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Alkali-extractable polysaccharides in marine sediments: Abundance, molecular size distribution, and monosaccharide composition

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Abstract—Carbohydrates from coastal sediments were characterized using differential extraction and ultrafiltration techniques. Carbohydrates adsorbed on sediment particles were extracted by various combinations of heating, ultrasonic radiation, and alkali treatment, and the extraction efficiencies of these treatments were compared. Heating (121°C, 60 min) in the presence of alkali (Na_2CO_3, 20 mM) followed by ultrasonication (20 min on ice) yielded maximal recovery. By repeating this extraction three times, 50% (at maximum) of total carbohydrate in the sediment could be extracted to solution. Carbon-based ratios of carbohydrate to total organic carbon in the extracts and residues were 19 to 28% and 10 to 13%, respectively. The major fraction (68–84%) of extracted carbohydrate was found in macromolecular fractions (>10 kDa). The extracted carbohydrates could be precipitated with 80% (v:v) ethanol with a recovery of 74 to 80%. The monosaccharide composition of the ethanol precipitable polysaccharides was then analyzed by capillary gas chromatography and compared with carbohydrates in the insoluble residue fraction. The molecular composition of residual polysaccharides was somewhat variable, presumably depending on whether the sediment was affected by terrestrially derived organic matter, whereas the composition of the extracted polysaccharides was strikingly similar irrespective of depositional environment. Implications of these data for mobility, reactivity, and origins of sediment organic matter are discussed. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

Carbohydrates are ubiquitous compounds in marine sediments, representing a significant fraction of total sediment organic carbon (usually 5–20%; Degens and Mopper, 1979; Cowie and Hedges, 1984; Bergamaschi et al., 1997). They are not only an important energy source for various heterotrophic organisms in the sediment (Decho, 1990; Tibbles et al., 1994) but also may be a potential precursor for refractory organic matter preserved in sedimentary rocks (Sinninghe Damsté et al., 1998). Marine primary producers produce polysaccharides as a major photosynthetic product, some of which have unique monosaccharide compositions and arrangements (Lewin, 1974; Kloareg and Quatrano, 1988; Hoagland et al., 1993). Thus, carbohydrates are potentially useful clues to the origin of organic matter in sediments (Böhm et al., 1980; Cowie and Hedges, 1984; Vreeland et al., 1987). However, exact physical and chemical states of carbohydrates in marine sediments, for example, mechanisms of binding and adsorption to sediment particles, molecular size distribution, and monosaccharide arrangements, are not well understood.

Sediment organic carbon in general is tightly or loosely associated with mineral particles, with only a small fraction (typically <1%) dissolved in pore waters. Because the concentration of dissolved organic carbon is usually higher in the pore water than in the overlying seawater, it has been postulated that organic molecules dissolved in pore waters or loosely associated with sediment grains are a possible source of refractory dissolved organic matter in the water column of deep ocean (Burdige et al., 1992; Martin and McCorkle, 1993; Guo and Santschi, 2000). The mobility of sediment organic matter is largely controlled by adsorption of organic molecules on sediment mineral particles (Mayer, 1994), as well as by interactions between organic molecules. However, the great diversity of organic molecules and the complexity of these organic–mineral and organic–organic interactions have hampered characterization of these interactions, and this is essential to understanding the behavior of sediment organic molecules during diagenetic processes and in the sediment–water interactions. Therefore, methodologies must be developed to appropriately fractionate sediment organic mixtures and to resolve the nature of the molecular interactions.

Most sediment organic molecules exist as polymeric forms in which monomer residues are bound to each other by specific chemical bonds. In the case of carbohydrates, monosaccharide residues are bound into a polysaccharide molecule by the glycosidic bonds, which can be hydrolyzed by hot acid digestion but are fairly stable in neutral and alkaline solutions. In contrast, the ester linkage, which often binds polysaccharides with other organic molecules, is easily destroyed by alkaline treatments. The *O*-glycosidic bonds between polysaccharides and proteins, which are common in proteoglycans produced by various marine invertebrates, are also hydrolyzed under alkaline conditions (Collins and Ferrier, 1995). The hydrogen and van der Waals bonds, which bind the organic macromolecules with each other and with sediment mineral particles, can be dissociated by heating. Therefore, treatments with alkali (Hitchcock, 1977) and heat (Handa and Tominaga, 1969) often have been applied to extract water-soluble polysaccharides adsorbed on natural particulate matter without significant destruction of polymer structure. Soluble polysaccharides, once released to water phase, can be precipitated through dehydration using a hydrophilic organic solvent such as ethanol or

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acetone (Handa and Tominaga, 1969; Underwood et al., 1995; Sun et al., 2000).

In this study, we applied heat and alkali treatments to extract organic molecules including carbohydrates from the bulk sediment. We compared extraction efficiencies of carbohydrates among various treatments to infer relative importance of binding mechanisms. The molecular-size distribution of the extracted organic carbon and carbohydrates was estimated to determine to which size class the carbohydrates were relatively accumulated. The major fraction of extracted carbohydrate was found in high molecular weight fractions (>10 kDa). Therefore, the extracted polysaccharides could be precipitated with 80% (v:v) ethanol with a good recovery. The monosaccharide composition of the ethanol precipitable polysaccharides was then analyzed by capillary gas chromotography (GLC) and compared with carbohydrates in the insoluble residue fraction. Based on these data, we discuss chemical properties and the possible origin and roles in organic diagenesis of the extractable carbohydrates in marine sediments.

2. MATERIAL AND METHODS

2.1. Sediment Samples

Sediment samples were obtained from three stations along the east coast of Japan: station A (slope off Bosoh Peninsula; 34°40′N, 139°53′E; depth, 2500 m), station B (Sagami Bay; 35°00′N, 139°12′E; 1500 m), and station C (Otsuchi Bay; 39°20′N, 141°57′E; 43 m). Sediment types of stations A, B, and C were oligotrophic mud with no visible land-derived debris, eutrophic mud containing traces of vascular plant fragments, and eutrophic sandy mud that was significantly affected by land-derived organic matter. Sediment was sampled at stations A and B using a multiple corer (80 mm in diameter, 60 cm long, 8 cores) at KT96 to 9 cruise of R/V Tansei-maru (3–9 June, 1996). Some cores were cut into 10-mm sections on board, and each section was separately packed in a polystyrene petri dish and immediately frozen at −20°C. For other cores, the top 8 to 10 cm of sediment was stocked in polypropylene containers and frozen. The sliced samples were used for vertical profile analyses below (data in Figs. 3 and 4), and the bulk samples were used for all the other experiments. Sediment of station C was sampled in May 1996 using a Smith-Macintyre grab sampler (Rigosha, Tokyo). Sample was packed in polypropylene containers without sectioning and stored frozen (−20°C) until analyses.

