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Decrease in molecular weight of photosynthetic products of marine phytoplankton during early diagenesis

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Abstract

Changes in the size and neutral aldose (NAld) composition of phytoplankton photosynthetic products during the early diagenetic process were experimentally examined using ^{13}C as a tracer. Most (94.7%) of the photosynthetically produced (P-) organic carbon (OC) was found to be particulate organic carbon (POC) after a 12-h light incubation. An increase in the concentration of P-dissolved organic carbon (DOC) was found after the shift to the dark incubation, probably due to a leakage of cellular constituents. An increase in the concentration of P-DOC was mainly due to an increase in the high molecular weight (HMW: >10 kDa) fraction that reached its maximum concentration on day 3 and then declined at a relatively high rate. The change in the concentration of the low molecular weight (LMW: <10 kDa) DOC fraction was less marked than that in the HMW fraction, and the rate of decrease was much slower, indicating a more biorefractory nature of P-LMW DOC. P-dissolved carbohydrates (DCHO) accounted for 38% and 50% in P-HMW DOC and P-LMW DOC, respectively, after a 12-h light incubation. The concentration of P-LMW DCHO showed a rapid decrease in the early stage of the dark incubation, whereas the concomitant increase in the concentration of P-LMW dissolved noncarbohydrate (DnonCHO) was noticed. The decrease in the contribution of CHO was noticed both in P-POC and P-HMW DOC fractions, but the decline rates were slower than that in P-LMW DOC fraction. On day 60, the remaining P-OC accounted for 4.6% of photosynthetic material originally produced. The distribution of the size fractions of the remaining P-OC on day 60 (POC, 14%; HMW DOC, 22%; LMW DOC, 64%) indicates that phytoplankton photosynthetic products were rapidly degraded to the less bioreactive LMW DOM during early diagenesis. The present results indicate that the annual global ocean production rate of semilabile DOC with lifetimes exceeding 2 months is 1.91 PgC yr^{-1}.

The composition, production, and recycling processes of marine dissolved organic matter (DOM) have recently received renewed attention because of the significance of DOM as an organic carbon reservoir on earth (Hedges 1992; Holmen 1992) and its possible relation to global change (Hedges 1992). However, the chemical composition, biogeochemical properties, and dynamics of DOM are still poorly understood (Williams 2000; Benner 2002).

The identification and quantification of organic molecules such as neutral aldoses (NAld, Ittekkot et al. 1981; McCarthy et al. 1996; Amon et al. 2001) and amino acids (AA, Lee and Bada 1977; Coffin 1989; McCarthy et al. 1996), all of which are abundantly found in organisms, have been carried out. Although NAld is so far the most abundant organic class molecularly identified as DOM, it accounts for roughly 10% of surface dissolved organic carbon (DOC) (Benner 2002). Although AA accounts for more than 50% of the cellular organic compounds (Wakeham et al. 1997), the contribution of AA to the extracellular pool is generally lower than that of NAld (Benner 2002). Thus, a major discontinuity is found in the contribution of these biomolecules to cellular material of microorganisms and that of the surrounding waters.

The discontinuity in the organic composition between marine microorganisms and DOM is also found in the molecular weight (MW) composition of organic matter. Microorganisms are mainly composed of high molecular weight (HMW) macromolecules such as polysaccharides and proteins, and low molecular weight (LMW) metabolites usually constitute a relatively small pool. In marine DOM, on the other hand, LMW DOM constitutes the major fraction, while the contribution of HMW DOM accounts for only a minor portion even in the surface layer (Benner et al. 1992; Ogawa and Ogura 1992; Guo et al. 1995).

Amon and Benner (1996) compared the size and bioreactivity of DOM from freshwater and marine systems and proposed a conceptual ''size-reactivity model'' whereby ''the bioreactivity of organic matter decreases along a continuum of size and diagenetic state.'' This model agrees with recent findings concerning the relationship between the bioreactivity and MW composition of marine DOM (Benner et al.

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1992; Amon and Benner 1994; Santschi et al. 1995; Guo et al. 1996). However, the shift in the size distribution of photosynthetic products from phytoplankton, which is the ultimate source of organic matter in the pelagic area, toward LMW DOM has not been experimentally elucidated. Furthermore, the time scale of this size-distribution shift remains unknown.

Lara and Thomas (1995) examined the change in the composition of photosynthetic products of marine diatom *Thalassiosira tumida* in early diagenesis. They fractionated ^{14}C-labeled dissolved products by XAD resin and observed that a substantial fraction of the photosynthetic products remained as hydrophobic DOM. Their results indicate structural rearrangements of photosynthetic products in early diagenesis and formation of refractory material but afford little information on the change in the MW composition of photosynthetic products.

In the present study, we collected natural plankton populations and labeled their photosynthetically produced organic compounds with ^{13}C. The samples were then incubated in the dark for 2 months, and the fates of the photosynthetic products were followed in the MW and NAld fractions by the combined methods of ^{13}C tracer, tangential flow, and gas chromatography/mass spectrometry (GC/MS) to elucidate the shift in the size distribution and NAld composition of phytoplankton photosynthetic products.

Materials and methods

Incubation—Natural phytoplankton populations were collected from eutrophic Hakata-Bay, Kyushu, Japan at 0600 h on 27 July 2001 during a bloom of the diatom *Skeletonema costatum.* Macrozooplankton were removed using a 100-μm nylon mesh screen; the sample was transferred to a polyethylene bag and enriched with KNO_3, KH_2PO_4, and Na_2SiO_3 to final concentrations of 23.8, 2.36, and 12.6 μmol L^{-1}, respectively. After the addition of $NaH^{13}CO_3$ (the ^{13}C atom percentage was 15% of the dissolved inorganic carbon [DIC]), phytoplankton populations were incubated under fluorescent light (160 μmol quanta m^{-2} s^{-1}) at 27°C, which corresponded to the surface temperature of the sampling site, to label the newly photosynthetically produced organic matter with ^{13}C. A subsample was collected at day 0.5, and the remaining sample was incubated in the dark for 60 d. Subsamples were collected at intervals of 1 to 30 d.

