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Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight

Lihini I. Aluwihare[a,*], Daniel J. Repeta[b], Robert F. Chen[c]

[a] Geosciences Research Division, Scripps Institute of Oceanography, 9500 Gilman Drive, La Jolla, CA 92093-0244, USA
[b] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[c] Department of Environmental, Coastal, and Ocean Sciences, University of Massachusetts Boston, 100 Morrissey Boulevard, Boston, MA 02125-3393, USA

Abstract

This study focuses on the chemical characterization of high molecular-weight dissolved organic matter (HMW DOM) isolated from the Middle Atlantic Bight in April 1994 and March 1996. Using proton nuclear magnetic resonance spectroscopy (^{1}HNMR) and monosaccharide analysis we compared both spatial and temporal variations in the chemical structure of HMW DOM across this region. Our analyses support the presence of at least two compositionally distinct components to HMW DOM. The major component is acyl polysaccharide (APS), a biopolymer rich in carbohydrates, acetate and lipid, accounting for between 50% and 80% of the total high molecular-weight dissolved organic carbon (HMW DOC) in surface samples. APS is most abundant in fully marine, surface-water samples, and is a product of autochthonous production. Organic matter with spectral properties characteristic of humic substances is the second major component of HMW DOM. Humic substances are most abundant (up to 49% of the total carbon) in samples collected from estuaries, near the coast, and in deep water, suggesting both marine and perhaps terrestrial sources. Radiocarbon analyses of neutral monosaccharides released by the hydrolysis of APS have similar and modern (average 71‰) $\Delta^{14}C$ values. Radiocarbon data support our suggestion that these sugars occur as part of a common macromolecule, with an origin via recent biosynthesis. Preliminary radiocarbon data for total neutral monosaccharides isolated from APS at 300 and 750 m show this fraction to be substantially enriched relative to total HMW DOC and DOC. The relatively enriched radiocarbon values of APS at depth suggest APS is rapidly transported into the deep ocean. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Ocean margins serve as transition zones between terrestrial and marine environments. For example, elevated nutrient concentrations in rivers help fuel primary production in these environments such that the standing stock of phytoplankton on ocean margins is often an order of magnitude higher than in the central ocean basins (Walsh, 1988). Coastal primary production is estimated to be $>8\times10^{15}$ g C/yr (Valiela, 1995), approximately 20% of whole ocean primary production, and burial of carbon in these regions is estimated to be three times greater than in the open ocean (Liu et al., 2000). In addition, ocean margins also can serve as conduits for the transport of organic carbon derived from terrestrial and freshwater environments to the open ocean. Hedges (1992), estimated that

*Corresponding author. Tel.: +1-858-534-3196; fax: +1-858-822-3310.

E-mail address: laluwihare@ucsd.edu (L.I. Aluwihare).

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0.4×10^{15} g C/yr, in the form of particulate and dissolved organic carbon (DOC), entered the marine environment via freshwater inputs.

Carbon budgets for the Middle Atlantic Bight (MAB) initially suggested that a significant fraction of the organic carbon fixed on the shelf was available for transport to the slope and export to the interior of the ocean. Consistent with this hypothesis, a major mid-slope 'depocenter' for organic carbon and sediment was shown to exist off Cape Hatteras. However, measurements of sinking particle flux (Anderson et al., 1994; Bacon et al., 1994; Biscaye and Anderson, 1994; Falkowski et al., 1994) and bacterial remineralization rates (Kemp et al., 1994) made as part of the shelf edge exchange process studies [SEEP I (July 1983–October 1984) and SEEP II (February 1988–June 1989)], found that $<4\%$ of annual production was exported off the MAB as particulate organic carbon (POC). Furthermore, radiocarbon measurements of sediment trap material and freshly deposited sedimentary organic matter indicated that much of the organic carbon at the mid-slope 'depocenter' was reworked from older material (Anderson et al., 1994), supporting the finding that there is little across-shelf transport of the fresh organic matter derived from in situ primary production on the shelf.

The SEEP program did not include the role of DOC when constructing the carbon budget for the MAB. The concentration of DOC in surface waters is one to two orders of magnitude greater than that of POC (Druffel et al., 1992), and a large portion of the carbon fixed on the shelf could be exported to the ocean's interior as DOC. DOC concentration in surface waters (Vlahos et al., 2002) increase from northeast to southwest, and from offshore to inshore. The north to south and inshore to offshore gradients in DOC concentration arise from the production and accumulation of DOC on the shelf as a result of in situ primary production and river inputs to a southwestward flowing water mass. The MAB receives a large supply of freshwater from the Hudson, Delaware and Chesapeake estuaries (0.005 Sv/yr; Biscaye and Anderson, 1994) that carries high loads of DOC (in the excess of 200 μM C). Much of this DOC is believed to be of terrestrial origin, consisting of refractory organic matter that can be transported across the shelf and into the open ocean. Several studies have attempted to quantify the flux of terrestrial organic matter to the open ocean using lignin phenols as molecular tracers (e.g., Ertel et al., 1986; Meyers-Schulte and Hedges, 1986; Goni et al., 1997; Mannino and Harvey, 2000a; Mitra et al., 2000). For example, by quantifying lignin oxidation products, Mannino and Harvey (2000a) estimated an annual flux of 2.0×10^{10} g terrestrial organic carbon per year from the Delaware River and Estuary to the coastal ocean. However, lignin oxidation products are only a fraction of the total dissolved organic matter (DOM) in estuarine and coastal-ocean systems. Repeta et al. (2002) demonstrated that a significant fraction of the DOM isolated from freshwater systems is chemically distinct from humic substances, but compositionally related to a marine DOM. Thus, by quantifying the flux of lignin oxidation products alone, we may underestimate the contribution of DOM from rivers to the coastal ocean. On the MAB, the large northeast to southwest and inshore to offshore gradients in DOC allow us to compare DOC that is primarily marine in origin to DOC that is strongly influenced by continental input and local shelf productivity. Using proton nuclear magnetic resonance (^{1}HNMR) spectroscopy and molecular level analyses, we investigated the spatial variation in chemical composition of DOM with molecular weights >1000 Daltons (1 kDa). Throughout the remainder of this manuscript, we refer to this fraction as high molecular-weight (HMW) DOM.

2. Methodology

2.1. Sampling

Sampling sites are shown in Fig. 1 and listed in Table 1. All surface samples were collected using a teflon-lined hose, and pumped onboard ship with a pneumatic, diaphragm pump (Lutz Pumps, Inc., Norcross, GA). Deep samples were collected via CTD rosette mounted 30-l Niskin bottles. Seawater samples were filtered through Whatman

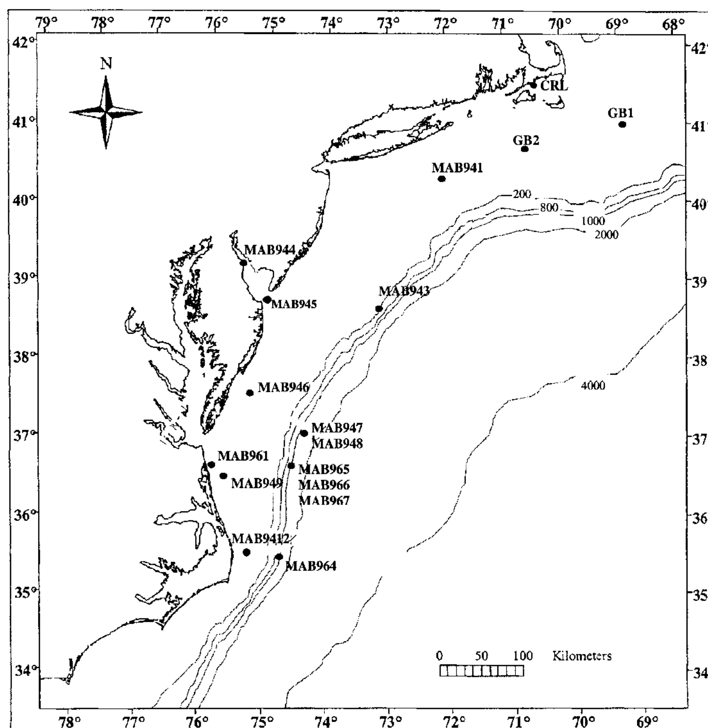

Fig. 1. The Mid-Atlantic Bight, showing the location of samples described in this study. Latitude (°N) and longitude (°W) and sampling depths are listed in Table 1. Depth contours (200, 600, 1000, 2000, 4000) are in meters.