2.2. Extraction Procedure

A polypropylene centrifuge tube (50 mL) was used for extraction reactor. A 10 to 15 g (wet weight) portion of sediment sample with 20 mL of 0.5 mol/L NaCl solution was placed in the reactor tube. Weights of the tube and contents were recorded. One or a combination of the following treatments was then applied: (i) soaking in alkali solution, (ii) heating, and (iii) ultrasonication (see Table 1 for abbreviations of extraction treatments used in text). To make the extraction solution alkaline, 1 mL of 0.5 mol/L Na_2CO_3 solution was added to the reactor tube and gently mixed by a vortex mixer (final concentration, 15–20 mM). Heating was done by autoclaving the tube (tightly capped) at 121°C for 60 min. Contents were cooled and mixed well with a vortex mixer before the next step of extraction. Ultrasonication was performed using an ultrasonic cell disrupter (VP-30s, TAITEC, Koshigaya, Japan; 300 W full output, 20 kHz). The disrupter was tuned to half of the full output and operated on ice at 1:1 pulse for 20 min. After all the treatments were finished, weight of the contents was adjusted to the original value by dropping Milli-Q water, because some evaporation occurred during extraction. The contents then were mixed well and centrifuged at 4°C and 2000 *g* for 20 min. The supernatant was recovered as extract. For some cases, the extraction procedure was repeated on the residual pellet, and the extracts of the repetitive treatments were analyzed separately. The tube with the residue was weighed and dried to the constant weight. The wet and dry weights were used for calculation of water contents in the original sediment and in the residue. Both the extract solution and the dried residue were used for analyses of total carbohydrate, organic carbon, and total nitrogen. The dried residue was also analyzed for monosaccharide composition.

Table 1. Abbreviations used for the combinations of extraction treatments.

Abbreviation	Treatments
N	Centrifugation[a] only
A1	Alkali[b] added before centrifugation
A2	Alkali added and stored overnight before centrifugation
S	Sonicated[c] before centrifugation
SA	Sonicated, alkali added, centrifuged[d]
AS	Alkali added, sonicated, centrifuged
H	Heated[e] before centrifugation
HA	Heated, alkali added, centrifuged
HS	Heated, sonicated, centrifuged
HSA	Heated, sonicated, alkali added, centrifuged
HAS	Heated, alkali added, sonicated, centrifuged
AH	Alkali added, heated, centrifuged
AHS0	Alkali added, heated, sonicated for 10 min, centrifuged
AHS1	Alkali added, heated, sonicated for 20 min, centrifuged
AHS2	Alkali added, heated, sonicated for 40 min, centrifuged
AHS3	Alkali added, heated, sonicated for 60 min, centrifuged
AHS4	Alkali added, heated, sonicated for 90 min, centrifuged

[a] Centrifugation was done at 2000 g, 4°C for 20 min, and the supernatant was recovered.
[b] Sodium carbonate was added to a final concentration of 20 mmol/L.
[c] Treated by a ultrasonic cell homogenizer (1:1 pulse) on ice for 20 min if not otherwise described.
[d] Treatments were done in described order.
[e] Autoclaved for 60 min at 121°C.

To isolate a macromolecular fraction of organic matter by precipitation from the extract, four volumes of 99.5% guaranteed reagent-grade ethanol was added to the extract solution in a 100 mL glass vial and mixed well. The mixture was settled in refrigerator (4°C) for 2 d, during which hydrophilic organic polymers were spontaneously aggregated and precipitated. The suspension was then centrifuged at 4°C and 8000 *g* for 30 min. The precipitate was washed twice with 99.5% ethanol and finally solubilized again in 3 to 6 mL of Milli-Q water for later analyses for total carbohydrate and monosaccharide composition. In some cases, the precipitation process was performed after pH of the extract solution was adjusted to 5.0 using acetic acid–sodium acetate buffer. The recovery of precipitate did not significantly change between the extracts with and without pH adjustment, and therefore we report only precipitation without pH adjustment.

2.3. Molecular-Size Fractionation

To obtain molecular weight (MW) fractions from the extract solutions, we used the ultrafiltration cartridge Amicon Microcon (Millipore, Bedford, USA.; nominal MW cutoff: 3000, 10,000, 100,000 Da). Before use, the cartridge was soaked in Milli-Q water overnight at 4°C and then gently rinsed twice with Milli-Q water to remove glycerol additive (humectant added by the manufacturer). A 500 μL portion of the extract was put on the cartridge and centrifuged at 4°C and the 80% maximal gravity assigned to each cartridge by the manufacturer. The filtrate was used for later analyses of total carbohydrate, organic carbon, and total nitrogen. Fractions of (nominal) <3000, <10,000, and <100,000 Da were measured directly, whereas fractions of 3000 to 10,000, 10,000 to 100,000, and >100000 Da were calculated by subtraction.

Accuracy of the MW cutoff of the ultrafiltration cartridges was checked using standard carbohydrates: glucose (MW 180), maltotriose (504), maltoheptaose (1153), and dextrans (average MW 5000, 10,500, 67,300, 473,000 and 2,000,000). Maltotriose was purchased from Wako Pure Chemical Industries (Osaka, Japan), and the other carbo-

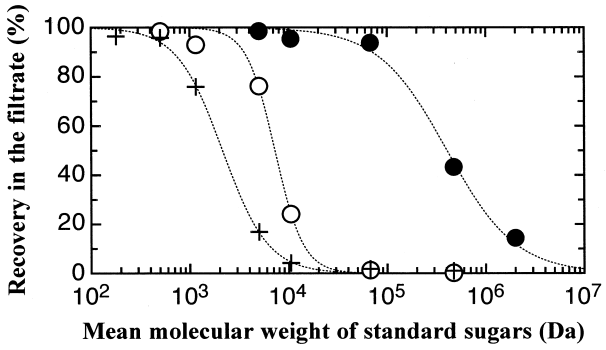

Fig. 1. Recovery of standard carbohydrates of known MW (glucose, maltooligosaccharides, dextrans) in the filtrate by ultrafiltration. Nominal MW cutoff values of the ultrafiltration cartridges are: 3000 (cross), 10,000 (open circle) and 100,000 (solid circle). Dotted lines are the best-fit hyperbolic tangent curves for respective cutoff values.

hydrates from Sigma-Aldrich Fine Chemicals (St. Louis, USA). Each standard was dissolved in alkaline solution containing 0.2 mol/L Na_2SO_4 and 0.02 mol/L Na_2CO_3. The results showed that possible loss of low MW polysaccharides by adsorption to the filters generally can be neglected. Recoveries of these standard carbohydrates in the filtrates (Fig. 1) were rather close to the values expected from the nominal cutoff for the cartridges of 3000 and 10,000 Da. In the case of the cartridge of 100,000 Da cutoff, significant fractions of the standards that had much larger MWs than the cutoff value passed through the cartridge filter, presumably because of the loose three-dimensional structure of the carbohydrate molecules. Hence, by using the ultrafiltration method, large MW fractions (i.e., >100,000 Da) of carbohydrate in natural samples are potentially underestimated, whereas the estimation for smaller MW fractions is relatively accurate.

2.4. Analytical Methods

Total carbohydrate concentration (TCHO) of the extract solutions (including MW fractions and ethanol precipitates) was determined by the phenol–sulfuric acid (PSA) method (Handa, 1972; Underwood et al., 1995). Briefly, 1 mL portions of the extract (or its dilution) were poured into two glass tubes. The 5% (w:v) phenol solution (1 mL) was added to one of the tubes, and Milli-Q water (1 mL) was added to the other. The tubes were shaken well, added with conc. H_2SO_4 (5 mL), shaken vigorously, and settled for cooling. Absorbance at 488 nm was measured using a spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). The latter tube was used as blank. The calibration curve was drawn using D-glucose as the standard.

TCHO of the dried residue was determined after hydrolysis. A weighed fraction of the sample (10–20 mg) was placed in a glass tube (10 mL), to which 12 mol/L H_2SO_4 solution (1 mL) was added. After 3 h at room temperature, 9 mL of Milli-Q water was added to the tube. The contents were mixed well and heated at 100°C for 4 h. After cooling, the tube was centrifuged (3000 *g*, room temperature, 10 min), and a portion of the supernatant was analyzed for carbohydrate by the PSA method.

