

A major biopolymeric component to dissolved organic carbon in surface sea water

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Organic carbon dissolved in sea water is an important component of the global carbon cycle¹. Concentrations of dissolved organic carbon (DOC) in the ocean's surface mixed layer are at least twice those in the deep sea^{2,3}, because of the production of soluble carbon compounds by marine algae in the euphotic zone^{4,5}. But very little is known about the chemical composition of DOC, and the connection between photosynthetic production and DOC accumulation is not well understood^{6,7}. Here we report the chemical characterization of macromolecular DOC at several sites in the Atlantic and Pacific oceans. Neutral sugars, acetate and lipids show similar distributions, suggesting that these constituents are linked together in a common macromolecular structure. Chemical linkage patterns between the oligosaccharide portions of dissolved organic matter subjected to ultrafiltration are highly specific, with little variation between ocean basins. We show that laboratory culture experiments on the decomposition of algal exudate produce macromolecular organic matter with similar compositions and linkage characteristics. We propose that a significant fraction of DOC in sea surface water consists of structurally related and biosynthetically derived acyl oligosaccharides that persist after more labile organic matter has been degraded.

Between 25% and 35% of marine DOC is in a high-molecular-weight fraction that can be recovered by ultrafiltration or dialysis⁸⁻¹⁰.

Table 1 Sample location, DOC concentration, and the relative abundance of major biochemicals in UDOM

Sample* (date)	Location	DOC (μM)	Carbon (relative %)		
			Carbohydrate†	Acetate	Lipid
Atlantic Ocean					
Georges Bank(Mar. 93)					
GB-1	40° 30' N, 70° 45' W	80	81	11	4
GB-2	40° 58' N, 68° 54' W	85	76	9	14
Mid-Atlantic Bight (Apr. 94)					
MAB-1	40° 32' N, 72° 09' W	95	73	10	15
MAB-5	38° 56' N, 75° 05' W	116	84	6	10
MAB-6	37° 43' N, 75° 24' W	99	75	9	16
MAB-7	37° 11' N, 74° 16' W	97	77	10	14
Woods Hole (Jul. 94)					
WH-1	41° 32' N, 70° 31' W	102	86	10	4
WH-2‡	41° 32' N, 70° 31' W	102	77	15	7
Oosterschelde (Apr.-Jun. 94)§					
Os-1	51° 36' N, 04° 07' E	260	84	11	4
Pacific Ocean					
Scripps Pier (Jun. 93)					
SP-1	32° 52' N, 117° 18' W	ND‡	81	12	6
Peru Upwelling (Nov. 92)					
PU-1	11° 04' S, 78° 04' W	ND	81	13	6
Hawaii (Jan. 95)	19° 40' N, 156° W	ND	85	7	8
Average composition (all samples)			80 ± 4	10 ± 2	9 ± 4

* Samples from Georges Bank, the Mid-Atlantic Bight, Woods Hole, Scripps Pier, Hawaii and Oosterschelde were collected with either an Amicon DC-10 or Filtron ultrafiltration system fitted with a 1K membrane. Samples were collected in all-Teflon system with cartridge filtration at 0.2 μm . Water was processed immediately with collection. Concentrates were de-salted by dialysis with deionized (Milli-Q) water. Carbon was monitored in feed, retentate and permeate, and mass balances were \pm 15%. All samples were collected between 1–15 m. Water from the Peru Upwelling area was collected at 100 m, frozen, and returned to the laboratory where the >1K fraction was isolated by dialysis against deionized water using a cellulose membrane. † Carbohydrate, acetate and lipids were determined by ^1H NMR after normalization for differences in C/H (carbohydrates 1; acetate 0.67; lipids 2.5), and assuming 30% deoxysugar content. Values are expressed relative to the summed total. NMR spectra were acquired on a Bruker AC-300 (MHz) spectrometer in D_2O , using water suppression. ‡ >10K sample. § Composite sample collected between 25 April and 8 June. || Ref. 19. ¶ Not reported/determined.