GF/F (0.7 μm) or Gelman A/E (1.0 μm) filters (293 mm, pre-combusted at 450°C for 12 h), followed by a 0.2 μm, Gelman Criticap 100 capsule (acid (0.1 N HCl) rinsed overnight). Filtered samples were stored (<5 h) in acid cleaned high density polyethylene barrels (Fluoropure, Chaska, MN) until ultrafiltration. All tubing and fittings were teflon except for the 293-mm filter holder (polycarbonate) and the Criticap capsule filters (polycarbonate).

2.2. Ultrafiltration

HMW DOM was isolated using an Amicon DC-10L pump fitted with two Amicon S10N1 spiral-wound polysulphone membrane cartridges in series. These membranes have a 1-nm pore diameter and are designed to have a nominal molecular weight cutoff of 1000 Da (1 kDa). A 200-l sample of seawater typically took between 8 and 12 h to concentrate to approximately 2 l. Concentrated samples were combined with a post-sample Milli-Q water rinse of the ultrafiltration system and frozen (−4°C) in pre-combusted glass bottles or acid-rinsed (concentrated HCl) teflon bottles.

New ultrafiltration cartridges and cartridges that were stored for over 3 weeks, were thoroughly cleaned before use. Cartridges were cleaned by sequentially passing 4 l of micro-detergent (1%), 0.01 N sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions (in Milli-Q water) through the system (Guo et al. 1994). Between each cleaning solution, the cartridges were rinsed with 20 l (5 × 4 l each) of Milli-Q water. After the last Milli-Q rinse, the pH of the permeate was measured to insure that it was between pH 5 and 6. While on board the ship, we used an abbreviated cleaning procedure for surface samples. Cartridges were rinsed only with base and acid solutions (e.g., MAB941, MAB943, MAB947, MAB961). When a deep sample followed a surface sample (e.g. before

Table 1
Locations, depths, C/N ratio, and the ratio of major biochemicals for the HMW DOM samples discussed in this study

Sample	Depth (m)	Latitude (°N)	Longitude (°W)	Date (M-Y)	DOC (μM)	C/N	%HMW DOC	APS (%C)	Humics (%C)	APS		
										Carbohydrate (%C)	Acetate (%C)	Lipid (%C)
GB-1	**3**	**40.6**	**70.6**	**3–94**	**80**					**81**	**11**	**8**
GB-2	3	41.0	68.9	3–94	85					77	9	14
CRL	**3**	**41.5**	**70.5**	**4–94**	**102**	**13**	**10**	**55**	**45**	**81**	**10**	**9**
MAB941	3	40.3	72.0	4–94	89	14	11	81	19	77	8	15
MAB943	**3**	**38.7**	**73.1**	**4–94**	**72**	**16**	**17**	**50**	**50**	**74**	**7**	**19**
MAB944	3	39.3	75.3	4–94	209	20	15	53	47	74	8	18
MAB945	**3**	**38.9**	**75.1**	**4–94**	**132**	**23**	**20**	**51**	**49**	**76**	**7**	**17**
MAB946	3	37.7	75.4	4–94	115			54	46	81	6	13
MAB947	**3**	**37.1**	**74.2**	**4–94**	**91**	**21**	**17**	**57**	**43**	**81**	**7**	**12**
MAB948	750	37.1	74.2	4–94	47	14	8	38	62	67	5	28
MAB949	**3**	**36.6**	**75.8**	**4–94**	**99**			**34**	**66**	**80**	**6**	**14**
MAB9412	3	35.6	75.4	4–94	112			32	68	76	6	18
MAB961	**3**	**36.8**	**75.0**	**8–96**	**160**	**12**	**23**	**63**	**37**	**87**	**8**	**5**
MAB964	750	35.5	74.7	8–96	50	13	8					
MAB965	**3**	**36.7**	**74.6**	**8–96**	**83**			**61**	**39**	**85**	**6**	**9**
MAB966	300	36.7	74.6	8–96	40	16	10	35	65	75	6	19
MAB967	**750**	**36.7**	**74.6**	**8–96**	**40**	**16**	**14**	**26**	**74**	**78**	**5**	**17**

Percent HMW DOC (%HMW DOC) was calculated from the concentration of DOC in the sample following ultrafiltration and dilafiltration. APS refers to the sum of percent carbohydrate + acetate + lipid carbon in each spectrum, and humic substances refer to the sum of baseline resonances in each spectrum. Also listed is the relative abundance of carbohydrate, acetate and lipid in APS for each sample (i.e. carbohydrate + acetate + lipid = 100%). See text for an explanation of the calculations.

MAB966 and MAB967), or when a shelf sample followed a freshwater sample (MAB944 and MAB945, MAB946), the full cleaning protocol was used. Membranes were preconditioned with 3–4 l of seawater before sample collection.

Samples were desalted in the laboratory by diafiltration against Milli-Q water according to the following procedure. Samples (approximately 2 l) were diluted with 2 l of Milli-Q water and concentrated by ultrafiltration to between 1.5 and 2 l. This dilution and ultrafiltration of the sample was repeated eight to ten times (using a total of 16–20 l of Milli-Q water). Diafiltered samples were stored frozen, for up to several months, until lyophilized for spectroscopic and molecular level measurements.

2.3. Desalting with ion exchange

Some samples were further desalted using ion exchange resins. Freeze-dried samples were dissolved in 1 ml of Milli-Q water and applied to a 6–8 mm ID glass column packed with AG 50W-X8 cation exchange resin (H^+ form, BioRad Laboratories, Hercules, CA) at 5 mg sample/2 ml of resin bed. The cation exchange resin was pre-rinsed three times with 5 ml of Milli-Q water prior to packing the column, and the sample was recovered by elution with three column volumes of Milli-Q water. Carbon recoveries after ion exchange were between 62% and 82%. Desalting by ion exchange improved the appearance of ^{1}HNMR spectra, but did not change results from spectral integration or molecular level analyses. Neutral monosaccharides (arabinose, xylose, glucose, galactose, mannose, rhamnose and fucose), uronic acids (galactouronic acid and glucosamine) and small proteins (Bovine Serum Albumin (BSA)) showed no losses ($>90\%$ carbon recovery) to the column during desalting. Desalted samples were freeze dried before further analyses.

