

## Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean's interior

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### Abstract

The peptidoglycan layer of the bacterioplankton cell wall contains four major amino acids (alanine, Ala; serine, Ser; aspartic acid, Asp; and glutamic acid, Glu) in a characteristic enantiomeric ratio (D/L ratio). It is assumed that bacterioplankton are the only biological source of significance for these four specific D-amino acid species in the ocean. The concentrations of these dissolved total enantiomeric amino acids were measured throughout the water column of the Faroe Shetland Channel (North Atlantic). Concurrently, the uptake of D- versus L-Asp and of L-leucine (as a measure of bacterial production) by bacterioplankton was determined. The D/L ratios of the dissolved total Ala, Asp, Glu, and Ser did not exhibit any particular trend with depth, averaging 0.49 for Ala, 0.42 for Asp, 0.15 for Glu, and 0.09 for Ser. The ratio of D-/L-Asp uptake by bacteria, however, increased from surface (D-/L-Asp uptake ratio of ~0.03) to deeper layers reaching a D-/L-Asp uptake ratio of close to 1 at 1,000 m depth, indicating that mesopelagic bacteria utilize D-Asp almost as efficiently as L-Asp. Subsequent laboratory experiments with surface-water bacterioplankton assemblages incubated in nutrient-amended artificial seawater confirmed that bacterioplankton, in the absence of other utilizable organic carbon, efficiently utilize D-amino acids. In these laboratory experiments, the D-/L-Asp uptake ratio increased within 8 d to values similar to those obtained for mesopelagic bacteria. Furthermore, the presence of flagellates stimulated the uptake of D-Asp probably via enhanced release of D-amino acids during bacterivory. Thus, our results indicate that dissolved D-amino acids might be an important substrate for mesopelagic bacterioplankton. The efficient uptake of D-amino acids in the deeper layers of the ocean might indicate that mesopelagic bacterioplankton are utilizing bacterial cell wall-derived organic matter efficiently.

The oceanic dissolved organic carbon (DOC) pool is considered to be mainly of phytoplankton origin (Aluwihare and Repeta 1999). This view has been challenged by the notion that the largest oceanic biomass, the bacterioplankton (Whitman et al. 1998), also considerably fuel this DOC pool (Heissenberger and Herndl 1994; Tanoue et al. 1995; McCarthy et al. 1998; Stoderegger and Herndl 1998). It has been demonstrated that bacterioplankton transform labile DOC into recalcitrant DOC (Brophy and Carlson 1989; Tranvik 1993; Ogawa et al. 2001). One of the most refractory compounds of the bacterial cell is its cell wall. Specific compounds of this cell wall, the peptidoglycan layer, have been shown to constitute a significant fraction of the oceanic DOC pool as indicated by the characteristic enantiomeric ratio (D/L ratio) of its dissolved total amino acids (McCarthy et al. 1998).

The main biotic source of D-amino acids in the sea is the peptidoglycan layer of the bacterial cell wall where four specific enantiomeric amino acids (alanine [Ala], glutamic acid

[Glu], aspartic acid [Asp], and serine [Ser]) are present (Schleifer and Kandler 1972; McCarthy et al. 1998). Abiotically, D-amino acids are formed by racemization, which converts the L-enantiomeric form of amino acids into the corresponding D-amino acids. This racemization is a significant source of D-amino acids only over geological time scales (Lee and Bada 1977). Whereas the production of D-amino acids in the sea is largely restricted to bacteria, L-amino acids are produced and released into the oceanic DOC pool by a large variety of organisms, but the most important source is phytoplankton (Fuhrman 1987). These phytoplankton-derived L-amino acids serve as an important substrate for bacterioplankton and are consequently turned over rapidly (Suttle et al. 1991).

In contrast to that, D-amino acids are generally considered to be refractory, as indicated by the increase in the ratio of D-/L-amino acids in DOC degradation experiments with surface-water DOC (Jørgensen et al. 1999; Amon et al. 2001). Therefore, it has been suggested that the ratio of D-/L-amino acids can be used as a diagenetic indicator of the bioreactivity of the oceanic DOC pool (Amon et al. 2001). Because the DOC pool becomes increasingly refractory from the surface layers to the deep waters (Bauer et al. 1992), one would expect that the D-/L-amino acid ratio of the DOC pool increases with depth as well. However, such an increase in the D-/L-amino acid ratio with depth has not been found in the DOC fraction larger than 1,000 Da (McCarthy et al. 1998) which represents about 20–30% of the bulk oceanic DOC (Benner et al. 1992).

In this study, we measured the concentrations of the four bacterial cell wall-derived enantiomeric amino acid species

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present in the DOC pool throughout the North Atlantic water column. Concurrently, we determined the uptake of D- versus L-Asp by bacterioplankton in the different water layers. Because major shifts in the D-/L-Asp uptake ratio of bacterioplankton from the surface to the deep mesopelagic layers were found, additional laboratory experiments were performed. In these experiments, the hypothesis was tested that bacterioplankton, in the absence of other utilizable organic matter, shift from a preferential L-amino acid uptake to a more efficient utilization of D-amino acids (i.e., bacterially derived DOC). Furthermore, the potential role of flagellates in the production of bacterial cell wall-derived dissolved amino acids was examined.

