

Ultraviolet radiation induces reversal of the bioavailability of DOM to marine bacterioplankton

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ABSTRACT: The effect of solar radiation on the bioavailability of dissolved organic matter (DOM) was investigated during a cruise in the Southern Ocean (~44.5° S, 63° E) in January and February 1999. Seawater was collected in the chlorophyll maximum layer (CML, 30 to 80 m depth) and in deep waters (DW, 200 to 5000 m depth), filtered through 0.2 µm pore-size filters, and exposed to surface solar irradiation for 8 h. The bioavailability of the irradiated DOM and of that in the corresponding dark treatment was subsequently tested in bioassay experiments performed in the dark. Seawater from the CML was also size-fractionated and the effect of solar radiation on the bioavailability of the <20 kDa DOM fraction was compared with that of the bulk DOM. Our results clearly demonstrate that the biological reactivity of DOM from both the CML and DW following exposure to solar radiation is inversely related to the initial bioavailability of the DOM prior to its exposure. The photochemically induced stimulation of the bacterial activity was ~2-fold higher in the <20 kDa fraction than in the bulk DOM. These results clearly indicate that initially bioreactive DOM can be rendered biologically more recalcitrant by exposure to solar radiation, while DOM of initially low bioavailability can be, at least partly, photochemically transformed to compounds of higher biological reactivity.

KEY WORDS: Dissolved organic matter · Ultraviolet radiation · DOM-photoreactivity · Bacterioplankton · Southern Ocean

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INTRODUCTION

Photochemical transformation of dissolved organic matter (DOM) can considerably affect its biological availability to bacterioplankton. The photochemical alteration of DOM and the subsequently released low molecular weight organic and inorganic compounds have been recognized as a potentially important substrate for bacterioplankton (see review by Moran & Zepp 1997). In contrast, recent evidence indicates that the bioavailability of irradiation-exposed DOM can also be reduced compared to non-exposed DOM (Benner & Biddanda 1998, Tranvik & Kokalj 1998, Obernosterer et al. 1999b). Loss of organic carbon due to the photochemical production of inorganic carbon species (Salo-

nen & Vähätalo 1994, Miller & Zepp 1995, Graneli et al. 1996), the photochemical production of reactive oxygen species such as hydrogen peroxide (Cooper et al. 1988), and photochemical transformation of DOM resulting in compounds that are biologically more recalcitrant might be responsible for the observed reduction in bacterial activity on irradiated DOM.

There are indications that the response of bacterioplankton to photochemically induced changes of DOM is related to the original biological availability of the DOM prior to exposure to solar radiation (Benner & Biddanda 1998, Obernosterer et al. 1999b). The bulk DOM can roughly be separated into 3 pools: a highly labile pool with turnover times of hours to days, a semi-labile pool with turnover times on a seasonal time scale, and a refractory DOM pool with a turnover of >1 yr (Kirchman et al. 1993). Only ~25 to 50% of the bulk DOM pool has been characterized on a molecular

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level so far, indicating that carbohydrates and amino acids account for an important part of the labile DOM (Suttle et al. 1991, Benner et al. 1992, Rich et al. 1996). Size fractionations of DOM have shown that low molecular weight DOM (<1000 Da), comprising ~70 to 80% of the bulk oceanic DOM (Carlson et al. 1985b, Benner et al. 1992, Guo et al. 1995), is mainly comprised of highly degraded material with low biological reactivity (Tranvik 1990, Amon & Benner 1994). The relative contribution of biologically recalcitrant DOM increases from surface to deep waters (Carlson & Ducklow 1995). However, this refractory DOM pool contains highly light-absorbing moieties, rendering it photochemically reactive (Kieber et al. 1989, Mopper et al. 1991).

The Antarctic Circumpolar Current is a unique global link connecting all major oceans. Most of the waters transported in the circumpolar current represent a mixture of waters formed in other parts of the world's oceans (Whitworth & Worth 1987, Orsi et al. 1995). Deep waters originating further north, such as the North Atlantic Deep Water Current, enter the sub-polar regime and mix with Antarctic shelf waters (Orsi et al. 1995). The transport of deep waters into the surface mixed layer of specific regions of the Southern Ocean could lead to large-scale photochemically induced transformation of DOM, thereby significantly influencing bacterial activity.