2.4. Elemental analysis

Samples were analyzed for carbon and nitrogen content using an EA 1108 elemental analyzer with Eager 200 data acquisition software (Fisons Instruments, Inc., Beverly, MA). Between 300 and 1000 μg of lyophilized DOM were weighed into 8×6 mm tin cups. Blank cups and known standards also were run in order to correct for the C and N associated with tin cups.

2.5. Nuclear magnetic resonance spectroscopy

All spectra were acquired using a Bruker AC300, 300 MHz spectrometer (Bruker Instruments, Inc., Manning Park, Billerica, MA) equipped with an Aspect 3000 data system (Spectrospin AG, Industriestrasse 26, CH-8117 Faellanden, Switzerland). Typically, 10 mg of HWW DOM was dissolved in 1–2 ml of D_2O (> 99.9 at%, Aldrich Chemical Company, Milwaukee, WI) and freeze-dried. This was repeated a second time in order to ensure that the exchangeable protons in each sample were saturated with deuterium. The sample was then freeze-dried and dissolved in 0.75–1 ml D_2O for NMR spectroscopy. Chemical shifts are reported in the standard 'δ' notation (units of ppm), relative to HDO ($\delta = 4.8$ ppm). All samples were run in 5-mm emperor glass NMR tubes (Wilmad Glass Co., Buena, NJ) and transferred using combusted glass NMR pipettes (Wilmad Glass Co.).

Quantitative functional group distributions in HMW DOM can be obtained by integrating the area under each peak in the NMR spectrum. To do this, spectra were photocopied, the peaks cut by hand, and weighed. Integrations were made in triplicate, and peak masses agreed to $+5\%$.

2.6. Lipid analysis

Several samples were analyzed for the presence of ester (MAB941–948, MAB9412, MAB961, MAB964, and MAB967) and ether (MAB944, MAB945 and MAB947) bound lipids (Fig. 1). All reactions were performed in sealed tubes under nitrogen, and all glassware was pre-combusted and pre-extracted with methanol and dichloromethane. Aqueous solutions of HCl and potassium hydroxide (KOH) were pre-extracted with ethyl ether or dichloromethane to remove free lipids. Samples (20–30 mg) were dissolved in Milli-Q water (10–15 ml), acidified to pH 3–4, and extracted with ethyl ether or dichloromethane to remove unbound lipids. Following extraction, samples were freeze-dried, and esters were released by acid hydrolysis (4 N HCl, 4–22 h at 100°C) or saponification (20% KOH, 24 h at 100°C) (Sidorczyk et al., 1983). HMW DOM ethers were released by treatment with boron tribromide (90°C for 4 h) according to Benton and Dillon (1942). In order to recover ether or ester bound lipids, sample pH was adjusted to pH 3–4, and extracted with dichloromethane, diethyl ether or hexane. The extracts were dried by rotary evaporation and dissolved in deuterated dichloromethane for ^{1}HNMR spectroscopy. Lipids were derivatized using *N,O*-bis [trimethylsilyl] trifluoroacetamide (BSTFA; Pierce, Rockford, IL) in pyridine (60°C, 15 min) for fatty acid analysis and with acetic anhydride and pyridine (room temperature, 15 min) for fatty alcohol analysis. Samples were then dried, dissolved in dichloromethane, and analyzed by gas chromatography using a DB-5 (J&W Scientific, Folsom, CA) column. The initial column temperature was 55°C. Five minutes after injection the column was heated at 4°C/min to a final temperature of 320°C, and held for an additional 10 min before ending the analyses.

Sub-samples of the organic extracts were dried under nitrogen and re-dissolved in water for low molecular weight lipid analysis. Lipids or small organic acids (formic acid, acetic acid, lactic acid, etc.) were analyzed in triplicate by ion chromatography on a Dionex Ion Chromatograph (AS11 column; Dionex electrochemical detector) with water as the eluant.

2.7. Monosaccharide analysis

The monosaccharide composition of HMW DOM was determined according to York et al. (1985). For the acid hydrolysis of HMW DOM, 300–700 μg of dry sample were dissolved in 0.5 ml of 2 M trifluoroacetic acid containing 20 μg of myo-inositol as an internal standard. This mixture

was heated in a tightly capped glass vial at 121°C for 2 h. Samples for uronic acid and amino sugar analyses were hydrolyzed in 4 M methanolic-HCl, at 90°C for 8 h. In all cases, samples were cooled to room temperature, and the acid was removed by drying under a stream of nitrogen. Neutral monosaccharides were reduced to alditol acetates using 250 μl of 0.3 M sodium borohydride (10 mg/ml) in 1 M NH_4OH, and incubated at room temperature for a minimum of 2–3 h. The reaction was quenched with glacial acetic acid (250 μl) to decompose the excess borohydride. Glacial acetic acid and trace amounts of water were removed by adding methanol (3 × 250 μl) and then drying under a stream of nitrogen. The dry alditols were per-acetylated with 100 μl of acetic anhydride and 20 μl of 1-methyl imidazole, and mixed thoroughly. After 15 min at room temperature, the excess acetic anhydride was quenched with 0.5 ml of Milli-Q water. After a further 10 min at room temperature, 0.5 ml of dichloromethane was added. The solution was vortex-mixed and the alditol acetates were extracted into dichloromethane. This organic layer was withdrawn, dried over anhydrous sodium sulfate, transferred to a clean vial, and the dichloromethane removed under a stream of nitrogen. The alditol acetates were then dissolved in 100 μl of methanol and analyzed using an HP 5890 gas chromatograph equipped with an on-column injector and FID, and fitted with a 30-m, DB-5 fused silica column (0.25 mm ID, and 0.20 μm film). The initial column temperature was 150°C. After 1 min the column temperature was increased by 2°C/min to a final column temperature of 240°C, which was held for an additional 15 min. Alditol acetates elute in 15–30 min (Fig. 2).

