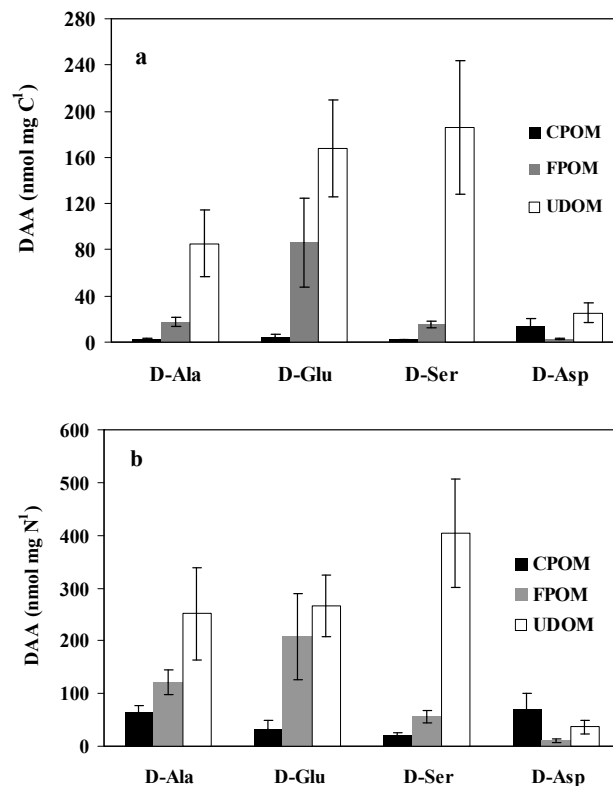
Fig. 6. Spatial-averaged C-(**a**) and N-(**b**) normalized yields of total D-amino acids (total DAA, the sum of D-Ala, D-Glu, D-Asp and D-Ser) in the coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and ultra-filtered dissolved organic matter (UDOM) size fractions based on values at the 13 sampling stations. Bars represent standard deviations.

Fig. 7. Spatial-averaged C-(**a**) and N-(**b**) normalized yields of individual D-amino acids (DAAs), including D-Ala (D-alanine), D-Glu (D-glutamic acid), D-Asp (D-aspartic acid), and D-Ser (D-serine) in the coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM) and ultra-filtered dissolved organic matter (UDOM) size fractions. Bars represent standard deviations.

0.3 %, and 1.2 %, respectively (Kaiser and Benner, 2005). The DAA concentrations were reported after reducing the D-enantiomers produced during acid hydrolysis. The concentration of THAA pools was analyzed by an ICS 3000 Dionex AAA-Direct System with an AminoPac PA10 analytical column ($2\,\text{mm} \times 250\,\text{mm}$, $8.5\,\mu\text{m}$) with guard column ($2\,\text{mm} \times 50\,\text{mm}$) and IPAD detector. The flow rate was $0.25\,\text{mL\,min}^{-1}$ and the elution temperature was $30\,°\text{C}$. Concentrations of the individual amino acids were calculated according to individual standard curves, produced from a mixture of the amino standard solution AA-S-18 (Sigma-Aldrich), to which ornithine (Orn), β-Ala, γ-Aba, and α-Aba were added. Twenty one amino acids were analyzed and the sum of them was used to represent the THAA concentration. In our study no corrections for the acid-hydrolysis efficiency were conducted (Benner and Kaiser, 2003).

3 Results

3.1 Bulk characteristics for CPOM, FPOM, and UDOM

The topographical characteristics of the sampling stations are shown in Table 1. Results of the yields of the three fractions with and without the treatment of freeze-drying and

sonication are listed in Table 2. ANOVA analysis showed that there were no significant differences between the yields of the three size fractions with and without the treatment of freeze-drying and sonication ($P < 0.05$), indicating that the effects of freeze-drying and sonication on the yields of the fractions were negligible.

