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Detailed Variations in Bioactive Elements in the Surface Ocean and Their Interaction with Microbiological Processes

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General Introduction

For a deep understanding of the way of biogeochemical cycles between the surface ocean and the lower atmosphere are linked, an important key is to elucidate the cycles of bioactive macro elements, especially C, N, P, Si, through the biological processes in the surface ocean. The cycles of these elements are driven by the inter-conversion between CO_2 plus nutrients and organic matter, however, detailed mechanisms still remain mostly unknown. For example, in the oligotrophic ocean, typically in the subtropical regions, the mechanism of nutrient supplies which sustain the primary production is not consistently understood. The real forms of the bioactive elements which are incorporated into organic matter are not exactly identified, and, furthermore, their decomposition processes still remains a "black box". Conventional research approaches only are insufficient to solve these subjects, and it is necessary to introduce new technologies. Thus, in order to elucidate the mechanism of the cycles of bioactive elements in the surface ocean, our research group has aimed to develop and apply advanced technologies to identify in detail the forms of nutrients, organic matter and the microbiological community, and to detect their detailed variation. Specifically, the following four topics (sub-themes) were studied: (1) detailed analyses of the dynamics of nutrients in the oligotrophic surface ocean using a high-sensitive colorimetric method (J. Kanda and F. Hashihama); (2) the development of an accurate and simple method for measuring particulate phosphorus and the investigation of the decomposition processes of dissolved organic phosphorus in terms of hydrolysis extoenzyme (M. Suzumura); (3) the development and examination of the method for measuring the concentrations of volatile organic carbon in seawater, and the characterization of decomposition processes of dissolved organic matter (H. Ogawa); and (4) the elucidation of the microbial degradation mechanism of submicron particles in the ocean (K. Kogure).

Topic 1: Detailed analyses of the dynam-

ics of nutrients in the oligotrophic surface ocean using a highly-sensitive colorimetric method (J. Kanda and F. Hashihama)

Introduction

In spite of the long history of biogeochemical studies at sea, our understanding of nutrient dynamics in the subtropical oligotrophic ocean has been considerably limited. This limitation apparently derived from the low concentrations of surface nutrients in the ocean, which are frequently below the detection limits of standard analytical methods. In order to reveal surface nutrient dynamics in the oligotrophic ocean, we need to design new analytical tools, and then to conduct new field observations using these tools. During this project, we developed a four-channel highly-sensitive analytical system to determine nanomolar concentrations of nitrate+nitrite (N+N), nitrite, soluble reactive phosphorus (SRP), and silicic acid, and we examined the surface distribution of these nutrients, and associated biogeochemical processes, over the oligotrophic waters of the Pacific and Indian Oceans.

Methods

Development of highly-sensitive colorimetry

A four-channel highly-sensitive nutrient analyzer was constructed by connecting a Liquid Waveguide Capillary Cell (LWCC, World Precision Instruments) to a gas-segmented continuous flow colorimetric system (AutoAnalyzer II, Technicon). The LWCCs have a long pathlength of 50 cm for N+N and nitrite, and 100 cm for SRP and silicic acid, respectively. The manifold configurations, flow diagrams, and preparation of analytical regents were based on the methods of Whitledge *et al*. (1981) for N+N and nitrite, and Hansen and Koroleff (1999) for SRP and silicic acid. The detection limit, defined as three times the standard deviation of the blanks, was 2 nM for nitrite, 3 nM for both N+N and SRP, and 11 nM for silicic acid. Seawater collected from the surface of the subtropical western North Pacific, and preserved for >1 year, was used as N+N and nitrite blanks, whose N+N and nitrite concentrations were determined by a chemiluminescent technique (Garside 1982; Kanda *et al*. 2007). The blanks for SRP and silicic acid were prepared by removing SRP and silicic acid from the western North Pacific surface water, based on the MAGIC (magnesium-induced co-precipitation) procedure (Karl and Tien 1992). More detailed information on the highly-sensitive colorimetry is described in Hashihama (2007) for N+N and SRP, and in Hashihama and Kanda (2010) for silicic acid.

Field observation

We conducted a continuous underway survey on cruise transects of the R/V *Hakuho-maru*, R/V *Tansei-maru*, and RT/V *Umitaka-maru*. These transects covered a broad area over the central and western Pacific Ocean and southern Indian Ocean. The survey was intermittently performed during the period from December 2004 to September 2008. This period includes a period prior to the project in order to integrate as much nutrient data as possible. During the project, a four-channel highly-sensitive nutrient analyzer was used to continuously measure the concentrations of N+N, nitrite, SRP, and silicic acid in seawater, which was pumped up from the ship's bottom. Prior to this project, a two-channel highly-sensitive analyzer for N+N and SRP was adopted. The spatial resolution of our nutrient analysis was at a horizontal scale of approximately 1 km at a cruising speed of 15 knots (27.8 km h^{-1}). Temperature, salinity, and *in vivo* chlorophyll fluorescence of the water were continuously measured by using a

thermosalinometer, and a fluorometer, throughout all cruises. Data for nutrient, temperature, salinity, and in vivo chlorophyll fluorescence were taken at 1-min intervals.

Along with the nutrient analysis, seawater samples were discretely collected from the pumped-up water for total dissolved phosphorus (TDP) analysis, microscopic observations, flow cytometry, and high-performance liquid chromatography (HPLC) algal pigment analysis. The TDP samples were filtered through a precombusted Whatman GF/F filter by gravity filtration, and the filtrate was immediately frozen for later analysis. The concentration of TDP was determined by a persulfate oxidation method using an automated flow analytical system (QuAAtro, SEAL Analytical). The standard analytical protocol of the manufacturer was adopted. The concentration of dissolved organic phosphorus (DOP) was determined by subtracting the SRP concentration from the TDP concentration.

The microscopic samples were fixed with glutaraldehyde, or neutralized formalin, at a final concentration of 1% (v/v). The fixed samples were concentrated by sedimentation. Micro-sized diatoms and diazotrophs (*Trichodesmium* and *Richelia*) in the concentrates were counted using an inverted microscope.

The samples for flow cytometry were fixed with 1% (v/v) glutaraldehyde. The fixed samples were frozen in liquid nitrogen and then stored at –80°C until analysis. We used a PAS-III flow cytometer (Partec) equipped with a 488-nm argon ion laser. *Prochlorococcus*, *Synechococcus*, and eukaryotes were identified using protocols established by Marie *et al*. (1999). In addition to identifying these phytoplankton groups, we identified nanoplanktonic unicellular cyanobacteria using the procedure described by Kitajima *et al*. (2009) and Sato *et al*. (2010). This nano-sized organism could potentially fix dinitrogen gas under nitrate-depleted surface waters (Zehr *et al*. 2001).

The seawater samples for HPLC were filtered through a Whatman GF/F filter. The filters were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Pigment analysis was conducted by the method described by Zapata *et al*. (2000) using a Shimadzu HPLC system. Ten biomarker pigments; monovinyl chlorophyll *a*, divinyl chlorophyll *a*, pheophytin *a*, pheophorbide *a*, peridinin, 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, myxoxanthophyll, and zeaxanthin, were detected using a photodiode array. These pigments were quantified from the peak area calibrated against that of standard pigments (DHI Water and Environment).

