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Production and neutral aldose composition of dissolved carbohydrates excreted by natural marine phytoplankton populations

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Abstract

Natural populations of diatoms were incubated for 4–12 h with $H^{13}CO_3^{-1}$. The production of particulate and dissolved fractions of organic carbon and neutral aldoses (NAld) was followed by gas chromatography/mass spectrometry. The extracellular production rate of dissolved organic carbon (DOC) ranged from 4.1% to 6.4% of total (particulate and dissolved) production rate. Glucose was a major component of the excreted dissolved neutral aldoses (DNAld), and galactose, rhamnose, fucose, xylose, and mannose were found as secondary components of the excreted DNAld. The comparison of NAld composition with cellular products suggests that glucose in the excreted DNAld is composed mainly of storage glucan. On the other hand, the high ratios of dissolved production to total (dissolved and particulate) production of galactose, rhamnose, fucose, xylose, and mannose probably reflect the active excretion of heteropolysaccharides by diatoms. By assuming steady-state concentrations, turnover rates of DOC and DNAld can be estimated from the phytoplankton production of ^{13}C-labeled material. The estimated turnover rates of DNAld through phytoplankton photosynthesis is 4.2–5.1 times higher than that of total DOC, which indicates the bioreactive nature of DNAld. This high turnover rate of DNAld mainly resulted from the high turnover rate of glucose, and it is likely that dissolved glucan is important as a carbon and energy carrier in the marine food web. The fact that the turnover rates of DNAld, which is considered to constitute heteropolysaccharides, are lower than that of glucose, would suggest that heteropolysaccharides are more resistant to biological degradation than glucan.

The importance of dissolved organic matter (DOM) in the biogeochemical cycle of ocean systems has recently been actively discussed (e.g., Kirchman et al. 1991; Carlson and Ducklow 1995; Yamanaka and Tajika 1997; Hansell and Carlson 1998; Karl et al. 1998). Intensive studies on DOM, such as the absolute concentration of dissolved organic carbon (DOC) (Sharp et al. 1995; Thomas et al. 1995; Chen et al. 1996) and molecular weight composition of DOM in relation to their bioreactivity (Amon and Benner 1994; Guo et al. 1995; Santschi et al. 1995), have been carried out. Recent studies revealed that high-molecular-weight (HMW) compounds are more labile than low-molecular-weight (LMW) compounds (Amon and Benner 1994; Santschi et al. 1995). One of the reasons why HMW compounds are labile is that they are composed of biomolecules, such as carbohydrates, to a greater extent than LMW DOC (Benner et al. 1997).

The concentrations of the total dissolved carbohydrate (DCHO) and colloidal carbohydrates (CCHO) in seawater have been determined by spectrophotometry. Variations with depth (Handa 1970; Pakulski and Benner 1994; Børsheim et al. 1999), time (Burney et al. 1981; Børsheim et al. 1999; Fajon et al. 1999), and oceanic region (Pakulski and Benner 1994) have been reported. These results point to high DCHO concentrations where high phytoplankton biomass occurs (Pakulski and Benner 1994; Børsheim et al. 1999; Fajon et al. 1999) and indicate that phytoplankton is the major source of DCHO. The spectrophotometric method, however, cannot determine the molecular composition of DCHO and thus offers little information on the origin and bioreactivity that are the key properties in the biogeochemical role of DCHO.

Gas chromatography (GC) (Brockmann et al. 1979; Janse et al. 1996; Sigleo 1996) and high-performance liquid chromatography (HPLC) (Rich et al. 1996; Skoog and Benner 1997) have recently been applied to the determination of the neutral aldose (NAld) composition of DCHO and CCHO in natural seawater. The NAld composition of DCHO and CCHO determined in natural seawater suggests that phytoplankton extracellular products and/or cell wall polysaccharides are the major source of DCHO and CCHO in the surface layer of the oceans (Sigleo 1996; Biersmith and Benner 1998; Aluwihare and Repeta 1999).

Excretion of photosynthetic products (Hellebust 1965; Mague et al. 1980; Karl et al. 1998) has been pointed out as one of the major sources of DOM in marine system. Culture studies can determine the composition of excreted organic matter, because organic compounds found in the culture media can be assumed to originate from phytoplankton photosynthesis. In natural environments, on the other hand, the measurement of the concentration of organic compounds in the ambient waters by GC or HPLC can not distinguish materials freshly produced by phytoplankton from ambient DOM.

Hama et al. (1987) applied a combination of a ^{13}C tracer

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Table 1. Changes in DOC and dissolved carbohydrate concentration in samples before and after desalinization for 2.5 h. No significant losses of DOC to carbohydrates were found.

Sample No.	Conductivity (ms cm^{-1})		DOC (μgC L^{-1})		Dissolved carbohydrate (μgC L^{-1})	
	Original	Desalted	Original	Desalted	Original	Desalted
1	32.8 (100)	3.0 (9.1)	2,510 (100)	2,380 (94.8)	392 (100)	384 (98.0)
2	39.3 (100)	3.2 (8.1)	2,760 (100)	2,710 (98.2)	666 (100)	639 (95.9)
3	35.5 (100)	3.0 (8.5)	4,250 (100)	4,170 (98.1)	622 (100)	610 (98.1)

technique with GC/mass spectrometry (GC/MS) to determine the production rate of organic molecules, such as monosaccharides of carbohydrates and amino acids of proteins, in the photosynthetic products of phytoplankton. When this method is applied to the study of the production process of DCHO, the production rates of the monosaccharides from phytoplankton excretion that constitute DCHO can be measured. Further, the isotopic ratio, which is measured for particular elements (C in the present study) and organic molecules in the stable isotope tracer technique, involves the combined information of concentration and production rates (Dugdale and Goering 1967; Hama et al. 1993). Thus, the turnover rates of DOC and monosaccharides of DCHO through phytoplankton excretion can be estimated and make it possible to compare the biogeochemical properties of DOC and DCHO at the molecular level.

