Major biochemical composition of dissolved high molecular weight organic matter in seawater

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Abstract

Ultrafiltered dissolved organic matter (UDOM) was isolated from surface, oxygen minimum, and deep waters of three ocean basins and its elemental as well as molecular-level aldose and amino acid compositions were determined. Surface concentrations account for 23–33% of total dissolved organic carbon, and are a factor of 2–3 greater than those in deeper waters. Oceanic UDOM has an extremely characteristic organic composition, clearly distinct from other marine materials such as fresh plankton, sinking particles or humic substances. Polysaccharides appear to be the major reactive component of UDOM. They have a distinctive aldose distribution rich in galactose and deoxy sugar that is almost ubiquitous regardless of depth or location, suggesting that UDOM carbohydrate is dominated by a very similar suite of polysaccharide throughout the ocean. In contrast, amino acids account for a relatively minor component of both total UDOM and of its organic nitrogen component. Amino acid distributions are similar to those from unfractionated seawater, and are not preferentially remineralized.

In O_2 minimum and deep ocean water, ultrafiltered material accounts for 18–25% of total dissolved organic carbon. Compositions are nearly invariant in these subsurface isolates, suggesting that ultrafiltered material is stable and unreactive throughout the subsurface ocean. Taken together with large compositional differences between UDOM and sinking particles, this observation suggests that dynamic aggregation is probably not an important formation or removal process for UDOM in the deep ocean. Amino acid and especially carbohydrate concentrations are lower in deep UDOM, but the overall molecular-level compositions remain similar to those from surface waters. This molecular-level homogeneity suggests that the UDOM biopolymers reflected in amino acid and carbohydrate data persist relatively unaltered in the deep ocean.

1. Introduction

Oceanic dissolved organic matter (DOM) represents one of the largest dynamic reservoirs of reduced carbon on Earth. At $\sim 10^{18}$ g carbon, the DOM reservoir is of the same magnitude as all living vegetation on the Earth's continents, and larger than the atmospheric CO_2 pool (Hedges, 1992). In surface ocean waters, DOM forms the base of microbial food webs with up to 40% of all primary production estimated to cycle through the dissolved pool (Azam et al., 1983). Advection and remineralization of DOM may also play key roles in maintaining oxygen and nutrient balances in the upper ocean (Michaels et al., 1994).

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Although DOM cycling is fundamental to many ocean processes, its composition and geochemistry remain poorly understood. A principal reason is the difficulty of chemically characterizing this highly dilute (~ 1 mg/l) and complex mixture of organic compounds. Direct analysis of unconcentrated DOM is only feasible for a few compound classes, such as amino acids and lipids, which generally constitute less than 15% of total DOM in surface waters (e.g., Ittekkot, 1982; Williams and Druffel, 1988) and a far smaller fraction from deep water. More complete DOM characterization is thus presently dependent on sample concentration. Efficient, representative isolation of DOM from a 10^4 greater abundance of sea salts has, however, proved difficult.

Recently, tangential flow ultrafiltration has been applied as a promising new method to recover samples of oceanic DOM (Benner, 1991; Benner et al., 1992; Santschi et al., 1995). Ultrafiltration isolates organic compounds based primarily on size rather than chemistry, without subjecting the sample to the extremes of pH (2–13) needed for resin isolations (Thurman, 1986). The method clearly isolates a larger fraction of total oceanic DOM than has been routinely achieved. Using ultra-filters with a nominal pore size of ~ 1 nm (~ 1000 daltons), about 30–35% of total DOM can be repeatably isolated from surface ocean water (Benner et al., 1992). Bulk chemical characteristics of ultrafiltered DOM (UDOM) show that it is markedly richer in biologically labile molecules than XAD resin isolates, suggesting that it is a more representative sub-sample of the bulk DOM pool (Benner et al., 1992).

Ultrafiltered DOM has also recently been designated by some authors as representing ocean "colloids" because material recovered with the ultra-filter membranes nominally corresponds to a colloidal size of 1 nm–1 μm (e.g., Hunter and White, 1987; Kepkay, 1994). Although both this and the traditional "dissolved" definition of DOM are purely operational, there may be important functional differences between the two size classes (e.g., Johnson and Kepkay, 1991; Kepkay, 1994). For example, aggregation of colloidal DOM may provide a conduit from the dissolved to the particulate carbon pool. This "colloidal pumping" may eventually form sinking particles, with important implications for cycling of trace metals and radionuclides (Honeyman

and Santschi, 1989). Such distinct processes should be reflected in both the concentration and biochemical composition of UDOM. Thus, these samples allow characterization of not only a larger and more representative fraction of total DOM than previously possible, but also the investigation of a potentially distinct geochemistry of marine colloids.

We present here the first detailed study of the molecular-level composition of ultrafiltered material in the world's oceans. We have isolated UDOM from surface, oxygen minimum, and deep waters in three oligotrophic ocean sites: the central Gulf of Mexico, the North Pacific Ocean, and the Sargasso Sea. A primary goal of this work is to establish the basic biochemical composition of UDOM throughout the open ocean, with this sample set allowing comparison of both geographic and depth-related compositional differences from widely separate open ocean sites. We focus on the molecular-level aldose and amino acid compositions of these samples, in the context of overall UDOM concentrations and elemental ratios. UDOM is in many respects a compositionally unusual mixture, whose reactivity may be dominated by relatively few, higher-molecular-weight biopolymers.

2. Methods

UDOM samples were collected from surface (2–10 m), oxygen minimum (750–900 m) and deep (2400–4000 m) waters on three separate cruises from April 1991 to May 1992 in the North Pacific, Sargasso Sea and Gulf of Mexico (Fig. 1). Ultrafiltration was conducted aboard ship as has been described previously (Benner, 1991; Benner et al., 1992). Briefly, sequential casts of a 12 or 24 rosette of 10–30 l Niskin bottles were used to collect total samples of 200–1200 l. The entire filtration process typically required 16–18 hours for the larger (> 1000 l) samples, for which casts were staggered to minimize time standing on deck. After each cast, the rosette was drained into Nalgene polypropylene barrels and immediately gently filtered through 0.2 μm Nuclepore filter cartridges to remove particles and microorganisms. The samples were then concentrated to ~ 1 l aboard ship using either an Amicon DC-10 or DC-30 tangential flow ultrafiltration sys-

tem, with Amicon 1000-Dalton spiral-wound poly-sulfone filters (nominal pore size = 1 nm). UDOM concentrates were de-salted by diafiltration using purified freshwater (Milli-Q). Carbon mass balances were determined by analyzing subsamples of original seawater, UDOM concentrate and permeate for total dissolved organic carbon (DOC). Desalted UDOM concentrates were frozen aboard ship and transported to Port Aransas where they were further reduced to dry powders using a vacuum centrifuge. All subsequent chemical analyses were performed on powdered samples.

DOC measurements were made using a Shimadzu TOC-5000 analyzer as described previously (Benner and Strom, 1993). Atomic C/N ratios were determined with a precision of approximately $\pm 2\%$ with a Carlo Erba 1106 or 1108 CHN analyzer, after vapor phase HCl treatment (Hedges and Stern, 1984).