Results and Discussion

Basin-scale distribution of surface N+N and SRP

The study area through the central and western Pacific Ocean, and the southern Indian Ocean, except for equatorial upwelling, was characterized by an overall depletion of N+N concentration below 20 nM (Fig. 1(a)), as has been reported in Hashihama *et al*. (2009). Although all cruises were conducted during different seasons, a consistent depletion of the N+N concentration was observed. Since nitrite concentrations were nearly equal to the detection limit of 2 nM, N+N concentrations were mostly ascribed to nitrate concentration.

In contrast, SRP concentrations varied dynamically between <3 nM and 300 nM throughout the study area (Fig. 1(b)). The SRP concentrations were generally lower (<50 nM) in the western North Pacific and the western Indian Ocean, and in the vicinity of Fiji, Hawaii, and Java, than in other areas including the western equatorial Pacific, the Coral Sea, and the central Indian Ocean (100–300 nM). Among the

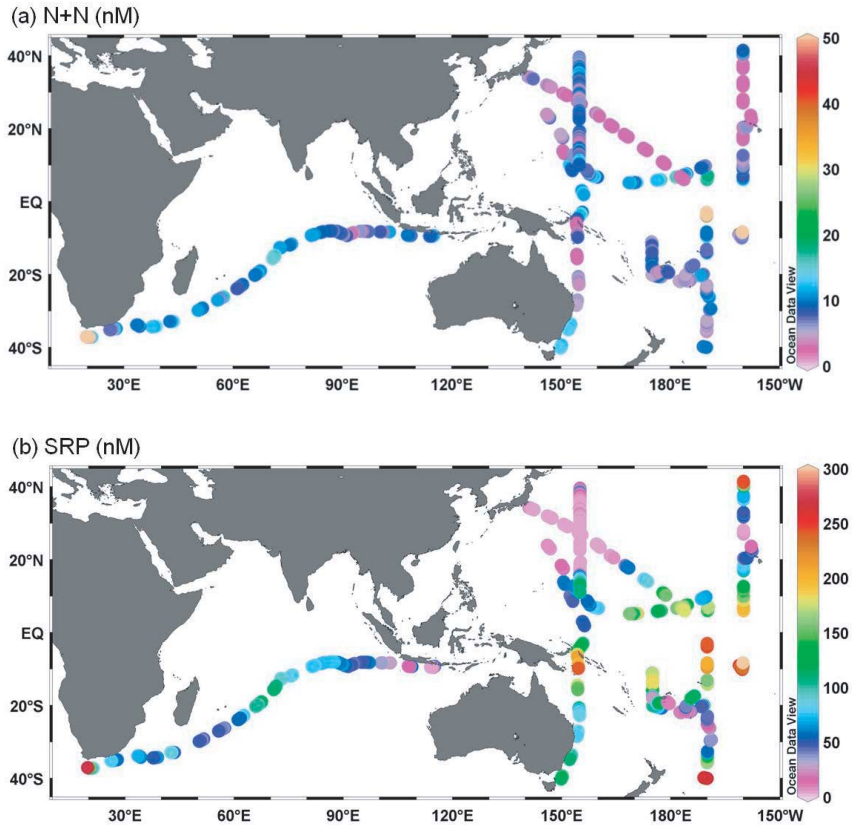

Fig. 1. Surface distribution of (a) N+N, and (b) SRP, in the central and western Pacific Ocean and the southern Indian Ocean.

lower SRP regions, a remarkable exception was found: an almost complete exhaustion of SRP (<10 nM) existed at a horizontal scale of >2000 km in the western North Pacific in both summer and winter. The SRP exhaustion was most likely associated with an elevated dinitrogen fixation and nanocyanobacteria abundance (Kitajima *et al*. 2009). We suggest that the macro-scale SRP exhaustion with an enhanced diazotrophic activity was driven by high dust deposition from the Asian continent to the surface of the western North Pacific (Hashihama *et al*. 2009). Since we also observed a relatively high abundance of *Trichodesmium*, *Richelia*, and nanocyanobacteria in other lower SRP regions as described below, SRP decreases in the surface waters of the subtropical ocean are suggested to be tightly coupled with diazotrophic activity. On the other hand, the higher SRP waters could be conservative due to an inadequate dust supply and little diazotrophic activity.

Diazotrophs can utilize several forms of DOP as an alternative to SRP (Dyhrman *et al*. 2006), despite the fact that DOP is not directly available for the most part. Surface DOP concentrations in the SRP-depleted western North Pacific were generally above 200 nM and DOP was more abundant than SRP. Although the signifi-

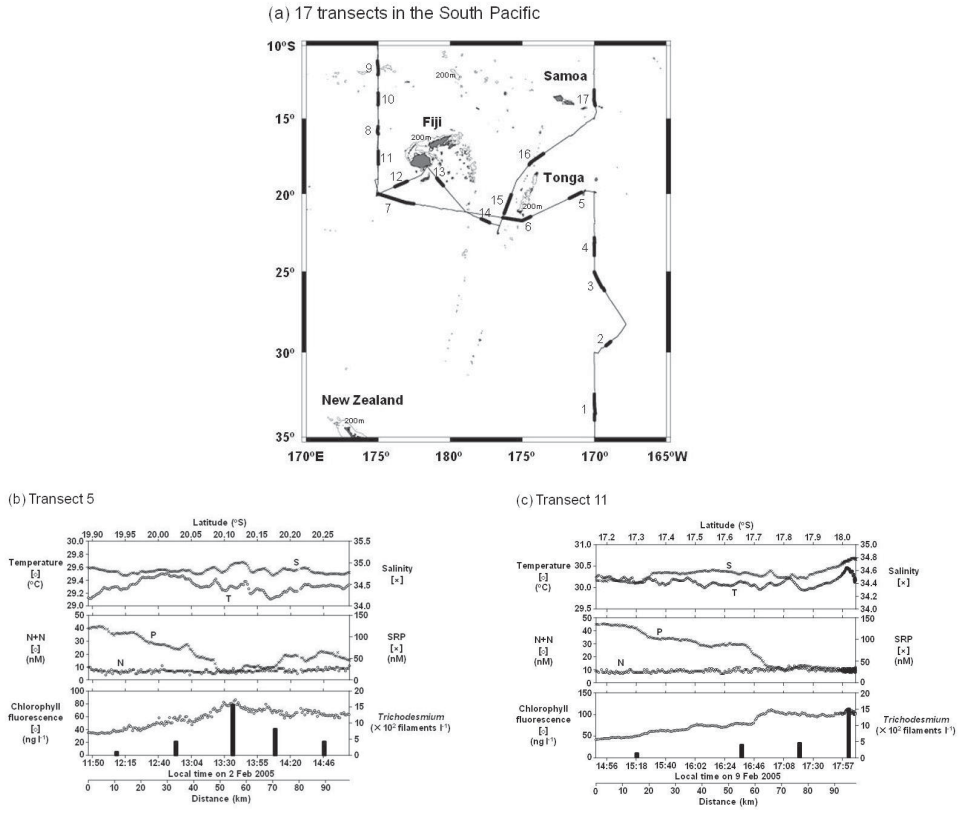

Fig. 2. (a) Cruise track in the vicinity of the South Pacific islands from January to February 2005. Thick lines with numbers denote transects where a continuous underway survey was done. Surface distributions of temperature, salinity, N+N, SRP, chlorophyll fluorescence, and *Trichodesmium* on (b) Transect 5, and (c) Transect 11. Abundance of *Trichodesmium* is shown by black bars. Modified from Hashihama *et al.* (2010).

cance of the DOP pool as a source of P for diazotrophs is still controversial (Moutin *et al.* 2008), we cannot exclude the possibility that the diazotrophic activity was supported by the bioavailable DOP.

