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Isotope dilution of intracellular amino acids as a tracer of carbon and nitrogen sources of marine planktonic bacteria

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ABSTRACT: Isotope dilution (ID) of intracellular amino acids in bacterial assemblages in the subarctic Pacific was measured in order to examine the relationship between dissolved free amino acids (DFAA) and other sources of amino acids for protein synthesis. When ^{3}H-amino acids were added at trace amounts to <0.8 µm filtrates, ID of single amino acids varied greatly. Isotope dilution of serine, glycine, threonine, arginine, alanine, and tyrosine was 1- to 4-fold, indicating that 25 to 100 % of bacterial biosynthetic requirements was met by uptake of these amino acids. Valine, phenylalanine, isoleucine, and leucine exhibited 8- to 50-fold ID indicating that only a minor fraction of bacterial biosynthetic requirements was met by uptake of these amino acids. These results were consistent with measurements of the fraction of bacterial biomass production supported by uptake of DFAA (8 to 42 %). Intracellular isotope dilution appeared to be inversely related to the extracellular concentration of the respective DFAA. Glutamate ID was always high (13- to 95-fold) and independent of its concentration. This high ID was probably due to substantial ammonium uptake, indicating that in addition to DFAA, ammonium was an important N-source for amino acid de novo synthesis and thus for protein production.

INTRODUCTION

Heterotrophic bacteria are important in the turnover of organic matter in aquatic ecosystems. In pelagic environments they are the predominant group of organisms that assimilate dissolved organic matter (DOM) and transform it into biomass, making it available to higher trophic levels. Some of the most important DOM components for bacterial growth are dissolved free amino acids (DFAA; Kirchman 1990). As a combined C and N source, they are immediate precursors for protein which comprises 60 % of bacterial biomass (Simon & Azam 1989). The importance of DFAA in supporting bacterial growth is usually evaluated by comparing DFAA uptake (DFAA concentrations × turnover of radiolabelled DFAA) with bacterial biomass production. These studies indicate that the fraction of bacterial production supported by uptake of DFAA can vary from <5 to 100 % in marine and freshwater environments depending on the substrate supply (Jørgensen 1987, 1990, Fuhrman 1990).

To examine the biosynthetic processes of bacterial assemblages in more detail, another approach is to study the contribution of DFAA to intracellular pools, through which all precursors for protein synthesis must pass (Anraku 1980). Amino acids can enter the intracellular pool via 4 possible pathways (Payne 1980): (1) direct uptake of DFAA; (2) uptake of oligopeptides (2 to 5 amino acids) and subsequent intracellular hydrolysis; (3) cell-surface-mediated hydrolysis of dissolved combined amino acids (DCAA) with coupled uptake of single amino acids or oligopeptides; (4) de novo synthesis of amino acids from ammonium and organic carbon. Recently, Simon & Azam (1989) introduced a method by which some of these biosynthetic pathways can be studied in natural assemblages of aquatic bacteria. They measured isotope dilution (ID) of ^{3}H-leucine in the bacterial intracellular pool by comparing the specific activity of ^{3}H-leucine in the bulk water with that in the intracellular bacterial pool. By extending this approach to other amino acids, one can differentiate among the various sources of amino acids contributing to the intracellular pool. High ID would indicate a low contribution of DFAA compared with other

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pathways (1). To further differentiate pathways (2), (3), and (4), the IDs of individual amino acids can be compared. If ammonium is the major N source and not DCAA, ID of glutamate should be significantly higher than ID of other amino acids since glutamate is a key metabolite in ammonium uptake together with α-ketoglutarate (Brown 1980), which, after transamination to glutamate, should dilute the specific activity of ^{3}H-glutamate.

I addressed these questions during a study in the subarctic Pacific as part of the NSF-supported SUbarctic Pacific Ecosystem Research (SUPER) program. The overall goal was to examine the role of heterotrophic bacteria in the N budget of these waters, which have high concentrations of inorganic N and P (Anderson et al. 1977, Wheeler & Kokkinakis 1990). The results of this study indicate that ID of single tritiated amino acids in the intracellular pool varied between no dilution and 95-fold ID, depending on the amino acid and extracellular concentration. High ID of glutamate suggested that ammonium uptake and de novo synthesis of amino acids was important for bacterial protein production.

