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Photochemical bleaching of fluorescent dissolved organic matter in the subtropical North Pacific Ocean

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We examined the molecular weight distribution of the fluorescence intensity of $FDOM_M$ and dissolved organic carbon (DOC) concentrations in the subtropical western North Pacific, in order to characterize photoreactivity of $FDOM_M$ and understand the relationship between $FDOM_M$ and DOC pool. DOC concentrations in a hydrophobic fraction (O-DOC), which is extracted by solid phase extraction, accounted for 34 and 38% of bulk organic carbon in the surface and deep layer. $FDOM_M$ in a hydrophobic fraction (O-$FDOM_M$) accounted for 78 and 37% of $FDOM_M$ in the surface and 1000-m depth seawater, respectively. Molecular weight distribution of O-$FDOM_M$ and O-DOC showed that ratio of the fluorescence intensity to the DOC concentration of each MW fraction increased with depth. This suggests that the contribution of O-$FDOM_M$ to O-DOC in the deeper seawater is higher than that in the surface layer. When deep-seawater samples were irradiated by natural sunlight for 5 days, the fluorescence intensity of O-$FDOM_M$ decreased to almost half and its molecular weight shifted to lower one, whereas the O-DOC concentration did not decrease. It implies that a part of O-$FDOM_M$ remains as photobleached $FDOM_M$ (no-fluorescence DOM) after photobleaching.

Keywords: marine humic-like fluorescent dissolved organic matter, photobleaching, solar irradiation, size distribution, hydrophobic/hydrophilic properties

Abbreviations: DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; DOM, dissolved organic matter; FDOM, fluorescent dissolved organic matter; $FDOM_M$, marine humic-like fluorescent dissolved organic matter; FPA, fluorescence peak area; HPSEC, high-performance size-exclusion chromatography; LU, luminescence units; MW, molecular weight; ODS, octadecylsilyl; O-DOC, dissolved organic carbon extracted by ODS cartridges; O-DOM, dissolved organic matter extracted by ODS cartridges; O-$FDOM_M$, marine humic-like fluorescent dissolved organic matter extracted by ODS cartridges; QSU, quinine sulfate units; R-DOM, bio-refractory dissolved organic matter; RT, retention time; SPE, solid-phase extraction; TOC, total organic carbon

INTRODUCTION

Marine dissolved organic matter (DOM) is one of the largest reduced carbon pools on the surface of the earth. Numerous studies have demonstrated the importance of DOM for the microbial food web (Azam *et al*., 1983; Nagata *et al*., 2000), and for carbon export in marine environments (Hansell and Ducklow, 2003; Jiao *et al*., 2010). Although marine DOM has been considered to be mainly derived from photosynthetic production by phytoplankton, whose almost organic matter is considered to be composed of labile biomolecules, most accumulated DOM is composed of uncharacterized and bio-refractory DOM (R-DOM) (Benner, 2002). R-DOM is considered to play an important role in fixing the reduced carbon in the ocean for the long term. An elucidation of the dynamics of R-DOM is needed to understand the carbon cycle in the ocean as well as on the surface of the globe.

Marine humic substances are traditionally defined as a fraction separated by hydrophobic resin (e.g., XAD resin) and are known to be one of the main components of R-DOM in the open ocean (Thurman, 1985). To understand the dynamics of DOM, it is essential to elucidate the processes of the production, degradation and accumulation of marine humic substances in marine systems. Marine humic substances are characterized by specific optical (absorbance and fluorescence) properties. DOM with the fluorescent property of marine humic substances is referred as marine humic-like FDOM ($FDOM_M$), which have a broad emission peak at 370–420 nm from excitation at around 310–320 nm (e.g., Coble, 1996; Coble *et al*., 1998, Stedmon *et al*., 2003). In open ocean, $FDOM_M$ is autochthonous material pro-

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duced by bacteria (Yamashita *et al*., 2007; Yamashita and Tanoue, 2008; Shimotori *et al*., 2009, 2010), phytoplankton (Romera-Castillo *et al*., 2011) and zooplankton (Urban-Rich *et al*., 2006).

Photobleaching, the loss of fluorescence of $FDOM_M$ due to solar radiation, has been considered to be the primary sink for $FDOM_M$. The fluorescence intensity of $FDOM_M$ in the surface layer is typically low due to photobleaching by sunlight irradiation (Chen and Bada, 1992; Hayase and Shinozuka, 1995; Helms *et al*., 2013). Previous irradiation experiment showed correlated relationships between the decrease in the fluorescence intensity of $FDOM_M$ and the increase in photochemically-produced dissolved inorganic carbon (DIC) and low molecular weight (MW) carbon species. This suggested that DIC and low-MW compounds were mainly derived from $FDOM_M$ (Mopper *et al*., 1991; Obernosterer *et al*., 1999). However, measuring the decrease of fluorescence we do not have information about the degradation to DIC or low molecular weight compounds. During photoirradiation experiments of bacteria-derived FDOM, no significant decrease in DOC concentration was observed, in contrast to a decrease in the fluorescence intensity (Kramer and Herndl, 2004; Shank and Evans, 2011). This finding implies that photobleached $FDOM_M$ would be able to survive over a substantial period of time.