## Materials and methods

*Study site and sampling*—Water was collected at six stations in the Faroe Shetland Channel of the North Atlantic (1°W, 62°N–5°W, 60°N) during the BIOPROCS cruise with the R/V *Pelagia* in the summer of 1999. The complex hydrography of the Faroe Shetland Channel was recently reviewed by Turrell et al. (1999). Briefly, there are two distinctive surface waters present: the North Atlantic water (NAW) and the modified North Atlantic water (MNAW) (Turrell et al. 1999). Beneath the MNAW, the Arctic intermediate/North Icelandic water (AI/NIW) occupies a depth of 400–600 m. Below the AI/NIW is the Norwegian Sea Arctic intermediate water (NSAIW) ranging from 600–800 m depth (Turrell et al. 1999). Below about 800 m depth, there is the Faroe Shetland Channel deep water originating from the Norwegian Sea deep water (Turrell et al. 1999).

Samples were collected from different depths with acid-cleaned NEOX bottles (12 liter) for bacterial production measurements, for dissolved organic carbon (DOC) and nitrogen (DON), and for dissolved total enantiomeric amino acids (DTEAA). For DTEAA analysis, the samples were filtered immediately through 0.2- $\mu\text{m}$  acid-rinsed polycarbonate filters (Nuclepore) and stored in combusted glass ampoules (450°C for 4 h) at  $-20^{\circ}\text{C}$  until analysis. At three out of the six stations, D- and L-Asp uptake by the natural bacterioplankton assemblage collected at the different depths was also measured as described in detail below.

*Laboratory experiments*—Laboratory experiments were performed to assess the role of flagellate grazing on the production of bacterial cell wall-derived DTEAA. Seawater dilution cultures were established with 900 ml of artificial seawater. To this artificial seawater, 100 ml of a 0.6- $\mu\text{m}$  filtered community containing only bacteria and collected from the coastal North Sea was inoculated for treatment. For the treatment containing both bacteria and flagellates, 100 ml of seawater was filtered through a 3- $\mu\text{m}$  polycarbonate filter (Nuclepore). Both treatments were amended with 100  $\mu\text{mol L}^{-1}$  glucose, 10  $\mu\text{mol L}^{-1}$  ammonium, and 1  $\mu\text{mol L}^{-1}$  phosphate. Duplicate cultures were incubated at 19°C in the dark for 8–10 d. Two sets of experiments, each in duplicate, were performed. Within 2 d, essentially all the glucose was taken up by the bacteria as determined by DOC analyses on 0.2- $\mu\text{m}$  filtered samples (data not shown). Cultures were sampled at 24-h intervals until day 6 and at 48-h intervals there-

after. Samples were taken for determining bacterial and flagellate numbers, bacterial production, D- and L-Asp uptake, and the concentration of DTEAA as described below.

*Bacterial production*—Bacterial production was estimated from rates of protein synthesis with [ $^{14}\text{C}$ ]L-leucine (Amersham, S.A., 319 mCi  $\text{mmol}^{-1}$ ) (Simon and Azam 1989). Duplicate samples and one formaldehyde-killed control were incubated with 20  $\text{nmol L}^{-1}$  (final concentration) of [ $^{14}\text{C}$ ]leucine. In the North Atlantic, 10–20-ml samples were incubated at in situ temperature for up to 3 h, whereas for the laboratory experiments, 5-ml samples were kept at 19°C for 1 h. All incubations were performed in the dark. Incubations were terminated by adding 2% formaldehyde (final concentration); subsequently, the samples were filtered through 0.45- $\mu\text{m}$  Millipore HAWP filters followed by three rinses with 5-ml iced-cold 5% trichloroacetic acid. Upon return to the laboratory, filters were dissolved in 1 ml ethyl acetate (Riedel de Haen), and after 10 min, 8 ml of scintillation cocktail (Insta-gel plus II, Canberra Packard) was added. The radioactivity of the filter was assessed after 18 h and converted into bacterial carbon production using the formula given elsewhere (Simon and Azam 1989).

*D- and L-Asp uptake by bacterioplankton*—To measure the bacterial uptake of D- and L-Asp, duplicate water samples and one formaldehyde-killed control were incubated with either D-[2,3- $^3\text{H}$ ]Asp or L-[2,3- $^3\text{H}$ ]Asp (Amersham, S.A.; D-Asp, 12.8 Ci  $\text{mmol}^{-1}$ ; L-Asp, 22.0 Ci  $\text{mmol}^{-1}$ ) at a final Asp concentration of 10  $\text{nmol L}^{-1}$ . All the other incubation conditions were the same as described for bacterial production. After terminating the incubations by adding 2% formaldehyde (final concentration), the samples were filtered through 0.45- $\mu\text{m}$  Millipore HAWP filters and rinsed twice with 5 ml of 0.2- $\mu\text{m}$  double-filtered seawater. The radioactivity of the filter was assessed and the disintegrations per minute converted into the actual amount of D- and L-Asp incorporated into the bacterial community.

In an additional set of experiments, we checked whether the D-/L-Asp uptake ratio of bacterioplankton is dependent on the concentration of the added radiolabeled enantiomeric Asp because we spiked the samples with 10  $\text{nmol L}^{-1}$  Asp, whereas the concentration of dissolved free Asp in the water column of the North Atlantic is  $<5 \text{ nmol L}^{-1}$  (data not shown because they could not be reliably detected with our method, *see below*). Two hundred milliliters of 3- $\mu\text{m}$  filtered North Sea seawater were inoculated into 1.8 liters of nutrient-amended artificial seawater (nutrients added as described above for the laboratory experiments) and held in the dark at 18°C for 8 d. The uptake rates of D- and L-Asp by bacterioplankton were determined twice during the 8-d incubation period by adding D-[2,3- $^3\text{H}$ ]Asp or L-[2,3- $^3\text{H}$ ]Asp at six different concentrations (0.1, 0.5, 1, 5, 10, and 20  $\text{nmol L}^{-1}$ ) to triplicate samples and one formaldehyde-killed blank. In the two experiments performed, the uptake rates of D- and L-Asp increased with increasing Asp concentrations (from 0.1  $\text{nmol L}^{-1}$  Asp to 10  $\text{nmol L}^{-1}$  Asp by a factor 10) (data not shown). The D-/L-Asp uptake ratios, however, were independent of the D- and L-Asp concentrations used (data not shown). The D-/L uptake ratio for Asp averaged over all the

