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Adsorption of dissolved free amino acids on colloidal DOM enhances colloidal DOM utilization but reduces amino acid uptake by orders of magnitude in marine bacterioplankton

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ABSTRACT: In an attempt to quantify abiotic adsorption of easily metabolizable dissolved free amino acids (DFAA) to colloidal dissolved organic matter (CDOM) in the sea, laboratory experiments were performed using dextran of various molecular size ranging from 4.4 to 2000 kDa or phytoplankton-derived CDOM (>1 kDa) as model CDOM, and leucine or a DFAA mixture. Abiotic leucine adsorption to dextran was found to be independent of the molecular size of the dextran. Adsorption of leucine to dextran and phytoplankton-derived CDOM was saturated at ~7.5 nmol mg^{-1} dextran-C; other DFAA species reached higher saturating concentrations (maximum: serine, 48.6 nmol mg^{-1} dextran-C). Adsorption of DFAA to dextran and phytoplankton-derived CDOM led to an approximately 3 times more efficient utilization of dextran and CDOM by marine bacterioplankton than colloidal matter to which no DFAA were adsorbed. Bacterial uptake of CDOM-adsorbed leucine, however, was reduced by 2 to 3 orders of magnitude as compared to the uptake of 'truly' free (non-adsorbed) leucine offered simultaneously at the same concentration. This finding might also be relevant for bacterial production measurements if leucine or thymidine are used as a tracer in colloidal-matter-rich micro-environments such as marine snow. All the CDOM-adsorbed DFAA (except glycine and threonine) were detectable without prior hydrolysis by *o*-phthalaldialdehyde (OPA) derivatization in the HPLC. Adsorption of labile DFAA on otherwise refractory CDOM also has important implications for the biogeochemical flux of CDOM by forming 'semi-labile' molecules which are more readily degradable by bacterioplankton and thereby reducing the flux of CDOM to the deep ocean.

KEY WORDS: Colloidal matter · Dissolved free amino acids · Leucine Bacterioplankton · Dissolved organic matter · Bacterial uptake · Bacterial production measurements

INTRODUCTION

Bacterioplankton are the principal consumers of dissolved organic matter (DOM) in the ocean (Azam & Cho 1987). This DOM can be taken up without prior cleavage by bacteria if the molecular weight of the DOM is below 600 (Gottschalk 1986). If the molecules are larger they have to be cleaved by bacterial ectoenzymes embedded in the capsular membrane or the periplasmatic space prior to the uptake (Gottschalk 1986). Nevertheless, it has been shown recently that marine bacterioplankton can utilize high molecular weight material more rapidly than the DOM fraction <1 kDa (Amon & Benner 1994, 1996). In their size-reactivity model, Amon & Benner proposed a gradual transformation from large, labile molecules to small, refractory DOM. While the marine bulk DOM fraction <1 kDa might be relatively refractory compared to larger molecules, there is a small pool of low molecular weight DOM such as dissolved free amino acids (DFAA) and monomeric carbohydrates which are taken up efficiently by bacterioplankton and, owing to

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their low concentrations, turnover times are short (generally <1 d) (Fuhrman & Ferguson 1986, Fuhrman 1987, Suttle et al. 1991, Rich et al. 1996). It is well documented that bacterioplankton can cover most of their carbon and nitrogen demand by the uptake of DFAA released by phytoplankton (Jørgensen 1987, Keil & Kirchman 1991b, Ducklow et al. 1993, Jørgensen et al. 1993, Kirchman et al. 1993, Middelboe et al. 1995).

Abiotic alterations of specific compounds of the DOM might change the availability for bacterioplankton. As shown by Keil & Kirchman (1993, 1994) labile proteins become refractory over time. This process is significantly enhanced by radiation, especially in the ultraviolet (UV) range (300 to 400 nm) (Keil & Kirchman 1994, I. Obernosterer, B. Reitner & G. J. Herndl unpubl., C. Pausz & G. J. Herndl unpubl.), while originally refractory DOM becomes labile upon UV exposure (Lindell et al. 1995, 1996, Wetzel et al. 1995, Granéli et al. 1996, Kaiser & Herndl 1997, Reitner et al. 1997). However, not only light alters the availability of labile DOM. It has been shown that labile DOM also becomes refractory due to sorption processes in sediments (Keil et al. 1994, Hedges & Keil 1995).

According to Gustafsson & Gschwend (1997) 'colloidal matter is any constituent that provides a molecular milieu into and onto which chemicals can escape from the bulk aqueous solution, while its vertical movement is not significantly affected by gravitational settling.' Colloidal dissolved organic matter (CDOM) is an efficient sorption site for charged molecules such as metals or radionuclides (Baskaran & Santschi 1993, Martin et al. 1995, Moran et al. 1996). In the aquatic environment significant amounts of CDOM are produced during the decay of phytoplankton blooms (Kepkay et al. 1993, Guo et al. 1994, Kepkay 1994, Wells & Goldberg 1994, Veronclark et al. 1995), by bacterioplankton (Tranvik 1993, Heissenberger & Herndl 1994, Stoderegger & Herndl in press) and by chemical reactions between different groups of molecules (Gustafsson & Gschwend 1997). This CDOM might serve as an important food source for protists (Sherr 1988, Tranvik 1994). Due to the adsorption of labile molecules on CDOM, this CDOM might become a more attractive nutrient source, leading to enhanced mineralization.