Sediment TOC content increased with the sediment size. Average TOC contents for UDOM, FPOM, and CPOM were 4.12, 8.95, and $11.0\,\text{mg\,C\,g}^{-1}$ dry weight, respectively. The largest average total N content was observed in the FPOM fraction ($2.1\,\text{mg\,N\,g}^{-1}$ dry weight) and CPOM had the lowest average total N content ($1.1\,\text{mg\,N\,g}^{-1}$ dry weight). The UDOM fraction had an intermediate total N value of $1.9\,\text{mg\,N\,g}^{-1}$ dry weight. The results indicated that CPOM was relatively enriched in organic C and depleted in N compared to the FPOM and UDOM fractions, whereas FPOM was enriched in N-containing molecules. C:N ratios varied greatly from 1.2 to 41 among the fractions of the samples (Table 3). For each sampling location, the highest C:N ratio value was in CPOM among the three fractions, whereas the lowest C:N ratio was in UDOM except for S6 and S7

Table 3. Total organic carbon (TOC) and total nitrogen (Total N) contents, C:N ratios (mole TOC:Total N), percentages of TOC and total N as total hydrolysable amino acids (THAA) in sediment samples of different size fractions.

Sampling station	Size fraction	TOC (wt %)	Total N (wt %)	C:N	%C as THAA	%N as THAA
S1	CPOM	0.25	0.017	17.2	2.91	47.57
	FPOM	0.73	0.185	4.6	57.13	34.75
	UDOM	1.41	0.620	2.7	10.45	12.82
S2	CPOM	2.21	0.063	40.9	8.18	50.46
	FPOM	1.00	0.144	8.1	4.04	31.15
	UDOM	1.15	0.450	3.0	6.19	14.29
S3	CPOM	2.55	0.254	11.7	21.83	37.89
	FPOM	1.09	0.290	4.4	3.72	15.73
	UDOM	0.39	0.300	1.5	27.53	29.46
S4	CPOM	0.59	0.067	10.3	7.49	36.27
	FPOM	0.80	0.129	7.2	23.24	49.90
	UDOM	0.30	0.090	3.9	15.75	21.83
S5	CPOM	1.53	0.109	16.4	17.14	46.08
	FPOM	0.72	0.172	4.9	14.87	51.91
	UDOM	0.11	0.070	1.8	24.76	29.82
S6	CPOM	0.46	0.047	11.4	6.58	30.74
	FPOM	0.43	0.134	3.7	27.20	46.12
	UDOM	0.29	0.060	5.6	18.44	58.30
S7	CPOM	0.37	0.038	11.4	2.21	20.09
	FPOM	0.60	0.136	5.1	38.29	56.65
	UDOM	0.46	0.100	5.4	11.88	49.47
S8	CPOM	1.13	0.163	8.1	21.67	50.60
	FPOM	0.88	0.195	5.3	4.23	14.67
	UDOM	0.35	0.150	2.7	14.23	30.20
S9	CPOM	0.55	0.050	12.8	2.26	20.30
	FPOM	0.95	0.230	4.8	26.06	30.43
	UDOM	0.46	0.340	1.6	28.46	35.16
S10	CPOM	0.98	0.113	10.1	19.01	57.34
	FPOM	0.79	0.207	4.5	12.52	28.94
	UDOM	0.08	0.040	2.3	14.93	21.09
S11	CPOM	0.38	0.041	10.8	2.43	22.39
	FPOM	1.10	0.184	7.0	14.62	14.62
	UDOM	0.16	0.140	1.3	30.44	13.96
S12	CPOM	0.79	0.174	5.3	9.18	28.51
	FPOM	1.31	0.370	4.1	18.87	19.28
	UDOM	0.10	0.050	2.3	7.31	13.78
S13	CPOM	2.44	0.274	10.4	15.56	31.08
	FPOM	1.24	0.291	5.0	5.69	24.41
	UDOM	0.10	0.100	1.2	17.97	1.36

CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; UDOM, ultrafiltered dissolved organic matter.

