Changes in the Chemical and Size Composition of Phytoplanktonic Organic Matter during the Microbial Decomposition Process

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Contents

Abbreviations

POM・・・・・・・・・・・・・・・・・・・・・・・・・Particulate Organic Matter

POC・・・・・・・・・・・・・・・・・・・・・・・・Particulate Organic Carbon

P-POC・・・・・・・・・・・・・・・・・・・・Photosynthetically produced POC

PON・・・・・・・・・・・・・・・・・・・・・・・・Particulate Organic Nitrogen

DOM・・・・・・・・・・・・・・・・・・・・・・・・Dissolved Organic Matter

P-DOM・・・・・・・・・・・・・・・・・・Photosynthetically produced DOM

DOC・・・・・・・・・・・・・・・・・・・・・・・・Dissolved Organic Carbon

P-DOC・・・・・・・・・・・・・・・・・・・Photosynthetically produced DOC

TOC・・・・・・・・・・・・・・・・・・・・・・・・Total Organic Carbon

P-TOC・・・・・・・・・・・・・・・・・・・Photosynthetically produced TOC

P-OM・・・・・・・・・・・・・・・・・Photosynthetically produced Organic Matter

P-OC・・・・・・・・・・・・・・・・・Photosynthetically produced Organic Carbon

HMW-DOM・・・・・・・・・・・・・・・・・・・High Molecular Weight DOM

HMW-DOC・・・・・・・・・・・・・・・・・・・High Molecular Weight DOC

P-HMW-DOC・・・・・・・・・・・・Photosynthetically produced HMW-DOC

LMW-DOM・・・・・・・・・・・・・・・・・・・Low Molecular Weight DOM

LMW-DOC・・・・・・・・・・・・・・・・・・・Low Molecular Weight DOC

P-LMW-DOC・・・・・・・・・・・・Photosynthetically produced LMW-DOC

THNA · Total Hydrolysable Neutral Aldoses

THAA · Total Hydrolysable Amino Acids

TFA · Total Fatty Acids

SFA · Saturated Fatty Acid

MUFA · Monounsaturated fatty acid

PUFA · Polyunsaturated fatty acid

Absract

Chapter 1) General introduction

The decomposition process of phytoplanktonic organic matter in aquatic environments, which is the main streams of elemental cycle in the earth's surface, was investigated in the present study. The organic matter supplied into water column by phytoplankton includes both of particulate organic matter (POM) and dissolved organic matter (DOM). The decomposition processes of POM and DOM play different roles in the elemental cycle. Thus, this study investigated the decomposition process of POM (Chapter 2) and DOM (Chapter 3), derived from phytoplankton.

Chapter 2) Decomposition process of POM derived from phytoplankton

POM produced by phytoplankton in surface layer of water column is exported to deep layer as "sinking particle". The sinking particle undergoes decomposition by bacteria as it sink and the export flux by sinking particle is affected by the organic composition of phytoplankton. Phytoplankton is mainly composed of carbohydrate, protein and lipid. Therefore, the study on the lability of these three groups of organic matter is especially significant for understanding of the relationship between the lability and molecular composition of phytoplankton. Thus, the change in the composition of three groups of organic matter in POM during the simulated microbial decomposition process of organic matter derived from phytoplankton (*Microcystis* spp. and Diatom) was investigated for 60 days.

The concentration of neutral aldoses decreased drastically relative to amino acids and fatty acids during the early phase (days 0–7) of decomposition process of phytoplanktonic organic matter. The decrease in glucose was especially remarkable

among neutral aldoses. Storage glucan is conceivable as the carbohydrate mainly comprised of glucose. Therefore, these results suggest that the storage glucan is one of the most labile organic matters in phytoplankton. In addition, it is suggested that storage lipid also has labile property. Therefore, the export flux of organic matter to deep layer in natural water column is expectable to decrease when the contribution of storage carbohydrate and lipid to phytoplanktonic organic matter is high. Although the compositional variety of organic matter among the phytoplankton species was clearly observed at the start of decomposition, it became obscure through the course of 60 days experiments. This indicates that while the organic composition of the labile fraction of phytoplanktonic organic matter varies depending on the phytoplankton groups, the refractory fraction has similar composition. The addition of bacterial organic matter is likely another reason for the similar composition of the remaining organic matter at the end of experiments because the compositional increases in bacteria-specific molecules in POM throughout the experiments were observed.

Chapter 3) Decomposition process of DOM derived from phytoplankton

Phytoplankton supplies DOM to water column directly through extracellular release and cell lysis, and indirectly via bacteria. DOM is transferred to deep layer by vertical mixing and the amount of exported DOM to deep layer likely depends on the lability of DOM. The lability of DOM probably vary with molecular composition of DOM. Low molecular weight (LMW-) DOM is known to be more refractory than high molecular weight (HMW-) DOM in the oceanic DOM, indicating that lability of DOM probably relates with its size composition. However, the relationship between the size composition and lability of “fresh” DOM is still unknown. In order to reveal such relationship, the change in the size composition of DOM during 60 days decomposition

experiments of phytoplanktonic organic matter was investigated.

DOM released from living phytoplankton or by cell lysis was mainly composed of LMW-DOM, but had very labile property. These results suggest that the relationship between the molecular size and lability observed in the oceanic DOM is not established in “fresh” DOM yet. DOM became more abundant in LMW compounds during the late phase (day 7–60) of decomposition in an experiment. This result suggests that refractory LMW-DOM was produced by bacteria, and DOM derived from phytoplankton becomes similar composition to oceanic DOM within a few months. This timescale is very short relative to the average age of oceanic DOM (4000–6000 years). The other experiments, however, showed that the HMW-DOM also had comparable contribution to DOM with LMW-DOM until day 60. Thus, the time scale, in which the size composition becomes similar to oceanic DOM, is sometimes longer than a few months. This time scale is likely affected by molecular composition of DOM, therefore, the studies on the change in molecular composition of DOM derived from phytoplankton is necessary for the further understanding of decay process of DOM.

Chapter 1.

General introduction

1.1. General introduction

Hydrosphere covers 70% of the earth's surface, thus the elemental cycle therein has very important impact on the elemental cycle of entire earth. One of the most important driving forces for elemental cycle is the dynamics of the organic matter; i.e. production, transfer, modification and decomposition processes. There are various systems in hydrosphere, such as fresh water, coastal ocean, and open ocean, and these systems are common in the point that phytoplankton is the major primary producer. The organic matter in some oligotrophic lakes is derived mainly from allochthonous source (Sakamoto 1975; Saunders et al. 1980). However, phytoplankton photosynthesis is responsible for the most organic matter source in eutrophic lakes (Sakamoto 1975; Saunders 1980) and in the ocean (Williams and Gordon 1970; Meyers-Schulte and Hedges 1986). Therefore, the dynamics of the organic matter derived from phytoplankton can be regarded as the main streams of elemental cycle in hydrosphere.

Organic matter in water is operationally fractionated into particulate organic matter (POM) and dissolved organic matter (DOM) by filtration using a filter whose pore size of 0.2–1.0μm. Although organic matter is continuum in size and thus the strict size fractionation is impossible, this method has been routinely attempted for the organic matter in water so far (Libes 1992; Hedges 2002). POM includes phytoplankton, bacteria, sinking particle and their fragment. On the other hand, DOM includes various organic matters released from phytoplankton, bacteria and their detritus, though a part of living particles such as phytoplankton and bacteria with small size are operationally fractionated into DOM.

The simplified diagram of dynamics of organic matter derived from phytoplankton is shown in Fig. 1-1. Organic matter in hydrosphere is primarily produced by

phytoplankton in surface layer (Fig. 1-1. I). Organic matter and O_2 are supplied to surface layer while CO_2 and nutrient such as inorganic nitrogen and phosphorus are removed from surface layer by the primary production. Primary production by phytoplankton has been reported to vary with nutrient condition from 0.05–0.2gC m^{-2} d^{-1} in oligotrophic system to >1gC m^{-2} d^{-1} in eutrophic system (Vollenweider 1968; Sakamoto 1975; Hamanaka 2002). The organic matter produced is mainly comprised of biomolecules such as carbohydrates (Hama and Handa 1992; Biersmith and Benner 1998; Hamanaka et al. 2002), proteins (Hamanaka et al. 2002) and lipids (Hama 1991; Hamanaka et al. 2002).

Organic matter produced by phytoplankton in the surface layer undergoes various fates; they are grazed by zooplankton, transported to deep layer as “sinking particle” and released to surface layer as DOM (Libes 1992; de la Rocha 2006). Zooplankton, such as flagellated and ciliated protozoan, consume a substantial amount of phytoplankton (Libes 1992; de la Rocha 2006; Fig. 1-1. II). A part of incorporated organic matter into zooplankton is passed up to higher trophic level (e.g. macrozooplankton or fish), supporting the food web in water column. Although the proportion of consumed organic carbon by zooplankton to primary production would vary with system, the average proportion will be about 12% of primary production from the estimation by Calbet (2001).

Organic matter of phytoplankton, which is not grazed by zooplankton, follows a different path from that of phytoplankton grazed. When phytoplankton becomes senescent, it tends to aggregate and makes a large particle. Such aggregates start to sink from surface layer to deep layer as “sinking particle” (Fig. 1-1. III). This process is referred as “biological pomp” which exports bioelements from surface to deep layer (Libes 1992; de la Rocha 2006). The sinking particle undergoes further decomposition

by bacteria as it sink (Wakeham et al. 1997; Lee et al. 2004), thus only a part of organic matter in sinking particle is exported to deep layer. The export flux of organic carbon from surface layer to deep layer by sinking particle has been estimated in various systems such as lake (Sakamoto 1975) and ocean (Falkowski 2000; Hamanaka et al. 2002). The export ratio (export flux/primary production) ranges about 20–30%, thus as much as 70–80% of organic matter produced by phytoplankton is decomposed in surface layer.

