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Inhibition by Peptides of Amino Acid Uptake by Bacterial Populations in Natural Waters: Implications for the Regulation of Amino Acid Transport and Incorporation†

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To investigate the regulatory interactions of amino acid transport and incorporation, we determined the effects of dipeptides on amino acid uptake by bacteria in an estuary and a freshwater lake. Dipeptides noncompetitively inhibited net transport and incorporation of amino acids into macromolecules but had no effect on the ratio of respiration to incorporation. Nearly maximum inhibition occurred at peptide concentrations of <10 nM. In contrast, the initial uptake rate of glycyl-[^{14}C]phenylalanine was not affected by glycine or phenylalanine. Net amino acid transport appeared to be inhibited by the increased flux into the intracellular pools, whereas the incorporation of labeled monomers into macromolecules was isotopically diluted by the unlabeled amino acids resulting from intracellular hydrolysis of the dipeptide. Chloramphenicol, sodium azide, and dinitrophenol all inhibited the initial uptake rate of leucine and phenylalanine. These results suggest that in aquatic environments amino acids are taken up by active transport which is coupled closely to protein synthesis.

Bacteria may process as much as 50% of the carbon fixed by phytoplankton in freshwater and marine ecosystems (reviewed by Azam et al. [2]). The calculated fraction of primary production consumed by bacteria is based on estimates of bacterial production (2) and oxygen utilization by various size fractions of the microbial assemblage in seawater (15). It has been implicitly assumed that the bacterial utilization of dissolved organic compounds (DOC) is coupled with bacterial growth and production. Since many of the growth rate estimates are based on rates of thymidine incorporation, it also has been assumed that the uptake and subsequent mineralization of DOC are coupled with DNA synthesis (3). Although this coupling must exist over some time scale, it is unclear in aquatic ecosystems how tightly the uptake of organic compounds is coupled with the synthesis of DNA and other macromolecules by the bacterial populations. If the coupling is not tight, then estimates of macromolecular synthesis would not adequately reflect the uptake and turnover of DOC. Measuring bacterial production, which reflects the sum of the rates of uptake of a large number of compounds, appears to be the best approach for estimating the turnover of the DOC pool. The direct approach of measuring the concentration and turnover of all compounds possibly used by bacteria is not feasible, even if all the compounds could be identified and their concentrations measured.

Microorganisms are in a "balanced growth" stage when the specific rates (absolute rates divided by the intracellular concentration) of the various metabolic processes are equal to the specific growth rate (11). Growth of bacteria in pure culture is balanced during most of the exponential growth phase (11). Growth of bacteria in natural aquatic ecosystems may not be balanced, since DOC concentrations and other parameters affecting growth can vary greatly. In unbalanced growth, natural bacterial populations may take up DOC but neither synthesize new macromolecules, such as protein and DNA, nor divide. Information about the growth stage—balanced versus unbalanced growth—will affect how we interpret the various measures of bacterial production in aquatic environments. More importantly, the relationship between DOC uptake and macromolecular synthesis is likely to determine the response of the bacterial assemblage to fluctuations in DOC concentrations and, ultimately, the contribution of bacteria to biogeochemical cycles and trophic dynamics.

One direct mechanism by which DOC uptake becomes uncoupled from macromolecular synthesis is the buildup of intracellular pools of low-molecular-weight precursors. If bacteria in natural aquatic environments are in balanced growth and if DOC uptake is in fact coupled to macromolecular synthesis, then we expect that DOC uptake would be affected by the flux into and out of the intracellular pools. To examine the relationships among DOC uptake, intracellular pools, and macromolecular synthesis, we chose to focus on amino acid uptake and protein synthesis. We hypothesized that if amino acid uptake and protein synthesis are tightly coupled, then the net transport of an amino acid should be (i) inhibited by an increased flux into the intracellular pool of that free amino acid and (ii) dependent on protein synthesis which removes amino acids from the intracellular pool.

We tested these hypotheses by examining the effect of peptides on amino acid uptake by the natural bacterial assemblages in an estuary (Fresh Creek, Andros Island, Bahamas) and a freshwater lake (Lake Oglethorpe, Georgia). The addition of peptides to a water sample increases the flux into the intracellular amino acid pools in such a way that we can examine the effect of the pools on net amino acid transport independently of the extracellular free amino acid concentrations. In species of bacteria examined to date, dipeptides and oligopeptides are transported by uptake systems distinct from those mediating amino acid transport (reviewed by Payne [9]). Once across the cytoplasmic membrane, the peptides are quickly cleaved by intracellular peptidases, thus contributing to the intracellular amino acid pool (9). Using this approach, we found that peptides added at nanomolar concentrations inhibited the uptake of extra-

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cellular amino acids. Our results suggest that the net transport of amino acids is tightly coupled with protein synthesis.

MATERIALS AND METHODS

Description of sample sites. The experiments discussed here were conducted with water samples from a freshwater lake in Georgia, Lake Oglethorpe, and an estuary at Andros Island, Bahamas. A detailed description of the microbiological and limnological features of Lake Oglethorpe is in preparation. Briefly, this eutrophic lake, located ca. 16 km south of Athens, has an annual primary production of 135 g of C m^{-2}. Dissolved organic carbon is fairly constant at 3.5 mg of C $liter^{-1}$, and bacterial abundance was about 1.5×10^6 cells ml^{-1} when the experiments described here were conducted. We know little about the Bahamian estuary except that chlorophyll concentrations were ca. 2 μg $liter^{-1}$, and salinity was 25% during this study. Subsurface samples were collected with polycarbonate bottles. The experiments were started less than 1 h after the water samples were collected and were done on the ship at Andros Island or in the laboratory for the samples from Lake Oglethorpe. All experiments with water from Lake Oglethorpe were conducted from November 1982 to January 1983 at the in situ temperature of ca. 12°C and in the dark. The experiments at Andros Island were conducted at ca. 30°C in October.