Organic carbon (OC) and total nitrogen (TN) concentrations of the extracts (including MW fractions) were determined as follows: A precombusted glass fiber filter (GF/D, 10 mm in diameter, Whatman, Maidstone, UK) was placed on the bottom of a precombusted glass vial and wetted with a small portion (usually 40 μL) of the extract. After drying at 60°C, the vial containing the filter was put in an airtight polypropylene container and treated by vapor of conc. HCl for 3 d to remove carbonate. The filter was then dried in vacuo, packed in a tin capsule, and subjected to element analysis using a CHN analyzer (NA-1500, Fisons, Milan, Italy).

OC and TN analyses of the dried residues were done as follows: A 10 to 30 mg portion of the residue was put in a silver capsule that was prewashed with acetone. With the mouth left open, the capsules were treated by HCl vapor for 3 d to remove carbonate. The capsules were then dried again, folded tightly, and analyzed using the CHN analyzer.

Monosaccharide compositions of carbohydrates were analyzed by GLC using the method of Walters and Hedges (1988) with minor modifications. In preparation of the ethanol precipitable, high MW fraction of extract, the precipitate dissolved in Milli-Q water was spiked with HCl to the final concentration of 0.5 N and heated at 105°C on dry block bath for 4 h to hydrolyze carbohydrate. In preparation of the dried residue, 20 to 50 mg of the residue was hydrolyzed by 10 mL of 0.5 N HCl at 105°C for 4 h. The hydrolysates were then desalted using an electrodialysis apparatus (MICRO ACILYZER, Asahi Chemical Co., Kawasaki, Japan) equipped with an ion exchange cartridge of the nominal MW cutoff of 100 Da. It usually took ca. 10 min to remove HCl sufficiently. For some samples, hydrolysis using 12 mol/L H_2SO_4 was applied to compare with that using dilute HCl. The ethanol precipitate dried at 60°C, as well as the dried residue, was soaked in 1 mL of 12 mol/L H_2SO_4 solution at room temperature for 3 h. The suspensions were then diluted with 9 mL of Milli-Q water and hydrolyzed further at 100°C for 4 h. Desaltation was done using an ion exchange cartridge of nominal MW cutoff of 300 Da instead of 100 Da. All the hydrolysates were spiked with internal standard D-allose (2 μmol per sample) to correct possible losses during the following treatments.

The desalted hydrolysates were treated with Na_2CO_3 followed by $NaBH_4$ as described by Walters and Hedges (1988). Excess $NaBH_4$ was destroyed by acidification with several drops of 1 N HCl. After ebullition of H_2 ceased, Na^+ and Cl^- were removed by electrodialysis using the 100-Da cutoff ion-exchange cartridge. The desalted solution was transferred to a gourd flask with 50 μL of 50% (v:v) glycerol (Mopper, 1977) and evaporated to dryness at 40°C. Excess boric acid was removed by adding and evaporating 30 mL of methanol twice. After the last evaporation, contents of the flask were dissolved in 1 mL of methanol, transferred to a glass ampoule, spiked with the second internal standard perseitol (2 μmol), and evaporated in a vacuum oven at 40°C. After methanol was evaporated, the ampoule was heated to 85°C for 2 h in vacuo, and then the derivatization protocol of Walters and Hedges (1988) was followed, in which alditols are acetylated and the carboxyl groups of aldonic acids are derivatized with *n*-hexylamine. After derivatization, solvents were evaporated under N_2 at 55°C, and the residue was redissolved in 500 μL of chloroform and injected (1 μL) to a capillary gas chromatograph (Hewlett-Packard 5890 equipped with a capillary column HP-1701 (30 m, 0.25 mm i.d., 0.25 μm film thickness) and a flame-ionization detector). Eight neutral sugars (rhamnose [Rha], fucose [Fuc], ribose [Rib], arabinose [Ara], xylose [Xyl], mannose [Man], galactose [Gal], glucose [Glc]) and two uronic acids (glucuronic acid and galacturonic acid) were measured. Concentrations of uronic acids often were too low to be detected. Uronic acids other than the two above were not detected. Peak identification was verified by GC/MS (Hewlett-Packard GC 6890 and MSD 5973 equipped with the same column as above).

The amount of total aldoses detected by GLC was always considerably lower than the amount of TCHO as determined by the PSA method (mol glucose equivalent). The following factors may be responsible for the observed low recoveries by GLC: (i) The sensitivity of the PSA method slightly varies between different monosaccharides, which reduces the accuracy of carbohydrate concentrations measured by this method compared with GLC. (ii) Some carbohydrates may not be hydrolyzed completely to monosaccharides by dilute HCl (e.g., cellulose). (iii) The samples might have contained carbohydrate-like (PSA-positive) substances that could not be detected by the GLC method we employed. (iv) Monosaccharide residues that have electric charges (e.g., uronic acids) might have been partially lost during the sample preparation for GLC by the electrodialysis. The first factor cannot explain the difference between GLC and PSA because the PSA method is known to be the most sensitive for glucose, which we used for calibration; therefore, we most likely underestimated total carbohydrate when measured by the PSA method. Preliminary tests showed that HCl-unhydrolyzable carbohydrates may constitute significant fractions of carbohydrates in the sediment of station C, but not in those of stations A and B. Even in the case of station C, the discrepancy between GLC and PSA could not be sufficiently explained by the presence of this fraction. Therefore, we postulate that some unidentified carbohydrates and the loss of charged sugars during sample preparation

Table 2. The ratio of carbohydrate extracted by various treatments to total carbohydrate and the fraction of ethanol precipitable polymeric carbohydrate to the total of the extracted carbohydrate (see Table 1 for abbreviations).

	Station A		Station B		Station C	
Treatment	Extraction efficiency (%)[a]	Ethanol precipitation efficiency (%)[b]	Extraction efficiency (%)[a]	Ethanol precipitation efficiency (%)[b]	Extraction efficiency (%)[a]	Ethanol precipitation efficiency (%)[b]
N	1.2	67.2	2.5	74.3	0.0	—
A1	2.4	61.4	3.7	98.7	0.3	29.4
A2	4.1	70.9	nd	nd	nd	nd
S	5.8	78.3	5.0	75.8	2.5	74.7
SA	6.2	76.7	6.2	86.1	2.7	74.4
AS	7.8	70.6	7.7	85.0	3.3	74.7
H	11.2	67.2	8.5	72.1	7.7	68.5
HA	10.4	69.0	9.0	76.3	nd	nd
HS	13.6	72.1	10.8	79.8	10.4	69.0
HSA	15.9	66.6	11.6	78.7	10.5	69.1
HAS	13.7	70.6	12.4	77.3	12.8	72.9
AH	21.0	70.5	21.9	80.9	10.5	68.6
AHS0	23.9	71.5	23.9	92.8	nd	nd
AHS1	28.9	70.9	26.2	81.5	13.3	72.6
AHS2	26.9	71.5	28.6	81.4	12.8	75.3
AHS4	29.5	79.4	nd	nd	nd	nd
Total HCl-hydrolyzable carbohydrate [μmol Glc-equiv. g^{-1}]	32.5	—	50.8	—	30.1	—

[a] Glucose equivalent ratio (%) of carbohydrate extracted by each treatment to total HCl-hydrolyzable carbohydrate. The latter was estimated by hydrolyzing dried sediments with 0.5 N HCl at 105°C for 4 h and measuring carbohydrate in the hydrolysates by the PSA method.