In this study, we tested the hypothesis that photochemically induced changes of the biological reactivity of DOM are directly related to the initial bioavailability of the DOM prior to exposure to solar irradiation. Experiments were performed with DOM originating from different water masses and depth layers during a cruise in the Southern Ocean. We further compared the biological and photochemical reactivity of the <20 kDa DOM fraction with that of the bulk DOM pool, and used bovine serum albumin (BSA) as a biologically labile model compound to elucidate the relationship between the biological and the photochemical reactivity of different components of the DOM pool.

MATERIALS AND METHODS

Sampling sites. The study was carried out during the ANTARES IV cruise on board the RV 'Marion Dufresne II' in January and February 1999 in the Indian sector of the Southern Ocean. The main survey area, the central Crozet Basin (~44.5° S, 63° E), is characterized by multiple fronts where the Agulhas Return Current and the Antarctic Circumpolar Current (ACC) converge. The ACC in this sector of the Southern Ocean mainly consists of the Subantarctic and the Subtropical Front

(Park et al. 1993). Samples were collected at 3 main stations, the Polar Frontal Zone south of the Subantarctic Front (Stn 3), the Subtropical Zone north of the Subtropical Front (Stn 7) and the Subtropical Zone north of the Agulhas Return Current (Stn 8, see Table 1). Samples were also collected across a transect between the Subantarctic Front and the Agulhas Return Current (Stns P3, P4, P5, G1, G2, G3: see Table 1). Generally, concentrations of inorganic nitrogen in surface waters varied between 0.1 and 17.9 μM , and in waters ≥ 200 m depth between 11.2 and 33 μM (courtesy S. Blain). Inorganic phosphate concentrations in this area have been reported to range between 0.3 and 1.1 μM in surface waters and between 0.5 and 2.4 μM at depths ≥ 200 m (Treguer et al. 1980). Chlorophyll a concentrations ranged between 0.13 and 0.87 $\mu\text{g l}^{-1}$ in surface waters (see Table 1).

Experimental design. Seawater samples were collected from the chlorophyll maximum layer (CML, 30 to 80 m depth) and from deep-water layers (DW, 200 to 5000 m depth) using 10 l Niskin bottles mounted on a CTD rosette. In order to determine the initial DOM bioavailability, raw seawater was subsampled (20 ml for CML, 40 ml for DW) for measurements of bacterial production as described below. For the determination of irradiation-induced changes of the DOM bioavailability, one fraction of the freshly collected seawater was immediately filtered through 0.2 μm polycarbonate filters (Nuclepore, 47 mm filter diameter) to obtain particle-free seawater, and the other fraction was filtered through 0.8 μm filters (Nuclepore, 47 mm filter diameter) to prepare the bacterioplankton inocula. The filters were pre-rinsed with 1 l Milli-Q water and 500 ml seawater before use. All glassware used was rinsed with 0.1 N HCl and subsequently with Milli-Q water. Exposure of the 0.2 μm filtrate from the different depth layers to surface solar irradiation was performed in quartz glass tubes (250 ml volume) sealed at both ends with Teflon foil (200 μm thick, Fluorplast, Amsterdam, The Netherlands). The Teflon foil was supported by PVC holders fitted to the tube with an O-ring. Respective dark controls were wrapped in aluminum foil. The quartz tubes were incubated in a water bath on the deck of the ship connected to a running seawater system to maintain *in situ* temperature conditions of the surface layers (10 to 17°C). All incubations were done from 09:00 to 18:00 h. To determine the biological reactivity of the DOM, the irradiated and the respective dark treatments (180 ml each) were inoculated with a natural bacterial assemblage (0.8 μm -filtered seawater; 20 ml) collected at the corresponding depths (CML and DW). The dilution cultures were kept in the dark at surface water temperature for 24 h before subsamples for bacterial production were taken.