different concentrations used was  $0.016 \pm 0.003$  at the beginning and  $0.61 \pm 0.22$  at the end of the incubation period, indicating an increased efficiency in utilizing D- over L-Asp toward the end of the incubation.

*Dissolved total enantiomeric amino acid analysis*—Samples were hydrolyzed by adding 2 ml of 38% HCl and 100  $\mu\text{l}$  of ascorbic acid ( $55 \mu\text{mol L}^{-1}$  final concentration) to 2 ml of sample and subsequently flushing with  $\text{N}_2$  for 20 min. Then, the pre-combusted glass ampoules were sealed and kept at  $110^\circ\text{C}$  for 24 h. The hydrolyzed samples were neutralized with 2.4 ml of borate buffer, and the pH was adjusted to 8.5 with 32% NaOH.

After hydrolysis, the concentration of the individual DTEAA species was determined by high-performance liquid chromatography (HPLC) after precolumn derivatization with *o*-phthalaldehyde and *N*-isobutyryl-D-cysteine (Fitznar et al. 1999). On selected samples, *N*-isobutyryl-L-cysteine was used as well for checking the identity of D-amino acids. Particular attention was paid to those DTEAA species originating from the bacterial cell wall (Ala, Ser, Asp, Glu), but other enantiomeric amino acids were detected as well (Pausz et al. unpubl. data). A Merck-Hitachi integrated HPLC system was used consisting of an L-6200 pump, an F-1050 fluorescence detector (set at Ex 330 nm; Em 445 nm), an AS-4000 intelligent autosampler for automatic derivatization, and a D-600 interface. For separation of the individual amino acids, we used a reversed-phase column (LichroCart 125-4 packed with Superspher 100, Merck) and eluents and a multistep gradient system as described in Fitznar et al. (1999). Eluent A was sodium acetate ( $25 \text{ nmol L}^{-1}$ , pH 7), eluent B was 100% HPLC-grade methanol, and eluent C was  $25 \text{ nmol L}^{-1}$  sodium acetate (pH 5.3) (Fitznar et al. 1999). All eluents were degassed with helium before use. For calibration, external standards (Fluka, Aldrich, Sigma) were used. The coefficients of variation between duplicate samples analyzed were 1–9%; the relative standard deviation for the individual amino acids and each run was  $<6\%$ . We also measured free enantiomeric amino acids. Owing to the detection limit of the method ( $\sim 5 \text{ nmol L}^{-1}$ ), we could not reliably determine free enantiomeric amino acids. The concentrations of the free enantiomeric amino acid species in the water column of the Faroe Shetland Channel were generally  $<5 \text{ nmol L}^{-1}$ .

## Results and discussion

*Distribution of DTEAA in the water column of the Faroe Shetland Channel*—The concentrations of the four DTEAA species indicative of cell wall-derived DOC (Asp, Glu, Ser, Ala) exhibited no particular trend with depth in the water column of the Faroe Shetland Channel (Table 1). Also, no trend with depth was discernible for the ratio of the D-/L-amino acid species (Table 1). The lack of any significant decrease in the concentrations of the DTEAA species with depth is not only in contrast to the commonly reported decline of amino acids but also of bulk DOC and DON with depth. The lack of any decreasing trend with depth for DTEAA corresponds with the rather stable DOC and DON concentrations observed throughout the water column of the

Faroe Shetland Channel. In the surface waters (5–100 m depth), DOC and DON concentrations averaged  $64.8 \pm 13.2 \mu\text{mol L}^{-1}$  ( $n = 70$ ) and  $7.8 \pm 3.1 \mu\text{mol L}^{-1}$  ( $n = 80$ ), respectively (Kramer et al. unpubl. data). In the layers below 600 m (Norwegian Sea Arctic intermediate water and Faroe Shetland Channel deep water), mean DOC and DON concentrations were  $50.3 \pm 2.8 \mu\text{mol L}^{-1}$  ( $n = 9$ ) and  $6.9 \pm 2.4 \mu\text{mol L}^{-1}$  ( $n = 7$ ), respectively (Kramer et al. unpubl. data). Thus, whereas DOC decreased on average 23% from the top 100-m layer to the  $>600$ -m layers, DON decreased only by 12%. The determination of DON (via persulfate oxidation) is independent of the HPLC-based method to determine DTEAA, and both parameters remained rather stable throughout the water column of the Faroe Shetland Channel. Thus, we are confident that the absence of any decline in DTEAA concentrations with depth is real and not caused by limitations associated with the HPLC method. The rather constant DTEAA concentrations are most likely due to the characteristic physical and chemical features of the five different water masses present in the Faroe Shetland Channel (Turrell et al. 1999). Based on the salinity and temperature characteristics of the water masses given in Turrell et al. (1999) and the salinity and temperature data in combination with the individual DTEAA concentrations obtained during the cruise, we can relate concentrations of DTEAA (sum of L and D enantiomeric amino acid species) to the specific water masses (Pausz et al. unpubl. data). The sum of the D-DTEAA concentrations was more stable over the different water masses than L-DTEAA. The sum of the L-DTEAA was about one third lower in the surface-water masses (NAW and MNAW) than in the AI/NIW (400–600 m depth). The AI/NIW is created as a mixture of Arctic and Atlantic waters during winter convection on the north Icelandic Shelf and is therefore relatively young. Beneath the AI/NIW, in the NSAIW (600–800 m depth), the L-DTEAA concentrations were around 20% lower than in the AI/NIW (Pausz et al. unpubl. data). The sum of the L-DTEAA in the water mass beneath the NSAIW, in the Faroe Shetland Channel bottom water was lower than in the NSAIW but almost identical to the concentrations in the surface-water masses (NAW and MNAW) (Pausz et al. unpubl. data). Because of the morphology of the Faroe Shetland Channel, current velocities are high, and lateral transport might be more important in this area than in more open areas of the Atlantic. This might result in a deviation from the expected water column profile of amino acids in the Faroe Shetland Channel from areas where vertical transport is more important than lateral transport.

