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Glutamine-Stimulated Amino Acid and Peptide Incorporation in *Bacteroides melaninogenicus*

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The uptake of a number of amino acids and dipeptides by cells and spheroplasts of *Bacteroides melaninogenicus* was stimulated by the presence of glutamine; 50 mM glutamine induced maximum uptake of glycine or alanine, and glutamine stimulated the uptake of glycine over a wide concentration range (0.17 to 170 mM). Glutamine stimulated the uptake of the dipeptides glycylleucine and glycylproline at significantly faster rates compared with glycine and leucine. The amino acids whose uptake was stimulated by glutamine were incorporated into trichloroacetic acid-precipitable material, and the inclusion of chloramphenicol or puromycin did not affect this incorporation. The uptake of glutamine by cells was concentration dependent. In contrast, in the absence of chloramphenicol 79% of the glutamine taken up by cells supplied with a high external concentration (4.4 mM) was trichloroacetic acid soluble. Glutamate and α-ketoglutarate were identified in the intracellular pool of glutamine-incubated spheroplasts. The amino acids and peptides were incorporated into cell envelope material, and a portion (30 to 50%) of the incorporated amino acids could be removed by trypsinization or treatment with papain. The effect of glutamine was depressed by inhibitors of energy metabolism, suggesting that glutamine-stimulated incorporation is an energy-mediated effect.

Bacteroides melaninogenicus, a microorganism with complex nutritional requirements, is usually cultured in a medium whose major component is Trypticase (BBL Microbiology Systems), a pancreatic digest of casein. Substitution of Trypticase with an acid hydrolysate of casein does not support growth (5), and the ability of Trypticase to support growth appears to be due to its content of peptides, although these peptides have not been studied in detail. Amino acids also are utilized by *B. melaninogenicus*, which thus derives its carbon and energy requirements from both peptides and amino acids. Further nutritional requirements are supplied by yeast extract, heme (13), and vitamin K_1 (8).

Although there have been many studies on the transport of amino acids by microorganisms, very few have employed obligate anaerobes, with the exception of *Veillonella alcalescens* (3). The major studies of peptide uptake have also employed aerobic or facultative anaerobic microorganisms (16). A study of peptide uptake in *Bacteroides ruminicola* has shown that oligopeptides are utilized, whereas amino acids are not (17). None of the microorganisms studied previously have a peptide requirement analogous to that of *B. melaninogenicus*.

Lev and Milford have shown that glutamine stimulates the incorporation of sphingolipid precursors into complete sphingolipids in washed cells of *B. melaninogenicus* (10) and that the effect of glutamine is energy mediated. In the present study I show that glutamine stimulates incorporation of amino acids and peptides into the cell envelope. A description of this system is presented.

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MATERIALS AND METHODS

Bacterial cultures. *B. melaninogenicus* subsp. *levii* (ATCC 29147; isolated from bovine rumen [10]) was used in the following experiments. Cells were obtained from cultures grown in a Trypticase yeast extract basal medium supplemented with 0.025% hemolysed erythrocytes and 0.1 μg of vitamin K_1 per ml. Cells were harvested from mid-log phase cultures at a turbidity of 125 to 145 Klett units (red filter) (2 to 2.5 mg [wet weight] per ml) after 10 to 16 h of growth. They were incubated at 37°C under an atmosphere containing 95% H_2 and 5% CO_2 in a specially designed apparatus (8) containing 200 ml of medium.

Spheroplast preparation. A 200-ml amount of cells was freshly grown in the anaerobic apparatus (7), and 30 ml was discarded. A solution of 30 g of sucrose dissolved in 30 ml of medium was added by using a 30-ml syringe and an 18-gauge needle. This sucrose solution was prewarmed under reducing conditions before addition. Penicillin was then added to give a final concentration of 500 μg/ml. The culture containing sucrose and penicillin was stirred for 10 min; spheroplast formation was complete after an additional 5 h of incubation. Conversion to spheroplasts was followed by phase-contrast microscopy. The cells increased in

diameter from 0.4 to 0.6 μm initially to 1.4 to 1.6 μm after spheroplasting, and more than 95% of the cells showed morphological change during this process. These spheroplasts were sensitive to osmotic rupture.

Spheroplasts were collected by centrifugation at 21,000 × *g* for 45 min, suspended in 50 mM sodium phosphate (pH 7.4) containing 10 mM Mg^{2+}, 1 mM dithiothreitol, and 15% sucrose, recentrifuged, resuspended in the same buffer at a concentration of 8 to 9 mg of protein per ml, and stored at −70°C. Under these conditions they maintained metabolic activity for 3 to 4 weeks. Before use, the spheroplasts were thawed by immersion in water at 37°C; energy was depleted by incubation for 2 h at 37°C under 95% H_2–5% CO_2, and the spheroplasts were diluted in sucrose buffer to 1 mg of protein per ml.