Analysis—After the organic matter (OM) was separated into particulate organic matter (POM) and DOM by filtration over a precombusted glass fiber filter (Whatman GF/F), the filtrates were desalted by electrodialysis (Microacilizer S-3, Asahi Chemical; Hama and Yanagi 2001). DOM in the desalted filtrates was fractionated into HMW (>10 kDa) and LMW (<10 kDa) fractions using a tangential flow cartridge (Millipore PLGC 10k). The recovered concentrations of DOC ranged from 94% to 111%, with a mean value of 102% ($\pm$7.4 as 1 σ, n = 7). The concentrations and ^{13}C atom percentage of particulate and dissolved organic carbon (POC and DOC, respectively) were measured by a mass spectrometer with an elemental analyzer (DELTAplus, Thermo Finnigan) with the methods described by Hama and Yanagi (2001).

Particulate and dissolved carbohydrates (PCHO and DCHO, respectively) were hydrolyzed with 1 N H_2SO_4 at 100°C for 5 h, and the concentration and ^{13}C atom percentage of neutral aldoses (NAlds) such as rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, and glucose were determined using GC/MS (Voyager, Thermo Finnigan). The detailed analytical procedures have been described elsewhere (Hama and Yanagi 2001).

Calculation—The concentration of photosynthetically produced organic matter (P-OM) on the sampling day was calculated as follows.

$$\text{concentration of P-OM } (\mu\text{g C L}^{-1}) = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times [\text{C}] \quad (1)$$

where a_{is} is the ^{13}C atom percentage in an incubated sample, a_{ns} is the ^{13}C atom percentage in a natural (nonincubated) sample, a_{ic} is the ^{13}C atom percentage in DIC, and [C] is the concentrations (μg C L^{-1}) of POC, DOC, and particulate or dissolved NAld (PNAld or DNAld, respectively) in the incubated samples (Hama and Yanagi 2001). The ^{13}C atom percentage of DIC was applied to dissolved products as well as particulate products, probably resulting in an underestimation of the production of dissolved organic components, especially the production of HMW compounds (Hama and Yanagi 2001). All determinations, including those of the ^{13}C atom percentage of POC, DOC, and NAld, were done in duplicate, and the coefficient of variation in the concentration of P-OM was generally less than 5% (Hama and Yanagi 2001).

Concentration of inorganic nutrients and chlorophyll a—The concentration of inorganic nutrients such as nitrate, phosphate, and silicate was determined by the methods described in Parsons et al. (1984). The concentration of chlorophyll *a* (Chl *a*) was determined by fluorometry after extraction with 90% acetone (Parsons et al. 1984).

Results

Chl a *and inorganic nutrients*—A rapid growth in the phytoplankton population occurred during light incubation, and the concentration of Chl *a* increased from 55 μg L^{-1} on day 0 to 112 μg L^{-1} on day 0.5. Inorganic nutrient concentration

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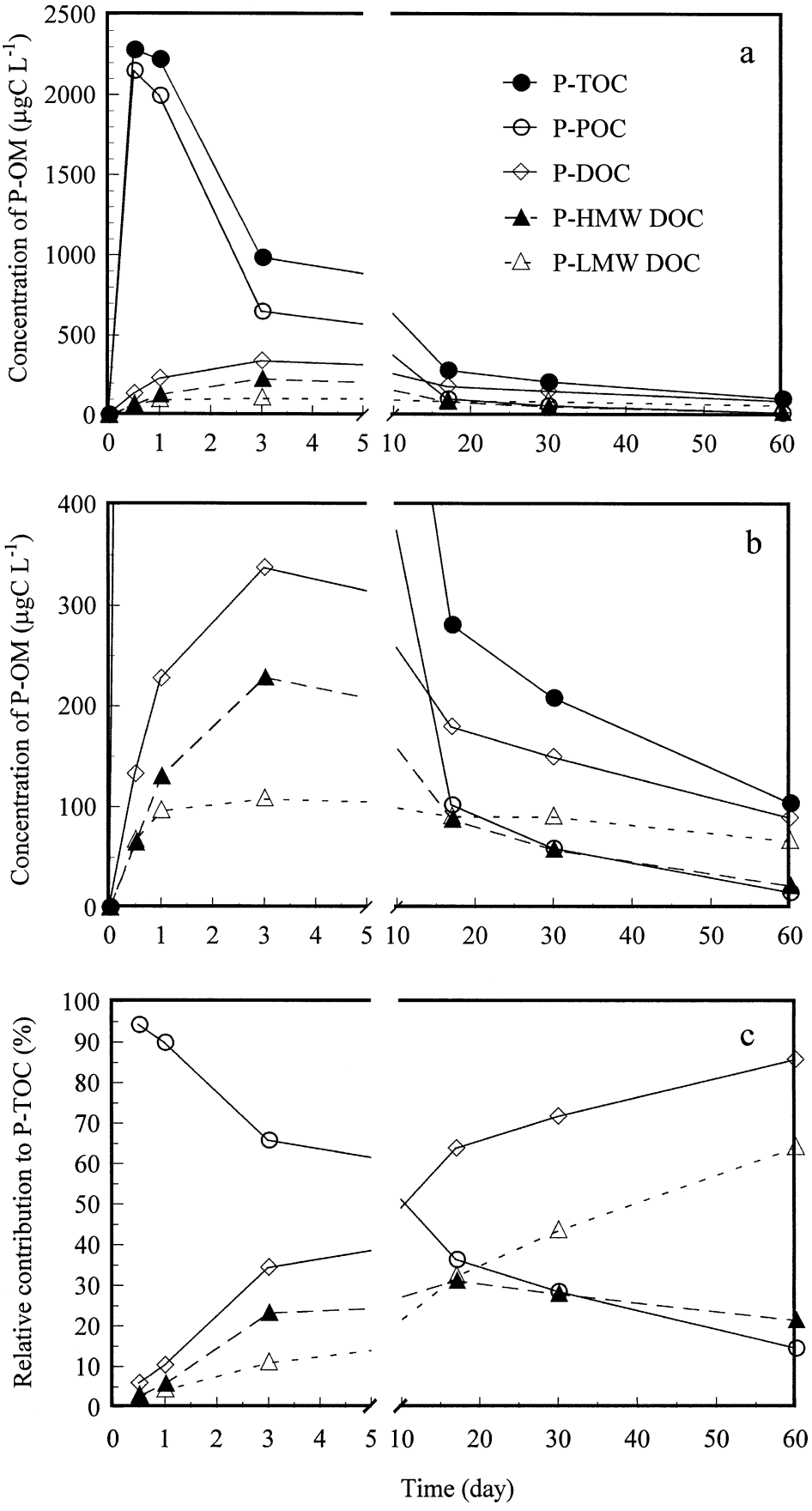
Fig. 1. Changes in the concentrations of photosynthetically produced total organic carbon (P-TOC), particulate organic carbon (P-POC), dissolved organic carbon (P-DOC), high molecular weight DOC (P-HMW DOC), and low molecular weight DOC (LMW DOC) during the incubation experiment (a and b; b enlarges a segment of a). (c) The relative contribution of each size fraction to P-TOC on the sampling day.