In our study we tested the hypothesis that CDOM efficiently adsorbs labile molecules in seawater, resulting in enhanced utilization of the CDOM by bacteria while the labile, adsorbed component is taken up less efficiently. Using dextran of various molecular sizes or the colloidal fraction of the photosynthetic extracellular release (PER) as model CDOM, and leucine or a DFAA mixture as the labile component, we determined the utilization efficiency of the CDOM and the adsorbed leucine or DFAA mixture. Thus, in other words, we investigated the importance of CDOM as an abiotic competitor with bacterioplankton for DFAA.

MATERIALS AND METHODS

Adsorption efficiency of leucine to dextran. The adsorption efficiency of [^{14}C]leucine [Amersham; specific activity (SA) = 319 mCi mmol^{-1}] to unlabeled dextran (Sigma Chemicals) of various molecular weight was determined in artificial (Parsons et al. 1984) and in aged (for 3 wk at 20°C in the dark) 0.2 µm filtered seawater (Millipore; polycarbonate) collected from the oligotrophic Mediterranean Sea off Villefranche-sur-Mer (France). Aged 0.2 µm filtered or artificial seawater (48 ml) was transferred into a dialysis tube (Spectrapor; 1000 MW cut off, pre-treated according to the recommendations of the manufacturer, see also Herndl et al. 1993) and 1 ml of dextran [prepared in HPLC grade water (Merck); conc. of the stock solution: 1 mg C ml^{-1}] and 1 ml of leucine (stock solution: 68.5 nmol ml^{-1}, [^{14}C]leu:cold leu = 2:3) added. The dialysis tube was suspended in 950 ml aged or artificial seawater, placed on a magnetic stirrer and held at 20°C. The time course of the radioactivity detectable outside the dialysis tube was followed over a period of 6 h by transferring 1 ml subsamples of the water in the jar into a scintillation vial and adding 8 ml of scintillation cocktail (Insta-Gel, Canberra Packard) as described in more detail below. After subsampling, the water was replaced. The experiments were performed with dextran of 8 different molecular weights (from 4.4 to 2000 kDa).

In order to calculate the adsorption of leucine to dextran over time, dialysis experiments were also run containing exactly the same concentration of [^{14}C]leucine but no dextran. From the total number of disintegrations per minute (DPM) detected in the water in which the dialysis tube containing only [^{14}C]leucine was suspended, the DPM detected in the surrounding water containing the dialysis tube filled with [^{14}C]leucine and dextran was subtracted and converted to nmol leucine adsorbed per mg dextran-C.

Adsorption efficiency of a DFAA mixture to dextran. In addition to the above adsorption experiments with leucine, a DFAA mixture (Sigma Chemicals) was used in one experiment consisting of 2 replicates; the DFAA mixture was composed of 17 amino acid species. To 1 l of artificial seawater, 748.55 nmol l^{-1} of the DFAA mixture (final conc.) and 2.32 mg dextran l^{-1} (or 1 mg dextran-C l^{-1}, 70 kDa) were added; this was incubated at 20°C in the dark for 5 h, filtered through an Amicon ultrafiltration system with a polysulfone cartridge (molecular weight cut-off of 30 kDa) at low pressure and flushed 3 times with 1 l of artificial seawater in order to remove the non-adsorbed DFAA. The reser-

voir and the cartridge of the ultrafiltration system were pre-cleaned and conditioned before the experiment by flushing with at least 5 l of Milli-Q water, circulating 0.05 M NaOH for at least 30 min and flushing again with 5 l Milli-Q water. Subsamples (10 ml) for amino acid analyses (described below) were taken from the filtrate and the water retained by the cartridge before and after the adsorption experiments to estimate the concentrations of DFAA adsorbed to dextran.

Bacterial growth on non-adsorbed leucine and dextran compared with dextran-bound leucine as substrate. Experiments on the bacterial utilization of leucine adsorbed to dextran were performed; control experiments were run using free dextran and leucine, respectively, at the same concentrations as bound. [^{14}C]leucine adsorbed to dextran was produced in the same way as described above using unlabeled dextran of 70 kDa. Based on the radioactivity measurements it was calculated that 9.58 nmol leucine was adsorbed per mg dextran-C. Thereafter, stocks of free leucine and dextran were prepared at concentrations of 9.58 nmol ml^{-1} and 1 mg dextran-C ml^{-1}, respectively. Seawater cultures with 3 different treatments were established by adding either 20 ml of the stock solution of leucine or dextran or the corresponding amount of leucine adsorbed to dextran to 950 ml of double 0.2 µm filtered (Millipore; polycarbonate) aged seawater. Subsequently, 50 ml of 0.8 µm filtered seawater were added to each of the 3 treatments. At various time intervals samples were withdrawn from the seawater cultures, which were held at 20°C in the dark. On subsamples of the 3 different treatments, bacterial abundance and [^{3}H]thymidine incorporation were determined as described below.