Uptake of labeled amino acids in the presence of peptides. Various concentrations of the dipeptide phenylphenylalanine (Phe-Phe) or leucylleucine (Leu-Leu) were added to samples along with either [^{3}H]leucine (47 Ci/mmol) or [^{3}H]phenylalanine (60 Ci/mmol). The final concentration of the added labeled compounds was 0.5 nM for leucine and 1.3 nM for phenylalanine. The peptides were added just before the addition of the labeled amino acids. Each peptide concentration was tested with duplicates in several experiments. At various times 10-ml aliquots were filtered through 0.45-μm membrane filters (Gelman Instrument Co.) and rinsed twice with filtered water from which the sample came. In those experiments with a single time point, the incubation period was 1 h, and abiotic adsorption of the label was corrected with Formalin-killed controls. The validity of using a single time point was tested in several experiments which demonstrated that uptake was linear at least during the first hour of the experiment (see below). The filters were radioassayed, and the quench correction was by the channels ratio method.

The production of $^{14}CO_2$ from the respiration of [^{14}C]phenylalanine (510 mCi/mmol) and [^{14}C]leucine (329 mCi/mmol) was measured by acidifying the water sample with H_2SO_4 after incubation and trapping the evolved $^{14}CO_2$ in an ethanolamine trap (4). The final concentration of the ^{14}C-labeled amino acids was 10 nM. The radioactivity associated with acid-insoluble material (macromolecules) was collected on membrane filters, rinsed twice, and radioassayed as described above.

We examined the relationship between amino acid uptake and macromolecular synthesis by measuring the incorporation of labeled amino acids into the trichloroacetic acid (TCA)-insoluble material. Peptides and labeled amino acids were added to water samples as described above. At various times 10-ml aliquots were removed and 10 mM unlabeled amino acids were added to stop the uptake of the labeled compounds. The aliquots then were cooled in an ice bath. Cold TCA was added such that the final concentration was 5%. After extraction for ca. 15 min, the TCA-insoluble material was collected on membrane filters and rinsed twice with cold 5% TCA before being radioassayed as described above.

Uptake of Gly-Phe. We tested whether the uptake of the dipeptide glycylphenylalanine (Gly-Phe) was inhibited by either glycine or phenylalanine. Gly-Phe was used because it was the only radiolabeled dipeptide that was available commercially. The dipeptide was uniformly labeled in the phenylalanine moiety at 2 mCi/mmol of dipeptide, but the glycine moiety was not labeled. Uptake of Gly-Phe was examined in two types of experiments. In one experiment with short incubation times, inhibition by glycine and phenylalanine was tested by adding these compounds at a final concentration of 1,000 nM. Phenylalanine was tested also at 50 and 100 nM. The dipeptide Gly-[^{14}C]Phe was added at a final concentration of 40 nM, and 10-ml aliquots were filtered every 15 or 30 min. The second experiment was conducted at lower concentrations, but the incubation period was increased to 10 h, and the volume filtered was 20 ml. The concentration of the added Gly-[^{14}C]Phe was 20 nM. Various concentrations of glycine plus phenylalanine added together were tested as well as unlabeled Gly-Phe; this was a typical Wright-Hobbie experiment (16). Abiotic adsorption for this endpoint determination was corrected for by using Formalin-killed controls.

Inhibitors of bacterial metabolic activity. Three inhibitors of metabolic activity were tested for their effect on amino acid uptake. These inhibitors were added 30 min before the addition of the labeled amino acids. The controls received the same solution as the inhibitor but without the inhibitor. Chloramphenicol, which inhibits the 50S subunit of procaryotic ribosomes, was tested at several concentrations. Dinitrophenol and sodium azide interfere with active transport. These compounds were added at 1 mM.

Sources of amino acids, peptides, and metabolic inhibitors. All unlabeled compounds were purchased from Sigma Chemical Co. The radioactive amino acids were supplied by New England Nuclear Corp., whereas Gly-[^{14}C]Phe was from Amersham Corp. All peptides were checked for contamination by free amino acids with reverse-phase liquid chromatography (6). Contamination was <0.1%. All of the amino acids and peptides used in these experiments were of the L form, and the monomers in the peptides were linked by α bonds.

RESULTS

Peptides inhibit amino acid uptake. We tested whether the dipeptides Phe-Phe and Leu-Leu inhibited the uptake of labeled free amino acids. The dipeptide Leu-Leu inhibited [^{3}H]leucine uptake, but Phe-Phe had no effect on [^{3}H]leucine uptake (the added concentration of [^{3}H]leucine was 0.5 nM) (Fig. 1A). The addition of 5 nM Leu-Leu inhibited [^{3}H]leucine uptake by ca. 50%. Similarly, Phe-Phe at 5 nM inhibited the uptake of [^{3}H]phenylalanine (the added concentration of [^{3}H]phenylalanine was 1.3 nM) by 50% (Fig. 1B). Leu-Leu did inhibit [^{3}H]phenylalanine uptake, but much higher concentrations of Leu-Leu were necessary to obtain the same inhibition as observed with Phe-Phe; [^{3}H]phenylalanine uptake was reduced by 7 and 49% when 5 and 100 nM, respectively, Leu-Leu were added (Fig. 1B). Nearly maximum inhibition of amino acid uptake was found at 5 nM, the lowest concentration tested. Inhibition by the peptides was immediate, as the uptake of the labeled amino acids was lower in the presence of the dipeptides during the shortest incubation periods we tested (<30 min; see Fig. 2 and 5, based on data from Andros Island and Lake Oglethorpe, respectively). Amino acid uptake was linear during the first 4

h of all experiments (see Fig. 5 and 6 for examples) except for one sample from Andros Island (see Fig. 2).