Sporadic decrease of SRP in the South Pacific

In addition to the basin-scale variation of SRP, mesoscale SRP decreases at a <100-km horizontal scale were observed during cruising. In particular, this was most significant in the vicinity of the South Pacific islands during the R/V *Hakuho-maru* KH-04-5 cruise from January to February 2005 (Hashihama *et al.* 2010). In this area, a total of 17 transects, whose lengths ranged between 42 and 271 km, were sampled by continuous survey (Fig. 2(a)). This area was characterized by an overall depletion of N+N as described above, but SRP varied from 7 to 192 nM.

In 7 out of the 17 transects, mesoscale SRP decreases were observed. The SRP decrease coincided with an elevation of *in vivo* chlorophyll fluorescence, which was accompanied by an increase in phytoplankton abundance, as revealed by microscopy, flow cytometry, and accessory pigments. This mirror-image relationship

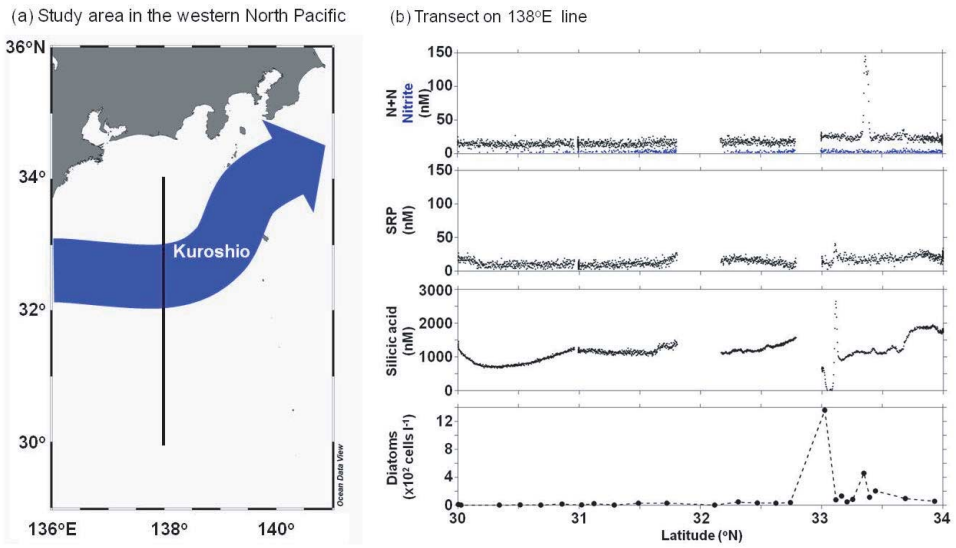

Fig. 3. (a) Cruise track in the western subtropical North Pacific from June to July 2007. A light gray arrow denotes the Kuroshio Current path, which was available from Quick Bulletin of Ocean Condition, published by the Hydrographic and Oceanographic Department of the Japan Coast Guard (http://www1.kaiho.mlit.go.jp/KANKYO/KAIYO/qboc/index_E.html). (b) Surface distributions of N+N, SRP, silicic acid, and diatoms, on the 138°E line.

between the SRP concentration and phytoplankton abundance was most apparent on both a 99-km transect east of Tonga, where the SRP concentration ranged from 17 to 125 nM (Transect 5; Fig. 2(b)), and on a 98-km transect west of Fiji, where the SRP concentration ranged from 23 to 136 nM (Transect 11; Fig. 2(c)). Both of the transects contained distinct blooms of *Trichodesmium* in areas with the lowest SRP concentrations. In contrast, no corresponding fluctuations in temperature and salinity occurred in areas of minimum SRP levels. The abundance of *Trichodesmium*, *Prochlorococcus*, *Synechococcus*, and all accessory pigments examined tended to be higher in the low SRP waters than in the high SRP ones. In particular, *Trichodesmium* occurred in low SRP waters (<25 nM). There was no significant relationship between SRP concentrations and nanocyanobacteria. These results suggest that surface SRP decreases are associated with phytoplankton utilization of SRP, and that the nitrogen supply from *Trichodesmium* may contribute to this utilization.

Sporadic decrease of silicic acid in the western North Pacific

Continuous observations for trace silicic acid in conjunction with the N+N, nitrite, and SRP observations, were performed in the surface waters of the western North Pacific along the 138°E line during the R/V *Tansei-maru* KT-07-15 cruise from June to July 2007. As described above, the concentrations of N+N, nitrite, and SRP, were almost depleted in the western North Pacific, while silicic acid concentrations were generally above 500 nM. However, we detected an unstable field of silicic acid and associated diatom dynamics in the northern edge of the Kuroshio Current during summer (Fig. 3(a)). Silicic acid concentrations between 33°N and

34°N along the 138°E transect showed highly-dynamic variations (Fig. 3(b)). A threshold concentration for net silicic acid uptake by silicon-starved diatoms is generally assumed to be approximately 1 μM (=1000 nM) (Paasche 1973). However, we detected a complete depletion of <11 nM at a 4-km horizontal scale around 33.1°N, and this concentration level was far below the threshold. Concentrations of N+N and SRP at the silicic acid depleted site were not so much different from those in the neighboring waters. A sporadic N+N elevation up to 145 nM was detected at 33.4°N. The temperature and salinity did not exactly correspond to these nutrient fluctuations.

At the silicic acid depleted site, cell densities of diatoms drastically increased up to 1358 cells l^{-1}. This diatom bloom mostly consisted of *Chaetoceros* spp. In contrast to the cell abundance, a diatom marker, fucoxanthin, was quite low at the silicic acid depleted site. This suggests that the synthesis of photosynthetic pigments was limited. Indeed, the concentration ratio of the chlorophyll derivative (pheophytin *a* + pheophorbide *a*) to chlorophyll *a* was apparently high at the silicic acid depleted site, implying that organic matter in the cells was toward degradation rather than synthesis. These results indicate that diatoms could exhaust silicic acid up to an extremely-low concentration level and that their cells were in a decline phase.

Conclusions

Our intensive observations first demonstrated the surface distribution of nanomolar nutrients in the central and western Pacific Ocean and the southern Indian Ocean. The main findings are as follows: (1) A basin-scale extremely-low P availability appeared to be unique to the western North Pacific. (2) Mesoscale decreases of the surface SRP, observed in the vicinity of islands, were likely produced through SRP uptake by phytoplankton, and sporadic *Trichodesmium* blooms might have played a significant role in the supply of new nitrogen for SRP uptake. (3) Our highly-sensitive colorimetry could detect dynamic fluctuations of silicic acid despite relatively consistent N+N, nitrite and SRP, and the unusual complete depletion of silicic acid was ascribed to a likely uptake by diatoms. The highly-sensitive nutrient analysis is found to be essential to biogeochemical studies in oligotrophic open-ocean systems. Further studies using this technique should reveal various types of temporal and spatial variations of nanomolar nutrients and their interrelationships with biogeochemical processes.