[b] Glucose equivalent ratio (%) of polymeric carbohydrate precipitated by ethanol (final 80% v:v) after extraction to the total extracted carbohydrate by each treatment.

nd = not determined.

are the main factors that led to underestimation for total carbohydrate concentrations by GLC.

3. RESULTS

3.1. Extraction and Ethanol Precipitation

The fractions of extracted carbohydrate to total carbohydrate and the fraction of ethanol (80%) precipitable carbohydrate to total extracted carbohydrate in the top 10 cm of the sediment cores (Table 2) are compared among various extraction protocols listed in Table 1. Carbohydrate originally dissolved in pore water is represented by the fraction extracted by saline water without additional treatments (N) and constituted only 0 to 2.5% of total HCl-hydrolyzable carbohydrate. Comparison of the efficiency of extraction by cold alkali soaking (A1, A2), ultrasonication (S), and heating (H) showed that heating was the most effective, and alkali soaking was the least effective. It may be supposed that the fractions extractable by heating, ultrasonication, and cold alkali soaking (subtracted by the fraction N) approximately represent the carbohydrates that are adsorbed to sediment particles by hydrogen bond or intermolecular dispersion force, physically trapped in micropores and intergranular spaces of sediment particles, and bound to other insoluble organic macromolecules by weak ester or *O*-glycosidic linkage, respectively. Therefore, the above results indicate that a significant fraction (6.0 to 10.0%) of sediment carbohydrate was intrinsically soluble but bound to mineral or organic insoluble particles by hydrogen or van der Waals bonds. The fraction of carbohydrate that was soluble but physically trapped in micropores or intergranular spaces appeared to be relatively small (2.5–4.6%).

Extraction efficiencies by some combinations of two extraction steps (SA, AS, HA, HS) were generally similar to the values expected from the sums of the efficiencies of respective steps subtracted by the double-counted fraction N. However, the extraction efficiency by hot alkali treatment (AH) was significantly (2.5–12.2%) higher than the sum of those by cold alkali soaking (A1) and heating (H). This difference can be interpreted by the presence of carbohydrates bound to other insoluble organic materials by relatively rigid ester (or *O*-glycosidic) linkages that could not be cut by cold alkali, but hot alkali, treatments. The ratio of the fraction of carbohydrates putatively bound by the hydrogen or van der Waals bonds (or both; fraction H − N) to that putatively bound by the ester or *O*-glycosidic linkages (AH − H) was higher in the shallow bay sediment (station C, 2.8) than in the deep ocean sediments (A, 1.0; B, 0.5). The ultrasonication after the hot alkali treatment further increased the extraction efficiencies, although the increments were predictable from the sum of the efficiencies of the respective treatments (HA + S–N). Extends of the period of ultrasonication did not significantly affect the extraction efficiency (Table 2).

The fraction of carbohydrate precipitable by 80% ethanol in the total extracted carbohydrate was, in most cases, 65 to 85%, irrespective of the extraction efficiency (Table 2). This result indicates that the majority of the extracted carbohydrates had relatively high MWs. The precipitable fraction was slightly higher for the sediment of station B than for the other stations.

Table 3. Molecular size fractionation (glucose equivalent %) of carbohydrate extracted by various treatments.

	Station A			Station B			Station C		
Treatment[a]	LMW[b]	MMW[b]	HMW[b]	LMW	MMW	HMW	LMW	MMW	HMW
AS	17.2	15.7	67.1	15.8	37.1	47.1	nd	nd	nd
H	31.3	32.0	36.7	28.4	70.6	1.0	nd	nd	nd
HAS	27.6	26.7	45.7	16.9	29.9	53.2	nd	nd	nd
AH	24.6	26.8	48.6	61.5	12.4	26.1	23.6	31.0	45.4
AHS0	24.6	26.9	48.5	nd	nd	nd	nd	nd	nd
AHS1	20.1	24.4	55.5	45.8	9.4	44.8	35.4	1.9	62.7
AHS2	20.1	27.6	52.3	nd	nd	nd	39.2	1.6	59.1
AHS3	17.6	25.4	57.0	nd	nd	nd	nd	nd	nd
AHS4	17.3	20.5	62.2	nd	nd	nd	nd	nd	nd

[a] See Table 1 for abbreviation.
[b] Nominal molecular weight cutoff of ultrafiltration: LMW < 10 kDa < MMW < 100 kDa < HMW.
nd = not determined.

The extracted carbohydrates may have acidic monosaccharide residues, such as uronic acids, which should dissociate in alkaline solutions and may affect the precipitation efficiency. However, even when the alkaline extracts were neutralized with acetate buffer (pH 5.0) before the addition of ethanol, the precipitable fraction did not significantly increase (data not shown). This suggests that the frequency of the acidic residues in the extracted macromolecular carbohydrates was not high.

3.2. Molecular-Size Fractionation

Molecular size of the extracted carbohydrates was estimated using ultrafiltration cartridges and classified into three fractions: LMW (low molecular weight; <10 kDa), MMW (medium molecular weight; 10–100 kDa), and HMW (high molecular weight; >100 kDa). The data (Table 3) show that the treatments with cold (AS) or hot (AH) alkali increased recovery of the HMW fraction of carbohydrates in the extracts compared with the heating treatment alone (H) in both stations A and B (station C was not tested). This result implies that carbohydrate molecules putatively bound to insoluble organic matter by ester or *O*-glycosidic bonds had relatively large MWs compared with those that were hydrogen bonded with organic or mineral particles.

Intensive extraction processes such as AH and AHS may have caused fragmentation of some of macromolecules (which could be extracted intact by milder treatments), leading to an underestimation of HMW and MMW fractions in the intensively extracted carbohydrate. Thus, although our data generally showed that the intensive extraction protocols enhanced the recovery of HMW carbohydrate, the original molecular size of the extracted carbohydrates might have been still larger than those shown by our data. On the other hand, longer ultrasonic treatments after the hot alkali extraction (tested only for station A) slightly increased relative recovery of the HMW carbohydrate (Table 3). Thus, ultrasonication did not cause fragmentation of carbohydrate molecules.

Comparing the ethanol precipitable fraction with the MW fractions, it was found that the amount of the ethanol precipitable carbohydrates was almost identical to the sum of the carbohydrates belonging to the HMW and MMW fractions (Fig. 2). This indicates that the carbohydrates with the MW >10 kDa can be precipitated by dehydration with 80% ethanol. There were a few remarkable exceptions in the data of station B, in which the ethanol precipitable fraction largely exceeded the sum of HMW and MMW fractions. The cause of this deviation is unknown at present.