a
P-TOC
P-POC
P-DOC
P-HMW DOC
P-LMW DOC
Concentration of P-OM (μgC L-1)
b
Concentration of P-OM (μgC L-1)
c
Relative contribution to P-TOC (%)
Time (day)

showed a rapid decrease concomitant with the increase in phytoplankton biomass. The remaining nutrient concentrations on day 0.5 were nitrate, 5.0; phosphate, 0.11; and silicate, 5.68 μmol L^{-1}.

Photosynthetically produced total, particulate, and dissolved organic carbon (P-TOC, P-POC, and P-DOC)—The time course in the concentration of P-OM shown in Fig. 1 is divided into three phases: phase 1 (day 0 to 0.5 in the light), phase 2 (days 0.5 to 3 in the dark), and phase 3 (days 3 to 60 in the dark).

Phase 1: The concentration of P-TOC was 2270 μg C L^{-1} at the end of phase 1, and 94.7% of the photosynthetic products were found to be P-POC. The excreted fraction during the 12-h incubation period was 5.3%, being almost comparable with the results using ^{14}C as a tracer (Baines and Pace 1991).

Phase 2: The concentrations of P-TOC and P-POC rapidly decreased during phase 2. The concentration of P-TOC decreased by 1300 μg C, equivalent to 57% of P-TOC on day 0.5, during this period. This decrease in P-TOC concentration was exclusively due to the decline in P-POC, and only 30% of initially produced P-POC remained as P-POC on day 3. Concomitant with the decrease in P-POC, P-DOC increased from 227 to 337 μg C L^{-1}. This increase corresponded to 13% of the decrease in P-POC during the same period.

Phase 3: The concentrations of P-TOC and P-POC continued to decrease throughout this phase, although their rate of decrease declined after day 3. The rate decrease in P-DOC concentration was slower than those of P-TOC and P-POC. As a result, the P-DOC concentration exceeded that of P-POC on day 17, with the predominance of P-DOC increasing toward the end of this phase. The concentration of P-TOC remaining on day 60 was 104 μg C L^{-1}, which corresponded to 4.6% of its concentration on day 0.5. In other words, 95.4% of P-TOC had been respired after 60 d. After 60 d, most of the P-OM was in dissolved form and P-POC only accounted for 14%.

Molecular weight composition of P-DOM—Phase 1: Almost equal concentrations of P-HMW DOC and P-LMW DOC were found in phase 1, showing that phytoplankton populations excreted photosynthetic products with a variable MW (Fig. 1).

Phase 2: The concentration of P-HMW DOC increased in phase 2 and reached a maximum of 229 μg C L^{-1} on day 3, which was 3.5 times higher than its value on day 0.5. This increase accounted for 80% of the P-DOC increase in this phase. Although P-LMW DOC as well as P-HMW DOC showed an increase in this phase, the formation rate of P-LMW DOC was lower than that of P-HMW DOC. P-HMW DOC and P-LMW DOC accounted for 68% and 32% of P-DOC, respectively, on day 3, when P-DOC reached its maximum concentration.

Phase 3: The concentration of P-HMW DOC rapidly decreased in phase 3 from 229 μg C L^{-1} on day 3 to 22 μg C L^{-1} on day 60. Only 10% of the maximum concentration found on day 0.5 remained on day 60. In contrast, the concentration of P-LMW DOC decreased very slowly in this phase, and its contribution exceeded that of P-HMW DOC on day 17. On day 60, the concentration of P-LMW DOC was three times higher than that of P-HMW DOC, accounting for 75% and 64% of P-DOC and P-TOC, respectively.

Photosynthetically produced carbohydrates (P-CHO)—Phase 1: CHO accounted for the major products in both P-POM and P-DOM (Fig. 2). Photosynthetically produced particulate carbohydrates (P-PCHO), which was calculated by summing up the concentrations of the eight NAlds, accounted for 58% of P-POC in this phase. CHO was also the main component of P-DOC, accounting for 45%, a value almost comparable with results obtained previously (Hama and Yanagi 2001). The aldose yield was higher in the LMW fraction than in the HMW fraction, accounting for 50% and 38%, respectively (Fig. 3).

A definite difference in the NAld composition was found between the P-HMW DCHO and the P-LMW DCHO (Table 1). Fucose, galactose, and glucose dominated in the HMW fraction. Fucose had the highest concentration in P-HMW DCHO but accounted for less than 1% in the LMW fraction. Glucose accounted for 78% of P-LMW DCHO.

Phase 2: The concentration of P-PCHO showed a drastic decrease during dark incubation, declining from 1240 μg C L^{-1} on day 0.5 to 194 μg C L^{-1} on day 3. This decrease was exclusively due to a decrease in glucose concentrations. The contribution of glucose to P-PCHO fell from 89% to 35% during this stage.