(Table 3). The C:N ratio range in CPOM was relatively wide (5.3–41), whereas the C:N ratio ranges in FPOM and UDOM were relatively narrower (3.7–8.1 and 1.2–5.6, respectively) (Table 3). The highest average C:N value was found in CPOM, whereas UDOM had the lowest average C:N ratios (Fig. 3), also indicating that the CPOM fraction was N poor relative to the FPOM and UDOM fractions.

3.2 Amino acid yields and compositions

The THAA compositions within each size fraction and sampling station were relatively depleted in acidic amino acids (Asp and Glu) (Table 4). β-Ala, γ-Aba, and α-Aba were depleted, while Lys and Arg were relatively enriched in the three size fractions (Table 4). The degradation index generally decreased with decreasing sediment fraction sizes (Dauwe et al., 1999) (Fig. 4). The percentages of total N as THAA were from 20 % to 57 % in CPOM, from 15 % to 57 % in FPOM, and from 1.36 % to 58 % in UDOM. The percentages of total N as THAA generally overlapped over a broad range, exhibiting a relatively large variability among the sediment samples (Table 3). The average percentages of total N as THAA were 37 %, 32 %, and 26 % in CPOM, FPOM, and UDOM, respectively (Fig. 5).

C- and N-normalized yields of specific and total D-enantiomers (D-Ala, D-Glu, D-Asp and D-Ser) varied among

Table 4. Mole percents (%) of 21 amino acids in sediment samples of different size fractions.

