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Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters

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Abstract—The high molecular weight fraction of dissolved organic matter in a suite of lakes, rivers, seawater, and marine sediment interstitial water samples was collected by ultrafiltration and characterized by molecular level and spectroscopic techniques. Proton nuclear magnetic resonance spectra of all samples show a high degree of similarity, with major contributions from carbohydrates, bound acetate, and lipids. Molecular level analyses of neutral sugars show seven monosaccharides, rhamnose, fucose, arabinose, xylose, mannose, glucose, and galactose, to be abundant, and to occur in comparable relative amounts in each sample. Previous studies have emphasized the distinctive composition of dissolved humic substances in fresh and marine waters, and have attributed these differences to sources and transformations of organic matter unique to each environment. In contrast we find a large fraction of freshwater high molecular weight dissolved organic matter (HMWDOM; > 1kD) to be indistinguishable from marine HMWDOM in bulk and molecular-level chemical properties. Aquatic HMWDOM is similar in chemical composition to biologically derived acylated heteropolysaccharides isolated from marine algal cultures, suggesting a biological source for some fraction of persistent HMWDOM. High molecular weight DOC contributes $51 \pm 26\%$ of the total DOC, and monosaccharides $18 \pm 8\%$ of the total HMWDOC in our freshwater samples. These contributions are on average higher and more variable, but not significantly different than for surface seawater (30% and 16% respectively). Biogeochemical processes that produce, accumulate, and recycle DOM may therefore share important similarities and be broadly comparable across a range of environmental settings. Copyright © 2002 Elsevier Science Ltd

1. INTRODUCTION

Dissolved organic matter (DOM) in fresh and marine waters has distinctive chemical and isotopic characteristics. Fresh water DOM sampled by adsorption onto XAD resins (fulvic and humic substances) is isotopically light ($\delta^{13}\text{C} \approx -27$ per mil), has a high C/N (40 to 50), is rich in aliphatic, aromatic, and carboxyl carbon (Hedges et al., 1992), has strong absorption in the near UV, and is often depicted with a high condensed, cyclic molecular structure. In contrast, marine DOM is isotopically heavier ($\delta^{13}\text{C} \approx -22$ per mil), has a low C/N ratio (15 to 20), is rich in alkoxy carbon, has weak absorption in the near UV, and is often depicted with a more open, linear molecular structure (Gagosian and Steumer, 1977; Francois, 1990; Leenheer, 1994). The dissimilar chemical characteristics of fresh water and marine DOM presumably arise from differences in the organic matter sources and formation processes of these two environments. Dissolved organic matter in rivers and lakes contains a large fraction of organic matter derived from terrestrial plants, while marine microalgae and bacteria synthesize the precursors to marine DOM. Fresh water and marine DOM are often considered to be very distinct carbon reservoirs, with different sources, cycling times, and fates (Hedges et al., 1997).

However, humic substances contribute only a fraction of the total dissolved organic matter in aquatic environments. A large fraction of DOM remains uncharacterized, and may therefore have sources, cycling times and fates different from humic substances. Up to one third of DOM in seawater can be recov-

ered by ultrafiltration. Chemical characterization of this high molecular weight dissolved organic matter (HMWDOM) has shown it to be chemically distinct from marine humic substances (Benner et al., 1992). We have recently compared the chemical properties of HMWDOM in surface seawater collected from geographically diverse locations, and shown that the spectroscopic and molecular level properties of this fraction are remarkably homogeneous (Aluwihare et al., 1997). Marine HMWDOM collected by ultrafiltration or dialysis is rich in carbohydrate and acetate, and has a very specific distribution of neutral sugars. Major neutral sugars include glucose, galactose, mannose, xylose, arabinose, fucose, and rhamnose, which occur in approximately equimolar abundance (Sakugawa and Handa, 1985; Malcolm, 1990; McCarthy et al., 1994, 1996; Aluwihare et al., 1997; Borch and Kirchman, 1998). There is little spatial or temporal variation in the relative abundance of these seven neutral sugars, and the ratio of carbohydrate:acetate:lipid in surface seawater, as determined by ^1H NMR, is also relatively constant (Aluwihare et al., 1997). High molecular weight DOM with molecular level and spectroscopic properties similar to seawater HMWDOM can be generated by phytoplankton in culture (Aluwihare et al., 1997; Aluwihare and Repeta, 1999). A large fraction of marine DOM may consist of structurally simple, but refractory biopolymers rather than the more structurally complex humic and fulvic acids.

