Anomalous distribution of dissolved organic carbon in the Sea of Japan

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(Received February 2, 2001; Accepted August 2, 2001)

Dissolved organic carbon (DOC) in the Sea of Japan was determined by the high temperature catalytic oxidation (HTCO) method. The chemical parameters related to DOC, i.e., the three-dimensional excitation-emission matrix spectrum, nutrients, chlorophyll a, dissolved oxygen and monosaccharides concentration were also determined. The vertical distribution of DOC in the northernmost sampling site (CM10; 44.1°N, 138.6°E) was not like that those in other sampling sites. An anomalously high DOC concentration was observed for the middle layer (250 to 1000 m) at CM10, which was two or three times higher than the concentration for the same layer at other sampling sites. Moreover, dissolved oxygen, nutrients and fluorescence intensity of marine humic-like substances were also unusual for the middle layer of CM10, compared to the same layer at other sampling sites. The amounts of Chl. a and monosaccharides between the surface and 100 m depth at CM10 were larger than those of other sampling sites. In the middle layer, the water mass structure of CM10 differed from that of other sampling sites. These results suggested that the cause of the DOC distribution anomaly at CM10 could be considered as due to the inflow of quite different water mass with incomplete oxidation of organic matter.

INTRODUCTION

The Sea of Japan is a marginal sea surrounded by the Japanese Islands, Siberia and the Korean Peninsula. The Sea of Japan is connected to the western Pacific and the Sea of Okhotsk by shallow channels (Tsushima, Tsugaru, Souya and Mamiya channels; maximum depths are less than 150 m). The deep water of the Sea of Japan has interesting properties such as the presence of a homogeneous water mass and a high dissolved oxygen content (Gamo et al., 1986). However, relatively little chemical research has been done on the Sea of Japan. Dissolved organic matter (DOM) constitutes over 90% of total organic matter in the ocean and plays an important role in the cycle of biogeochemical compounds. It is considered that the DOM in the ocean originates from deposition of atmospheric organic matter, terrestrial DOM passing through the ocean (Siegenthaler and Sarmiento, 1993) and degradation of dying phytoplankton (Sharp, 1977). However, the processes of production and decomposition of DOM in the ocean are largely unknown.

It is thought that most unidentified compounds such as humic substances are contained in DOM (Bauer et al., 1992; Druffel et al., 1992). A DOM fraction with fluorescence properties is present in the humic substances in natural waters. Hence, fluorescence analysis is considered an effective technique to investigate the characteristics of humic substances (Mopper and Schultz, 1993). It is possible to detect differences in fluorescence properties such as the intensity of a fluorescence peak and spectrum shape in the sample by three-dimensional excitation-emission matrix spectroscopy (3D-EEM). Coble (1996) reported on the fluorescence properties in a wide variety of water sam-

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amples from river, coastal and marine environments with 3D-EEM and found tyrosine-like, tryptophan-like, protein-like, and marine humic-like fluorescence properties. Therefore, 3D-EEM is an effective method for the characterization of fluorescent organic matter in natural waters (Suzuki et al., 1998).

In this study, we have observed an anomalous vertical distribution of DOC in the northern Sea of Japan. We focused on the processes of DOM decomposition to explain this anomaly. Therefore, we investigated the distribution of fluorescence intensity obtained from 3D-EEM and nutrients and dissolved oxygen associated with the decomposition of DOM. We also investigated the distribution of monosaccharides and chlorophyll $a$ associated with the production of DOM.