The extent of the export flux by sinking particle has significant impact on the ecosystem in both of surface and deep layer because export flux determines biogeochemical environments of water column of aquatic ecosystems. An increase in the export flux of organic matter, which is composed of bioelements such as carbon, nitrogen and phosphorus, will result in the decrease in the concentration of dissolved CO_2 and nutrient in surface layer. A decrease in the concentration of CO_2 in surface layer leads to a decrease in the partial pressure of CO_2 in surface layer. Depleted partial pressure of CO_2 might enhance the dissolution of CO_2 from the atmosphere to hydrosphere (Martin et al. 1990; Takahashi et al. 2002). Thus, this process closely relates to the global carbon cycle. The increase in the export flux of organic matter also means the increase in the supply of organic matter and consumption of dissolved O_2 in deep layer. The increase in supply of organic matter to deep layer would enhance the activity of abyssal heterotrophic organisms. Too much supply of organic matter, however, will result in the O_2 deficiency in the deep layer and bottom sediment, thus inhibits the activity of organisms (Cloern 2001; Gilbert et al. 2005). Therefore, it is very important to study the factors which determine the export flux, because the change in export flux has critical effect on elemental cycle in hydrosphere.

One of the factors, which affect the export flux by sinking particle, is assumed to be

the organic composition of phytoplankton, because individual component of the organic matter should have a different lability. For example, when the phytoplankton produce a large proportion of labile organic matter, export ratio is possibly suppressed. Therefore, it is very important to reveal the relationship between the lability and molecular composition of phytoplankton for the further understanding of elemental cycle in hydrosphere.

Phytoplankton is mainly composed of carbohydrate, protein and lipid (Wakeham et al. 1997; Benner 2002a; Lee et al. 2004). Therefore, the study on the lability of these three groups of organic matter is especially significant for the understanding of the relationship between the lability and molecular composition of phytoplankton. The individual lability of these organic matters can be expected by the distribution of organic matters through the water column. It can be considered that proteins are more labile than carbohydrates because the decrease in the ratio of proteins to carbohydrates in sinking particle with water depths has been observed in oceanic system (Wakeham et al. 1997; Lee et al. 2004). This observation suggests that the decomposition rate of proteins is relatively faster than carbohydrates. Therefore, when phytoplankton has large amount of protein relative to carbohydrate, the phytoplanktonic organic matter likely have relatively labile property and export flux perhaps become lower. Analysis of *in situ* sinking particle, however, cannot reveal the temporal change in chemical composition of sinking particle because the collected sinking particles from different depths did not necessarily have same source. It is uncertain, therefore, that the hypothesis that the protein decomposes faster than carbohydrate is appropriate or not.

In order to reveal the actual change in chemical composition of sinking particle during its decomposition process, laboratory experiment is considered to be effective, in which POM derived from phytoplankton is decomposed by bacteria under the dark

condition. The analysis of decomposition process of particle, which has the same source, is possible in this method. However, such experimental approaches have not been carried out so far. Therefore, the change in the composition of carbohydrate, protein and lipid during the simulated decomposition process of POM derived from was studied in Chapter 2. The result of this study will reveal the actual relationship between the lability and chemical composition of phytoplankton.

Another fate of the organic matter produced by phytoplankton, other than consumption by zooplankton or sink to deep layer as sinking particle, is the dissolution to ambient waters in surface layer as DOM (Libes 1992; Carlson 2002; de la Rocha 2006). Phytoplankton supplies DOM to ambient water through various mechanisms; i.e. extracellular release (Fogg 1966; Baines and Pace 1991; Carlson 2002; Fig. 1-1. IV) and cell lysis (Libes 1992; Carlson 2002; Fig. 1-1. V). Living phytoplankton release photosynthetically produced organic matter as DOM (Fogg 1966; Baines and Pace 1991; Carlson 2002). The proportion of extracellular release to primary production is known to vary with average proportion as 26% (Baines and Pace 1991). On the other hand, the release of DOM by cell lysis is estimated to be 10–50% of primary production (Libes 1992). Additionally, bacteria, which incorporate phytoplanktonic DOM, also released DOM to surrounding water (Ogawa et al. 2001; Calson 2002; Kitayama et al. 2007; Fig. 1-1. IV). Thus, phytoplankton supply three types of DOM to water column directly and indirectly; extracellular released DOM from living phytoplankton, DOM released by phytoplankton cell lysis, and DOM indirectly produced through bacterial community.

DOM supplied to water column by these processes does not sink towards bottom like sinking particle, but it is transferred with the movement of water mass instead (Ogawa and Tanoue 2003). Surface water in tropical and subtropical area is hardly mixed with

deep water because pycnocline, which is formed by the substantial differences in water temperature between surface and deep water, inhibits the vertical mixing (Ogawa and Tanoue 2003; Carlson 2002). Thus, DOM released from phytoplankton is accumulated in surface layer in these areas, instead of sinking to deep layer. On the other hand, surface water in high latitudinal area is well mixed with deep water because of absent of pycnocline, thus DOM will exported to deep layer (Ogawa et al. 1999). Surface DOM in temperate area is accumulated on summer due to the development of the seasonal pycnocline, but is transferred to deep layer by winter mixing (Carlson et al. 1994). The export flux of organic matter to deep layer by this process (Fig. 1-1. VII) is estimated to be comparable to the export flux by sinking particle in the ocean (Carlson et al. 1994; Ogawa and Tanoue 2003). The change in the export flux by this process, therefore, has a huge impact on the aquatic ecosystem in the same way to sinking particle as mentioned above.

Most of the released DOM from phytoplankton is known to be readily consumed by bacteria (Petit 1999), but some of them escape bacterial attack and remains in water column for long time (Hansell and Carlson 1998; Hama et al. 2004). This DOM remained in the surface water likely contributes the export flux to deep layer. The amount of remained DOM is assumed to vary with molecular composition of DOM derived from phytoplankton, because the lability of the individual component of DOM varies each other. Therefore, it is significant to elucidate the relationship between the lability and molecular composition of DOM derived from phytoplankton.

As the information about the molecular composition and lability of DOM, the relationship between size composition and lability of DOM in the ocean has been well studied. DOM is generally fractionated by the molecular weight of 1–10 kDa, into low molecular weight (LMW-) DOM and high molecular weight (HMW-) DOM using

ultrafiltration, in the studies of size composition of DOM. LMW-DOM is known to be more abundance, poorer in biomolecules, biologically more refractory and older, than HMW-DOM in the oceanic DOM (Amon and Benner 1996; Ogawa 2000; Benner 2002b; Carlson 2002). From these properties of oceanic DOM, Amon and Benner (1996) proposed size-reactivity continuum model; "the bioreactivity of organic matter decreases along a continuum of size (from large to small) and diagenetic state (from fresh to old)". Thus, the lability of DOM derived from phytoplankton probably relates with its size composition.

"Size-reactivity continuum model", however, proposed by the analysis of oceanic DOM most of which is comprised of very refractory compounds. Thus, the relationship between the size composition and lability of "fresh" DOM is still unknown. In order to reveal such relationship, laboratory experiment is conceivable, in which phytoplanktonic organic matter is decomposed by bacteria under the dark condition and the temporal change in size composition of DOM derived from phytoplankton is analyzed. There are however, few experimental studies on the relationship between the size composition and lability of "fresh" DOM derived from phytoplankton. Therefore, the changes in the size composition of DOM derived from phytoplankton during the simulated decomposition process of phytoplanktonic organic matter were studied in Chapter 3.

These studies lead to the understanding the impact of chemical and size composition of phytoplankton on the elemental cycle in hydrosphere. Moreover, the findings in these studies provide significant information for the deep understanding of elemental cycle in the earth's surface.

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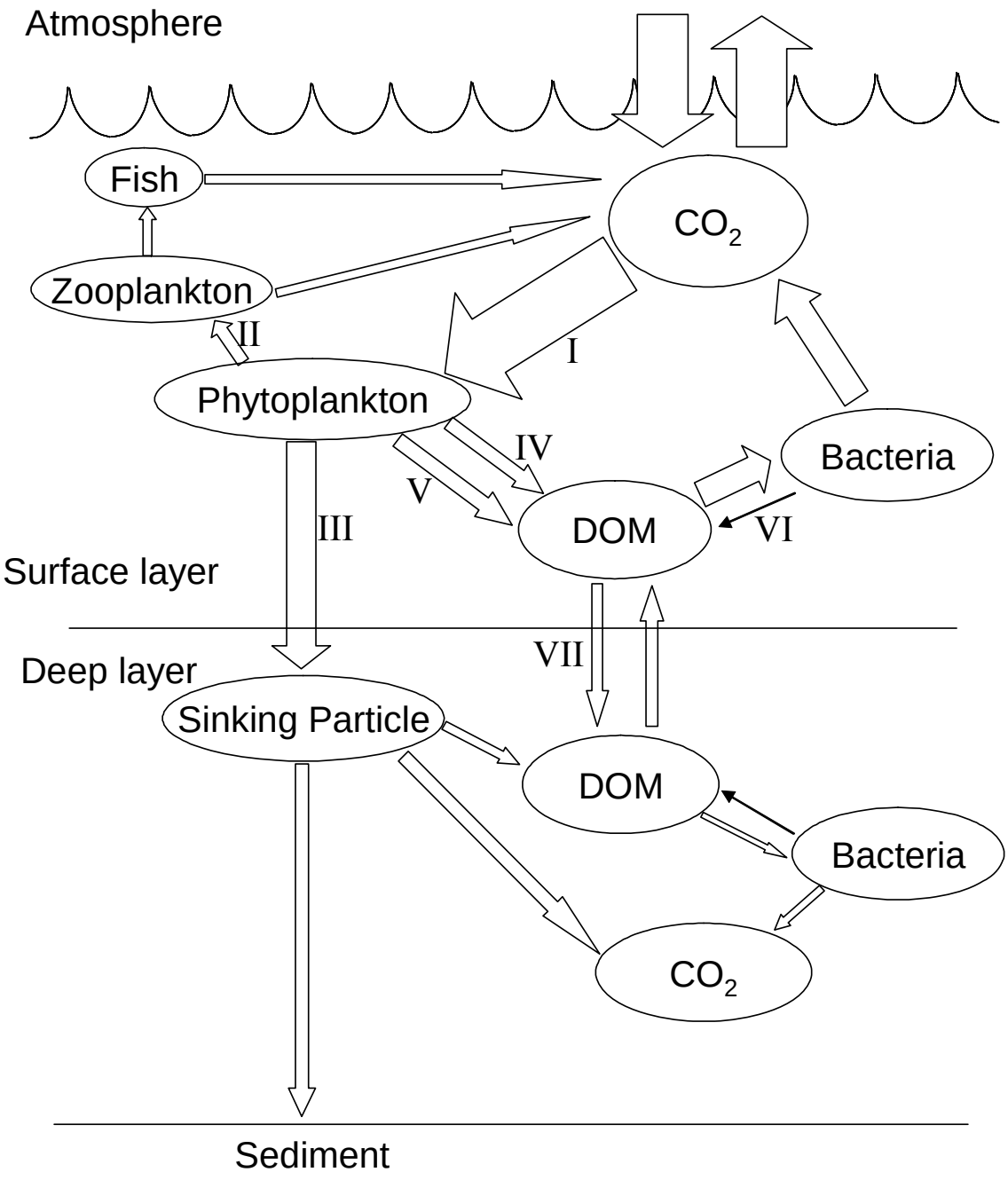