Concomitant with the decrease in P-PCHO in this phase, P-HMW DCHO increased at a rate that accounted for 27% of the total increase in the concentration of P-HMW DOC. The increase in P-HMW DCHO in this phase was mainly due to the increase in the concentration of fucose, xylose, mannose, and galactose, and they totally accounted for 80% of P-HMW DCHO.

The concentration of P-LMW DCHO decreased in this phase, although P-LMW DOC showed a slight increase. Thus, the aldose yield in the P-LMW DOC fraction decreased from 50% on day 0.5 to 13% on day 3. The decrease in aldose yield was due solely to the rapid reduction in glucose concentration. The dominance of glucose had disappeared by day 3, and the NAld composition of P-LMW DCHO on that day was roughly comparable to that of P-HMW.

Phase 3: The concentration of P-PCHO decreased to 3.0 μg C L^{-1} on day 60, which only accounted for 0.24% of the maximum concentration of P-PCHO on day 0.5. The contribution of P-PCHO to P-POC decreased to 22% in the early period of phase 3 and showed no significant change through the rest of phase 3.

The concentration of P-DCHO showed a rapid decrease

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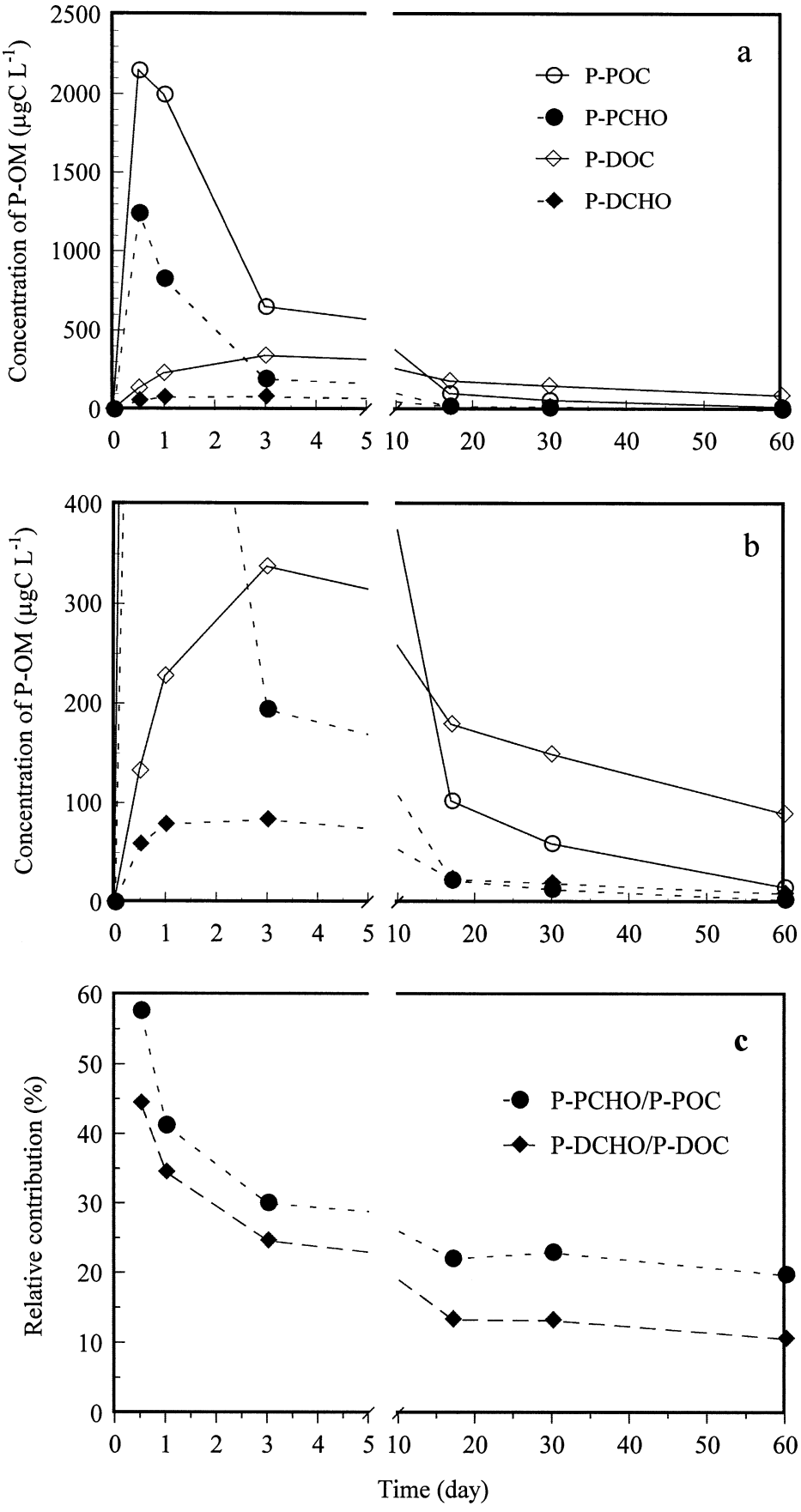
Fig. 2. Changes in the concentrations of P-POC, photosynthetically produced particulate carbohydrate (P-PCHO), P-DOC, and photosynthetically produced dissolved carbohydrate (P-DCHO) (a and b; b enlarges a segment of a). (c) P-PCHO/P-POC and P-DCHO/P-DOC.


a
P-POC
P-PCHO
P-DOC
P-DCHO
Concentration of P-OM (μgC L-1)
2500
2000
1500
1000
500
0
0 1 2 3 4 5 10 20 30 40 50 60
b
Concentration of P-OM (μgC L-1)
400
300
200
100
0
0 1 2 3 4 5 10 20 30 40 50 60
c
P-PCHO/P-POC
P-DCHO/P-DOC
Relative contribution (%)
60
50
40
30
20
10
0
0 1 2 3 4 5 10 20 30 40 50 60
Time (day)