**METHODS**

**Sampling and sample treatment**

Sampling locations are shown in Fig. 1. Seawater samples were collected at four stations during the cruise of the R/V Hakuho Maru from July 15 to August 14, 1998 (cruise KH98-3). Seawater samples at various depths were collected with a CTD-CMS (CTD-Carousel Multi Sampling system) equipped with 12-l Niskin bottles while surface seawater samples were collected with a bucket. All Niskin bottles were completely coated inside with Teflon to prevent seawater contamination during sampling. Seawater samples were transferred directly to glass bottles (250 ml) which had been rinsed three times with the same seawater. About 200 ml of seawater samples were immediately filtered through a precombusted glass-fiber filter (Whatman GF/F filters) by vacuum filtration. After the initial three aliquots of the filtrate had been discarded, 5 ml of filtrate was transferred to a precombusted glass ampoule with a Pasteur pipette.

This filtrate for DOC analysis was acidified with 50 µl of 6N-HCl and high purity air was bubbled through to remove inorganic carbon (Sumitomoseika Co. Ltd.), before it was frozen and stored in a refrigerator (–80°C).

The filtrate for 3D-EEM analysis was transferred to a precombusted glass ampoule (5 ml) and frozen and stored in a refrigerator (–80°C). All glassware and glass fiber filters (GF/F) were precombusted in an electric oven at 450°C for 4 hours before use.

**Dissolved organic carbon analysis**

Concentrations of DOC were measured by the high temperature catalytic oxidation (HTCO) method (Sharp, 1997) with a Shimadzu TOC-5000 analyzer (Shimadzu Co. Ltd.). The instrument calibration was done by the injection of a glucose standard solution (Kishida Co. Ltd., highly pure grade) which had been dried at 60°C for 4 hours. A working standard solution (8.3 mM) was prepared by dilution of a stock solution (83.3 mM) each week with MilliQ-TOC water.

The ampoules containing the acidified filtrate samples were placed in an ice-water bath (below 5°C) and the samples were quickly thawed. After thawing, the glass ampoules were shaken and opened just before analysis. Four initial aliquots (250 µl each) of a sample were used to wash the micro-syringe and line. After washing, four or five aliquots (250 µl) of the sample was injected into the quartz combustion column. The column was
packed with Pt-alumina catalyst (0.5% Pt on alumina) and operated at 680°C.

The analytical blank (instrument blank + Milli-Q water blank) varied slightly (32.8 ± 7.1 µM) and average analytical precision was 2.1% C.V. in all analyses. The average precision of duplicate samples was 1.4% C.V.

3D-EEMs analysis

The ampoules containing the filtrate samples were thawed in an ice-water bath (below 5°C). After thawing, these samples were left at room temperature (22 ± 2°C) for 1 hour before analysis.

3D-EEM was done with a Hitachi model F-4500 fluorescence spectrophotometer equipped with a 150 W xenon lamp and high sensitivity cellholder with an attached mirror (Hitachi Ltd.). Excitation spectra were collected by scanning wavelengths from 200 to 500 nm, and emission spectra were collected from 250 to 550 nm at 5 nm intervals. The scanning speed was 2400 nm per minute with excitation and emission bandwidths of 5 nm and 10 nm, respectively. All spectra were normalized as relative fluorescence intensity (R.F.I.) and expressed in terms of quinine sulfate units (QSU). Ten QSU corresponds to the fluorescent intensity of standard quinine sulfate (10 µg/l, in 0.1 M sulfuric acid) at excitation wavelength 350 nm/emission wavelength 450 nm.

Raman scattering peak did not appear at a fixed peak position and it did not show the same intensity in all seawater samples. Moreover, the Raman scattering peak did not overlap the marine-humic like substances peak. Therefore, no correction was made for the Raman scattering effect in this study.