There is little knowledge that $FDOM_M$ is mineralized by photobleaching, because $FDOM_M$ has been quantitatively assessed only by its fluorescence intensity. Bulk DOM is a mixture containing $FDOM_M$ and other DOM, and it is difficult to estimate the carbon amount for $FDOM_M$. In this study, we examined the molecular weight distribution of the fluorescence intensity of $FDOM_M$ and DOC concentrations in the subtropical western North Pacific, in order to understand the relationship of $FDOM_M$ and DOC pool. Hydrophobic DOM was then fractionated from bulk DOM by hydrophobic solid-phase extraction (SPE) which was able to efficiently extract $FDOM_M$ from DOM (Amador *et al*., 1990). We measured the fluorescence intensity and carbon concentration of hydrophobic DOM at the MW level using high-performance size-exclusion chromatography (HPSEC). We further assume the fate of photobleached $FDOM_M$ in the surface layer.

Sample Description

The fluorescence intensity and organic carbon concentration of seawater were determined at the depths of 10, 150 and 1000 m in the western North Pacific Ocean (25°N, 137°E) in June 2007 during a cruise of the R/V *Keifu-Maru* (Japan Meteorological Agency). Seawater used for a sunlight irradiation experiment was collected from a depth of 1000 m at 30°N, 137°E. The samples collected from each depth using the CTD rosette system were transferred into acid-cleaned polycarbonate bottles (1 L). The water samples were not filtered to avoid possible contamination during filtration (Yoro *et al*., 1999; Lee *et al*., 2008; Omori *et al*., 2010), and were stored at –20°C until analysis.

SPE was performed to prepare the DOM samples for HPSEC analysis using octadecylsilyl (ODS) cartridges (C18 Sep-Pak, Waters Corp) (Amador *et al*., 1990; Omori *et al*., 2011). In brief, seawater samples were acidified to pH 2.4 using hydrochloric acid, and were loaded into pretreated cartridges. After loading, the retained DOM by the ODS phase was eluted using methanol. The extract was evaporated to eliminate methanol and redissolved in Milli-Q water. The extracts were filtered through 0.45-μm pore size cartridge filters. Hereafter, we will refer to the DOM fraction extracted by ODS cartridges as "O-DOM". Measurements of DOC concentrations and the fluorescence intensity and HPSEC analyses were performed for O-DOM (Omori *et al*., 2011). $FDOM_M$ contained in the O-DOM fraction was termed O-$FDOM_M$.

Analytical Methods

The total organic carbon (TOC) and DOC concentrations were analyzed by the high-temperature catalytic-oxidation method (Shimadzu, TOC-5000A). Water samples were acidified with hydrochloric acid and purged with CO_2-free pure air to remove inorganic carbon. The system was standardized before the measurements with potassium hydrogen phthalate. Consensus reference materials from the Hansell Research Laboratory (http://www.rsmas.miami.edu/groups/biogeochem/CRM.html) were analyzed as reference samples in each analytical run of the water samples (45.9 ± 1.8 μM, $n = 14$).

The fluorescence intensities of $FDOM_M$ and O-$FDOM_M$ were measured by a fluorescence spectrometer (Hitachi F-4500) equipped with a 150 W ozone-free xenon lamp. After the samples were allowed to adjust to room temperature, the fluorescence spectra were determined in duplicate for each sample using a 1-cm quartz cell. Excitation and emission slit widths were set to 10 nm. Emission scan were recorded from 250 to 500 nm, every 5 nm, with sequentially excitation every 5 nm between 240 and 390 nm. The fluorescence spectrum of Milli-Q was subtracted from each measurement to eliminate a Raman scatter peak. In this study, the fluorescence intensity at Ex/Em = 310/410 nm was adapted to determine the quantities of $FDOM_M$ and O-$FDOM_M$ (Omori *et al*., 2010, 2011). The fluorescence intensity was normalized with quinine sulfate units (QSU) after that of Milli-Q was subtracted.

MW distribution of O-$FDOM_M$ was measured by the method reported in the previous study (Omori *et al*., 2011). In brief, the HPSEC system consisted of an Agilent