3.3. Comparison With Total Organic Carbon

Vertical changes in the ratio of carbohydrate carbon to total organic carbon in both the extracts, which were obtained by combination of hot alkali extraction and ultrasonication (AHS1 in Table 1; referred to as AHS fraction below), and the residues (R) were compared for sediment cores of stations A and B (Figs. 3, 4). Organic carbon (OC) contents were 5 to 10 times

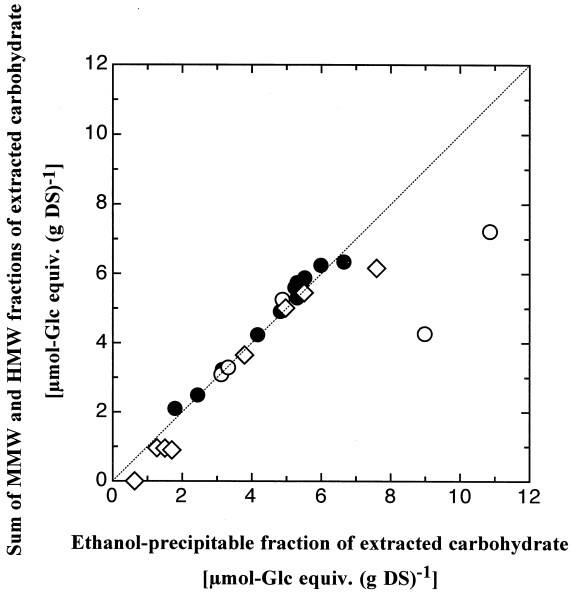

Fig. 2. Relationship between the ethanol precipitable fraction and the macromolecular (MMW + HMW) fraction of carbohydrates extracted by various methods from sediments. Concentrations are normalized to μmol glucose equivalent per g dry weight of original sediment (DS). Symbols: solid circle = station A, open circle = station B, diamond = station C.

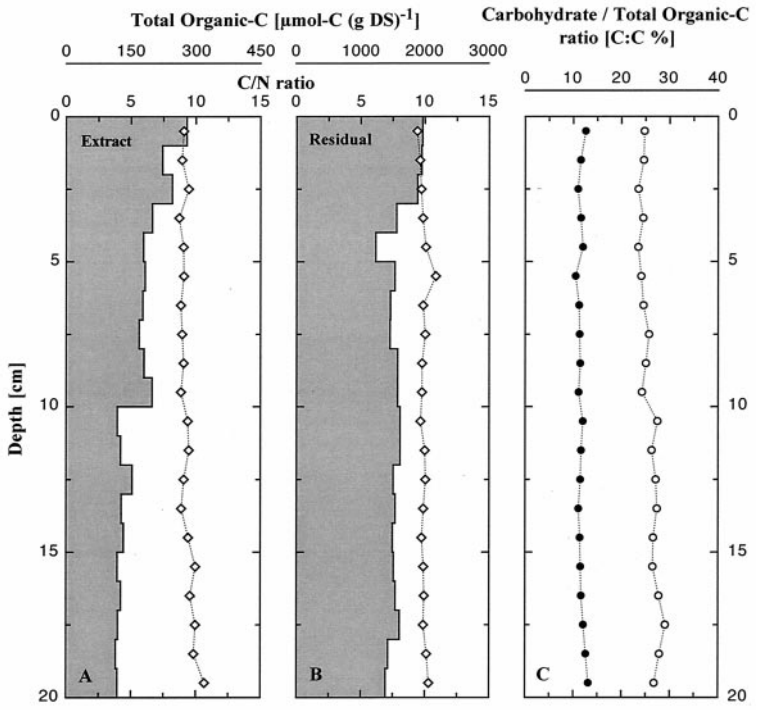

Fig. 3. Vertical profiles of total organic carbon (shaded area) and C:N atomic ratio (diamond) in the extracted (A) and residual (B) fractions of sediment organic matter and fraction (C:C %) of carbohydrate in total organic carbon (C) in the extracted (open circle) and residual (solid circle) fractions in the sediment core of station A. Concentrations are normalized to μmol-C per g dry weight of original sediment (DS). Carbohydrate carbon is calculated as 6 times of the glucose equivalent carbohydrate concentration measured by the PSA method.

higher in the R fraction than in the AHS fraction. The ratio of AHS–OC/R–OC was 0.14 at the top layer of the station A core and gradually decreased downward to 0.08 at 20 cm. This ratio was also higher (0.16–0.21) in the eutrophic (2.2–2.7 mmol OC g^{-1}) sediment of station B than in the oligotrophic (1.4–2.2 mmol OC g^{-1}) sediment of station A. These trends indicate that the AHS–OC represents relatively fresh and labile OC in the sediments. The C/N ratio slightly increased with depth, being significantly higher in the residual fraction than in the extracted fraction for both stations ($p < 0.0001$, two-tailed *t*-test). It was significantly higher in station B (10.4 $\pm$ 0.2) than station A (9.9 $\pm$ 0.3) for the R fraction ($p < 0.0001$) but not significantly different for the AHS fraction (9.3 $\pm$ 0.5 in station A, 9.3 $\pm$ 0.2 in station B; $p = 0.90$).

The ratio of carbohydrate carbon to total OC did not significantly change with depth for both stations and for both AHS and R fractions (Figs. 3C, 4C). This ratio was almost twice as high in the AHS fraction as in the R fraction. In station A, carbohydrates constituted 25.8 $\pm$ 1.6% (mean $\pm$ S.D.) of the AHS–OC, which was significantly higher ($p < 0.0001$) than in station B (20.4 $\pm$ 0.8%). The ratio in the R fraction was not significantly different ($p = 0.57$) between station A (11.6 $\pm$ 0.6%) and station B (11.5 $\pm$ 0.4%). The absolute concentration of carbohydrates was significantly higher ($p < 0.0001$) in station B than in station A for both the AHS and R fractions.

The carbohydrate-C to total OC ratio was compared for a few AHS samples from stations A and B among molecular size classes: LLMW (<3 kDa), LMW (3–10 kDa), MMW (10–100 kDa), HMW (>100 kDa); see Table 4. Carbohydrates were concentrated in total OC in the following order: LLMW < LMW ($\approx$ residue) < MMW < HMW. In the HMW fraction, 31 to 42% of organic carbon were carbohydrates. On the concentration basis, 17 to 26%, 23 to 38%, and 42 to 56% of the AHS carbohydrates were found in the LLMW + LMW, MMW, and HMW fractions, respectively, which suggests that carbohydrates were preferentially enriched in high MW organic matter.

C/N ratio of the AHS fraction of organic matter was the higher in the larger molecular size fraction (8.3 $\pm$ 0.7 for LLMW + LMW, 9.0 $\pm$ 0.6 for MMW, 12.8 $\pm$ 1.7 for HMW; $n = 14$). However, it may be noted that the LLMW fraction might have included inorganic nitrogen, although ammonia should have been lost during sample preparation, which was executed under alkaline conditions.

3.4. Monosaccharide Compositions

Monosaccharide compositions after mild hydrolysis using dilute HCl were compared between the ethanol precipitable fraction of the extracted (AHS) carbohydrates (referred to as fraction E) and the residual (insoluble) carbohydrates after extraction (fraction R) for each station (Table 5). In fraction E, hexoses were generally more abundant than pentoses and methyl pentoses (deoxy sugars), and the orders of abundance were Gal > Glc > Man for hexoses, and Xyl > Fuc > Rha > Ara > Rib for pentoses and methyl pentoses. Although the general trend of abundance in fraction R did not differ greatly from that in fraction E, variations among stations were rela-