Dissolved carbohydrates are also abundant in lakes, rivers, and estuaries (Thurman, 1985; Sigleo, 1996; Mannino and Harvey, 2000); however, the molecular and spectroscopic properties of HMWDOM from different freshwater systems have not been compared. Some evidence suggests that HMWDOM with a neutral sugar distribution and carbohydrate:acetate:lipid

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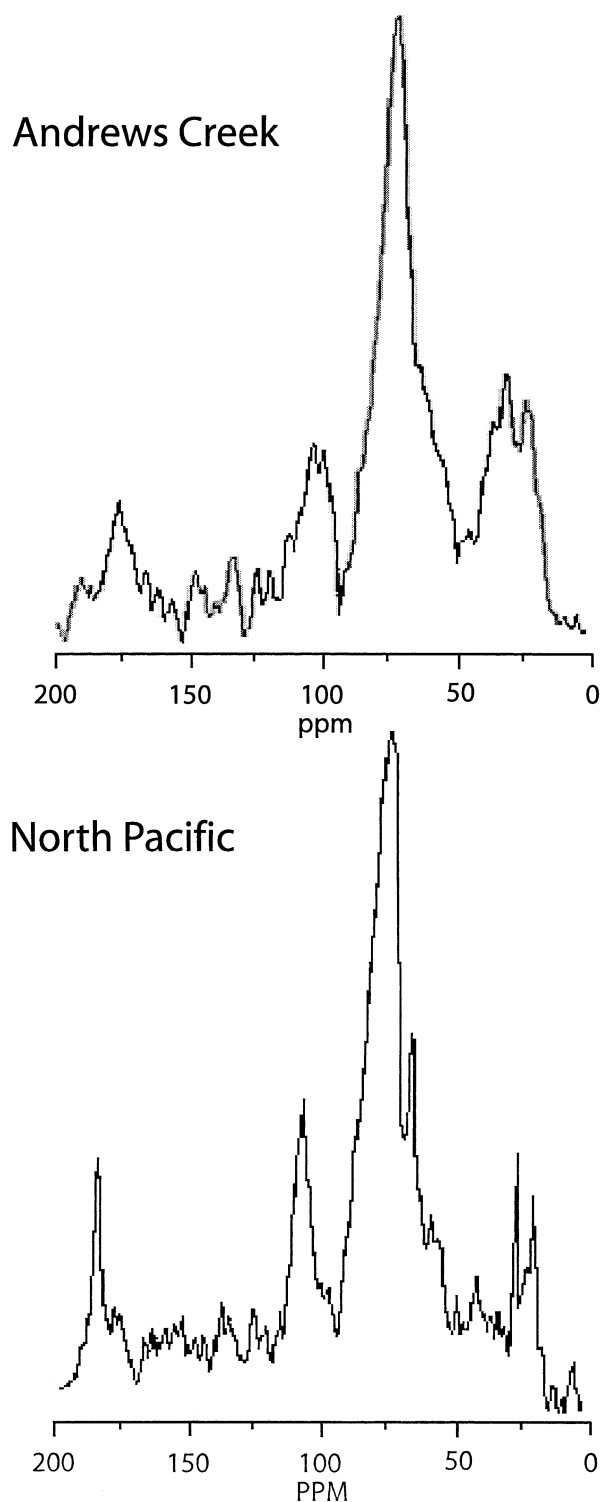


Fig. 1. ^{13}C NMR spectra of HMWDOM from the North Pacific Ocean (5 m, $31^{\circ}00.00'\text{N}$; $159^{\circ}00.00'\text{W}$) and Andrews Creek, Rocky Mountain National Park (CO, USA). The seawater spectrum was collected in water with 50 Hz line broadening applied. The Andrew's Creek spectrum was collected with solid state, cross-polarization magic angle spinning as described by McKnight et al., 1997. Some of the fine structure in the North Pacific Ocean spectrum may be due to the greater resolution of the liquid state spectrum. The chemical shift and integral abundance of the major resonances are similar in the two spectra. Some

ratio similar to seawater is abundant in non-marine waters. Sweet and Perdue (1982) reported polysaccharides in the Williamson River (OR, USA) to have approximately equal amounts of arabinose, xylose, mannose, glucose, and galactose, and further observed that the "relative abundance of arabinose, xylose, mannose, glucose, and galactose does not exhibit significant temporal or spatial variation." Monosaccharide analysis of DOM collected from White Clay Creek (PA, USA), and Lake Nakanuma (Japan) show the same suite of seven neutral sugars observed in seawater, and like seawater these sugars occur in nearly equal amounts (Ochiai and Hanya, 1980a, 1980b; Gremm and Kaplan, 1997). Gremm further noted that the composition of dissolved total sugars in White Clay Creek showed a "remarkable similarity to compositions reported for small streams in Japan (Ochiai and Nakajima, 1988), an intermediate sized river in North America (Sweet and Perdue, 1982) and large South American rivers (Depetris and Kempe, 1993; Hedges et al., 1994)."

Proton nuclear magnetic resonance (NMR) spectra of HMWDOM from the Illinois, Arkansas, and Mississippi Rivers display the same major resonances, and have a similar carbohydrate:acetate:lipid ratio observed in seawater HMWDOM (Leenheer, 1994, Aluwihare et al., 1997). Likewise, the ^{13}C NMR spectra of colloidal organic matter from two lakes and two streams in Rocky Mountain National Park, Colorado are very similar to ^{13}C NMR of marine HMWDOM (McKnight et al., 1997). Figure 1 compares the ^{13}C NMR of HMWDOM (>100kD) from Andrews Creek, a small alpine creek at 3400m elevation in Rocky Mountain National Park (McKnight et al. 1997), to surface seawater collected in the North Pacific Ocean. Both spectra display major resonances at 180 ppm (COOH), 110 ppm (OCO), 70 ppm (HCOH), and 20 to 30 ppm (CH₂, CH₃). Quantitatively, the ratio of these four resonances (COOH:OCO:HCOH:CH_x) for the Andrews Creek sample is (0.1:0.2:1:0.4), nearly identical to Woods Hole surface seawater (0.1:0.2:1:0.5) and surface seawater from the N. Pacific Ocean (0.2:0.2:1:0.4).