Monosaccharides analysis

The concentration of dissolved monosaccharides was measured by the MBTH (3-methyl-2-benzothiazolinon hydrazon hydrochloride) method as follows (Johnson and Sieburth, 1977; Johnson et al., 1981). A seawater sample (3 ml) was poured into a test tube. Two percent potassium borohydride solution (0.15 ml) was added to the test tube to reduce the monosaccharides to alditol. This reaction was carried out in a dark room for 4 h. Then, 0.7 M HCl solution (0.15 ml) was added to the sample, and reaction was allowed for 10 min. After this, periodic acid (0.3 ml) was added to the sample to oxidize terminal glycol groups of alditol. Then 0.25 M sodium arsenite solution (0.3 ml) was added to terminate the oxidation reaction; the mixture was left to stand overnight. Then, the sample was acidified with 2 M HCl (0.6 ml) and 0.6 ml of MBTH reagent (2.76 g of MBTH per 100 ml of 0.1 N HCl) were added to the sample. The test tube was tightly sealed and reacted at 110°C for 10 min. The sample containing the test tube was immediately placed in a water bath. Once cooled, 5% ferric chloride solution (0.6 ml) was added to the sample, which was reacted for 30 min in a dark room. After color development, acetone (3.0 ml) was added to the sample. The absorbance was measured at 635 nm with a Hitachi U1100 spectrophotometer (Hitachi Ltd.).

The calibration of dissolved monosaccharides was done by using glucose standard solution. All concentrations were expressed in carbon equivalents. The reproducibility of monosaccharides determination was < 5% C.V.

RESULTS

Water mass structure

Hydrographic data on water temperature and salinity were published in a preliminary report on the KH98-3 cruise (Nozaki, 1998). Vertical profiles of water temperature and salinity at the four stations in the Sea of Japan are shown in Fig. 2. At the southernmost sampling site, CM20, the temperature and salinity of the water were high from the surface to a depth of 300 m. The layer of maximum salinity was observed at 100 m. These characteristics might be influenced by the Tsushima warm current, which branches from Kuroshio current at the East China Sea. A difference of water mass structure, i.e., lower temperature and salinity were observed from the surface to 300 m at CM12. It is thought that the water mass in the Sea of Japan is divided into the northern water mass
and southern water mass with their boundary being near 40°N. In the middle layer (250 to 1000 m), the water temperature and the salinity in CM10 were slightly higher than those in other sampling sites, suggesting the water mass structure in the middle layer of CM10 was significantly different from that of other sampling sites.

In deep water (below 1000 m), a homogeneous water mass with a constant potential temperature (0.248 ± 0.049°C) and salinity (34.067 ± 0.001) was observed at all the sampling locations. The uniformities of water temperature and salinity are characteristic of the deep water in the Sea of Japan (Gamo et al., 1986).
Vertically profiles of DOC

At CM20, CM18 and CM12, a high concentration of DOC was observed in the surface (Fig. 3). The concentration of DOC decreased gradually from the surface to 1000 m. A submaximum of DOC concentration was observed at the depth of 2000 m at CM20, and the concentration of DOC from 1000 m to 2000 m increased slightly.

At CM10, the concentration of DOC decreased sharply from the surface to 200 m. The DOC concentrations in the surface layer (surface to 200 m) were two or three times higher than those at the same depth in other sampling sites. High DOC concentrations were observed in the middle layer (250 to 1000 m). The maximum concentration was 248 µM at the depth of 600 m, which was almost four times higher than values at other sampling sites. At depths below 1000 m, the DOC concentration increased with depth. The values at CM10 were higher than those at other sampling sites.

3D-EEM analysis and vertical profile of R.F.I.

The 3D-EEM spectrum at CM10 (depth: 3500 m) is shown in Fig. 4. The fluorescence maxima were observed at almost the same excitation and emission wavelengths in all samples (except the surface samples), which was similar to the marine humic-like fluorescence property reported by Coble (1996). The fluorescence peak was excited in the UV region (300–330 nm) and had an emission maximum between 380 to 420 nm. The fluorescence maximum peak position in the deep layer (below 1000 m) was slightly shifted (5–20 nm) towards longer wavelengths (red shift) compared to the surface layer (surface to 200 m). There was no shift of peak position in terms of sampling locations.