Sampling station	Size fraction	Asp	Thr	Ser	Glu	Gly	Ala	Val	Cys	Met	Ile	Leu	Tyr	Phe	β-Ala	α-ABA	γ-ABA	Orn	Lys	His	Arg	Pro
S2	CPOM	1.33	0.00	0.00	0.00	0.00	0.00	1.74	1.12	0.97	0.00	2.90	3.17	5.94	0.00	0.00	3.46	5.80	40.78	0.82	31.98	0.00
	FPOM	0.00	0.00	0.00	0.00	0.94	0.19	0.16	2.93	0.00	0.00	8.70	0.96	9.16	8.57	0.00	0.00	3.59	30.46	0.20	34.15	0.00
	UDOM	0.21	0.01	0.00	0.00	0.04	0.00	0.39	4.51	0.04	0.00	11.23	0.73	8.35	2.47	0.00	0.00	2.35	25.60	0.51	39.54	4.02
S3	CPOM	0.00	0.20	0.32	0.16	1.52	1.73	3.27	0.00	2.14	7.64	17.98	4.52	12.84	2.03	0.00	1.98	2.68	24.13	1.88	14.98	0.00
	FPOM	0.00	0.00	0.12	1.62	0.10	0.00	0.00	3.15	8.66	0.00	0.00	0.00	13.56	0.00	0.00	0.00	4.16	0.00	0.11	62.75	5.77
	UDOM	0.02	0.00	0.03	3.65	0.00	0.74	0.54	3.28	10.34	0.00	0.00	0.00	14.67	0.03	0.00	0.00	5.46	5.65	0.95	52.26	2.38
S4	CPOM	1.76	6.48	7.44	6.20	16.92	13.86	6.75	0.00	0.40	3.70	8.35	1.99	3.90	0.83	0.00	0.88	0.60	4.77	0.16	2.73	12.27
	FPOM	0.00	14.58	1.78	2.74	11.09	5.47	5.65	0.00	1.31	6.66	15.67	3.15	8.62	2.09	0.00	1.31	1.09	11.34	0.64	6.81	0.00
	UDOM	0.31	16.78	3.57	0.99	9.48	1.26	4.61	0.00	2.66	6.84	17.59	4.23	1.20	3.14	0.00	1.84	1.97	13.67	1.54	7.46	0.86
S5	CPOM	0.49	6.16	7.80	7.87	20.04	14.43	8.69	0.00	0.19	4.73	9.69	1.97	4.92	1.36	0.00	0.69	0.72	6.01	0.32	3.93	0.00
	FPOM	0.00	0.44	0.13	0.00	0.11	0.00	0.25	1.00	3.17	0.00	0.00	1.30	5.19	0.00	0.00	0.00	5.71	12.92	0.48	68.16	1.14
	UDOM	1.21	0.24	6.53	0.00	0.68	0.04	0.03	2.08	3.99	0.00	0.00	1.05	5.84	0.00	0.00	0.00	6.88	14.61	0.64	50.16	6.02
S6	CPOM	3.83	5.87	12.66	5.25	21.93	22.62	6.16	0.14	0.00	3.32	4.89	0.50	2.07	0.00	0.00	0.00	0.00	2.58	1.45	1.56	5.16
	FPOM	0.27	6.59	8.37	1.44	15.19	10.74	9.03	0.11	1.93	5.32	6.89	0.97	3.51	2.24	0.08	3.27	6.53	5.90	0.44	2.82	8.36
	UDOM	0.36	7.53	5.92	1.03	6.42	6.44	10.25	0.09	3.66	8.46	8.98	1.27	1.97	3.16	0.00	3.58	7.82	8.19	0.32	5.94	8.61
S7	CPOM	0.13	0.00	0.10	0.11	0.16	0.00	0.30	0.09	0.00	0.00	0.00	4.59	7.36	1.56	0.05	2.27	4.54	36.58	2.42	34.62	5.13
	FPOM	0.00	0.47	0.00	1.32	0.47	0.00	1.06	0.62	0.66	0.32	2.03	4.92	10.87	0.00	0.00	0.68	3.19	26.63	2.47	44.29	0.00
	UDOM	0.05	0.69	0.09	1.78	0.83	0.00	1.63	0.89	1.37	0.06	3.64	4.98	11.23	0.00	0.08	0.28	1.29	21.37	2.59	46.31	0.84
S8	CPOM	1.20	8.12	8.71	3.63	19.85	16.20	8.04	0.25	0.26	4.57	7.34	0.25	3.23	1.32	0.89	0.00	0.39	4.30	0.76	1.83	8.85
	FPOM	0.12	6.59	8.98	0.00	21.14	16.06	9.27	0.17	1.23	5.29	6.91	0.74	3.32	0.00	0.00	0.00	0.00	6.14	0.37	3.14	10.53
	UDOM	0.06	0.63	9.49	0.00	21.67	15.27	11.33	0.11	1.35	6.33	5.62	1.15	3.11	0.00	0.00	0.00	0.00	7.61	10.56	5.42	0.29
S9	CPOM	0.73	8.15	9.31	2.60	19.49	16.91	8.04	0.00	0.22	4.60	7.73	0.21	3.17	0.00	0.00	0.00	0.00	5.57	0.91	2.97	9.38
	FPOM	2.42	5.69	12.14	4.91	18.36	13.42	7.04	0.16	0.27	4.09	5.91	1.16	3.17	2.43	0.06	2.17	4.26	3.34	0.26	1.92	6.82
	UDOM	0.04	14.05	16.18	2.31	4.79	8.55	6.37	0.14	0.29	3.78	3.37	0.83	3.49	2.14	0.00	2.59	2.57	1.59	0.16	24.13	2.63
S10	CPOM	1.09	0.00	4.60	1.03	0.00	2.10	2.73	0.00	0.00	11.99	4.74	8.03	7.18	0.00	0.00	0.00	39.91	13.35	0.00	3.25	0.00
	FPOM	0.09	0.33	0.14	0.00	0.18	0.12	0.20	0.99	0.00	0.00	3.34	0.44	3.94	5.26	0.00	0.00	4.67	30.63	0.35	48.47	0.86
	UDOM	2.11	0.84	0.06	0.00	0.36	0.33	0.01	1.57	0.00	0.00	2.33	0.18	1.16	6.38	0.00	0.00	2.95	31.68	0.57	45.11	4.36
S11	CPOM	0.35	6.99	8.52	1.79	20.15	15.73	9.24	0.39	1.63	5.64	7.77	0.41	3.17	0.00	0.00	0.00	0.00	6.21	0.75	2.40	8.89
	FPOM	1.68	3.51	9.61	2.51	12.24	10.01	5.31	0.95	0.00	2.56	4.25	1.49	2.62	16.23	0.00	0.00	14.43	2.14	0.74	0.97	8.75
	UDOM	3.58	1.36	10.88	2.64	5.25	7.46	3.29	2.61	0.00	0.68	1.12	1.96	0.85	14.23	0.04	0.00	8.62	1.92	0.84	20.94	11.73
S12	CPOM	0.23	0.00	0.00	0.38	0.24	0.00	0.94	0.00	0.32	1.17	2.07	4.17	5.92	1.42	0.00	0.98	4.34	32.00	4.00	41.81	0.00
	FPOM	0.07	0.12	0.12	0.33	0.09	0.00	0.12	0.00	0.13	0.00	0.00	4.68	7.02	2.78	0.07	2.48	4.87	39.66	2.89	30.13	4.44
	UDOM	0.18	0.94	0.22	0.00	0.02	0.00	0.28	0.00	0.06	0.00	0.00	6.35	9.02	3.79	0.00	2.67	4.99	40.01	1.53	29.49	0.45
S13	CPOM	1.32	6.77	7.65	4.00	18.66	15.70	9.64	0.00	0.44	6.10	9.66	0.97	4.80	0.00	0.00			6.39	0.10	2.90	4.91
	FPOM	1.18	7.81	7.98	3.36	18.68	15.41	8.01	0.20	0.18	4.48	7.65	0.13	2.82	1.63	0.00	1.73	1.18	5.20	0.60	2.96	8.79
	UDOM	1.05	8.76	9.05	3.98	19.30	16.42	4.75	0.03	0.01	2.09	6.19	0.01	0.06	2.19	0.07	0.04	0.73	3.67	12.26	8.91	0.43

CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; UDOM, ultrafiltered dissolved organic matter.

the three fractions (Table 5). The highest average C- and N-normalized yields of total DAA were found in UDOM, whereas the lowest ones in CPOM (Fig. 6a, b).

Both the spatially averaged C- and N-normalized yields of D-Ala, D-Glu, D-Ser increased with the sediment OM sizes (Fig. 7a, b). In contrast, the highest average C-normalized yield of D-Asp was found in UDOM and the lowest in CPOM, whereas the highest average N-normalized yield of D-Asp was found in CPOM and the lowest in UDOM (Fig. 7a, b).

4 Discussion

4.1 Diagenetic trends for sediment OM of different size classes

Bacteria are important agents for sediment OM degradation. As microbial decomposition progresses, the reactive components of sediment OM are selectively consumed, leading to an enrichment of less reactive sediment OM components (Wakeham et al., 1997). N-containing bio-molecules exhibit different dynamics during sediment OM diagenesis, thus these N-containing molecules can be used as good indicators of OM "freshness" and diagenetic state (Lomstein et al., 2006; Keil et al., 2000; Vandewiele et al., 2009). Specifically, amino acids and the respective D-enantiomers have been reported to display different reactivities at different stages of OM diagenesis (Dauwe and Middelburg, 1998; Lomstein et al., 2006; Vandewiele et al., 2009). Distribution patterns observed with amino acids and their D-isomers can be employed for the assessment of their relative reactivity. In the present study, compositional features that seemed sensitive to different stages of diagenesis were amino acid based diagenetic index (DI) (Dauwe and Middelburg, 1998), C:N ratio, percentage of total N as THAA, C-and N-normalized yields of total DAA, and C- and N-normalized yields of D-Ala, D-Glu, D-Ser.