In additional sets of experiments (see Table 1), the photoreactivity of the <20 kDa DOM fraction and photo-induced changes in the bioavailability of an originally labile compound were determined. In order to determine the photoreactivity of the DOM fraction <20 kDa, the 0.2 μm filtrate from the CML was passed through a combusted (450°C for 4 h) and pre-rinsed (1 l Milli-Q water) 0.02 μm aluminum oxide filter (Anopore, 47 mm filter diameter). The photo-induced changes in the bioavailability of an originally labile compound were followed by amending the 0.2 μm filtrate of the different depth layers with the protein bovine serum albumin (BSA; final conc. 416 μM C) prior to irradiation. Exposure to surface solar irradiation and the subsequent preparation of the bacterial batch cultures of both, the <20 kDa DOM fraction and the BSA-amended treatments followed the protocol described above. Ten ml samples for DOC analyses were taken from the 0.2 μm and the 0.02 μm filtrate, fixed with 50 μl HgCl_2 (10 mg l^{-1} final conc.), and kept in sealed pre-combusted ampoules until analysis. DOM fluorescence was measured on the 0.2 μm and the 0.02 μm filtrates immediately after filtration and after exposure to solar irradiation and in the respective dark treatments, as described below.

Bacterial production measurements. Bacterial production was measured by [^3H]leucine (specific activity: 136 Ci mmol^{-1} ; Amersham, UK) incorporation. Bacterial production on raw seawater samples was measured in 20 ml samples from the CML (final conc. 10 nM) and in 40 ml samples from the DW (final conc. 5 nM). Twenty ml were used to measure bacterial production (final conc. 10 nM) in the dilution cultures. Incubations were done in triplicate with 1 blank at *in situ* temperature in the dark; blanks were fixed with formaldehyde (final conc. 4%, v/v) 10 min before adding the tracer. Incubation times for raw seawater samples were 1 h for samples originating from the CML and 200 m depth, 3 h for samples from 500 m depth, and 10 h for samples from ≥ 1000 m depth. All samples from the dilution cultures were incubated for 1 h. Following incubation, the samples were filtered through 0.2 μm cellulose nitrate filters (Millipore GSWP, 25 mm diameter filter) and rinsed twice with 10 ml ice-cold 5% trichloroacetic acid (Prolabo RP Normapur) for 5 min. The filters were dissolved in 1 ml ethylacetate, and after 10 min 8 ml of scintillation cocktail (PCS Amersham) was added. The radioactivity incorporated into bacterial cells was counted in a Canberra Packard Tri-carb 2000, and the disintegrations per minute were converted to the actual amount of substrate incorporated.

DOC analysis. DOC measurements were performed on a Shimadzu TOC-5000A with a quartz combustion column filled with 1.2% Pt on silica pillows with an

approximate diameter of 2 mm (Cauwet 1994). Modifications of the unit have been described in Yoro et al. (1997). DOC samples were acidified with 10 μl of 85% H_3PO_4 to a pH of ~ 2 , and sparged with CO_2 -free air for 10 min to remove inorganic carbon. One hundred μl of sample were subsequently injected in triplicate, and the analytical precision was on average $\sim 3\%$. Standards were prepared with potassium hydrogen biphthalate (Kanto Chemical Co Inc. Kyoto, Japan).

DOM fluorescence. Fluorescence of the DOM was measured at an excitation wavelength of 350 nm and an emission wavelength of 450 nm on a Perkin Elmer MPF-66 fluorescence spectrophotometer using a 1 cm quartz cuvette. DOM fluorescence is expressed as quinine sulfate units (1 QSU = 1 ppb quinine sulfate in 0.05 M H_2SO_4).

Statistical analysis. Differences among treatments were tested by analysis of variance (2-way ANOVA) and by the Tukey-test for pairwise comparison. The Wilcoxon test for matched-pairs was used to check for treatment effects (irradiation vs dark) on variables in the BSA-addition experiments. Statistics were performed with SYSTAT 5.2 (Wilkinson 1990).