These results suggest that the dipeptides specifically inhibited the uptake of their respective monomers and that dipeptides did not effectively inhibit the uptake of amino acids not present in the dipeptide. The inhibition of [^{3}H]phenylalanine uptake by high concentrations of Leu-Leu appears to be due to a mechanism different from that of Phe-Phe inhibition, as argued more thoroughly below. The results presented in Fig. 1 are from experiments conducted with water from Lake Oglethorpe in January, but similar results were obtained consistently in all experiments in which this effect was tested. However, the quantitative degree of inhibition varied significantly from experiment to experiment. We do not have enough information to evaluate this variation.

We tested whether tripeptides were more effective than dipeptides in inhibiting amino acid uptake. The tripeptide leucylleucylleucine (Leu-Leu-Leu) was slightly more effective than the dipeptide Leu-Leu in inhibiting leucine uptake (Fig. 2). This experiment was conducted with a water sample from Andros Island.

To determine the type of inhibition by the peptides of amino acid uptake, [^{3}H]leucine uptake was measured in the presence of different concentrations of unlabeled leucine and Leu-Leu (16). We examined the effect of Leu-Leu on the kinetic parameters of leucine uptake: $(K + S)$ is the sum of the half-saturation constant (K) and the natural leucine concentration (S), V_{max} is the maximum velocity of uptake, and T_t is the turnover time of leucine. The apparent $(K + S)$ for leucine uptake estimated in this experiment with samples from Lake Oglethorpe was ca. 20 nM and did not change significantly ($P > 0.05$) as the added Leu-Leu concentration was increased from 0 to 20 nM (Fig. 3). However, V_{max} decreased significantly from 0.62 to 0.42 nmol liter^{-1} h^{-1} with the addition of 20 nM Leu-Leu ($p < 0.05$; t-test). As a result predominantly of the decrease in V_{max}, T_t of leucine increased significantly from 28 to 40 h with the addition of 10 nM Leu-Leu ($P < 0.05$; t-test).

These results indicate that the inhibition by the peptides was noncompetitive. If the inhibition were competitive, $(K + S)$ would have increased with increasing Leu-Leu concentrations, but V_{max} would not have been affected (14).

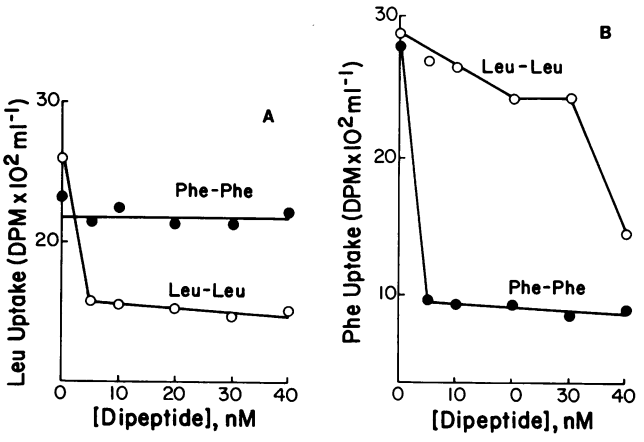

FIG. 1. (A) Uptake of [^{3}H]leucine in the presence of the dipeptides Phe-Phe and Leu-Leu; (B) uptake of [^{3}H]phenylalanine in the presence of the same dipeptides. These experiments were conducted with water samples from Lake Oglethorpe in January. The incubation time was 1 h, and the added concentrations of [^{3}H]leucine and [^{3}H]phenylalanine were 0.5 and 1.3 nM, respectively.

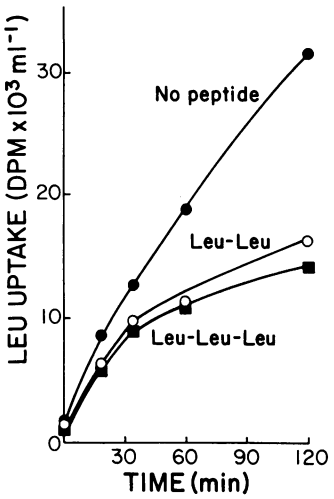

FIG. 2. Uptake of [^{3}H]leucine in the presence of the dipeptide Leu-Leu and the tripeptide Leu-Leu-Leu. This experiment was conducted with water samples from Andros Island. The added concentration of [^{3}H]leucine was 0.5 nM.

Transport of amino acids and dipeptides by different permeases. The distinction between competitive and noncompetitive inhibition is important. Noncompetitive inhibition suggests that dipeptides and amino acids are not transported by the same uptake system in natural bacterial populations and that the inhibition of amino acid uptake by peptides is the result of intracellular regulation of net amino acid transport. Competitive inhibition, on the other hand, would have suggested that these compounds were transported by the same permease. To test this more directly, we measured the uptake of Gly-[^{14}C]Phe in the presence of glycine, phenylalanine, and Gly-Phe added to samples from Lake Oglethorpe in January. The labeled dipeptide was added at 40 nM. Glycine added at 1 μM had only a small effect on Gly-[^{14}C]Phe uptake. Phenylalanine added at 1 μM did not significantly inhibit the initial (<30-min) uptake rate of Gly-[^{14}C]Phe but did block the subsequent uptake of the labeled dipeptide (Fig. 4A and B). The inhibition by high phenylalanine concentrations after the initial uptake of Gly-[^{14}C]Phe suggests that Gly-Phe was hydrolyzed intracellularly to glycine and phenylalanine, as shown in experiments with pure bacterial cultures (9). Low concentrations of phenylalanine (50 and 100 nM) had relatively little effect on peptide uptake (Fig. 4B). When unlabeled Gly-Phe was added at 1 μM, the uptake of Gly-[^{14}C]Phe (added at 40 nM) was negligible (Fig. 4A). Low concentrations (<100 nM) of glycine and phenylalanine added together did inhibit Gly-[^{14}C]Phe uptake during long incubations (Fig. 4C), but this effect was relatively small compared with the effect on labeled amino acid uptake of unlabeled dipeptides added at the same concentrations (Fig. 4C).