Molecular-level aldose compositions were determined as described in Cowie and Hedges (1984a). Briefly, UDOM powder corresponding to 0.5–2 mg of organic carbon was pre-treated with 72 wt.% sulfuric acid at room temperature for 2 hours, then hydrolyzed at 100°C for 3 hours in diluted (1.2 M) acid. The hydrolysate was neutralized with $BaSO_4$, and the $BaSO_4$ precipitate subsequently removed by centrifugation. The supernatant solution was then deionized by passing over a mixed-bed cation/anion exchange column. The sample was dried and redissolved in a pyridine/$LiClO_4$ solution, and then held at 60°C for 48 hours to equilibrate aldose anomers. Trimethylsilyl derivatives were formed by adding Regisil (bis-trimethylsilyl-trifluoroacetamide) directly to the equilibrated solution. Individual aldoses were quantified versus an adonitol internal standard by capillary gas chromatography (GC) with flame
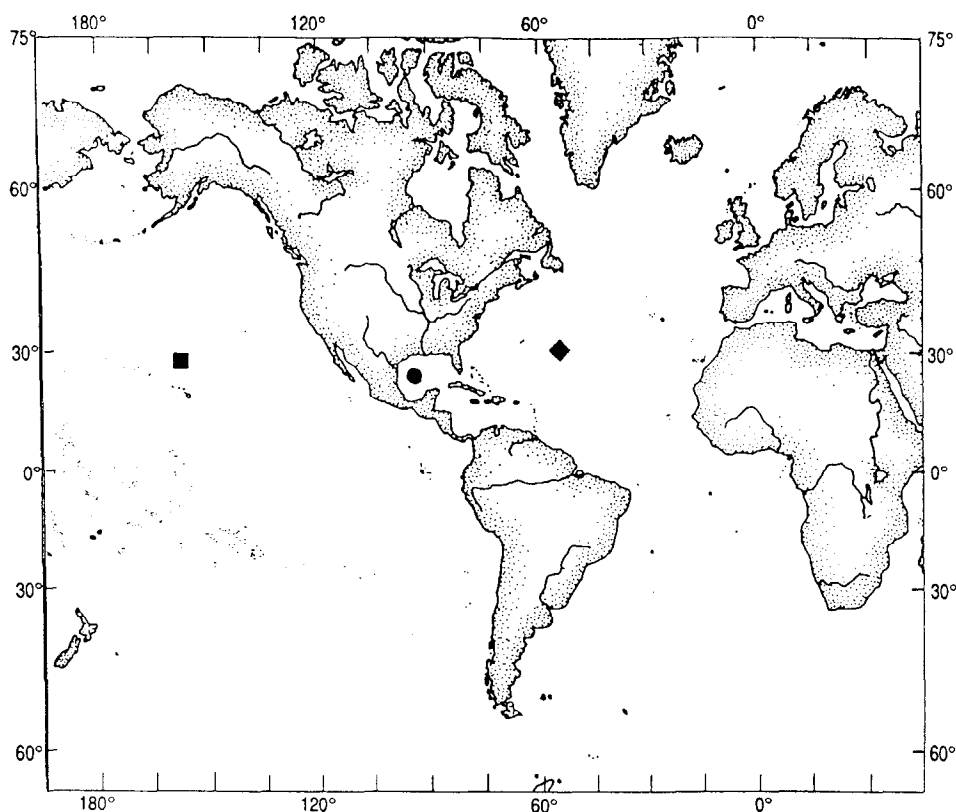

Fig. 1. UDOM sampling sites. North Pacific samples (■ = JGOFS Hawaii time series station, 22°45′N, 158°00′W) were collected in April 1991 from 10, 765 and 4000 m. Gulf of Mexico samples (● = 27°06′N, 95°31′W) were collected in August 1991 at 10 and 750 m. Sargasso Sea samples (◆ = JGOFS Bermuda time series station, 31°36′N, 64°23′W) were collected from 2, 900 and 2400 m in May 1992.

Table 1
UDOM recoveries and bulk characterization

Depth	North Pacific			Sargasso Sea			Gulf of Mexico	
(m)	10	765	4000	2	900	2400	10	750
DOC (μM)	82	38	41	72	47	46	95	48
UDOM (μM C)	27	10	9	16	9	11	29	12
%DOC	33	25	22	23	18	23	30	24
(C/N)$_a$	15.3	22.5	19.6	17.1	15.6	18.1	17.0	15.8

North Pacific data from Benner et al., 1992. Abbreviations: DOC = dissolved organic carbon; %DOC = percent of DOC > 1000 daltons recovered as UDOM; (C/N)$_a$ = atomic C/N ratio.

ionization detection. GC peaks were identified by comparison of retention times to authentic standards, and verified by GC-mass spectrometry. Typically at least one replicate analysis was included in each sample set. Average mean deviations for all UDOM replicates were ±7% for carbon normalized total

Table 2
UDOM amino acid yields

Depth (m)	North Pacific			Sargasso Sea			Gulf of Mexico	
	10	765	4000	2	900	2400	10	750
TDAA	278	51	109	178	122	89	262	150
THAA	10.2	5.16	12.1	14.7	14	6.5	8.7	13.1
%AA-N	17.4	14.2	28.3	28.7	21.9	11.5	16.6	24.3
Asp	11.4	9.1	10.4	10.2	9.9	13.0	12.2	10.9
Glu	17.8	11.9	14.5	15.2	17.9	17.0	15.8	17.7
Ser	9.7	6.2	11.7	9.1	6.9	7.6	11.3	7.4
Gly	15.5	20.9	18.1	15.1	15.5	19.8	17.3	16.2
Thr	6.6	3.9	4.0	7.5	5.2	5.2	8.5	5.4
Ala	13.0	14.9	10.1	15.8	14.3	17.7	15.6	12.4
Tyr	1.6	1.9	3.8	1.9	2.2	1.7	2.4	2.2
Met	1.7	0.0	2.5	0.1	0.9	0.0	0.5	1.2
Val	4.8	1.9	2.0	4.1	4.7	3.3	2.8	4.0
Phe	2.1	0.8	1.3	2.6	2.7	2.0	2.0	2.3
Ile	3.1	5.2	6.1	4.3	3.9	3.2	2.1	2.8
Leu	4.5	5.1	0.9	4.7	5.1	3.2	2.8	4.7
His	0.0	1.5	2.7	1.0	1.6	0.7	nd	1.1
Arg	4.3	2.0	4.5	5.6	7.5	4.5	3.0	7.3
Lys	2.4	10.1	4.7	nd	nd	nd	1.8	2.5
β-Ala	1.3	4.3	1.8	1.1	1.4	1.2	1.8	1.3
γ-Aba	0.1	0.3	0.8	1.7	0.0	nd	0.2	0.1
Aba	0.3	nd	nd	nd	0.2	nd	nd	0.1
Orn	nd	nd	nd	nd	nd	nd	nd	0.4