Bacterial uptake of non-adsorbed versus dextran-adsorbed leucine offered simultaneously. In another set of seawater cultures, 50 ml of 0.8 µm filtered and concentrated (~10 times) natural bacterial consortia were inoculated in 950 ml double 0.2 µm filtered aged seawater amended with 1 ml of [^{14}C]leucine bound to dextran (stock solution: 9.58 nmol leucine mg^{-1} dextran-C ml^{-1}) and 1 ml of [^{3}H]leucine (SA = 125 Ci mmol^{-1}; 9.58 nmol ml^{-1}). Thus, exactly the same concentration of dextran-bound and non-adsorbed leucine was added to triplicate seawater cultures using aged seawater. The natural bacterial community was concentrated by filtering 1 l of 0.8 µm filtered freshly collected seawater onto a 0.2 µm polycarbonate filter (Millipore; 47 mm filter diam.) and rinsing off the bacteria collected on the filter with 50 ml of the filtrate. The seawater cultures were incubated for 90 min at room temperature, which was close to *in situ* temperature. Within the 90 min incubation period, 8 subsamples were taken from each culture to determine bacterial abundance and uptake of dextran-bound [^{14}C]leucine and free [^{3}H]leucine as described below. In preliminary experiments with natural bacterial assemblages, uptake rates of [^{14}C]leucine were compared with [^{3}H]leucine uptake rates; no significant difference was found.

Bacterial utilization of phytoplankton-derived CDOM and adsorbed leucine. A *Chaetoceros muelleri* culture was grown in 2 l of 0.2 µm filtered (Millipore; polycarbonate), autoclaved f/2 medium (Parsons et al. 1984) at an irradiance of ~100 µE m^{-2} s^{-1} (12 h light: 12 h dark cycle, white cool lamps) at 18°C. Subsamples of 10 ml were taken once a day to measure the optical density at 550 nm in a spectrophotometer. At the onset of the exponential growth and in the late exponential phase the culture was amended with 4 µCi of [^{14}C]sodium bicarbonate. At 24 h after the second labeling, the culture was filtered onto a Whatman GF/F filter, the filtrate was acidified to pH < 2 with 2 N HCl and bubbled with N_2 for 24 h and the radioactivity of the [^{14}C]PER was measured in 2 ml subsamples with 8 ml scintillation cocktail (Insta-Gel) added. Subsequently, the pH of the filtrate was adjusted to the original pH of 8.2 with 1 N NaOH and 200 ml portions of the [^{14}C]PER were transferred to dialysis tubes, with a molecular weight cut off of 1 kDa suspended in 2 l of artificial seawater and placed on a magnetic stirrer at room temperature to remove all the phytoplankton-derived PER <1 kDa. The artificial seawater in which the dialysis tubes were suspended was replaced every 2 h for a total period of 24 h.

Thereafter, a mixture of unlabeled leucine and [^{3}H]leucine (final conc. 147 nmol l^{-1}) was added to each of the 3 dialysis tubes each containing 200 ml of the [^{14}C]CDOM (CDOM > 1 kDa), and the dialysis procedure was repeated as described above for 6 to 8 h. At 30 to 60 min intervals, 2 ml subsamples were taken from the artificial seawater in which the dialysis tubes were suspended, the radioactivity was assessed and the entire volume of the artificial seawater was replaced. The dialysis procedure was stopped after 6 to 8 h when significant [^{3}H]radioactivity was no longer detectable in the artificial seawater, indicating that all the free [^{3}H]leucine had been removed. Then, the specific activity per ml of the water in the dialysis tubes containing [^{3}H]leucine adsorbed to [^{14}C]CDOM was assessed.

Of this water, 45 ml was transferred to 120 ml biological oxygen demand (BOD) bottles and inoculated with 5 ml of 0.8 µm filtered freshly collected natural bacterial consortia (concentrated ~10 times, as described above) to determine the bacterial incorporation of the [^{14}C]CDOM and [^{3}H]leucine and the respiration of the [^{14}C]CDOM. Bacterial incorporation and respiration of the [^{14}C] CDOM with [^{3}H]leucine adsorbed was compared with the other 2 treatments to which either the same concen-

tration of [^{14}C]CDOM or [^{3}H]leucine was added. All 3 treatments were run in duplicate with 1 formalin-fixed control per treatment and held at 20°C in the dark for 8 h. A total of 3 experiments were performed. Thereafter, bacterial abundance, incorporation and respiration were measured for each flask as described below.

Enumeration of bacteria. Formalin-fixed samples (5 ml; 4% final conc.) were stained with DAPI (4',6-diamidino-2-phenylindole; 1 µg ml^{-1} final conc.) for 10 min (Porter & Feig 1980) and then filtered onto a black 0.2 µm pore size polycarbonate filter (Millipore; 25 mm filter diam) supported by a cellulose nitrate filter (0.45 µm pore size). Then, the filter was mounted on a slide and embedded in paraffin oil. Bacteria were enumerated on duplicate samples using an epifluorescence microscope (Leitz Laborlux S) at 1250× magnification. At least 300 bacteria were counted per sample. Bacterial abundance was converted to carbon biomass assuming a mean carbon content per bacterium of 20 fg C (Lee & Fuhrman 1987).