There was no lag in the uptake of Gly-[^{14}C]Phe (Fig. 4A and B). In experiments similar to those with labeled amino acids (see below), nearly 100% of the label from Gly-[^{14}C]Phe uptake was found in the cold TCA-insoluble material, and 50% of the total uptake was found in the hot acid-insoluble material after 30 min of incubation.

The uptake of Gly-[^{14}C]Phe in the presence of different concentrations of unlabeled Gly-Phe (Fig. 4C) was analyzed

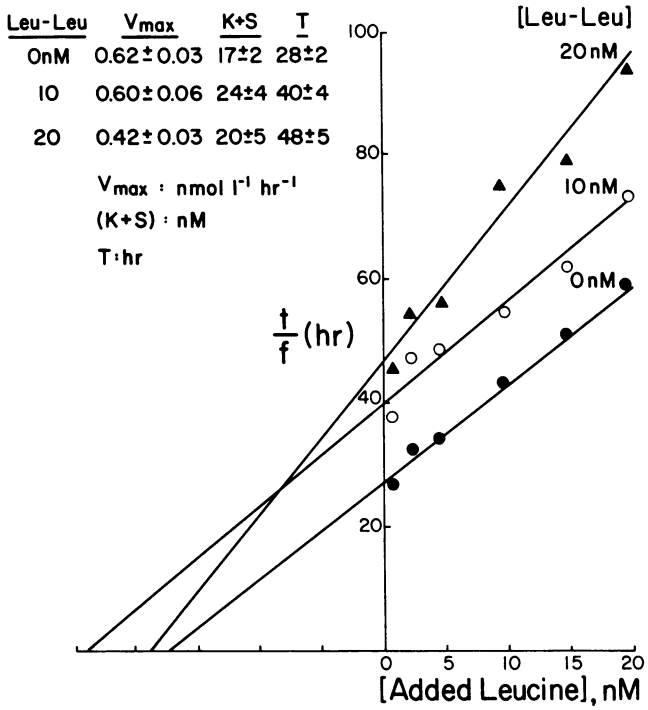

FIG. 3. Uptake of [^{3}H]leucine in the presence of different concentrations of unlabeled leucine and Leu-Leu. The parameters estimated from this analysis are V_{max}, (K + S), and the turnover time (T), and their respective standard errors. The label of the vertical axis, t/f, is the incubation time (t) divided by the fraction of added radioactivity taken up (f). This experiment was conducted with water samples from Lake Oglethorpe.

by the Wright-Hobbie approach (16). We calculated that V_{max} was 0.33 ± 0.023 nmol of Gly-Phe liter^{-1} h^{-1}. The data in Fig. 4C cannot be used to calculate (K + S) precisely because the estimated value of 6.2 ± 31 nM is not significantly different from zero. The uptake of Gly-Phe was apparently saturated by the addition of Gly-[^{14}C]Phe alone, the lowest addition possible (20 nM). The incorporated radioactivity from Gly-[^{14}C]Phe decreased in direct proportion to the addition of unlabeled Gly-Phe.

Coupling of amino acid uptake and macromolecular synthesis. Since peptides inhibited the total uptake of labeled amino acids, net transport into the cells must have been inhibited. However, it was unclear whether other cellular processes were affected by the presence of the polymers. We examined the effect of unlabeled dipeptides on the proportion of the labeled amino acid that was used for either energy production (production of $^{14}CO_2$) or macromolecular synthesis (incorporation into acid-insoluble material).

The absolute amount of $^{14}CO_2$ respired from [^{14}C]leucine decreased with increasing concentrations of Leu-Leu (Table 1; these data are from Andros Island). $^{14}CO_2$ production from the uptake of [^{14}C]leucine added at 10 nM decreased with the addition of 10 to 50 nM Leu-Leu (Table 1) as shown in previous experiments (Fig. 1). Unlike the previous experiments with low concentrations of peptides, the uptake of [^{14}C]leucine into the acid-insoluble material was higher in the presence of high concentrations of Leu-Leu (50 and 100 nM) compared with low concentrations of Leu-Leu (10 to 30 nM); similar results were found in other experiments with high peptide concentrations. In any case, the proportion of total uptake evolved as $^{14}CO_2$ (percent respiration) did not change significantly with the addition of Leu-Leu, except in the presence of the dipeptide at 100 nM. At this concentration, respiration was 17% compared with 9.5% at the other concentrations tested. Similar results were obtained with samples from Lake Oglethorpe and with the uptake of [^{14}C]phenylalanine in the presence of Phe-Phe, although the increase in percent respiration was not observed at high Phe-Phe concentrations (data not shown). Respiration of [^{14}C]phenylalanine was ca. 10%.

These results suggest that unlabeled peptides did not isotopically dilute the utilization of labeled amino acids for energy production. It appears that most of the leucine and phenylalanine taken up by the bacteria in short incubations was used for macromolecular synthesis (presumably protein synthesis), since percent respiration was very low.

To examine the effect of the peptides on macromolecular synthesis, we tested whether the unlabeled peptides affected the incorporation of labeled amino acids into macromolecules, i.e., the TCA-insoluble material. Most of the [^{3}H]phenylalanine (and [^{14}C]phenylalanine) taken up by the bacteria appeared in the macromolecular fraction (80 to 98%; Fig. 5 and Table 2). Furthermore, the appearance of radioactivity in macromolecules was very fast, and we observed no lag between transport and incorporation into macromolecules, at least during the shortest incubation period tested (10 min). The proportion of labeled amino acids taken up and appearing in the TCA-insoluble fraction did not change significantly with time (Table 2). It appears that the TCA-insoluble radioactivity was associated with protein. Nearly all of this radioactivity was also insoluble in hot TCA. Approximately 90% of the TCA-insoluble radioactivity was solubilized when treated with the protease pepsin.