While studies of dissolved humic substances have emphasized the differences in DOM composition between marine and non-marine environments, existing data on HMWDOM in streams, rivers and lakes suggests this fraction of fresh water DOM is compositionally distinct from humic substances and may be closely related to marine DOM. Here we report the results of chemical and spectroscopic analyses made on a suite of samples collected from seawater, marine porewaters, rivers and lakes to test this hypothesis.

2. EXPERIMENTAL METHODS

2.1. Sampling

Samples were collected from eight sites, including two freshwater lakes, Lake Superior ($47^{\circ}10'\text{N}$, $90^{\circ}30'\text{W}$), and Nobska Pond (Woods

(Opposite) differences in chemical shift probably result from the differences in spectrum calibration. The Andrews Creek spectrum is used with kind permission from Diane McKnight and Kluwer Academic Publishers, copyright 1997. The original data appears as Figure 6 in: McKnight et al. (1997) Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale watershed, Rocky Mountain National Park.

Table 1. Elemental, Molecular, and Spectroscopic Properties of HMWDOM in Rivers, Lakes and Seawater.

Sample	[DOC]	%HMW ^a	C/N	%Mono ^b	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Σ^c CH ₂ O	Σ Acetate	Σ Lipid
Delaware River	214	32	14	15	13 ± 0.2	14 ± 0.6	6 ± 0.09	9 ± 0.1	14 ± 0.3	24 ± 0.2	19 ± 0.6	67	3	30
Eel River	80	76	22	ND ^d	10 ± 2	11 ± 1	10 ± 1	13 ± 1	14 ± 0.7	16 ± 1	26 ± 2	71	3	26
Mississippi River	181	87	15	9	13 ± 2	15 ± 0.9	9 ± 0.3	12 ± 0.6	13 ± 0.3	19 ± 1	20 ± 1	65	3	32
Lake Superior	117	34	18	30	14 ± 0.5	19 ± 0.6	6 ± 0.3	15 ± 1	12 ± 1	20 ± 1	13 ± 0.2	61	3	36
Nobska Pond	550	24	6	20	17 ± 2	15 ± 1	8 ± 3	12 ± 0.3	13 ± 0.4	17 ± 0.3	16 ± 0.7	68	3	30
West Neck Bay	2200	14	9	38	11 ± 1	10 ± 1	3 ± 0.2	4 ± 0.6	8 ± 0.9	19 ± 2	44 ± 2	74	7	19
Salt Pond	ND	ND	12	8	20 ± 0.7	22 ± 0.3	9 ± 0.1	10 ± 0.2	11 ± 0.4	16 ± 1.3	13 ± 0.9	80	3	16
Peru	ND	ND	9	19	17 ± 0.7	18 ± 0.3	7 ± 0.2	9 ± 0.6	14 ± 0.01	23 ± 3	13 ± 1	85	9	7
Seawater	80	30	15	16	16 ± 2	15 ± 1	7 ± 2	12 ± 2	13 ± 2	20 ± 3	16 ± 3	80	10	10

^a %HMW is the percent of total DOC recovered by ultrafiltration using a nominal 1kD filter.

^b %Mono is the percent HMWDOC recovered as the seven neutral monosaccharides listed above.

^c Σ CH₂O Σ Acetate Σ Lipid were determined by integration of ¹HNMR as described by Aluwihare et al. (1997).

^d Not determined.

Hole, MA; 41°31'N, 70°40'W), and three rivers, the Mississippi River (near Venice, LA; 29°17'N, 89°21'W), Eel River (near Scotia, CA; 40°29'N, 124°06'W), and the Delaware River (near Philadelphia, PA; 39°37'N; 75°33'W). Surface sediments were collected by grab sampler in West Neck Bay (4 m, Long Island, NY; 41°04'N, 72°21'W), Salt Pond, a small coastal marine basin (5m, Falmouth, MA; 41°32'N, 70°38'N), and within the oxygen minimum zone of the Peru Continental Shelf (100 m, 11°04'S, 78°04'W). Interstitial water was recovered by inserting a wire mesh cylinder into the sediments and allowing them to drain overnight.

All samples were filtered through a 0.2 μ m polycarbonate filter capsule to remove bacteria and small particles. Pore water samples and the sample from the Delaware River were prefiltered through a Whatman GF/F glass microfiber filter to remove large particles. Subsamples were collected for high temperature chemical oxidation analysis of DOC, and the > 1kD (nominal molecular weight) size fraction of colloidal organic matter was recovered by ultrafiltration using an Amicon spiral wound cellulose cartridge filter (Amicon S10N1). The samples (12 to 40 L) were concentrated to approximately 1L, desalted with deionized, low carbon (Milli-Q) water, and lyophilized to recover HMWDOM as a dry powder.