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Topic 2: Development of an accurate and simple method for measuring particulate phosphorus and investigation of decomposition processes of dissolved organic phosphorus in terms of hydrolysis extoenzyme (M. Suzumura and coworker: N. Yamada)

Introduction

Phosphorus (P) is the essential nutrient sustaining marine primary production. Regeneration of bioavailable dissolved P from particulate matter is an important process maintaining P availability in the marine ecosystem (Karl and Björkman 2002; Paytan *et al*. 2003; Benitez-Nelson *et al*. 2007), and the bioavailability of P compounds in seawater is largely dependent on their chemical forms. While orthophosphate is known to be an overwhelmingly important P compound due to its high bioavailability, other forms of P including particulate, and dissolved, organic P (POP and DOP) have been considered as potential P sources. However, few studies have been conducted on the spatial and temporal variability of particulate P pools in pelagic environments (Loh and Bauer 2000; Suzumura and Ingall 2004; Yoshimura *et al*. 2007). Particulate organic carbon (POC) and nitrogen (PON) are frequently measured as a part of routine chemical analysis in marine observations, whereas POP determination is not often

included. This is most likely because a POP determination requires an additional volume of sample water as well as additional analytical procedures and costs. Also, as to the dissolved fraction, little is known about the chemical composition of DOP. Like C and N, P is the key element in the marine biogeochemical cycle. It is an important issue to identify, quantify and characterize organic P in marine environments for a better understanding of the dynamics of bioelements in the surface ocean ecosystems.

The objective of this study was to make a breakthrough in the study of marine P. One approach was to develop a new method for the determination of POP. The conventional high temperature dry combustion (HTDC) methods show high recoveries (100 ± 5%) of standard organic P compounds (Solözano and Sharp 1980; Nedashkovskiy *et al*. 1995; Kérouel and Aminot 1996; Ormaza-González and Statham 1996; Monaghan and Ruttenberg 1999). In contrast, persulfate chemical wet oxidation (CWO) methods show low P recoveries varying greatly among the protocols (Kérouel and Aminot 1996; Monaghan and Ruttenberg 1999; Raimbault *et al*. 1999; Lampman *et al*. 2001). The CWO method, however, is unquestionably simpler and less time consuming than the HTDC method. Weighing this advantage against the fact that CWO can produce low and variable P recoveries, a persulfate CWO method has been improved in this study and then compared with a conventional HTDC method.

Another approach was to examine the effects of environmental conditions on the process of organic P regeneration in the ocean surface ecosystems. In the regeneration of organic nutrients, enzymatic hydrolysis is an essential process sustaining primary productivity in the ocean. For example, alkaline phosphatase (AP) is the major contributor to the remineralization of organic P to liberate orthophosphate (Hoppe 2003; Dyhrman and Ruttenberg 2006 and cited therein). In this study, the effects of pH variation on AP activities were examined by laboratory experiments, since pH is a particularly important factor regulating enzyme activities, and it is now evident that increases in atmospheric CO_2 concentration have changed pH of ocean surface waters (Caldeira and Wickett 2005). Also, field observations and shipboard experiments were executed to investigate the depth profiles of various dissolved P pools and AP kinetic parameters in the euphotic zone across the coastal to oceanic environmental gradient in the western North Pacific Ocean.

Methods

Particulate P determination

The HTDC method used in this study was based on the protocols of Aspila *et al*. (1976) and Solözano and Sharp (1980). Samples were added with $Mg(NO_3)_2$ and combusted at 470°C for 90 min. Phosphate was extracted with 1 M HCl. In the CWO method tested here, samples were added with $K_2S_2O_8$ solutions prepared from a low-P source (Wako Chemical, Japan) and ranging from 0.5% to 3% (w/v) $K_2S_2O_8$. The samples were then heated at 120°C for 30 min using an autoclave. Soluble reactive P concentrations in the HTDC extract and CWO solution were measured by means of a standard colorimetric method. The HTDC and CWO methods were compared using the commercially-available P compounds, and various natural analogues of particulate matter including riverine suspended particle matter (SPM), coastal and pelagic marine sediments, net-collected and cultured plankton, geochemical reference materials, and estuarine and pelagic SPM.

Laboratory and field experiments for AP kinetic studies

Acidification experiments for enzyme

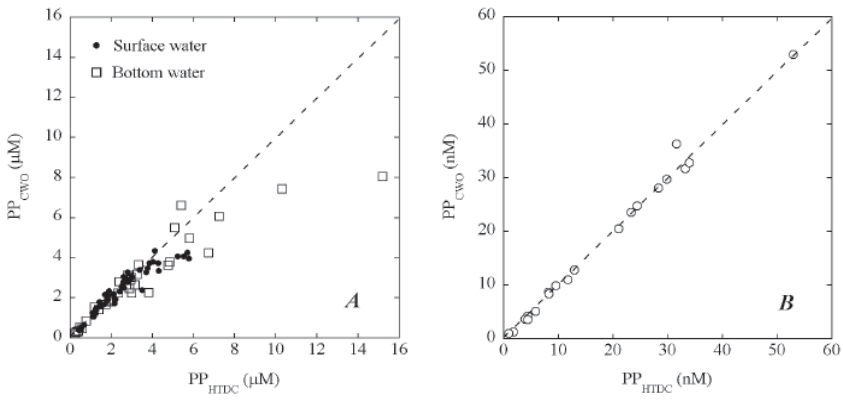

Fig. 4. (a) Plots of particulate P concentrations of HTDC versus CWO in the estuarine samples. The dashed line represents CWO/HTDC = 1. (b) Plots of particulate P concentrations of HTDC versus CWO in the pelagic samples. The dashed line represents CWO/HTDC = 1. Reprinted from Suzumura (2008), Limnol. Oceanogr. Methods, 6, 626, copyright (2008) by the Association for the Sciences of Limnology and Oceanography, Inc.

activities were carried out using surface seawater samples collected from four locations from a highly-eutrophic site of the innermost part of Tokyo Bay, the transition area between coastal and open ocean environments in Sagami Bay and Suruga Bay, and the oligotrophic open-ocean environment outside the Kuroshio Current. Among them, AP activity was measured in the samples from Tokyo Bay and Sagami Bay. Enzyme activities were determined as the hydrolysis rates of fluorogenic substrates (methylumbelliferyl phosphate (MUF-P)) according to the protocols of previous studies (Hoppe 1983; Fukuda *et al*. 2000).

Field observations and shipboard experiments to investigate various dissolved P-pools and AP kinetic parameters were conducted during the KT10-13 cruise of the R/V *Tansei-maru* in July 2010. Samples were collected from a coastal station in Sagami Bay and three pelagic stations along a transect outside the Kuroshio Current. Vertical profiles of SRP, DOP and AP hydrolyzable DOP (APH-DOP) in the euphotic zone were investigated using the method of Suzumura *et al*. (1998). To obtain complete kinetic parameters of the hydrolytic reaction via AP activity, multi-concentration measurements of MUF-P from 50 to 1000 nM equivalent to the *in situ* DOP level were executed (Thingstad *et al*. 1993; Sebastián and Niell 2004).