Total dissolved amino acids (TDAA) is the nM concentration of individual amino acids in the UDOM size fraction; total hydrolyzable amino acid yield (THAA) is the amino acid content of UDOM itself, expressed as mg amino acid per 100 mg OC; percent amino acid nitrogen (%AA-N) is on a weight basis. Individual amino acids are mole percentages of total yield. Compound abbreviations: Asp = aspartic acid; Glu = glutamic acid; Gly = glycine; Thr = threonine; Ala = alanine; Try = tyrosine; Met = methionine; Val = valine; Phe = phenylalanine; Ile = isoleucine; Leu = leucine; His = histidine; Arg = arganine; Lys = lysine; Aba = α-aminobutyric acid; Orn = ornithine. It should be noted that these values, as well as literature values discussed in the text, were obtained using conventional wet chemical hydrolysis which has recently been shown to yield lower concentrations than a vapor phase hydrolysis technique in some samples (Keil and Kirchman, 1991).
nd = not detected.

aldose yields, and ranged from ±5–10 wt.% for individual sugars, except xylose which had higher variability (±14%). These values are similar to those reported by Cowie and Hedges (1984a).

Total hydrolyzable amino acids were quantified using charge-matched recovery standards following the method of Cowie and Hedges (1992a). After the recovery standard mixture was added, a UDOM sample corresponding to approximately 0.2 mg organic carbon was hydrolyzed in 6 M HCl under N_2 (150°C for 70 min). Solids were removed by centrifugation and subsequent filtration, and the hydrolysate was evaporated to dryness in a vacuum centrifuge. The residue was redissolved in 0.8 N boric acid buffer at pH 10.5, and the solution loaded into a reverse-phase high pressure liquid chromatograph system equipped with an autoinjector. Fluorescent o-phthaldialdehyde derivatives were formed by the autoinjector immediately before injection, and individual amino acid yields were quantified relative to the appropriate charge-matched standard. Analytical precision for this procedure is ±10% or less for

yields of individual amino acids (Cowie and Hedges, 1992a).

3. Results

UDOM concentrations in all the analyzed seawater samples ranged from 9 to 29 μM C (Table 1), accounting for 18–33% of total DOC. Surface concentrations were consistently higher than O_2 minimum and deep water values, by approximately a factor of three in the North Pacific and Gulf of Mexico, and by approximately a factor of two in the Sargasso Sea. In addition, a larger *percentage* of total DOC was always present as UDOM in surface waters, consistent with the previous observation that the size spectrum of oceanic DOM is weighted toward smaller material in deeper waters (Benner et al., 1992).

Atomic C/N ratios of UDOM samples ranged from 13.4 to 22.5 (Table 1), with little consistent trend between surface and deeper waters. We had
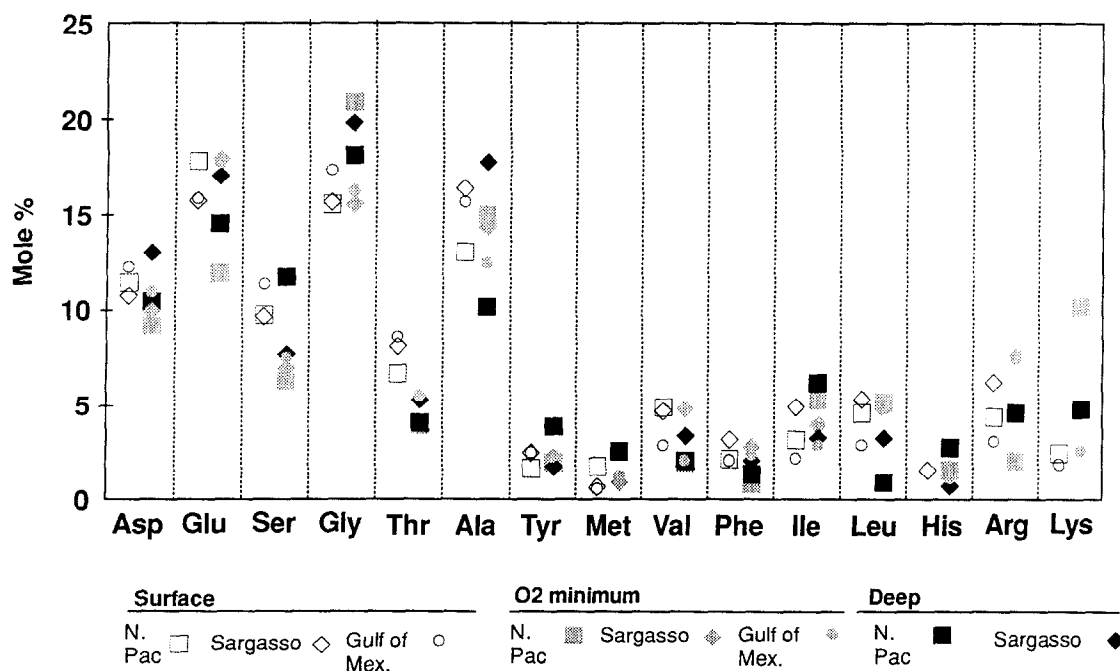

Fig. 2. UDOM amino acid composition. Mole% values for individual amino acids. Amino acids are arranged according to functionality (from left to right): acidic, neutral and basic. Data for each amino acid are grouped according to surface (*open symbols*) and subsurface samples (oxygen minimum and deep = *filled symbols*). *Abbreviations* are as in Table 3.

observed previously at the North Pacific station a substantial increase in $(C/N)_a$ between a surface value of 15.3, and O_2 minimum value of 22.5 (Benner et al., 1992). At the Sargasso Sea and Gulf of Mexico stations, however, there was much less difference between surface and O_2 minimum or deep water $(C/N)_a$ values (Table 1). In general, most UDOM isolates had similar $(C/N)_a$ ratios near 17. UDOM from surface ocean was only slightly enriched in nitrogen (average $(C/N)_a = 16.5$) compared to deep isolates [average $(C/N)_a = 18.3$]. Seawater DOM is clearly depleted in dissolved organic nitrogen relative to the Redfield value of about 7 for "average" marine plankton, yet it is distinctly enriched in N relative to dissolved marine humic substances collected on XAD resins, for which $(C/N)_a$ values fall in the range of 30–50 (Hedges et al., 1992).

Total hydrolyzable amino acid yields from the UDOM samples ranged from 5 to 14 mg amino acid per 100 mg OC (Table 2). The range of amino acid yield in surface waters (8.7–14.7 mg/100 mg OC) is similar to that in deep ocean UDOM (5.6–14.0 mg/100 mg OC). The percentage of total organic nitrogen composed of amino acids (%AA-N) ranged from 11.5 to 28.7 wt.% (Table 2), and also showed no consistent relationship to sample depth.

In all samples 65–80 mole% of amino acids are accounted for by six compounds: aspartic and glu-

tamic acid, serine, glycine, threonine, and alanine (Table 2; Fig. 2). Although the amino acid composition varied through the water column at each sample site, no general correlation between depth and composition held across the data set. Overall, the average amino acid composition of UDOM from all three ocean basins was remarkably homogeneous; the 2% standard deviation in the average mole% of the individual compounds was comparable to analytical variability.