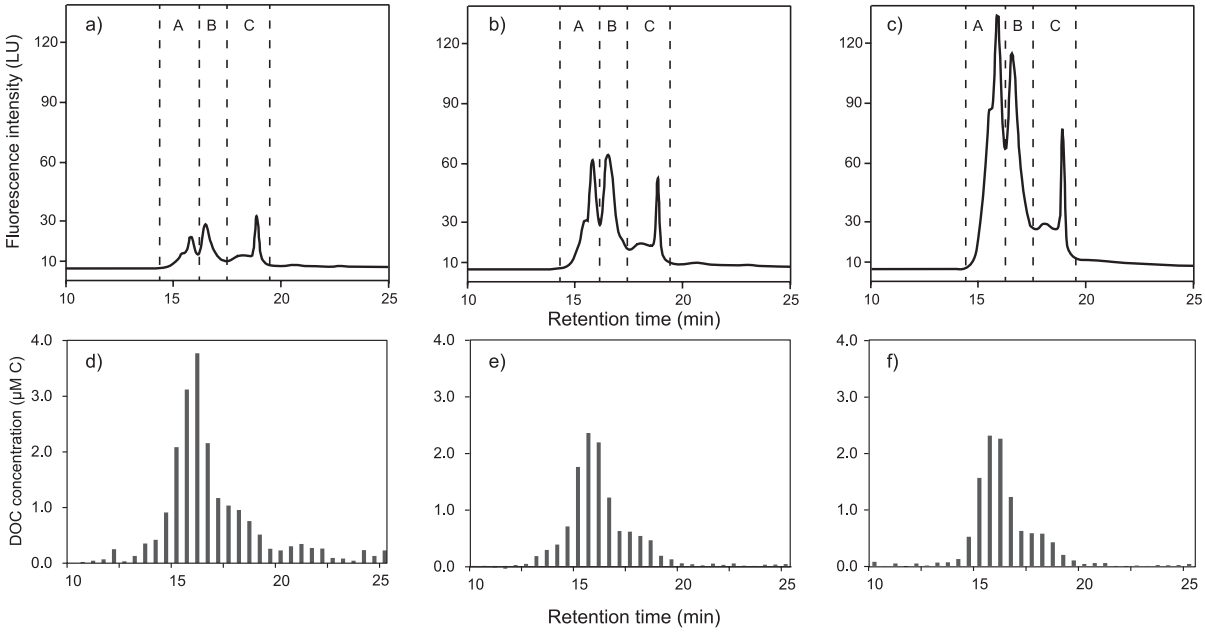

Fig. 1. Size exclusion chromatograms of O-FDOM$_M$ (a–c) and O-DOC concentrations (d–f) at 10 m (a, d), 150 m (b, e) and 1000 m (c, f).

1100 pump, column oven, fluorescence detector and autosampler. SEC separation was performed using a polymer gel column (Shodex OH Pak 803: 8.0 mm × 300 mm ID), which was able to separate an upper limit of approximate 400 kDa. A volume of 100 μl of O-DOM was injected into the HPSEC column at a flow rate of 0.5 ml min^{-1}. The mobile phase consisted of a 0.01 M $NaHCO_3$ buffer with 0.01 M sodium chloride (pH 8.5). The column was calibrated with polystyrene sulfonates (PSS) whose MWs were 1.8, 4.6, 8.0 and 18 kDa. Fluorescence was monitored at Ex/Em = 310/410 nm to determine the MW distribution of O-FDOM$_M$. The fluorescence intensity of O-FDOM$_M$ in the size-exclusion chromatogram was represented by a unit outputted from the fluorescence detector: luminescence units (LU).

For the analysis of the MW distribution of O-DOC, HPSEC elutes were collected in glass vials (precombusted at 450°C) each for 30 seconds by the fraction collector. HPSEC was run 7 times to collect 3.5 ml of the each fraction. The DOC concentration of the collected fractions was measured by TOC-5000A (Shimadzu). The DOC concentration of the buffer with no sample was subtracted from each measurement as baseline of the MW distribution of O-DOC. O-DOM samples for MW distribution analysis were concentrated 200-fold by SPE. O-DOC concentration of each fraction was converted to original value using the concentration factor. All molecular distributions of O-FDOM$_M$ and O-DOC in Fig. 1 were analyzed for a single sample collected from each depth (10, 150 and 1000 m).

Sunlight Irradiation Experiment

We examined changes in the fluorescence intensity and the DOC concentrations of bulk DOM and O-DOM under sunlight irradiation. The seawater sample collected from a depth of 1000 m was filtered through a 0.2-μm pore size alumina filter (Anodisc, Whatman) to sterilize it. The O-DOM fraction in the filtered seawater was obtained by ODS extraction and diluted with Milli-Q to reach the original volume of the seawater sample. Both the filtered seawater and the O-DOM fraction were poured into each of three quartz bottles (1 L) and allowed to stand in a water tank at a constant temperature (25°C) for 5 days under natural sunlight at the University of Tsukuba. Additional dark samples of the filtered seawater (n = 2) and the O-DOM fraction (n = 2) were prepared under the same conditions. They were wrapped in aluminum foil to avoid exposure to light. Experiments of the bulk seawater and the O-DOM fractions were started on 9 and 17 September 2009, respectively, and were continued for 5 days. Solar irradiances of UV (250–400 nm: UV-A and -B) and PAR (400–700 nm) were monitored by Apogee, SU-100 and SQ-100, respectively. Subsamples were collected from each bottle several times, and were measured for the fluorescence intensity and the DOC concentration of both DOM and O-DOM. MW distributions of both O-FDOM$_M$ and O-DOC were determined for samples at the start and end of the experiments. Photo-irradiated O-DOM fractions were extracted from the filtered seawater after the irradiation.