The addition of 10 or 25 nM Phe-Phe significantly lowered the proportion of radioactivity appearing in the TCA-insoluble fraction (Fig. 5 and Table 2). The actual proportion of total uptake that appeared in the macromolecular fraction and the effect of dipeptides on this proportion varied significantly from experiment to experiment. For example, in one experiment the addition of the dipeptide lowered the TCA-insoluble radioactivity from 100 to 90% (Fig. 5), whereas in another experiment the percentage dropped from 80 to as low as 51% (Table 2). Although it is not clear why these percentages vary, the experiments do suggest that unlabeled Phe-Phe can isotopically dilute the incorporation of labeled phenylalanine into macromolecules.

Amino acid uptake stopped by metabolic inhibitors. To examine more closely the relationship between protein synthesis and the inhibition of amino acid uptake by peptides, we tested the effect of blocking protein synthesis on amino acid uptake. Chloramphenicol, an antibiotic that blocks procaryotic protein synthesis, inhibited the initial uptake rate of both [^{3}H]leucine and [^{3}H]phenylalanine (Fig. 6 and Table 3). The uptake of [^{3}H]leucine was inhibited by 80% at 20 μg of the antibiotic ml^{-1}, whereas at 1 μg ml^{-1} inhibition was 39% (Table 3). Furthermore, Phe-Phe added at 25 nM had no effect on the uptake of [^{3}H]phenylalanine that continued in the presence of chloramphenicol (Fig. 6). These results suggest that amino acid uptake was coupled to protein synthesis and that the distribution of radioactivity involved in other metabolic processes (chloramphenicol-insensitive uptake) was not affected, as suggested by the experiments on the respiration of labeled amino acids in the presence of the dipeptides.

As further evidence that chloramphenicol inhibits protein synthesis of natural bacterial populations, we found that

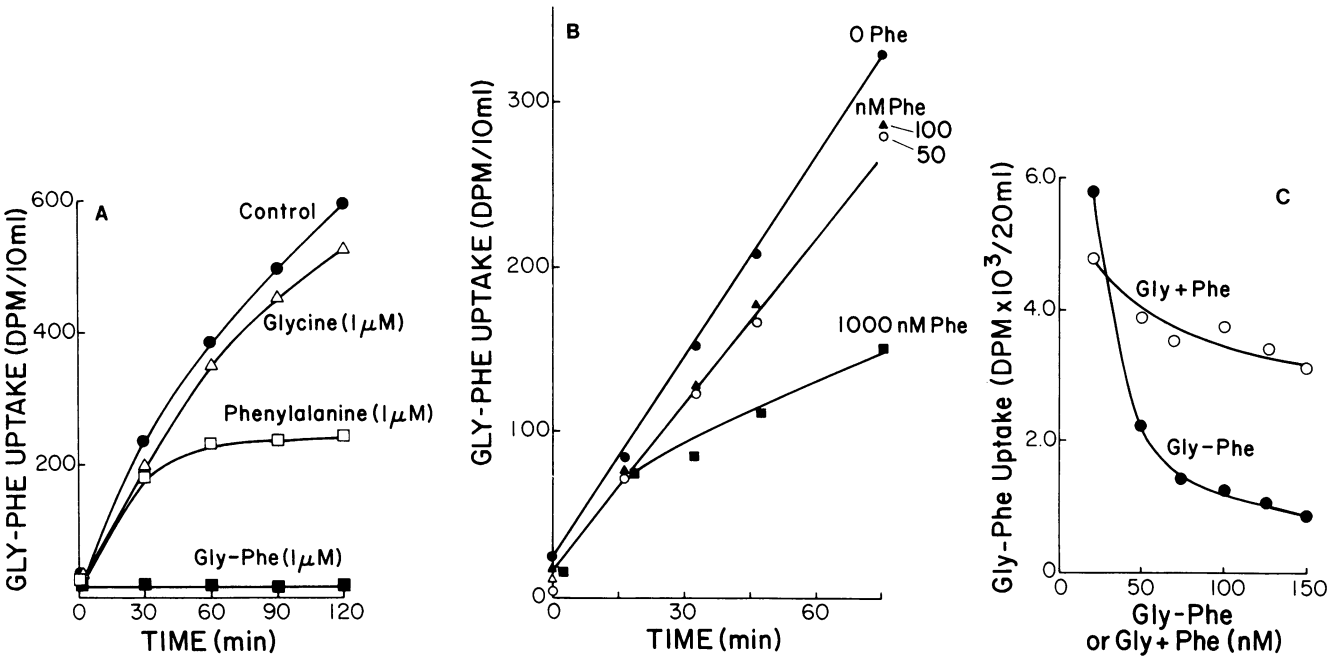

FIG. 4. (A) Uptake of the dipeptide Gly-[^{14}C]Phe in the presence of glycine, phenylalanine, and unlabeled Gly-Phe; (B) uptake of Gly-[^{14}C]Phe in the presence of different concentrations of phenylalanine; (C) uptake of Gly-[^{14}C]Phe (20 nM) in the presence of different concentrations of glycine and phenylalanine added together or unlabeled Gly-Phe. This experiment was conducted with water samples from Lake Oglethorpe.

$^{35}SO_4^{-2}$ incorporation into the hot TCA-insoluble fraction and dark $^{14}CO_2$ uptake were significantly lower in the presence of chloramphenicol in samples from Lake Oglethorpe. In contrast, light-dependent $^{14}CO_2$ fixation was unaffected by chloramphenicol (data not shown).