Total neutral sugar yields averaged 27 mg aldose/100 mg OC from surface UDOM, ranging from 31 in the North Pacific to 25 in the Gulf of Mexico and Sargasso Sea (Table 3). In O_2 minimum and deep waters, aldose yields were three- to five-fold lower than in shallow water (4.9–8.6 mg/100 mg OC). There was no consistent difference in aldose yields between oxygen minimum and deep water UDOM.

For molecular-level aldoses, in general all UDOM samples yielded comparable mole percentages of galactose, glucose, mannose, fucose, rhamnose and xylose (Table 3; Fig. 3), which together made up over 90% of aldoses. Lyxose was always a minor constituent, and in any case may be an epimerization product of xylose formed during hydrolysis (Cowie and Hedges, 1984a). Ribose was always near or below detection limits. Despite the lack of dramatic compositional differences between depths, all sam-

Table 3
UDOM aldose yields

Depth (m)	North Pacific			Sargasso Sea			Gulf of Mexico	
	10	765	4000	2	900	2400	10	750
TCH$_2$O	31	5.7	4.9	25	6.0	8.6	25	5.7
Lyx	1.3	1.2	2.0	1.1	1.1	1.5	2.7	1.8
Ara	8.9	8.7	7.1	8.9	6.9	9.9	10	11
Rib	nd	1.6	11.6	0.5	1.1	1.8	nd	2.7
Rha	12.3	13.5	9.3	13.2	18.6	17.2	14.4	12.9
Fuc	16.4	18.3	15.6	15.1	18.1	18.8	17.3	18.5
Xyl	12.2	10.7	5.2	13.1	7.7	10.5	9.6	9.2
Man	12.9	7.7	12.9	13.0	11.3	9.8	14.1	10.8
Gal	19.1	9.7	10.2	20.4	14.2	13.1	18.6	13.9
Glc	16.9	28.6	26.0	14.7	21.0	17.3	13.3	19.6

Total aldose yield (TCH$_2$O) is expressed in mg aldose per 100 mg UDOM OC. Individual monomers are given as mole percentages of total yield. Abbreviations: Lyx = lyxose; Ara = arabinose; Rib = ribose; Rha = rhamnose; Fuc = fucose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.
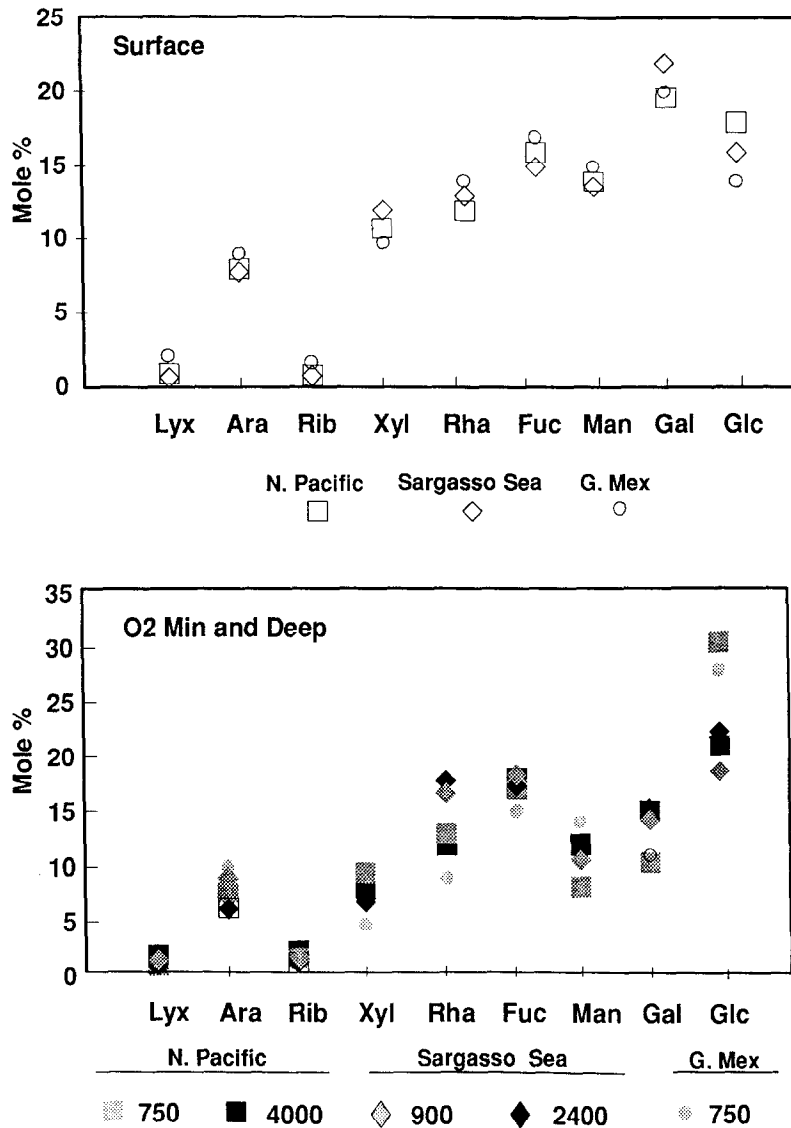
Fig. 3. UDOM aldose composition. Mole% values for individual aldoses, for surface and subsurface UDOM. Aldoses are arranged according to carbon number and functionality (from left to right): pentoses, deoxy sugars and hexoses. *Abbreviations* are as in Table 2.

ples can be clearly distinguished as either "surface" (< 10 m) or "subsurface" (> 750 m) based on consistent differences in some aldoses. The most pronounced difference was the relative abundance of galactose versus glucose. Whereas galactose was the most abundant sugar in surface waters, glucose predominated at depth. Within each depth range (surface or deep) neutral sugar compositions were extremely

uniform, and thus can be described by average molar percentages. In surface samples, galactose comprised 19–20% of total aldose, followed by smaller amounts of glucose, mannose, fucose, and rhamnose (13–17%). Xylose and arabinose made up 9–13% each, with less than 3% lyxose. In subsurface samples, glucose accounted for 20–29% of total aldose. Rhamnose and fucose were generally next most

abundant (9–19%), followed by galactose, mannose, xylose and arabinose (5–14%). Lyxose and ribose remained minor constituents at depth.

4. Discussion

Large-volume tangential flow ultrafiltration is a relatively new tool in marine organic geochemistry. Although the technique itself is not new, common application to the study of oceanic samples has begun only recently (Benner et al., 1992). Operationally defined, UDOM spans the region between particulate and truly dissolved organic matter. It is not yet clear to what degree UDOM is functionally representative of either, or whether it has a distinct "colloidal" composition and geochemistry. Comparison of UDOM composition to the traditionally studied water-column organic carbon pools of biomass, sinking particles, and DOC provides a geochemical context in which to examine UDOM composition.