Bacterial thymidine incorporation. Bacterial cell production was assessed by measuring the incorporation of [^{3}H]thymidine (Amersham; SA = 85 Ci mmol^{-1}, 20 nM final conc.) (Fuhrman & Azam 1982). Subsamples (5 ml) were incubated in triplicate with 2 formalin-killed controls at *in situ* temperature in the dark for 30 min. After incubation, the subsamples were filtered onto cellulose nitrate filters (Millipore, 0.45 µm, 25 mm filter diameter) and rinsed 3 times with 5% chilled trichloroacetic acid (TCA) for 5 min. Radioactivity of these filters was assessed after adding 1 ml ethylacetate (Riedel-de-Haen) and 8 ml scintillation cocktail (Insta-Gel). After allowing the samples to sit for 10 h in the dark, radioactivity was measured using a Packard Tri-Carb 2000 scintillation counter with the external standard ratio technique for sample quenching. [^{3}H]Thymidine incorporation was converted into bacterial cell production by using the conversion factor of 1.1×10^{18} cells produced mol^{-1} TdR (Fuhrman & Azam 1982). Whenever the dual labeling approach was used the DPM of the samples was assessed using a dual label channel of the Tri-Carb scintillation counter.

Bacterial incorporation of non-adsorbed [^{3}H]leucine and dextran-adsorbed [^{14}C]leucine. Subsamples (5 ml) were taken in triplicate over a time course of 90 min, filtered onto cellulose nitrate filters, rinsed with 5% chilled TCA and the filters transferred to scintillation vials and ethylacetate and scintillation cocktail added as described above for the thymidine incorporation. The radioactivity of [^{3}H] and [^{14}C] was assessed using a dual label channel on a Tri-Carb 2000 scintillation counter. DPM were converted to fmol leucine incorporated h^{-1} and normalized to the bacterial abundance.

Protocol for measuring incorporation and respiration of [^{14}C]CDOM and [^{3}H]leucine incorporation. After 8 h, the bacterial incorporation and respiration in the BOD bottles was stopped by injecting 5 ml of 6 N H_2SO_4 (pH < 1.5) into the 50 ml of water with a syringe through the rubber membrane sealing the bottle. Then, 0.1 ml of β-phenethylamine (Sigma Chemicals) was added with another syringe to the filter wick (made out of Whatman # 1 filter) contained in a well attached to the rubber membrane. The BOD bottles were allowed to sit for 24 h and adsorb the respired $^{14}CO_2$ to the phenethylamine-soaked filter wick. Thereafter, the filter wick was placed in a scintillation vial and the water filtered onto cellulose nitrate filters. Generally, the same approach was used for the treatment containing only [^{3}H]leucine; respiration, however, was not assessed in this treatment. All the wicks and the filters were radioassayed as described above using the dual label channel to facilitate comparison between the different treatments.

DFAA analysis. Subsamples (10 ml) for DFAA analysis were filtered through combusted glass fiber filters (480°C for 4 h; Whatman GF/F, 25 mm diameter) and kept frozen (–20°C) in combusted ampoules until analysis. DFAA were measured by HPLC (Merck-Hitachi) using the *o*-phthaldialdehyde (OPA)-reverse phase chromatography method (Mopper & Lindroth 1982). All DFAA determinations were performed in duplicate.

RESULTS

Adsorption of leucine and a DFAA mixture to dextran

Adsorption of leucine to dextran was not correlated with the molecular weight of dextran (Fig 1). A linear increase in leucine adsorbed to dextran was detected for a period of 5 h; thereafter, no further adsorption was detectable.

Adsorption experiments were also performed with a DFAA mixture using an ultrafiltration system (molecular weight cut-off of 30 kDa) instead of dialysis tubes to separate non-adsorbed DFAA from dextran-adsorbed DFAA and dextran with 70 kDa. As shown in Table 1, from the originally added 748.6 nmol l^{-1} DFAA, 45.8% adsorbed to dextran within 5 h. With 2 exceptions (glycine and threonine), all the dextran-adsorbed DFAA species were detectable without prior hydrolysis, indicating that the binding strength of OPA (in the derivatization) is stronger than that of dextran or that different groups are involved. 100% of valine and tryptophane bound to dextran, suggesting that the originally added concentrations of these 2 DFAA species were too low to saturate adsorption or that they have different adsorption properties. Leucine adsorption (6.8 nmol mg^{-1} dextran-C) in the DFAA mixture