Table 1. Fluorescence intensity and organic carbon concentration of bulk seawater and O-DOM in the western North Pacific Ocean at 25°N, 137°E

Sample	Fluorescence intensity (QSU)			OC concentration (μM C)			QSU/OC	
	$FDOM_M$	O-FDOM	O-$FDOM_M$/$FDOM_M$ (%)	TOC	O-DOC	O-DOC/TOC (%)	$FDOM_M$/TOC	O-$FDOM_M$/O-DOC
10 m	0.15	0.12	78.0	77.4	27.9	36.1	1.9×10^{-3}	4.2×10^{-3}
150 m	0.86	0.21	24.8	56.9	19.3	33.9	15.1×10^{-3}	11.1×10^{-3}
1000 m	1.10	0.41	37.0	42.7	16.2	37.9	25.8×10^{-3}	25.1×10^{-3}

RESULTS

Relationships between the fluorescence intensity and the carbon concentration of O-DOM

The fluorescence intensity and organic carbon concentrations of the bulk sample and O-DOM were shown in Table 1. The fluorescence intensities of $FDOM_M$ and O-$FDOM_M$ in 1000 m-depth seawater were higher than those in the surface seawater. The concentrations of TOC and O-DOC were conversely lower in 1000 m seawater than those in the surface seawater. The contribution of O-$FDOM_M$ to $FDOM_M$ in surface seawater was the highest in accordance with our previous report (Omori *et al*., 2011). The ratio of the fluorescence intensity to the TOC and DOC concentration (QSU/TOC and QSU/DOC) of both the bulk sample and O-DOM fraction was higher with increasing depth (Table 1). The QSU/DOC of the O-DOM fraction was higher than that of bulk seawater at the depth of 10 m, and was almost comparable to that at a depth of 1000 m.

Figure 1 shows the HPSEC chromatograms monitored by the fluorescence intensity (Ex/Em = 310/410 nm) of O-$FDOM_M$ obtained from depths of 10, 150 and 1000 m. Four major peaks appeared at RTs of 15.5, 15.9, 16.6, and 19.0 min. The O-$FDOM_M$ were separated into 3 fractions depending on their RTs; the first fraction (Fraction A) was composed of twin adjacent peaks with the shortest RTs from 15.5 and 15.9 min, the second contained the independent peak centered at the RT of 16.6 min (Fraction B), while the last fraction comprised the sharp peak around the RT of 19.0 min with the preceding indefinite broad peak (Fraction C) (Figs. 1a–c). The fluorescence intensity of each MW fraction was higher in the 1000-m depth seawater than that the surface seawater (Table 2), as was mentioned in Omori *et al*. (2011).

The MW distributions of O-DOC were shown as the DOC concentrations of a fraction for every 30 seconds. (Figs. 1d–f). Like that of O-$FDOM_M$, high O-DOC concentrations were detected at RT ranging from 13.5 to 20 min. The O-DOC concentrations started to increase from an RT of 13.5 min, and reached their maximum at around 15.5–16 min. The O-DOC concentrations decreased from an RT of 16 min, and reached around 0 nM at an RT of around 20 min, although O-DOC distribution of the 10-m depth sample showed a small peak after an RT of 20 min. Those characteristics of the elution pattern of O-DOC were almost the same for all samples.

The peak of O-DOC was detected at a longer RT than that of the PSS standard with 1.8 kDa (14.8 min). Thus, the MW of O-DOC in the subtropical North Pacific was regarded as less than 1.8 kDa. The DOC concentrations integrated from an RT of 14–24 min were 19.0, 11.2, and 10.6 μM C in the 10, 150, and 1000-m depths of seawater, respectively. Those integrated concentrations in the HPSEC elution accounted for 68, 69, and 65% of the O-DOC concentrations in seawater at each depth, respectively. The O-DOC concentration of each MW fraction was higher in the 10 m-depth seawater than that in 1000 m-depth seawater (Table 2).

The ratio of a fluorescence peak area (FPA) to DOC concentration (FPA/DOC) in each MW fraction increased with depth (Table 2), as well as the QSU/TOC and QSU/DOC of bulk seawater and O-DOM, which indicated a higher contribution of O-FDOM to O-DOM in deeper seawater. At all of depths, the FPA/DOC values of fractions at longer RTs were higher than those at shorter RTs. The FPA/DOC of Fraction A were 0.21, 0.78, and 2.70, and those of Fraction C were 0.67, 2.07, and 3.53 at depths of 10, 150, and 1000 m, respectively. We plotted FPA and DOC concentrations of O-DOM which were the averages for every 30 seconds ranging from 14 to 23 min in HPSEC chromatograms at each depth (Fig. 2). The plots were scattered over a wider range ($r^2 = 0.58$, $p < 0.01$) in the 10 m-depth seawater samples than those in deeper waters. In the 1000 m-depth seawater, the DOC concentrations significantly correlated with the levels of FPA ($r^2 = 0.91$, $p < 0.01$).

Changes in the fluorescence intensity and DOC concentrations in the sunlight irradiation experiments

Figure 3 shows changes in the fluorescence intensity of $FDOM_M$ and O-$FDOM_M$ in the 1000 m-depth seawater during the sunlight irradiation experiments; the intensity is plotted against the integrated irradiance of UV-A and B radiation. The fluorescence intensity of $FDOM_M$ immediately decreased from 1.03 to 0.70 QSU (68% of its initial intensity) at 340 kJ m^{-2} of integrated UV irradiation for 7 hours, and then gradually declined over the