Generally, D-Ala exhibited the highest concentrations among the four D-DTEAA species reported here followed by D-Asp, D-Glu, and D-Ser (Table 1) corresponding to the sequence of concentrations of these four D-amino acid species reported recently by Dittmar et al. (2001) for Arctic waters. Ala also exhibited the highest D/L ratios of the four DTEAA species, ranging between 0.28 and 0.69 ( $0.49 \pm 0.11$ , mean  $\pm$  SD;  $n = 54$ ) over the different stations and depths. The D/L ratios of Asp varied between 0.19 and 1.25 ( $0.42 \pm 0.2$ ) and of Glu and Ser between 0.04 and 0.54 ( $0.15 \pm 0.11$ ) and 0.03 and 0.25 ( $0.09 \pm 0.04$ ), respectively (Table 1). The lack of any trend with depth in the D/L ratios of these four

Table 1. Concentrations and D-/L ratios of the four dissolved total D-amino acids—aspartic acid (Asp), alanine (Ala), serine (Ser), and glutamic acid (Glu)—at different stations and depths in the North Atlantic.

Station	Depth (m)	Concentration (nmol L <sup>-1</sup> )							
		D-Asp	D-Ala	D-Ser	D-Glu	D-/L-Asp	D-/L-Ala	D-/L-Ser	D-/L-Glu
60°23'55"N 5°12'49"W	10	13.7	29.4	14.6	5.78	0.35	0.55	0.25	0.09
	30	25.5	29.3	11.5	7.6	0.64	0.49	0.17	0.12
	60	16.3	23.3	9.4	5.4	0.35	0.40	0.13	0.10
	100	14.9	28.9	5.9	6.1	0.33	0.34	0.06	0.09
	200	17.1	34.1	5.5	7.5	0.45	0.48	0.09	0.15
	300	31.9	26.5	6.1	5.5	0.84	0.35	0.08	0.09
	400	20.7	30.4	13.1	5.5	0.53	0.56	0.19	0.11
	500	14.7	27.2	16.2	6.8	0.45	0.46	0.22	0.14
	600	17.1	33.1	8.2	7.9	0.41	0.52	0.10	0.11
	800	15.1	34.6	6.4	5.2	0.48	0.58	0.09	0.10
60°16'56"N 5°03'24"W	900	16.9	32.2	8.0	5.8	0.39	0.50	0.09	0.10
	10	14.5	30.6	5.5	5.7	0.26	0.34	0.04	0.12
	30	14.7	27.3	6.7	2.4	0.35	0.65	0.16	0.08
	60	30.9	22.3	5.4	4.6	0.32	0.48	0.10	0.15
	100	9.0	16.3	4.8	4.8	0.26	0.29	0.06	0.12
	150	13.5	22.4	5.2	2.8	0.33	0.49	0.09	0.08
	250	14.0	25.0	8.1	4.6	0.31	0.30	0.06	0.11
	450	20.3	23.1	10.7	3.3	0.58	0.42	0.14	0.11
	500	12.0	24.1	7.7	3.3	0.35	0.49	0.11	0.09
	600	18.0	30.6	6.2	4.7	0.35	0.32	0.03	0.10
60°15'35"N 5°01'46"W	800	10.1	28.9	6.1	3.2	0.29	0.38	0.05	0.09
	930	8.7	23.4	5.0	2.3	0.25	0.37	0.05	0.09
	30	9.4	24.9	4.3	5.3	0.30	0.63	0.09	0.13
	100	14.2	21.3	4.8	2.7	0.53	0.55	0.10	0.07
	150	18.2	37.2	7.3	5.4	0.46	0.69	0.11	0.09
	250	11.7	24.5	3.4	6.6	0.37	0.59	0.06	0.13
	400	16.7	16.7	4.1	7.9	0.39	0.47	0.10	0.22
	500	12.8	27.0	3.9	4.7	0.34	0.57	0.07	0.10
	800	10.7	27.1	3.3	3.6	0.34	0.56	0.06	0.09
	61°10'26"N 3°26'58"W	10	16.2	44.9	7.4	17.6	0.29	0.64	0.07
20		14.7	34.8	6.1	19.6	0.32	0.67	0.10	0.39
30		17.5	42.2	8.5	13.3	0.33	0.58	0.07	0.17
40		16.2	37.4	5.7	10.1	0.36	0.65	0.09	0.19
400		12.7	36.1	6.7	20.4	0.29	0.60	0.07	0.33
550		12.9	32.7	4.9	11.3	0.36	0.63	0.08	0.22
60°57'36"N 3°08'48"W	1,100	13.9	34.0	5.1	12.9	0.39	0.64	0.10	0.24
	20	21.1	26.4	5.6	14.7	0.36	0.48	0.06	0.17
	30	21.3	26.3	7.2	20.4	0.36	0.49	0.10	0.26
	40	21.4	27.8	5.5	21.1	0.41	0.56	0.07	0.54
	60	17.5	42.7	4.7	22.7	0.34	0.66	0.06	0.33
	100	20.9	29.7	7.0	9.3	0.41	0.55	0.10	0.14
	150	16.9	23.6	5.6	18.8	0.41	0.54	0.08	0.30
	350	18.5	25.8	3.8	13.2	0.30	0.28	0.04	0.06
	450	16.4	24.1	4.9	15.6	0.38	0.46	0.08	0.48
	600	15.9	25.0	6.1	17.9	0.36	0.43	0.09	0.42
61°53'15"p N 1°35'46"p W	30	40.1	20.6	4.0	5.3	1.25	0.45	0.07	0.13
	40	37.9	32.4	6.2	4.7	1.01	0.55	0.09	0.10
	60	33.9	31.4	3.7	4.3	0.80	0.49	0.04	0.06
	100	29.6	21.8	3.4	5.5	0.96	0.54	0.07	0.13
	150	12.9	23.9	3.5	3.0	0.29	0.40	0.04	0.05
	250	31.3	23.8	3.2	2.7	0.54	0.44	0.04	0.04
	400	17.3	20.0	5.2	4.9	0.19	0.28	0.04	0.04
	700	8.6	19.9	4.2	4.8	0.21	0.38	0.04	0.06
	1,300	9.9	20.0	2.4	4.0	0.32	0.58	0.05	0.10