The inhibition of the initial rate of amino acid uptake by chloramphenicol was unexpected since, if amino acids were taken up by active transport, chloramphenicol should have had no effect on the initial uptake rate. To test directly whether phenylalanine is taken up by active transport, we measured the uptake of [^{3}H]phenylalanine in the presence of 1 mM dinitrophenol and 1 mM sodium azide. Both compounds inhibited the initial uptake rate by bacteria in samples from Lake Oglethorpe (Table 3). Sodium azide inhibited uptake by 65%, whereas inhibition by dinitrophenol was 84%. These results are consistent with the models based on pure culture experiments which have demonstrated conclusively that amino acids are taken up by active transport (reviewed by Anraku [1]).

Possible uptake of amino acids and peptides by phytoplankton. Until now we have assumed that bacteria were entirely responsible for the uptake of amino acids and peptides as measured in samples from Fresh Creek, Andros Island, and from Lake Oglethorpe. That chloramphenicol, an antibiotic

TABLE 1. Incorporation and respiration of [^{14}C]leucine in the presence of the dipeptide Leu-Leu in water samples from Andros Island, Bahamas

Leu-Leu concn (nM)	dpm/10 ml		% Respiration[a]
	Incorporation	CO_2	
0	4,948	549	10
10	3,539	398	10
20	3,347	381	9.8
30	2,713	303	9.9
50	3,412	282	7.6
100	4,128	831	17

[a] Percent respiration = CO_2/(incorporation + CO_2) × 100. The concentration of the added [^{14}C]leucine was 10 nM.

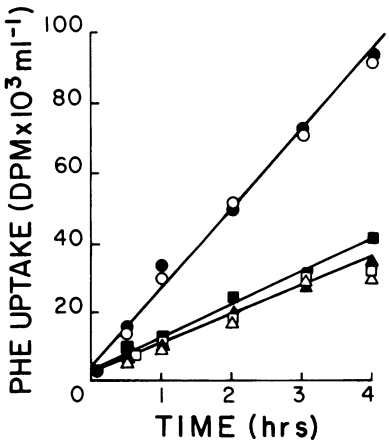

FIG. 5. Total uptake of [^{3}H]phenylalanine (solid symbols) and incorporation of [^{3}H]phenylalanine into TCA-insoluble material (open symbols) in the presence of the dipeptide Phe-Phe. This experiment was conducted with samples from Lake Oglethorpe. Symbols: ● and ○, no Phe-Phe; ■ and □, 10 nM Phe-Phe; ▲ and △, 25 nM Phe-Phe.

TABLE 2. Percentage of total [^{3}H]phenylalanine uptake that was incorporated into macromolecules (TCA-insoluble material) with Phe-Phe added to water samples from Lake Oglethorpe, Georgia

Incubation time (min)	% of [^{3}H]phenylalanine uptake incorporated[a]	
	No Phe-Phe	Phe-Phe (10 nM)
10	83	66
20	85	53
30	83	51

[a] Each percentage is the mean of two replicate measurements. The uptake rate of phenylalanine was 6,370 dpm ml^{-1} h^{-1} in the absence of Phe-Phe and 6,033 dpm ml^{-1} h^{-1} in the presence of 10 nM Phe-Phe. The concentration of the added [^{3}H]phenylananine was 1.3 nM.

effective against procaryotic ribosomes, inhibited amino acid uptake suggests that most of the amino acid uptake was by bacteria. As mentioned above, chloramphenicol had no effect on light-dependent $^{14}CO_2$ uptake, suggesting that the antibiotic did not affect phytoplankton processes. Furthermore, the effect of Leu-Leu on leucine uptake by the <3.0-μm-sized fraction, which contained little chlorophyll but nearly all of the bacteria (Table 4), was similar to the effect measured in a whole water sample from Lake Oglethorpe. To examine more thoroughly the possible uptake of amino acids and peptides by phytoplankton, we added [^{3}H]leucine (1 nM) or Gly-[^{14}C]Phe (300 nM) to water samples from Lake Oglethorpe. After incubation for 3 h, subsamples were gravity filtered through Nuclepore filters with a pore size of 3.0 μm to measure the uptake by the >3.0-μm-sized fraction. Subsamples were also filtered through 0.2-μm Gelman filters to determine total uptake. A similar experiment examined the uptake of $^{14}CO_2$.

The <3.0-μm-sized fraction from Lake Oglethorpe water had little chlorophyll (16% of total) and only 5% of the total light-dependent $^{14}CO_2$ fixation (Table 4). Even so, this small-sized fraction, which contained nearly all of the bacteria, accounted for 72 and 70% of leucine (added at 1 nM) and Gly-Phe (added at 300 nM) uptake, respectively (Table 4). When 50 nM leucine was added, uptake by the small-sized fraction dropped slightly to 64%.

These data indicate that phytoplankton accounted for only a relatively small proportion of total amino acid and peptide uptake in Lake Oglethorpe when low (<10 nM) concentrations were used. If the uptake by attached bacteria and by the bacteria held on the 3.0-μm filter is ignored, at most 30% of the total uptake of leucine and Leu-Leu could have been due to phytoplankton.

DISCUSSION

Bacteria in natural waters are likely to encounter a complex mixture of low-molecular-weight compounds and polymers present at nanomolar concentrations. The lack of a lag in the uptake of Gly-[^{14}C]Phe and in the inhibition by dipeptides of amino acid uptake suggests that the potential for dipeptide uptake is a natural feature of the bacterial communities of both Lake Oglethorpe and Andros Island. Our results also suggest that polymers of amino acids are taken up by natural bacterial populations in preference to the corresponding monomer; very low concentrations of dipeptides (e.g., 5 nM) inhibited the uptake of the corresponding free amino acids, whereas the accumulation of labeled Gly-[^{14}C]Phe was inhibited only by very high concentrations of phenylalanine (1 μM) and was unaffected by glycine. Bacteria may "prefer" peptides over the free amino acids because a given concentration of a peptide provides more carbon and energy, at a similar energetic "cost" per nanomole transported, than does the same concentration of an amino acid monomer (9). Bacteria studied in pure culture often grow more rapidly on peptides than on similar concentrations of free amino acids (9). An energy-limited environment may select for those species that minimize energetic costs in transporting nutrients, since this process can account for 50% of total metabolic costs during growth (p. 87 of reference 13).