The uptake of amino acids or peptides by spheroplasts was demonstrated as follows. A reaction mixture containing 55 mM glutamine, the radioactive substrate, and spheroplasts (0.5 mg of protein) in 0.5 ml of sucrose-phosphate buffer containing Mg^{2+} and dithiothreitol was placed into an anaerobic jar containing water at 37°C and incubated at 37°C under 95% H_2–5% CO_2. After incubation, the reaction was stopped by the addition of 4.5 ml of ice-cold sucrose buffer; spheroplasts were filtered through type EH membranes (0.5 μm; Millipore Corp.) and quickly washed three times with 5 ml of buffer. The membranes were placed in vials and counted in a dioxane-based scintillation fluid. The radioactivity was plotted, and slopes were determined by linear regression. The temperature of the washing buffer did not affect the uptake of amino acids; ice-cold, room temperature, and 37°C buffers gave similar results.

In other experiments, 0.5 ml of 10% trichloroacetic acid was added to the reaction mixture, and the precipitate was diluted with 5% cold trichloroacetic acid-phosphate buffer and filtered. The filters were washed extensively with 5% trichloroacetic acid-phosphate buffer, and the membranes were placed in scintillation vials and counted.

Uptake studies with intact cells were performed in the manner described above for spheroplasts, except that cells were suspended in 50 mM sodium phosphate.

Because this microorganism is an obligate anaerobe, most experiments were performed in an H_2-CO_2 atmosphere. Subsequently, it was found that amino acid incorporation proceeded as well aerobically, and initial rates of uptake were determined under aerobic conditions. In these aerobic experiments spheroplasts and glutamine were incubated for 15 min at 37°C, and reactions were initiated by the addition of labeled amino acid or peptide.

Trypsinization of spheroplasts after glycylleucine, leucine, or valine uptake was performed as follows. Spheroplasts were incubated with 1 μCi of [1-^{14}C]glycylleucine plus 55 mM glutamine for 10 min or with 1 μCi of leucine or valine for 40 min. They were diluted in sucrose-phosphate buffer and washed three times by centrifugation. The sedimented spheroplasts from the last wash were suspended in sucrose buffer, and the suspension was divided into two parts. To one-half, 1% (wt/vol) trypsin was added, and both samples were incubated for 2 h at 37°C. An equal volume of 10% trichloroacetic acid was added to both samples, which were then filtered, washed with 5% trichloroacetic acid-phosphate buffer, and counted.

Incorporation of D-alanine into peptidoglycan was determined by the procedure of Mirelman and Nuchamowitz (14). Spheroplasts (0.5 mg of protein) were incubated for 40 min with 1 μCi of D-alanine in the presence of 55 mM glutamine. The reaction mixture was treated with 1.5 ml of hot (100°C) 4% sodium dodecyl sulfate for 5 min. The insoluble material was collected by filtration through a 0.22-μm membrane filter (Millipore Corp.).

The content of the intracellular pool of spheroplasts after glutamine uptake was examined as follows. Spheroplasts (0.5 mg of protein) were incubated with [^{14}C]glutamine (5 μCi; 22 μmol) for 40 min, after which they were collected on a 0.45-μm Millipore filter and washed twice with 5 ml of sucrose-phosphate buffer. The membrane filter was placed in a tube containing 2 ml of water, and the tube was immersed in boiling water for 5 min (1). The filter was removed, and the solution was centrifuged. The supernatant was reduced in volume under a vacuum, and a sample was applied to a 0.1-mm cellulose thin-layer plate. This was developed with propan-2-ol-formic acid-water (10:2:10) (2), and the locations of the radioactive spots were determined by radioautography, as described previously (10).

The radioactive spot corresponding to α-ketoglutarate was scraped off the plate, eluted with phosphate buffer, and incubated in a reaction mixture (0.5 ml) containing 50 mM NH_4Cl, 20 mM NADH, and 200 U of glutamate dehydrogenase. After incubation the reaction mixture was heated in boiling water and centrifuged, and the supernatant was applied to a thin-layer plate and developed as described above. Location of the radioactivity was determined by radioautography.