RESULTS

Bacterial response to DOM of different origin exposed to solar radiation

Initial bacterial activity in the raw seawater samples were ~ 1 to 2 orders of magnitude higher in the CML than in the water layers from 200 to 5000 m depth. In contrast, DOC concentrations varied only by a factor of up to 2 between the mixed surface layer and 5000 m depth (Table 1). This resulted in DOC-normalized leucine incorporation ranging from 0.16 to 1.23 $\text{pmol leu h}^{-1} (\mu\text{mol DOC})^{-1}$ in the CML and from 0.0014 to 0.0276 $\text{pmol leu h}^{-1} (\mu\text{mol DOC})^{-1}$ in DW, clearly indicating the substantially lower bioavailability of the DOM originating from ≥ 200 m depth. DOC-normalized fluorescence of the DOM was significantly higher (by $\sim 40\%$) in samples from DW than in samples from the CML (ANOVA, $p = 0.008$, $n = 8$; Table 1). Diel integrated surface solar UVB radiation on the days when the experiments were performed varied between 2.3 and 5.2 kJ m^{-2} (NASA, Goddard Space Flight Center, New York). Exposure of the DOM from the different water layers to surface solar irradiation resulted in variable changes in the DOM bioavailability to bacterioplankton (Fig. 1). The ratio of the bacterial activity in the irradiation-exposed/dark treatment (I/D ratio) was inversely related to the initial DOC-normalized bacterial activity for the DOM from the CML as well as from the DW. In the CML, I/D ratios ranged

Table 1. Stations sampled during ANTARES IV cruise with corresponding sampling depth, concentration of DOC, DOM fluorescence (as quinine sulfate units, QSU) prior to exposure to solar irradiation, and chlorophyll *a* concentration. nd: not determined. Superscripted numbers for Stns 7 and 8 indicate individual experiments performed at one station

Stn	Latitude (°S)	Longitude (°E)	Depth (m)	DOC (μM)	DOM-fluorescence (QSU, μM ⁻¹ DOC)	Chl <i>a</i> (μg l ⁻¹)
Chlorophyll maximum layer						
3 ^a	46° 01.74'	62° 45.66'	30	70	0.0039	0.27
P5 ^a	43° 49.76'	64° 30.01'	60	67	0.0074	0.13
G1	45° 11.54'	63° 04.65'	80	64	0.0063	0.27
G2	44° 50.18'	62° 52.67'	35	80	0.0050	0.67
P3 ^a	44° 37.92'	62° 49.37'	50	64	nd	0.55
G3	44° 28.85'	62° 43.99'	45	65	0.0069	0.24
P4 ^b	45° 19.95'	61° 52.63'	40	63	0.0059	0.87
7 ^{1, ab}	44° 11.48'	63° 24.44'	30	65	0.0075	0.73
7 ^{3, ab}	43° 56.13'	64° 47.97'	40	73	0.0071	0.79
8 ^{1, b}	43° 00.94'	62° 55.96'	50	73	0.0087	0.41
8 ²	42° 54.80'	63° 04.10'	30	73	0.0083	0.34
Deep water						
3 ^a	46° 01.74'	62° 45.66'	200	60	0.0047	0.01
P5 ^a	43° 49.76'	64° 30.01'	500	52	0.0061	<0.01
G2	44° 50.18'	62° 52.67'	1000	49	0.0119	<0.01
7 ^{1, a}	44° 11.48'	63° 24.44'	1000	47	0.0110	<0.01
7 ²	44° 00.12'	64° 09.91'	200	67	0.0079	0.01
7 ³	43° 56.13'	64° 47.97'	200	58	0.0106	0.01
8 ¹	43° 06.81'	62° 29.63'	3000	50	0.0169	<0.01
8 ²	43° 00.94'	62° 55.96'	5000	47	0.0149	<0.01

^aStations where experiments with BSA-amended DOM were performed
^bStations where size fractionation of DOM was performed

from 0.48 to 6.82 and were therefore similar to I/D ratios from DW (0.70 to 10.52). In DW, the highest I/D ratios were detectable for samples from ≥500 m depth (Fig. 1).