DTEAA species in the water column of the Faroe Shetland Channel is in agreement with the few data available for other oceanic regions obtained with different methods (Lee and Bada 1977; McCarthy et al. 1998). Dittmar et al. (2001), however, found slightly higher D-/L-Ala ratios in deep Arctic waters compared to surface waters. Generally, the D-/L ratios of dissolved total Ala, Asp, Glu, and Ser reported by Dittmar et al. (2001) are very similar to the ratios we obtained (Table 1).

Based on the mean D-Ala concentration of  $27.9 \pm 6.3$  nmol L<sup>-1</sup> ( $n = 54$ ) averaged over all the stations and depths and assuming that all the dissolved total D-Ala originates from the peptidoglycan layer of bacterioplankton, we can estimate the contribution of bacterioplankton-derived peptidoglycan to the bulk DON pool. It has been shown that the D-Ala concentration in peptidoglycan is relatively constant, at least for culturable nonmarine bacteria (D-Ala-N  $\times 5.7$ ; Rogers 1983). Based on these directly measurable hydrolyzable D-Ala and the DON concentrations (data not shown), bacterioplankton-derived peptidoglycan contributes about 2–2.5% to the DON pool of the study site. Although the average D-Ala concentration we report here is almost identical to the concentration found by Dittmar et al. (2001) for open Arctic waters, our estimated peptidoglycan contribution to the DON pool is lower than the estimate of Dittmar et al. (2001) because of the higher DON concentration in our study area compared to the open Arctic waters. There is evidence from recent NMR studies that most of the oceanic DON not recoverable on a molecular level consists probably of nonhydrolyzable amino acids (McCarthy et al. 1997). Thus, because only ~10% of the DON pool is hydrolyzable amino acids (Dittmar et al. 2001), it follows that dissolved peptidoglycan contributes ~20–25% to the pool of hydrolyzable dissolved amino acids in the study area. This confirms earlier studies showing that bacterioplankton cell-derived peptidoglycan comprises an important fraction of the oceanic dissolved total amino acid (DTAA) pool (McCarthy et al. 1998; Jørgensen et al. 1999; Dittmar et al. 2001). These figures should be considered a rough estimate only because the above calculation is based on the D-Ala concentration in peptidoglycan obtained from cultured nonmarine, mostly biomedically important, bacteria (Rogers 1983). The contribution of D-Ala on the peptidoglycan of marine bacteria might be more variable than that reported by Rogers (1983), considering their phylogenetic and functional diversity.

**Bacterioplankton uptake of D- versus L-Asp**—In order to estimate the potential of bacterioplankton to utilize bacterial cell wall-derived dissolved D-amino acids, we compared D- and L-Asp uptake of natural bacterial communities collected from different depth layers. The ratio of bacterial D-/L-Asp uptake increased exponentially from 0.03 in the surface layers to about 1 at 900 m depth (Fig. 1). These Asp uptake rates were measured by adding 10 nmol L<sup>-1</sup> Asp to the samples, which is ~2–10 times the free Asp concentration in the water column of the study area (data not shown). Nevertheless, the trend in D-/L-Asp uptake ratios observed with depth should not be affected by the Asp concentration added as shown in the laboratory experiments with Asp concentra-

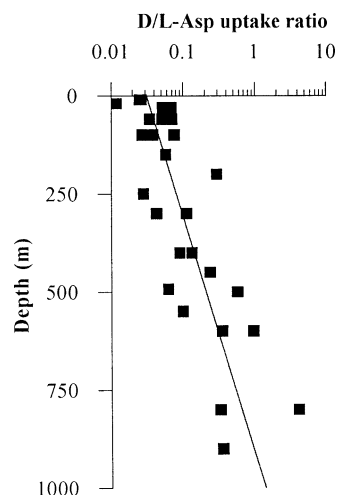


Fig. 1. Depth dependence of the uptake ratio of D-/L-aspartic acid (Asp) by bacterioplankton in the water column of the Faroe Shetland Channel of the North Atlantic. The D-/L-Asp uptake ratio increased exponentially with depth ( $x = 0.031 \times 10^{0.002y}$ ;  $r^2 = 0.63$ ).

tions ranging from 0.1 and 10 nmol L<sup>-1</sup> (see *Material and methods*).