Fig. 1-1. Simplified diagram of dynamics of organic matter in hydrosphere. The width of each arrow represents the relative magnitude of carbon flux. The explanations for Roman numerals are in text

Chapter 3.

Decomposition process of DOM derived from phytoplankton

3.1. Introduction

The decomposition process of the POM derived from phytoplankton is investigated in Chapter 2. There is, however, another fate of phytoplanktonic organic matter; dissolved to the ambient water as DOM during decomposition process, and then decomposed by bacteria (Libes 1992; Ogawa 2000; Carlson 2002). DOM does not sink to bottom but remains in water column and diffuses by concentration difference or moves with water body (Carlson et al. 1994; Hansell at al. 1997). Therefore, the production and decomposition process of DOM have different effect from these of POM on the elemental cycle in hydrosphere.

DOM derived from phytoplankton is released to water column by some process; extracellular release by living phytoplankton (Baines and Pace 1991; Nagata 2000; Carlson 2002), cell lysis of phytoplankton (Gobler et al. 1997; Furman 1999; Carlson 2002) and release from bacteria which incorporates phytoplanktonic organic matter (Ogawa et al. 2001; Carlson 2002). Most of released DOM from phytoplankton is readily consumed by bacteria as mentioned in chapter 2. Some of them, however, resists bacterial attack and remains in water column for long time (Hansell and Carlson 1998; Hama et al. 2004). This remained DOM is possibly exported to deep layer by vertical mixing (Carlson et al. 1994; Ogawa et al. 1999). Therefore, the study of decomposition process of DOM is needed for the deep understanding of elemental cycle. DOM in fresh water is removed by not only biological decomposition but also outflow of water while DOM in the ocean is hardly removed without decomposition process. Thus, the decomposition process of DOM is especially important in the oceanic system rather than in the fresh water system. Consequently, the decomposition process of DOM derived from oceanic phytoplankton is studied in Chapter 3.

It is estimated that as much as 700GtC of DOM exists in the whole ocean and this value corresponds to more than 99% of oceanic organic carbon, thus the percentage of POC is only less than 1% (Schimel et al. 2000; Hedges 2002; Ogawa and Tanoue 2003). Considering that the POM accounted for about 50% in lake Kasumigaura (Yanai and Tonooka 2003), the proportion of DOM in the ocean is very large. Moreover, DOM in the ocean is one of the largest and dynamic carbon pool on the earth's surface, comparable to CO_2 in atmosphere (Siegenthaler and Sarmiento 1993). Thus, DOM in the ocean must play very important role not only in hydrosphere but also in the global elemental cycle.

Although there are some processes which supply DOM to the ocean, most of the oceanic DOM is considered to be derived from the oceanic phytoplankton. This concept is supported by the finding that the delta ^{13}C value of oceanic DOC is close to phytoplankton rather than terrestrial plant (Williams and Gordon, 1970). Moreover, organic matter used as indicator of terrestrial source such as lignin is scarcely detected in the ocean (Meyers-Schulte and Hedges, 1986), also suggesting that the oceanic DOM is derived from phytoplankton. The oceanic DOM, however, is known to have very different chemical composition from phytoplankton. Phytoplankton is mainly composed of biomolecules such as carbohydrate, protein and lipid as observed in chapter 2, while these components are accounted for only 1–11% of oceanic DOC (Benner 2002a, b), thus unidentifiable organic matter dominates this pool.

Instead, the information about molecular weight composition is accumulated for DOM in the ocean (Ogawa 2000; Benner 2002a, b). LMW-DOM (<1–10 kDa) is known to be more abundance, poorer in biomolecules, biologically more refractory and older than HMW-DOM (>1–10 kDa) in the oceanic DOM (Amon and Benner 1996; Ogawa 2000; Benner 2002b; Carlson 2002). Therefore, the relationship between molecular

weight and lability in oceanic DOM is well-defined, despite that it is contradictory because bacteria directly incorporate only LMW-DOM through membrane (Carlson 2002). From these observations, size-reactivity continuum model is proposed; “the bioreactivity of organic matter decreases along a continuum of size (from large to small) and diagenetic state (from fresh to old)” (Amon and Benner, 1996). However, the understanding of the process in which such DOM is produced from the phytoplanktonic organic matter is far from enough.

The decomposition experiment of phytoplanktonic organic matter which analyzes the changes in the amount and size composition of DOM will be helpful in the understanding of this process. Hama et al. (2004) conducted the decomposition experiment of ^{13}C labeled photosynthetically produced organic matter (P-OM), and revealed that DOM derived from phytoplankton survives as LMW-DOM rather than HMW-DOM. This result shows that the relationship between lability and size composition is established at the time scale of a few months. This finding, however, is yet proved to be general, thus the study using phytoplankton from various system is needed. Although Hama et al. (2004) used phytoplankton from eutrophic system, primary production in global ocean is mainly occurred in mesotrophic system (Field et al. 1998). Therefore, decomposition experiment of P-OM using phytoplankton from mesotrophic system will be valuable for the further understanding of: 1) the relationship between the DOM size compositions and its production mechanism (extracellular release by living phytoplankton, cell lysis of phytoplankton and release from bacteria); 2) the mechanism and time scale by which the size composition of newly produced DOM become rich in LMW-DOM like oceanic DOM.

In the present study, decomposition experiments of P-OM by phytoplankton were carried out. Phytoplankton communities were collected from mesotrophic system in

coastal and open ocean. Changes in the concentrations and size composition of photosynthetically produced DOC (P-DOC) were determined to assess the relationship between the size composition of DOM and the decomposition process.

3.2. Materials and methods

3.2.1. Sampling and incubation experiment

Coastal water for incubation experiments was collected at offshore Shimoda (St. Sh; 34º39'N, 138º56'E, depth 30.5m), located in Izu peninsula, Japan on 24 April in 2001. Pelagic samples were collected from the two stations in Southern Ocean (Sts. SO1; 54º45'S, 140º02'E, depth 3291m on 18 January in 2002 and SO2; 40º12'S, 109º98'E, depth 4612m on 25 January in 2003; Fig. 3-1) at St. 18 of the Hakuho-Maru (Ocean Research Institute at the University of Tokyo) KH-01-3 cruse, and St. PP4 of the cruise of Umitaka-Maru (Tokyo Fisheries University), respectively. The surface waters were collected using a sampling bucket and transferred into acid cleaned polycarbonate bottles (20 l). After the addition of ^{13}C-$NaHCO_3$ (the final ^{13}C atom% of dissolved inorganic carbon was 18.1%, 15.9% and 16.5% at Sh, SO1, and SO2, respectively), these samples were incubated under a natural light and temperature condition for 24h in outdoor incubator in order to label the photosynthetic products of phytoplankton with ^{13}C. The samples were subsequently incubated in the dark at 21°C for about 60 days. The subsamples (1.2 l) were collected from the cultures at intervals of 1 to 30 days to follow the fate of organic matter during the experiments.

3.2.2. Analysis

Each subsample was filtered through glass fiber filters (GF/F, Whatman) that were precombused at 450°C for 4 hours, as soon as collected. The filter samples were used for the analyses of POM, while the filtrate samples were used for the measurement of

DOM. The filters and filtrates were stored at –20°C until analysis.

The filtrates were sawed at room temperature and desalted by electrodialysis (Micro Acilyzer S3, Asahi Chemical). DOM was subsequently, fractionated into HMW-DOM (>1 kDa) and LMW-DOM (<1 kDa) by ultrafiltration (Proflux M12 Tangential Flow Filtration System, Millipore; Prep/Scale-TFF 2.5ft^2 Cartridge).

For each size fraction (POM, HMW-DOM and LMW-DOM), the concentration and ^{13}C atom% of organic carbon were measured. The concentration of POC and the ^{13}C atom% of POC, High Molecular Weight DOC (HMW-DOC) and Low Molecular Weight DOC (LMW-DOC) were measured by elemental analyzer/mass spectrometer (EA1108, Fisons/Delta Plus, Finnigan; Hama and Yanagi 2001). The HMW-DOC and LMW-DOC concentration were measured by high temperature combustion method (TOC5000A, Shimadzu).