Chemicals. ^{14}C-labeled amino acids and peptides were obtained from the following sources. L-[*U*-^{14}C]-phenylalanine (355 mCi/mmol), L-[*U*-^{14}C]proline (260 mCi/mmol), L-[U-^{14}C]valine (301 mCi/mmol), L-[^{14}C]-threonine (205 mCi/mmol), L-[*U*-^{14}C]leucine (309 mCi/mmol), L-[*U*-^{14}C]arginine (112 mCi/mmol), and 5-amino-[1-^{14}C]valeric acid (3 mCi/mmol) were obtained from Schwarz Bio Research, Inc., Orangeburg, N.Y.; DL-[3-^{14}C]serine (49.6 mCi/mmol), α-amino-[1-^{14}C]isobutyric acid (49.25 mCi/mmol), L-[*U*-^{14}C]glutamate (230 mCi/mmol), L-[*U*-^{14}C]glutamine (235 mCi/mmol), [1,2-^{14}C]taurine (56.6 mCi/mmol), acetyl-[1-^{14}C]alanine (1 mCi/mmol), [1-^{14}C]glycine (57 mCi/mmol), L-[*glycine*-2-^{3}H]glutathione (2.48 mCi/mmol), and L-[^{35}S]methionine (500 Ci/mmol) were obtained from New England Nuclear Corp., Boston, Mass.; and L-[*U*-^{14}C]alanine (105 mCi/mmol), D-[1-^{14}C]alanine (21 mCi/mmol), [*carboxyl*-^{14}C]sarcosine (1.4 mCi/mmol), and L-[*U*-^{14}C]asparagine (140 mCi/mmol) were from California Biochemical Corp., Burbank, Calif. [1-^{14}C]glycyl-L-leucine (20 mCi/mmol) and glycyl-L-[*U*-^{14}C]leucine (16 mCi/mmol) were from Amersham Corp., Arlington Heights, Ill. [1-^{14}C]glycyl-L-proline (12.7 mCi/mmol) was a gift from F. Naider. [1-^{14}C]-ethionine (1.7 mCi/mmol) was from ICN, Irvine, Calif. *N,N'*-dicyclohexyl carbodiimide and carbonyl cyanide-*m*-chlorophenyl hydrazone were from Sigma Chemical Co., St. Louis, Mo. *p*-Trifluoromethoxyphenylhydrazone was a gift from A. Finkelstein. 6-Diazo-5-oxo-L-

norleucine was donated by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Glutamate dehydrogenase (EC 1.1 1.3) was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Cellulose-coated thin-layer plates (0.1 mm; type MN300) were obtained from Brinkmann Instruments, Westbury, N.Y.

RESULTS

Amino acid uptake. The addition of glutamine to washed cells of *B. melaninogenicus* resulted in a marked stimulation of [^{14}C]glycine and L-[^{14}C]leucine uptake. The radioactivity was found in trichloroacetic acid-precipitable material, and chloramphenicol (100 μg/ml) did not reduce this incorporation. The glutamine-stimulated incorporation resulted from uptake by the cells and incorporation into macromolecules. In most experiments spheroplasts were used since they showed incorporation activity similar to intact cells, and they retained this activity for considerably longer periods when stored at −70°C.

Figure 1 shows the effect of glutamine on glycine incorporation. Starting approximately 5 min after the addition of glutamine, there was a linear incorporation for the 40-min experimental period. In the absence of glutamine, a very low level was incorporated, and this level did not increase with time. This glutamine-stimulated incorporation was found also with L-[^{14}C]alanine and L-[^{14}C]leucine. The 5-min lag in glutamine-stimulated glycine uptake was eliminated by preincubation of the spheroplasts with glutamine before the addition of labeled glycine. The initial rate of glycine incorporation was linear from zero time to 9 min (see below).

Figure 2 shows the incorporation of glycine in the presence of increasing concentrations of glutamine. Maximum incorporation occurred at approximately 50 mM, and similar results were obtained for L-[^{14}C]alanine incorporation.

Glutamine stimulated glycine uptake at concentrations ranging from 0.17 to 170 mM (Fig. 3), although at the highest concentration the stimulating effect of glutamine was reduced. However, over a 10^3-fold increase in glycine concentration, the stimulating effects of glutamine were similar.

The uptake of L-[^{14}C]glutamine was examined at concentrations ranging from 0.044 to 440 mM (Fig. 4). Uptake was linear with respect to external concentration from 0.044 to 4.4 mM, and the concentration of glutamine taken up was approximately equal to the external concentration, assuming that the water content of the spheroplast preparation was 2 μl.