In the present study, the DI values generally decreased from the larger to smaller sizes of OM, and the DI for CPOM was significant higher than those for FPOM and UDOM (Fig. 4). The DI is a reliable and robust index for the extent of diagenetic alteration in sediments (Dauwe and Middelburg, 1998; Keil et al., 2000; Lomstein et al., 2006; Vandewiele et al., 2009). Our study showed that the DI could be used

Table 5. C- and N-normalized yields of individual (D-Ala, D-Glu, D-Asp and D-Ser) and total D-amino acids (total DAA, the sum of D-Ala, D-Glu, D-Asp and D-Ser) in sediment samples of different size fractions.

Sampling station	Size fraction	D-Ala	D-Glu	D-Asp	D-Ser	D-Ala	D-Glu	D-Asp	D-Ser	Total DAA	Total DAA
		nmol mg C^{-1}				nmol mg N^{-1}				nmol mg C^{-1}	nmol mg N^{-1}
S1	CPOM	2.61				225				2.61	225
	FPOM										
	UDOM										
S2	CPOM	7.11				227				7.11	227
	FPOM		4.44				68.2			4.44	68.2
	UDOM	70.7				362				70.7	362
S3	CPOM										
	FPOM	11.6			13.9	203			122	25.4	325
	UDOM		130	49.4	1481		167	63.4	1903	1660	2134
S4	CPOM				10.2				122	10.2	122
	FPOM		40.4				184			40.4	184
	UDOM				441				1446	441	1446
S5	CPOM	8.78				117				8.78	117
	FPOM	25.8	18.5	4.83		461	165	43.1		49.1	669
	UDOM	708	378	207		2243	599	328		1294	3170
S6	CPOM				8.74				80.7	8.74	80.7
	FPOM	9.98		22.4	13.8	68.2		76.6	47.3	46.2	192
	UDOM		243		355		1176		1719	598	2894
S7	CPOM										
	FPOM		13.7	2.18	9.28		37.5	5.99	25.5	25.1	69.0
	UDOM										
S8	CPOM	1.55				16.7				1.55	16.7
	FPOM	8.53				99.2				8.53	99.2
	UDOM										
S9	CPOM	3.15			2.25	120			42.7	5.40	162
	FPOM	44.7			29.0	214			69.4	73.7	283
	UDOM		63.2		136		85.9		185	199	271
S10	CPOM	8.91			3.56	125			25.0	12.5	150
	FPOM		9.89	4.06	65.8		46.6	19.1	310	79.8	376
	UDOM										
S11	CPOM	0.49		2.41		26.3		64.6		2.90	90.9
	FPOM	21.4	23.5		25.1	88.0	48.4		51.7	70.0	188
	UDOM		284	73.1			322	82.8		357	404
S12	CPOM		57.3	15.0			431	112		72.3	543
	FPOM	104	1009		43.6	445	2151		92.9	1157	2689
	UDOM										
S13	CPOM		165				747			165	747
	FPOM										
	UDOM	328	1084			670	1107			1412	1777

CPOM, sediment coarse particulate organic matter; FPOM, fine particulate organic matter; UDOM, ultra-filtered dissolved organic matter; D-Ala, D-alanine; D-Glu, D-glutamic acid; D-Asp, D-aspartic acid; D-Ser, D-serine; the blank, below detection limit.

to characterizing the diagenetic state of sediment particulate and dissolved OM. The C:N ratio was also found to consistently decrease with declining particle size, demonstrating that the bulk parameters could be used to indicate the diagenetic states of sediment particulate and dissolved parameter. CPOM was enriched in terrestrial organic C and depleted in N. The lowest C:N ratio in UDOM suggested that C-rich OM was degraded and some low molecular weight OM rich in N was produced as microbial degradation progressed. The consistent trend of the DI and C:N ratio with the OM sizes showed that the bulk elemental parameter could also be used to indicate the diagenetic history of OM.