In the present study, we applied the ^{13}C/GC/MS method to the determination of the dissolved NAld (DNAld) composition of excreted DCHO by a natural phytoplankton population in eutrophic Hakata Bay, Kyushu, Japan, to assess the compounds of excreted DCHO and the origin of DCHO in the seawater. The turnover rates of DOC and DNAld through phytoplankton excretion are compared to evaluate the variability of bioreactivity among DNAld.

Materials and methods

Incubation experiment—The incubation experiment was carried out in Hakata Bay, which is located in the northern part of Kyushu, Japan. Hakata Bay is one of the most eutrophic bays in Japan, and the yearly average primary productivity is estimated to be 1000 mgC m^{-2} d^{-1} (Yanagi and Onitsuka 2000). A 60-liter water sample was collected from the water surface at Sta. E-6, whose water depth was 7–8 m at 0600 h on 22 July 1997, and transferred into a polyethylene bag. The sample was enriched with KNO_3 and KH_2PO_4 to a final concentration of 22.3 μM NO_3^- and 2.25 μM PO_4^{3-}. After the addition of ^{13}C-$NaHCO_3$ (15% ^{13}C in dissolved inorganic carbon), the sample was incubated under white luminescent light (160 μE m^{-2} s^{-1}). Water temperature was controlled at 27°C, which was close to the surface water temperature at the sampling site.

Aliquots of samples (4 liters) were recovered 0, 4, 8, and 12 h after incubation and filtered through precombusted (450°C, 4 h) glass-fiber filters (Whatman GF/F). Filters and filtrates were stored at −20°C until analysis.

Analytical procedures—Electrodesalinization: Filtrates were desalted by electrodialysis (Microacilizer S-3, Asahi Chemical) for 2.5 h prior to further analyses. The ability to desalinate seawater and the recovery of DOC and DCHO through electrodialysis was examined by use of the GF/F filtrates. The concentrations of DOC and DCHO-carbon were measured by wet combustion (Menzel and Vaccaro 1964) and phenol-sulfuric acid methods (Handa 1966), respectively. The examinations were applied to three samples from Hakata Bay, and the recoveries of DOC and DCHO after 2.5 h of desalting ranged from 95% to 98% and 96% to 98%, respectively, whereas the conductivity decreased to 8%–9% of the initial conductivity (Table 1). These results confirm that desalinization for 2.5 h desalted seawater effectively without significant loss of DOM.

Concentration and ^{13}C atom% of DOC: The desalted filtrates of the samples incubated for 0, 4, 8, and 12 h were concentrated to 20 ml by a rotary evaporator at <45°C. HCl was added to a 2-ml aliquot of concentrate to decrease the pH to 2. The concentrate was purged with N_2 gas for 3 min to remove the dissolved inorganic ^{13}C. The concentration and ^{13}C atom% of DOC absorbed onto the glass-fiber filters (Whatman GF/F) were measured by a mass spectrometer (TracerMAT, Finnigan MAT) combined with an elemental analyzer (EA 1108, FISONS Instruments). The concentrations of DOC in the concentrated sample ranged from 79% to 86% of the original filtrates, which implies a possible loss of 10%–16% of DOC during the concentration and drying processes.

Concentration and ^{13}C atom% of NAld constituting DCHO: An aliquot of the desalted and concentrated sample was hydrolyzed with 1N H_2SO_4 at 100°C for 5 h. NAld (rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, and glucose) were converted to acetyl derivatives according to the method of Handa and Tominaga (1969). The NAld concentration was determined by a GC system (GC14A, Shimadzu) equipped with a fused silica capillary column (OV-1701, 25 m × 0.25 mm inner diameter) and FID detector, with N_2 as the carrier gas. The ^{13}C atom% of NAld was estimated by GC/MS (DX-302, JEOL). Separation of NAld was the same as for GC, but He was used as the carrier gas. Chemical ionization was applied by use of ammonium as a reagent gas to obtain the mass spectrum. The ^{13}C atom% of each NAld was estimated from the differences in the relative intensities of isotopic peaks between nonincubated and incubated samples (Kouchi 1982; Hama and Handa 1986). The detailed analytical conditions of GC and GC/MS can be found in Hama et al. (1987).

Concentration and ^{13}C atom% of particulate organic carbon (POC) and NAld constituting particulate carbohydrates

Table 2. Concentrations of Chl *a*, particulate organic carbon (POC) and inorganic nutrients (NO_3^-, NH_4^+ and PO_4^{3-}) during the incubation experiment.

Incubation length (h)	Chl *a* (μg L^{-1})	POC (μgC L^{-1})	NO_3^-	NH_4^+	PO_4^{3-}
			(μM)		
0	54.7	2,690	22.3	1.8	2.25
4	83.1	3,490	8.2	1.7	0.86
8	110	4,960	0.2	1.2	0.25
12	132	6,100	0.05	1.4	0.05

(PCHO): The concentration and ^{13}C atom% of POC collected onto glass-fiber filters were determined by a mass spectrometer (TracerMAT, Finnigan MAT) according to the method of Hama et al. (1983). The concentration and ^{13}C atom% of NAld of PCHO were determined by GC and GC/MS after hydrolysis with 1N H_2SO_4 at 100°C for 5 h.

Calculation of production rates of particulate and dissolved organic materials: Production rates of POC and particulate neutral aldose (PNAld) were calculated according to the method of Hama et al. (1983) and (1987), respectively. The procedures use the percentage of ^{13}C of dissolved inorganic carbon (DIC), because the ^{13}C atom% of newly synthesized organic matter by photosynthesis reflects that of DIC.

$$\text{Production rate } (\mu\text{gC L}^{-1}\text{ h}^{-1}) = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times \frac{[C]}{t} \quad (1)$$

where a_{is} is the ^{13}C atom% in an incubated sample, a_{ns} is the ^{13}C atom% in a natural (nonincubated) sample, a_{ic} is the ^{13}C atom% in inorganic carbon, t is the incubation length (h), and [C]: concentration (μgC L^{-1}) of POC or NAld in the incubated sample.