POM and DOM, which were produced during the light incubation, are defined as photosynthetically produced POM (P-POM) and photosynthetically produced DOM (P-DOM), respectively. Because P-POM and P-DOM should have same ^{13}C atom% as dissolved inorganic carbon in the culture, the photosynthetically produced POC (P-POC) and P-DOC concentration can be calculated by this equation

$$P_t = C_t * (a_{is} - a_{ns})/(a_{it} - a_{ns}) \quad \text{(Hama et al. 1983)} \qquad (1)$$

in which, P_t is the concentration of P-POC or P-DOC at day t (μgC l^{-1}), C_t is the concentration of POC or DOC at day t (μgC l^{-1}), a_{is} is the ^{13}C atom% of POC or DOC at day t, a_{ns} is the ^{13}C atom% of natural organic matter and a_{it} is ^{13}C atom% of inorganic carbon in culture medium.

Chl *a* on a filter was extracted with N,N-dimethylformamide, and its concentration

was measured by fluorometry (Suzuki and Ishimaru 1990).

3.3. Results

The concentrations of Chl *a* and organic carbon in the samples used for the incubation experiments are shown in Table 3-1. The Chl *a* concentrations were 0.26–0.51 μgC l^{-1}, and higher phytoplankton biomass was noticed in the sample from Shimoda than Southern Ocean. The concentration of total organic carbon (TOC = POC + HMW-DOC + LMW-DOC) of the samples ranged from 937 to 1289 μgC l^{-1}. The smallest TOC concentration was observed in SO1. LMW-DOC (541–805 μgC l^{-1}) was the largest fraction of TOC (58–62%) in each experiment, and HMW-DOC (256–342 μgC l^{-1}) were the second most important fraction accounting for 27–29%. POC (140–149 μgC l^{-1}) were the smallest fraction (11–15%) among the three size fractions. Although the Chl *a* concentration was different among 3 stations, the concentration of POC was comparable.

3.3.1. Size composition of photosynthetically produced organic matter

The concentration of photosynthetically produced organic carbon (P-OC) was calculated using the concentration and ^{13}C atom% of the organic carbon. The concentration of P-POC was 30.9 μgC l^{-1} in Sh, 11.4 μgC l^{-1} in SO1, and 13.6 μgC l^{-1} in SO2 at the end of light incubation (Table 3-2, Fig. 3-2, 3-3, 3-4).

In Sh and SO1 experiments, the contribution of P-DOC was small relative to P-POC; P-DOC accounted for 5% (1.6 μgC l^{-1}) and 16% (2.2 μgC l^{-1}) of P-POC in Sh and SO1 experiment, respectively. The concentration of photosynthetically produced HMW-DOC (P-HMW-DOC) and photosynthetically produced LMW-DOC (P-LMW-DOC) was 0.5 and 1.1 μgC l^{-1} in Sh, and 1.2 and 1.0 μgC l^{-1} in SO1, respectively (Table 3-2,

Fig. 3-2, 3-3).

On the other hand, P-DOC dominated largely 51% of P-OC (14.3 μgC l^{-1}) at the end of light incubation in SO2 experiment. This P-DOC was dominated by P-LMW-DOC (13.7 μgC l^{-1}), accounting for 96% of P-DOC (Table 3-2, Fig. 3-4). Thus, LMW-DOC was measured as the important fraction of P-DOC in the SO2 experiment.

3.3.2. Changes in concentration of photosynthetically produced carbon

P-POC showed large decrease as soon as the dark incubation started in Sh experiment (Fig. 3-2). The concentration of P-POC decreased from 30.9 to 8.5 μgC l^{-1} during the first 2 days, thus 73% of P-POC on day 0 disappeared in this period. The increase in the P-DOC was observed during the first 3 days, concomitant with the decrease in P-POC. The increase from day 0 to 3 (4.4 μgC l^{-1}) accounted for 20% of the disappeared P-POC in the same period. The increase in the concentration of P-DOC was almost attributed to that in P-LMW-DOC, whose concentration increased from 1.1 to 5.1 μgC l^{-1} from day 0 to 3, accounting for 93% of the increase in P-DOC. The P-LMW-DOC, however, decreased to 1.2 μgC l^{-1} during day 3 to 7. After day 7, the concentration of P-POC showed slight decrease, while the concentration of P-LMW-DOC exhibited a slight increase. The concentration of P-HMW-DOC did not show a significant change throughout the experiment.

The rapid decrease in P-POC was also observed in SO1 (Fig. 3-3). The concentration of P-POC decreased to 4.3 μgC l^{-1} during the first 6 days and the decrease corresponded to 62% of P-POC on day 0. The increase in the concentration of P-LMW-DOC during the same period was 1.4 μgC l^{-1}. This value corresponded to 19% of the vanished P-POC. The concentration of P-POC and P-LMW-DOC declined after day 6, while the

concentration of P-HMW-DOC increased reaching comparable value (1.3 μgC l^{-1}) to that of P-LMW-DOC (0.9 μgC l^{-1}) on day 66.

Incubation experiment was conducted in duplicate in SO2, thus average concentrations of subsamples from two incubations were shown in Fig. 3-4. The coefficients of variation in the concentrations were 0.6–20% and 9.7–88% in P-POC and P-DOC, respectively. The P-POC concentration declined rapidly in SO2 experiment (by 59% during the first 7 days) just like the Sh and SO1 experiments. The concentration of P-LMW-DOC also decreased rapidly from 13.7 to 0.9 μgC l^{-1} during the first 1 day. However, the P-LMW-DOC concentration turned to increase to 3.1 μgC l^{-1} during day 1 to 2, but then it declined to 1.2 μgC l^{-1} again until day 3. The concentration of P-POC showed gradual decrease (from 8.7 on day 3 to 2.6 μgC l^{-1} on day 61) after that, while that of P-DOC remained constant.

A part of P-OC survived for about 60 days in each experiment. The concentration of photosynthetically produced TOC (P-TOC) which survived for about 60 days corresponded to 12.6% (P-POC, 4.9%; P-HMW-DOC, 2.0%; P-LMW-DOC, 5.8%), 27.1% (P-POC, 10.8%; P-HMW-DOC, 9.2%; P-LMW-DOC, 7.0%) and 16.6% (P-POC, 9.4 %; P-HMW-DOC, 3.7 %; P-LMW-DOC, 3.5%) of P-TOC on day 0 in Sh, SO1 and SO2, respectively (Table 3-3). Thus, 73–87% of photosynthetically produced organic carbon was used by the microbial community during the experimental periods.

3.3.3. Changes in size composition of photosynthetically produced carbon

The changes in the size composition of P-OC in 2 experiments were shown in Fig. 3-5. P-DOC accounted for small fraction in Sh and SO1 at the start of dark incubation. However, its proportion increased during the first 6–7 days, to 29% and 40% in Sh and

SO1, respectively, which were mainly due to the decrease in P-POC. Although P-DOC accounted for larger than 50% of P-OC on day 0 in SO2 experiment, its proportion fell to 12% during the first 1 day. Subsequent proportion of P-DOC increased just like the other experiments reaching 24% on day 7.

The increases in the proportion of P-DOC continued during the late phase of the dark incubation (days 6 or 7–60), with final proportion of 61%, 60% and 44% in Sh, SO1 and SO2, respectively. P-LMW-DOC was larger fraction (46%) than P-HMW-DOC (16%) at the end in Sh experiment. On the other hand, the proportion of P-LMW-DOC and P-HMW-DOC was comparable in SO1 (P-LMW-DOC 26%; P-HMW-DOC 34%) and SO2 (P-LMW-DOC 21%; P-HMW-DOC 22%) at the end of the experiment.

3.4. Discussion

The Chl *a* concentrations of sampling sites (Table 3-1) suggest that these are mesotrophic system (0.1–1 μg l^{-1} range is assumed for mesotrophic system, Field et al 1998). The primary production in mesotrophic area is estimated to be responsible for 56% of the global oceanic primary production (Field et al 1998), thus the biogeochemical cycle of bioelements including the decomposition process of the organic matter investigated in this study, plays an important role on the global elemental cycle.

3.4.1. Relationship between *in situ* DOC concentration and photosynthetically produced DOM

The concentrations and size distributions of the organic matter collected from three sites are comparable with those observed in ocean surface layer (Benner 2002a, Ogawa and Tanoue 2003), though the DOC concentration at SO1 was lower than those at the other two sites. It is possible that the differences in the production and decomposition rates of P-DOC affect the concentration of DOC in the ambient water. The concentration of P-DOC released during the light incubation, however, was not lower at SO1 than those observed in two other sites (Table 3-2). In addition, the percent of residual P-DOC which remained until day 60 to P-TOC on day 0 was the highest in SO1 (Table 3-3), meaning that the DOM derived from phytoplankton photosynthesis in this site is not especially labile. Thus, the lowest DOC concentration in SO1 dose not necessarily implies the low production rate and the high decomposition rate of DOC.

The comparison of daily production rate of P-DOC with the ambient concentration of

DOC suggests a limited contribution of P-DOC to ambient DOC pool. The daily release rate of the photosynthetic products ranged from 1.6 to 14.3 μgC l^{-1} d^{-1} in three sites and these only accounted for 0.16–1.2% of the ambient DOC concentration in each site (797–1149 μgC l^{-1}). This low contribution of P-DOC, therefore, probably results in a little effect of the extracellular release of organic carbon on the ambient concentration of DOC. Moreover, even in SO2 experiment, in which the largest extracellular production of DOC (14.3 μgC l^{-1}) was observed, most (89%) of released DOC was disappeared within a day likely due to the microbial consumption as mentioned below. Thus, it is conceivable that the high release rate of P-DOC is not necessarily resulted in the high ambient DOC concentration.