2.2. Analysis

Samples were analyzed for total carbon, nitrogen, and sulfur using a Fissions model 1108 elemental analyzer. Proton and carbon nuclear magnetic resonance spectra (¹H, ¹³CNMR) were collected on a Bruker 300 or 500 MHz NMR spectrometer. For ¹HNMR spectra 3 to 5 mg subsamples were freeze-dried in D₂O to reduce HDO, dissolved in D₂O and the solvent suppressed (Bruker PRESAT) during acquisition. Results are reported relative to water at 4.8 ppm. For ¹³CNMR, samples (100mg) were dissolved in 10%D₂O/90%H₂O. Results are reported relative to COOH at 180 ppm.

Monosaccharides released by the hydrolysis of HMWDOM were analyzed in triplicate as alditol acetates. Briefly 1 to 2 mg subsamples were hydrolyzed with 2M trifluoroacetic acid (TFA) at 120°C for 2 h. Myo-inositol (20 μ g) was added as an internal standard. Cooling the solution to room temperature quenched hydrolysis, and the TFA was removed under a stream of nitrogen. The sample was dissolved in 250 μ L isopropanol and taken to dryness twice to remove residual TFA. Samples were dissolved in 250 μ L of 1M ammonium hydroxide containing 10 mg/mL sodium borohydride and allowed to react for 1 h at room temperature. The reduction was quenched by drop-wise addition of glacial acetic acid until no effervescence was observed. Water was removed by drying under a stream of nitrogen, and borates removed by repeated evaporation (4x) with 0.5 mL of methanol:acetic acid (9:1, v/v) followed by methanol (3x, 0.5 mL each).

Alditols were acylated in 100 μ L acetic anhydride and 20 μ L 1-methyl imidazole for 15 min at room temperature. Water (0.5 mL) was added, and after 10 min the samples were extracted with methylene chloride (0.5 mL, 2x). Alditol acetates were quantified by capillary gas

chromatography using a 30 m, SP-2330 column (0.25mm ID, 0.20 μ m film; Supelco, USA), a temperature program of 80(2)/30/170/4/240(10) (initial T°C(hold time)/ramp(°C/min)/T°C/ramp(°C/min)/final T°C(hold time) with helium as the carrier gas. Monosaccharides were identified by coelution with authentic standards and by gas chromatography/mass spectrometry. Peak areas were normalized to myoinositol and reported as mole percent of the total monosaccharides.

3. RESULTS

Data for the concentration of dissolved organic carbon, recovery of HMWDOC, and C/N ratio are given in Table 1. There was a wide range in the concentrations of dissolved organic carbon (DOC) in our samples, from 80 μ M in the Eel River, to 2200 μ M in porewater samples from West Neck Bay. The percent carbon recovered as HMWDOC was also quite variable, with highest recoveries for the Mississippi and Eel River samples (87 and 72%) and the lowest recovery for the West Neck Bay porewater sample (14%). Carbon to nitrogen ratios for Lake Superior and river samples were similar to values typical of surface seawater (15 \pm 2), while C/N ratios for marine sediment porewater samples and Nobska Pond were significantly lower (9 and 6, respectively).

Proton NMR spectra for all samples were characterized by major resonances for carbohydrates (5.5 to 5.0 ppm, anomeric H; and 4.5 to 3.5 ppm), bound acetate (hereafter acetate)(2 ppm) and lipid (1.3 to 1.0 ppm), superimposed on a baseline with a broad maximum at 2.3 ppm (Fig. 2). Total carbohydrate, acetate and lipid were determined after baseline subtraction. The shape of the baseline in the samples is similar to ¹NMR spectra of the humic substances fraction isolated in fresh and marine waters. The HMW fraction of freshwater samples in particular includes a significant fraction of humic substances. Treatment of the samples with 1M HCl (17 h, 105°C) followed by solvent extraction shows acetic acid to be the major (>60%) compound liberated by acid hydrolysis (Aluwihare et al., 1997). Aromatic resonances (8.0 to 7.0 ppm) are present in the spectra of all three river samples and Nobska Pond HMWDOM, but are absent from NMR spectra of Lake Superior and marine porewaters. The ratio of carbohydrate:acetate:lipid in seawater HMWDOM is relatively constant at 80 \pm 10:10 \pm 1:10 \pm 2 (mean \pm 1 SD)(Aluwihare et al., 1997). Marine porewater samples have a very similar