The effect of glutamine on the uptake of a number of amino acids and related compounds

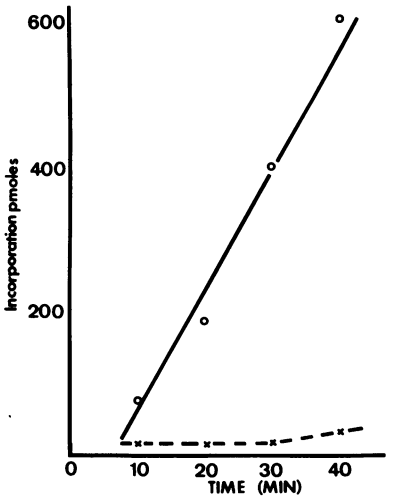

FIG. 1. *Stimulation of glycine incorporation by glutamine. Spheroplasts (0.5 mg of protein) were incubated in a water bath at 37°C in an anaerobic jar under an atmosphere containing 95% H_2 and 5% CO_2 with 18 nmol of glycine plus 55 mM glutamine, as described in the text. The reaction was stopped by dilution with cold sucrose-phosphate buffer, and the solution was filtered rapidly. Symbols: O, glutamine; ×, no glutamine.*

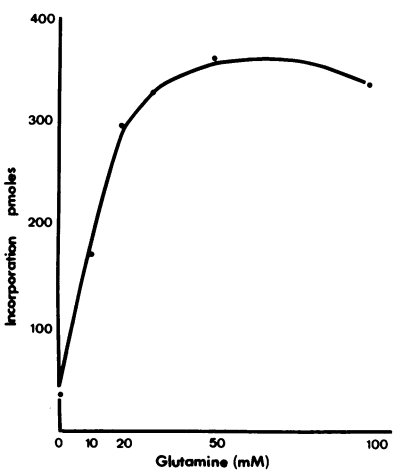

FIG. 2. *Incorporation of glycine as a function of glutamine concentration. Spheroplasts were incubated with 18 nmol of [^{14}C]glycine (118 mCi/nmol) and increasing concentrations of glutamine for 40 min, as described in the text.*

was then examined. The amino acids fell into the following three groups (Table 1): (i) those showing significant stimulation of incorporation by glutamine; (ii) those showing a lower stimulation with glutamine; and (iii) those not stimulated by glutamine. Of interest was the finding that the degree of D-alanine incorporation was similar to the degree of L-alanine incorporation.

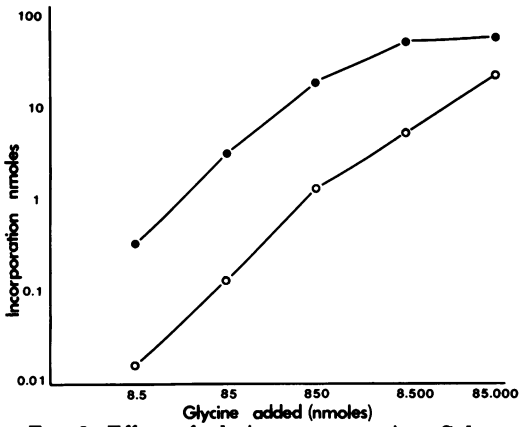

FIG. 3. *Effect of glycine concentration. Spheroplast preparations (0.5 mg of protein) containing from 8.5 nmol to 85 μmol of glycine (0.17 to 170 mM) and 1 μCi of [^{14}C]glycine were incubated for 40 min at 37°C. The preparations were then filtered, washed, and counted as described in the text. Symbols: ●, with 55 mM glutamine; ○, without glutamine.*

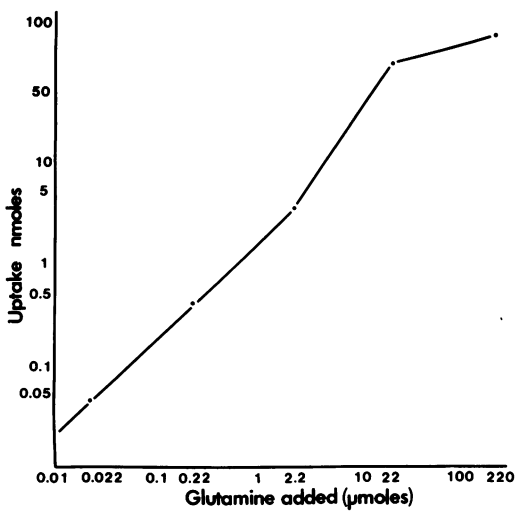

FIG. 4. *Uptake of glutamine. Spheroplast preparations (0.5 mg of protein) containing from 0.022 to 220 μmol of glutamine (0.044 to 440 mM) and 5 μCi of glutamine were incubated for 40 min at 37°C. The preparations were filtered, washed, and counted.*