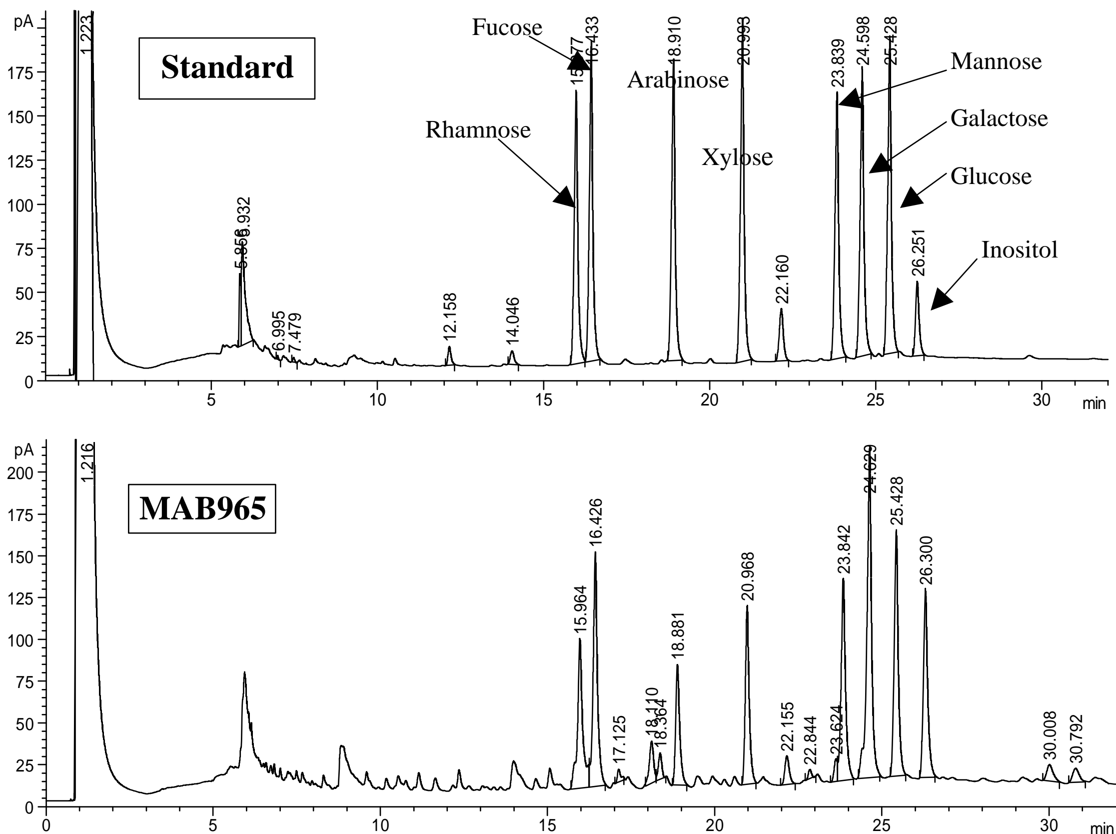

Fig. 2. Two raw gas chromatographs of monosaccharides derivatized as alditol acetates. The *x*-axis is time in minutes, and the *y*-axis shows relative response in mA (milli amperes). Myo-inositol is added as an internal standard.

2.8. Radiocarbon analyses

Samples were hydrolyzed in 2 M trifluoroacetic acid at 121°C for 2 h as performed for routine monosaccharide analyses. Following hydrolysis and desalting by ion exchange, the total monosaccharide fraction in each sample was purified from unhydrolyzed material using cation exchange high performance liquid chromatography (HPLC) on a sulphonated styrene-divinylbenzene gel in the silver form. Two different column manufacturers were used, Supelco (Supelcogel™ Ag, Supelco, Inc., Bellefonte, PA) and Alltech (Benson carbohydrate, BC-100, Ag^+, Alltech Associates, Inc., Deerfield, IL). The performance and retention time of monosaccharide standards on these two columns were identical. Monosaccharides were eluted with Milli-Q water using a flow rate of 0.5 ml/min with the column heated to 80°C. The total weight of the sample injected onto the column did not exceed 1 mg/injection. In some cases (MAB961, MAB967), samples were re-injected onto the Ag^+ column to purify the monosaccharide fraction further from non-carbohydrate components. Monosaccharide fractions were lyophilized immediately after collection. For MAB966 and MAB967, no subsequent purification of individual monosaccharides was attempted due to the small amounts of sample. For MAB961, monosaccharides were further purified via HPLC using an aminopropyl silyl-bonded phase (5 μm Supelcosil LC-NH_2, Supelco; Adsorbosphere NH_2, 3 μm or carbohydrate from Alltech). Columns were operated at room temperature using a mobile phase of 75/25 acetonitrile/water (v/v) at a flow rate of 1 ml/min. Samples were dissolved in the mobile phase prior to injection. After sample collection, the acetonitrile was removed by evaporation under nitrogen using a TurboVap LV Evaporator (Zymark) at 50°C. The remaining water was lyophilized, and a fraction (5–10%) of each sample was re-injected onto the amino column to quantify total sugar and to monitor sample purity.

The remaining fraction (90–95%) of each sample was dissolved in 1–2 ml of Milli-Q water and transferred to the bottom of 8″ Vycor quartz tubes (9 mm OD, 7 mm ID, Anderson Glass Co, Inc., Fitzwilliams, NH) containing copper oxide. The quartz tubes and added copper oxide (300–500 mg; Elemental Microanalysis, Manchester, MA) had been combusted at 850°C for 5 h prior to the addition of each sample. In addition to CuO, 100 mg of silver (silver shot, Alfa Aesar, Ward Hill, MA) was added to the quartz tubes containing total HMW DOM samples. The silver reacts with sea-salt contaminants that may be present in the sample. All samples, in quartz tubes, were lyophilized overnight. Lyophilized samples contained between 10 and 100 μg C.

Dry samples were placed under vacuum and evacuated for at least 1 h prior to sealing using a butane/oxygen torch. During the sealing, the bottom 2″ of each tube was placed in a liquid nitrogen bath to avoid any sample loss through volatilization. Sealed samples were combusted at 850°C for 5 h. The CO_2 generated during the combustion was quantified using the ideal gas law ($n = PV/RT$; where n is the number of moles of CO_2 and R is the gas constant) by measuring the partial pressure (P) of the gas in a pre-calibrated volume (V) at a known temperature (T). Quantified CO_2 samples were then transferred to 6 mm Pyrex tubes (Anderson Glass Co., Fitzwilliams, NH) and flame-sealed. Subsamples (10% of CO_2) of HMW DOM were removed for $\delta^{13}C$ measurements.

Carbon dioxide was converted into graphite for microscale accelerator mass spectrometry (AMS) radiocarbon measurements according to the protocol of Pearson et al. (1998). In all cases, CO_2 was reduced to graphite on a 325-mesh spherical cobalt catalyst cleaned at 400°C for 0.5 h under 0.7 atm H_2. The procedure requires that a carbon:cobalt ratio of 60 μg/1 mg be used. We used no less than 1 mg of Co, so that smaller samples have a lower carbon to cobalt ratio. The graphite was pressed into targets and analyzed by AMS along with standards and process blanks. Two primary standards NBS Oxalic Acid I (NIST-SRM-4990) and Oxalic Acid II (NIST-SRM-4990C) are used during all ^{14}C measurements. The process blank material was a Johnson–Mathey 99.9999% pure graphite powder.

3. Results and discussion

Sampling site information, characteristics, and other ancillary data for each sample are listed in Table 1. The samples analyzed in this study spanned a wide range of DOC concentrations (40–210 μM). Carbon to nitrogen ratios in the HMW DOM samples average 16 (±4; 1 SD), and range from 12 to 23. This range is larger than previously observed for open-ocean samples by Benner et al. (1992) and McCarthy et al. (1996) (15–22), but similar to the range in C/N ratios observed by Guo et al. (1996) and Mitra et al. (2000) for the southern MAB (11–25), and Mannino and Harvey (1999) for the Delaware River and Estuary.

The average amount of HMW DOC isolated in our study was 16(±5)% for surface samples and 10(±5)% for deep samples (Table 1). These values are for carbon recoveries following ultrafiltration, diafiltration, and lyophilization. We observed marked losses in HMW DOC (>50% in some cases) following diafiltration. Other investigators also have noted carbon losses during diafiltration, for example, Guo and Santschi (1996) observed that 35–39% of the HMW DOC was lost during diafiltration. Prior to diafiltration, we isolated 24(±8)% of the total DOC as HMW carbon and this is consistent with other estimates (between 20% and 30%; Carlson and Mayer, 1985; Benner et al., 1992).