Using these techniques, organic matter was sampled at sites in the Atlantic and eastern tropical Pacific oceans (Table 1). Proton NMR of ultrafiltered dissolved organic matter (UDOM) yields very similar spectra for all samples, with well resolved resonances from carbohydrates (5.5–5 p.p.m. (anomeric), 4.3–3.4 p.p.m. (CH), and 1.3 p.p.m. (CH_3); 55% total carbon), acetate (2.0 p.p.m.; 7% total carbon), and low-molecular-weight lipids (1.3 (CH_2) and 0.9 (CH_3) p.p.m.; 6% total carbon) (Fig. 1a). Carbohydrate, acetate and lipids determined by ^1H NMR are in good agreement with ^{13}C NMR data for Woods Hole sea water (L.I.A. and D.J.R., unpublished results) and for the North Pacific Ocean⁸. The abundance of major biochemicals in UDOM is relatively constant in all samples, and has an average carbohydrate/acetate/lipid carbon ratio of 8/1/1 (Table 1). Compositional similarities of UDOM are also evident in the monomer components of the carbohydrate. Monosaccharide analysis of hydrolysed UDOM in six samples spaced across the US Mid-Atlantic Bight, between Cape Cod and Cape Hatteras (WH-1, MAB 1–7), Hawaii, and in the eastern North Atlantic (Oosterschelde) show a fixed ratio of major neutral sugars (Fig. 2). Galactose (0.20 \pm 0.03 mol%) is the most abundant monosaccharide in the samples. Xylose (0.12 \pm 0.02), rhamnose (0.16 \pm 0.02), fucose (0.15 \pm 0.1), glucose (0.16 \pm 0.03) and mannose (0.13 \pm 0.03) are only slightly less abundant. Arabinose (0.07 \pm 0.02), the least abundant sugar, is 35% of the concentration of galactose. This complex distribution of neutral sugars is similar to UDOM neutral sugar distributions in the Gulf of Mexico, Sargasso Sea, and North Pacific Ocean (Fig. 2)^{11,12}. Data presented in Table 1 and Fig. 2 span a range of oceanographic settings, geographical areas and collection dates. DOC concentrations vary threefold, from 80 μM to 260 μM , at the collection sites.

Polysaccharide linkage analysis¹³ of UDOM recovered from the eastern and western North Atlantic and Pacific oceans was used to investigate the degree of polymerization and crosslinking of the carbohydrate fraction. Quantitatively, 40% of all sugars have terminal linkages, 40% are linked without branching, and 20% are

crosslinked with one branch point. The ratio of terminal/non-branched/branched linkages yields an average polymer consisting of five sugars (DP5), having a minimum (nominal) relative molecular mass of 1,000 (1K), has one branch point, and is acetylated at two sites (carbohydrate/acetate molar ratio of 5:2). Algal polysaccharides are synthesized with a wide range of linkages, including (1,2), (1,3), (1,4) and (1,6) for non-branched sugars, and (1,2,3), (1,2,4), (1,3,4), (1,3,6), (3,4,6) for branched sugars. However, within specific polysaccharides, a much smaller number of linkages are observed¹⁴. UDOM is likewise characterized by a limited number of specific linkages and is highly branched. A fraction of all sugars have terminal linkages (Table 2). Only a few sugars are branched, and with a single exception (3, 4, 6 galactose in the Oosterschelde sample), only 1, 3, 4 branching is observed. For non-branched sugars, major linkages include 1, 3 arabinose (100%), 1,3 rhamnose (50–80%), 1,3 fucose (90–100%), 1,4 xylose (30–80%), 1,2 mannose (40–100%), 1,3 glucose (50–100%) and 1,3 galactose (90–100%).

The relatively fixed ratio of major biochemicals and simple oligosaccharide linkage patterns observed in UDOM suggests that a large fraction of macromolecular DOC in surface sea water is not the complex, heterogeneous polymers expected from geopolymerization of simple biomolecules^{15,16}. Rather, our results show that most macromolecular organic matter is a mixture of structurally related acyl-oligosaccharides. Carbohydrate, acetate and lipid portions of UDOM are linked together in a common macromolecular structure, giving rise to the relatively fixed ratio of major biochemicals measured by our analyses. The carbohydrate portion of this macromolecule is also characterized by a distinctive and very heterogeneous distribution of seven neutral sugars. Processes responsible for the formation of these macromolecules must be operative on a global scale. Observed variations in monosaccharide distributions, and in the ratio of carbohydrate, acetate and lipid, may result from differences in UDOM sources and removal processes between our different sampling sites. Indeed, UDOM