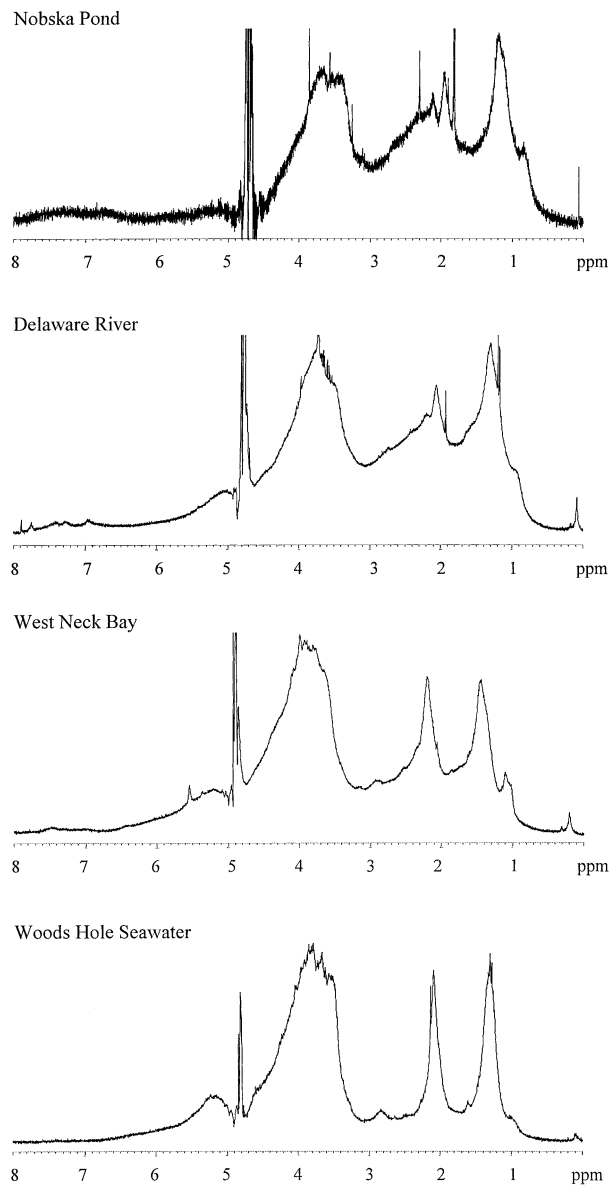


Fig. 2. ¹H NMR spectra for Nobska Pond, Delaware River, West Neck Bay (Long Island, NY) and surface seawater (Woods Hole, MA, USA). Spectra were collected in D₂O with water presaturation at 4.8 ppm (sharp peak). Each spectrum has resonances at 5.5, 3 to 4, and 1.3 ppm from polysaccharides, 2 ppm from acetate, and 1 to 1.3 ppm from lipids. The Delaware River and Nobska Pond samples have much higher contributions from unresolved resonances between 1 and 5 ppm. Aromatic resonances (7 to 8 ppm) are minor in all spectra.

ratio of $80 \pm 8:8 \pm 1:13 \pm 8$, while freshwater samples all had a much higher proportion of lipid resonances (1.3 to 1.0 ppm) $66 \pm 3:3 \pm 0:31 \pm 3$, suggesting a higher relative amount of hydrophobic (humic and fulvic) material. Carbohydrate, acetate, and lipid resonances account for 34 to 51% of the total HMWDOC as determined by ¹H NMR spectra, somewhat lower than the 70% total HMWDOC observed for surface seawater.

All samples were analyzed in triplicate for neutral monosaccharides, and the results are presented in Figure 3. Neutral

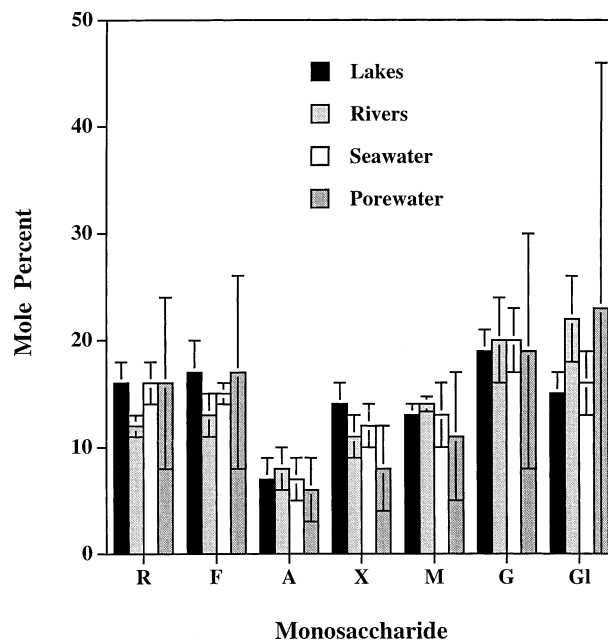


Fig. 3. Distribution of Rhamnose, Fucose, Arabinose, Xylose, Mannose, Galactose, and Glucose, normalized to total neutral sugars, in average lake, river, seawater, and marine sediment porewater HMWDOM samples. Error bars are 1 SD.

monosaccharides, quantified as alditol acetates by gas chromatography, contribute between 5 and 38% of the total carbon in the samples, somewhat less than the 20 to 30% total carbohydrate estimated by ¹H NMR. Seven major sugars: rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose were present in all samples in the same relative abundance. These sugars have also been identified as the most abundant neutral sugars in seawater HMWDOM (Sakugawa and Handa, 1985; McCarthy et al., 1994, 1996; Aluwihare et al., 1997). West Neck Bay pore water HMWDOM had the most anomalous distribution of monosaccharides, with higher amounts of glucose relative to other samples. Environmental setting has no apparent effect on the monosaccharide distribution. We observe no significant difference in the monosaccharide distribution between seawater, rivers, and lakes. If glucose is excluded, no difference is observed in the relative monosaccharide distribution between anoxic marine sediment porewaters and other samples (Fig. 4).