3.1. Nuclear magnetic resonance data

Based on the chemical shifts in the ^{1}H and ^{13}C NMR spectra (Aluwihare, 1999), we assigned peaks to particular functional groups. These functional groups were interpreted to be present in three major biochemicals. As can be seen in Fig. 3, major resonances in the ^{1}HNMR spectra arise from carbohydrates (4.9–5.5 ppm (anomeric; OC**H**OH), 3–4.0 ppm [C**H**OH], and 1.3 ppm [C**H**$_3$]), acetate (2.0 ppm [C**H**$_3$CO]), and lipids (0.9 ppm [C**H**$_3$] and 1.3 ppm [C**H**$_2$]). We have previously suggested these biochemicals are linked together in a common macromolecular structure, termed acylated polysaccharide (APS) (Aluwihare et al., 1997). We define APS as having a carbohydrate/acetate/lipid carbon ratio of 8/1/1 and a specific composition of seven neutral monosaccharides. The resonance centered at 4.8 ppm in all ^{1}HNMR spectra arises from partially deuterated water (HDO) and is used as the reference peak.

In our MAB samples, the ^{1}HNMR resonances characteristic of APS sit atop an unresolved baseline between 0.8 and 4.6 ppm, which is assumed to arise from humic substances (see below). We can calculate the amount of APS in HMW DOC by multiplying the integrated area of carbohydrate, acetate and lipid by their C/H ratios (1, 0.67, 0.5, respectively). By this approach, APS (carbohydrates + lipids + acetate; Table 1) accounted for 50–80% (except in the case of MAB949 and MAB9412) and 26–38% of the HMW DOC in surface and deep waters. As shown in Table 1, the average carbohydrate:acetate:lipid carbon ratio is 79(±4):8(±2):13(±4) ($n = 13$) in surface waters, and 73(±6):5(±1):21(±6) ($n = 4$) in the deep ocean. Lipid carbon (1.3 ppm) was corrected for a contribution from deoxy sugars as determined by GC analyses of monosaccharides.

Overall, the relative ratio of major biochemicals (Table 1) in APS as identified by ^{1}HNMR spectroscopy is similar in both coastal- and open-ocean stations. Even estuarine samples such as MAB944 and MAB945 show ratios very similar to those of open-ocean samples (e.g. MAB947), consistent with the observations of Repeta et al. (2002). Lipids show the greatest variation in relative abundance and this variation may arise because these compounds are an important constituent of both APS and humic substances.

We were also interested in identifying any distinct features in the ^{1}HNMR spectra that were influenced by freshwater- and estuarine-derived DOM. Fig. 4 compares ^{1}HNMR spectra for MAB941, taken north of the Delaware River, MAB944, taken in the Delaware River, and MAB947, taken south of the river. As mentioned above, a featureless baseline lies beneath the major APS resonances in our ^{1}HNMR spectra. This baseline is similar in appearance to ^{1}HNMR spectra of humic substances (Stuermer and Payne, 1976; Malcolm, 1990). Humic substances from both freshwater and marine environments are rich

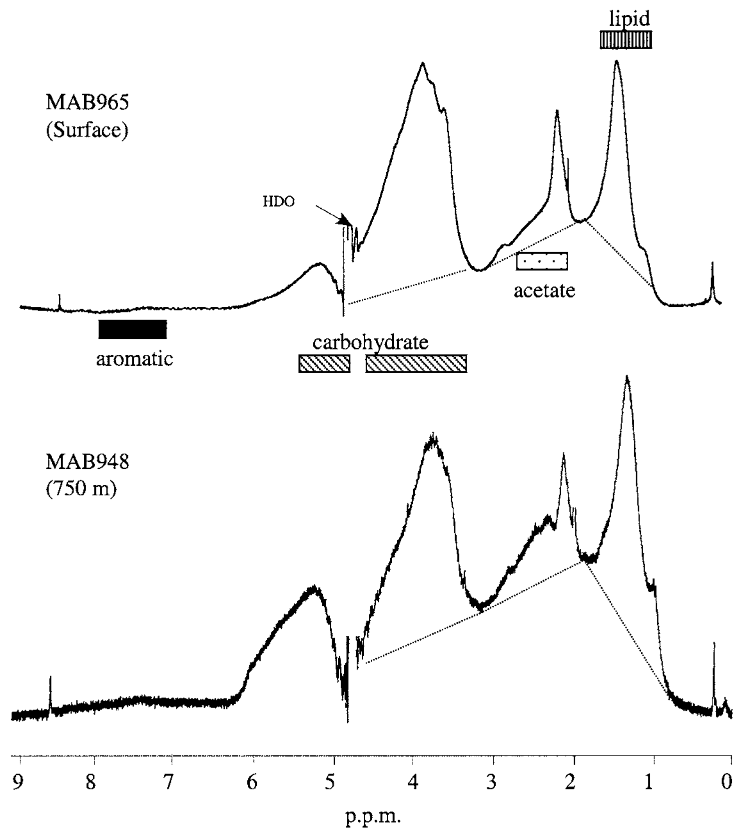

Fig. 3. Two ^{1}HNMR spectra from surface North Atlantic (MAB965) and deep North Atlantic (HMW DOM). HDO is partially deuterated water, the reference standard. Resonances corresponding to aromatic, carbohydrate, acetate, and lipid protons are identified in the figure. Dashed lines represent where baselines were drawn for each peak. Areas of the peaks above the baseline are summed to give APS carbon and areas below the baseline are summed for humic substance carbon. See text for proton to carbon conversions.

in alkyl carbon and their ^{1}HNMR spectra typically have a strong featureless baseline resonance between 1 and 4 ppm, underlying a distinct resonance at 1.3 ppm (Stuermer and Payne, 1976; Malcolm, 1990; Mcknight et al., 1997). This shape of the humic substance ^{1}HNMR (in the region 1–3 ppm) closely resembles the enhanced unresolved baseline in many of our nearshore surface-water samples (e.g., MAB944, Fig. 4). The contribution of humic substances to HMW DOC can be calculated by multiplying the unresolved baseline between 0.8 and 3 ppm, and between 3 and 4.6 ppm by the C/H ratios 2 and 1, respectively. These data are presented in Table 1. Based on these assumptions, humic substances only account for 19% of the carbon in MAB941, but account for >40% of the carbon in MAB943 and MAB947. The higher abundance of humic substances in MAB944 and MAB947 (for example) may reflect a relatively higher contribution of terrestrially derived HMW DOM to our inshore surface seawater samples. Higher contributions from humic substances are also observed for deep-ocean HMW DOM relative to surface ocean samples. These increased relative amounts of humic substances may simply reflect the presence of aged or highly degraded DOM.

In ^{1}HNMR spectra, terrestrial humic substances often show enhanced aromatic resonances in the region of 7 ppm. Aromatic resonances are present in some central MAB samples (e.g., MAB944) but not others (MAB941; Fig. 4). We can estimate the