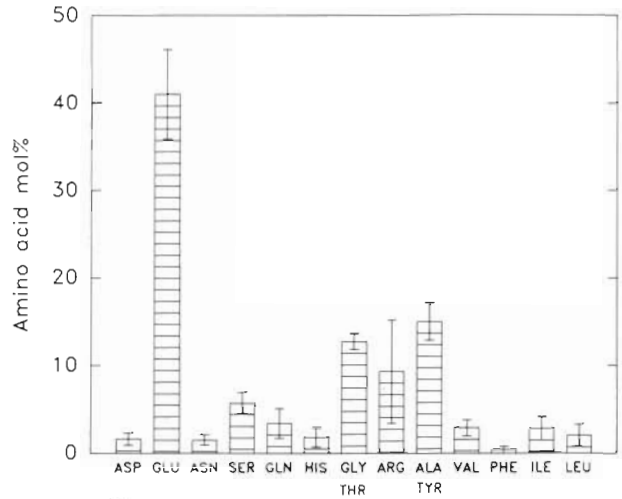

Fig. 1. Mol% of amino acids in the bacterial intracellular pool. Mean of all samples analyzed (N = 7) and standard deviation. Asp: aspartate; Glu: glutamate; Asn: asparagine; Ser: serine; Gln: glutamine; His: histidine; Gly: glycine; Thr: threonine; Arg: arginine; Ala: alanine; Tyr: tyrosine; Val: valine; Phe: phenylalanine; Ile: isoleucine; Leu: leucine. Gly/Thr and Ala/Tyr co-eluted in the HPLC analyses

MATERIALS AND METHODS

Experiments were carried out during May 1988 at Station P (145° W, 50° N) in the Gulf of Alaska. Water was collected in 30 l Teflon-lined GoFlo bottles with Kevlar line. The basic experiments consisted of adding various ^{3}H-DFAAs at tracer concentrations to water samples and measuring uptake rates and isotope dilution of amino acids in the intracellular pool of bacteria (see below). This was compared to bacterial protein, C, and N production rates measured by incorporation of ^{3}H- or ^{14}C-leucine (Leu; see below). Bacterial numbers were counted with the standard acridine orange technique (Hobbie et al. 1977).

Amino acid analysis. High performance liquid chromatographic (HPLC) analysis of DFAA after *o*-phthaldialdehyde pre-column derivatization (Lindroth & Mopper 1979) in water samples and the extracted pool was carried out according to Simon & Azam (1989). A Rainin HPLC system was used. Although most samples were analyzed in the lab after the cruise, some were analyzed on board ship immediately after or within 48 h of the experiment. All analyses gave comparable results irrespective of the time the samples were kept frozen at −20 °C. Replicate analyses usually agreed within 5 %. To estimate SA_{ext} or SA_{int} (see below), 1 ml fractions (flow rate = 1 ml min^{-1}) were collected after separation by HPLC and radioassayed.

Isotope dilution. Isotope dilution was measured as described by Simon & Azam (1989) with the following modifications. Water was filtered through 0.8 µm Nuclepore filters by gravity, removing essentially all chlorophyll *a* and cyanobacteria but only 3 % of heterotrophic bacteria (Kirchman et al. 1989). A mixture of L-^{3}H amino acids (mean specific activity 57 Ci mmol^{-1}, NET-250), L-(3,4 ^{3}H) glutamate (69.7 Ci mmol^{-1}), or L-(4,5 ^{3}H) leucine (60 Ci mmol^{-1}) were added at final concentrations of 0.5 or 10 nM to triplicate 250 ml samples in polycarbonate bottles. All radiolabels were from New England Nuclear. A subsample was withdrawn at the start of the incubation for HPLC analysis of DFAA concentration and extracellular specific activity. After incubation at in situ temperature in the dark, uptake was stopped after 1.5 to 4 h by rapid filtration through a 0.2 µm, 47 mm Nuclepore filter. Filters were dipped in boiling HPLC-grade water for 4 min to extract the intracellular pool. Extraction times between 30 s and 4 min yielded similar amino acid pool concentrations (Simon & Azam unpubl.). Longer extraction resulted in protein hydrolysis which led to enhanced amino acid concentrations and mol% distributions significantly different from that of the intracellular pool (Fig. 1). Five minute extraction already increased the amino acid concentration by a factor of 4 and reduced the glutamate mol% to <20 %. The extracted samples were placed on ice and kept frozen (−20 °C) until HPLC analysis within 4 wk (see above). Isotope dilution (ID) was calculated as ID = SA_{ext}/SA_{int}, where SA_{ext} is specific activity of the amino acid in the bulk water (external pool); SA_{int} is specific activity of the amino acid in the intracellular pool. Specific activity was calculated as the ratio of the radioactivity coeluting with a given amino acid to the amino acid concen-