4. DISCUSSION

Most studies describing the chemical properties of DOM in fresh waters have focussed on humic substances (humic and fulvic acids) isolated by adsorption onto XAD resins. Humic substances are hydrophobic, structurally complex macromolecules that are products of abiotically mediated "geopolymerization" reactions. The chemical characteristics of humic substances can most readily be assessed by NMR spectroscopy. Carbon NMR spectra of soil humic substances show a wide range of functionalized carbon, but characteristically include high amounts of aromatic carbon. Aromatic carbon in DOM may come from aromatic precursors (lignin, tannin, etc.) or

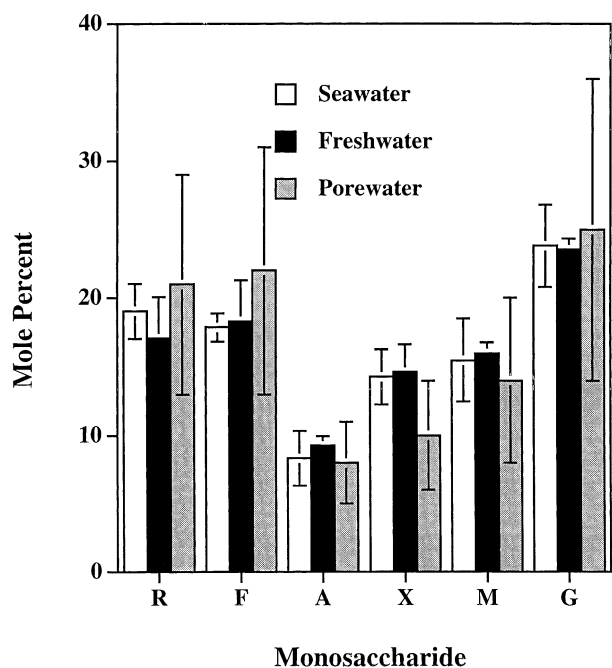


Fig. 4. The normalized distribution of neutral sugars excluding glucose in freshwater, seawater, and marine sediment porewater samples. Error bars are 1 SD.

may be the result of geopolymerization reactions that transform biologically derived precursors into more complex and presumably refractory DOM. In contrast, NMR spectra of marine humic substances show little aromatic carbon, but have relatively high amounts of aliphatic carbon (Stuerner and Payne, 1976; Hatcher et al., 1980). A large fraction of the carbon in both types of samples is present as carboxylic acid. NMR spectra of humic substances in rivers and lakes are highly variable in the relative amounts of the different functional groups, but in general display spectra intermediate of soils and marine DOM (Malcolm, 1990). Spectral and molecular level data for fresh water humic substances can be interpreted as representing a mixture of terrestrial and aquatic components, or a distinct class of DOM which incorporates carbon sources and pathways common to both environments.

Differences in the chemistry of terrestrial and marine humic substances are well established, and most likely arise from organic matter sources and formation pathways unique to each environment (Stuerner, 1975; Malcolm, 1990). Vascular plant material, which serves as the precursor of terrestrial humic substances is more aromatic, has a higher C/N, and is isotopically depleted in ^{13}C relative to marine organic matter, which has higher amounts of protein (lower C/N), and more aliphatic lipids (Francois, 1990). Formation of terrestrial humic substances occurs in soils, and highly colored products may result. In contrast, carbohydrates, proteins, and lipids produced by algae and bacteria serve as precursors for the abiotic formation of humic substances in the aquatic environments (Gilliam and Wilson, 1985). Humification reactions in aquatic systems often occur in the presence of sunlight, and yield products that are largely colorless. Humic substances, and by inference terrestrial and marine DOM, are considered to be unique products of

the environment in which they are formed. As such, they are often assumed to be cycled by different processes and to have different fates within the ocean.

The apparent uniqueness of humic substances in soils, freshwater, and marine environments contrasts sharply with the uniformity of spectral and molecular level properties of freshwater and marine HMWDOM recovered by ultrafiltration and dialysis.

Proton nuclear magnetic resonance spectra of seawater HMWDOM (>1 kD) shows this fraction of DOM to be compositionally uniform throughout the Atlantic and Pacific Oceans. The NMR spectrum of marine HMWDOM is dominated by resonances characteristic of carbohydrates, acetate, and lipids, which appear in a relatively fixed ratio. Analyses of acid hydrolyzed HMWDOM also shows abundant monosaccharides, and a characteristic distribution of seven neutral sugars (Fig. 3). Aminosugars (glucosamine, galactosamine) and uronic acids (glucouronic acid and galactouronic acid) are present at concentrations equal to or less than the concentration of neutral sugars (Aluwihare et al., 1997). Molecular level characterization of HMWDOM by direct temperature resolved mass spectrometry (DT-MS) corroborate wet chemical monosaccharide analyses (Boon et al., 1998). Multivariate analyses of DT-MS spectra show abundant HMWDOM carbohydrates, with a significant fraction of neutral and *N*-acetyl amino sugars.