4.1. Amino acids in UDOM

4.1.1. Dissolved amino acid concentrations in UDOM size fraction

Total dissolved amino acid concentrations (TDAA) in the UDOM size fraction are similar in range to those measured previously for hydrolysates of whole seawater samples. TDAA for a given sample can be approximated by dividing the amino acid content of the UDOM isolate by the total water volume filtered. TDAA for surface UDOM ranges from 178 nM (Sargasso Sea) to 278 nM (North Pacific; Table 3). Atlantic and Pacific deep ocean values are both near 100 nM, while oxygen minimum values range from 51 nM in the North Pacific to 150 nM in the Gulf of Mexico. In comparison, whole seawater TDAA for surface non-bloom environments have generally been reported from 300 to 1000 nM (e.g., Lee and Bada, 1975, 1977; Ittekkot, 1982; Henrichs and Williams, 1985; Hubberten et al., 1994), and a more constant 150–200 nM in the deep ocean (Lee and Bada, 1975, 1977; Hubberten et al., 1994). Since UDOM represents 20–30% of total DOM, comparison suggests that at least with respect to amino acid concentrations, UDOM isolates are representative of total DOM.

Between surface and deep waters there is a consistent 40–60% decrease in UDOM amino acid concentrations. However, these changes are generally proportional to changes in total DOC. There is no indication that amino acids are preferentially utilized in surface UDOM, as might be expected of a nitrogen-rich and labile compound class. Instead, in both surface and deep waters amino acids make up a small but relatively consistent ~ 1% of DOC and ~ 4% of UDOM organic carbon. In spite of a range of absolute concentrations, geographic variations (Table 3) also largely disappear when amino acid concentrations are normalized to DOC. For example, surface Sargasso Sea UDOM-AA in particular is much lower than either the North Pacific or Gulf of Mexico concentrations (Table 3), but as a percentage of DOC the variation is small (1% of DOC in surface Sargasso Sea vs. 1.4% of DOC in the North Pacific).

4.1.2. Proportion of amino acid nitrogen in UDOM

Another way to look at UDOM is as a distinct substance instead of a sample of the DOM pool, analogous to particulate, colloidal, or humic fractions. Although the total nitrogen content of UDOM (C/N$_a$ ratio ~ 17) is much higher than that of marine humic fractions (C/N$_a$ ~ 30–50; Meyers-Schulte and Hedges, 1986), it is substantially depleted in nitrogen relative to Redfield ratios. In terms of amino acid content, UDOM is also substantially depleted relative to marine particles or biomass. Fresh marine plankton, sediment trap materials and even surficial sediments give total amino acid yields typically within the range of 50 to 100 mg AA/100 mg OC, with amino acids accounting for 40–80% of total nitrogen (Cowie and Hedges, 1992b,1994). In UDOM, which at least in surface waters must have a considerable fresh component, amino acid yields and percent amino acid nitrogen are less than half these values (Fig. 4). UDOM thus is clearly distinct in its organic nitrogen composition from fresh marine biomass and sinking particles. Since the larger part is not amino acid, UDOM must be highly enriched in some other type of nitrogenous material.

4.1.3. Molecular-level amino acid distributions

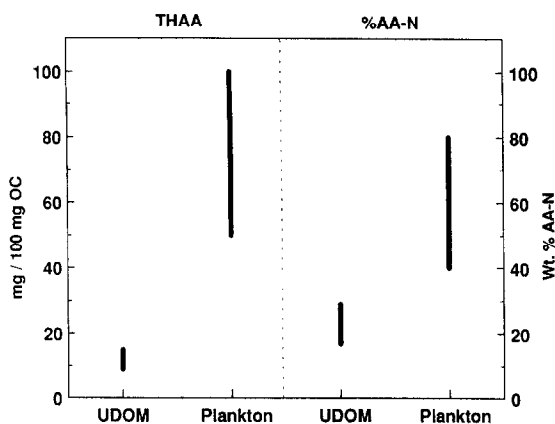The almost invariant molecular-level amino acid composition of UDOM (Fig. 2) is similar to that of

Fig. 4. THAA and %AA-N ranges: UDOM vs. phytoplankton. Range of total hydrolyzable amino acids (THAA: mg amino acids per 100 mg OC) and %AA-N values in UDOM from all depths compared with ranges from phytoplankton and bacteria (Cowie and Hedges, 1984a,b; Cowie, 1990). *Abbreviations* are as in Table 3.

unfractionated DOM (e.g., Lee and Bada, 1975; Henrichs and Williams, 1985; Coffin, 1989; Hubberten et al., 1994), as well as particles and biomass (Cowie and Hedges, 1992b). Serine and threonine appear to have small elevations in surface UDOM relative to deep samples (Fig. 2), but overall there is comparable variability among surface and deep water compositions. Thus, there appears to be little compositional difference between amino acids within different size fractions of oceanic DOM. Similar amino acid compositional uniformity has also been reported among different DOM size classes in an estuary (Coffin, 1989).

4.1.4. Non-protein amino acids

Two non-protein amino acids, β-alanine (β-ala) and γ-aminobutyric acid (γ-aba) are present in almost all UDOM samples. Non-protein amino acids are produced from the breakdown of specific protein amino acids, and their presence has generally been used as an indicator of organic matter aging or diagenesis (e.g., Cole and Lee, 1986; Cowie and Hedges, 1994). Dissolved non-protein amino acids have rarely been measured in seawater. β-ala and γ-aba make up a small, but relatively constant 1–3 mole% of UDOM amino acids from both surface and deep water samples (Table 3). In comparison, Henrichs and Williams (1985) tentatively identified β-ala

in surface water off the coast of Baja California, and found it to account for 1–5% of total dissolved amino acids in unfractionated subsurface water. The additional non-protein amino acids α-aminobutryic acid (α-Aba) and ornithine (Orn), also found in sediments, were not detected in most UDOM samples. Although it is doubtful that concentration-age relationships observed in sediments would be meaningful in the dissolved phase, the generally similar concentrations of non-protein amino acid content in both surface and deep UDOM is still surprising given the consistent increases in β-ala + γ-aba concentrations with advancing age and degradation in organic matter from many other environments (Cowie and Hedges, 1994). Most likely non-protein amino acids simply do not accumulate in UDOM, or else proteinaceous material in UDOM would have to be of similar age and extent of degradation in both surface and abyssal waters.

4.2. UDOM carbohydrate

Hydrolyzable aldoses together make up about 10–15% of organic carbon in surface UDOM. Although this is comparable to many previous estimates of "total" carbohydrate in unfractionated DOM (e.g., Burney et al., 1979; Ittekkot, 1982; Sakugawa and Handa, 1983), such a comparison is more questionable than for amino acids due to wide variability of carbohydrate yield with hydrolysis and detection method (Pakulski and Benner, 1994; Bergamaschi, 1995). In addition, since colorimetric detection methods (such as MBTH) used in most previous studies of "total" marine carbohydrate are non-specific, they can measure charged sugar residues in addition to aldoses (Pakulski and Benner, 1992).