tration. Since the specific activity is calculated by 2 variables the standard error of the triplicate determination is ca 50 %.

Incorporation of amino acids and estimates of bacterial production. Incorporation rates of a mixture of L-^{3}H amino acids, L-^{3}H glutamate (specifications see above), and L-^{14}C Leu (342 mCi mmol^{-1}, New England Nuclear) into the ice-cold trichloroacetic acid (TCA) precipitate were measured according to Kirchman et al. (1985). The amino acid mixture and glutamate were added at 0.5 nM and Leu was added at 10 nM final concentration. Samples were incubated at surface seawater temperature in the dark (triplicates or duplicates and a killed control) and uptake was stopped after 2 to 3 h by filtration onto 0.45 µm cellulose membrane filters. Total DFAA incorporation rates were calculated from ambient DFAA concentrations and the turnover rates of ^{3}H-amino acids. DFAA incorporation rates were converted to C assuming 60 g C (mol DFAA)$^{-1}$ (50 % of the mean formula weight) and to N by a calculated C:N ratio of 2.6 for the added DFAA mixture.

Leucine incorporation (leu_{inc}) was converted to bacterial protein production (BPP) according to Simon & Azam (1989): BPP (g C l^{-1} h^{-1}) = $leu_{inc} \times 100/7.3 \times FW \times ID \times F$ where leu_{inc} is exogenous leucine incorporated into the ice-cold TCA precipitate (mol l^{-1} h^{-1}), ID is isotope dilution (see above), FW is formula weight of leucine (131.2 g mol^{-1}), F is fraction of leu_{inc} in the hot TCA precipitate (0.9). Protein production was further converted to total bacterial C production assuming a C:protein ratio of 0.86 for marine bacteria (Simon & Azam 1989) and to N assuming a C:N ratio of 4 (Nagata 1986, Lee & Fuhrman 1987).

RESULTS

Amino acid isotope dilution in the intracellular pool

To examine sources of amino acids for biosynthetic requirements, IDs of various amino acids in the bacterial intracellular pool were measured. Samples for these experiments were taken from the surface and 40 m where bacterial production rates were often at a maximum (Fig. 2). Bacterial numbers in these depths ranged between 5.7×10^8 and 1.0×10^9 cells l^{-1} (Fig. 2). In situ concentrations of total DFAA were between 5 and 89 nM (Table 1). During pre-filtration and sample manipulation DFAA concentrations in the <0.8 µm filtrates increased substantially as compared to the in situ concentrations (Table 1). Serine, glycine+threonine, arginine, and alanine+tyrosine comprised >70 % of total DFAA concentrations in situ as well as in the <0.8 µm filtrates.

To determine the amino acid concentrations in the bacterial intracellular pool, the volume of the total bacterial assemblage was estimated from the bacterial abundance assuming a mean cell volume of 0.07 µm^3 (Lee & Fuhrman 1987) and a water content of 64 % (Simon & Azam 1989). Amino acid amounts in the intracellular pool samples were thus divided by the total bacterial pool volume. Pool concentrations ranged from 28 to 135 mM (Table 1). The intracellular pool was dominated by glutamate which comprised 41 mol % of all amino acids (Fig. 1). Other amino acids did not exceed 15 mol% each.

Isotope dilution of amino acids in the intracellular pool was rather variable and depended on the amino acid and the extracellular concentration (Table 2).