The coefficient of variation of the determination of the photosynthetic production rate is generally within 5% (Hama et al. 1993). Equation 1 is also applied to the calculation of the production rates of DOC and DNAld by excretion, but [C] is the concentration of DOC or DNAld in the incubated sample. The concentrations of DOC and DNAld in the desalted and concentrated sample are used for the calculation of the production rate because the ^{13}C atom% of DOC and DNAld are determined for the desalted and concentrated sample. Thus, the estimated production rate of DOM is possibly underestimated, because the recovery of DOC in the desalted and concentrated samples ranged from 79% to 86% of the original filtrate. No correction is made for the possible loss of DOM during the desalting and concentration processes. Furthermore, the application of ^{13}C atom% of DIC to estimate the excretion rate also implies an underestimation, because part of the excreted organic compounds has a lower ^{13}C atom% than that in DIC, as proposed in the specific activity of the excreted materials in the ^{14}C tracer method (Storch and Saunders 1978; Mague et al. 1980; Jensen 1985).

Turnover rates of particulate and dissolved organic matter through phytoplankton photosynthesis are calculated as follows (Hama et al. 1988, 1993).

$$\text{Turnover rate } (\text{h}^{-1}) = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{is})} \times \frac{1}{t} \quad (2)$$

where t is the incubation length (h). The use of ^{13}C atom% of dissolved inorganic carbon in Eq. 2 implies that the turnover rate of DOM resulted in an underestimation from the standpoint of the ^{13}C atom% of excreted materials as described in the calculation of the production rate.

Concentration of chlorophyll *a* and inorganic nutrients: The concentration of Chl *a* was determined by fluorometry after extraction with 90% acetone (Parsons et al. 1984). The concentration of inorganic nutrients including nitrate, ammonium, and phosphate were measured by the methods described in Parsons et al. (1984).

Results

Concentration of Chl a*, POC, and inorganic nutrients*—The concentration of Chl *a* and POC showed a marked increase during the 12-h incubation (Table 2). Specific growth rates based on the concentration of Chl *a* and POC are estimated as 1.8 and 1.6 d^{-1}, respectively. The similarity of the growth rate obtained from the concentration of POC with that estimated from Chl *a* indicates that phytoplankton constituted a major part of POC. Diatoms such as *Skeletonema costatum* and *Chaetoceros* sp. dominated throughout the experiment, accounting for almost all of the phytoplankton population in cell numbers.

The nitrate and phosphate concentrations showed a rapid decrease during incubations. Only very low concentrations were detected after 12 h of incubation.

Production of total POC—A rapid increase in the ^{13}C atom% of POC was found in the first 8 h of the incubation, and it reached 9.86 atom% after 12 h (Table 3). The photosynthetic production rates for POC measured by the ^{13}C tracer method were calculated as 206, 333, and 319 μgC L^{-1} h^{-1} in the 4-, 8-, and 12-h incubated samples.

Table 3. ^{13}C atom % and production rate (μgC L^{-1} h^{-1}) of POC, DOC and total organic carbon (sum of POC and DOC) during the incubation with 4, 8, and 12 h.

Incubation length (h)	POC production			DOC production			Total production rate (μgC L^{-1} h^{-1})
	^{13}C atom %	Production rate (μgC L^{-1} h^{-1})	% of total production	^{13}C atom %	Production rate (μgC L^{-1} h^{-1})	% of total production	
4	4.44	206	95.9	1.29	8.8	4.1	215
8	8.58	333	94.8	1.89	18.3	5.2	351
12	9.86	319	93.6	2.56	21.9	6.4	341

Table 4. ^{13}C atom % and production rate (μgC L^{-1} h^{-1}) of eight neutral aldoses constituted particulate carbohydrate during 4, 8, and 12 h.

Incubation length (h)	Rha	Fuc	Rib	Ara	Xly	Man	Gal	Glc	Total**	PNAld/POC***
4										
^{13}C atom %	3.76	2.75	6.88	4.09*	2.44	3.88	4.09*	5.63		
Production rate	2.02	1.74	7.53	1.47	1.43	2.58	5.75	51.8	74	0.360
8										
^{13}C atom %	5.22	5.98	7.49	4.75	6.09*	6.09*	6.09*	11.96		
Production rate	1.21	2.43	2.96	0.77	2.09	2.44	3.33	146	163	0.490
12										
^{13}C atom %	5.60	6.46	7.02	8.46	6.77*	6.77*	6.77*	11.68		
Production rate	0.97	2.02	1.92	1.26	1.61	2.11	4.37	153	167	0.521

Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.
* Calculated as the weighted mean value of other aldoses except glucose in the same incubation length (see text in detail).
** Sum of the production rate of eight aldoses.
*** Ratio of total aldose production to POC production.