We propose the following model as a working hypothesis to explain the peptide-mediated inhibition of amino acid uptake by natural bacterial populations. Peptides are taken up independently of free amino acids and are cleaved intracellularly to the monomers. This increases the intracellular pool of the corresponding free amino acid when protein synthesis does not increase concomitantly. As the intracellular pool reaches a critical concentration, net transport of the amino acids is inhibited. Our hypothesized relationship between transport and protein synthesis as mediated by the intracellular pools is based on the effect of peptides on the proportion of radioactivity, added as free amino acids, that appears in the TCA-insoluble fraction. The percent TCA-insoluble radioactivity is reduced in the presence of peptides, which can be explained by isotope dilution (lower specific activity) of the intracellular pools. Furthermore, that chloramphenicol inhibits amino acid uptake suggests a close coupling between amino acid uptake and protein synthesis.

At least two alternative models could be proposed to explain the inhibition by peptides of amino acid uptake, but the data presented in this paper as well as published data from other experiments with pure cultures support our hypothesis and not the alternative models. One alternative explanation, although not supported by published studies with pure cultures, is that peptides inhibit amino acid uptake because these compounds are taken up by the same transport system; the inhibition of Gly-[^{14}C]Phe uptake by phenylalanine is consistent with this hypothesis. However, neither glycine nor phenylalanine significantly inhibited the initial rate of Gly-[^{14}C]Phe uptake, which is a more accurate

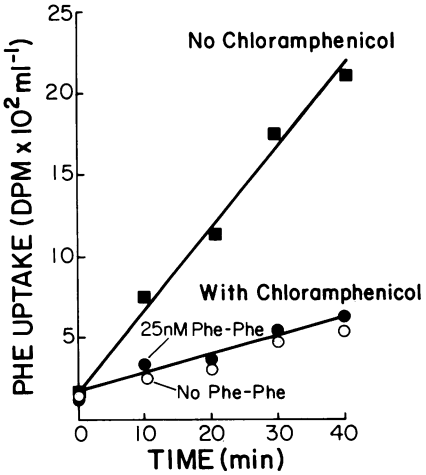

FIG. 6. Uptake of [^{3}H]phenylalanine in the presence of the protein synthesis inhibitor chloramphenicol and the dipeptide Phe-Phe. This experiment was conducted with samples from Lake Oglethorpe.

measure of the transport rate. We hypothesize that the inhibition of Gly-[^{14}C]Phe uptake by 1 μM phenylalanine is due to a mechanism similar to one that operates in the inhibition of phenylalanine uptake by Phe-Phe; that is, the high concentration of unlabeled amino acids in the intracellular pool prevents any incorporation of the labeled amino acids into proteins. That Leu-Leu noncompetitively inhibits [^{3}H]leucine uptake is further evidence that these compounds are transported by separate permeases.

The second alternative model is that the peptide is transported into the cell and quickly cleaved to unlabeled amino acids, which are transported out of the cell and, thus, isotopically dilute the uptake of the labeled amino acids. In experiments with pure cultures, amino acids are released rapidly by the cells after peptide uptake and hydrolysis (8). However, the noncompetitive nature of Leu-Leu inhibition of leucine uptake is evidence against this alternative model. Furthermore, isotope dilution by excretion of unlabeled amino acids resulting from peptide hydrolysis could not have been important when the dipeptide was added at only 10 nM. For example, the extracellular specific activity of labeled leucine would have to be diluted by 35 nM unlabeled leucine (based on the kinetic data given in Fig. 3) to result in the same decrease in the uptake of labeled leucine observed when Leu-Leu is added at 10 nM. In other words, more than 100% (350%) of Leu-Leu would have to be taken up, cleaved to leucine, and released by the cell for this alternative hypothesis to explain our results. The same conclusion is reached when similar calculations are done on data from experiments in which inhibition of phenylalanine uptake by Phe-Phe is examined.

The types of competition discussed in this paper are based on models derived from single-enzyme systems (14). Even though amino acid and peptide uptake systems surely involve more than one protein, these models of competition are useful approximations for uptake by pure cultures of bacteria in the laboratory and by bacterial populations in natural aquatic environments. The effect of peptides on V_{max} of amino acid uptake is clear, and the data demonstrate that peptides are taken up by permeases distinct from those mediating amino acid transport, suggesting that the model for noncompetitive inhibition applies. However, we cannot rule out the possibility that the interactions between peptide uptake and amino acid uptake are more complicated than suggested by the noncompetitive model. These interactions would appear as affecting the ($K + S$) term, which characteristically had the greatest error in our experiments. Furthermore, Payne and Gilvarg (10) suggested that peptides may induce the synthesis of amino acid permeases and thus enhance amino acid uptake. Amino acid uptake by natural bacterial assemblages can be greater in the presence of high concentrations of peptides (Table 1).

Further experiments are needed to examine the mechanism by which net amino acid uptake is inhibited by peptides. There are at least two possible mechanisms: (i) transport across the membrane is inhibited by the buildup, as the peptides are hydrolyzed, of the intracellular pool of free amino acids; (ii) transport into the intracellular pool is not inhibited, but rather the efflux from the pool increases as the intracellular pool increases in concentration (10). Either mechanism also needs to take into account the de novo synthesis of amino acids. It is conceivable that in some aquatic environments peptide uptake, and the concomitant release of free amino acids from peptide hydrolysis, may slow down de novo synthesis. At least in pure cultures of bacteria, the presence of amino acids blocks the synthesis of those amino acids. If this is so, net uptake of the free amino acid may continue unaffected by the peptides in some aquatic environments. However, at least in the two environments we examined here, peptides inhibited leucine and phenylalanine uptake, suggesting that de novo synthesis of these amino acids did not contribute significantly to protein synthesis.