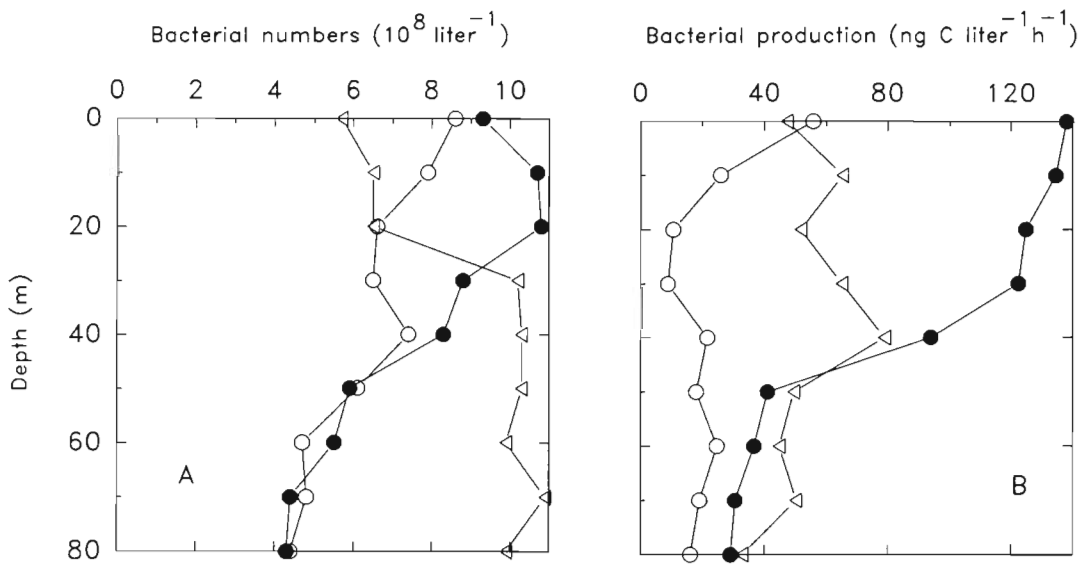

Fig. 2. (A) Bacterial abundance and (B) production on 15 May (○), 17 May (●), and 28 May 1988 (△) at Station P (145° W, 50° N) in the subarctic Pacific

Table 1 Total DFAA concentration in situ, in <0.8 µm filtrates and in the bacterial intracellular pool of the isotope dilution experiments. Leucine and glutamate were added at 0.5 and 10 nM final concentration, and the amino acid mixture (AAmix) at 0.5 nM final concentration of each individual amino acid. nd: not determined

	15 May	17 May	28 May	
	40 m	0 m	0 m	40 m
In situ (nM)	nd	5	89	10
<0.8 µm filtrates				
Leucine (nM)	55	392	202	239
Glutamate (nM)	126	nd	nd	111
AAmix (nM)	nd	nd	206	150
Intracellular pool (mM)	28	40	135	36

Radioactivity in the 1 ml fractions could not be attributed always to a single amino acid, since 2 or 3 amino acids were present in 4 of the 22 1-ml fractions collected (Table 2). The radiolabels of the 6 amino acids dominating the <0.8 µm filtrate (see above) exhibited 1- to 4.4-fold ID. Their concentrations in < 0.8 µm filtrates were between 30 and 65 nM. Other ^{3}H-amino acids added at 0.5 nM were diluted much higher by non-labelled amino acids (Table 2). Their concentrations in the filtrate always were <4.5 nM except for glutamate which had concentrations between 5.5 and 13 nM. Highest ID occurred with glutamate and valine/phenylalanine. Mean ID for all amino acids added as a mixture on 28 May was 9.7 at 0 m and 15.5 at 40 m.

Interestingly, when ^{3}H-glutamate was added at 10 nM it was diluted by non-labelled glutamate in the intracellular pool nearly as much as when added at 0.5 nM. In contrast, ^{3}H-Leu was diluted 12-fold when added at 0.5 nM but only 2.2-fold when added at 10 nM (Table 2). Isotope dilution of Leu added at 10 nM was relatively constant in all experiments (mean = 2.6 ± 0.52).

Comparison of DFAA incorporation and bacterial production

Incorporation of DFAA into the ice-cold TCA precipitate was compared with total bacterial biomass production in order to estimate the contribution of the DFAA pool to bacterial protein synthesis. On 17 May at 0 m, incorporation of DFAA accounted for 8 % of bacterial protein production, 5 % of C, and 7.5 % of N production (Table 3). On 28 May at 0 m, respective percentages were 34 %, 20 %, and 31 %, and at 40 m 42 %, 24 %, and 37 %. This indicates that on these dates and depths the majority of amino acids for bacterial biosynthesic requirements did not come from incorporation of DFAA.