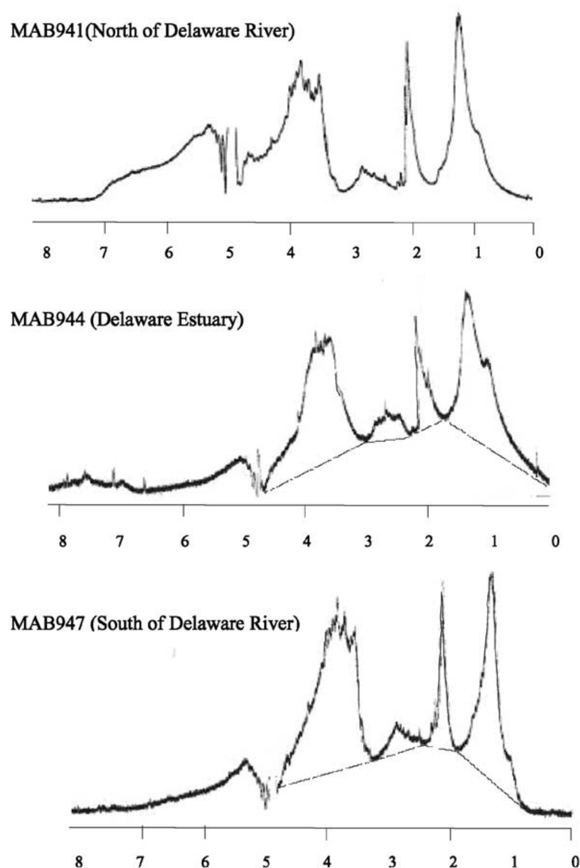

Fig. 4. Proton NMR spectra from three sites in the Central MAB. MAB941 was taken north of the Delaware River, MAB944 was isolated from the Delaware River, and MAB947 was isolated on the slope break, south of the river. Drawn in dotted lines are the baselines for each of the three major peaks: carbohydrates, lipids, and acetate. The areas beneath the dotted lines are identified as humic substances in the text.

amount of aromatic carbon present in MBA944 and MAB945 using the C/H ratio of 1.2 (mono-substituted benzene). Based on this ratio, between 6% and 12% of the carbon in these samples is present in aromatic compounds. If aromatic DOC is further substituted (as with lignins; Hedges and Ertel, 1982) or there is a significant amount of polycyclic aromatic carbon substitutents, the amount of aromatic carbon in our samples may be higher (up to 30% C assuming that four of the six carbon atoms are substituted). This is consistent with ^{13}C NMR data (Aluwihare, 1999) and is within the estimates of Mannino and Harvey (2000b), who found that as much as 40% of HMW DOC consisted of lignin phenols at a site near MAB944. In general, aromatic carbon does not represent a large fraction of HMW DOC despite the significant freshwater inputs to this region of the MAB. Selective removal of aromatic carbon within HMW DOM may occur preferentially, perhaps through photo-oxidation of colored dissolved organic matter.

The presence of DOM from freshwater and/or terrestrial sources in the MAB is supported by stable and radiocarbon data from the central MAB (Bauer and Druffel, 1998; Bauer et al., 2001). Surface-water DOM in this area is more enriched in radiocarbon (–109‰ to –37‰) and depleted in ^{13}C (−22.1‰ to –22.7‰) compared to their northern and southern counterparts. Based on their isotopic data, Bauer et al. (2001) conclude that ^{13}C-depleted, terrestrial and freshwater organic matter contributes the majority of the ^{14}C-enriched DOC to this region of the MAB. Bauer and Druffel (1998) estimate that in the central MAB, approximately 33% of the DOC in surface waters of the slope could be of terrestrial origin with $\Delta^{14}C$ values as enriched as +311‰. If the relatively higher abundance of baseline resonances in coastal samples is a result of the input of organic matter from terrestrial and freshwater sources, then up to 50% of the total C in some HMW DOM samples may be of non-marine origin.

3.2. Molecular level analyses

Hydrolysis of HMW DOM was accompanied by the appearance of sharp peaks in the ^{1}HNMR spectra, especially in the region of 2.0 ppm. This peak, assigned as bound acetate in non-hydrolyzed samples, could be transferred, following hydrolysis, by solvent extraction into an organic phase. Nuclear magnetic resonance spectroscopy of the organic phase shows a sharp singlet at 2.0 ppm (acetic acid). The presence of acetic acid in the organic layer was further confirmed by ion chromatography. In all cases, the amount of acetic acid determined by ion chromatography was between 70% and 80% of the acetate estimated by ^{1}HNMR spectroscopy. Resonances between 0.9

and 1.3 ppm (CH_3 and CH_2 from lipids) were absent from the ^{1}HNMR spectrum of the organic extract, showing that the contributions from other lipids to HMW DOM are small. In agreement with the NMR data for organic extracts, gas chromatography of the derivatized extracts did not show the presence of lipids. No ether-bound lipids were detected in the samples treated with BBr_3.

Other investigators have analyzed for the presence of lipids in HMW DOM (Mannino and Harvey, 1999). Their study showed that saturated fatty acids (C_{16} and C_{18}), uncharacterized neutral hydrocarbons (C_{17}–C_{29}), and sterols accounted for up to 2% of the total carbon in HMW DOM isolated from the Delaware River and Estuary. Low molecular weight fatty acids (C_9–C_{13}) also were detected in HMW DOM following thermochemolysis with tetramethyl ammonium hydroxide (TMAH) (Mannino and Harvey, 2000b). Our analytical procedures differed from these studies in that we discarded the free lipid fraction by pre-extracting the samples prior to hydrolysis and saponification. Our analyses target ether- or ester-bound lipids and do not include free lipids that are weakly associated with HMW DOM. In general, hydrolyzable lipids make up a very small fraction (<2% C, excluding acetate) of the total HMW DOM. Certainly, no increase in lipid content correlating with the increase in lipid resonances (both the peak at 1.3 ppm and the lipid baseline discussed in the NMR section) was observed for coastal samples. This finding suggests that increased lipid-baseline resonances do not arise from ester- or ether-bound lipids, and must be derived from other aliphatic components.

The mole fraction distributions of major neutral sugars in HMW DOM are shown in Table 2. All samples have the same suite of neutral

Table 2
The mole percent distribution of monosaccharides in HMW DOM

Sugars	**MAB941**	MAB943	**MAB944**	**a**MAB945	**MAB946**	MAB947
Rhamnose	**0.14**	0.14	**0.17**	0.15	**0.16**	0.15
Fucose	**0.13**	0.15	**0.15**	0.12	**0.12**	0.14
Arabinose	**0.05**	0.05	**0.07**	0.08	**0.07**	
Xylose	**0.12**	0.14	**0.14**	0.1	**0.14**	0.13
Mannose	**0.13**	0.11	**0.11**	0.16	**0.13**	0.11
Galactose	**0.17**	0.2	**0.14**	0.19	**0.14**	0.15
Glucose	**0.17**	0.13	**0.15**	0.08	**0.21**	0.12
2-Methyl rhamnose	**0.01**		**0.02**	0.01		0.02
Gluocuronic Acid	**0.04**	0.03	**0.02**	0.03	**0.1**	0.04
Galacturonic acid	**0.02**	0.03	**0.02**	0.05		0.03
N-Acetyl glucosamine	**0.02**	0.02	**0.01**	0.03		0.03
N-Acetyl galactosamine				0.01		0.01
	MAB961	MAB964	MAB965	MAB967	Average	Deviation
Rhamnose	0.2	**0.17**	0.14	**0.18**	**0.17**	0.02
Fucose	0.17	**0.2**	0.16	**0.16**	**0.16**	0.02
Arabinose	0.07	**0.09**	0.09	**0.09**	**0.08**	0.02
Xylose	0.11	**0.13**	0.11	**0.1**	**0.13**	0.02
Mannose	0.13	**0.12**	0.15	**0.17**	**0.14**	0.02
Galactose	0.21	**0.18**	0.23	**0.16**	**0.19**	0.03
Glucose	0.1	**0.12**	0.12	**0.14**	**0.14**	0.04

We were unable to detect any arabinose in MAB946; however, the carbon content of this sample was low and arabinose may have been present below the detection limit. Average refers to the average mole percent distribution of each neutral monosaccharide for the entire data set. In the case of MAB941, MAB943, MAB944, MAB945, MAB946 and MAB947, the mole percent distribution was re-calculated without uronic acids, amino sugars or methyl sugars in order to compute the average distribution for the data set. Deviation is +1 SD. Glucose distributions varied ±25% of their mean distribution, arabinose distributions varied ±20%, and all other sugars varied between ±13% and 15%. Replicate monosaccharide analyses varied ±5%.