A number of other compounds, including serine and asparagine, were examined for their capacity to stimulate incorporation of glycine or L-leucine by spheroplasts. Asparagine possessed approximately 40% of the activity of glutamine, whereas serine was inactive. This effect of asparagine was distinct from that of glutamine since 5 mM 6-diazo-5-oxo-L-norleucine, a glutamine antagonist, completely inhibited the effect of glutamine but did not affect the stimulation by asparagine. Asparagine (50 mM) also did not stimulate glutamine or glutamate uptake. 6-Diazo-5-oxo-L-norleucine (5 mM) did not significantly reduce glutamine uptake. ATP, AMP, adenosine, and ribose 1-phosphate, which together with glutamine are active in stimulating sphingolipid synthesis from a precursor (10), were inactive in stimulating amino acid incorporation.

Peptide incorporation. Since peptides play an important role in the nutrition of *B. melaninogenicus* (5), experiments were performed with the dipeptide glycylleucine. Incorporation of glycylleucine was not linear but followed a curve which reached a maximum at 40 min (Fig. 5). The level of glycylleucine taken up was significantly greater than the level of glycine or leucine taken up (Fig. 1 and Table 1). The initial rates of incorporation of glycine-labeled glycylleucine and glycine were examined. The spheroplast preparation was preincubated for 15 min with glutamine, and incorporation was determined after the addition of glycine or glycylleucine. The initial rate of incorporation of the peptide was approximately 15 times that of the amino acid (Fig. 6). A similar result was found when the initial rates of uptake of glycyl-[^{14}C]leucine and leucine were compared. The uptake of glycylproline in the presence of glutamine proceeded at a rate similar to the uptake of glycylleucine.

TABLE 1. *Effect of glutamine on the incorporation of amino acids*

Degree of incorporation[a]	Amino acid	Concn (pmol)
Significant	Glycine	360
	L-Alanine	428
	D-Alanine	416
	L-Valine	85
	L-Serine	162
	L-Threonine	158
	L-Leucine	175
Low[b]	L-Asparagine	25–50
	L-Arginine	25–50
	L-Methionine	25–50
None	L-Proline	<10
	L-Phenylalanine	<10
	Ethionine	<10

[a] Spheroplasts (0.5 mg of protein) were incubated at 37°C for 40 min anaerobically with 20 nmol (1 μCi) of the above compounds with or without 55 mM glutamine. The spheroplasts were then washed on a Millipore filter and counted as described in the text. The values are corrected for the control (no glutamine) incorporation.

[b] Asparagine, arginine, and methionine incorporations were to a significant degree equal with or without glutamine. A number of compounds were not incorporated by spheroplasts; these included L-glutamate, D-leucine, acetylalanine, α-aminoisobutyric acid, δ-aminovaleric acid, taurine, sarcosine, and glutathione.

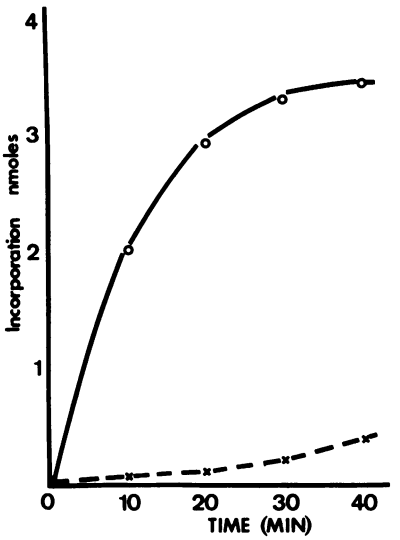

FIG. 5. *Stimulation of [1-^{14}C]glycylleucine incorporation by glutamine. Spheroplasts were incubated in a solution containing 50 nmol of glycylleucine (20 mCi/mmol) and 55 mM glutamine* (○) *or no glutamine* (×). *Conditions were as described in the legend to Fig. 1.*

The nature of glutamine-stimulated peptide uptake was examined by the following inhibition experiments (Table 2). When nonradioactive glycylglycine or glycine was included in reaction mixtures at 10 times the molar concentration of [^{14}C]glycylleucine, the glycylglycine caused a marked inhibition of glycylleucine uptake, and the glycine caused a smaller but nonetheless significant inhibition. In contrast, the inclusion of leucine or leucylleucine caused no inhibition. In a similar experiment, the uptake of glycine was inhibited by glycylglycine and to a lesser degree by glycylleucine, whereas leucine and leucylleucine had little or no effect. The inhibition of glycylleucine uptake by glycine suggests that after uptake, hydrolysis of the peptide occurs before incorporation.