3.4.2. Photosynthetically production during the light incubation

The P-DOC concentration at the end of the light incubation can be regarded as extracellular production by living phytoplankton. Over the past few decades, a considerable number of studies have been conducted on the extracellular release of organic matter by phytoplankton (e.g. Wheeler et al. 1996; Vernet et al. 1998; Karl et al. 1998). The percent extracellular release (PER) to total primary production has been reported to vary with several factors, such as nutrient availability (Wangersky 1978; Wood and Valen, 1990), productivity (Fogg et al. 1965; Anderson and Zeutschel 1970) or cell size (Bjørnsen 1988), ranging from 0 to 80% (see review by Carlson 2002). In this study, PER in Sh and SO1 experiments are comparable with those of the recent studies as summarized by Baines and Pace (1991), but relatively high value was observed in SO2 experiment (Table 3-2). In addition, very high contribution of P-LMW-DOC in P-DOC was observed in SO2, while the comparable contributions of P-

HMW- and P-LMW-DOC were observed in Sh and SO1.

Nutrient availability has been known to affect PER or size composition of DOM released from living phytoplankton. Fractionation studies of photosynthetic products revealed that non-nitrogenous compounds such as carbohydrate are disproportionally accumulated in phytoplankton cell (Hama 1988; Hama et al. 1988). When phytoplankton cannot retain all of them as intra-cellular component, some part of accumulated product is released (Fogg et al. 1966; Wood and Valen 1990; Myklestad 1995). This release is referred as "overflow" mechanism (Fogg et al. 1966). When storage glucan, accumulated in phytoplankton cell as observed in chapter 2, is released, they likely be fractionated into HMW-DOM because the molecular weight of storage glucan is reported to be >6000 Da (Myklestad 1988). Thus, the predominance of LMW-DOM in the extracellular products in SO2 leads to the idea that phytoplankton population in SO2 is not limited by nutrients. Although the nutrient concentrations of the sample waters were not available in this study, those of same areas determined by other studies are referred in Table 3-4. Assuming that the nutrient incorporation of phytoplankton follows the stoichiometric ratio of Redfield; carbon: nitrogen: phosphorus is 106:16:1 (Redfield et al, 1963), the amounts of incorporated nitrogen and phosphorus are estimated as shown in Table 3-4. The calculated values (0.17–0.41μMN and 0.011–0.026μMP) are considerably lower than *in situ* nutrient concentration (NO_3 1.2–23μM; PO_4 0.25–1.5μM) in each experiment. Thus, nutrient depletion not likely occur in each experiment. Therefore, the other factors will affect on PER and size composition of released DOM.

Another mechanism of extracellular release is "passive diffusion", in which LMW-DOM such as monosaccharide or free amino acid is released through cell membrane by permeation (Fogg et al 1966; Bjørnsen 1988). The release of LMW-DOM in SO2

experiment suggests that “passive diffusion” might dominate as release mechanism. In this mechanism, PER is assumed higher in small cell because the proportion of surface area to volume of phytoplankton cell is large in small cell (Bjørnsen 1988). Therefore, cell size likely affected the PER observed in this study, though the detailed information of size composition of phytoplankton is not available.

The extracellular DOM observed in SO2 is probably very labile because the concentration of P-DOC drastically dropped on day 1 (Fig. 3-4). It is consistent with a very labile nature of LMW-DOM such as monosaccharides (Skoog et al. 1999) or free amino acids (Keil and Kirchman 1999) which are possibly released by “passive diffusion”. Although “passive diffusion” would dominate in SO2 because the LMW-DOC was the major components of P-DOC, it is possible that the utilization of P-DOC is also intensive even when they are released by “overflow”; the susceptibility of storage glucan, which probably main components of P-DOC through “overflow” mechanism, has been ascertained (Hama and Yanagi 2001; Chapter 2). Thus, major part of the extracellular released DOM from phytoplankton is probably removed from water column within a short time, and molecular weight composition of extracellular released DOM would has a little effect on the size composition of DOM in the ambient water. Indeed, the proportion of LMW-DOC to bulk DOC at SO2 is not especially high (70%), being similar value with Sh (67%) or SO1 (68%), despite the high contribution of LMW-DOM in released DOM. Although oceanic DOM is generally rich in LMW-DOM, this study revealed that DOM released from living phytoplankton is not the direct source of oceanic LMW-DOM because of the labile properties of the released organic compounds. Therefore, other mechanisms should determine the size composition of oceanic DOM.

3.4.3. DOM released through phytoplankton lysis

During the early phase of dark incubation, the P-POC concentration decreased drastically as soon as the sample has transferred into the dark condition, while the concentration of P-DOC showed increase in each experiment (Fig. 3-2, 3-3, 3-4). The rapid decline of POC during the early period of phytoplankton decay process is in agreement with the results of Chapter 2 and reference therein. Berges and Falkowski (1998) observed that the number of active phytoplankton cell started to decrease during a few days when phytoplankton was incubated under the dark condition because of inhibition of photosynthesis. Therefore, the decrease in P-POC and the increase in P-DOC during the early phase of dark incubation suggest the release of intracellular constituents as P-DOC concomitant with the phytoplankton lysis.

However, the increases in P-DOC only accounted for 19–20% of the decrease in the concentration of P-POC in the same period. It is uncertain that the P-DOC accumulated in the incubation medium in SO1 was decomposed immediately because the subsample just after day 6, when the maximum concentration of P-DOC was observed, was not collected in this experiment (Fig. 3-3). However, most of the accumulated P-DOC was immediately disappeared during a few days in Sh and SO2 (Fig. 3-2, 3-4). These results clearly show that most of the DOC released through phytoplankton cell lysis is very labile compounds. Considering that phytoplankton cell is mainly composed of carbohydrate, protein, and lipid (Wakeham et al. 1997; Chapter 2), it is apparent that the labile DOC released through cell lysis mainly comprises of such biomolecules. This rapid disappearance of the released DOC can be attributed to the efficient utilization by bacteria as discussed in chapter 2. Brussaard et al. (1995) observed the correlation between the dissolved algal esterase activity, which is an indicator of the frequency of

phytoplankton cell lysis, and the bacterial production in surface water of the North Sea. This finding supports the fact that the DOM released by phytoplankton cell lysis is good substrate for bacteria in natural environments. Thus, these coupling between the release of DOM through phytoplankton cellular lysis and the efficient uptake by bacteria probably prevent the accumulation of labile DOM in the ambient waters.

The labile P-DOC released by cell lysis observed in Sh and SO2 experiment mainly comprised of P-LMW-DOC (93–96%; Fig. 3-2, 3-4). These results are seemed to contradict the nature of oceanic DOM, in which low molecular weight fraction is reported to be more refractory and have older age than high molecular weight fraction (Amon and Benner 1996; Ogawa 2000; Benner 2002b; Carlson 2002). This contradiction suggests that the relationship between molecular size and lability generally confirmed in the oceanic DOM is not accordance with the lysis-induced DOM. The reason why the oceanic DOM does not reflect the property of lysis-induced DOM should be the large amount of the oceanic DOM (797–1149 μgC l^{-1} in this study) relative to lysis-induced DOM (0.7–4.4 μgC l^{-1} in this study only accounting for 0.1–0.4% of DOC concentration in ambient water). Although the DOM released by cellular lysis of phytoplankton was mainly composed of LMW-DOM just like the oceanic DOM, the lysis-induced DOM had very labile property contrast to oceanic DOM. This result suggests that the most part of DOM released by cellular lysis of phytoplankton should not remain in water column for long time. Thus, it is apparent that the size composition of the oceanic DOM, which is abundant in LMW-DOM, does not simply reflect the size composition of DOM released by phytoplankton lysis. Therefore, the size composition of the oceanic DOM will be determined after the early phase of decomposition process.

3.4.4. Decline in molecular weight of photosynthetically produced DOM

The concentration and composition of P-OC continued to change during the late phase (day 7–60; Fig. 3-2, 3-3, 3-4, 3-5). The proportion of P-POC to P-TOC showed decrease as decomposition proceeded in each experiment, in contrast to the proportion of P-DOC (Fig. 3-5). These results imply that the organic matter derived from phytoplankton tend to remain as dissolved form rather than particulate form. This finding is very important because it can explain the reason why there is much larger DOM pool than POM pool in the ocean (Schimel et al. 2000; Hedges 2002; Ogawa and Tanoue 2003).

The reason for the recalcitrant nature of residual P-DOM is likely the result of the transformation from biomolecules into refractory DOM by abiotic or biotic process. The exposure to UV irradiation has been known as the abiotic formation process of refractory DOM (Benner and Biddanda 1998; Tranvik and Kokalj 1998). Dark incubation, however, was conducted in this study, thus it is impossible that photochemical transformation was concerned with the production of the refractory P-DOM in this experiment. Therefore, biotic process is conceivable for the formation process of refractory P-DOM in this study and bacteria is possible producer of refractory P-DOM. Ogawa et al. (2001) reported the release of refractory DOM by bacteria, which was fed by labile organic matter. In fact, some specific bacterial cellular organic compounds have been identified as the components of the refractory DOM from oceanic environments (Tanoue et al. 1995; McCarthy et al. 1998; Kitayama et al. 2007), though these organic components were not analyzed in this study. Kaiser and Benner (2008) estimated that 25% of oceanic DOC is derived from bacteria, suggesting that bacteria community have substantial effect on the oceanic DOC. Considering these findings, production of refractory P-DOM by bacteria likely occurred in this study.

Although three experiments showed the increase in P-DOC contribution to P-OC, the temporal changes in the contribution of P-LMW-DOC and P-HMW-DOC were different among the experiments. P-LMW-DOC became dominant fraction among P-DOC until day 60 in Sh experiment (Fig. 3-5). The residual P-DOM at the end of the experiment is likely composed of non-labile compounds because they survived for 60 days against microbial activity. Considering that the oceanic DOM is also mainly composed of LMW-DOM, this result implies that the photosynthetically produced organic matter became similar size composition to the oceanic DOM within a few months. This time scale is very short, referring that the average age of the oceanic DOM is estimated to be 4000–6000 years (Williams and Druffel 1987).