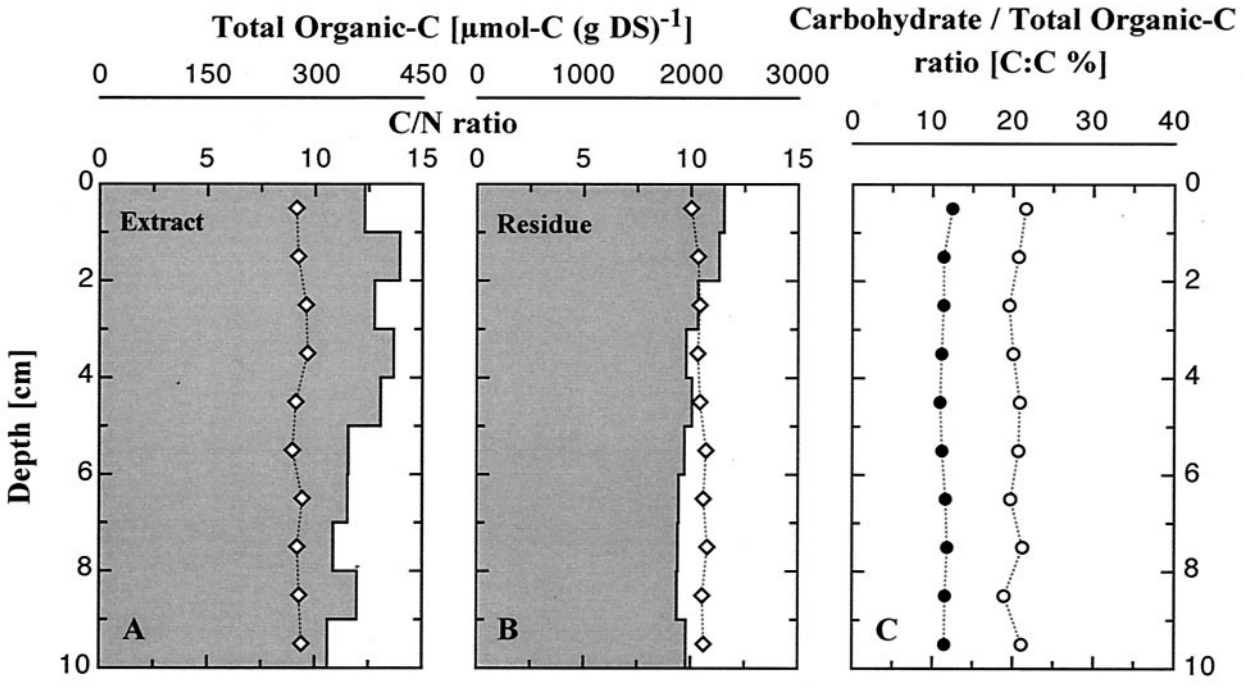

Fig. 4. Same as Figure 3 but in the sediment core of station B.

tively large. One of the conspicuous differences in fraction R from fraction E is higher abundance of arabinose than methyl pentoses (especially station C).

To quantify the difference in aldose composition, we introduce a similarity index S_{ij} defined as follows:

$$S_{ij} = \left(\sum_{\text{aldose}} p_i p_j\right) \Big/ \sqrt{\sum_{\text{aldose}} p_i^2 \cdot \sum_{\text{aldose}} p_j^2}$$

where p_i and p_j mean the relative abundance of an aldose p in the samples i and j, respectively, and the summation is over all the aldoses measured (8 neutral sugars in this case). The value S is 1 if the relative abundances are identical, and 0 if the samples contain no common substance. The results of comparison between stations areas follows: for fraction E, S_{AB} = 0.995, S_{BC} = 0.990, and S_{CA} = 0.988; for fraction R, S_{AB} = 0.976, S_{BC} = 0.938, and S_{CA} = 0.947. These results indicate that the aldose composition is strikingly similar among stations for fraction E, whereas significant variability is present for fraction R. The aldose composition of fraction R of station C, where terrestrial input was large, showed poor similarity to those of stations A and B. The similarity indices between fractions E and R are 0.981, 0.972, and 0.935 for stations A, B, and C, respectively. These results suggest that the aldose composition of fraction R is affected by terrestrially derived carbohydrates and that the similarity between fractions E and R increases with decreasing input of terrestrial carbohydrates.

The extraction protocol AHS1 was repeated three times for the sediment of station C. The total extracted carbohydrates in the second and third extractions were 74 and 23% of that of the first extraction (Table 6). The sum of carbohydrates obtained by three times of extraction amounted to 49.5% of total (12 mol/L H_2SO_4-soluble) carbohydrate in the original sediment. Comparison of the monosaccharide composition of the ethanol precipitable carbohydrates in each extract (Table 6) shows that the relative abundances of rhamnose, fucose, and xylose consistently decreased from the first to the third extractions and further to the insoluble final residues, whereas those of arabinose and glucose increased. The sum of the amount of each aldose (HCl-hydrolyzable fraction) in the three extracts and the final residue was consistent (within an error of 7%) with the amount of the aldose in carbohydrates directly hydrolyzed by HCl from dry sediment without extraction. The only exception was ribose, the recovery of which was significantly reduced when dried sediment was used. Thus, possible alteration of monosaccharide composition during the extraction treatments can be ruled out. The similarity indices with respect to the first extract are: 0.985, 0.963, 0.860, and 0.582 for the second extract, the third extract, and the HCl-hydrolyzable and unhydrolyzable residual fractions, respectively. Therefore, two end members of monosaccharide composition may be discerned: One represents the most easily extractable fraction of sediment carbohydrate, which is enriched in methyl pentoses and xylose; the other is the least extractable carbohydrate, which is enriched in arabinose and glucose.

4. DISCUSSION

The mobility of sediment organic matter is a geochemically important factor affecting both burial and preservation of organic carbon and benthic carbon flux across the ocean floor. It is controlled by the physicochemical properties of organic matter, such as molecular size, hydrophobicity, electric charge, and mechanisms of organic–mineral and organic–organic interactions. Although the properties of bulk sediment organic matter are little known, dissolved organic carbon (DOC) in

Table 4. Molecular size fractionation of extracted organic matter and the C:N ratios and carbohydrate contents of respective size fractions and the residue. Concentration is normalized to mol-C per g dry weight of original sediment (DS).

	Total Organic-C [μmol (g DS)$^{-1}$]					C/N atomic ratio					Carbohydrate/Total Organic-C ratio[a] [C:C %]				
	Extract					Extract					Extract				
Sample	LLMW[b]	LMW[b]	MMW[b]	HMW[b]	Residue	LLMW	LMW	MMW	HMW	Residue	LLMW	LMW	MMW	HMW	Residue
(Station A)															
0–1 cm	104	40	56	79	1962	7.5	7.3	9.4	14.6	9.4	9.4	13.9	37.3	41.8	12.8
6–7 cm	52	23	45	56	1462	8.3	7.1	8.5	11.0	9.9	9.9	16.6	25.1	41.1	11.3
(Station B)															
0–1 cm	123	75	85	86	2307	7.5	9.1	9.5	12.5	10.0	9.8	10.8	30.7	38.8	11.9
6–7 cm	78	73	72	122	1888	7.5	9.2	9.0	11.8	10.6	9.4	9.7	22.1	30.9	11.3

[a] Carbohydrate carbon was calculated as 6 times mol-glucose equivalent determined by the phenol–sulfuric acid (PSA) method.
[b] Nominal molecular weight cutoff by ultrafiltration: LLMW $<$ 3 kDa $<$ LMW $<$ 10 kDa $<$ MMW $<$ 100 kDa $<$ HMW.

Table 5. Comparison of monosaccharide compositions of the ethanol precipitated polymeric carbohydrates in extracts and the carbohydrates in extract residues. Concentration is presented as μmol of each monosaccharide per g dry weight of original sediment (DS).