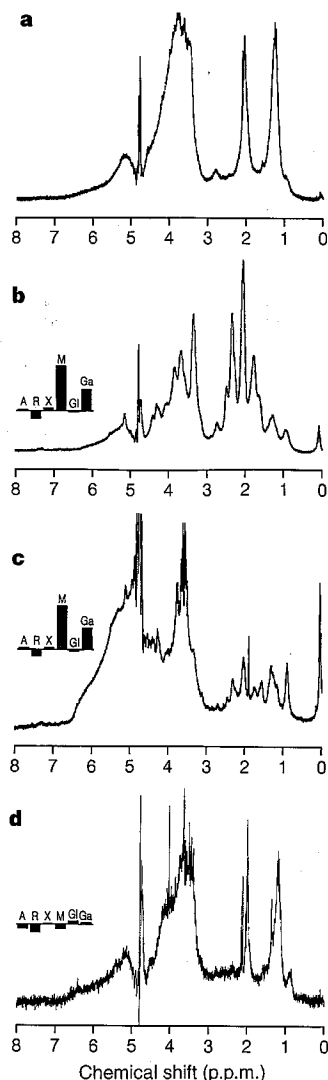


Figure 1 **a**, Proton NMR of UDOM from sample Woods Hole sea water showing major resonances from carbohydrate (5.5–5.0 (anomeric), 4.3–3.4 (CH) and 1.3 p.p.m. (CH_3 from deoxysugars), acetate (2.0 p.p.m.), and low-molecular-weight lipids (1.3 and 0.9 p.p.m.). These resonances from major biochemicals are superimposed on a featureless background of overlapping resonances that accounts for the remaining 30% carbon in our samples. Aromatic constituents (6.8–8.0 p.p.m.) contribute <1% of the total carbon in our samples, with slightly higher values observed in coastal seawater samples that are heavily influenced by freshwater inputs from nearby estuaries. Acetate and lipids are not extractable from UDOM (redissolved in water, pH 1) with organic solvents. However, after hydrolysis with 4 M HCl (90°C; 30 h), acetate and lipids are recoverable in the organic extract. The identification of acetate was confirmed by ion chromatography, which yielded 94% of the expected amount measured by NMR. **b**, NMR spectrum, collected immediately after algae were removed from the culture, showing major resonances from carbohydrates, proteins and lipids. Monosaccharide distributions (inset); where A is arabinose, R is rhamnose, X is xylose, M is mannose, Gl is glucose and Ga is galactose) are given as the difference between culture and average seawater data (Fig. 2) normalized to fucose. Positive deviations correspond to a higher monosaccharide relative abundance in the culture sample. **c**, NMR spectrum showing the selective degradation of dissolved proteins (2.8–1.5 p.p.m.) after 19 d incubation. **d**, After 37 d, the distribution of major biochemicals and monosaccharides is similar to UDOM collected from sea water (**a**). An incubated control sample of permeate (<1K DOC) without added algae shows no significant UDOM fraction, and a control sample poisoned (HgCl_2) after the removal of algae likewise shows no change with incubation from NMR spectrum **b**. Samples were dissolved in D_2O , and chemical shifts are expressed relative to water at 4.8 p.p.m.

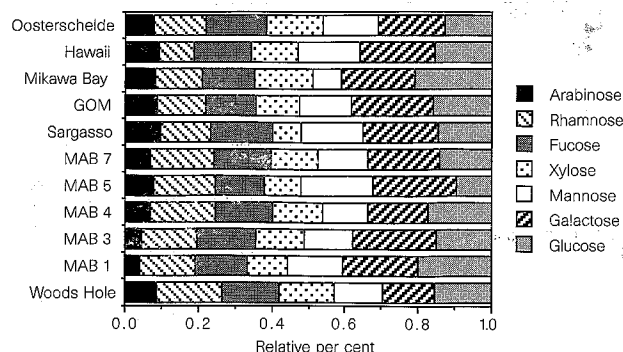


Figure 2 Normalized distribution of monosaccharides in UDOM samples collected from Woods Hole, the Mid-Atlantic Bight (MAB), Hawaii and Oosterschelde. Data from Gulf of Mexico (GOM), Sargasso Sea (Sargasso), and Mikawa Bay surface water are given for comparison^{11,12}. Gluco- and galactouronic acid and amino sugar (not shown) distributions are more variable than neutral sugars, but contribute <12% of total monosaccharides in our samples. Data were generated for unpurified samples using a variety of analytical methods for hydrolysis and monomer quantitation. The comparison therefore includes differences in the data due to the presence of other UDOM constituents and the inherent biases of the respect analytical techniques. Sample locations for MAB-3 and MAB-4 are 37°40' N, 73°25' W and 39°16.6' N, 75°21.2' W respectively.

carbohydrates include the same monosaccharides identified in transparent exopolysaccharides from marine snow, suggesting one possible removal mechanism¹⁷. Acyl-oligosaccharides in UDOM are probably formed by direct biosynthesis, and persist after the removal of more labile constituents.

To simulate the production and accumulation of the oligosaccharide in sea water, we conducted a simple laboratory experiment to monitor changes in UDOM during decomposition of algal exudate. Water (180 l) collected from 750 m depth (DOC, 45 μM) at a site offshore of Cape Hatteras, was filtered to remove particles, and ultrafiltered to remove UDOM. Nutrients (N, P, Si), trace metals, vitamins, and a seed culture of the marine diatom *Thalassiosira weissflogii* were added to the permeate (<1K) which was then incubated under artificial light at room temperature. After the algae reached stationary phase growth, and DOC values began to decline, the culture was filtered to remove algae and incubated again in the dark. After 19 days, 21 of Vineyard Sound sea water (pre-screened to 53 μM , Woods Hole, MA) was added to inoculate the sample with a natural consortium of bacteria and protozoa.