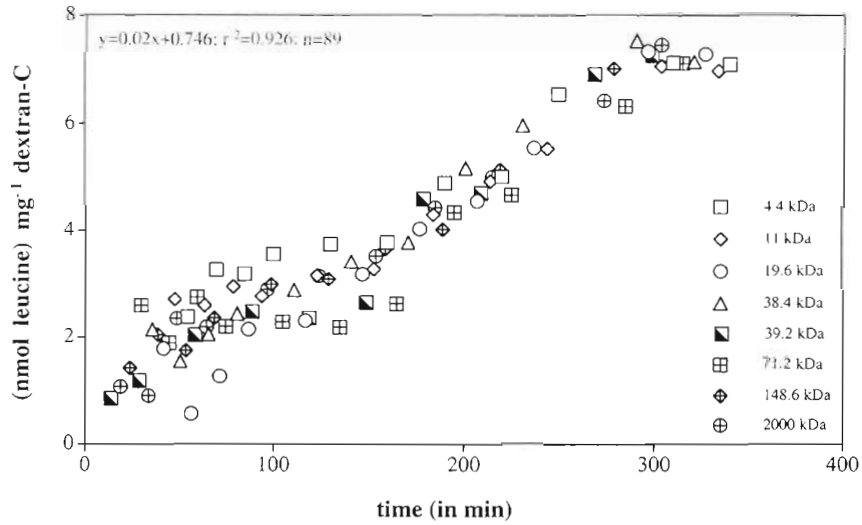

Fig. 1 Development of the adsorption of [^{14}C]leucine to dextran of various molecular sizes in artificial and aged seawater in the experimental system over time. Adsorption rates of leucine to dextran were calculated by measuring the radioactivity of leucine outside the dialysis in the surrounding water. The radioactivity detected in the water in which the dialysis tubes containing dextran and leucine were suspended was subtracted from the radioactivity detected in the water in which a dialysis tube containing only leucine was suspended. For further details see text

(Table 1) was exactly the same as in the time course experiment (Fig. 1) after the same incubation period of 5 h (based on the equation given in Fig. 1).

Table 1 Adsorption of a dissolved free amino acid (DFAA) mixture to 1 mg dextran-C l^{-1} (70 kDa) in 1 l of artificial seawater at 20°C after 5 h. DFAA adsorbed to dextran were detectable by OPA derivatization without prior hydrolysis (except glycine and threonine). Details of the experimental procedure are given in the text. ASP: asparagine; GLU: glutamic acid; SER: serine; HIS: histidine; GLY: glycine; THR: threonine; ALA: alanine; ARG: arganine; TYR: tyrosine; MET: methionine; VAL:valine; TRY: tryptophane; PHE: phenylalanine; ILE: isoleucine; LEU: leucine; ORN: ornithine; CYS: cystine. nd: not detectable. No significant alterations in the concentration of DFAA were detectable over the incubation period in controls without dextran

DFAA	Initially added (nmol l^{-1})	Adsorbed (nmol l^{-1} and nmol mg^{-1} dextran-C)	% adsorbed
ASP	70.7	34.6	48.9
GLU	40.2	15.3	38.0
SER	122.5	48.6	39.7
HIS	44.3	14.9	33.6
GLY	59.0	nd	–
THR	32.3	nd	–
ALA	50.3	19.0	37.8
ARG	41.0	15.7	38.2
TYR	34.3	12.4	36.0
MET	45.1	16.7	37.0
VAL	23.7	23.7	100.0
TRY	25.7	25.7	100.0
PHE	29.9	9.3	31.0
ILE	39.1	15.3	39.2
LEU	20.7	6.8	33.9
ORN	43.1	16.6	38.5
CYS	267.0	9.9	36.7
Sum of DFAA	748.6	284.2	45.8 ± 21.6

Bacterial growth on non-adsorbed leucine and dextran compared with dextran-bound leucine as substrate

Bacterial growth in seawater cultures amended with 192 nmol leucine either bound to 20 mg dextran-C l^{-1} or freely (192 nM) available resulted in about twice the bacterial abundance when grown on non-adsorbed leucine as compared to dextran-bound leucine (Fig. 2a). Dextran addition (also 20 mg C l^{-1}) to aged seawater did not promote significant growth. Bacterial production in the presence of non-adsorbed leucine was up to 3 times higher (mean: 0.76 ± 0.08 µg C l^{-1} h^{-1}; n = 4) than at the same concentration of leucine bound to dextran (mean: 0.37 ± 0.17 µg C l^{-1} h^{-1}; n = 4) (Fig. 2b). Bacterial production in the treatment amended with only dextran was more than 1 order of magnitude lower than in the other treatments (mean: 0.01 ± 0.004 µg C l^{-1} h^{-1}; n = 4).

Bacterial utilization of non-adsorbed versus dextran-bound leucine offered simultaneously

In order to more directly determine the uptake of leucine in its non-adsorbed versus dextran-bound form, we simultaneously offered [^{14}C]leucine bound to dextran and [^{3}H]leucine at the same concentration to a natural bacterial community. As shown in Fig. 3, bacteria used non-adsorbed leucine preferentially over dextran-bound leucine. Bacterial uptake of non-adsorbed leucine was 11.7 amol bacterium^{-1} h^{-1} while dextran-bound leucine was taken up at a 3 orders of magnitude lower rate (0.11 amol bacterium^{-1} h^{-1}; calculated from the equations given in Fig. 3).