Results and Discussion

Development of the CWO method for particulate P determination

The recoveries of P relative to those of the HTDC method were 102 ± 6.7% from standard organic, and inorganic, P compounds, and 100 ± 3.8% from natural particulate matter analogues including riverine suspended particulate matter (SPM), sediments, plankton, and geochemical reference materials of rock. However, low recoveries of 14% to 69% were observed for reference samples of clay minerals.

A comparison, using many samples of estuarine and pelagic SPM, showed that the CWO method produced PP values consistent with those obtained by the HTDC method, except for some estuarine samples enriched in inorganic matter (Figs. 4(a) and

(b)). The low recoveries were ascribed to the presence of an inorganic P fraction highly recalcitrant to chemical digestion. The procedural blank was lower for the CWO method than for the HTDC method. The filter blanks showed a large variation among the filter materials tested; aluminum oxide membrane filters were shown to contain a considerable amount of P that could cause significant contamination in both methods. Analytical precision was equivalent between the two methods. Considering its simplicity and its less time-consuming nature, the CWO method presented here is suitable for PP determination in aquatic environments (Suzumura 2008).

AP activity in various environmental conditions

AP activities showed relatively minor variations over the pH range tested. However, the trends of acidification effects on AP activity were opposite at the sites located in Tokyo Bay and Sagami Bay. With confidence levels of 99%, AP activity increased slightly with acidification in Tokyo Bay, whereas it decreased in Sagami Bay. The effect of acidification on AP activities differed between the coastal and semipelagic samples, and this was likely due to a freshwater influence at the nearshore station. As well as the effects of acidification on AP activity, those on the activities of leucine aminopeptidase, α- and β-glucosidase, and lipase, were examined with being important promoters of the degradation of marine organic matter, including proteins, carbohydrates, organic phosphorus compounds, and lipids. The effects of acidification on enzyme activity appear to vary depending on enzyme type and location, but we conclude that acidification will cause changes in the cycling of organic matter in marine ecosystems, in particular to proteinous and lipid substances (Yamada and Suzumura 2010).

In the field observations of various dissolved P-pools, it was found that SRP concentrations at/above the Chl *a* maximum layer (CML) depth were quite low around the detection limit (20 nM) at all stations, suggesting a P deficient condition. Below the CML, to 200-m depths, SRP concentrations increased rapidly with depth to 107–194 nM at the pelagic stations and to 1100 nM at the coastal station. DOP was the dominant P pool at/above the CML accounting for 62 to 100% of the total dissolved P. The lowest concentration of DOP (30 nM) was found in the coastal surface waters (0- and 10-m depths) and, below this zone, DOP rapidly increased to 260 nM. DOP depth profiles at the pelagic stations showed minimum concentrations (48–81 nM) at the subsurface (10–20 m), increasing with depth more gradually than that at the coastal station. The concentrations of APH-DOP were low, ranging from 20 to 54 nM. APH-DOP composed a considerable fraction of the total DOP (50–81%) only above the CML at the coastal station where the lowest DOP concentration was observed. APH-DOP was rather a minor constituent, accounting for 14–48% of the total DOP in the pelagic samples. A large part of the ambient DOP in ocean waters was unreactive to AP, and, hence, could be low in bioavailability as a P source.

In some depth samples from all stations, AP kinetic parameters, including the potential maximum hydrolysis rate (Vmax), the apparent half-saturation constant (Km), and the turnover time of APH-DOP (TA), were characterized to evaluate the importance of DOP remineralization in the P cycle. The highest Vmax in the pelagic stations was observed at the surface and rapidly decreased to constantly low values throughout the sampling depths. At the coastal station, the highest Vmax was observed at the subsurface (10 m) and gradually decreased with depth. Comparing the data at the CML depth, there were considerable differences across

the environmental gradient from coastal to open-ocean stations. The *in situ* hydrolysis rate that was estimated from the ambient APH-DOP concentration and TA decreased considerably, whereas TA and Km increased. Although the concentrations of DOP and APH-DOP were relatively comparable across the environmental gradient, a high efficiency in DOP remineralization by AP activities observed only in the coastal environment, could sustain P requirement from the high Chl *a* biomass therein. The role of DOP as a potential P source is quite complicated and its importance varies greatly among the environmental conditions.

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Topic 3-1: Development and examination of the method for measuring the concentrations of volatile organic carbon in seawater (H. Ogawa)

Introduction

Dissolved organic carbon (DOC) in the ocean is a huge carbon reservoir in the Earth's surface system. Its standing stock amounts to 680 Gt which is approximately equivalent to the pool of CO_2 in the atmosphere (760 Gt) or land biomass (570 Gt). Little is known about the chemical composition and structure of DOC. Recently, the molecular-weight distribution of DOC has been investigated, and these studies demonstrated that low-molecular-weight (LMW) fraction (<1 k Da) accounts for a major part of DOC (60–80% of the bulk DOC; Ogawa and Tanoue 2003). However the chemical composition at the molecular level is entirely unknown. On the other hand, some specific compounds dissolved in seawater having LMW and a low boiling point have been determined as volatile organic compounds, such as DMS, halocarbon, methane and other hydrocarbons. Although the carbon amount of these specific compounds in total is less than 1% of DOC, it is possible that there might be more kinds of volatile compounds in seawater that remain still unidentified, considering that most of DOC is LMW. If it is true, our understanding of carbon flux to the atmosphere from the surface ocean should be dramatically changed. Thus, it is necessary to determine the bulk concentration of volatile organic carbon (VOC) in seawater, but, in fact, there have been few studies of this. About 30 years ago, one study tried to determine VOC concentrations using a purge and trap method (Mackinnon 1979). It was concluded that VOC accounts for 1–6% of the total organic carbon (TOC). It appears that this result did not have a great impact on the trend of research because, at that time, the problem of climate change, such as global warming and its relation to the oceanic carbon cycle, received less attention than at present. However, the result means that 10–40 Gt of the VOC pool exists in the ocean, which exceeds considerably the biomass carbon in the ocean (*ca.* 3 Gt) and cannot be bypassed in terms of the oceanic carbon cycle. Furthermore, as the analytical precision of carbon (as CO_2) has greatly improved compared with that of 30 years ago, it is possible that we can estimate the pool size of VOC in the ocean more precisely. Thus, we have sought to develop and examine the method for measuring the bulk concentrations of VOC in seawater.

Methods

First, a purge and trap method to extract VOC components from seawater was

tested. The analytical system was all homemade on the basis of the previous study (Mackinnon 1979). The carbon concentrations were measured by a non-dispersive infrared analyzer (NDIR) after converting VOC into CO_2 through high-temperature combustion. Next, a distillation method was examined. It is based on the measurement of TOC concentrations in distillate from seawater by using a rotary evaporator. A TOC analyzer (Shimadzu TOC-5000) was used for TOC measurements.