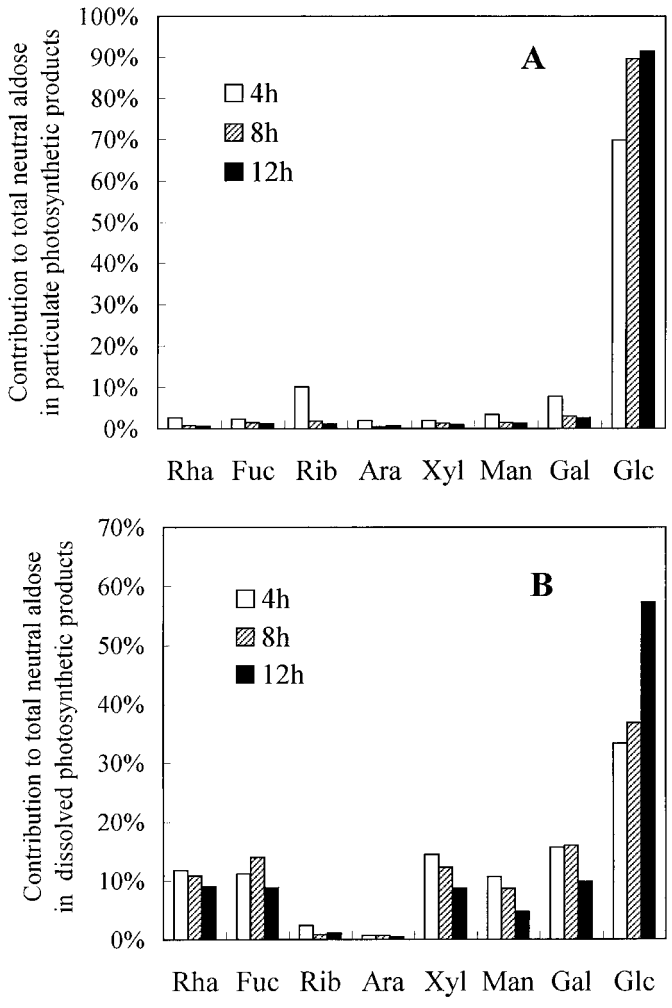

Fig. 1. Relative NAld composition of particulate (A) and dissolved (B) photosynthetic products after varying incubation times. Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; and Glc, glucose.

Production of PNAld—The ^{13}C atom% of NAld, which were obtained by the hydrolysis of PCHO, was measured by a GC/MS as shown in Table 4. However, the ^{13}C atom% of some NAld could not be directly measured by GC/MS. The ^{13}C atom% of mannose of the sample for 4, 8, and 12 h and those of galactose for 8 and 12 h could not be estimated because the chromatographic peaks of these aldoses could not be separated from the adjacent glucose peak, which increased markedly with incubation length. The ^{13}C atom% of arabinose in the sample for 4 h and xylose in the samples for 8 and 12 h also could not be calculated because of the serious effects of noise peaks on the mass spectra. For PNAld, whose ^{13}C atom% could not be calculated by GC/MS analysis, the weighted mean value of ^{13}C atom% of other PNAld of the same sample was applied. On this occasion, glucose was excluded from the calculation of the weighted mean value of ^{13}C atom% of PNAld, because glucose always showed much higher values than other seven PNAld (Hama 1988; Hama et al. 1988). For example, the weighted mean ^{13}C atom% of rhamnose, fucose, ribose, xylose, and mannose in the 4-h incubated sample was applied as the ^{13}C atom% of arabinose and galactose in 4-h incubation.

The ^{13}C atom% of PNAld tended to increase with incubation length. Ribose and glucose showed a more rapid increase than the other PNAld, which implies the incorporation of carbon at higher rates. These PNAld, however, showed a slight decrease after 8 h. Other PNAld showed increases in ^{13}C atom% up to 12 h.

The production rates of eight PNAld are summarized in Table 4. Among them, glucose showed the highest production rate of up to 153 μgC L^{-1} h^{-1} in the 12-h incubated sample. Although galactose, mannose, ribose, and fucose are found to be important secondary PNAld, the difference between the production rate of glucose and the other PNAld is quite obvious in particulate photosynthetic products.

Glucose accounted for 70%–91% of total carbohydrate carbon, showing a higher contribution with a longer incubation length (Fig. 1). Other PNAld accounted for $<$10% of the total PNAld in every sample. The contribution of ribose was $\sim$10% of total PNAld production in a 4-h incubation,