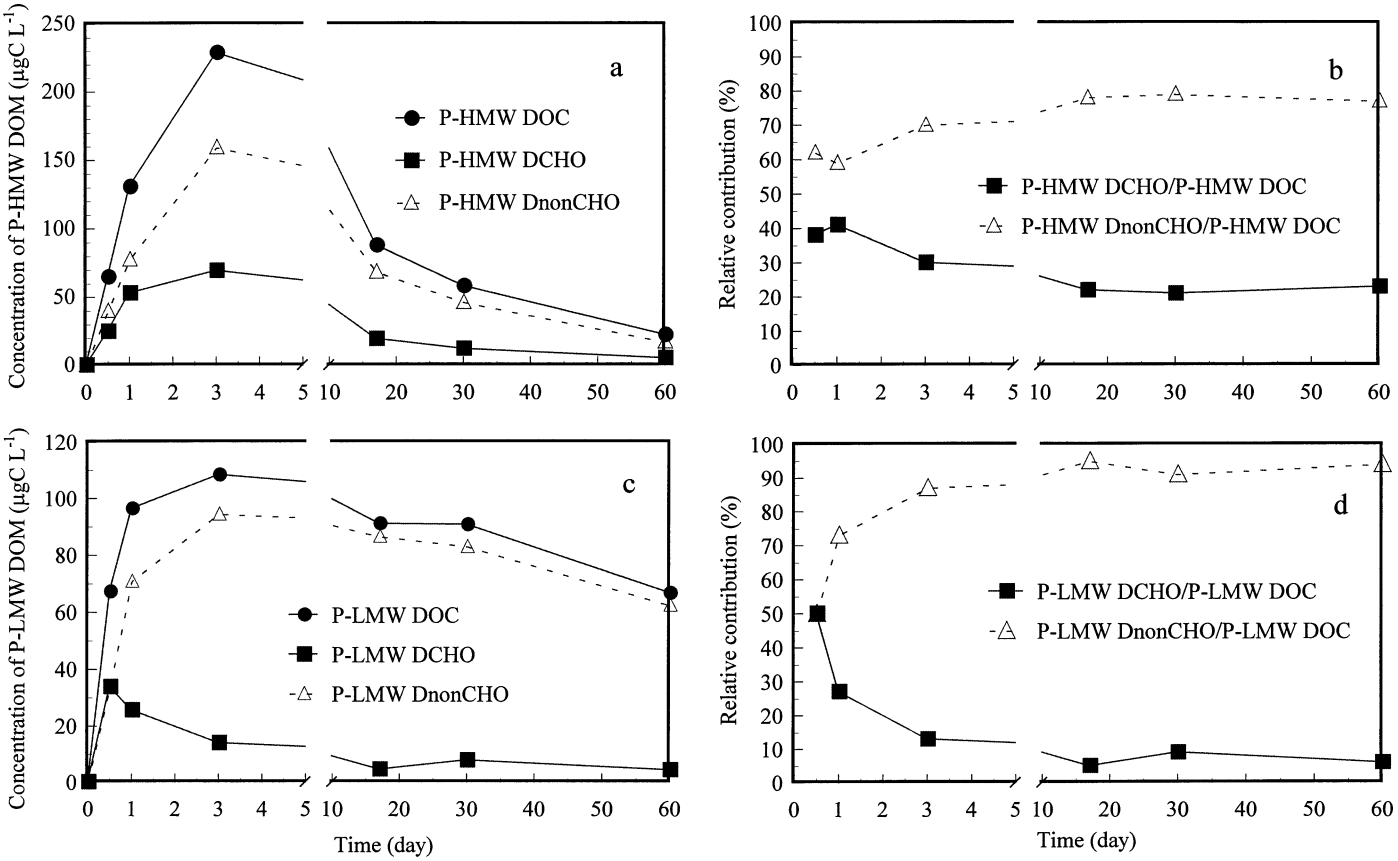


Fig. 3. Changes in the concentrations of P-DOC, P-DCHO, and photosynthetically produced dissolved noncarbohydrate (P-DnonCHO), obtained for the HMW and LMW fractions (a and c, respectively), and the relative contributions of P-DCHO and P-DnonCHO to P-DOC, for HMW and LMW fractions (b and d, respectively).

in the early stage of phase 3 from 83.4 μg C L^{-1} on day 3 to 23.9 μg C L^{-1} on day 17 and showed a slower decrease to 9.5 μg C L^{-1} by day 60. The concentration of P-HMW DCHO also showed a considerable decrease in this phase. The concentration of P-LMW DCHO showed a decrease from day 3 to day 17 but remained constant thereafter. No clear changes in the NAld composition of P-HMW and P-LMW DCHO were found in phase 3. On day 60, the aldose yield in the DOC fraction had decreased to 10%. The aldose yield in the HMW fraction was relatively high (23%), while the aldose yield in the LMW fraction was low (6.4%).

Turnover time of P-OM—The turnover time of P-OM for each size class and organic compound class was calculated by dividing the maximum concentration (day 0.5 for P-POM and day 3 for P-DOM) by the degradation rates in the periods subsequent to the time of maximum concentration (days 0.5–3 for P-POM and days 3–17 for P-DOM), as shown in Table 2. Very short turnover times of around 3 d were estimated for particulate products, indicating that photosynthetically incorporated carbon was rapidly respired and dissolved during dark incubation.

In contrast, P-DOC had about 10 times longer turnover time than P-POC. Within the P-DOC pool, P-HMW DOC had a shorter turnover time than P-LMW DOC, indicating it was more bioreactive than P-LMW DOC. We can define a fraction called P-HMW dissolved noncarbohydrate (P-HMW DnonCHO), calculated as the difference between P-HMW DOC and P-HMW DCHO carbon. A P-LMW DnonCHO can be defined in a similar way. In the HMW fraction, the turnover time of P-HMW DnonCHO was similar to P-DCHO. In other words, P-HMW DnonCHO had bioreactive properties comparable to P-HMW DCHO. In contrast, the turnover times of P-LMW DCHO and P-LMW DnonCHO were strikingly different from each other (6 and 167 d, respectively). The turnover time of bulk P-LMW DOC did not reflect the bioreactive properties of P-LMW DCHO because of the low contribution of DCHO to LMW DOC. Thus, the refractory character of P-LMW DOC was due to the P-LMW DnonCHO components.