Our experiments suggest that the specific activity of the intracellular pool reaches equilibrium with the extracellular pool rapidly, since the proportion of labeled leucine and phenylalanine taken up and appearing in the TCA-soluble pool was low and remained constant over the incubation period. Isotopic equilibrium may explain why chloramphenicol inhibited the initial uptake rate of amino acids, which was an unexpected result, since the proteins mediating active transport should have been already synthesized. However, once the intracellular pool reaches isotopic equilibrium with the extracellular pool, protein synthesis is the only mechanism by which the cell could accumulate radioactivity, a process that chloramphenicol would inhibit.

TABLE 3. Effect of metabolic inhibitors on amino acid uptake in water samples from Lake Oglethorpe, Georgia

Inhibitor	% Inhibition[a]
Chloramphenicol (μg ml^{-1})[b]	
0	0
1	39
5	58
10	74
20	80
Dinitrophenol (1 mM)[c]	84
Sodium azide (1 mM)[c]	65

[a] Percent inhibition was calculated by comparing the initial uptake rates in duplicate treated and untreated samples over a 1.0-h incubation period with time points taken every 10 or 15 min.

[b] The uptake of [^{3}H]leucine in the untreated sample was 185 dpm ml^{-1} h^{-1}. The concentration of added leucine was 0.5 nM.

[c] The uptake of [^{3}H]phenylalanine in the untreated sample was 646 dpm ml^{-1} h^{-1}. The concentration of added phenylalanine was 1.3 nM.

TABLE 4. Contribution of the small size fraction to heterotrophic and autotrophic processes in Lake Oglethorpe, Georgia

Processes	Total[a]	% <3.0 μm
Heterotrophic		
Leucine uptake at:		
1 nM[b]	7,510 dpm/10 ml	71
50 nM	3,940 dpm/10 ml	64
Gly-[^{14}C]Phe uptake at 300 nM	7,580 dpm/10 ml	70
Bacterial abundance	2.1 × 10^{6} cells/ml	95
Autotrophic		
CO_2 uptake[c]	12,370 dpm/30 ml	5
Chlorophyll	28.5 U	16

[a] The total for each measurement is the actual data used to calculate percent <3.0 μm. The rates are not corrected for incubation time, which was 3 h. The chlorophyll concentration is given in arbitrary fluorescence units as measured in acetone extracts with a Turner fluorometer. All data are means of three replicates from samples taken in December 1983. The experiment was conducted at the in situ temperature of 12°C.

[b] The added concentration of leucine or Gly-Phe.

[c] Light-dependent uptake. Dark CO_2 uptake, which amounted to 15% of the total light uptake, was subtracted from the uptake in illuminated samples.

Exchange between extracellular and intracellular pools may explain the nonspecific inhibiton of Leu-Leu on phenylalanine uptake. High leucine concentrations can be toxic to *Escherichia coli* cells, since the LIV-I transport system exchanges extracellular leucine for intracellular isoleucine, resulting in decreased viability due to isoleucine limitation (12). Likewise, in our experiments with natural bacterial assemblages, the increased flux into the intracellular pool of leucine resulting from Leu-Leu hydrolysis may cause an increased efflux of intracellular isoleucine out of the cell. The decrease in the isoleucine pool would limit protein synthesis and thus lead to inhibition of phenylalanine uptake via the buildup of the phenylalanine intracellular pool.

Our model argues for the importance of the intracellular pool in controlling net amino acid uptake, but we have no data on the actual size of the pools of natural bacterial assemblages. It is also not entirely clear why there should be a maximum size to the intracellular pools of leucine and phenylalanine, as suggested by our data. Direct measurements of the intracellular pools are being undertaken. However, the accuracy of these measurements will always be compromised by the presence of other microorganisms with large intracellular pools, most importantly phytoplankton, which do not significantly utilize amino acids or peptides at low concentrations. We believe that the experiments described here minimize perturbations of the water sample while maximizing the effectiveness of our efforts to examine the relationships between amino acid uptake and protein synthesis by bacteria, exclusive of other microorganisms.

Amino acids and peptides are not representative of the entire DOC pool. Amino acids and peptides probably interact differently than do leucine, phenylalanine, and their polymers. With this caveat in mind, we would like to point out the ecological significance of our experiments.

The peptide-mediated inhibition of amino acid uptake has important implications for understanding the regulation of amino acid uptake by bacteria in natural waters. Net amino acid transport appears to be closely coupled with protein synthesis. This implies that, if exposed to an abrupt increase in an amino acid, the bacteria will be able to utilize that concentration increase only to the extent that protein synthesis can be increased. The capacity of the intracellular pools of leucine and phenylalanine to expand appears to be limited. "Luxury" uptake of these two amino acids was not observed in our experiments. The bacteria of Lake Oglethorpe and of Fresh Creek, Andros Island, did not appear to shunt an increased supply of leucine and phenylalanine to energy production, since peptides had no effect on percent respiration, except at high concentrations (100 nM).

Although our data are consistent with the hypothesis that DOC uptake and macromolecular synthesis are tightly coupled, the synthesis of other macromolecules and the role of precursor pools for these macromolecules need to be examined. Previous studies have discussed thoroughly the importance of intracellular pools of precursors for DNA and RNA synthesis (3, 5, 7). However, these studies have focused on the problem of measuring the specific activity of radiolabeled precursors. The question of the coupling of nucleic acid synthesis to the uptake of DOC and to other cellular functions should be considered. Understanding the linkage between DOC uptake and macromolecular synthesis is essential for examining how DOC is processed by bacteria and transferred to higher trophic levels in marine and freshwater ecosystems.

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