Although we have measured only free D- versus L-Asp uptake, it is likely, albeit untested, that this pattern holds also for other amino acid species. Generally, the uptake patterns of individual L-amino acids by bacteria are similar (Jørgensen 1992). If the other three amino acid species are taken up at similar D-/L-uptake ratios as Asp, then the high uptake ratio of D-/L-Asp in the mesopelagic zone indicates an adaptation of the mesopelagic bacterioplankton community to utilize D-amino acids relatively more efficiently than bacteria in the euphotic zone. In the euphotic zone, phytoplankton activity supplies mainly L-amino acids, which are taken up by bacteria efficiently and, according to our data (Fig. 1), preferentially over D-amino acids. Thus, in the euphotic zone, the supply ratio of D-/L-amino acids might be similarly as low as the D-/L-Asp uptake ratio we measured. In the mesopelagic zone, however, because of the absence of phytoplankton production and the preferential use of L-amino acids in the surface layers, the bioavailable DOC, including the freshly produced DOC, supports a supply ratio of D-/L-amino acids that is considerably higher than in the euphotic layer. In short, we speculate that the observed shift in bacterial uptake ratios of D-/L-amino acids with depth reflects the shift in the production of bioavailable D-/L-amino acids from the surface layers to the mesopelagic zone. Such a close coupling between the supply ratio and the uptake ratio would explain the rather constant D enantiomer concentrations and D/L ratios of the four DTEAA species throughout the water column. Also, one has to keep in mind that the D-/L supply ratio, as well as the D/L uptake ratio, refers to the supply and uptake of free amino acids. We have measured, however, the concentrations of DTEAA, of which the free amino acids comprise only a rather small fraction (usually <5% of DTAA). Bacterioplankton-mediated ectoenzymatic hydrolysis of combined amino acids is generally considered to be the rate-limiting step in the bacterial uptake of amino acids (Hoppe et al. 2002). Once combined amino

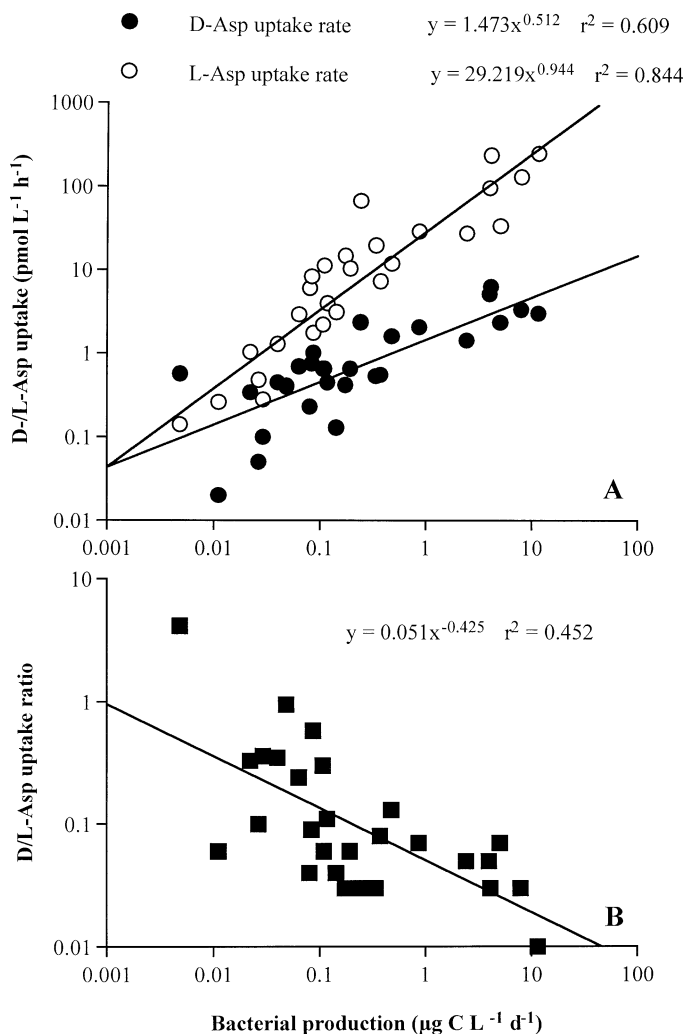


Fig. 2. Relation between bacterial production and (A) the D-/L-Asp uptake rate and (B) the D-/L-Asp uptake ratio obtained from measurements on the bacterioplankton in the water column of the Faroe Shetland Channel in the North Atlantic.

acids are hydrolyzed, the cleaved free amino acids are readily taken up, keeping the pool of free amino acids low and rather uniform.