Hama et al. (2004) also observed the comparable result to this study using the phytoplankton assemblage from a eutrophic system. It had been uncertain that, however, the result of Hama et al. (2004) was applicable for mesotrophic or oligotrophic area, because nutrient condition has been known to alter the chemical composition of photosynthetic products. For example, Hama (1988) observed that the ratio of carbohydrate to protein in photosynthetic products increased with the decrease in the nutrient concentration in ambient water. The lipid composition in phytoplankton is also affected by nutrient availability. Storage lipid is known to accumulate in phytoplankton cell in low nutrient (Hama et al. 1992; Kuwata et al. 1993). These findings indicate that the organic composition available for bacteria is affected by the nutrient concentration. The changes in substrate composition for bacteria likely lead to the alteration of the size composition of DOM released from bacteria.

The primary production in mesotrophic area is estimated to be responsible for 56% of the global oceanic primary production while eutrophic area for only 19% (Field et al 1998). Thus the decomposition process of photosynthetically products in mesotrophic

area has more important effect on the global dynamics of DOM than that in eutrophic area. It is important, therefore, that this study proves that the size composition of organic matter derived from the phytoplankton in mesotrophic system also shifts toward LMW-DOM rich composition similar to the oceanic DOM within a relatively short time scale.

On the other hand, P-HMW-DOC showed increases in the contribution to P-OC during the late phase in the SO1 and SO2 experiments, being comparable contribution with P-LMW-DOC at the end of the experiments (Fig. 3-5). These results indicate that the size composition of organic matter derived from phytoplankton is not always dominated by LMW-DOM within 2 months. The bulk lability of phytoplanktonic organic matter reflects its chemical composition as has been discussed in Chapter 2, thus the lability of each size fraction of DOM derived from phytoplankton also could be affected by chemical composition. Hama et al. (2004) reported that residual P-LMW-DOC which remained until day 60 was composed of organic compounds with lesser amount of carbohydrate relative to residual P-HMW-DOC. Their result suggests that the unidentifiable organic matter by biomolecules such as carbohydrate, molds the bulk refractory nature of DOM. Unidentifiable organic matter also tend to remain in the refractory POM as observed in Chapter 2. Therefore, it is necessary to examine the changes in concentration and composition of carbohydrate or other biomolecules and unidentifiable organic matter during the decomposition process. Such analysis will reveal the reason for the variation in the contribution of HMW-DOM and LMW-DOM to refractory DOM derived from phytoplankton among the sampling sites. These analyses will lead to the deeper understanding on the mechanism of the determination of the size composition of organic matter derived from phytoplankton, and further on the elemental cycle in hydrosphere.

3.4.5. Conclusion

This study investigated the decomposition and transformation processes of DOM derived from phytoplankton, with special reference to changes in molecular weight of organic matter. The microbial lability has been considered to be lower in LMW-DOM than HMW-DOM in the oceanic DOM. Such relationship is, however, not established in the “fresh” DOM such as extracellular released DOM from living phytoplankton and DOM released through cellular lysis of phytoplankton. After the labile DOM is decomposed, the diagenetic process by bacterial community probably produces the DOM whose size composition is similar to the oceanic refractory DOM. It is proved that the decrease in molecular weight of DOM derived from phytoplankton proceeds as soon as 2 months.

3.5. References

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Table 3-1. Concentration of Chl *a* (μg l^{-1}) and organic carbon (μgC l^{-1}) in the samples used for incubation experiments

Experiment	Chl *a*	TOC	POC	HMW-DOC	LMW-DOC
Sh	0.51	1159	149	333	677
SO1	0.32	937	140	256	541
SO2	0.26	1289	142	342	805

Table 3-2. Concentration of photosynthetically produced organic carbon (μgC l^{-1}) during the light incubation

Experiment	P-TOC	P-POC	P-DOC	P-HMW-DOC	P-LMW-DOC
Sh	32.6	30.9	1.6	0.5	1.1
SO1	13.7	11.4	2.2	1.2	1.0
SO2	27.9	13.6	14.3	0.6	13.7

Table 3-3. Proportion of residual P-OC concentration which remained until day 60 to P-TOC concentration at day 0 (%)

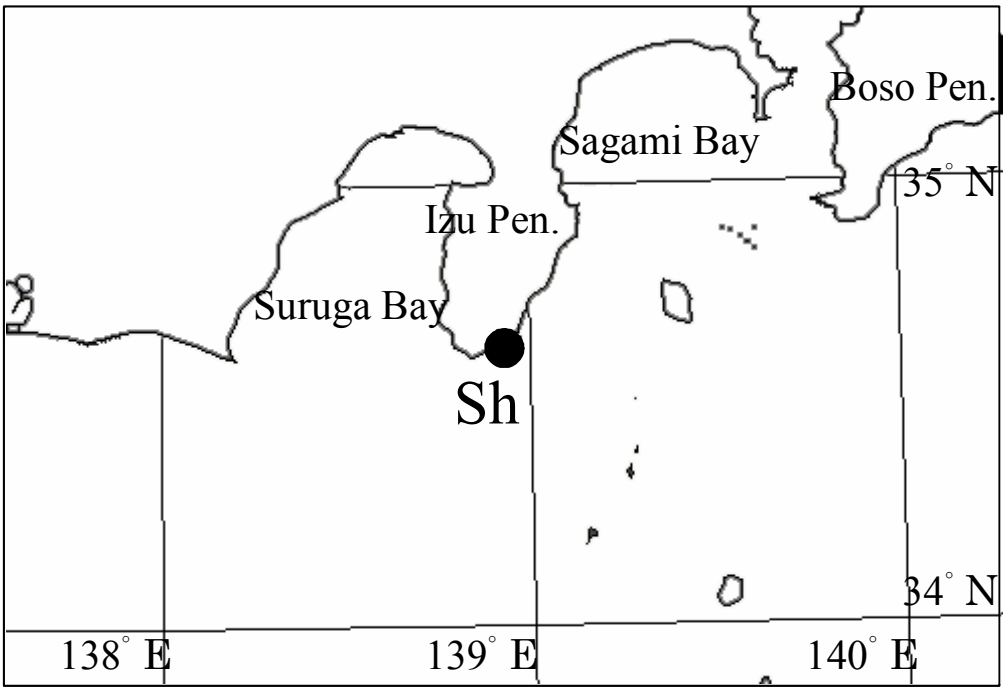
Experiment	P-TOC	P-POC	P-HMW-DOC	P-LMW-DOC
Sh	12.6	4.9	2.0	5.8
SO1	27.1	10.8	9.2	7.0
SO2	16.6	9.4	3.7	3.5

Table 3-4. Nutrient incorporation by phytoplankton during the light incubation and nutrient concentration in sampling sites

Experiment	P-TOC at day 0 (μgC l^{-1})	Incorporated concentration (μM)*		Nutrient concentration of sampling site (μM)	
		N	P	NO_3	PO_4
Sh	32.59	0.410	0.026	1.23**	0.25**
SO1	13.66	0.172	0.011	22.7***	1.47***
SO2	27.91	0.351	0.022	4~5****	0.5~0.6****

*Calculated assuming that nitrogen and phosphorus was incorporated with C:N:P molar ratio of 106:16:1

The data for nutrient are reference values from **Iwasaki (personal communication), ***Ogawa (personal communication) and ****Garcia et al. (2006)