On 28 May at 40 m, the incorporation rate of glutamate was also measured to determine how much of the glutamate requirement for protein synthesis was met by incorporation of dissolved free glutamate. Incorporation of glutamate was 4.8 ng (32 pmol) l^{-1} h^{-1} and accounted for 5 % of total protein production (Table 3). To compare glutamate incorporation with the glutamate requirement, protein production was converted into moles of amino acids by dividing grams protein by 120, which is the average formula weight of amino acids. This results in 770 pmol protein amino acids l^{-1} h^{-1}. Assuming a mol% of 11.5 for glutamate in protein of marine bacteria (Simon & Azam 1989), the glutamate requirement would thus be 89 pmol l^{-1} h^{-1}. Hence, uptake of free glutamate accounted for 36 % of the glutamate requirement for protein synthesis. The requirement for the intracellular glutamate pool is <5 % of that for protein synthesis.

Table 2. Isotope dilution of ^{3}H-amino acids (specific activity in the bulk water:specific activity in the bacterial intracellular pool). Amino acids were added as a mixture (AAmix) at 0.5 nM final concentration each or individually as glutamate or leucine at 0.5 or 10 nM final concentration to 0.8 µm filtrates. Pooled amino acids indicate that their radioactive peaks were not resolved within 1 ml fractions of the HPLC effluent. nd: not determined

Amino acids		15 May	17 May	28 May	
		40 m	0 m	0 m	40 m
AAmix					
Aspartate		nd	nd	8.4	6.0
Glutamate		nd	nd	13.3	32.0
Serine		nd	nd	1.0	1.0
Glycine/threonine/histidine		nd	nd	1.3	4.4
Arginine/alanine/tyrosine		nd	nd	1.6	3.0
Valine/phenylalanine		nd	nd	30.3	53.0
Isoleucine/leucine		nd	nd	11.8	8.0
Glutamate	0.5 nM	95.0	nd	nd	22.5
	10 nM	77.6	nd	nd	nd
Leucine	0.5 nM	11.8	nd	nd	nd
	10 nM	2.2	2.7	3.3	2.2

Table 3. Bacterial protein, carbon, and nitrogen production and incorporation rates of a mixture of amino acids into the ice-cold TCA precipitate added at trace amounts (0.5 nM). C production is 86 % and N production is 21.5 % of protein production. Rates are ng l^{-1} h^{-1}

	17 May	28 May	
	0 m	0 m	40 m
Bacterial production			
Protein	160.4	55.0	91.9
Carbon	138.0	47.3	79.0
Nitrogen	34.5	11.8	19.8
Incorporation of amino acids			
Amino acids	13.4	18.5	38.6
Amino acid C	6.7	9.3	19.3
Amino acid N	2.6	3.6	7.4

DISCUSSION

The purpose of this study was to examine the role of the DFAA pool versus other sources in supplying amino acids for protein synthesis by the bacterial assemblage in the subarctic Pacific. The approach was to study the isotope dilution (ID) of ^{3}H-amino acids in the intracellular bacterial pool. It was found that ID varied greatly depending on the amino acid and its extracellular concentration. Highest IDs occurred with glutamate, valine/phenylalanine and isoleucine/leucine (Table 2), indicating that sources other than uptake of the respective DFAA contributed 87 to 98 % to intracellular pool requirements. Concentrations of valine and phenylalanine in the < 0.8 µm filtrates were always < 2.5 nM, those of isoleucine and leucine < 4.5 and < 2 nM, respectively. In contrast to these amino acids, IDs of glycine/threonine and arginine/alanine/tyrosine were much lower, < 4.4-fold. Serine did not show any ID. Concentrations of serine and glycine/threonine in the < 0.8 µm filtrates were > 30 nM, those of arginine and alanine/tyrosine > 14 nM. Thus, ID appeared to vary inversely with the DFAA concentration. ID seemed to be reduced substantially if the concentration was sufficient to maximize DFAA uptake. This conclusion is supported by the experiment in which Leu was added at trace amounts (1.3 nM final concentration) and 10 nM. When added at trace amounts ID of Leu was 5-fold higher as compared to the 10 nM addition (Table 2).