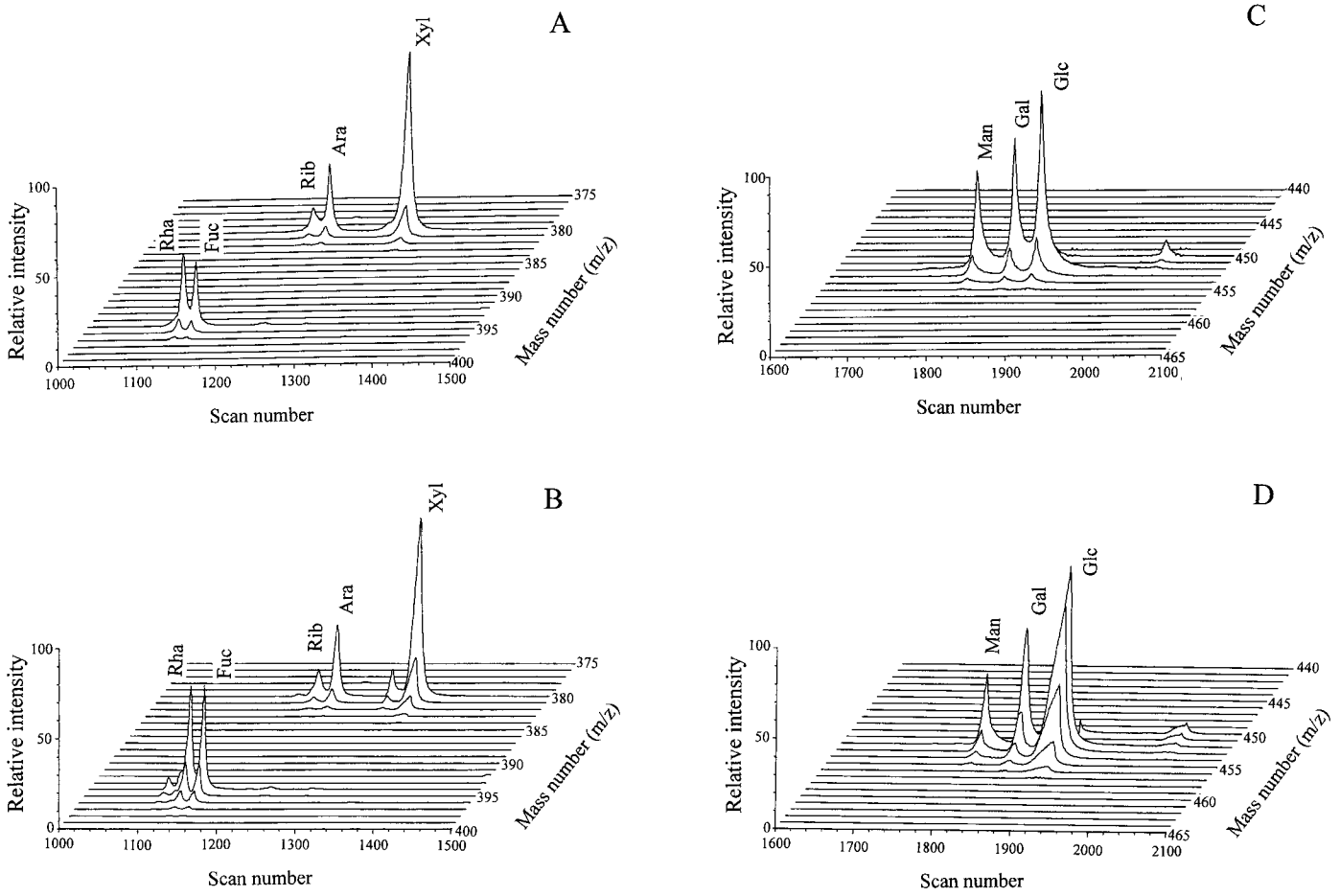

Fig. 2. Three-dimensional chromatograms showing the abundance of DNAld in samples before and after incubation. The degree of incorporation of ^{13}C into the carbon skeleton of DNAld can be estimated by the increase in the relative intensities of isotopic peaks. (A and B) Chromatograms of rhamnose, fucose, ribose, arabinose, and xylose (A, nonincubated sample; B, sample incubated for 12 h). (C and D) Chromatograms of mannose, galactose and glucose (C, nonincubated sample; D, sample incubated for 12 h).

and it decreased to 1.8% and 1.1% in 8- and 12-h incubations, respectively.

Production of total DOC—The ^{13}C atom% in DOC increased as the incubation length increased and reached 2.56 atom% after 12 h, which confirms the excretion of photosynthetically produced organic compounds (Table 3). The production rate of excreted DOC was calculated to be 8.8 μgC L^{-1} h^{-1} in the 4-h incubated sample, and it increased up to 21.9 μgC L^{-1} h^{-1} in the 12-h incubated sample. This production rate accounted for 4.1%–6.4% of the total (POC and DOC) primary production, showing an increasing trend with incubation length. These contributions of excretion are in the range determined for a natural marine phytoplankton population by the ^{14}C tracer method (Hellebust 1965; Mague et al. 1980; Baines and Pace 1991).

Production of DNAld—The ^{13}C atom% of NAld obtained from the hydrolysate of the filtrates of incubated samples was estimated by GC/MS. The three-dimensional chromatograms of nonincubated and 12-h incubated samples are shown in Fig. 2. The quasimolecular ion peaks (m/z, 394 for acetyl derivatives of rhamnose and fucose; m/z, 380 for derivatives of ribose, arabinose, and xylose; and m/z, 452 for derivatives of mannose, galactose, and glucose) were found as the base peaks both for nonincubated and 12-h incubated samples. The degree of the incorporation of ^{13}C into carbon skeleton of DNAld can be estimated by the increase in the relative intensities of isotopic peaks. The most obvious increases in isotopic peaks were found in glucose; the relative intensities of isotopic peaks such as m/z 453, 454, 455, and 456 in the 12-h incubated sample (Fig. 2D) are much higher than those in nonincubated sample (Fig. 2C), which reflects the rapid incorporation of ^{13}C to glucose. This result clearly shows that carbohydrates containing glucose with high ^{13}C content are excreted in the dissolved form. On the other hand, little increase was found in the relative intensities of isotopic peaks of ribose and arabinose between the nonincubated sample (Fig. 2A) and the 12-h incubated sample (Fig. 2B). These variations in the increases in relative intensities of isotopic peaks among DNAld reflect the differences in the relative production rates of DNAld through the excretion of photosynthetic products.

The ^{13}C atom% of dissolved NAld, as calculated from

Table 5. ^{13}C atom % and production rate (μgC L^{-1} h^{-1}) of eight aldoses constituted dissolved carbohydrate during 4-, 8-, and 12-h incubations.

Incubation length (h)	Rha	Fuc	Rib	Ara	Xly	Man	Gal	Glc	Total*	DNAld/DOC**
4										
^{13}C atom %	2.31	2.18	1.43	1.13	1.53	1.87	2.03	2.93		
Production rate	0.61	0.57	0.12	0.03	0.74	0.55	0.80	1.71	5.13	0.586
8										
^{13}C atom %	3.76	3.79	1.79	1.30	2.85	2.80	3.28	8.47		
Production rate	1.01	1.30	0.08	0.06	1.14	0.80	1.48	3.43	9.29	0.509
12										
^{13}C atom %	4.95	4.72	2.47	1.36	3.33	2.91	4.23	10.51		
Production rate	0.94	0.92	0.11	0.05	0.90	0.49	1.03	5.96	10.4	0.474

* Sum of the production rate of eight aldoses.
** Ratio of total aldose production to DOC production.

mass spectra, is shown in Table 5. Analogous to what was found in the particulate fraction, glucose showed the largest increase in ^{13}C atom% in the dissolved fraction; hence, the largest production of all sugars in the dissolved phase. Rhamnose, fucose, and galactose showed relatively high values of >4 ^{13}C atom% after 12 h. Ribose and arabinose were rapidly produced in the particulate phase (6.88 and 4.09 ^{13}C atom%, respectively, in a 4-h incubated sample), whereas the production of these sugars in the dissolved phase was much lower (1.43 and 1.13 ^{13}C atom%, respectively, in a 4-h incubated sample). Arabinose had the lowest dissolved production of all neutral aldoses.

Glucose shows the highest production rate in the dissolved and the particulate phase (Table 5). The production of dissolved glucose increased rapidly from 8 to 12 h, although the production rate of other DNAld did not exhibit such an increase. Glucose accounted for 33%–57% of the total DNAld products (Fig. 1), being significantly lower than its level in PNAld products. Although rhamnose, fucose, and xylose were only minor components of produced PNAld (each accounting for 0.6%–2.7% of total PNAld production), they showed significant contributions to the dissolved products, i.e., 8.7%–11.8%, 8.8%–14%, and 8.7%–14.4%, respectively. In contrast, ribose accounted for only a minor portion of dissolved products (0.9%–2.4%), although it showed a high contribution of >10% in particulate products in a 4-h incubation.