Photochemical reactivity of <20 kDa DOM fraction

The <20 kDa DOM fraction accounted for ~94 ± 5% (n = 4, range 86 to 98%) of the bulk DOM (measured as DOC). However, bacterial activity in the treatments kept in the dark was significantly lower (15 to 83%; ANOVA, p = 0.034, n = 4) in the <20 kDa DOM fraction than in the bulk DOM. Carbon-normalized bacterial activity in the <20 kDa DOM fraction was, on average, lower by a factor of 2 (n = 4, range 1.2 to 5.7) than bulk DOM. The response of bacterial activity to exposure of the <20 kDa DOM fraction to surface

solar irradiation exhibited a trend similar to that obtained for the corresponding bulk DOM (Fig. 2). However, the stimulation of bacterial activity by photochemically induced transformation of the DOM pool was ~2-fold higher for the <20 kDa DOM fraction than for the bulk DOM (Fig. 2).

Response of the bacterial activity to BSA-amended, irradiated DOM

The addition of BSA to DOM originating from the CML and subsequent exposure to surface solar irradiation resulted in a significant increase in bacterial activity compared to the corresponding dark control (mean I/D ratio = 2 ± 0.65; Wilcoxon, p = 0.022, n = 5; Fig. 3). Exposure of the corresponding unamended DOM resulted in a mean I/D ratio of 1.5 ± 0.72; however, no significant difference was detectable between the irradiated and the dark treatments. A contrasting pattern was observed for DOM originating from DW. While unamended, irradiated DW showed significantly higher bacterial activity in the solar irradiation-exposed DOM (mean I/D ratio = 2.2 ± 0.73; Wilcoxon, p = 0.05, n = 3); BSA-amended, irradiated DOM from DW resulted in a decrease in bacterial activity compared to the corresponding dark control (mean I/D ratio = 0.35 ± 0.06; Wilcoxon, p = 0.05, n = 3; Fig. 3).

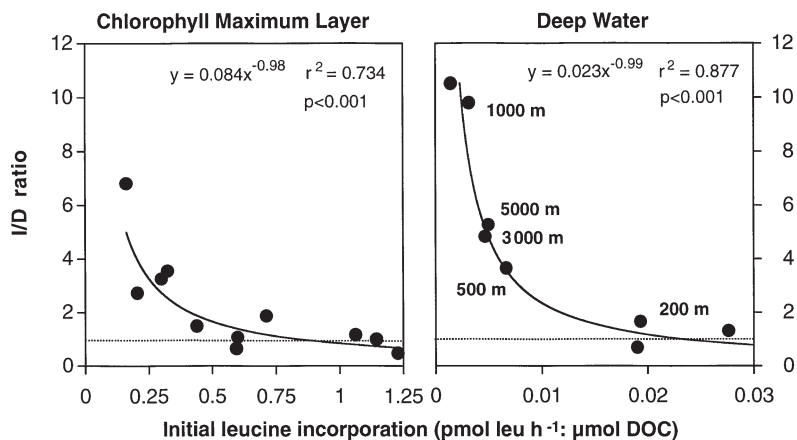


Fig. 1. Relationship between initial DOC-normalized bacterial activity of DOM originating from the chlorophyll maximum layer and deep waters and I/D ratio (ratio between bacterial activity in the irradiation-exposed sample and corresponding dark control). Dotted line indicates I/D ratio of 1

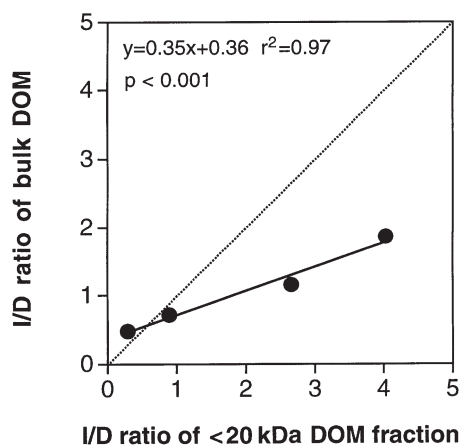


Fig. 2. Relationship between I/D ratio of the <20 kDa fraction and I/D ratio of the bulk DOM of samples collected in the chlorophyll maximum layer. Dotted line indicates 1:1 relationship