Further experiments were then performed with leucine-labeled glycylleucine. As Table 2 shows, neither glycine nor glycylglycine was inhibitory; significant inhibition was found when leucine and leucylleucine were used.

Incorporation of amino acids and peptides into trichloroacetic acid-precipitable material. With [^{14}C]glycine or [^{14}C]leucine all of the glutamine-stimulated uptake was found in the trichloroacetic acid-precipitable fraction after a 40-min incubation, and the addition of 100 μg of chloramphenicol per ml to the reaction mixtures did not reduce incorporation into trichloroacetic acid-precipitable material. In other experiments the growth of *B. melaninogenicus* was completely inhibited by 2 μg of chloramphenicol per ml. Higher levels of chloramphenicol (200 and 400 μg/ml) and puromycin (50 μg/ml) reduced incorporation by 20 to 40%; in these experiments no glycine or leucine pool was detected.

An experiment on the uptake of [^{14}C]glycine

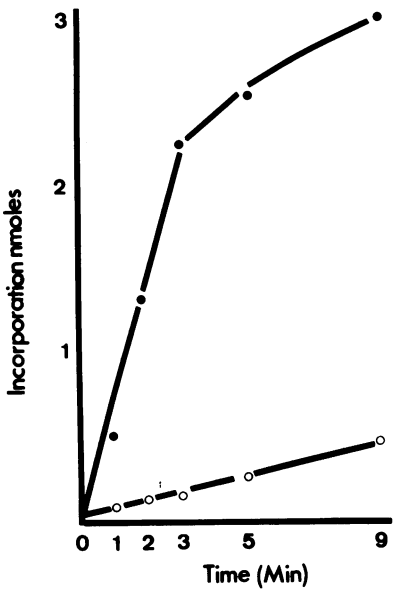

FIG. 6. *Initial rates of glycylleucine and glycine incorporation. Spheroplast preparations (0.5 mg of protein) were preincubated with 55 mM glutamine (15 min, 37°C) under aerobic conditions. The reactions were started by adding 50 nmol of [1-^{14}C]glycylleucine or 50 nmol of glycine at the same specific activity (20 mCi/mmol). The reactions were incubated, filtered, washed, and counted. Symbols:* ○, *glycine;* ●, *glycylleucine.*

TABLE 2. *Inhibition of glycylleucine and glycine incorporation*[a]

Inhibitor	% Inhibition		
	[1-^{14}C]-glycyl-leucine	Glycyl-L-[U-^{14}C]leu-cine	[^{14}C]gly-cine
Glycylleucine	51	59	62
Glycylglycine	73	0	75
Glycine	22	0	8
Leucylleucine	0	55	0
Leucine	0	45	9

[a] Inhibition is expressed as percent depression of [^{14}C]glycylleucine or [^{14}C]glycine incorporation; inhibitors were added at 10 times the molar concentration of glycylleucine (50 nmol; 100 mM) or glycine (18 nmol; 36 mM) to suspensions containing deenergized spheroplasts (0.5 mg of protein) and 50 mM glutamine. Reactions were incubated for 40 min at 37°C.

at increasing concentrations of glycine in the presence of glutamine (similar to the experiment shown in Fig. 4) showed that more than 95% of the radioactivity was trichloroacetic acid precipitable at a concentration of 0.17 mM, but this value decreased to 72% at a concentration of 170 mM. This experiment was performed in the absence of chloramphenicol. In contrast, trichloroacetic acid precipitation of spheroplasts incubated with glutamine (5 μCi; 22 μmol) and without chloramphenicol resulted in a loss of 79% of the total radioactivity after filtration.

Effect of inhibitors of energy metabolism on glutamine-stimulated incorporation. The effects of a number of inhibitors on the glutamine-stimulated incorporation of L-alanine, L-leucine, and [1-^{14}C]glycylleucine were determined (Table 3). Carbonyl cyanide-*m*-chlorophenyl hydrazone and *p*-trifluoromethoxyphenyl hydrazone were the most effective, whereas *N,N'*-dicyclohexyl carbodiimide and others were less inhibitory.