Turnover rates of DOC and DNAld through phytoplankton excretion—The turnover rate of DOC through phytoplankton excretion varied from 0.0038 to 0.0099 h^{-1} and showed distinctive variations among DNAld (Fig. 3). Glucose showed a markedly high turnover rate, ranging from 0.038 to 0.18 h^{-1}, and tended to increase with incubation length. Turnover rates of rhamnose, fucose, xylose, mannose, and galactose were estimated within the range of 0.02–0.03 h^{-1}, which was

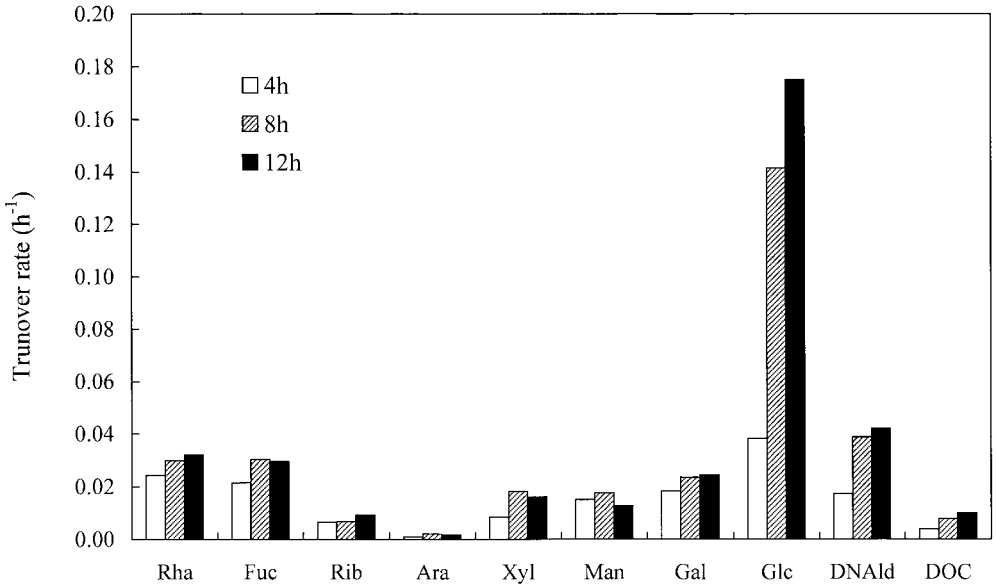

Fig. 3. Turnover rates of individual DNAld, total DNAld, and DOC estimated from phytoplankton excretion rates and assumption of concentration steady state. Turnover rate for total DNAld is estimated as the weighted mean value of the rates of the eight individual DNAld.

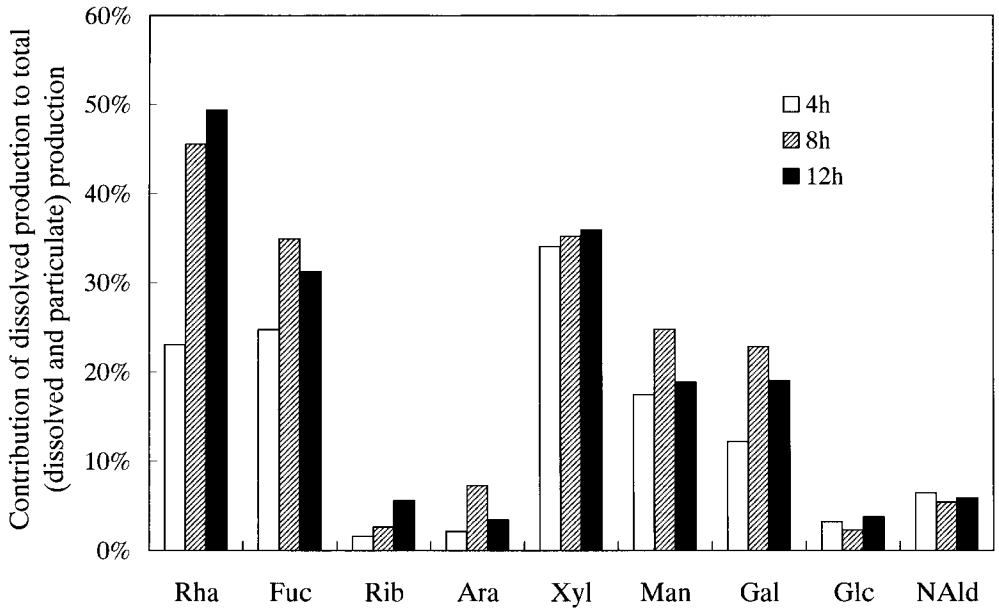

Fig. 4. Contribution of the dissolved production to the total (particulate and dissolved) production for individual NAld and total NAld after varying incubation times.

much lower than that of glucose but higher than those of ribose and arabinose. The turnover rates of ribose and arabinose in DNAld were quite low because of their low production rates. The turnover rates of total DNAld ranged from 0.017 to 0.042 h^{-1}, being 4–5 times higher than those of DOC.

Discussion

NAld composition of excreted CHO and their source—The analysis of DCHO produced by phytoplankton photosynthesis showed that glucose was predominant, ranging from 33% to 57% of total DNAld estimated from the sum of eight NAld. The high ^{13}C atom% of glucose in DCHO reflected the high atom% in cellular glucose. The contribution of glucose to cellular products increased to 91% of total carbohydrate after a 12-h incubation, and this likely reflects the accumulation of storage carbohydrates under nutrient-depleted conditions (Hama 1988; Hama et al. 1988). In the present study, although nitrate was added prior to the incubation with 22.3 μM, it was exhausted until 8 h, showing the serious nitrogen limitation after 8 h. Diatoms, which dominated in the present study, have been known to produce β-1,3-D-glucan as storage polysaccharides (Handa 1969; Myklestad and Haug 1972; Craigie 1974; Myklestad 1989). The high ^{13}C atom% of dissolved glucose found in the incubated samples suggests the excretion of storage glucan by diatoms that dominated in the sample.