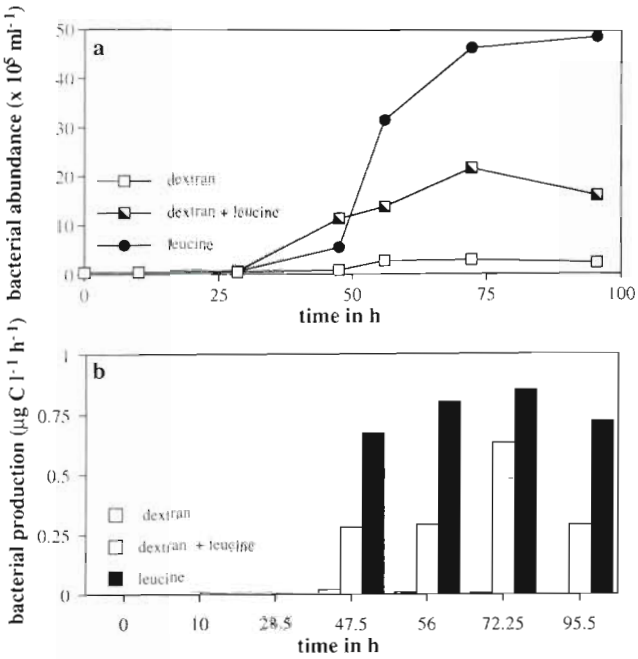

Fig. 2. Bacterial growth in seawater cultures. Aged seawater was amended either with free 20 mg dextran-C l^{-1} or 192 nmol leucine l^{-1} or in bound form (192 nmol leucine l^{-1} bound to 20 mg dextran-C l^{-1}). (a) Development of bacterial abundance in the 3 different treatments over time and (b) bacterial production measured by [^{3}H]thymidine incorporation

Influence of adsorbed leucine on the utilization of phytoplankton-derived CDOM

Additionally to dextran, phytoplankton-derived CDOM (>1 kDa) was used as a model colloidal material to determine the role of adsorption of leucine on the utilization of CDOM. Leucine adsorbed to CDOM (8.75 nmol leucine mg^{-1} CDOM) led, on average, to a 3 times higher incorporation rate of CDOM than CDOM without adsorbed leucine of the same concentration. The respiration rate for CDOM to which leucine was adsorbed, however, was less than half of the respiration rate of the CDOM without leucine adsorbed (Fig. 4). Total bacterial uptake (incorporation + respiration) was about twice as high for leucine-adsorbed CDOM than for CDOM without adsorbed leucine. Bacterial growth yield [bacterial production/(bacterial production + respiration) × 100] was 82% for the leucine-adsorbed CDOM and 40% for CDOM without leucine. Similar to the experiments above where leucine was offered simultaneously in non-adsorbed and dextran-adsorbed form (Fig. 3), incorporation of leucine was more than 20 times lower when offered in CDOM-adsorbed form (Fig. 4).

DISCUSSION

In the present study we found that amino acids efficiently adsorb to dextran and phytoplankton-derived colloidal matter. This adsorption of DFAA leads, on the one hand, to an approximately 3 times higher utilization of dextran and CDOM than of CDOM to which no amino acids are adsorbed. On the other hand, bacterial uptake of CDOM-adsorbed leucine is reduced by 2 to 3 orders of magnitude as compared to the uptake of 'truly' free leucine.

We used dialysis tubes and ultrafiltration to separate free from CDOM-adsorbed leucine and DFAA mixtures, respectively. The efficiency of this separation was checked with [^{14}C]dextran of 70 kDa. Loss of dextran through the dialysis tubes (molecular weight cut off 1 kDa) was found to be less than 2% while ultrafiltration (30 kDa molecular weight cut off) resulted in a loss of ~8%. Thus the DFAA concentrations given in Table 1 are likely to slightly underestimate adsorption, since no attempts were made to correct the leucine concentrations measured for potential dextran loss through the cartridge. Nevertheless, the error introduced due to inefficient retention by the dialysis tubes and the ultrafiltration cartridge is probably small and does not alter the general conclusions from these experiments. Data obtained from leucine adsorption using dialysis tubes (Fig. 1) agreed well with the leucine adsorption in the DFAA mixture experiment

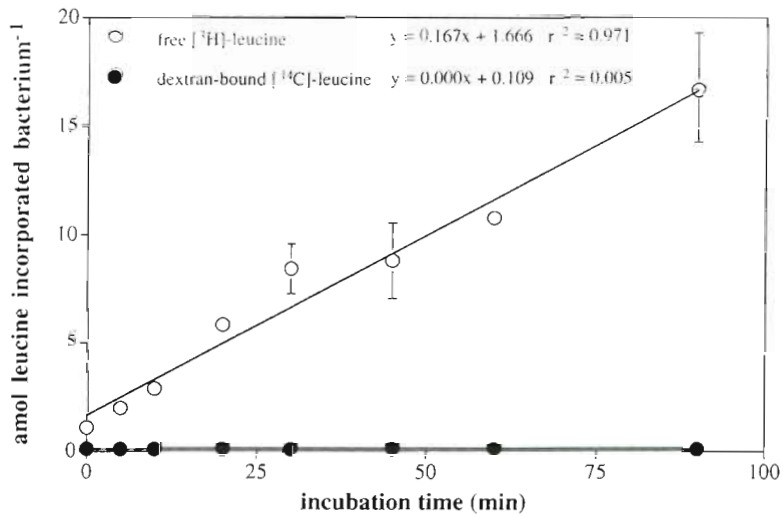

Fig. 3. Time course of bacterial incorporation of leucine offered simultaneously in the same concentration (9.6 nmol l^{-1}) in free ([^{3}H]leucine) and in dextran-bound ([^{14}C]leucine) form; amol = 10^{-18} mol