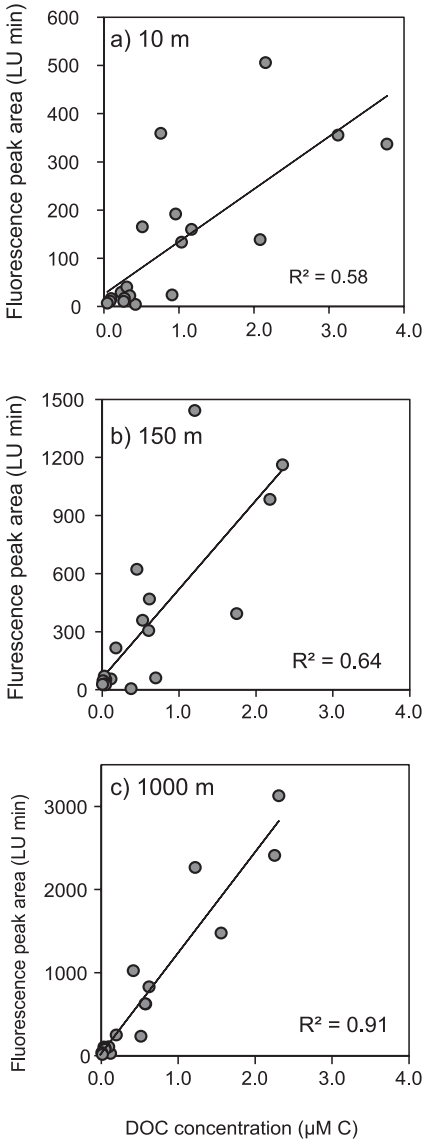

Fig. 2. Relationships between FPA and DOC concentrations of each 30-seconds fraction in the MW distribution of O-DOM at depths of a) 10 m, b) 150 m and c) 1000 m. The DOC concentrations were averaged for each 30 seconds from 14 to 23 min in the chromatograms.

course of the experiments. The final intensity on day 5 (0.48 QSU) was equivalent to 47% of the initial intensity. Though the fluorescence intensity of O-FDOM$_M$ decreased, its decline rate was more gradual than that of FDOM$_M$. The final intensity (0.37 QSU) of O-FDOM$_M$ was equivalent to 56% of the initial intensity (0.66 QSU) at the start of the experiment. There was little change in the intensity of both FDOM$_M$ and O-FDOM$_M$ in dark samples throughout the experiments; the intensity changed from 0.99 to 1.16 and 0.64 to 0.72 QSU, respectively (data not shown).

Table 2. Fluorescence peak area (FPA) and DOC concentration of Fraction A, B and C in MW distribution of O-DOM at each depth. The values in parenthesis in FPA and DOC columns were composition ratios for three Fractions A, B and C.

Sample	Fraction A			Fraction B			Fraction C			Total		
	FPA[a] (LU*min)	DOC (μM C)	FPA/DOC[b]	FPA[a] (LU*min)	DOC (μM C)	FPA/DOC[b]	FPA[a] (LU*min)	DOC (μM C)	FPA/DOC[b]	FPA[a] (LU*min)	DOC (μM C)	FPA/DOC[b]
10 m	1.4 (21.7)	6.5 (38.1)	0.21	2.7 (41.7)	7.1 (41.4)	0.38	2.4 (36.6)	3.5 (20.6)	0.67	6.5	17.2	0.38
150 m	4.1 (26.7)	5.2 (46.8)	0.78	7.2 (47.7)	4.0 (36.2)	1.81	3.9 (25.6)	1.9 (17.0)	2.07	15.2	11.0	1.37
1000 m	12.2 (37.4)	4.5 (43.1)	2.70	13.8 (42.4)	4.1 (39.1)	3.36	6.6 (20.2)	1.9 (17.8)	3.53	32.5	10.5	3.10

[a]*FPA was represented as original value dividing by concentration ratio.*
[b]*FPA/DOC was a ratio of FPA to DOC concentration of each and total fraction.*

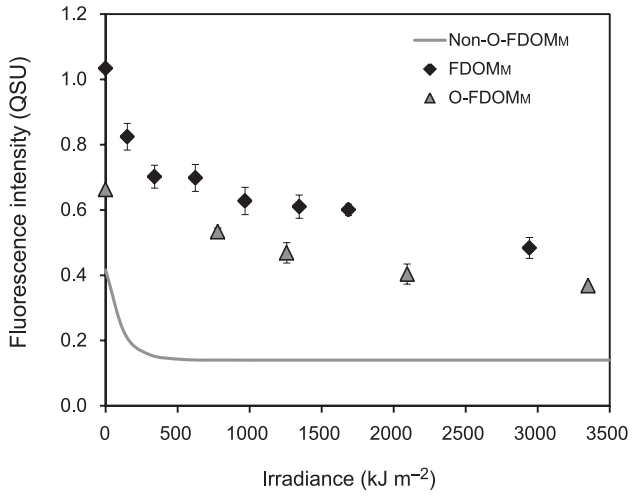

Fig. 3. Changes in the fluorescence intensity of $FDOM_M$, O-$FDOM_M$ and non-O-$FDOM_M$ in sunlight irradiation experiments. The fluorescence intensity of non-O-$FDOM_M$ was estimated by subtracting the intensity of O-$FDOM_M$ from that of $FDOM_M$. Error bars of $FDOM_M$ and O-$FDOM_M$ represent standard deviations (n = 3) and ranges (n = 2), respectively.