Figure 4 illustrates the proportion of the extracellular production to the total (intracellular and extracellular) production calculated for each NAld. Although the extracellular production of glucose was the highest among NAld, the proportion of extracellular production to the total production of glucose was low, only accounting for 2.3%–3.8%. In other words, almost all of the photosynthetically produced glucose was retained in the phytoplankton cells, and only a small part was excreted in dissolved form. The disproportionate accumulation and excretion of storage carbohydrate by phytoplankton is considered to occur when more carbohydrate is produced than required by the structural demands of the cell under nutrient-depleted condition (Smetacek and Pollehne 1986; Biersmith and Benner 1998). Undoubtedly, the production of cellular storage glucan was extremely high in our experiment considered from the standpoint of PNAld composition, and it is possible that the concentration of storage glucan exceeded the cellular capacity. Thus, the excretion of storage glucan is regarded as an ''overflow'' of cellular components and not an essential component of the diatom metabolism.

Although storage glucan is considered as the most probable source of glucose in the hydrolysate of excreted DCHO as discussed above, it is possible that extracellular DCHO contains monomer and/or oligomers of glucose. No attempts were made to determine the MW distribution of excreted DCHO in the present study, and the contribution of monomer and oligomer cannot be estimated. Hama and Handa (1992) determined the NAld composition of the water-extractable fraction of natural phytoplankton cells, which corresponds to the metabolic pool and likely contains LMW PCHO (mono- and oligosaccharides). They found that ribose and arabinose were the major constituents of water-extractable LMW PCHO as well as glucose. When LMW PCHO constituting the metabolic pool in phytoplankton cells is excreted as DCHO, the ^{13}C atom% of ribose and arabinose in DCHO fraction should increase. The very low ^{13}C atom% of ribose and arabinose in DCHO in the present study (Table 5) demonstrates that both NAld are scarcely excreted and suggests that the excretion of LMW CHO is insignificant in our experiments. Thus, the main part of glucose found in excreted DCHO perhaps exists as polymerized glucan. More

studies that use gel-filtration or ultrafiltration are needed to assess the MW distribution of excreted DCHO composed of glucose.

Galactose, rhamnose, fucose, xylose, and mannose were found to be important but secondary NAld species among extracellular carbohydrates. These NAld were found to be the major constituents of carbohydrates in culture media of phytoplankton (Allan et al. 1972; Haug and Myklestad 1976; Hoagland et al. 1993; Biersmith and Benner 1998).

Diatoms are known to produce extracellular polymeric substances (EPS) in the form of stalks, adhering films, and cell coatings, and these substances are conspicuous components of diatom growth (Hoagland et al. 1993). A considerable amount of EPS materials is likely to be soluble in water and to pass through filter paper. These materials are operationally defined as DOM (Decho 1990). The polysaccharides composed of rhamnose, fucose, xylose, mannose, and galactose (heteropolysaccharides) probably correspond to EPS. The contributions of dissolved production to total production of these NAld were considerably high, ranging from 12% to 34%, 23% to 46%, and 19% to 49% in the 4-, 8-, and 12-h incubated samples, respectively (Fig. 4). This result strongly suggests that the newly produced heteropolysaccharides composed of these NAld are actively excreted from phytoplankton as EPS with a role in colony formation, habitat stabilization, and so on, as summarized by Decho (1990) and Hoagland et al. (1993).

Comparison with NAld composition of natural seawater—The NAld composition of DCHO and CCHO in natural seawater has been determined. In the last 5 yr especially, our knowledge of the molecular nature of DCHO and CCHO has been greatly advanced (McCarthy et al. 1996; Rich et al. 1996; Sigleo 1996; Borch and Kirchman 1997; Skoog and Benner 1997; Aluwihare and Repeta 1999). Although minor variations are found depending on the area, season, depth, and isolation methodology, the NAld spectra of DCHO and CCHO are generally comparable, i.e., glucose, galactose, mannose, rhamnose, fucose, and xylose are the main components, usually accounting for ~80% of the total DNAld determined. These are followed by arabinose, and the contribution of ribose is quite low regardless of the sampling site (McCarthy et al. 1996; Sigleo 1996; Skoog and Benner 1997). The NAld composition of excreted photosynthetic products of a natural phytoplankton population measured in this study is almost comparable with the results reported for the DCHO and CCHO in seawater except for arabinose, whose contribution to excreted carbohydrates is even lower than ribose in this study. Biersmith and Benner (1998) determined the NAld composition of ultra-filtered DOM (>1,000 Da) in culture media of marine phytoplankton and found it generally compatible with the composition of CCHO in the surface layer of seawater. They concluded that heteropolysaccharides produced as extracellular products by phytoplankton are a major source of CCHO in the surface layer. The present study measured the NAld composition of excreted carbohydrates by a natural phytoplankton population and confirmed the importance of the excreted heteropolysaccharides as the source of DCHO. This is ascertained by the fact that the contribution of the dissolved fraction to total production was high in NAld, which most likely constitute the heteropolysaccharides (Fig. 4).

Although the reported NAld composition of DCHO and CCHO was relatively constant, the contributions of glucose exhibited considerable time and spatial variations. The fraction of glucose in excreted DNAld (33%–57%) found in the present study is higher than the recent DCHO and CCHO measurements (McCarthy et al. 1996; Sigleo 1996; Borch and Kirchman 1997). A predominance of glucose comparable with our result was reported by Ittekkot et al. (1981), who determined the NAld composition of DCHO from the surface layer of the northern North Sea during a bloom of phytoplankton. They found a high contribution of glucose (>60% of total carbohydrate) in the combined form of DCHO at the peak of the phytoplankton biomass, and it coincided with nutrient depletion. An almost comparable contribution of glucose (>50%) was reported by Brockmann et al. (1979) during the development of a natural phytoplankton population in an outdoor tank. This high contribution of glucose also coincided with a high phytoplankton biomass. These results indicate that an accumulation of storage glucan in phytoplankton cells under nutrient-depleted conditions (Hama 1988; Hama et al. 1988) likely resulted in the excretion of glucan as DCHO.