Incorporation of amino acids into the cell envelope. (i) Incorporation of D-alanine into peptidoglycan was determined by incubation of cells with D-alanine in the presence of 55 mM glutamine. The reaction mixture was then treated with 4% sodium dodecyl sulfate at 100°C for 5 min and filtered. A control was incubated with D-alanine and glutamine, trichloroacetic acid precipitated, and filtered through a 0.45-μm membrane. Compared with the control, 75 to 79% of the D-alanine label was retained after treatment with hot sodium dodecyl sulfate. (ii) The distribution of the label after rupture of the cells was determined. Cells were incubated with [^{14}C]glycine or [^{14}C]leucine in the presence of glutamine, washed three times with 0.05 M phosphate buffer, and ruptured by sonication. The membranes were then sedimented by centrifugation (40,000 × *g* for 15 min); 30 to 35% of the ^{14}C label was associated with the pellet, and 79% of the cell protein was in the supernatant. (iii) Removal of incorporated glycylleucine, leucine, and valine with trypsin was examined by using spheroplasts or intact cells which had been incubated with [1-^{14}C]glycylleucine, leucine, or valine in the presence of glutamine and treated as described above. The results of this procedure were variable, and a loss of 30 to 50% of the label was found after trypsinization and precipitation with trichloroacetic acid. A similar loss of label was found after spheroplasts incubated with glycylleucine plus glutamine were treated with 10 mg of papain per ml (15).

TABLE 3. *Effect of inhibitors of energy metabolism on glutamine-stimulated incorporation of L-alanine, L-leucine, and [1-^{14}C]glycylleucine*[a]

Inhibitor	Concn	% Inhibition		
		L-Alanine	L-Leucine	Glycylleucine
Azide	1 mM	ND[c]	86	48
DNP	50 mM	ND	9	7
DCCD	20 μM	60	78	55
CCCP	20 μM	92	91	74
FCCP	20 μM	93	91	72

[a] Spheroplasts (0.5 mg of protein) were incubated for 40 min at 37°C in solutions containing amino acids or peptide plus inhibitor and 55 mM glutamine. They were then filtered and counted as described in the text. Activity is expressed as percent inhibition of the L-[^{14}C]alanine, L-[^{14}C]leucine, and [^{14}C]glycylleucine control values (350 pmol of L-alanine, 75 pmol of L-leucine, and 3.0 nmol of glycylleucine incorporated).

[b] DNP, 2,4-Dinitrophenol; DCCD, *N,N'*-dicyclohexyl carbodiimide; CCCP, carbonyl cyanide-*m*-chlorophenyl hydrazone; FCCP, *p*-trifluoromethoxyphenyl hydrazone.

[c] ND, Not done.

Metabolic fate of glutamine. After incubation of a spheroplast preparation with [^{14}C]glutamine, thin-layer chromatography of a boiling water extract revealed two radioactive spots. The major spot cochromatographed with glutamate and contained 70% of the radioactivity. The minor spot ran close to the front, as does α-ketoglutarate in this system. The minor spot was identified as α-ketoglutarate as follows. It was extracted and incubated with NADH, NH_4Cl, and glutamate dehydrogenase. Subsequent chromatography revealed a radioactive spot which cochromatographed with glutamate.

Attempts to produce active vesicles. A number of attempts were made to produce membrane vesicles which would show glutamine-stimulated amino acid uptake. Vesicles prepared by sonication or French pressure cell rupture showed no activity. The procedure of Konings and Kaback (4), which is adapted to anaerobically grown bacteria, also produced inactive vesicles in a number of experiments. Spheroplasts which were subjected to a mild osmotic shock by dilution of 1 g of spheroplasts in 3 ml of 0.05 M phosphate buffer and which did not show complete lysis also did not show glutamine-stimulated amino acid uptake.

DISCUSSION

Glutamine stimulated incorporation of a number of amino acids and dipeptides by cells and spheroplasts of *B. melaninogenicus*. Previously, glutamine was shown to stimulate incorporation of a sphingolipid precursor in this microorganism (10). There is no available evidence on the participation of glutamine in stimulating the assimilation of substrates by other microorganisms.

Maximum uptake of glycine and L-alanine was found with 50 mM glutamine, and this level of glutamine stimulated glycine incorporation over a wide glycine concentration range. This glycine system was saturable at high glycine concentrations. The uptake of glycine without glutamine was concentration dependent and may represent facilitated diffusion. The uptake of glutamine was also concentration dependent but differed significantly from the uptake of glycine. At the concentrations used to stimulate amino acid incorporation, approximately 80% of the glutamine accumulated in a trichloroacetic acid-soluble form in an experiment performed in the absence of chloramphenicol. Glycine at similar concentrations did not accumulate in a trichloroacetic acid-soluble form to a significant degree. This accumulation of [^{14}C]glutamine was the only indication of an amino acid pool found in these studies.