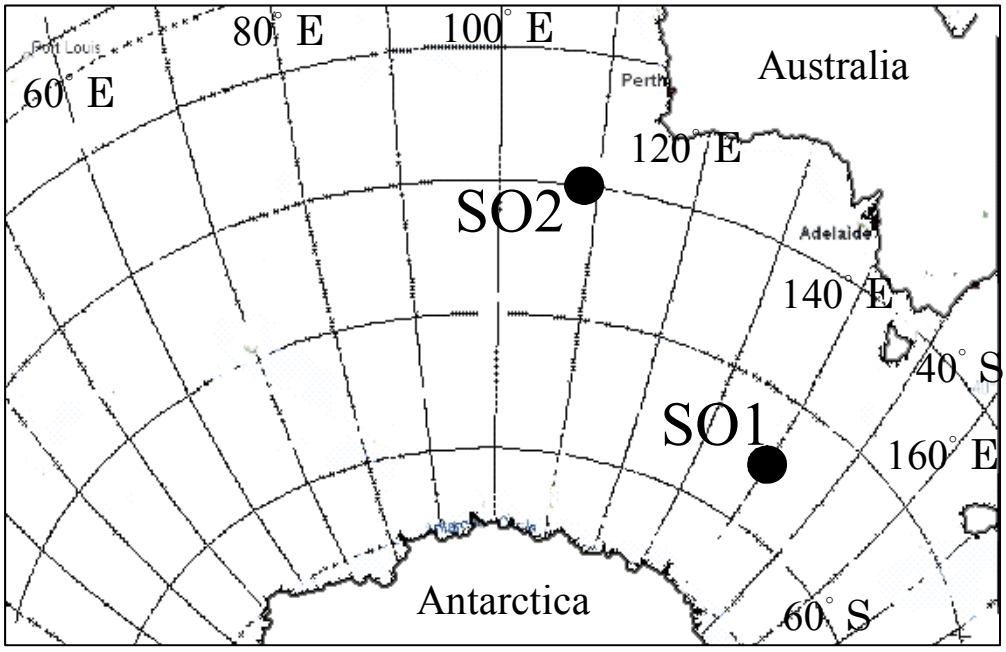


Fig. 3-1. Location of the sampling sites

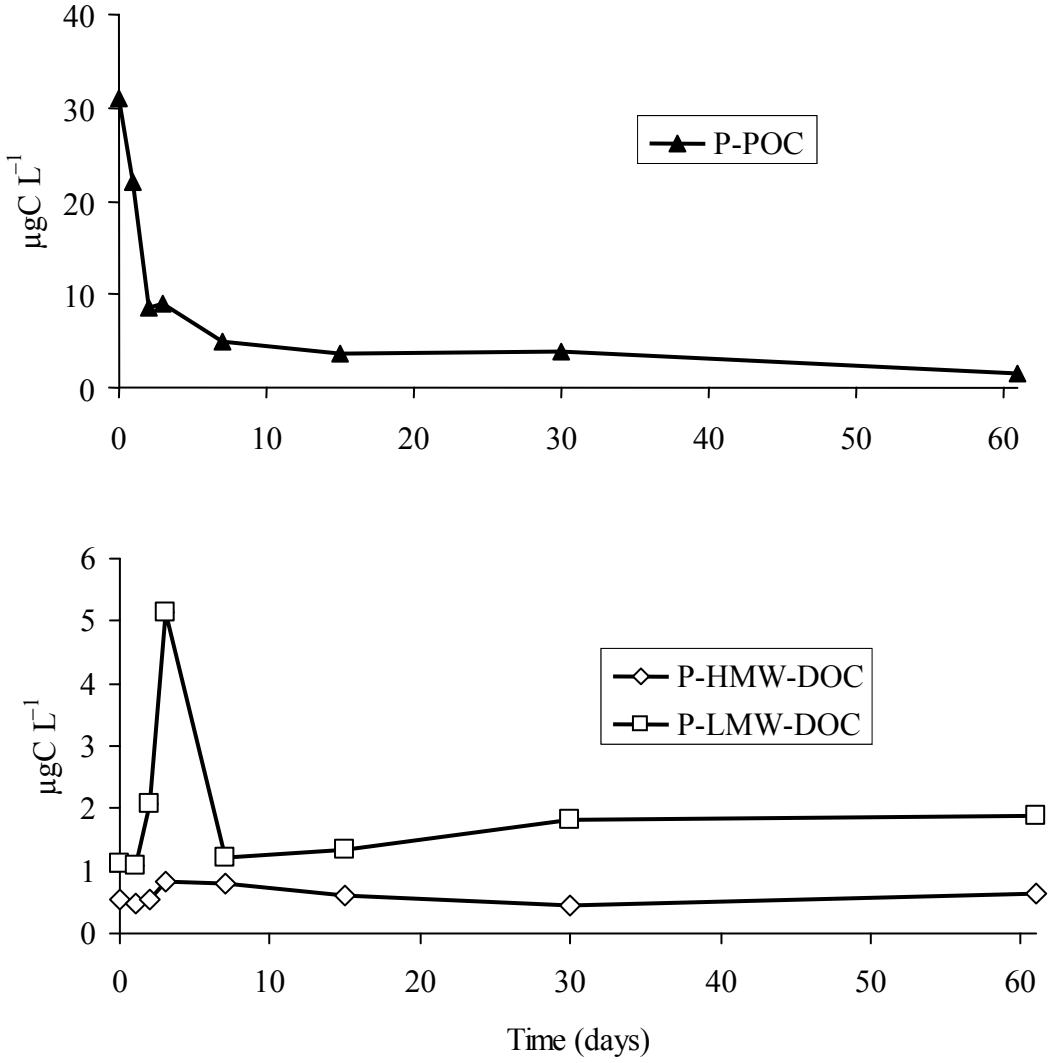


Fig. 3-2. Changes in the concentration of P-OC during Sh experiment

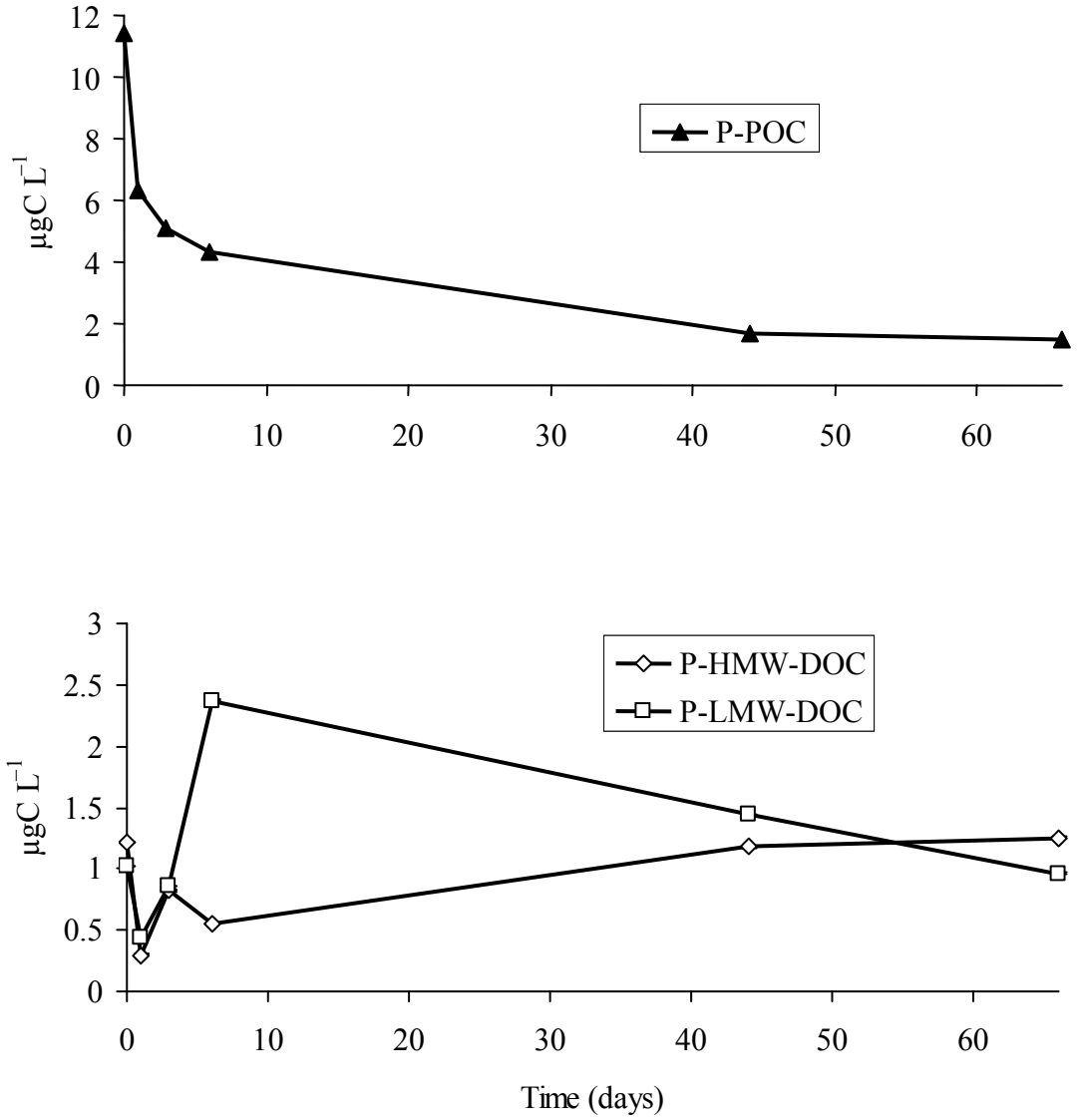


Fig. 3-3. Changes in the concentration of P-OC during SO1 experiment

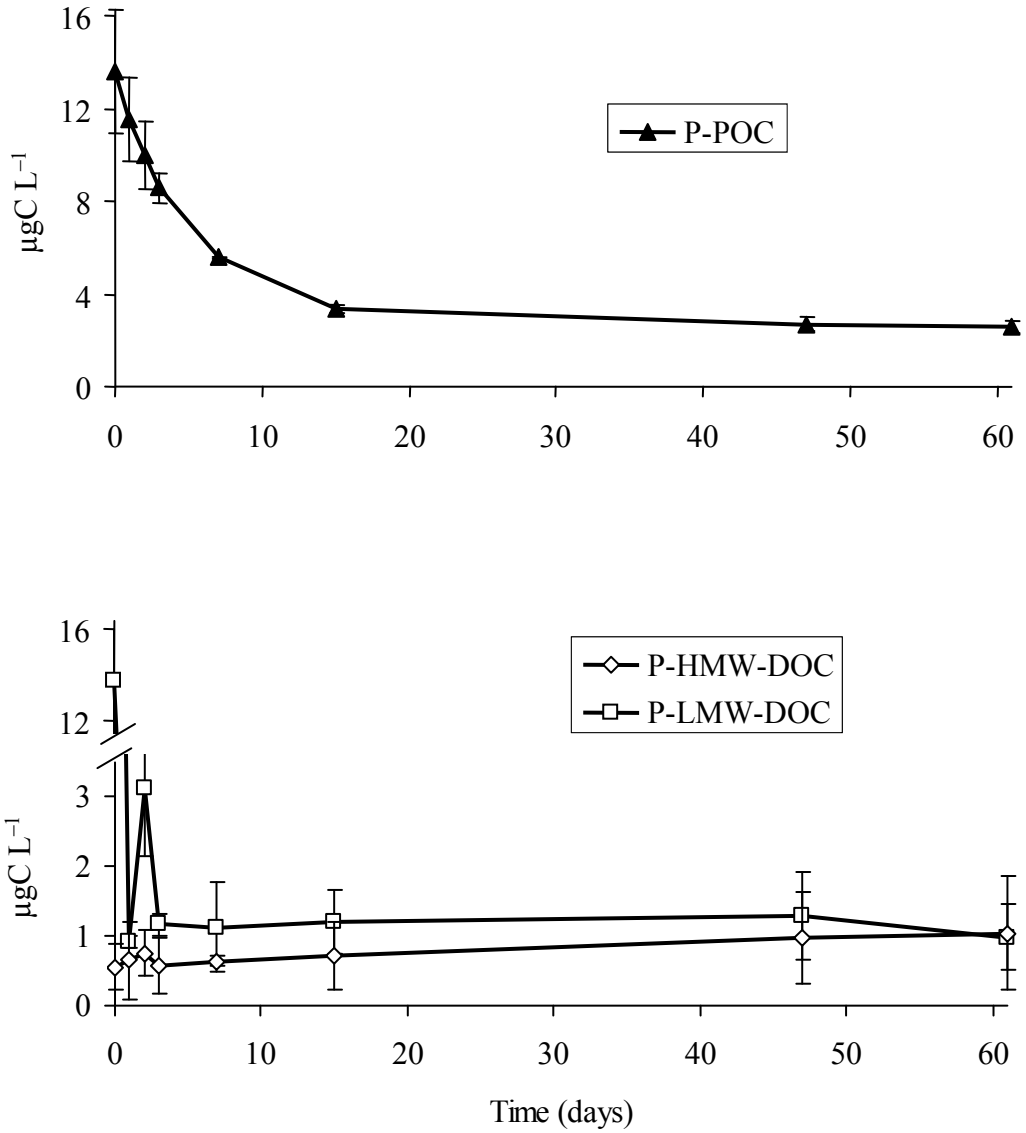


Fig. 3-4. Changes in the concentration of P-OC during SO2 experiment

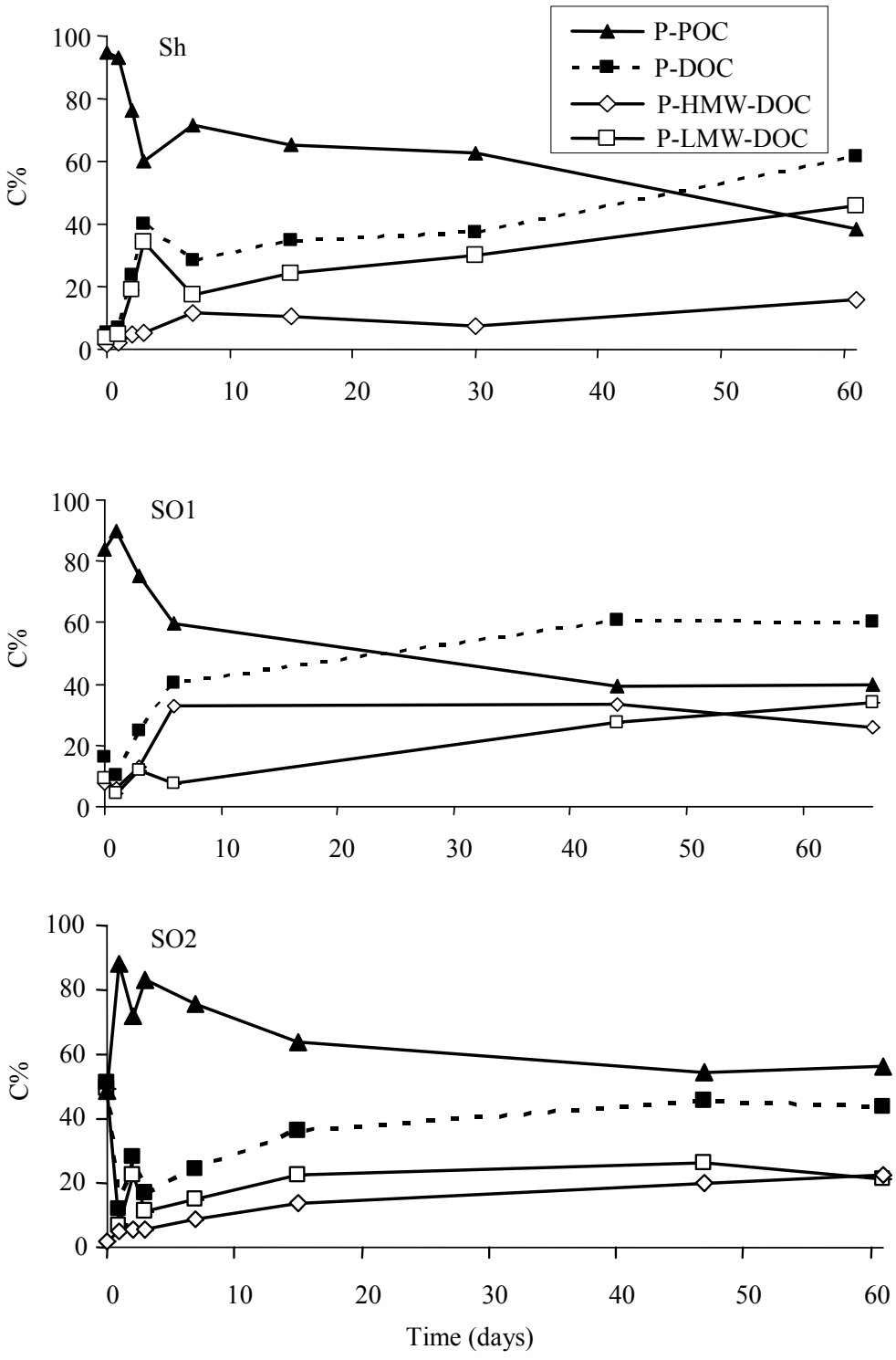


Fig. 3-5. Changes in the contribution of each size fraction of P-OC to P-TOC during experiments

Chapter 4.

General conclusion

4.1. General conclusion

The decomposition process of POM and DOM derived from phytoplankton by bacteria was investigated in this study. It is important process in the dynamics of organic matter in hydrosphere, and further, in the global biogeochemical cycle. Both of POM and DOM consist of various components and individual component has a different lability from each other depending on the structure of organic molecule, thus organic matter composition must have critical impact on the lability of POM and DOM. The relationship between organic matter composition and lability can be expected from *in situ* distribution of them. However, a little experimental research on the relationship has been conducted so far. The present study reveals the importance of organic matter composition in the material cycle of in aquatic environments.

4.1.1. Relationship between chemical composition and lability of POM

Nitrogenous organic matter has been considered more labile than non-nitrogenous organic matter, but carbohydrate, especially storage glucan, is shown to be much more labile relative to protein during the earliest phase of phytoplankton decomposition process. Therefore, it is indicated that high content of carbohydrate as organic constitute possibly makes phytoplankton organic matter more labile.

These findings suggest that the contribution of storage carbohydrate in phytoplankton cell have critical importance on the export flux of organic matter from surface layer to deep layer. When phytoplankton cell with high contribution of storage carbohydrate sinks to middle-deep layers, the fraction decomposed in surface layer will be high due to rapid decomposition of storage carbohydrate. Therefore, the export ratio, moreover,

elemental cycle in water column from surface to bottom is possibly altered by the storage carbohydrate content of phytoplankton cell. The amount of carbohydrate in phytoplankton is affected by nutrient availability (Hama 1988; Hama et al. 1988), thus nutrient condition in the surface layer likely have a great effect on the export ratio.

4.1.2. Relationship between size composition and lability of DOM