While preparing the < 0.8 µm filtrates DFAA concentrations increased substantially. This probably decreased ID of amino acids since an inverse relationship between ID and extracellular concentration of amino acids was observed. The elevated DFAA concentrations in the < 0.8 µm filtrates thus would lead to overestimating the importance of the DFAA pool for supplying amino acids to the intracellular pool, yielding an upper limit for DFAA as a C and N source. However, the impact of the enhanced amino acid concentration appears to be small. The mean intracellular ID of 4, excluding glutamic acid and valine/phenylalanine, would predict that DFAA were accounting for about 25 % of bacterial protein production. Based on a direct comparison of DFAA incorporation with bacterial production measurements in unfiltered water, DFAA supplied < 8 % (17 May) to 42 % (28 May) of bacterial biomass production (Table 3), which is in the range the ID measurements would predict. These percentages are also in the range reported by other studies comparing amino acid uptake and bacterial production (Billen & Fontigny 1987, Jørgensen 1987, 1990, Fuhrman 1990).

Isotope dilution of glutamate was always high irrespective of the added glutamate concentration. Glutamate is a key metabolite for ammonium uptake and transamination (Brown 1980). Hence the high ID of glutamate is probably because ammonium uptake by bacteria was high, which is consistent with direct measurements with ^{15}N-ammonium in the subarctic Pacific (Kirchman et al. 1989). The high ID of glutamate also is an indirect indication that other amino acids were synthesized de novo from ammonium and organic carbon. If only glutamate uptake rate, which explains 36 % of the glutamate requirements for protein synthesis, had been measured, the key role of glutamate for ammonium uptake would not have been elucidated.

High ID of amino acids also could have occurred if isotopic equilibrium in the intracellular pool had not been reached (King & Klug 1982). This would have been the case if the incubation time was substantially shorter than the amino acid pool turnover time. To calculate the pool turnover time the same cell size (0.07 μm^3) and water content (64 %) as for calculating the pool concentration were assumed (see 'Results'), giving a pool size of 45×10^{-18} l per bacterium. Since this assumes that all cellular water is in the amino acid pool, it results in the largest pool size possible and thus the longest turnover time. Taking 40 mM as the amino acid pool concentration (Table 1) yields a pool size of 2×10^{-18} mol total amino acids. The pool turnover time is then the pool size divided by the amount of amino acids taken up for protein synthesis assuming steady state conditions. Converting the protein production rates from g protein into mol amino acids as above (see last paragraph of 'Results') and per bacterium this corresponds to 6×10^{-19} moles of total amino acids produced per cell on 28 May. This yields a pool turnover time of 3 h, which is close to the incubation times. Turnover times of individual amino acids were somewhat longer or shorter according to their mol% distribution in the intracellular amino acid pool and bacterial protein.

Isotope dilution factors, therefore, might have been affected by the amino acid pool turnover times. The impact, however, was presumably small because ID factors of 1, which were determined for several amino acids, can only occur if isotopic equibrium has been reached. The amino acid pool turnover time in Simon & Azam (1989; Table 4) calculated with the same assumptions as above is 1 h. Simon & Azam showed that isotopic equilibrium of ^{3}H-leucine in the intracellular pool of marine bacteria was reached after 30 min, i.e. is half of the pool turnover time. This is a further indication that the ID factors were affected only slightly by pool turnover times close to incubation times. Isotope dilution factors close to 1 also indicate that a large fraction of the bacterial assemblage was actively incorporating amino acids (Simon 1990).

The 2.6-fold ID of Leu added at 10 nM concentrations (Table 2) has implications for estimating protein and biomass production by bacteria with the leucine method (Kirchman et al. 1985, Simon & Azam 1989). The reliability of this method is based on a low ID of Leu (see 'Material and Methods'). Simon & Azam (1989) found a 2-fold ID of Leu in the Southern California Bight of the Pacific. The 2.6-fold ID I found in the subarctic Pacific is 1.3-fold higher. Considering the standard deviation and experimental errors involved in this method, this ID is a valuable confirmation of the ID found by Simon & Azam in a rather different environment. This suggests that 2- to 3-fold ID might be valid in marine oligo- to mesotrophic waters if Leu incorporation is maximized and if the incubation time is $\geq$ the turnover time of the intracellular amino acid pool (see above). However, for measuring bacterial protein production in unknown aquatic ecosystems I still suggest determining ID for Leu or using the most conservative assumption (ID=1) and ensuring that leucine incorporation is maximized.