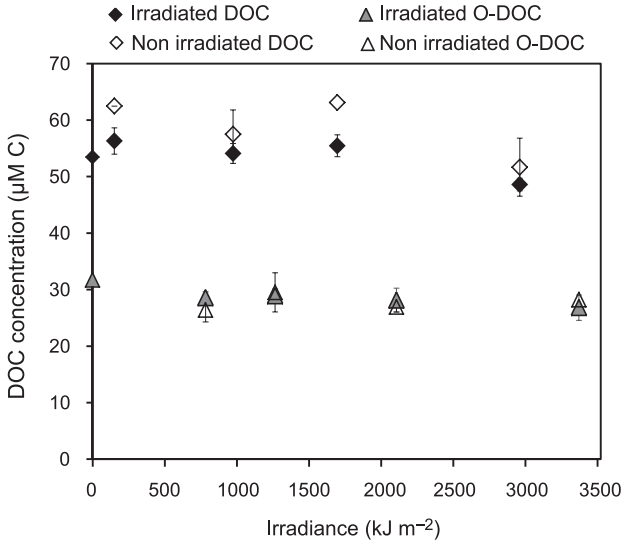

Fig. 4. Changes in the DOC concentrations of DOM and O-DOM in sunlight irradiation experiments. Error bars represent range of samples (n = 2).

The fluorescence intensity of $FDOM_M$ decreased faster than that of O-$FDOM_M$ in the irradiation experiments, suggesting that a fraction other than O-$FDOM_M$ (non-O-$FDOM_M$) is more labile to photobleaching than O-$FDOM_M$. To estimate a photobleaching change in non-O-$FDOM_M$ in the experiments, we obtained differences in the fluorescence intensity between O-$FDOM_M$ and total $FDOM_M$ (Fig. 3). When the fluorescence intensity of O-$FDOM_M$ at the end of the irradiation experiment was the constant, the change in the intensity of O-$FDOM_M$ owing to photobleaching was fit to an exponential decay curve ($r^2 = 0.92$);

$$\text{fluorescence intensity of O-FDOM}_M = 0.37 + 0.33e^{-1.01E\text{-}3 \cdot I} \quad (1)$$

where, I is irradiance (kJ m^{-2}). Non-O-$FDOM_M$ was estimated to exponentially decline (Eq. (2)):

$$\text{fluorescence intensity of non-O-FDOM}_M = 0.14 + 0.20e^{-1.12E\text{-}2 \cdot I} \quad (2)$$

as shown in Fig. 3. The absolute value of the coefficient of exponential function is used as index of the decay rate constant when $FDOM_M$ exponentially decreases as a result of photoirradiation (Skoog *et al*., 1996). A higher coefficient value indicates a higher decay rate. Non-O-$FDOM_M$ with a higher coefficient (1.12E-2) is photobleached faster than O-$FDOM_M$ with a lower coefficient (1.01E-3). This means that non-O-$FDOM_M$ is more labile with respect to photoirradiation than hydrophobic $FDOM_M$.

The DOC concentrations of the irradiated seawater were slightly lower than those of the dark samples throughout the period of exposure to sunlight irradiation (Fig. 4), though the difference was not statistically significant ($p > 0.05$). The O-DOC concentrations of both irradiated and non-irradiated samples varied little with time, and averaged 28.8 ± 1.8 and 28.6 ± 2.1 μM C, respectively (Fig. 4); the difference between them was not statistically significant ($p > 0.05$).

Sunlight irradiation affected the MW distribution of O-$FDOM_M$ in HPSEC chromatograms (Table 3, Fig. 5). The FPA of the higher MW fractions decreased more significantly in response to irradiation than the lower MW fractions. Fractions A, B, and C decreased to 44.2, 55.6, and 73.0% of their initial FPA on day 0, respectively. The MW composition of the original deep seawater that had been composed of 39.8, 35.5, and 24.7% of Fractions A, B and C, respectively, changed to 30.7, 36.2, and 33.1% following irradiation.

The O-DOC concentration of higher MW fractions decreased slightly due to sunlight irradiation, while the concentrations of the middle and lower MW fractions increased (Table 3, Fig. 5). The O-DOC concentrations of Fractions A, B, and C changed to 87.0, 107.5, and 105.5% to their initial concentrations on day 0 (100%). The change in the MW distribution of O-DOC due to light irradiation was less than that of O-$FDOM_M$; the O-DOC was initially composed of 48.9, 31.6, and 19.6% of Fractions A, B, and C, respectively, and changed to 43.8, 35.0, and 21.3% due to sunlight irradiation.

Table 3. FPA and DOC concentration of Fraction A, B and C in MW distribution of O-DOM before and after photoirradiation in sunlight irradiation experiments. The values in parenthesis in FPA and DOC columns were composition ratios for three Fractions A, B and C.