The contribution of DNAld to DOC in the extracellular products ranged from 47% to 59% (Table 5) and was considerably higher than the values in the ambient DOC (16%–18%; detailed results are not shown). This difference in the DNAld contribution between the dissolved photosynthetic products and ambient DOC is probably due to decomposition of bioreactive DNAld after excretion. Lower contribution of DNAld in DOC than our measurements has been reported in the surface water in the open ocean (<10%; McCarthy et al. 1996; estimated from the reported concentration of DOC, colloidal DOC, and CNAld). High phytoplankton standing stock and high primary productivity likely resulted in the higher contribution of DNAld in DOC in a eutrophic bay than in the open ocean.

Turnover of DOC and DCHO—The turnover rate of dissolved organic constituents has been estimated both by the concentration decrease (Kirchman et al. 1991; Amon and Benner 1994) and the microbial uptake rate of ^{14}C- or ^{3}H-labeled organic molecules (Rich et al. 1996; Skoog et al. 1999). The present study attempted to estimate the turnover rates of DOC and DNAld through phytoplankton excretion of photosynthetic products. Because phytoplankton excretion is not the sole source of DOM, the estimated turnover rate probably underestimates the ''true'' turnover rate, which should ideally be determined by a comparison of the concentration with the ''total'' production or removal rates. However, we consider that a comparison of the turnover rate of DOC with DNAld through phytoplankton excretion is valuable in the assessment of the relative bioreactivity among dissolved organic constituents.

The turnover rate of DOC ranged from 0.0038 to 0.0099 h^{-1}, tending to increase with incubation length. Little information has been available on the turnover rate of DOC through the excretion of photosynthetic products of natural phytoplankton. Hama and Handa (1987) estimated the turn-

over rate of organic constituents through phytoplankton photosynthesis in a eutrophic lake and obtained the rate of 0.0030 h^{-1} for DOC, which roughly coincides with the present result. In an experiment that was independent of phytoplankton excretion, Kirchman et al. (1991) estimated the turnover rate of DOC from the decrease in the DOC concentration in incubated samples (spring phytoplankton bloom, North Atlantic) and reported a rate in the range of 0.025–0.363 d^{-1}, which corresponds to 0.0010–0.015 h^{-1}. In a similar experiment with surface water from New Mexico, Amon and Benner (1994) estimated the turnover time of two DOC size fractions. They reported 6 and 26 d for HMW (>1000 Da) and LMW (<1000 Da) DOC, which corresponds to turnover rates of 0.0069 and 0.0016 h^{-1}, respectively. The rate obtained in the present study may not be directly compared with the rates reported so far, because our estimate took into consideration phytoplankton excretion as the sole source of DOC. Nevertheless, it is worth noting that the rates estimated here are comparable with those of Kirchman et al. (1991) and Amon and Benner (1994).

The turnover rates obtained for total DNAld (weighted mean value of eight DNAld) were 0.017–0.042 h^{-1}, which is 4.2–5.1 times higher than those of DOC. This result strongly suggests that carbohydrates constitute a bioreactive fraction of DOM. Concerning the bioreactivity of marine DOM, its relation with MW fractions has recently been studied, and HMW DOM was confirmed to have a more bioreactive nature than LMW DOM (Amon and Benner 1994; Santschi et al. 1995). The difference in the bioreactivity between the two MW fractions is probably due to the difference in the contribution of biomolecules such as carbohydrates (Benner et al. 1992; Amon and Benner 1994). The high turnover rate of carbohydrates found in the present study suggests that the greater bioreactivity of HMW DOM (Amon and Benner 1994; Santschi et al. 1995) reflects the property of DCHO, which are probably one of the major constituents in HMW DOM (Hama and Handa 1980; Benner et al. 1992; McCarthy et al. 1996).

The turnover rate of dissolved glucose showed the highest value (0.038–0.18 h^{-1}) among DNAld. The turnover rates of rhamnose, fucose, xylose, mannose, and galactose, which probably constituted the heteropolysaccharide of extracellular carbohydrates of diatoms, were almost comparable to each other and were considerably lower than that of glucose. The difference found in the rates between glucose and DNAld constituting heteropolysaccharides implies a difference in the bioreactivity of dissolved glucan and heteropolysaccharides in seawater. Phytoplankton storage glucan is considered one of the most bioreactive compounds, as has been observed in the vertical profile in the ocean (Handa and Yanagi 1969) and in decomposition experiments (Handa 1969; Matsunaga 1981). Considering the high contribution of glucose in extracellular DOM as found in the present study, it should be expected that the excreted glucan plays an important role in the biogeochemical cycle in the marine euphotic layer as a carrier of carbon and energy through a marine food web. Heteropolysaccharides, on the other hand, are possibly more resistant than glucan, judging from their lower turnover rate. Aluwihare and Repeta (1999) examined the compositional change in HMW DCHO through the decomposition process and reported the more resistant property of the heteropolysaccharides relative to total polysaccharide. The similarity in NAld composition between heteropolysaccharides from phytoplankton and those in DCHO and CCHO in surface seawater implies that heteropolysaccharides are more resistant to microbial degradation than glucan and hence can accumulate in seawater. Thus, the biogeochemical properties of dissolved glucan and heteropolysaccharide agree well with the range of turnover rates obtained in the present study.

This study suggests that the DCHO excreted by phytoplankton populations is mainly composed of two groups, glucan and heteropolysaccharides, and that the relative proportions of the two groups probably varies depending on factors such as phytoplankton biomass and nutrient availability. The apparent bioreactivity of excreted carbohydrates is likely related to the relative proportions of the two groups. Carlson et al. (1998) observed that the bioreactivity of DOM produced by a phytoplankton bloom differed in the Ross Sea polynya and the Sargasso Sea. The results obtained in the present study suggest that such differences in the bioreactivity of DOM originating from a phytoplankton bloom is due to the difference in the relative proportion of glucan and heteropolysaccharides.

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