DOM fluorescence

Initial DOC-normalized fluorescence was significantly higher for DOM originating from DW than for DOM from the CML (ANOVA, $p = 0.008$, $n = 8$; Table 1). No significant difference in the initial fluorescence of the bulk DOM and the <20 kDa DOM fraction was detectable. A significant decrease in DOM fluorescence during exposure to surface solar irradiation for 8 h was observed for DOM from the CML (by ~5%) and DW (by ~10%; Table 2). The loss of fluorescence was, on average, 2-fold higher in irradiation-exposed DOM from DW than from the CML. No decrease in DOM fluorescence, however, was observed in the <20 kDa DOM

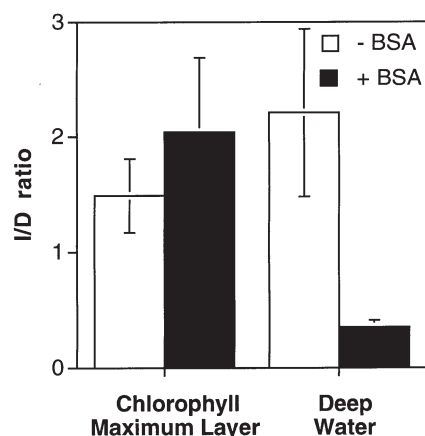


Fig. 3. Ratio (means \pm SE) between bacterial activity in the irradiation-exposed sample and corresponding dark control (I/D ratio) of DOM originating from the chlorophyll maximum layer and the deep-water layers and of the corresponding bovine serum albumin (BSA)-amended treatments; 416 μ M C (final conc.) were added

fraction during exposure to solar irradiation. Also, BSA-amended samples originating from the CML and DW exposed to surface solar irradiation did not show an alteration in the DOM fluorescence compared to the corresponding dark incubations (Table 2).

DISCUSSION

In the present study, the effect of surface solar irradiation on the bioavailability of DOM originating from both the CML and the DW was inversely related to the initial DOM bioavailability. These results confirm recent findings on the relationship between the bioavailability and photochemically induced changes of DOM from the Mediterranean Sea (Obernosterer et al. 1999b), and indicate therefore that this relationship is a common phenomenon of oceanic DOM. These solar irradiation-induced alterations can be assessed by comparing the bacterial activity in the irradiation-exposed/dark treatment (I/D ratio). In the Mediterranean Sea, I/D ratios were similar to those in the present study. However, a pronounced difference in the I/D ratios of DOM from the CML, being consistently <1, and mesopelagic waters, being >1, was observed in the Mediterranean Sea (Obernosterer et al. 1999b).

Photoreactivity of DOM from CML and from DW

Exposure of DOM from the CML to surface solar irradiation resulted in either stimulated or reduced bacterial activity following exposure compared to the treatments in the dark (Fig. 1). In contrast, irradiated DOM from

Table 2. DOM fluorescence (means \pm SD) of water originating from chlorophyll maximum layer and from deep water after exposure to surface solar irradiation for 8 h, as percentage of fluorescence measured in corresponding samples in the dark. +BSA: 416 μ M C (final conc.) bovine serum albumin added; n: number of experiments. No significant differences between initial DOM fluorescence and corresponding samples in the dark were detected. *Significant difference between irradiated sample and corresponding dark control (ANOVA, $p < 0.05$)

	Chlorophyll maximum layer Bulk DOM <20 kDa DOM fraction	Deep water bulk DOM
	95* ± 14 (n = 10)	99 ± 13 (n = 4)
+BSA	112 ± 19 (n = 4)	97 ± 10 (n = 4)