Discussion

Shift in MW distribution—Knowledge concerning the size distribution of marine DOM and its relation to bioreactivity has rapidly accumulated during this decade. Marine DOM is mainly composed of LMW DOM, whereas HMW DOM accounts for only a minor portion (Benner et al. 1992; Ogawa and Ogura 1992; Guo et al. 1995; Amon and Benner 1996; Benner 2002). The refractory property of bulk LMW DOM has been established by decomposition experiments (Amon and Benner 1994), turnover time due to phytoplankton ex-

Table 1. Neutral aldose (NAld) composition of photosynthetically produced particulate carbohydrates (P-PCHO), dissolved carbohydrates (P-DCHO), high molecular weight (HMW), and low molecular weight (LMW) DCHO. (Percent of total NAld in each fraction.)

	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total NAld (μg C L^{-1})
Day 0.5									
POM	0.5	1.1	1.6	0.1	1.4	2.1	4.0	89.2	1240
DOM	2.8	14.5	1.4	0.5	2.3	7.5	15.0	56.0	58.7
HMW	5.0	33.4	0.3	0.3	3.5	8.2	22.8	26.4	24.9
LMW	1.2	0.5	2.2	0.6	1.5	7.1	9.2	77.8	33.8
Day 1									
POM	1.2	2.3	3.0	0.3	2.4	4.1	5.6	81.1	823
DOM	5.2	34.9	2.3	1.0	3.8	4.8	21.8	26.2	78.6
HMW	6.5	50.5	0.4	0.5	3.4	4.9	30.1	3.7	53.0
LMW	2.5	2.6	6.0	2.1	4.5	4.5	4.7	73.1	25.6
Day 3									
POM	7.2	8.3	7.9	1.2	7.5	15.0	18.1	34.8	194
DOM	11.1	24.2	1.6	3.1	14.0	19.1	20.2	6.8	83.4
HMW	10.9	25.7	0.4	2.4	13.8	18.9	21.8	6.1	69.5
LMW	12.0	16.7	7.3	6.4	15.1	19.9	12.4	10.1	13.9
Day 17									
POM	3.6	8.5	8.4	3.8	15.8	10.8	9.1	40.0	22.5
DOM	13.2	12.6	2.3	8.3	18.5	15.5	17.5	12.1	23.9
HMW	9.9	11.6	0.9	7.8	20.8	16.8	19.1	13.1	19.4
LMW	27.0	16.7	8.2	10.5	8.7	10.3	11.0	7.6	4.5
Day 30									
POM	6.4	13.4	13.1	1.7	18.7	14.6	11.9	20.2	13.6
DOM	14.5	12.6	4.0	12.4	16.9	12.6	13.9	13.1	19.9
HMW	11.9	11.6	1.1	9.4	19.4	14.0	15.6	17.0	12.1
LMW	18.5	14.1	8.5	17.1	12.9	10.4	11.3	7.2	7.8
Day 60									
POM	8.6	4.7	4.5	8.3	13.5	14.8	14.5	31.1	3.0
DOM	11.8	19.1	6.2	11.9	13.9	10.3	16.0	10.8	9.5
HMW	11.6	15.2	1.5	9.1	15.5	12.2	17.8	17.1	5.2
LMW	15.0	23.9	11.8	15.3	12.0	8.0	13.8	6.2	4.3

cretion (Hama 2000), vertical distribution (Benner et al. 1997), and the ^{14}C age (Santschi et al. 1995). These recent results strongly suggest that LMW DOM is the possible end product of microbial diagenetic processes in the water column. The present study clearly shows that the size shift from newly produced, particulate and HMW, photosynthetic products toward LMW DOM proceeds rapidly during early diagenesis.

Amon and Benner (1996) estimated the bioreactivity of size-fractionated DOM collected from various aquatic environments by comparing the oxygen consumption, bacterial abundance, and bacterial production among the different size fractions. The higher lability of HMW DOM was indicated in all samples, leading the authors to propose a ''size-reactivity model,'' according to which labile HMW OM changes successively to refractory LMW OM along a diagenetic continuum. This model, however, was conceptual and has not been confirmed by time-series experiments on the changes in MW composition and bioreactivity in the diagenetic processes. The present study is the first that experimentally demonstrates the shift from fresh, phytoplankton, particulate, photosynthetic products toward LMW DOM during early diagenetic processes, thus confirming the model proposed by Amon and Benner (1996).

Most of the freshly produced organic matter is composed of particulate cellular biopolymers. The concentration of POC generally accounts for 1–10% of TOC in ocean waters (Williams 1971), even in the surface layer where the abundance of microorganisms is the highest through the water column. The size distribution (POC, 14%; DOC, 86%) obtained on day 60 shows that the size distribution of P-OM rapidly approximates that of OM in the ocean surface layer. Furthermore, the MW distribution of P-DOC (HMW, 25%; LMW, 75%) on day 60 is roughly comparable to the MW distribution of DOC in the surface layer. Thus, the present study demonstrates that the size distribution of phytoplankton photosynthetic products rapidly shifts toward that commonly observed in surface oceanic waters.

Composition and bioreactivity of P-HMW DOM—The present study indicates that the major part of HMW DOM exists in a transitional stage in the diagenetic process of phy-

Table 2. Turnover time (in days) of photosynthetically produced organic matter in each size fraction. Turnover times are calculated by dividing the maximum concentrations (day 0.5 for the P-POM and day 3 for the P-DOM) by the declining rates in the following periods (days 0.5–3 for P-POM and days 3–17 for P-DOM).