Results and Discussion

Examination of the analytical method

A variety of analytical conditions for the purge and trap method, such as sample volume, materials of adsorbent, purging time and temperature, were examined. However, the analytical blank which was obtained from the measurement for ultra pure water (Milli-Q) was often comparable to the values of seawater samples. And the recoveries for some standard compounds of low boiling points that were spiked into seawater, were not satisfactory. Finally, it was concluded that the purge and trap method was difficult to apply to the measurement of the bulk VOC in seawater. Next, the blank, and recovery by the distillation method, were tested for ultra-pure water and some standard compounds, respectively. Consequently, the results were much better than the purge and trap method. So, in this study, the distillation method was considered acceptable for measuring the bulk VOC concentrations in seawater. However it is possible that a very volatile fraction, that is rapidly purgeable, might be missed when the sample is acidified and bubbled to purge CO_2 in the TOC measurements. In contrast, relatively less volatile (semi-volatile) compounds, due to their hydrophilic properties, might be included in this measurement because part of them are possibly recovered in the distillate from seawater. Thus, further examination is necessary, and the definition and chemical significance of the organic fraction obtained from this method should be clearer. At least it may be interesting considering it as a possible source of organic compounds in rain water derived from sweater distillation.

The vertical distributions of VOC in the ocean measured by the distillation method

The measurement of VOC concentrations by the distillation method was first applied to the samples collected in the subtropical areas on the KH-06-2 expedition of R/V *Hakuho-maru*. The VOC concentrations varied relatively largely from 5 to 20 μM in the surface layer (<100 m), whereas those in the deep waters between 200–3000 m were relatively constant around 10–15 μM. This suggests that both production and consumption might occur in the surface layers and a biologically refractory fraction of VOC might remain in the deep layers.

Topic 3-2: Characterization of decomposition processes of dissolved organic matter (by H. Ogawa)

Introduction

The concentrations of DOC in the ocean are higher and more variable in the surface waters, ranging normally from 50 to 90 μM, than in the deep waters (around 35–45 μM). Previous studies using a ^{14}C dating approach to oceanic DOC have demonstrated that the fraction of DOC that exists with a uniform concentration throughout the deep ocean has a long life (up to 6,000 yrs), while that accumulating only in the surface layers has a relatively short residence time. The former is called "refractory DOC; R-DOC" and the latter is "semi-labile DOC; S-DOC". Some recent studies have reported that the concentrations of S-DOC vary significantly with

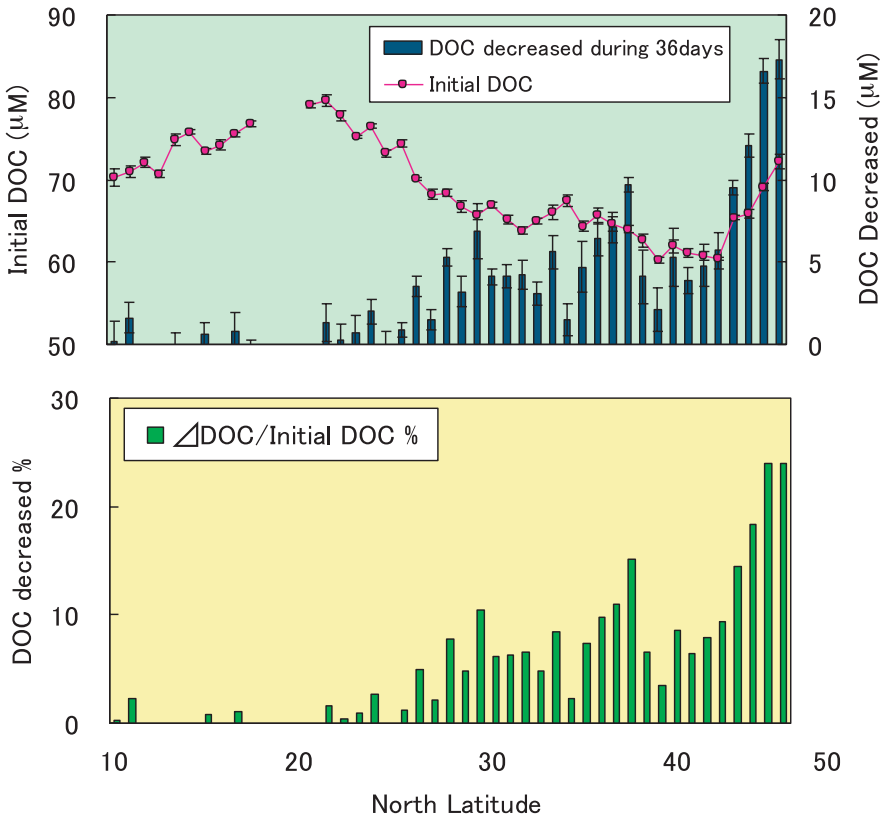

Fig. 5. Distribution of bacterially-degradable DOC in surface waters along the meridian section of 160°W in the central North Pacific.

region. In particular, those in the subtropical region, where strong stratification develops within the surface water column, are much higher than those in the high-latitudes or the equatorial regions with an upwelling system. This is explained by the hydrographic stability of surface water mass mainly regulating the concentrations of S-DOC (Ogawa and Tanoue 2003). However, little is known about the regional variation in their quality, especially the microbiological degradability. Dissolved organic matter (DOM) includes nitrogen (DON) and phosphorus (DOP) as well as carbon (DOC), which are essential elements for biogeochemical cycles in the ocean. The decomposition of the semi-labile DOM (S-DOM) leads to the supply of nutrients into the euphotic layers, as well as CO_2 production. Therefore, it is possible that the microbial degradability of S-DOM might be one of the key factors which control biological production in the oligotrophic environments of the subtropical region. Thus, this study has sought to elucidate the decomposition process of S-DOM in the surface ocean.

Methods

The samples obtained through the KH-05-2 cruise of the R/V *Hakuho-maru* in the

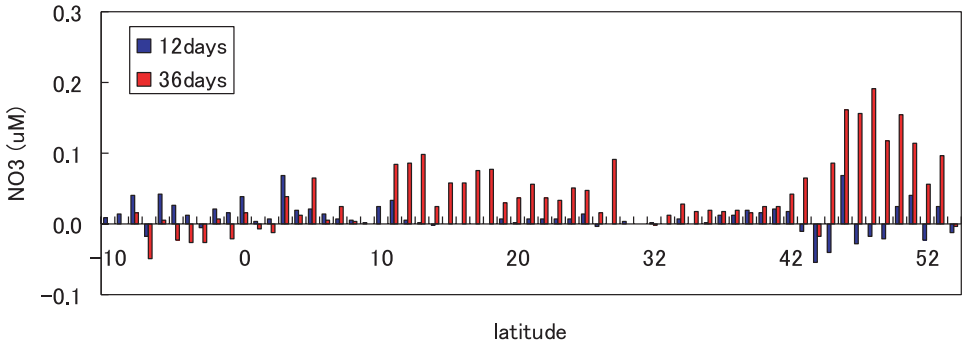

Fig. 6. The changes in nitrate concentrations during the degradation experiment of surface seawater collected along the meridian section of 160°W.