	Station A			Station B			Station C		
	Extract[a]	Residue[b]	Extracted fraction	Extract	Residue	Extracted fraction	Extract	Residue	Extracted fraction
Monosaccharide	[μmol (g DS)$^{-1}$]		(%)	[μmol (g DS)$^{-1}$]		(%)	[μmol (g DS)$^{-1}$]		(%)
Rhamnose	0.52 ± 0.01	1.00	34.5	0.81	1.32	38.2	0.45 ± 0.01	0.75 ± 0.05	37.8
Fucose	0.61 ± 0.01	0.95	38.9	0.94	1.65	36.4	0.42 ± 0.01	0.71 ± 0.04	36.9
Ribose	0.18 ± 0.00	0.58	23.7	0.25	0.86	22.7	0.13 ± 0.01	0.94 ± 0.12	11.9
Arabinose	0.37 ± 0.01	1.10	25.0	0.56	1.37	28.8	0.40 ± 0.02	2.32 ± 0.12	14.8
Xylose	0.71 ± 0.03	1.75	28.8	1.20	2.35	33.8	0.56 ± 0.02	1.57 ± 0.07	26.2
Mannose	0.58 ± 0.05	1.60	26.7	1.21	1.17	50.8	0.59 ± 0.02	1.13 ± 0.03	34.5
Galactose	0.98 ± 0.05	2.35	29.5	1.82	2.54	41.7	0.78 ± 0.03	2.13 ± 0.08	26.9
Glucose	0.66 ± 0.04	2.12	23.8	1.25	1.96	38.9	0.72 ± 0.03	2.14 ± 0.05	25.2
Glucuronic acid	nd	0.19	nd	0.41	nd	nd	0.16 ± 0.01	0.18 ± 0.02	46.7
Galacturonic acid	nd	0.21	nd	0.38	nd	nd	0.17 ± 0.00	0.29 ± 0.02	36.5

* Values with "±*d*" are average ± standard deviation ($n = 3$); values without "±*d*" are data of single analyses; nd = not determined.
[a] Ethanol precipitable fraction of the extract was hydrolysed with 0.5 N HCl at 105°C for 4 h.
[b] Extract residue was dried and hydrolyzed with 0.5 N HCl at 105°C for 4 h.

pore waters has been relatively well characterized (e.g., Krom and Sholkovitz, 1977; Orem et al., 1986; Chin and Gschwend, 1991; Burdige and Gardner, 1998; Thimsen and Keil, 1998), and importance of the pore water DOC in the benthic carbon flux has been postulated (Burdige et al., 1992; Alperin et al., 1999; Guo and Santschi, 2000). Burdige et al. (2000) reported that DOC in pore waters was relatively rich in carbohydrates (10–55%) compared with particulate organic carbon (POC) of bulk sediments (5–9%). They hypothesized that dissolved carbohydrates are intermediates that appear during the decomposition process of POC in bulk sediments. Although the dissolved carbohydrates in pore water are intrinsically labile, it also has been postulated that a part of them may gradually undergo chemical and biochemical modifications producing low MW, refractory DOC. Arnosti and coworkers showed that the enzymatic hydrolysis of macromolecular polysaccharides into mono- and oligosaccharide fragments in marine sediment is rapid and potentially overwhelms the terminal oxidation of organic carbon (Arnosti et al., 1998, Arnosti and Holmer,1999), which also implies that these carbohydrate fragments may temporarily accumulate in pore water and play a complex role in the overall organic diagenesis in the sediment. Thus, carbohydrates may be one of the quantitatively important organic substances linking POC in bulk sediments to pore water DOC, as well as pore water DOC to DOC in the overlying water column. However, DOC in pore waters usually constitutes <1% of total organic carbon in bulk sediments, and the relationships between the pore water DOC and the bulk POC have not been resolved in detail.

In our study, we examined a practical method by which the bulk sediment POC could be fractionated by solubility and binding properties. The organic molecules that could be extracted intact by chemical and physical treatments were characterized in terms of molecular size and monomer compositions. With combination of the extraction techniques, 8 to 17% of the bulk sediment POC could be extracted to solution by a single extraction (Figs. 3, 4). Using this method, we demonstrated that the relatively mobile fraction of sediment POC, that is, organic carbon extractable by alkali–heating–sonication (referred to as AHS fraction; Table 1), contained a large amount of carbohydrate (19–28%) compared with the immobile (unextractable) fraction (10–13%). Although the sediments we used seem somewhat richer in carbohydrate than those used by Burdige et al. (2000), the general trend that carbohydrates are enriched in the more mobile fractions is consistent in these two studies. The relatively higher "mobility" of carbohydrates is probably related to the fact that many carbohydrate molecules are hydrophilic and have relatively loose and flexible three-dimensional structure compared with other natural macromolecules, such as proteins and lipids.

Comparing the extraction efficiency among a few extraction protocols, it was indicated that the extractable carbohydrates could be separated into two fractions. The one could be extracted by merely heating (H in Table 1) and was putatively comprised of the carbohydrate molecules bound to sediment mainly by the hydrogen bond, the dispersion force, or both. The other could not be extracted without addition of alkali (AS or AH − H), and appears to include carbohydrates bound to sediment particles by alkali-vulnerable bonds such as ester and *O*-glycosidic bonds. Both the fractions were similar in abundance. However, the latter seemed richer in the high MW carbohydrate (>100 kDa) than the former (Tables 2, 3). This difference in size distribution suggests that alkali-vulnerable bonds are preferentially broken during the early stage of remineralization of macromolecular carbohydrates; then decomposition goes on to cleavage of alkali-resistant bonds (e.g., glycosidic bond), leaving relatively small fragments of carbohydrate molecules for which noncovalent bonds are the sole means of binding to insoluble sediment particles.

In this context, it may be notable that pore water DOC in oxic sediments is usually constituted of low molecular weight (LMW; e.g., < 3 kDa) organic carbon as the major component (Krom and Sholkovitz, 1977; Chin and Gschwend, 1991; Burdige and Gardner, 1998). To explain this size distribution, Burdige and Gardner (1998) proposed the size-reactivity model in which production and consumption of DOC intermediates during mineralization of POC are assumed to yield progressively smaller and less reactive organic molecules, eventually

Table 6. Monosaccharide compositions of ethanol precipitated polymeric carbohydrates in serial extracts and residual carbohydrates from the sediment of station C. Concentration is present as μmol of each monosaccharide per g dry weight of original sediment (DS).

	Ethanol-precipitable fraction of extracts[a]						Residues			
	First extract		Second extract		Third extract		HCl-hydrolyzable[a]		HCl-resistant[b]	
Monosaccharide	[μmol (g DS)$^{-1}$]	RA[c] [%]	[μmol (g DS)$^{-1}$]	RA [%]	[μmol (g DS)$^{-1}$]	RA [%]	[μmol (g DS)$^{-1}$]	RA [%]	[μmol (g DS)$^{-1}$]	RA [%]
Rhamnose	0.441 ± 0.021	11.3	0.212 ± 0.011	9.3	0.076 ± 0.032	8.3	0.310 ± 0.043	4.0	0.150 ± 0.050	2.5
Fucose	0.485 ± 0.021	12.5	0.214 ± 0.013	9.4	0.075 ± 0.026	8.2	0.290 ± 0.033	3.8	0.133 ± 0.015	2.2
Ribose	0.163 ± 0.005	4.2	0.062 ± 0.003	2.7	0.025 ± 0.009	2.7	1.322 ± 0.481	17.2	0.000 ± 0.000	0.0
Arabinose	0.513 ± 0.033	13.2	0.324 ± 0.017	14.2	0.174 ± 0.049	19.0	1.606 ± 0.126	21.0	0.534 ± 0.076	8.9
Xylose	0.612 ± 0.034	15.7	0.305 ± 0.017	13.4	0.111 ± 0.033	12.2	0.946 ± 0.077	12.3	0.580 ± 0.066	9.7
Mannose	0.327 ± 0.019	8.4	0.234 ± 0.013	10.2	0.089 ± 0.050	9.8	0.507 ± 0.034	6.6	0.507 ± 0.005	8.5
Galactose	0.735 ± 0.036	18.9	0.450 ± 0.026	19.7	0.166 ± 0.052	18.1	1.219 ± 0.098	15.9	0.498 ± 0.069	8.3
Glucose	0.497 ± 0.032	12.8	0.375 ± 0.019	16.4	0.160 ± 0.075	17.5	1.266 ± 0.085	16.5	3.586 ± 0.044	59.9
Glucuronic acid	0.052 ± 0.007	1.3	0.049 ± 0.006	2.1	0.017 ± 0.006	1.9	0.035 ± 0.050	0.5	nd	
Galacturonic acid	0.065 ± 0.005	1.7	0.056 ± 0.006	2.5	0.021 ± 0.015	2.3	0.164 ± 0.053	2.1	nd	
Total neutral and acidic sugars	3.89		2.28		0.91		7.67		5.99	
Total carbohydrate[e]	7.0 ± 0.2 8.8 ± 0.3[e]		5.0 ± 0.1 6.5 ± 0.1[e]		1.7 ± 0.2 2.0 ± 0.2[e]		11.8 ± 0.5		5.8 ± 1.1	