High molecular weight DOM from lakes and rivers has ^1H NMR spectra similar to HMWDOM from surface seawater. The distribution of neutral monosaccharides between river, lake, and marine HMWDOM samples is also similar, irrespective of lake or river size, catchment area, or geographic location. All lake and river HMWDOM samples analyzed in our study have spectroscopic and molecular level properties characteristic of acylheteropolysaccharides (APS) previously isolated from seawater and described by Aluwihare et al. (1997). Our data show that APS is a major component of HMWDOM in all aquatic environments, whether freshwater and marine. The agreement of our data with literature data on ^1H and ^{13}C NMR, and monosaccharide distribution in other lakes and rivers demonstrates a compositional uniformity in aquatic HMWDOM on a much broader geographic scale than represented by our set of samples.

Laboratory culture studies have shown that marine microalgae produce APS (Aluwihare et al., 1997; Aluwihare and Repeta, 1999). Although these studies focussed on marine algae, all algal classes studied in culture have both freshwater and marine species. The presence of APS in lakes and rivers therefore suggests freshwater species have the biosynthetic capacity to synthesize APS. Factors that lead to the accumulation of APS in the environment are unknown. Aluwihare and Repeta (1999) have shown that the remineralization of APS by microorganisms is slow in laboratory culture, perhaps due to the heterogeneity of the polysaccharide. Similar results were obtained by Ochiai and Hanya (1980b) who monitored the change in monosaccharide distribution of lake water with microbial degradation. After one month, the distribution of neutral sugars evolved from a glucose rich mixture to a mixture very similar in composition to APS. The ubiquity of APS, and the relatively fixed molecular properties of APS across different environments implies that molecular structure may contribute to the recalcitrance of HMWDOM.

Each year, 0.2 to 1.0 GT carbon is introduced to the ocean by rivers in the form of DOC (Mantoura and Woodward, 1983; Hedges et al., 1997). Global input rates for riverine DOM are calculated from average riverine DOC concentrations and river discharge fluxes, and under the assumption that DOC is conservative in estuaries. Riverine DOC has an isotopic value ($\delta^{13}\text{C} \approx -28$ per mil) similar to the isotopic value of (C_3) vascular plants, and the humic fraction of riverine DOM, as discussed above, is chemically distinct from the humic fraction of marine DOM. Both observations have led to the inference that riverine DOC is principally derived from higher plant carbon, and as such is not extensively degraded or removed in estuaries (van Heemst et al., 2000). The annual input of riverine DOC is sufficient to support the measured radiocarbon age of marine DOC, but the distinctive isotopic and chemical composition of terrestrial and marine DOC suggests terrestrial DOC is rapidly remineralized once it is introduced to the ocean (Hedges et al., 1997). However, the chemical uniqueness of freshwater and marine humic substances may not be representative of freshwater and marine DOM in general. Isotopically, freshwater aquatic organisms are known to synthesize organic matter with a wide range of carbon isotopic values (-24 to -31 per mil; Spiker, 1980; Fry and Sherr, 1984; France, 1995), and it is difficult to assign a specific isotopic end member characteristic of autochthonous freshwater production. Further, it has been shown that both C_3 plants, which synthesize isotopically enriched organic matter ($\delta^{13}\text{C} \approx -15$ per mil), and C_4 plants ($\delta^{13}\text{C} \approx -27$ per mil) contribute to POM in coastal marine sediments. It is likely that C_3 plants also contribute to terrestrial DOM as well (Goni et al., 1997).

Our observation that a significant fraction of riverine DOM is chemically similar to marine DOM, and is probably autochthonous in origin, challenges one of the assumptions used to infer a terrestrial origin for riverine DOM. Humic substances only contribute a fraction of the total DOM in rivers and lakes. In situ photoautotrophic production or heterotrophic secondary production also contributes a substantial fraction of recalcitrant DOM to fresh waters. In our samples from lakes and rivers, between 10 to 35% of the total carbon can be attributed to APS. Current models which consider the fate of terrestrial organic matter in the ocean may therefore overestimate the amount of terrestrial organic matter delivered to the sea (Hedges et al., 1997), and the importance of the ocean as a sink for this carbon.

A significant fraction of sediment pore water DOC is in a high molecular weight fraction, although little is known of its molecular composition (Burdige and Gardner, 1998). High molecular weight DOM from three samples of marine porewaters from anoxic surficial (0 to 10cm) sediments was analyzed by ^1H NMR and for monosaccharides. Like surface seawater, the NMR spectrum of marine porewaters is dominated by carbohydrate, acetate, and lipid resonances, which occur in a ratio similar to seawater. The spectra have much lower proportions of unresolved baseline resonances than river and lake HMWDOM samples (Fig. 2). Molecular level analyses of monosaccharides yield the same seven neutral sugars in each sample, again in a ratio similar to seawater HMWDOM monosaccharides (Fig. 4). The major difference between porewater and surface seawater samples is the much higher relative abundance of glucose, principally in the West Neck Bay (L.I.) sample where it contributes 44% of the total neutral sugars.

Glucose polysaccharides are major energy storage products in marine algae (laminarin, starch, etc.), and the high relative abundance in marine porewaters is not surprising. If the distribution of monosaccharides in porewaters and seawater are compared without glucose (Fig. 4) then no difference between the sample types is observed. The agreement in monosaccharide distribution exclusive of glucose suggests the presence of both APS and glucose homopolysaccharides in porewater samples.