Sample	Fraction A			Fraction B			Fraction C		
	FPA[b] (LU*min)	DOC (μM C)	FPA/DOC[c]	FPA[b] (LU*min)	DOC (μM C)	FPA/DOC[c]	FPA[b] (LU*min)	DOC (μM C)	FPA/DOC[c]
Before irradiation[a]	19.6 (39.8)	8.5 (48.9)	2.3	17.5 (35.5)	5.5 (31.6)	3.2	12.2 (24.7)	3.4 (19.6)	3.6
After irradiation[a]	8.3 (30.7)	7.4 (43.8)	1.1	9.7 (36.2)	5.9 (35.0)	1.7	8.9 (33.1)	3.6 (21.3)	2.5

[a]*Sample before irradiation: O-DOM extracted from the 1000 m-depth seawater before irradiation. Sample after irradiation: O-DOM extracted from the 1000 m-depth seawater after irradiation for 5 days.*
[b]*FPA was represented as original value dividing by concentration ratio.*
[c]*FPA/DOC was a ratio of FPA to DOC concentration of each and total fraction.*

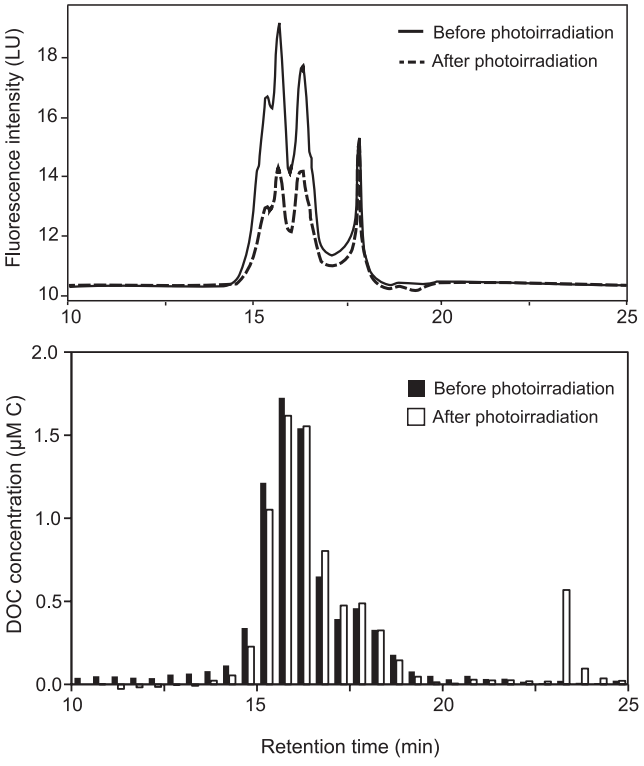

Fig. 5. Size exclusion chromatograms of a) O-FDOM$_M$ and b) O-DOC in sunlight irradiation experiments.

DISCUSSION

Photoreactivity of FDOM$_M$

The fractionation by SPE using XAD resin and ODS is among the most widely used methods to characterize DOM in aquatic environments (Thurman, 1985; Amador *et al*., 1990; Dilling and Kaiser, 2002). This method is able to divide bulk DOM into hydrophobic and non-hydrophobic (hydrophilic) fractions, although the boundary between hydrophobic and hydrophilic fractions depends on the function of the solid phase. In this study, O-FDOM$_M$ and Non-O-FDOM$_M$ which are separated by ODS SPE are referred to as hydrophobic and hydrophilic FDOM$_M$, respectively.

The increase in the contribution of hydrophobic FDOM$_M$ to bulk FDOM$_M$ and the lower decay rate observed for hydrophobic FDOM$_M$ than for hydrophilic FDOM$_M$ in the sunlight irradiation experiment (Fig. 3) suggests that hydrophobic FDOM$_M$ is more refractory to photoirradiation than hydrophilic FDOM$_M$. Some reports indicate that FDOM and chromophoric DOM are composed of fractions with different susceptibilities to photoirradiation (Skoog *et al*., 1996; Vodacek *et al*., 1997). Bertilsson and Bergh (1999) showed that the number of photochemical reactions induced per photon absorbed is 2–3 times higher in the hydrophilic than in the hydrophobic fraction of terrestrial DOM in a humic lake. Although the FDOM$_M$ in this study is different from the terrestrial DOM in its origin and major chemical characteristics, its hydrophobic/hydrophilic characteristics may be among the most important factors determining the photoreactivity of autochthonous DOM. We also suggest that hydrophilic FDOM$_M$ produced in the surface layer readily disappears from the FDOM$_M$ pool due to sunlight irradiation and a resistant fraction among hydrophobic FDOM$_M$ remains as a large part of an FDOM$_M$ pool in the surface layer. This agrees well with the high contribution of hydrophobic FDOM$_M$ to FDOM$_M$ (78%) in surface waters (Table 1 and Omori *et al*., 2011).

Hydrophobic fraction of DOM and FDOM$_M$

We showed that the QSU/DOC of O-DOM and the FPA/DOC in MW distribution increased with increasing depth (Tables 1 and 2), suggesting that the contribution of O-FDOM$_M$ to O-DOC in the deeper seawater is higher than that in the surface layer. This similar characteristic with bulk FDOM$_M$ (Table 1) indicates that O-FDOM$_M$ as well as bulk FDOM$_M$ decreases due to photobleaching in the surface layer and accumulates due to microbial metabolism in the deep layer (Chen and Bada, 1992; Hayase

and Shinozuka, 1995; Yamashita and Tanoue, 2008).