The vertical profile of relative fluorescence intensity (R.F.I.) at the four stations in the Sea of Japan is shown in Fig. 5. In the surface layer (surface to 200 m), the R.F.I. values were low; then, the value of R.F.I. increased with depth. The vertical profile shapes at CM12 and CM18 were similar. However, the minimum layer for R.F.I. was observed at 500 m for CM10 which contrasted with the DOC maximum layer. The maximum layer for R.F.I. was also observed from 125 m to 300 m for CM20.
The vertical distribution of monosaccharides and Chl. a

The distinct concentration maximum layer of monosaccharides was observed at 50 m for CM10 (Fig. 6). At CM12, CM18 and CM20, the concentrations were almost uniform. The monosaccharides concentration at 50 m for CM10 was almost five times higher than concentrations at the same depth for CM12, CM18 and CM20.

The concentration of Chl. a has been described in a preliminary report on the KH98-3 cruise (Nozaki, 1998). The maximum concentration of Chl. a was observed from 30 to 50 m at all sampling sites. The concentration maximum layer of Chl. a was shifted to greater depth, as the observation point was moved south (Fig. 6).

DISCUSSION

The vertical distribution shape of DOC, except at CM10, was similar to the results of other investigators in various open ocean regions. However, the DOC concentrations for the deep layer (below 1000 m) in the Sea of Japan were higher than those in the equatorial Pacific Ocean (Peltzer and Hayward, 1996), Indian Ocean (Doval and Hansell, 2000) and equatorial Atlantic Ocean (Thomas et al., 1995).

An anomalous of DOC concentration was found in the northernmost sampling site (CM10). This meant representing that the concentration and vertical distribution of DOC had high values in the surface layer (surface to 200 m) and the maximum value in the middle layer (250 to 1000 m) was significantly different from values in other open ocean and sampling sites of the Sea of Japan.

Phytoplankton activity is one factor which controls the distribution and behavior of DOM. Generally, Chl. a is used as an indicator of phytoplankton abundance, and monosaccharides are closely related to the phytoplankton metabolism (Sellner and Nealley, 1997). The integrated amounts of Chl. a from the surface to 100 m were 54 (CM10), 38 (CM12), 47 (CM18) and 33 mg/m² (CM20). This result suggested that CM10 had the highest phytoplankton abundance, almost 1.6 times that of CM20. Moreover, the concentrations of monosaccharides were higher at CM10 than other sampling sites. The integrated amounts of monosaccharides from the surface to 100 m were 0.37 (CM10), 0.24 (CM12), 0.24 (CM18) and 0.20 (CM20) M/m². The phytoplankton activity of CM10 was higher than that at other sampling sites and excretion of DOM was much greater. Therefore, the anomalously high concentration of

Fig. 6. Vertical profiles of Chl. a and monosaccharides in the Sea of Japan. △ CM20, × CM12, ● CM18, ○ CM10.
DOC in the surface layer (0–200 m) of CM10 might be influenced by phytoplankton activity.

The relative fluorescence intensity (R.F.I.), apparent oxygen utilization (AOU) and nutrients was investigated as an indicator of the oxidation and alteration of DOM (Figs. 7 and 8). The distribution of R.F.I. was approximately similar to that of nutrients. This distribution pattern coincided with that reported by Hayase and Shinozuka (1995) and Mopper and Schultz (1993). A positive correlation was observed between R.F.I. values and nutrients for the Sea of Japan (phosphate; $r = 0.813$, nitrate + nitrite; $r = 0.803$, $n = 83$). In general, AOU is also useful as a parameter of oxygen consumption by remineralization of organic substances in the water column. In addition to these facts, AOU had a positive correlation with the R.F.I. value for the Sea of Japan ($r = 0.844$, $n = 83$). The fluorescent organic matter was bleached photochemically by sunlight in the euphotic zone (Kieber et al., 1989; Mopper et al., 1991). Hayase and Shinozuka (1995) confirmed that fluorescent organic substances were regenerated in the water column by oxidation of organic substances, as in nutrients at the equatorial Pacific. The vertical distribution of R.F.I. correspondence to the consumption of AOU and regeneration of nutrients implied that fluorescent organic matter might be produced in alteration and oxidation processes of DOM. Therefore, fluores-
cent organic matter could be useful to investigate the alteration and oxidation mechanism of DOM in the Sea of Japan.