The sequence of NMR spectra in Fig. 1b–d traces the production of dissolved organic matter and the accumulation of acyl-oligosaccharides. At the onset of stationary phase growth, DOC concentrations had reached 430 μM . The NMR spectrum of UDOM (Fig. 1b) shows major contributions from polysaccharides (4.3–3.2 p.p.m.), proteins (2.8–1.5 p.p.m.) and minor amounts of lipid (1.5–0.8 p.p.m.). After 19 days of degradation, the amounts of major biochemicals changed considerably (Fig. 1c); sugars and lipids are relatively more abundant, but a significant protein fraction remains. Finally, 18 days after the addition of Vineyard Sound sea water (37 days total incubation), the NMR spectrum of UDOM (Fig. 1d) is similar to UDOM in sea water (Fig. 1a). The monosaccharide composition of our first incubation sample is dominated by mannose (42%) and galactose (26%), but all other major sugars measured in UDOM are present (Fig. 1b–d, inset). After 19 days, the relative abundances of mannose and galactose were unchanged (41% and 27%, respectively). After 37 days, mannose and galactose decreased to respectively 8% and 21% of the total sugar, and the ratio of major neutral sugars approached UDOM values. Oligosaccharide linkage patterns at the end of our experiment were highly specific and nearly identical to UDOM isolated from sea water (Table 1).

Table 2. Oligosaccharide linkages for culture and surface seawater UDOM

	Culture			Oosterschelde			Hawaii			Woods Hole		
	T*	B	NB	T	B	NB	T	B	NB	T	B	NB
Arabinose (f)†	100	0	0	100	0	0	100	0	0	100	0	0
Arabinose (p)‡	25	33 (1, 3, 4)	42 (1, 3)	31	0	68 (1, 3)	18	48 (1, 3, 4)	34 (1, 3)	55	0	45 (1, 3)
Rhamnose	22	4 (1, 3, 4)	43 (1, 3) 27 (1, 4)	54	9 (1, 3, 4)	22 (1, 3) 17 (1, 4)	60	27 (1, 3, 4)	10 (1, 3) 3 (1, 4)	78	0	11 (1, 3) 11 (1, 4)
Fucose	36	24 (1, 3, 4)	12 (1, 3) 26 (1, 4)	34	29 (1, 3, 4)	37 (1, 3)	47	4 (1, 3, 4)	43 (1, 3) 6 (1, 4)	72	9 (1, 3, 4)	19 (1, 3)
Xylose	50	24 (1, 3, 4)	3 (1, 3) 12 (1, 4) 10 (2, 3)	24	54 (1, 3, 4)	2 (1, 3) 6 (1, 4) 13 (2, 3)	28	30 (1, 3, 4)	9 (1, 3) 33 (1, 4)	79	10 (1, 3, 4)	4 (1, 3) 6 (1, 4)
Mannose	11	30 (1, 3, 4)	31 (1, 2) 6 (1, 4) 14 (1, 6) 9 (3, 6)	9	29 (1, 3, 4)	25 (1, 2) 6 (1, 4) 3 (1, 6) 25 (3, 6)	20	15 (1, 3, 4)	21 (1, 2)	19	25 (1, 3, 4)	16 (1, 2)
Galactose	33	29 (1, 3, 4)	10 (1, 3) 28 (1, 6)	9	24 (1, 3, 4) 55 (3, 4, 6)	6 (1, 3) 5 (1, 6)	25	36 (1, 3, 4)	29 (1, 3)	55	33 (1, 3, 4)	12 (1, 3)
Glucose	21	64 (1, 3, 4)	15 (1, 3)	18	56 (1, 3, 4)	25 (1, 3)	7	0	80 (1, 3) 14 (1, 2)	39	28 (1, 3, 4)	32 (1, 3)

Each entry shows the percentage per sugar of a particular linkage type, followed (for branched and non-branched types) by numbers in parentheses describing the linkages.

* T, terminal; B, branched; NB, non-branched.

† Furanose form.

‡ Pyranose form.

Previous studies of DOC composition and cycling have emphasized the role of geopolymerization reactions in the production of structurally complex, metabolically resistant organic matter. The recent report of bacterial porin-like proteins dissolved in sea water demonstrates the potential for a contribution from resistant biopolymers¹⁸. Our study extends this concept to a quantitatively significant fraction of DOC, where a family of closely related acyl-oligosaccharides formed by direct biosynthesis contributes up to 70% of UDOM and 20% of total DOC in surface sea water. The very similar monosaccharide composition of surface and deep-sea UDOM suggests that some fraction of these oligosaccharides may survive over long timescales and contribute a significant fraction of the total marine DOC^{12,18}. Further work detailing the chemical structure, potential sources, and metabolism of these oligosaccharides is needed to understand better the link between algal production of DOC, and its accumulation in surface sea water. □

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