			HMW fraction (>10 kDa)			LMW fraction (<10 kDa)		
P-POC	P-PCHO	P-DOC	P-DOC	P-DCHO	P-DnonCHO	P-DOC	P-DCHO	P-DnonCHO
3.6	3.1	30	23	19	25	88	6	167

toplankton photosynthetic products. The increase in the concentration of P-HMW DOC within a few days of dark incubation was concomitant with the rapid decrease in P-POC concentration. This suggests that the newly synthesized cellular macromolecules are released as P-HMW DOM, probably due to the lysis of phytoplankton cells. Thus, the organic composition of P-HMW DOM in phases 1 and 2 should highly resemble that of P-POM. Carbohydrates are major components of fresh P-HMW DOM, accounting for 30–40% in phases 1 and 2, which agrees with the high CHO contribution in P-POM in the same period (38–58%). Although the contribution of photosynthetically produced proteinaceous substances was not estimated in the present study, proteins may be another major category of photosynthetic products. This suggests that proteins are likely the main components of noncarbohydrate P-HMW DOM. P-HMW DCHO and P-HMW DnonCHO have similar turnover times, and this is probably due to the fact that biomacromolecules constitute the major part of P-HMW DOM.

Composition and bioreactivity of P-LMW DOM—In contrast to P-HMW DOC, the concentration of P-LMW DOC showed no drastic change during dark incubation. However, this does not imply that the production and decomposition of P-LMW DOM did not occur. P-LMW DCHO accounted for 50% of P-LMW DOC on day 0.5 and showed a marked concentration decrease during phase 2. P-LMW DnonCHO was calculated from the difference between P-LMW DOC and P-LMW DCHO. Hence, it was the slight increase in P-LMW DOC during this period, concomitant with a decrease in P-LMW DCHO, that indicated an increase in P-LMW DnonCHO. P-LMW DnonCHO, thus, became the main component of P-LMW DOC instead of CHO.

The estimated turnover time of P-LMW DOC is much longer than that of P-HMW DOC, and this agrees well with the lower bioreactivity of LMW DOC compared to HMW DOC (Amon and Benner 1994; Benner 2002; Carlson 2002). It is apparent that the low bioreactivity of P-LMW DOC is exclusively due to P-LMW DnonCHO; the high bioreactivity of P-LMW DCHO is not reflected in the property of total P-LMW DOC due to its low contribution. The high contribution of uncharacterized compounds to LMW DOC can probably be generalized to aquatic environments (Hama and Handa 1987; Benner 2002), and it characterizes the low bioreactivity of LMW DOC.

Only a little information has been accumulated on the chemical characteristics of the transformed organic compounds from phytoplankton photosynthetic products. Lara and Thomas (1995) fractionated DOC in the culture medium of nonaxenic culture of *T. tumida,* by XAD resin. During the first 20-d light incubation with ^{14}C, the hydrophobic fraction, especially hydrophobic acid and neutral fractions, accounted for the major part of $DO^{14}C$. These fractions are operationally defined as ''humic'' materials, and their results indicate that humic materials are produced on relatively short time scales. The combination of the traditional fractionation with XAD resin and the prevailing MW fractionation can give significant information on the chemical properties of noncharacterized DOM, which appeared in the early diagenesis of phytoplankton organic carbon.

Transformation to P-LMW DnonCHO—The present study clearly shows that the low bioreactivity of LMW DOC was established only a few days after the photosynthetic organic carbon production. The concentration of P-LMW DnonCHO, which exhibited the longest turnover time, increased from 33.4 μg C L^{-1} on day 0.5 to 94.4 μg C L^{-1} on day 3. It is unlikely that the LMW metabolites, as constituents of phytoplankton cells, have low bioreactivity. Thus, it is suggested that the transformation from compounds with high bioreactivity, which are abundant in phase 2, to compounds with low bioreactivity occurs during this period.

The participation of bacteria in the transformation of simple organic compounds to refractory ones has been experimentally suggested (Brophy and Carlson 1989; Tranvik 1993; McCarthy et al. 1998; Ogawa et al. 2001). Brophy and Carlson (1989) observed that ''high'' MW (0.7–1.4 kDa; note that this molecular weight range is included in the LMW fraction in the present study) DOM was biologically derived from simple molecules such as glucose and leucine. This transformation of dissolved products reportedly occurred in as few as 3 d from the addition of glucose or leucine to the natural microbial population. Other experiments (Stoderegger and Herndl 1998; Ogawa et al. 2001) also showed the release of refractory DOM within a few days after the addition of glucose or free amino acids. These time scales are comparable with the results obtained in the present study.

Brophy and Carlson (1989) found that bacterially transformed products persisted for 6 months, indicating their biorefractory nature. The refractory property of bacterial products was recently ascertained by the experiments of Ogawa et al. (2001). Their findings are comparable with the results obtained in this study, showing that the turnover time of P-LMW DnonCHO was the longest among the organic fractions. Thus, it is possible that the increase in P-LMW DnonCHO found in phase 2 reflects the bacterial transformation of organic matter produced by phytoplankton photosynthesis. It is a matter of speculation whether bacteria are involved in the production of P-LMW DnonCHO in the pre-

sent study. Nevertheless, recently accumulated knowledge on the bacterial role in the production of refractory DOM (McCarthy et al. 1998; Dittmar et al. 2001) strongly suggests that bacteria play an important role in the transformation of organic materials in the early diagenetic processes.

Bioreactivity of DCHO—The present study clearly shows that the NAld composition and bioreactivity of DCHO differ between the HMW and LMW fractions. The composition of the HMW fraction was dominated by fucose, rhamnose, xylose, mannose, and galactose, suggesting that dissolved carbohydrates in the HMW fraction were mainly composed of heteropolysaccharides from diatoms (Hama and Yanagi 2001). The MW of these heteropolysaccharides is higher than 10 kDa, since such NAlds were rarely found in P-LMW DCHO. Heteropolysaccharides are probably produced as extracellular polymeric substances (EPS) by photosynthesizing diatoms (Decho 1990; Hama and Yanagi 2001). The NAld composition of P-HMW DCHO in phase 2, when the concentration of P-HMW DCHO increased, was also comparable with the structural CHO of diatoms (Handa 1969; Allan et al. 1972; Hama and Handa 1992). Thus, the increase in the P-HMW DCHO may reflect the dissolution of structural components of phytoplankton due to cell lysis.