DW stimulated subsequent bacterial growth, albeit at highly variable rates (Fig. 1). Photochemically induced stimulation of the bacterial activity (i.e. I/D ratios >1; Fig. 1) followed the same trend for DOM originating from the CML and the DW, resulting in similar I/D ratios. Based on the photochemical production of low molecular weight carbonyl compounds and carbon monoxide, it has been concluded that deep-water DOM is ~5 to 10 times more photoreactive than DOM of surface waters (Mopper et al. 1991). In the present study, the loss in fluorescence which has been shown to be related to the photochemical degradation of DOM (Kieber et al. 1990, Graneli et al. 1996) was about 2-fold higher in DW-DOM than in DOM from the CML (Table 2). The increased bacterial activity on irradiation-exposed DOM compared to DOM in the dark can be attributed to the release of organic and/or inorganic nutrients from refractory moieties. The importance of these photoproducts in stimulating bacterial activity probably depends on the substrate or element limiting bacterioplankton growth. DW-DOM is dominated by biologically recalcitrant matter; however, deep waters are usually replete in inorganic nutrients. In contrast, DOM present in surface waters is characterized by a higher degree of bioreactivity, while inorganic nutrient sources frequently limit growth, at least during daytime when competition with phytoplankton for inorganic nutrients is intense (Thingstad & Rassoulzadegan 1995, Rivkin & Anderson 1997, Kuipers et al. 2000). In a recent study, Skoog et al. (1999) report that bacterial growth is limited by the availability of inorganic nutrients in the CML, while above and below the CML bacterial growth is limited by organic carbon. These authors suggest a depth-dependent alteration in factors limiting bacterial growth.

Thus, the photochemical production of initially limiting nutrient sources will significantly determine the overall effect of irradiation on the bioavailability of the DOM. Assuming that bacterioplankton growth on DOM originating from the CML is limited in nitrogen or phosphorus rather than in carbon (Skoog et al. 1999, Kuipers et al. 2000), the photochemically induced release of labile nitrogen or phosphorus species will enhance bacterial activity. If a C:N:P ratio of 45:9:1 is assumed for bacterioplankton (Goldman et al. 1987), nitrogen- or phosphorus-limited bacterial growth can be significantly stimulated by a comparatively low increase in the concentration of these nutrients, while relatively more carbon is needed for bacterial growth. Thus, even at several-fold lower photoproduction rates of surface water DOM, compared to DOM from deep waters (Mopper et al. 1991), exposure of surface-water DOM to solar irradiation could result in an enhancement of subsequent bacterial growth similar to that observed in the present study. This also implies that

the photochemical production of biologically available substrates may not necessarily lead to a stimulation of bacterial activity following exposure of DOM to solar irradiation. Several studies quantifying the microbial uptake of specific photochemically produced substances support this notion. These studies have shown that the photochemical production of bioavailable carbon compounds exceeds their bacterial utilization (~3- to 30-fold) (Kieber et al. 1989, Bertilsson & Tranvik 1998, Obernosterer et al. 1999a).

Reduced bacterial activity on DOM exposed to surface solar irradiation

The decreased bacterial activity on irradiated DOM compared to the dark control observed in the present study (Fig. 1) has also been reported in other studies (Benner & Biddanda 1998, Tranvik & Kokalj 1998, Obernosterer et al. 1999b). Polymerization reactions and humification (Harvey et al. 1983), which can also be photochemically induced (Kieber et al. 1997), have been shown to render parts of the DOM biologically less labile (Carlson et al. 1985a, Keil & Kirchman 1994).