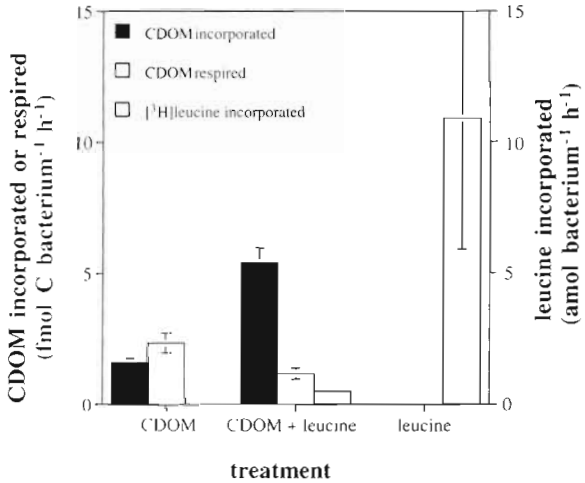

Fig. 4. Bacterial incorporation and respiration of phytoplankton-derived colloidal DOM (CDOM; 16.8 mg C l^{-1} or 1.4 mmol C l^{-1}) in the presence and absence of adsorbed leucine (147 nmol l^{-1}, corresponding to 8.75 nmol leucine mg^{-1} CDOM) and, for comparison, the uptake of free leucine (147 nmol l^{-1}) in the absence of phytoplankton-derived CDOM. Bars represent means ± SD of 3 experiments each performed in duplicate. Note that the incorporation and respiration of CDOM is in fmol C bacterium^{-1} h^{-1} while the leucine incorporation is given in amol bacterium^{-1} h^{-1} (amol = 10^{-18} mol)

using ultrafiltration (Table 1) further indicating that both methods give comparable results.

Using dextran and phytoplankton-derived CDOM as model colloids and leucine as a model substance which is readily utilizable for bacterioplankton we determined the importance of adsorption processes on the utilization of CDOM. DFAA readily adsorb to CDOM as shown in Fig. 1 and Table 1. The adsorbed leucine, however, is barely utilized if non-adsorbed leucine is available as well (Fig. 3). If DFAA are available in their free, non-adsorbed form then they are preferentially used. Similar results were obtained for phytoplankton-derived CDOM (Fig. 4), indicating that dextran serves as a good model to study the adsorption of labile components and its influence on the utilization of CDOM by marine bacterioplankton.

Our results indicate that under *in situ* conditions, CDOM, owing to its adsorptive capacity, competes with bacterioplankton for 'free' DFAA. Since polysaccharides have been shown to be the dominant chemically characterizable compound of the oceanic DOM pool (Benner et al. 1992, Pakulski & Benner 1994, McCarthy et al. 1996, Aluwihare et al. 1997), this competition between abiotic adsorption and bacterioplankton uptake might be of considerable biogeochemical significance. Nagata & Kirchman (1996) calculated the surface area of colloids to be in the range of 10 m^2 m^{-3} (derived from data given in Koike et al. 1990 and Wells & Goldberg 1991) in the upper mixed layer of the ocean. Similar to our findings, Nagata & Kirchman (1996) found lower utilization rates of proteins (bovine serum albumin, BSA) adsorbed to sub-micrometer particles. Due to its smaller size, the surface area of CDOM used in this study probably exceeds the surface area calculated by Nagata & Kirchman (1996). This implies that, for a DFAA molecule released from a source such as a phytoplankton cell, it is much more likely to become adsorbed to a CDOM molecule than get in contact with bacteria due to the at least 4 orders of magnitude lower numbers and even lower surface area of bacteria. The concentration of single DFAA species in oceanic waters is usually in the range of 5 to 50 nM, depending on the amino acid species and the trophic stage of the water body (Mopper & Lindroth 1982, Søndergaard et al. 1985, Fuhrman & Ferguson 1986, Keil & Kirchman 1991a, Müller-Niklas et al. 1994). The extent to which these DFAA concentrations are really free in the pelagic environment remains unknown since the OPA derivatization technique to detect DFAA does not distinguish between truly free (non-adsorbed) and adsorbed DFAA, as shown in Table 1. Jørgensen & Søndergaard (1984) conducted size exclusion chromatography to determine the molecular weight structure of the DFAA detected by OPA derivatization and HPLC and concluded that most of the DFAA are truly free and not adsorbed. Our study, however, points to the possibility of efficient adsorption of DFAA to CDOM and thereby significantly reducing the availability of these adsorbed DFAA for bacteria. Due to adsorption of highly labile DFAA to refractory CDOM, the CDOM-DFAA complex becomes 'semi-labile' and therefore more easily degradable (Figs. 2 & 4).

Oceanic dissolved organic carbon (DOC) concentrations range from ~100 µM in surface waters to ~45 µM in the deep ocean, and about 10 to 50% of this DOC pool is considered as colloidal DOC (Kepkay et al. 1993, Guo et al. 1994). The major fraction of this colloidal DOC is comprised of polysaccharides (Benner et al. 1992). Thus in surface waters, colloidal DOC is present in concentrations between ~8 and ~40 µM (100 to 480 µg C l^{-1}). Based on the mean adsorption of DFAA to dextran of 284 nmol DFAA mg^{-1} dextran-C (Table 1), between 28 and 136 nmol DFAA could be adsorbed to colloidal DOC in oceanic surface waters, assuming that this colloidal DOC has the same adsorption capacity as dextran and phytoplankton-derived CDOM used in this study. Typically, the sum of DFAA in the ocean ranges between 70 and 100 nM (Suttle et al. 1991). This would mean that between ~11 and 55% of the DFAA detectable by HPLC can be adsorbed to CDOM in surface waters.