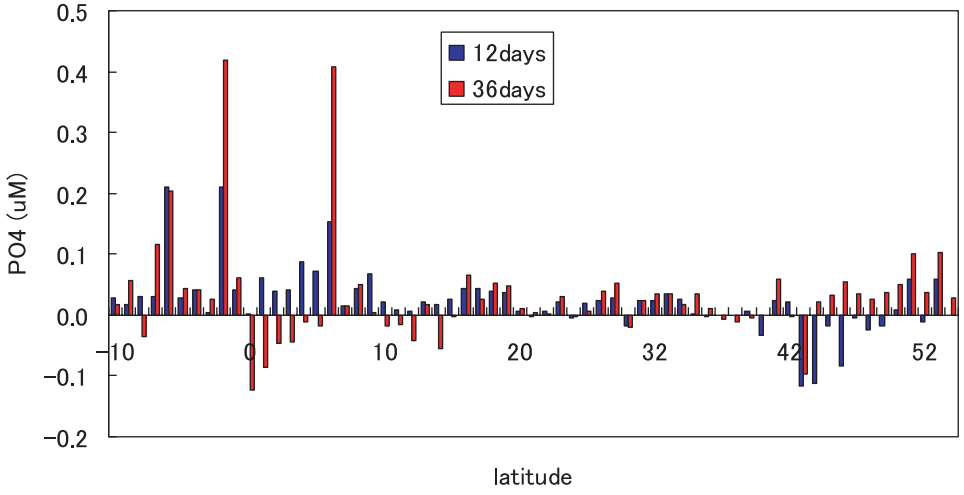

Fig. 7. The changes in phosphate concentrations during the degradation experiment of surface seawater collected along the meridian section of 160°W.

central North Pacific were used for the decomposition experiment of DOM. The surface waters were collected along the meridian transect of 160°W at intervals of 1 degree between 54°N and 10°S. The sample, filtered through a Whatman GF/F filter, was poured into a glass ampoule and then sealed with a torch. The sample for determining the initial concentrations of DOC and DON was frozen immediately. That for the degradation experiment was incubated in the dark at room temperature, and then frozen after 1 month. The measurement of DOC and DON concentrations was conducted with a high-temperature combustion method (Ogawa *et al*. 1999). Nutrients produced by the degradation of DON and DOP during the incubation were measured with an autoanalyzer (AACS III, BRAN+LUEBBE).

Results and Discussion

The north-south distribution of the surface DOC concentrations along the meridian section of 160°W showed a clear trend indicating that they were significantly high (70–80 μM) in the subtropical region compared with those in the subarctic region (60–70 μM, Fig. 5). The DOC reduction by bacterial respiration during the 1-month incubation were 5–15 μM (10–25% of the initial DOC) in the subtropical gyre, while less than 5 μM (<5% of the initial) in the subarctic region. It demonstrates that the microbial degradability of S-DOC could vary largely with regions and most of S-DOC accumulated in the subtropical surface could not be easily utilized by heterotrophic microbes.

The degradations of DON and DOP were evaluated from the production of nitrate (Fig. 6) and phosphate (Fig. 7), respectively. Overall, the degradability of both DON and DOP is relatively higher in the subarctic than in the subtropical area; however, the difference was not so large as compared with the case of DOC. In the subtropical gyre, approximately 20–100 nM (1–3% of the initial DON) of nitrate and 0–50 nM (<15% of the initial DOP) of phosphate were produced by microbial oxidation of DOM during the 1-month incubation. It suggests that DON and DOP would be potentially important sources of nutrients which support primary production in the oligotrophic environment of the subtropical gyre. Furthermore, it is possible that S-DOM in the subtropical region might function efficiently as the carbon fixation through a selective recycling of nitrogen and phosphorus relative to carbon.

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Topic 4: Microbial degradation of submicron particles in the ocean (by K. Kogure and coworkers: Y. Seo, T. Suga, H. Kondo, M. Nishimura)

Introduction

In the surface layer of the ocean, there are approximately one million prokaryotic cells. Most of them are present in a free-living state. In this layer, there are also so called submicron particles (SMPs) (Koike *et al*. 1990), of which concentrations usually exceed that of prokaryotic cells by one to two orders of magnitudes. Therefore, prokaryotic cells may constantly collide with those SMPs. The origin, chemical composition and fate of SMPs are, however, not yet clear (Yamasaki *et al*. 1998), primarily because technically it is not easy to collect them, sort them depending on particular characteristics and analyze them chemically and quantitatively. Therefore, current knowledge has been obtained mostly by a particle counter or electron microscopy. It is assumed that the characteristics of SMPs may vary, depending on the environments, season, and various biological components. For instance, viral particles are one component of SMPs, and their numbers may fluctuate with those of the host cells.

SMPs are considered as part of DOC and, generally of a biological origin. As they may be composed of relatively labile organic materials, they potentially serve as organic substrates for heterotrophic marine microorganisms. The understanding of the origin, turnover, and degradation mechanisms is quite important for clarifying the material cycles in the ocean water column (Ogawa and Tanoue 2003). So first, how do prokaryotic cells degrade SMPs? According to the textbooks, they are first degraded into smaller molecules or building blocks (Obayashi and Suzuki 2005) outside of the cells. This may be accomplished by extracellular enzymes or enzymes associated with the surface of the cells. The molecular weight should be less than several hundred prior to the uptake. As a second step, degraded components are transported into the cells through appropriate transport systems that are generally quite specific and selective.

Although this basic concept of microbial biodegradation has been well established, it was developed by the experimental examinations conducted in the laboratory, which is characterized by high concentrations of both cells and organic matter. It is easily assumed that the concentrations of extracellular enzymes may be high, so that they effectively degrade high-molecular compounds into smaller molecules. However, the application of this concept to the natural environment is difficult primarily because the concentrations of both cells and organic compounds are by far less than those in the test tubes. In that case, the cells may have two potential problems: first, cells have to recognize the compositions of organic compounds in order to synthesize proper enzymes, otherwise, cells may waste their energy and cellular components; and second, even if the cells synthesize a proper enzyme, the extracellular release may lead to the diffusion of degraded molecules. If the harvest is smaller than the investment to synthesize enzymes, cells may not be able to maintain themselves.

We proposed a new concept for the microbial degradation of SMPs in the ocean, i.e., the cells first retain SMPs around the cells, synthesize proper enzymes, degrade while SMPs are still retained on the cell surface, and uptake with minimum diffusion. By retaining on the cell surface, the cells may be able to recognize the chemical compositions of SMPs. This step is crucial for minimizing the cellular energy required for the biodegradation and subsequent uptake. The purposes of this investigation were, first, to collect prokaryotic cells that may potentially have the ability of "particle capturing" (PC) and second, to clarify the ecological significance of those PC prokaryotic cells.

Methods

Two methods, an optical method using atomic force microscopy (AFM) and a microbiological method, were employed. For AFM, seawater samples were fixed with formaldehyde (1%, final conc.), filtrated through GS (Millipore, pore size 0.2 μm) and observed by AFM (mode. SPM-9500 J2, Shimadzu). The AFM was equipped with a microfabricated and oxide-sharpened Si_3N_4 cantilever (OMCL-AC160TS-C1, Olympus) with a pyramidal tip and a force of 42 Nm^{-1}. Prokaryotic cells were differentiated from non-living particles on the basis of their size, shape and cross-section (Nishino *et al.* 2004). Cells with SPMs were counted and the relative numbers of particle-possessing cells among the total number of bacterial cells was determined. At least 100 cells were observed in each sample. The latter method was employed for collecting cells with the PC ability and subsequent community structure analyses. Cells with PC ability were collected by using a magnetic separation method (Seo *et al.* 2007). In