Loss of DOC due to the photochemical production of carbon gases has also been suggested to account for the decreased bacterial activity on irradiation-exposed DOM compared to dark controls (Gobler et al. 1997). However, in recent studies of Tranvik & Kokalj (1998) and Pausz & Herndl (1999) using algal-derived DOC, the loss of DOC due to photo-oxidation decreased the DOC concentration by less than 1% during an exposure period of ≤16 h, while microbial activity was inhibited by 15 to 20%. Also, in humic lakes, photochemical production of inorganic carbon has been shown to account for only up to a ~4% decrease in the initial DOC concentration (Graneli et al. 1996, Reitner et al. 1997). Thus, the effect of photochemical carbon gas production on bacterioplankton activity was probably insignificant in our study as well. The photochemical production of toxic substances, such as hydroxyl radicals, might also lead to reduced bacterial activity on irradiation-exposed DOM (Lund & Hongve 1993, Anesio et al. 1999). However, to our knowledge, no direct evidence of photo-induced formation of toxic substances and their effect on bacterioplankton activity has been presented for marine systems thus far.

Photoreactivity of DOM of different molecular weight and biological reactivity

In the present study, photo-induced stimulation of the bacterioplankton activity was twice as high in the <20 kDa DOM fraction than in the bulk

DOM (Fig. 2). As indicated by the ~2-fold lower DOC-normalized bacterial activity in the <20 kDa DOM fraction compared to the bulk DOM of the samples in the dark, the <20 kDa DOM fraction is composed of biologically refractory material. According to the relationship established in the present study (Fig. 1), the effect of solar radiation on the bioavailability of the DOM might therefore be more pronounced for the <20 kDa DOM fraction than for the bulk DOM. We further suggest that compounds present in the >20 kDa DOM fraction might counteract the pronounced photochemically-induced stimulation of the bacterial activity observed in the <20 kDa DOM fraction. The model protein BSA used in this study has a molecular weight of ~60 kDa and is highly labile. Addition of BSA to DOM from the CML and subsequent exposure to surface solar irradiation increased bacterial activity compared to the dark control (Fig. 3). This photo-induced stimulation of bacterial activity was similar to that observed in the corresponding unamended treatments. However, BSA-amended, irradiated DW-DOM resulted in reduced bacterial activity compared to the respective dark control. This reduction in the bacterial activity on BSA-amended, irradiated DOM from deep waters has been described recently for Mediterranean mesopelagic waters also (Obernosterer et al. 1999b). Similarly, in a study on XAD-8-fractionated DOM from coastal marine sites, increased bacterial activity was observed in the non-humic, BSA-amended, irradiated DOM fraction compared to the respective dark treatment, while decreased bacterial activity was observed in the humic, BSA-amended DOM fraction compared to the dark control (Obernosterer & Herndl 2000). These results suggest that biologically labile DOM can be photochemically transformed, resulting in compounds that are biologically less available. BSA does not absorb in the wavelength range >300 nm. This further indicates that high molecular weight DOM, such as BSA, is, at least to a certain extent, transformed indirectly, via photosensitizers (Zepp et al. 1985). This is consistent with recent findings of Tranvik & Kokalj (1998), who reported reduced bioavailability of irradiated algal-derived DOC in the presence of dissolved humic substances. In the present study, the higher DOM fluorescence of DW compared to that of the CML could indicate that the amount of precursors of photosensitizers is higher at depths ≥ 200 m. This might explain the differential response of bacterial activity to BSA-amended, irradiated DOM of varying depth layers. According to our results, the <20 kDa DOM fraction was mainly responsible for the photochemically induced stimulation of bacterial activity, while reduced bacterial activity was observed for irradiated BSA-amended DOM. This is consistent with the notion that the

bioavailability of originally refractory low molecular weight DOM (Amon & Benner 1994) increases upon exposure to surface solar irradiation, while bioactive, largely high molecular weight DOM is rendered more refractory upon exposure to solar radiation.

In the present study, we presented further evidence of an inverse relationship between the initial biological reactivity of DOM and its bioavailability following exposure to surface solar radiation. Our results confirm the pattern previously described for the Gulf of Mexico (Benner & Biddanda 1998) and the Mediterranean Sea (Obernosterer et al. 1999b), indicating that photochemical transformation can render labile DOM more refractory, while the bioavailability of originally recalcitrant DOM is increased upon irradiation. This suggests that the described relationship between the biological and the photochemical reactivity of DOM is also a common phenomenon in the Southern Ocean.

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