While comparison of different studies is problematic, UDOM analysis by different methods suggests that total UDOM carbohydrate is substantially larger than indicated by aldoses alone. In addition to molecular-level aldoses, MBTH and ^{13}C NMR have been used to characterize the surface North Pacific UDOM sample. ^{13}C NMR is a valuable addition because it provides an upper estimate of the carbohydrate content in a sample without the need for any hydrolysis or molecular-level quantification. The MBTH (charged sugar + aldose) estimate (Pakulski and Benner, 1992) is about double aldose alone, and

the NMR estimate is about four times higher (Benner et al., 1992). Both ^{13}C NMR and ^{1}H NMR analysis of surface UDOM samples from other sites is consistent with this result, indicating that a much larger fraction (40–60%) of UDOM is carbohydrate-like material (Benner et al., 1992; McCarthy et al., 1993; Aluwihare et al., submitted). While each method has uncertainty, the trend to larger estimates of carbohydrate-like material with increasingly broad-based methods suggest the possibility that (1) hydrolysis resistance and (2) substantial charged sugars content may be major characteristics of UDOM polysaccharides.

4.2.1. Molecular-level aldose composition

UDOM aldose composition (Fig. 3) is a departure from previous molecular-level results for seawater DOM. Analysis of unfractionated seawater has indicated that glucose and other hexoses dominate aldose distributions (Ittekkot et al., 1981; Sakugawa and Handa, 1983; Sakugawa and Handa, 1985a,b), in contrast to the consistent galactose and deoxy-rich distributions of UDOM. Moreover, separations of carbohydrates from bulk seawater have shown that a mixture of distinct sugars is generally present (Sakugawa and Handa, 1983), and that the abundance of different sugars can vary both with time and location (Ittekkot et al., 1981; Sakugawa and Handa, 1985a). UDOM composition, however, remains very similar at widely separate open ocean sites and depths suggesting compositional homogeneity, distinct from unfractionated DOM carbohydrate.

4.3. Potential contamination from living cells

Both amino acid and carbohydrate results indicate little or no contamination from rupture of living cells in the first stages of the filtration process, a particular concern in particle-rich surface waters. If cell lysis accounted for a significant fraction of material isolated, UDOM should resemble the biochemical makeup of phytoplankton cellular contents. In addition, these components should be enriched almost exclusively in surface UDOM. However, UDOM isolates do not resemble phytoplankton cell contents, and there are no large compositional differences with depth. For example, amino acids make up 50–80%
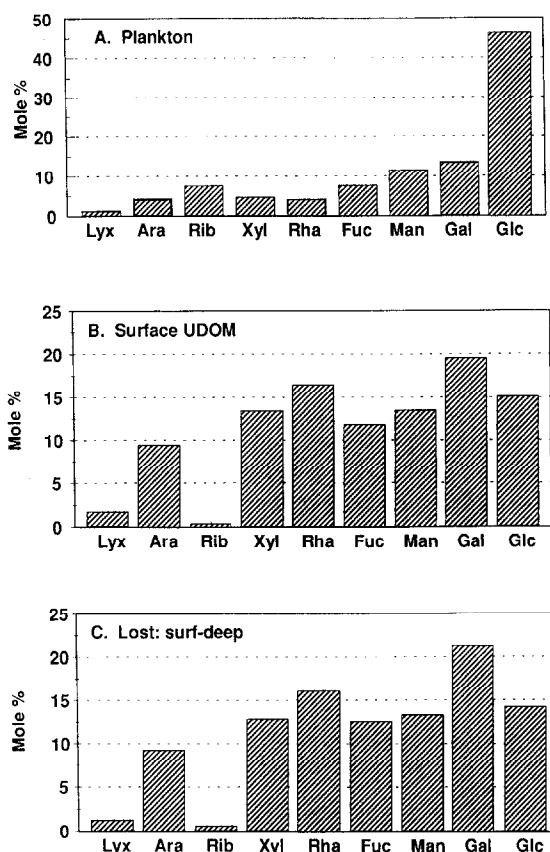

Fig. 5. Comparative UDOM aldose distributions.
A. UDOM average surface aldose composition. Abbreviations are as in Fig. 3.
B. Plankton aldose composition is an average from bulk net phytoplankton tows analyzed by G. Cowie (Cowie and Hedges, 1984a,b; Cowie, 1990).
C. Average composition of UDOM aldose removed between surface and oxygen minimum waters, calculated as described in the text.

of nitrogen in phytoplankton cell contents, yet both total amino acids and percent amino acid nitrogen are low in UDOM. Similarly, the aldose composition of phytoplankton cellular contents are highly enriched in glucose (Fig. 5A, B; Cowie and Hedges, 1996) versus UDOM which has relatively low glucose content. Although bacterial cells would not be expected to be lysed, aldose compositions also do not indicate contamination from bacterial sources; bacterial carbohydrates are characterized by unusually high ribose content (10–60 mole%; Cowie and

Hedges, 1984b), while UDOM aldose has only trace or undetectable ribose levels.

4.4. UDOM sources

Possible sources of surface DOM are numerous and include direct leakage of organic compounds by phytoplankton (Williams, 1981), release by zooplankton grazing (Jumars et al., 1989) and degradation of sinking particles (Wakeham and Lee, 1993). The elevated surface concentrations of total UDOM, as well as both aldoses and amino acids (Tables 2 and 3) indicate a local shallow water origin, but reveal little about specific pathways. Although molecular-level amino acid compositions are too similar in most organisms to provide source information (e.g., Sigleo et al., 1983; Cowie and Hedges, 1992b), the distinct monomer compositions that characterize many polysaccharides can be useful in narrowing potential sources.

UDOM carbohydrate is clearly distinct from fresh detrital material. Phytoplankton biomass has a relatively predictable aldose composition that is dominated by glucose and the other hexoses (Fig. 5A; Cowie and Hedges, 1984b), markedly different from the glucose-poor, galactose and deoxy-sugar rich composition of UDOM (Fig. 5B). Within the different carbohydrate classes, planktonic storage carbohydrates are predominately laminarans and other glucose polymers (Painter, 1983), and thus are unlikely as major sources. Structural polysaccharides from algal cells, however, are more likely to be important sources. They are both high molecular weight as well as more resistant to degradation, and some specific classes (e.g., the galactans) would provide a major galactose signal (De Leeuw and Largeau, 1993). Analysis of whole algal cell walls have also yielded aldose compositions similar to UDOM. Diatom cell wall carbohydrates, for example, contain heteropolysaccharides enriched in galactose, mannose, and rhamnose (Hecky et al., 1973; Haug and Myklestad, 1976; Cowie and Hedges, 1996). Bacteria are also an important potential source. As noted previously, bacterial aldose distributions are often characterized by high ribose content (Cowie and Hedges, 1984b), and thus the trace ribose in UDOM suggests that bacteria may be a less important source for aldoses. Nevertheless, bacterial biopolymers, such as

lipopolysaccharide and mureins, contain a wide variety of non-aldose monosaccharides (De Leeuw and Largeau, 1993), and these, as well as bacterial exopolysaccharide, may help account for the greater carbohydrate concentrations suggested by MBTH and NMR analyses.