Intracellular amino acid concentrations were in the mM range and were comparable to cultured bacteria (Tempest et al. 1970) and to other marine bacterial assemblages (Simon & Azam 1989). They were also comparable to marine algae (Admiraal et al. 1986, Haberstroh & Ahmed 1986). I did not determine the bacterial cell size for estimating the cell volume and water content but assumed a mean cell volume often found in marine bacterial assemblages (Lee & Fuhrman 1987) and calculated the water content according to Simon & Azam (1989). Glutamic acid dominated the intracellular pool by a proportion (41 mol%) also found in other marine bacterial assemblages (Simon & Azam 1989) and dilution cultures of bacterial assemblages growing on ammonium as the major N source (Simon unpubl.). In cultured bacteria grown under glucose or ammonium limitation, the intracellular amino acid pool is even more dominated by glutamate, between 53 and 89 mol% (Tempest et al. 1970). When a culture of *Aerobacter aerogenes* was grown under phosphorus limitation the glutamate mol%, however, was reduced to 19 % and alanine dominated the intracellular amino acid pool, making up 50 % (Tempest et al. 1970). This also holds true for natural aquatic bacterial assemblages. When amino acids are their major nitrogen sources the mol% of glutamate in the intracellular amino acid pool is reduced to < 20 % (Simon unpubl.).

The estimates of the contribution of DFAA to bacterial biosynthetic requirements explaining < 37 % of the C and N production suggest that other C and N sources are utilized as well. Potentially important other sources of amino acids are DCAA and de novo synthesis from other C and N sources such as nucleic acids, alkylamines, mono- and polysaccharides, and ammonium. Although it has been shown that nucleic acids are utilized by aquatic bacterial assemblages (Paul et al. 1987) there is no information about their relevance in the subarctic Pacific. Addition of alkylamines has little effect on the growth of marine bacterial assemblages (Höfle 1984, Kirchman 1990). Turnover of proteins (Hollibaugh & Azam 1983) and DCAA (Coffin 1989) by bacterial assemblages has been found to be comparable to turnover of DFAA. Also, bacteria have a preferential uptake for oligopeptides as compared to DFAA (Kirchman & Hodson 1986). Thus, DCAA such as oligopeptides may be another source of amino acids for bacterial growth. Addition of proteins, however, did not stimulate bacterial growth in the subarctic Pacific whereas addition of DFAA did (Kirchman 1990). This observation and the greatly varying ID of the various amino acids suggest that in this study DCAA were not a large source of amino acids for the bacterial intracellular pool. The question of sources for DFAA remains open, however The high glutamate concentration in the intracellular pool and the high ID of glutamate independent of the added concentration suggests that amino acid de novo synthesis from ammonium and organic carbon presumably was the most important source of amino acids in the bacterial intracellular pool. These data are consistent with ^{15}N-ammonium uptake rates measured by Kirchman et al. (1989) at the same site. They measured uptake rates of about 30 ng ammonium-N l^{-1} h^{-1} by the < 0.8 µm size fraction, comparable to the bacterial N production rates estimated from protein synthesis (Table 3).

In conclusion, this study has shown that measuring ID of amino acids in the bacterial intracellular pool is a valuable approach to elucidating the contribution of DFAA and other sources of amino acids to biosynthetic requirements of aquatic bacterial assemblages. Since amino acids are major C and N sources (Jørgensen 1987, 1990, Kirchman 1990), it is important to obtain detailed insight into pathways of amino acids entering

the bacterial cell and associated biosynthetic processes. The amino acid mol% composition of the intracellular pool together with ID measurements of amino acids also allows examination of the importance of ammonium uptake for bacterial N supply. This is of special interest in environments with high ammonium concentrations such as the subarctic Pacific (Anderson et al. 1977) but might also be a useful approach in other ecosystems.

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