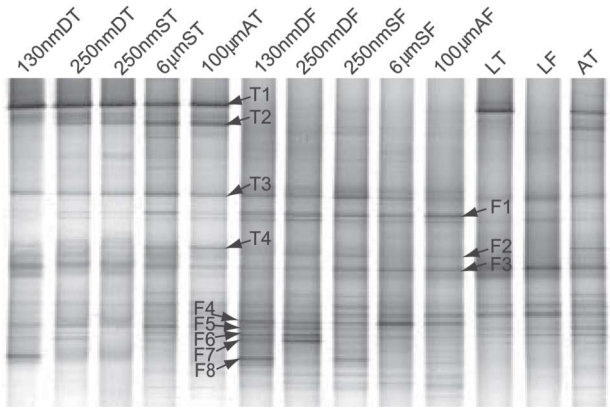

Fig. 8. Community structure analyses of cells collected by using different paramagnetic beads. Each lane indicates a sample obtained with paramagnetic beads of different size and materials. The numbers indicate the size of particles. D; dextran, S; silica, T; total population, F; filtered samples.

brief, particles of an appropriate size and composition were added into 1 mL of sample sweater, gently mixed for one hour and collected with a magnet. The cells with particles were treated for DNA extraction, PCR amplification for 16S rDNA and DGGE analyses. The fluctuation of bacterial cells with PC ability were observed in a mesocosm experiment conducted at the International Coastal Research Center, Ocean Research Institute, the University of Tokyo (ICRC-ORI) in Otsuchi, Iwate Prefecture from May to June in 2008. Two water tanks were filled with 200 l of 200 μm filtered seawater from each sampling station and amended with inorganic nutrients ($NaNO_3$, final conc. 44.1 μM and NaH_2PO_4, final conc. 4.4 μM). The two tanks were placed in an open air pool, to which surface seawater of the bay was introduced to maintain the temperature. On Day 5, both tanks were covered with a blackout curtain to accelerate the degradation process of phytoplankton. The seawater samples were taken with acid-washed polycarbonate bottles after mixing the seawater in the mesocosm.

Results and Discussion

Collection and characterization of prokaryotic cells with PC ability

The cells with PC ability were collected from seawater samples taken from Tokyo Bay, Sagami Bay, and offshore environments by a magnetic beads method of different sizes and materials. The relative numbers of such cells, and their community structures were analyzed in comparison with those of total cells. The results showed the followings: first, generally the numbers of prokaryotic cells collected with the paramagnetic beads were approximately one tenth of the total bacterial cells. Those cells are regarded as those which have the ability to absorb the paramagnetic beads around the cell surface during the one-hour incubation time. Therefore, it is regarded that those cells potentially have the ability to capture SMPs. Second, the community structures of those with PC ability were analyzed by the DGGE method (Fig. 8). They were different from those of total cells in the seawater. This indicates that there are unique populations that have PC abilities in seawater. Third,

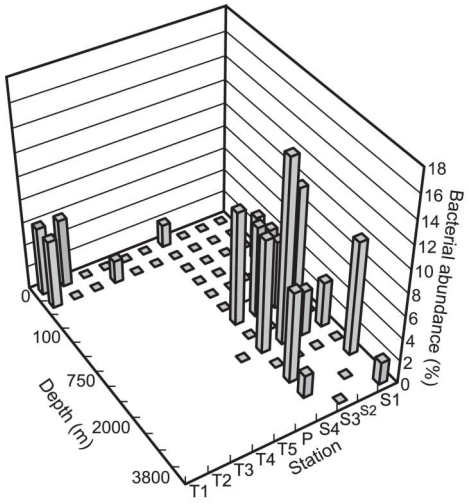

Fig. 9. Relative number of cells with PC abilities. T; Tokyo Bay, P; Sagami Bay, S; offshore stations.

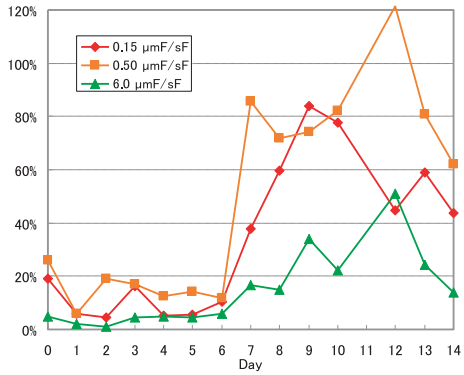

Fig. 10. Change in the relative number of prokaryotic cells with PC ability during the course of the mesocosm experiment. Three lines indicate results obtained with particles of different size.

the apparent community structures varied with the paramagnetic beads used. Although both size and materials affected the results, it seems the size was more influential. For instance, when particles larger than 6 μm were used, the apparent community structures were considerably different from those collected with particles less than 250 nm. For prokaryotic cells, the 6 μm particles may serve as a substrate for attachment, not capturing because they are much larger than the cell size. Therefore, this result indicates that abilities of attachment and capturing are not always same, and different populations might be involved in attachment or capturing. Fourth, the cell-specific aminopeptidase activity was measured for the cells with PC ability, and others. The activities of the former was generally 4 to 7 times higher than those of the latter, indicating that particle-capturing activity is coupling with high-degradation activities.

Observation of cells with PC ability by using AFM

Although the method using magnetic beads provide us with information on prokaryotic cells with possible PC ability, it is critical to observe cells directly and to confirm the occurrence of SMPs around the cells. However, both electron microscopy and epifluorescent microscopy have a limitation for this purpose. Tedious preparations are required for the former and such treatments may cause artifact. Although the sample preparation is much easier for the latter, the resolution is not sufficient for recognizing SMPs on the cell. Therefore, AFM was used for the observation of cells and SMPs on them. The following results were obtained by this approach. First, the presence of prokaryotic cells possessing particles around the surface in marine environments was confirmed. Second, the relative numbers of those cells varied horizontally and vertically (Fig. 9). Generally relatively high numbers were observed in highly eutrophic areas. In the open ocean, however, the numbers were higher in deep layers (500–2,500 m). These results indicate that the relative numbers of prokaryotic cells with PC ability may reflect the concentration and availability of organic matter in the environments. However, further investigation is required to confirm this result.

Dynamic variation of prokaryotic cells with PC ability in the mesocosm

The change in the number and community structure of cells with PC ability was investigated by using meso-scale seawater tanks in Otsuchi Bay. The two tanks filled with natural seawater were placed in an open space and amended with nutrients to induce phytoplankton bloom. The tanks were exposed to natural light at the beginning, and then covered with black curtain for initiating degradation steps. The field observations clarified the followings: First, the relative numbers of cells with PC ability sharply increased following algal bloom, and then declined (Fig. 10). Second, the community structures of cells with PC ability were different from those of total populations in the mesocosm.

Conclusions

1. It was confirmed that by using paramagnetic beads and subsequent collection with a magnet, cells with the ability to capture submicron particles are recovered from natural seawater. Their community structures are different from those of the total populations, indicating that there are specific groups with higher PC ability.

2. The relative numbers of cells with PC abilities varied horizontally and vertically in marine environments. In addition, the numbers also changed with phytoplankton bloom in the mesocosm tank. It is assumed that the numbers change with the concentration of organic matter, quantity, and quality of SMPs and other environmental parameters.

3. This is the first conceptual model of how prokaryotic cells may degrade SMPs in marine environments. Further investigation should clarify the mechanism of capturing and also the ecological significance of cells with PC abilities.

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