The minimum values of nutrient, R.F.I. and AOU were observed from 250 to 1000 m at CM10, corresponding to the DOC maximum layer. The regeneration of nutrients involved the decomposition of dissolved organic nitrogen and dissolved organic phosphorus (Maita and Yanada, 1990; Hopkinson et al., 1997). Redfield et al. (1963) provided an equation which expressed the stoichiometry of the regeneration of nutrients by oxidation of organic substances. In their equation, 138 moles of dissolved oxygen are consumed to produce 1 atom of phosphorus, 16 atoms of nitrogen and 106 atoms of inorganic carbon. Concentration differences of DOC at the same depth in the middle layer between CM10 and CM18 were calculated as ΔDOC. The ΔAOU, Δphosphate, Δnitrite + nitrate and ΔR.F.I. were also calculated in the same way as ΔDOC. A good negative correlation ($r = -0.966, n = 7$) was obtained between ΔDOC and ΔAOU (Fig. 9). In this correlation, the slope of the regression line (ΔDOC/ΔAOU; -0.67) was close to the Redfield ratio (C/O₂ = 106/138 = 0.77). Additionally, the correlations between ΔAOU and other parameters (Δphosphate, Δnitrite + nitrate and ΔR.F.I.) were also represented by good positive correlations ($r = 0.975; n = 8$, $r = 0.982; n = 8$ and $r = 0.903; n = 6$; Fig. 9). The slopes of the regression line for Δphosphate and Δnitrite + nitrate were 0.01 and 0.12, respectively. These slopes were approximately close to the Redfield ratio (P/O₂ = 1/138 = 0.08, N/O₂ = 16/138 = 0.12). These results for the remineralization processes between CM10 and CM18 suggested that the anomalous maximum concentration of DOC and minimum concentration of AOU and nutrients in the middle layer of CM10 might be due to the limited DOM oxidation.

Along northern shores in the Sea of Japan, sur-
face water is frequently cooled in winter and has sufficient density to sink (Nitani, 1972). Therefore, a rapid turnover time for surface water of the Sea of Japan (ca. 100 years) was reported (Kumamoto et al., 1998). This value is smaller than the mean residence time of average deep water throughout the world. It is thought that the high concentration of DOC in the deep layer (below 1000 m) is characteristic of the Sea of Japan and it might be caused by rapid circulation of deep water. However, the anomalous DOC concentration at CM10 could not be explained by the short surface turn over time of seawater.

The water mass structure from 250 to 1000 m was significantly different at CM10 than that at the same depth for other sampling sites (Fig. 10). High temperature and high salinity were observed at 250 to 1000 m depth at CM10. In a previous study on the circulation mechanism in the northern part of the Sea of Japan, a warm eddy appeared and moved southwestward (Ostrovskii et al., 1994). The high salinity and high temperature water at CM10 might be effects of this. In 1984, no minimum layer of nutrient and AOU were observed at Stn. AN-7 near CM10 during the cruise of the R/V Hakuho Maru (Sakai, 1984). The anomalous water mass characteristic recognized at CM10 in 1998 does not appear elsewhere. Consequently, the cause of the DOC anomaly recognized for the middle layer at CM10 could be attributed to the inflow of a quite different water mass (warm eddy) with incomplete oxidation of organic matter.

Acknowledgments—Authors are grateful to Professor Y. Nozaki of the Ocean Research Institute, the University of Tokyo and Professor T. Gamo of the Hokkaido University for planning and leading on the KH98-3 cruise. We thank the captain and crew of the R/V Hakuho Maru for assistance with sample collection.

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