Because bacterioplankton are thought to be the main source of the four D-amino acids, one might tentatively assume a close relation between bacterial production and D-amino acid concentration. The remarkably constant dissolved total D-Ala and D-Asp concentrations over a wide depth range are in sharp contrast, however, to the decline in

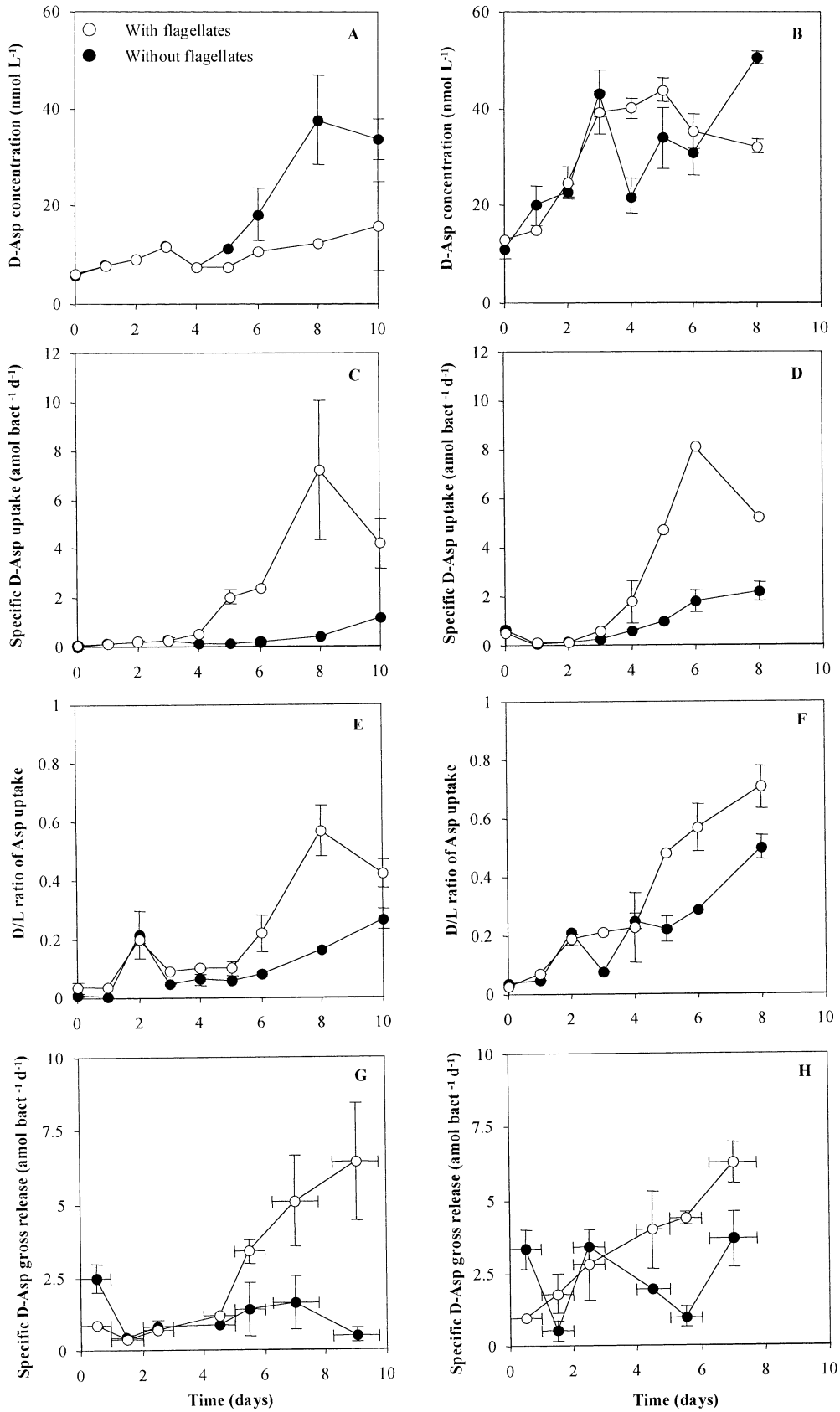
bacterial production from the near surface ( $0.17 \pm 0.15 \mu\text{mol C L}^{-1} \text{d}^{-1}$ ) to the deep mesopelagic layer by three orders of magnitude (Herndl unpubl. data). Bacterial production was positively correlated with D- and L-Asp uptake (Fig. 2A) and negatively correlated with the D-/L-Asp uptake ratio (Fig. 2B). The steeper slope for L-Asp than for D-Asp uptake causes the increasing D-/L-Asp uptake ratios with decreasing bacterial production. The question remains to be solved as to whether these relatively higher uptake rates of D-Asp compared to L-Asp in the deeper layers of the water column are due to specific species of prokaryotes inhabiting the mesopelagic zone.

Major shifts in the bacterial community composition between surface and mesopelagic waters have been reported by Moeseneder et al. (2001) for the Mediterranean Sea. These authors found that ~50% of all the phylotypes of bacteria present in the mesopelagic zone are specific for this layer, as determined by terminal-restriction fragment length polymorphism (Moeseneder et al. 2001). Thus, there is accumulating evidence that the prokaryotic community changes significantly with water column depth, and it is likely that these shifts in the prokaryotic community composition are responsible for the shifts in the uptake ratio of D-/L-Asp from the euphotic layer toward the deep mesopelagic zone.

*Interaction between flagellate bacterivory and bacterioplankton in the dynamics of dissolved D-amino acids*—Bacterioplankton represent by far the largest living surface area of the ocean, in general, and in the mesopelagic zone, in particular (Cho and Azam 1988). It is therefore reasonable to assume that grazing on bacteria might release substantial amounts of bacterial cell wall-derived amino acids to the oceanic DOC pool. This could occur either directly, via the solubilization of the peptidoglycan layer and the subsequent release of amino acids into the ambient water by flagellates, which are the most important consumers of bacterial biomass (Andersson et al. 1985; Nagata and Kirchman 1991), or indirectly, by providing a suitable substrate for further bacterioplankton solubilization of the particulate matter and subsequent uptake (Nagata and Kirchman 1997). In any case, bacterivory by flagellates would result in the production of DOC with a higher D-/L-amino acid ratio compared to phytoplankton-derived DOC.