The much higher concentration ($>200 \mu\text{M C}$) of APS in porewaters than found in surface seawater ($16 \mu\text{M}$) implies a release or production (from bacteria) of APS during organic matter remineralization in sediments. Acyl-heteropolysaccharide can be recovered from the extracellular HMWDOM fraction of algal culture media, but the high concentrations of APS in pore waters suggest a large intracellular reservoir is also present. The ^{13}C NMR spectrum of seawater HMWDOM (Fig. 1) compares favorably with the solid state CP-MAS ^{13}C NMR spectra of porewaters and a marine sapropel from Mangrove Lake, Bermuda (Hatcher et al., 1983; Orem et al. 1986) and sediment from Great Bay, New Hampshire (USA) (Orem and Hatcher, 1987) which have major resonances at 180 ppm (COOH), 140 ppm (C=C), 110 ppm (OCO), 70 ppm (HCOH), and 20 to 30 ppm (CH_x). The resonance at 140 ppm is relatively minor, and contributes only 10 to 15% of the total carbon in these samples. The other resonances (COOH:OCO:HCOH: CH_x ; 90% total carbon) occur in the ratio 0.2:0.2:1:0.6, similar to their ratio (0.2:0.2:1:0.5) in surface seawater. These data suggest APS may contribute a significant fraction of algal particulate matter. The possible relationship between APS and structural polysaccharides in microalgae warrants further investigation.

The monosaccharide composition of insoluble algal material and marine suspended particulate matter is heterogeneous but all seven of the neutral monosaccharides present in APS have been reported (Haug and Mykelstad, 1976). For example, McCarthy et al. (1994) report a monosaccharide distribution for water column particulate matter much like the distribution of monosaccharides we find in our porewater samples, with high concentrations of glucose and low but relatively equal concentrations of galactose, mannose, xylose, arabinose, fucose, and rhamnose. If glucose is excluded from the comparison, there is little difference in the relative abundance of these sugars in particulate matter and APS. A second potential source of APS in porewaters may be the flocculation of DOM into large rapidly sinking particles (macroaggregates) that are incorporated into sediments (Alldredge and Silver, 1988). Degradation of carbohydrate-rich macroaggregates in sediments could yield high concentrations of APS in porewaters. Marine snow particles are rich in polysaccharides and analyses of flocculent organic matter particles generated in laboratory culture experiments show compositional similarities to APS (Passow et al., 1995; Mopper et al., 1995).

5. CONCLUSIONS

High molecular weight dissolved organic matter recovered by ultrafiltration from rivers and lakes has spectroscopic and molecular level properties characteristic of APS previously described in marine HMWDOM. Like surface seawater, car-

bohydrates contribute the largest fraction of the total carbon to freshwater HMWDOM. Hydrolysis of HMWDOM yields a suite of seven neutral sugars: glucose, galactose, mannose, xylose, arabinose, fucose, and rhamnose, which are present in approximately equal amounts. High molecular weight DOC contributes $51 \pm 26\%$ of the total DOC, and monosaccharides $18 \pm 8\%$ of the total HMWDOC in our freshwater samples. These contributions are on average higher but more variable than for surface seawater (30 and 16% respectively). Dissolved organic matter in fresh and marine waters is a mixture of humic substances and APS, the proportions of which may vary with environmental factors. Previously published NMR and molecular level data for lake and river DOM is consistent with the presence of APS in a much broader suite of samples than analyzed in our study, and support the conclusion that APS may be a ubiquitous component of HMWDOM in all aquatic systems. The persistence of APS in many different aquatic environments implies a widespread biosynthetic capacity of microorganisms to manufacture APS. Knowledge of the biosynthesis, structure and fate of APS in any aquatic system is relevant to studies of HMWDOM cycling in all aquatic systems.

Acyl-heteropolysaccharides also contribute most of the carbon in the > 1 kD fraction of DOM recovered from porewaters of anoxic marine sediments. Concentrations of APS in porewaters may be in excess of $200 \mu\text{M}$, an order of magnitude higher than typically measured in surface seawater. High concentrations of APS in marine porewaters are probably the result of APS solubilization during remineralization of POM, or its direct synthesis by bacteria. In one sample (West Neck Bay) we also measured very high concentrations of glucose, which may arise from additional glucose storage polysaccharides. Efforts should be made to identify APS precursors in particulate organic matter.

Models of carbon exchange between the continents and the sea often assume that all riverine DOC is derived from higher plants. This assumption is based in part on the unique chemical properties observed for terrestrial and marine humic substances. Our observation of a chemically uniform fraction of HMWDOM in fresh water and marine environments challenges this assumption. Dissolved organic matter in rivers includes both terrestrially derived humic substances, and soluble organic matter introduced as a by-product of in situ production. Biopolymers produced in situ are resistant to degradation, and cycle on time scales long enough to be transported into the ocean. Current estimates of the delivery of terrestrial carbon to seawater are therefore too high, and need to be adjusted to include a contribution of autochthonous DOC.

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