[a] Hydrolyzed with 0.5 N HCl at 105°C for 4 h.
[b] Residue after hydrolysis with HCl was further hydrolyzed with 12 M H_2SO_4 at room temperature for 3 h and 1 M H_2SO_4 at 100°C for 4 h.
[c] Relative abundances.
[d] Determined by the phenol–sulfuric aicd (PSA) method (μmol glucose equivalent (g DS)$^{-1}$).
[e] Sum of the ethanol precipitable and nonprecipitable carbohydrates in the extract.
nd = not detected.

resulting in accumulation of refractory LMW DOC in pore water. Furthermore, Burdige et al. (2000) showed that carbohydrate concentration in pore waters was essentially independent of concentration of the LMW DOC. To explain this fact, they hypothesized that dissolved carbohydrates were the initial intermediates of POC remineralization and the precursors for the LMW DOC and predicted that carbohydrates would be found mainly in the high molecular weight (HMW) fraction of the pore water DOC. Although our present study was not directly related to pore water DOC, the data we obtained are generally consistent with their interpretation because, as they predicted, the molecular size distribution of the extractable (AHS) carbohydrates was significantly biased to HMW fraction (i.e., >100 kDa) compared with the total AHS TOC (Table 4). Thus, it is possible that the AHS carbohydrates are the early intermediary products from which smaller (and possibly more refractory) AHS POC and pore water DOC are produced, and thus the size-reactivity model of Burdige et al. (2000) may be extended to include the relatively mobile POC.

However, the AHS POC, as opposed to pore water DOC, still contained a sizable amount of HMW organic matter (>100 kDa, 23–35% of total), which necessitates that extra factors be added to the model. The following two factors may be relevant to this apparent difference between the two fractions of sediment organic carbon. First, as exemplified by the MW distribution of extractable carbohydrates, the AHS HMW OC may comprise to some degree of organic macromolecules bound to sediment particles by ester or *O*-glycosidic bonds. These molecules would not appear in pore water without the bonds disrupted chemically or biochemically. Second, organic molecules that interact with sediment particles solely by the noncovalent bonds are presumably in adsorption equilibrium with pore water DOC. However, because the adsorptive force is generally stronger for larger molecules, relatively smaller organic molecules would be accumulated in pore water, whereas most of larger molecules remain adsorbed. As a result of these factors, the LMW DOC could predominate the pore water DOC pool rather than the AHS POC pool.

We cannot extend a similar interpretation to the residual (unextractable) POC in the sediment, for which the MW distribution could not be determined. However, comparison of monosaccharide composition of the residual carbohydrates between stations suggested that this fraction is significantly affected by the origin of sediment organic matter, for example, by the presence of terrestrially derived organic substances. This is most evident in the serial extraction experiment for the sediment of the near-shore station C (Table 6), which shows progressive enrichment in arabinose and depletion of methyl pentoses in the less extractable carbohydrates. In extensive studies to evaluate the usefulness of monosaccharide composition for identification of carbohydrate sources, Cowie and Hedges (1984) and Boschker et al. (1995) have shown that methyl pentoses are much more enriched in plankton, bacteria, and sestonic detritus than in vascular plants and litters, whereas arabinose (and lyxose) is enriched in vascular plants (note that the alditol acetate-GLC method we employed cannot resolve lyxose from arabinose). Our data are apparently consistent with these trends, because arabinose was more enriched in the near-shore station C (19.8 mol.% in neutral sugars) than in the offshore stations A and B (9.6 and 10.4%), whereas methyl pentoses were most depleted in station C (12.5%, compared with 17.1 and 22.4% in stations A and B). Thus, it may be hypothesized that the residual carbohydrates represent the allochthonous organic matter that has survived diagenetic alteration.

In contrast, the monosaccharide composition of the extractable fraction of carbohydrates is similar between stations despite the different depositional environments (similarity index > 0.98). This similarity of composition may imply the common origin of the extractable carbohydrates, for example, de novo synthesis by indigenous microorganisms in sediments. This interpretation is consistent with the above-mentioned hypothesis that the extractable macromolecular carbohydrates are the initial intermediary products of remineralization of sediment organic matter. The observation that the overall remineralization rate of polysaccharides in marine sediments is not limited by the rate of the initial enzymatic hydrolysis by microorganisms (Arnosti and Holmer, 1999) also suggests the dynamic nature of the sediment carbohydrate pool in which polysaccharides can be both decomposed and regenerated by sediment microorganisms. However, further studies are needed to evidence the origin of the extractable carbohydrates.

The above interpretation also implies that monosaccharide compositions reported for bulk sediment samples may largely reflect the composition of bacterially produced carbohydrates. Thus, much caution should be taken when the origin of sediment organic matter is inferred from the data of monosaccharide composition of bulk sediment. One possible way to circumvent this difficulty is to use the data of monosaccharide composition of appropriately fractionated sediment organic matters, instead of bulk sediments, when investigating possible origins of organic matter and postdepositional alterations. In this study, we presented some of the promising techniques to separate sediment organic matter into geochemically meaningful fractions. Although the scope of the present study was limited within carbohydrate moieties, this method can be extended to other organic components such as peptides, nucleic acids, and lipids. However, there is still much room to improve the method. For example, concentration of alkali in the extraction solutions, extraction temperature, and output of ultrasonic treatment can be changed so that polysaccharide molecules bound to sediment particles with different binding strength may be differentially extracted. By using stronger reagents and repeating the extraction (e.g., Table 6), larger fraction of sediment organic matter may be recovered in solution and allowed to determine chemical properties such as MW distribution, isoelectric point, and adsorption isotherm. Extraction efficiency may be improved simply by increasing the liquid–solid ratio in the extraction reactor (Tao and Lin, 2000). On the other hand, Underwood et al. (1995) and Winder et al. (1999) utilized EDTA to improve extraction efficiency of acidic polysaccharides from sediments. Because bacterially produced carbohydrates often contain acidic residues (uronic acids; Sutherland, 1985; Decho, 1990), this modification may be useful to selectively extract bacterial polysaccharides from sediment mixture. Further studies are still needed to standardize the extraction and fractionation methods for carbohydrates and organic matter in general in marine sediments.

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