To determine the potential of the bacterioplankton community to adapt to the efficient utilization of D-amino acids and to investigate the role of bacterivory of flagellates on the production of bacterial cell wall-derived DTEAA, laboratory experiments were performed. Surface-water bacterial communities were inoculated in nutrient-amended artificial seawater in the presence and absence of flagellates. Gener-

Fig. 3. Time course of the concentration of dissolved total D-Asp (A, B), the specific D-Asp uptake rates (C, D), the D-/L-Asp uptake ratios (E, F), and the specific D-Asp gross release rates (G, H) of surface-water bacteria incubated in nutrient-amended artificial seawater with and without flagellates in two experiments (one experiment per column), each performed in duplicate. The specific gross release was calculated as the sum of the specific net release rates and the specific uptake rates (derived from panels C, D). Specific net release rates were calculated as the difference of D-Asp concentration during a specific sampling interval ( $t_2 - t_1$ ) divided by the mean bacterial abundance at  $t_1$  and  $t_2$ . Specific uptake rates were also averaged for time  $t_1$  and  $t_2$ . Horizontal bars indicate period of measurements. Symbols represent the mean  $\pm$  range of duplicate flasks. If vertical bars are not visible, the range is smaller than the size of the symbol.



ally, dissolved total D-Asp concentrations increased during the course of the experiments regardless of whether flagellates were present or not (Fig. 3A,B). In the absence of flagellates, dissolved total D-Asp reached higher concentrations than in the presence of flagellates in one experiment (Fig. 3A) but not in the other (Fig. 3B). Cell-specific D-Asp uptake rates increased only slightly in the flagellate-free controls but increased substantially in the presence of flagellates (Fig. 3C,D). Maximum specific bacterial D-Asp uptake rates were four to six times higher (7.2 and 8.2 amol Asp bacterium<sup>-1</sup> d<sup>-1</sup>) in the presence of flagellates compared to the flagellate-free treatment (1.2 and 2.2 amol Asp bacterium<sup>-1</sup> d<sup>-1</sup>; Fig. 3C,D). Corresponding to that, D-/L-Asp uptake ratios were consistently higher in the presence of flagellates compared to the treatments without flagellates after the added glucose was consumed (after 2 d) (Fig. 3E,F). Moreover, in the presence of flagellates, bacterial D-/L-Asp uptake ratios reached similar values toward the end of the incubation period at day 8 (Fig. 3E,F), as detected in the deep mesopelagic waters of the North Atlantic (D-/L-Asp uptake ratio 0.6–0.7; Fig. 1). Thus, there is evidence that even surface-water bacterioplankton respond with an enhanced D-/L-Asp uptake ratio if other organic substrates are in short supply. Bacterivory of flagellates stimulates D-Asp uptake by the bacterioplankton community. The more pronounced increase in the uptake ratio of D-/L-Asp over time in the presence of flagellates indicates that bacterivory of flagellates enhances the release of bacterial cell wall-derived D-amino acids, as indicated by the substantially higher specific D-Asp gross release from bacteria (Fig. 3G,H).

These laboratory experiments indicate that even surface-water bacterioplankton have the potential to utilize D-amino acids efficiently if other organic substrates are not available. It is likely that substantial shifts in the species composition of the inoculated bacterioplankton community took place in the laboratory experiments over the 8–10-d incubation period. The presence of flagellates might have further magnified these shifts in the bacterioplankton community composition by selective grazing and by keeping the bacterioplankton in a juvenile stage.

From these laboratory experiments, we can further conclude that flagellate grazing stimulated greatly the specific gross release of D-Asp from bacteria (Fig. 3G,H) and the bacterial utilization of D-Asp. However, even in the absence of flagellates, bacterioplankton release D-Asp as indicated in Fig. 3A. This D-Asp release could have been induced by viruses, which constitute, besides flagellates, the main biotic control agents of bacterioplankton, or by autolysis of bacterial cells. Certainly, the role of viruses and bacterial autolysis in the release of cell wall-derived organic matter needs to be further investigated.

We have shown, for the first time ever, that mesopelagic prokaryotic communities are capable of utilizing D-amino acids as efficiently as L-amino acids. Furthermore, as indicated by our laboratory experiments, surface-water bacterioplankton also have the potential to utilize D-amino acids efficiently if other organic substrates are in short supply. These findings have several important implications. The use of D-/L-amino acid ratios as an indicator of the diagenetic state of DOC requires caution because D-amino acids are

more bioavailable than hitherto assumed, especially in the mesopelagic zone. For the microbial ecology of the mesopelagic realm, our results indicate that specific prokaryotic communities are present that utilize D-amino acids as efficiently as L-amino acids. The phylogeny of the prokaryotes responsible for this efficient D-amino acid utilization in the mesopelagic environment is unknown, but even surface-water bacterioplankton have the physiologic capacity to utilize these D-amino acids efficiently if other organic nutrient sources are not sufficiently available. Whether this efficient utilization of D-amino acids represents a common bacterioplankton strategy to utilize them as a supplementary carbon, nitrogen, and energy source when other suitable organic substrates are scarce in the mesopelagic and deep waters remains to be investigated.

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