The finding that DFAA adsorb efficiently to CDOM, and, as a consequence, CDOM-adsorbed DFAA are

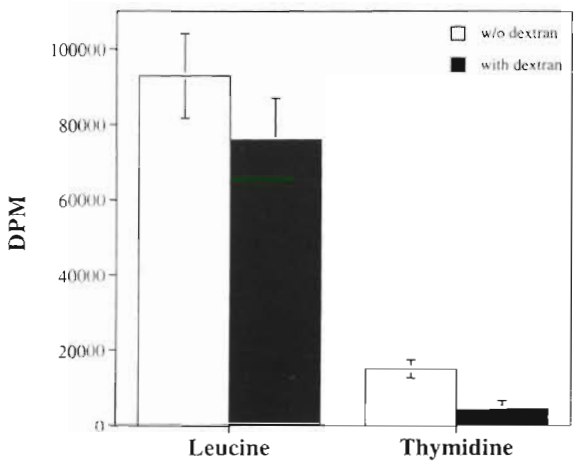

Fig. 5. Effect of dextran addition (1 mg dextran-C l^{-1} final conc.) on bacterial thymidine or leucine incorporation using 20 nM of either [^{3}H]leucine or [^{3}H]thymidine (final conc.) and freshly collected seawater from the NIOZ pier (Marsdiep, The Netherlands, coastal North Sea). Samples were incubated in triplicate with 2 formalin-killed blanks for 30 min in the dark. Vertical lines represent the SD from the mean of 3 replicate measurements. The difference between the leucine incorporation to which dextran was added and the treatment to which no dextran was added was not significant (Mann-Whitney, $p = 0.127$); significant differences were detectable between the 2 different treatments for the thymidine incorporation (Mann-Whitney, $p = 0.049$). DPM: disintegrations per minute

incorporated into bacteria at a significantly lower rate, also has important implications for bacterial production measurements. The most commonly used methods for bacterial production measurements are the incorporation of radiolabeled thymidine into bacterial DNA and leucine into bacterial protein (Kemp et al. 1993). In a preliminary experiment to test the presence of CDOM on bacterial production measurements, we added 20 nM [^{3}H]leucine or [^{3}H]thymidine, respectively, to 1 mg dextran-C l^{-1} (all final conc. in 5 ml subsamples) and compared the incorporation rates with those obtained by adding 20 nM of leucine or thymidine only. The presence of 1mg dextran-C l^{-1} significantly reduced the bacterial incorporation of radiolabeled leucine and thymidine (Fig. 5). The effect of tracer adsorption to dextran was much more pronounced for thymidine than for leucine, leading to underestimation of the actual bacterial production. Tracer adsorption to CDOM might bias bacterial production measurements, especially in environments with high concentrations of CDOM such as in marine snow. In fact, the polysaccharidic matrix of marine snow efficiently binds radiolabeled thymidine as well as leucine added for bacterial production measurements (Herndl unpubl. data). Discrepancies have been found in marine snow between bacterial growth measured by thymidine and leucine uptake and ectoenzymatic activity (Karner & Herndl 1992, Smith et al. 1992). In both studies it was concluded that ectoenzymatic activity on marine snow is higher than the demand for macromolecular synthesis to sustain the measured bacterial growth. As shown in Fig 3, if all possible adsorption sites are occupied, substrate addition leads to a linear increase over time. This saturation of possible adsorption sites has to be tested before bacterial production measurements are performed on marine snow, although this is often difficult in practice due to the limited number of samples available.

The biogeochemical implication of the finding that DFAA efficiently adsorbs to refractory CDOM is that CDOM becomes more accessible to bacterial degradation. The number of colloidal particles has been shown to rapidly decline with depth, indicating that most of the colloids are decomposed in the euphotic zone (Koike et al. 1990, Wells & Goldberg 1991, 1994). Based on our data, adsorption of labile, freshly released DFAA by phytoplankton or via sloppy feeding of zooplankton on phytoplankton (Lampert 1978, Riemann et al. 1986) might be responsible for the observed rapid decline of colloids in the upper layers of the ocean and thereby reduce the amount of CDOM transferred into the deeper layers. Upon adsorption of labile DFAA to CDOM, this high molecular weight CDOM is more efficiently utilized by bacteria, shifting, ultimately, the size spectrum of the oceanic DOM towards low molecular weight DOM, which has been shown to be comparatively old (Guo et al. 1996) and utilized by bacterioplankton at low rates (Amon & Benner 1996).

In summary, we have shown that DFAA readily adsorb to CDOM and particularly to polysaccharides, and thereby making them more easily available for bacterioplankton utilization while reducing the availablility of the adsorbed compared to non-adsorbed DFAA by orders of magnitude. This finding also has implications for bacterial production measurements, since the underlying assumption of these measurements is that the added tracer is taken up at the same rate as the unlabeled molecules present in the water Since an unknown amount of tracer might adsorb to CDOM, this assumption might be violated. This might be of particular relevance in situations where colloidal matter is abundant, such as in marine snow, and if high colloidal matter environments are compared with low colloidal matter environments, such as surface versus deep water Clearly, more awareness of this potential problem is necessary and more emphasis has to be put on the abiotic interactions of molecules and the influence of these interactions on bacterial transformation of DOM in the ocean.

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