The knowledge on the relationship between lability and size composition has been accumulated for DOM in the ocean as “size-reactivity continuum model” (Amon and Benner 1996). In this model, LMW-DOM in the ocean is thought to be more refractory than HMW-DOM. However, in this study, the DOM released by living phytoplankton or cell lysis was labile even in the case that LMW-DOM dominated. Therefore, the relationship between lability and size composition observed in oceanic DOM is not established in the early phase of decomposition. It is conceivable that the fresh LMW-DOM released phytoplankton is mainly composed of biochemical compounds such as carbohydrate, proteins and lipids and it is probably the reason why the fresh LMW-DOM is easily decomposed by bacteria.

The contribution of DOM which is mainly composed of LMW and refractory nature like oceanic DOM was found to increase in late phase of phytoplankton decomposition likely due to the bacterial activity. This process could occur within 2 months from production by phytoplankton. This time scale is long relative to the generation and decomposition process of the DOM released by living phytoplankton or cell lysis, but very short from the average age of oceanic DOM is 4000–6000 years.

4.1.3. Further study

Although it was shown in Chapter 3 that the size composition of P-OM can be determined within 2 months, it dose not be generalized as the common result of all experiments. In order to reveal the mechanism in which the size composition of P-OM is determined, we need to understand what kind of organic matter contributes the refractory property of DOM.

Analysis of indicators for particular organisms or its components are likely helpful information for this subject. For example, odd number fatty acids (Mancuso et al. 1990), D-amino acid (Kitayama et al. 2007), and porin protein (Tanoue et al. 1995) have been known as the indicator of bacterial organic matter, thus their amounts in DOM should be used as indicators of contribution of bacteria. In the same way, analyses of the other indicators possibly reveal which organisms or its components tend to contribute the production of recalcitrant DOM.

The most of refractory organic matter is, however, possibly composed of unidentifiable organic matter (Hama et al. 2004; Chapter 2). Therefore, the analysis of unidentifiable organic matter during decomposition experiments of P-OM is needed. The solid phase extraction by sorbent such as XAD resin (Benner 2002) is conceivable to characterize the unidentifiable DOM. Solid phase extraction had been developed for the extraction of colored DOM from lake water. Hydrophobic DOM extracted by this method is defined as humic substance. Although the structure of each molecular is not able to determined by this method, DOM can be fractionated depending on the chemical property (hydrophobic or hydrophilic). The analysis of $^{13}C/^{12}C$ ratio conducted in Chapter 3 is available after the fractionation of DOM into humic and non-humic substances. Therefore, the decomposition process of organic matter derived from

photosynthesis of phytoplankton can be investigated. These studies should afford the significant information of the chemical properties of refractory organic compounds.

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