Moreover, the relationships between the DOC concentrations and the FPA in MW distributions of O-DOM were stronger with increasing depth (Fig. 2). This also suggests the higher contribution of O-$FDOM_M$ to O-DOC in the deeper seawater. The strong linear relationship with the seawater sample obtained from a 1000 m-depth (Fig. 2c) implies that quantitative variations in O-$FDOM_M$ are accompanied by those of DOC, and that O-$FDOM_M$ is one of the main components of the hydrophobic fraction of DOC in deep seawater. However, the linear relationship does not evaluate the quantitative contribution of $FDOM_M$ to the hydrophobic DOC pool. Thus, we cannot rule out that O-$FDOM_M$ is a minor component of O-DOC.

Little has been examined the contribution of $FDOM_M$ to DOC pool in the open ocean. Although Hayase and Shinozuka (1995) assumed that carbon-based contribution of FDOM to DOC was 1% using fulvic acid extracted from coastal sediment, no one has known the exact contribution yet. Further study need to examine the contribution of $FDOM_M$ to DOC. Some incubation experiments showed that the hydrophobic DOM extracted by XAD resin (Lara and Thomas, 1995) and $FDOM_M$ (Kramer and Herndl, 2004; Shimotori *et al*., 2009) remained in DOM pool during transformation of labile DOM into R-DOM by bacterial activity. These results suggest that R-DOM formed by microbial activity is characterized by hydrophobic and/or fluorescent properties. The examination of the relationship between $FDOM_M$ and DOC of the hydrophobic DOM formed in the incubation experiment can give significant information on the contribution of $FDOM_M$ to DOC pool.

$FDOM_M$ after photobleaching

The DOC and O-DOC concentrations were not significantly changed by sunlight irradiation, although the fluorescence intensity of $FDOM_M$ and O-$FDOM_M$ were decreased by almost half (Figs. 4 and 5). The difference of their changes was similar with a recent study that 38% of DOC and 84% of fluorescence intensity of the concentrated DOM in mesopelagic seawater were lost during 68 days of the irradiation (Helms *et al*., 2013). No decrease in the DOC concentration in our experiment seems to be due to the shorter irradiation term (5 days) than the previous study.

The higher MW fraction (Fraction A) of O-$FDOM_M$ was photobleached to a greater extent than the lower MW fractions, which is consistent with previous studies in the natural environment (Omori *et al*., 2011). It has also been reported that photoirradiation shifts the MW of terrestrial and autochthonous humic substances to lower MW compounds (Lepane *et al*., 2003; Dalzell *et al*., 2009). Although the change in the MW distribution of O-DOC was less than that of O-$FDOM_M$, photoirradiation decreased the higher MW fraction of O-DOC to 87% and increased its lower MW fractions to 108% (Table 3, Fig. 5). Because the total O-DOC concentration did not decrease (Fig. 4), a part of O-DOM was likely to be phototransformed to the lower MW compounds without mineralization. These results suggest that photoirradiation bleaches the higher MW fraction of O-$FDOM_M$ without mineralization. Based on our assumption that O-$FDOM_M$ is one of main components of O-DOC in deep seawater, O-$FDOM_M$ after photobleaching might remain as DOM without the chemical structure responsible for fluorescence (photobleached $FDOM_M$). As indicated above, however, the contribution of O-$FDOM_M$ to the hydrophobic DOC pool has not been quantitatively evaluated yet. If O-$FDOM_M$ is a minor component of hydrophobic DOC, the decrease in the O-DOC concentration might not be detected. It is necessary to determine the carbon amounts of the photobleached $FDOM_M$.

Fates of $FDOM_M$ after photobleachig are important in marine biogeochemical cycle. A sunlight irradiation experiment showed that photochemical production rates of CO and low MW carbon species from DOM were highly correlated with the fluorescence intensity of DOM, suggesting that $FDOM_M$ was photochemically degraded to CO and low MW carbon species (Mopper *et al*., 1991). Moreover, previous studies have shown that DOM was transformed into various specific low-MW carbonyl compounds by sunlight that stimulated the growth and activity of microorganisms (Kieber *et al*., 1989; Benner and Biddanda, 1998; Obernosterer *et al*., 1999). In addition, this study assumes that a part of $FDOM_M$ after photoirradiation might exist as the photobleached $FDOM_M$. This is supported by the previous study that the fluorescence intensity of FDOM produced by bacteria decreased substantially due to photoirradiation, although only minor changes in both the concentration and bioavailability of DOC (Kramer and Herndl, 2004). If the photobleached $FDOM_M$ is refractory to microbial degradation, it might accumulate as constituents of the surface DOM pool.

Conclusion

This study provides information on the difference of photoreactivity of hydrophobic/hydrophilic $FDOM_M$ and the molecular weight distribution of O-$FDOM_M$ and O-DOC. The relationship between the fluorescence intensity and the DOC concentration in MW distribution implies a possibility that O-$FDOM_M$ is one of main components of hydrophobic DOM in the carbon base. Our sunlight irradiation experiments showed no decrease in O-DOC and a gradual decrease in O-$FDOM_M$, implying that hydrophobic $FDOM_M$ remains as DOM without fluorescence after photobleaching. However, this study cannot

quantitatively evaluate the contribution of O-$FDOM_M$ to O-DOC pool and the photobleached $FDOM_M$. As next step, the examination of the relationship between $FDOM_M$ and DOC of bacteria-derived DOM in the incubation experiment should give significant information on the contribution of $FDOM_M$ to DOC pool.

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