The turnover time of P-HMW DCHO was considerably longer than that of P-LMW DCHO, indicating heteropolysaccharides are less bioreactive than P-LMW DCHO. Hama and Yanagi (2001) estimated the turnover rates of DNAld through the excretion by phytoplankton. DNAlds, which were components of heteropolysaccharides, had lower turnover rates than glucose by roughly one order of magnitude. In decomposition experiments, on the other hand, Aluwihare and Repeta (1999) measured the compositional change in HMW DCHO and reported that heteropolysaccharides are more refractory than total polysaccharides. Thus, the more refractory character of P-HMW DCHO compared with P-LMW DCHO is consistent with the previous reports.

In contrast, glucose accounted for the largest fraction of P-LMW DCHO in the early phases of the experiments. A high fraction of glucose in excreted DCHO was also observed by Hama and Yanagi (2001), who suggested that storage glucan was excreted as an overflow of cellular components under nutrient-depleted conditions. Hama and Handa (1992) reported that the MW of hot-water-extractable polysaccharides from a natural phytoplankton population dominated by *S. costatum* and *Heterosigma* sp. ranged from 1 to 6 kDa. Thus, in the present study, storage glucan is presumably contained in the LMW ($<$10 kDa) fraction. In addition to glucose polymers, monomers and oligomers of glucose are probably included in this fraction, although their relative composition was not estimated in the present study.

The high bioreactivity of dissolved glucose and/or its polymers is clearly shown in the present study. Photosynthetically produced dissolved glucose in the LMW fraction was nearly undetectable by day 3, with only 5% of the maximum concentration on day 0.5 remaining on day 3. When the turnover time is estimated using the concentration on day 0.5, when the concentration of P-LMW DCHO showed a maximum, and the rate of decline from day 0.5 to 3, a quite short turnover time of 2.6 d is obtained for P-LMW DCHO. This turnover time is shorter than those of P-POC and P-PCHO. Thus, P-LMW dissolved glucose (possibly including oligomers and polymers) is possibly one of the most labile and important compounds supporting bacterial production and the microbial food web (Rich et al. 1996; Hama and Yanagi 2001).

The biogeochemical importance of the labile organic compounds such as glucose and/or its polymers cannot be elucidated by the measurements of their concentrations in seawater. Their concentrations are usually very low due to their high turnover rates, but that does not imply insignificant roles in the biogeochemical cycle. Their importance can be demonstrated by estimates of the production rate and the assimilation rate from the dissolved pool as in the present study. However, more frequent sampling in the early stage of the experiments is likely suitable to determine dynamics of labile molecules with turnover time of less than a day.

Although the aldose yields in the P-POC and P-HMW DOC fractions tended to decrease with time, the high yields were maintained during early diagenesis and were as high as 20% even at the end of the experiment. In contrast, the aldose yield in the P-LMW DOC fraction showed a drastic decrease in the early part of the experiment from 50% on day 0.5 to 4.9% on day 17. The resultant higher aldose yields in the P-POC and P-HMW DOC fractions than in the P-LMW DOC fraction lead to higher bioreactivity of the POC and HMW fractions. This indicates that the difference in the abundance, composition, and bioreactivity of carbohydrates among each size fraction is a major factor for the decrease in the bioreactivity of OM along a continuum of size and diagenetic state.

Estimate of global production of semilabile DOM—When we regard the concentration of photosynthetic products on day 0.5 as 100%, 4.6% of the organic photosynthetic products would remain after 60 d. P-DOC predominated the remaining P-OC on day 60, accounting for 3.9% of P-OC on day 0.5, while P-HMW DOC and P-LMW DOC accounted for 1.0% and 2.9%, respectively. DOC with a residence time of months to a year has been referred to as semilabile DOC (Carlson and Ducklow 1995), and its importance in the global marine carbon cycle, in processes such as vertical transport of carbon (Carlson et al. 1994) and horizontal advection (Hansell and Waterhouse 1997), has been suggested. P-OM remaining on day 60 can be called semilabile OM because its lifetime is more than 2 months. However, this fraction probably also includes refractory OM with a lifetime of more than a year.

When we posit that the annual global ocean productivity is 48.5 PgC yr^{-1} (Field et al. 1998), and that the partitioning of primary products in each size fraction during the early diagenetic processes obtained in the present study is applicable throughout the world's oceans, the following production rates of OC with lifetimes exceeding 2 months are estimated: TOC, 2.23; POC, 0.32; DOC, 1.91; HMW DOC, 0.48; and LMW DOC, 1.43 PgC yr^{-1}. It is noteworthy that our DOC value is roughly comparable to the estimated net DOC production in the upper layer of the world's oceans (1.2 PgC yr^{-1}, Hansell and Carlson 1998).

There is a considerable difference between the production

rates of DOM with a lifetime of more than 2 months (1.91 PgC yr^{-1}) and the refractory DOM (~0.1–0.2 PgC yr^{-1}), which is calculated by dividing the amount of carbon in the global DOC pool (685 PgC; Hansell and Carlson 1998) by the ^{14}C age of deep water DOC (4000–6000 yr; Williams and Druffel 1987; Druffel et al. 1992). The large difference in the production rates between these two pools implies that about 90% of the DOM exported to the ocean's interior will further decompose over an extremely long time. It goes without saying that although this process cannot be experimentally followed, the elucidation of the molecular structure of refractory DOM, the microbiological group(s) involved in its transformation, and the mechanisms protecting DOM against microbial activity would greatly improve our understanding of the marine carbon cycle and the role of DOC as the carbon reservoir of the earth.

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