monosaccharides, and on average, the relative distribution of neutral monosaccharides was similar among samples. Galactose was the most abundant monosaccharide (19±3 mol% total neutral sugars, Table 2), with glucose, mannose, xlyose, rhamnose, and fucose being only slightly less abundant and arabinose, the least abundant (8±2 mol%) neutral monosaccharide. Analysis of some of the samples (MAB941, MAB943, MAB944, MAB945, MAB946 and MAB947) for uronic acids and amino sugars showed that gluc- and galacturonic acids and amino sugars comprised <12% of the HMW monosaccharide C, and 2% of HMW DOC (see Table 2). Monosaccharide yields, as alditol acetates, were between 15% and 20% of HMW DOC in each surface sample. Deep-ocean samples showed lower monosaccharide yields, between 4% and 9% of total HMW carbon. This depth-associated decrease in monosaccharide yield is consistent with the ^{1}HNMR data showing a 50% decrease in carbohydrate resonances with depth. The monosaccharide yields from this study are comparable to other published data (6–20% of total HM WDOC in surface waters vs. 1.6–3.5% in deep water; McCarthy et al, 1996; Skoog and Benner, 1997). Polysaccharide data for HMW DOM isolated from the Delaware River and Estuary show comparable results to our coastal samples, between 7.5% and 19% of the HMW DOC (Mannino and Harvey, 2000a).

Data for neutral sugars in Table 2 are plotted in Fig. 5 to illustrate better the mole percent monosaccharide distribution. Despite the difference in monosaccharide yield between surface and deep waters, the mole fraction distribution of monosaccharides is remarkably similar for all samples and compares well to distributions observed by other investigators (Sakugawa and Handa, 1985; McCarthy et al., 1996, Aluwihare et al., 1997; Borch and Kirchman, 1997; Skoog and Benner, 1997). The higher concentration of monosaccharides in surface samples suggests that carbohydrates in HMW DOM have an upper ocean source. Aluwihare et al. (1997) have proposed that the major neutral monosaccharides, acetate, and some component of the lipid fraction are bound together in a common biomolecule as an acylated polysaccharide (APS). HMW DOM, similar in composition to APS, has been shown to be produced

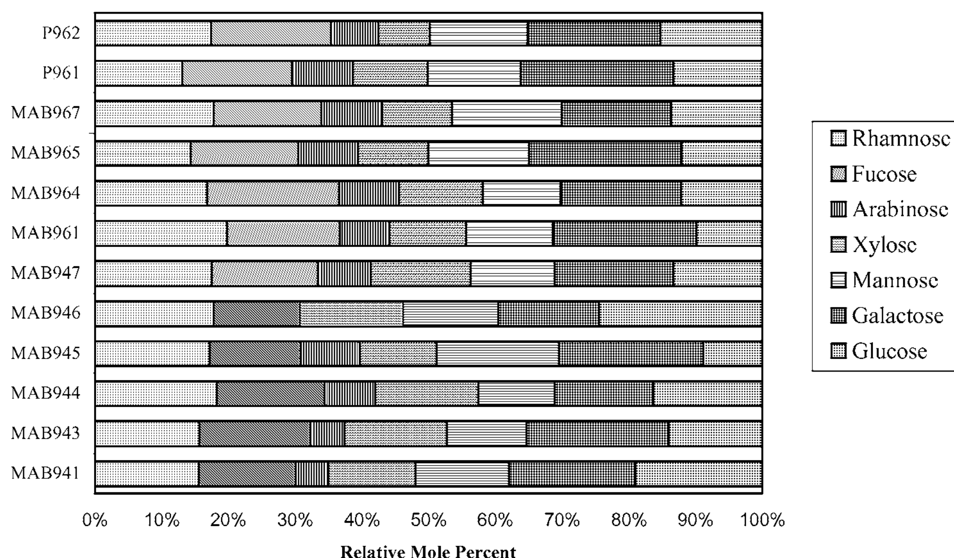

Fig. 5. Relative mole percent distribution of neutral monsaccharides in HMW DOM. Sample locations are noted in Table 1 except in the case of P961 (surface) and P962 (1600 m) which were isolated from the Central North Pacific (34.83°N, 123.15°W) and are shown here for comparison.

by algae in culture (Aluwihare et al., 1997; Aluwihare and Repeta, 1999), supporting an upper-ocean contribution directly from primary production. The APS in our deep-water samples could be supplied by a deep-ocean source (e.g., microbial production), or be transported to depth by advection or particle disassociation following production in surface waters.

Boon et al. (1998) analyzed the chemical composition of some of the MAB samples presented in this manuscript using direct temperature resolved mass spectrometry (DT-MS) following chemical ionization (NH_3) of the samples. Their data confirm the presence of the monosaccharides described above. In addition, these authors also interpret the presence of an acetamide peak in the their mass spectra to show the presence of acetylated amino sugars consistent with our identification of acetate in these samples.

The data in Fig. 5 visually demonstrate the similarity in monosaccharide composition of diverse samples. However, we were interested in determining whether there were any subtle compositional differences between our riverine-influenced coastal and open-ocean samples (e.g. MAB944 vs. MAB941), and whether differences in the composition of offshore samples (MAB941 vs. MAB943 vs. MAB947) could be distinguished as the shelf water flows southwestwards. We also were curious to determine whether there were any discernible differences between surface- and deep-water neutral monosaccharide distributions.

In order to investigate these relationships, we statistically compared the covariance in the mole fraction monosaccharides distribution of two samples. For example, in order to examine differences between freshwater influenced samples and open-ocean samples, we compared the monosaccharide distribution of MAB944 with MAB941, MAB943, and MAB947. We found that the monosaccharide distribution of the Delaware Estuary sample, MAB944, was similar to that of MAB941 (correlation coefficient of 0.8), MAB943 (0.8) and MAB947 (0.9) demonstrating that there are few discernible differences between estuarine and open-ocean samples. However, there appeared to be subtle differences between samples that were north or south of the Delaware River inputs. For example, the monosaccharide distribution in MAB941 (north of the Delaware River) was more closely related to MAB943 (0.85; east of the river) than MAB947 (0.8; south of the river); and MAB943 was more closely correlated with MAB947 (0.95) than MAB941 (0.85). In general, all open-ocean samples were very closely related (>0.7). Interestingly, MAB945 (mouth of the Delaware River) was not closely related to MAB944 (in the Delaware Estuary; 0.3), or open-ocean samples collected in 1994. The low correlation between these samples is consistent with measured differences in both the DOC concentration and the C/N ratio of HMW DOM at these two sites (Table 1).

In order to investigate the relationship between surface- and deep-water monosaccharide distributions, we compared MAB967 (750 m) to open-ocean surface samples (MAB941, MAB943, MAB946, MAB947 and MAB965). In all cases, correlation coefficients were $\leqslant 0.7$, indicating that there are differences between deep- and surface-ocean distributions. Deep-water monosaccharide distributions from sites in both the MAB and Pacific Ocean (Aluwihare, 1999), however, are well correlated (>0.85) with each other. Due to the increased relative abundance of fucose in MAB964, this deep sample was poorly correlated with all, surface (<0.4) and deep (0.5–0.6) samples.