Both physical and biological mechanisms may act to concentrate these heteropolysaccharides in UDOM. Solubility may have a role, since large heteropolysaccharides tend to be less soluble than smaller glucans. Preferential remineralization of glucose-rich polymers is likely as glucose is the most rapidly utilized aldose in both marine sediments and sinking particles (e.g., Cowie and Hedges, 1984b; Hernes et al., 1996). In the surface ocean glucose is by far the most abundant free aldose (e.g., Mopper et al., 1980; Ittekkot et al., 1981). There is also growing evidence from diverse environments that structural carbohydrates can be preferentially preserved. In sediment trap samples (Hernes et al., 1996) and sediments from the Peru margin (Bergamaschi, 1995) carbohydrate with an aldose distribution similar to cell wall polysaccharide (as well as UDOM) is concentrated during degradation.

4.5. UDOM reactivity

The sharp decreases in concentration of UDOM with depth (Fig. 6) to constant subsurface concentrations ($9–12$ μM) suggest that about two thirds of the surface UDOM is actively cycled, supporting recent evidence that higher molecular weight DOM is an important substrate for both bacteria (e.g., Amon and Benner, 1994; Kepkay, 1994), and possibly flagellates (Sherr, 1988).

4.5.1. Composition of reactive carbohydrate

Total UDOM aldose concentration decreases more rapidly with depth than bulk UDOM concentrations (Fig. 6), indicating that polysaccharides are remineralized preferentially to other components. This is consistent with previous ^{13}C NMR data for two of our sample sites, indicating that loss of bulk carbohydrate accounts for almost all compositional changes between surface and deep water UDOM (Benner et al., 1992; McCarthy et al., 1993). Although concentration decreases alone do not demonstrate remineralization, it seems unlikely that physical processes
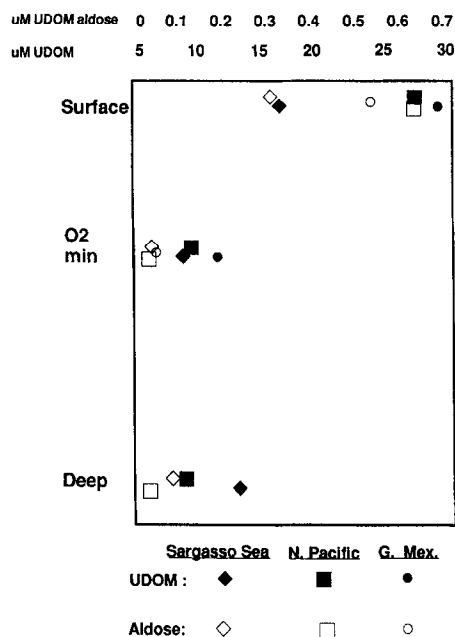
Fig. 6. UDOM and UDOM aldose concentrations. Total UDOM concentrations plotted with UDOM total aldose concentrations from each depth range. UDOM aldose concentration is calculated from total yield of aldose for each UDOM sample.

would selectively deplete the carbohydrate component. In addition, Amon and Benner (1994) recently used oxygen consumption and bacterial mass balances to demonstrate explicitly that UDOM from a coastal site was rapidly utilized by bacteria.

Coincident with a large concentration decrease, aldose compositional differences might be expected between surface and deep samples that would reflect the makeup of more refractory components. To accentuate any compositional trends, we have calculated the average differences between surface and sub-surface aldose distributions. Because the molar distributions within each depth range are similar, a representative average composition can be calculated in terms of aldose concentration (μM) for "surface" and "subsurface" UDOM from all three sites. Subtraction ($\mu M_{surface} - \mu M_{deep}$), and then conversion back to molar percentage, yields an approximation of the composition of the carbohydrate "lost" between surface and deep waters (Fig. 5C).

Surprisingly, the aldose composition of this "lost" carbohydrate is extremely similar to, and in fact

slightly *poorer* in glucose and richer in galactose than the bulk surface material (Fig. 5C). Based on usual trends in marine particles and sediments (e.g., Hernes et al., 1996), glucose would be expected to be preferentially utilized even if UDOM was relatively poor in this sugar. Moreover, the general similarity between surface (Fig. 5A) and "lost" (Fig. 5C) aldose may imply that instead of the broad mixture of polysaccharide types expected, reactive UDOM carbohydrate is relatively homogeneous. Although the lack of molecular-level changes could also result from diverse compounds being equally degraded, this seems unlikely. The observed compositional uniformity strongly suggests that a very similar suite of carbohydrate polymers is the major reactive UDOM component throughout the open sea. If this is correct, these polysaccharides would be some of the most abundant and geochemically important molecules in the oceanic carbon cycle.

4.5.2. UDOM amino acid reactivity

While overall UDOM amino acid concentrations decrease in upper waters, both total amino acid content and percent amino acid nitrogen (%AA-N) remain fairly consistent (Fig. 7), indicating little selectivity in remineralization of UDOM nitrogen
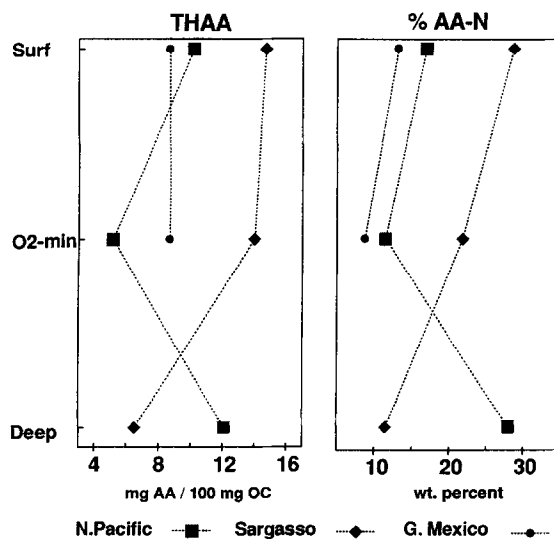

Fig. 7. THAA and %AA-N variation with depth. Total hydrolyzable amino acids (THAA) and %AA-N in UDOM from surface, oxygen-minimum and deep waters at each sample site.

forms. In contrast, because amino acids are generally a reactive compound class, a steady decrease in %AA-N is diagnostic of degradation in marine particles and sediments (Cowie and Hedges, 1994). One interpretation for the low and consistent %AA-N values of UDOM is that the organic nitrogen has already undergone significant degradation. However, since both amino acids and total UDOM are clearly reactive, consumption of UDOM amino acids must occur at rates generally similar to those of other components, even at oligotrophic ocean sites. It is unclear why amino acids would behave so differently than in other environments in the absence of some special protective mechanism.