The percentages of total N as THAA decreasing with the sediment particle sizes indicates that amino acids are relatively more reactive than TOC or bulk N in the sediments. The percentages of total N as THAA are diagenetically sen-

sitive and have generally been observed to fall with progressive degradation, for example with depths in marine sediment cores (e.g., Henrichs et al., 1987; Lomstein et al., 2006; Keil et al., 2000; Pedersen et al., 2001). Cowie and Hedges (1992) found a decrease of the percentage of total N as THAA with the sediment depth and demonstrated the potential of this parameter as a valuable diagenetic indicator. It has been reported that percentages of total N as THAA for most living organisms and tree leaves are >50 % but decrease as degradation progresses to near 10 % for ancient sediments (Cowie and Hedges, 1994). Although diagenetic alterations of amino acids are variable, observations on the general trend toward low percentages of total N as THAA with declining particle sizes support the feasibility of the parameters as a diagenetic indicator.

The observed C- and N-normalized yields of total DAA trends were in the order of CPOM < FPOM < UDOM, which agreed with the observed patterns of degradation alteration in the sediment samples, with CPOM being the least processed and UDOM the most. Therefore, CPOM was the most reactive with the largest size, and UDOM was the least reactive with the smallest size among the three size fractions in our study. Similarly, the percentage of total dissolved organic C as total DAA has been shown to increase during the decomposition of fresh, algal-derived DOM from an Arctic ice floe (Amon et al., 2001). Moreover, Tremblay and Benner (2009) found that % DAA (the ratio of summed mole of DAA to THAA) increased with OM in the Amazon River system. These studies indicate that C- and N-normalized yields of total DAA are useful diagenetic indicators. C- and N-normalized yields of individual D-enantiomers other than those of D-Asp show consistent diagenetic trends towards the decreasing physical size of OM, suggesting the selective preservation of the three DAAs other than D-Asp. The absence of the trend for D-Asp suggested a different diagenetic pathway of D-Asp in our study area. A possible reason to explain the discrepancy in the patterns of the four D-enantiomers is that other bacterial macromolecules with reactivity different from petidoglycan may have contributed to D-Asp (Kaiser and Benner, 2008). Nevertheless, the size-specific trends in our study showed the potential of individual D-enantiomers yields to be used as diagenetic indicators. The relatively higher D-Ser in UDOM suggested that D-Ser was much more preserved than other D-enantiomers as the diagenesis of sediment OM progressed. The high concentration of D-Ser in UDOM might be attributed to the variability in biopolymer structures and in the microbial community compositions in the sediments.

4.2 The "size-reactivity continuum" model for sediment OM

The "size-reactivity continuum" model has been used to relate bioavailability to size and diagenetic state of OM for dissolved OM in various water environments (Amon and Benner, 1996; Tremblay and Benner, 2009). Generally, diagenetically young OM seems to be most bio-reactive. OM becomes less reactive and smaller in physical size as biodegradation proceeds. The "size-reactivity continuum" model indicates that the major pathway of OM decomposition is a continuous change from bio-reactive organic particles and macromolecules to small refractory organic molecules (Amon and Benner, 1996).

Our incubation study on sediment CPOM and FPOM showed that the bio-reactivity of CPOM was significantly higher than that of FPOM ($P < 0.05$). The diagenetic pathway of larger POM in sediments is towards smaller POM and subsequent dissolved OM. Our study showed that the "size-reactivity continuum" model was applicable to sediment particulate and dissolved OM.

5 Concluding remarks

Distributions of amino acids and their D-isomers among the sediment fractions indicated that the amino acid-based diagenetic index, C:N ratio, and percentage of total N represented by total hydrolysable amino acids could be used as diagenetic indicators. Furthermore, the diagenetic state of sediment OM could also be characterized by the C- and N-normalized yields of total D-amino acids, and C- and N-normalized yields of D-alanine, D-glutamic acid, D-serine. The "size-reactivity continuum" model developed for the water column was supported by our results of the bio-reactivity of sediment particulate OM of varying size fractions. Our study showed that the "size-reactivity continuum" model was applicable to sediment particulate and dissolved OM.

Edited by: J. Middelburg

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