Despite increased DOC concentrations on the MAB during the 1996 cruise relative to 1994 (Vlahos et al., 2002), monosaccharide distributions in coastal samples collected in 1994 (e.g., MAB943, MAB947, etc.) were very similar to the suite of samples collected in 1996 (MAB961, MAB965 etc.). In general, the similarity in monosaccharide distributions over spatial and temporal scales indicates that carbohydrates have a similar source in all areas of the MAB.

Hydrolysis of HMW DOM and subsequent monomer analyses showed quantitative differences in the yield of major biochemicals compared with NMR data. Neutral monosaccharides quantified as alditol acetates accounted for 15–20% of the total HMW DOC in surface waters compared with the 37–62% yield expected by NMR spectroscopy. Amino sugars and uronic acids of glucose and

galactose accounted for an additional 1–2% of the total HMW carbon, while lipid analysis showed only acetate as the major lipid in HMW DOM, accounting for approximately 5–8% of HMW DOC. Amino acids, recovered from the acid hydrolysis of HMW DOM accounted for a further 4% of the carbon (McCarthy et al., 1996; Aluwihare, 1999). Altogether, a maximum of 34% of the total carbon in surface-water HMW DOM can be accounted for by molecular level analyses.

3.3. Radiocarbon in HMW DOC and APS

The radiocarbon content in total HMW DOC and APS was determined for three samples from the southern MAB. The results are summarized in Table 3. Surface HMW DOC has a Δ^{14}C value of –10.2‰, intermediate between Δ^{14}C-DIC (59‰; Bauer, personal communication) and total Δ^{14}C-DOC (−32‰; Bauer, personal communication). The Δ^{14}C enrichment of coastal HMW DOC is consistent with the data of Guo et al. (1996), who found surface Δ^{14}C-HMW DOC values in the Chesapeake Bay to be -1 ± 14‰. Since the HMW DOC at MAB961 is substantially more enriched in radiocarbon than DOC from truly open-ocean surface waters (e.g., –238‰ for Sargasso Sea surface waters; Druffel et al.,1992), this site must receive an increased contribution from freshly produced organic matter (with a radiocarbon age close to 59‰), and/or a terrestrial/freshwater contribution (riverine endmember DOC is approximately 200‰, Raymond and Bauer, 2001).

Table 3
Radiocarbon values for different fractions of carbon isolated from the Southern Mid-Atlantic Bight

MAB 961	Δ^{14}C
DIC	59
Total DOC	−32
HMW DOC	−10
Rhamnose	49
Fucose	72
Xylose	92
MAB966	Δ^{14}C
DIC	−32
Total DOC	−414
HMW DOC	−375
Total Sugars	−120
MAB967	Δ^{14}C
DIC	−33
Total DOC	−405
HMW DOC	−255
Total Sugars	−59

MAB961 was isolated from surface waters near the mouth of the Chesapeake Bay, while MAB966 (300 m) and MAB967 (750 m) were isolated from deep waters on the slope of the southern MAB. See Table 1 for sampling locations. Values for DIC and DOC are from J. Bauer and E. Druffel (personal communication).

As discussed earlier, APS is a large fraction of the newly synthesized HMW DOC in surface waters and may contribute to the radiocarbon-enriched component in HMW DOC. We measured the radiocarbon content of monosaccharides released by the hydrolysis of HMW DOC to determine the Δ^{14}C value of APS. Radiocarbon values for rhamnose, fucose and xylose, from MAB961 HMW DOC average 71 ± 30‰ (Table 3), similar to Δ^{14}C-DIC, but are substantially enriched relative to the total HMW DOC fraction. The Δ^{14}C-HMW DOC value for MAB961 (10‰) in combination with the fact that neutral monosaccharides account for approximately 15% of the sample yield a Δ^{14}C value of −24‰ for the remaining 85% of HMW DOC in this sample. This value is similar to Δ^{14}C-DOC. Individual sugars have comparable Δ^{14}C values, consistent with our hypothesis that these neutral monosaccharides are part of a common macromolecule.

The enriched Δ^{14}C values for DOC and HMW DOC on the shelf contrast sharply with the depleted Δ^{14}C values of these components in deep waters of the slope. At sites MAB966 and MAB967 (300 and 750 m, respectively), radiocarbon values for DOC and HMW DOC average –410‰ (DOC; Bauer et al., 2001) and –314‰ (–255‰ to –375‰), respectively (Table 3). These Δ^{14}C values demonstrate that HMW DOC contains a radiocarbon-enriched component relative to total DOC. These results differ from the results of Guo et al. (1996), who found Δ^{14}C-HMW DOC values similar to our total DOC fraction (−403‰ at 750 m; Guo et al., 1996) at a site close to MAB966 and MAB967. However, Santschi et al.

(1995) found that the >10 kDa fraction of DOC was enriched in radiocarbon relative to the >1 kDa (or HMW DOC) fraction.

As shown in Table 3, the radiocarbon values for partially purified polysaccharides from MAB966 and MAB967 show significant enrichment relative to total HMW DOM, (–120‰ and −59‰). The large differences in $\Delta^{14}C$ values for polysaccharides between the two samples most likely reflect a difference in the extent of purification. HPLC analyses of the total monosaccharide fraction show peak asymmetries indicative of unresolved contaminants. Further HPLC purification of surface-water HMW DOM-derived monosaccharides (MAB961) shows the presence of such contaminants in the total monosaccharide fraction. Even small differences in the amount of DOC with a $\Delta^{14}C$ of –410‰ included in this fraction would result in large changes in the measured $\Delta^{14}C$ value.

The high concentrations of APS in surface water, and our preliminary radiocarbon data, suggest that APS is part of the recently synthesized fraction of HMW DOM. The radiocarbon enrichment of APS relative to total DOC persists into the deep ocean, consistent with either advective transport, or introduction by the dissolution of large, rapidly sinking, surface-derived particles.

4. Conclusions

Chemical characterization of HMW DOM isolated from several areas of the MAB in both 1994 and 1996 show that carbohydrates are abundant (27–62% of the total carbon based on ^{1}HNMR data) in this fraction of DOM in both the surface and deep ocean. Furthermore, ratios of the major biochemicals (carbohydrates:acetate:lipid carbon) in HMW DOM are highly conserved from one site to the next (79($\pm$4):8($\pm$2):13($\pm$4) for surface waters, and 73($\pm$6):5($\pm$1):21($\pm$6) for deep waters). Estuarine HMW DOM samples are comparable to open-ocean samples in the relative ratios of carbohydrate to acetate, but also have aromatic carbon and an enhanced lipid contribution. Although lipids appear to be abundant in HMW DOM based on NMR, we detect only acetate in high quantities following hydrolysis of ester and ether bonds.

Radiocarbon data for the southern MAB (MAB961) are consistent with a high input of marine primary production but do not rule out the possible input of DOM from terrestrial/freshwater sources as well. The radiocarbon data presented here also show that HMW DOM is substantially enriched (by over 100‰) in radiocarbon relative to the total DOM at the slope sites examined in this study. This enrichment indicates that HMW DOM contains a relatively higher fraction of 'fresh' material than the total DOM pool. Radiocarbon values of the polysaccharide fraction are even more enriched and show near-surface water $\Delta^{14}C$-DIC values. Thus polysaccharides are part of the 'fresh' DOM that is added to the surface DOC inventory as a result of primary production and persist even in the DOM isolated from 750 m depth.

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