The similarity of %AA-N in surface and deep UDOM is also surprising because it implies the major fraction of UDOM nitrogen cycling is driven by non-amino acid compounds. Although it is difficult to speculate on what nitrogen forms might be so abundant and reactive, the concurrent decreases of nitrogen and carbohydrate suggest basic sugars as one possibility. If amino sugars were a significant component of UDOM nitrogenous material, this might result in at least partially coupled nitrogen-carbohydrate decrease in upper waters. In addition, amino sugars might help to explain the hydrolysis resistance of UDOM polysaccharide, as well as some of the glucosamine-like methyl peaks present in solid state ^{13}C CPMAS spectra (McCarthy et al., 1993). Pyrolysis products from our N. Pacific samples imply that chitin is not a major UDOM component, but also are suggestive of lipopolysaccharide and peptidoglycan content (Van Heemst et al., 1993). Whatever nitrogen-containing biopolymers prove to be dominant, it is clear that cycling of non-amino acid nitrogen is a central aspect of DOM geochemistry, and identification of these compounds is an important goal.

4.6. UDOM in the mid- and deep ocean

UDOM remains relatively abundant in the deeper waters, accounting for a substantial portion (18–25%) of DOC. Concentrations are essentially identical (9–12 μM) in both oxygen minimum and deep waters at all stations. This homogeneity suggests that UDOM is well mixed and cycles on time scales similar to unfractionated DOM whose deep water ^{14}C "ages"

(4000–6000 yr; Williams and Druffel, 1987; Bauer et al., 1992) imply long-term resistance to physical and biological removal. Recent radiocarbon measurements specifically on deep UDOM in the Gulf of Mexico (800–1600 m) and middle Atlantic Bight support this interpretation, yielding ^{14}C "ages" of ~ 4000–4500 yr B.P. for > 1000 dalton material (Santschi et al., 1995; J. Bauer, unpubl. data).

This similarity in cycling between UDOM and bulk DOM would not necessarily be expected if UDOM were subject to different processes resulting from its colloidal size. One hypothesis concerning oceanic colloids (nominally UDOM-sized material) is that dynamic coagulation of smaller particles acts to create a material pathway between dissolved and sinking material (Honeyman and Santschi, 1989). However, if most UDOM were being "pumped" into larger particles at even very slow rates, it should be extremely depleted in abyssal waters. A dynamic deep UDOM pool would have to be closely coupled to surface sources and be replenished at remarkably similar rates (precluding "old" ^{14}C ages) to produce such homogeneous worldwide concentrations. In addition, the chemical characteristics discussed previously (C/N ratios, amino acid and carbohydrate content) probably rule out substantial material interchange with sinking particles, as it is unlikely that physical transformations, such as coagulation or breakup, could create the observed contrasts between POM and UDOM composition.

An explanation for deep water UDOM's apparent long-term preservation is not obvious from its chemical composition. Although proportions of biochemicals differ, surface and deep UDOM composition is similar in many respects, including C/N ratio, amino acid content, and the presence of a substantial carbohydrate component. Moreover, molecular-level amino acid and aldose compositions are also similar in both surface and deep water. While there may be differences not apparent from hydrolysis products, these broad similarities suggest that portions of the same material which is consumed in surface waters is preserved largely intact in deep UDOM. It is possible that these biochemical remnants, while unchanged on the molecular-level, are present within larger macromolecules. However, the cycling differences between UDOM carbohydrates and amino acids, as well as the clear compositional differences

between deep UDOM and oceanic humic isolates (Benner et al., 1992) suggest that deep UDOM is a mixture of physically separate components which in some way are preserved over multiple ocean mixing cycles.

One possibility is that stability in the colloidal size range may in itself deter microbial utilization of suspended organic matter, due to decreased encounter rates (Johnson and Kepkay, 1991). Several characteristics of deep UDOM suggested by ^{13}C NMR analyses (McCarthy et al., 1993), such as branched aliphatic structure and high concentration of negative charge, are used in the manufacture of commercial colloids to retard coagulation and create physically stable suspensions (e.g., Hirtzel and Rajagopalan, 1985). Biochemicals in the UDOM size fraction may thus become "trapped" there by association with carboxyl-rich aliphatic moieties. There is also laboratory evidence that subtle changes in organic association can alter microbial lability. Keil and Kirchman (1994) observed that "abiotic modification" occurs in proteins aged in sterile seawater, resulting in substantial reduction in substrate potential, even as molecular-level compositions remain unaltered. Importantly, "abiotic modification" did not occur in organic-free seawater, suggesting complexation with other organics was crucial. If a similar mechanism is important for deep UDOM preservation, it implies the ability to not only slow utilization of fresh materials, but also to facilitate preservation over very long time scales.

5. Overview

The compositional similarity of UDOM from diverse sites suggests that the higher molecular-weight fraction of DOM has similar sources and reaction histories throughout the ocean. Polysaccharides, as revealed by the aldose as well as ^{13}C NMR (Benner et al., 1992; McCarthy et al., 1993) and ^{1}H NMR (Aluwihare et al., submitted), seem to be the major reactive component in the roughly half of surface UDOM that turns over in the upper water column. The nearly constant aldose ratios suggest relatively few structural heteropolysaccharides, accounting for 10–14% of the overall surface DOC pool, may largely drive upper water UDOM dynamics. Because

export of nitrogen-poor DOM may be important in closing upper water column carbon budgets (Sambrotto et al., 1993; Carlson et al., 1994; Michaels et al., 1994; Peltzer and Hayward, 1996), these UDOM polysaccharides may themselves play a central role in maintaining carbon balance in the upper ocean.

The contrast between these carbohydrates and the less dynamic nitrogen component of UDOM suggests that the cycling of different UDOM components may be largely independent. While carbohydrate is depleted more rapidly and seems to be enriched in higher molecular-weight DOM, the lower and more constant amino acid concentrations and elemental ratios, as well as the similarity between amino acids in UDOM and unfractionated seawater, suggest less rapid removal and a more uniform size distribution. These differences are consistent with other observations (e.g., deep UDOM concentrations and apparent age, as well as upper water column composition and gradients) which suggest that UDOM cycling is broadly similar to that of the larger DOC pool. The diverse dynamics of different UDOM biochemicals also emphasize that, like the larger DOC pool, UDOM is not a uniform substance but a heterogeneous mixture of independently cycling components.

Overall, UDOM composition suggests that at least the larger fractions of DOC may consist largely of relatively unaltered biochemicals rather than a mostly unresolvable mixture of highly degraded materials. Despite the lower concentrations and apparent long-term stability of UDOM in the deep ocean, the generally similar surface and deep molecular-level aldose and amino acid compositions suggest that a portion of the material produced in surface waters persists mostly unaltered at depth. The reasons for this apparent low reactivity, in an environment where few of the proposed mechanisms for organic preservation (e.g., low oxygen, surface adsorption, chemical alteration) are apparent, is an open question. One possibility is that a limited number of comparatively refractory biopolymers dominate UDOM composition, similar to the situation proposed for some sedimentary environments (e.g., De Leeuw and Largeau, 1993) and recently suggested for specific dissolved proteins in seawater (Tanoue, 1995). However, the selected preservation of a few biochemicals cannot

be the whole story, since the same materials seem to be in part removed in the upper ocean. A more detailed understanding of the composition of deep UDOM may point to the broader biological and physiochemical selectivities governing the removal processes for much of the ocean's DOC pool.

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