The incorporation of peptides differed from the incorporation of amino acids both in kinetics and in the level incorporated. The fact that glycine can compete with glycine-containing peptides and vice versa for incorporation indicates that the incorporation step occurs after uptake and hydrolysis of the peptide.

The glutamine-stimulated uptake does not represent solely transport across the cell membrane since the amino acids and glycylleucine were incorporated into the trichloroacetic acid-insoluble fraction. Chloramphenicol at 100 μg/ml did not reduce this incorporation. These results could indicate that chloramphenicol and puromycin do not inhibit protein synthesis or that glutamine stimulates amino acid incorporation by a special mechanism at the post-translational level. However, in *B. melaninogenicus* protein synthesis was inhibited by both antibiotics when incorporation by growing cultures was examined. Thus, puromycin (50 μg/ml) completely inhibited incorporation of [^{14}C]leucine (9), and in other experiments chloramphenicol (100 μg/ml) completely inhibited incorporation of [^{14}C]valine. High levels of chloramphenicol and puromycin did inhibit incorporation of amino acids to a small degree. However, this reduction in incorporation did not result in the accumulation of an amino acid pool.

These are several lines of evidence which indicate that glutamine stimulates incorporation primarily into the cell envelope. A significant proportion of glycine or leucine is associated with the envelope fraction after breakage, even though this procedure has been shown to result in the loss of some membrane components, such as the membrane-associated enzyme 3-ketodihydrosphingosine synthetase (9). Glutamine also stimulates incorporation of D-alanine into peptidoglycan, and furthermore, significant proportions of the amino acids and glycylleucine incorporated via the action of glutamine were removed by trypsin or papain, indicating that the site of incorporation is at or near the cell surface. The incorporated amino acids which were removed by trypsin were trichloroacetic acid soluble, indicating digestion of the incorporating protein.

An analogous system for amino acid incorporation has been described by Vambutas and Salton (19), who showed that glycine incorporation into membrane proteins of *Micrococcus lysodeikticus* was not inhibited by chloramphenicol.

The other compound found to date which stimulates amino acid incorporation is asparagine. The function of asparagine differs from that of glutamine since the action of the latter is inhibited by 6-diazo-5-oxo-L-norleucine, whereas that of asparagine is not. 6-Diazo-5-oxo-L-norleucine, which inhibits amido transfer reactions of glutamine, does not inhibit uptake significantly.

Glutamine most probably acts as an energy source for amino acid and peptide incorporation since inhibitors of energy metabolism, such as carbonyl cyanide-*m*-chlorophenyl hydrazone, *p*-trifluoromethoxyphenyl hydrazone, and *N,N'*-dicyclohexyl carbodiimide, inhibit the effect of glutamine. The generation of glutamate and α-ketoglutarate from glutamine suggests a mechanism for energy generation after formation of succinyl coenzyme A via an α-ketoglutarate thioclastic reaction (12). Succinate is an important factor in the metabolism of certain subspecies of *B. melaninogenicus* (6, 18).

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LITERATURE CITED

1. **Clark, V. L., D. E. Peterson, and R. W. Bernlohr.** 1972. Changes in free amino acid production and intracellular amino acid pools of *Bacillus licheniformis* as a function of culture age and growth media. J. Bacteriol. **112**:715–725.
2. **Jones, R., and J. G. Heathcote.** 1966. The rapid resolution of naturally occurring amino acids by thin-layer chromatography. J. Chromatogr. **24**:106–111.
3. **Konings, W. N., J. Boonstra, and W. DeVries.** 1975. Amino acid transport in membrane vesicles of obligately anaerobic *Veillonella alcalescens*. J. Bacteriol. **122**: 245–249.
4. **Konings, W. N., and H. R. Kaback.** 1973. Anaerobic transport in *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. U.S.A. **70**:3376–3381.
5. **Lev, M.** 1977. Casamino Acids enhance growth of *Bacteroides melaninogenicus*. J. Bacteriol. **129**:562–563.
6. **Lev, M., K. C. Keudell, and A. F. Milford.** 1971. Succinate as a growth factor for *Bacteroides melaninogenicus*. J. Bacteriol. **108**:175–178.

7. **Lev, M., and A. F. Milford.** 1971. Apparatus for metabolic studies with anaerobes. Appl. Microbiol. **21**:555–556.
8. **Lev, M., and A. F. Milford.** 1972. Effect of vitamin K depletion and restoration on sphingolipid metabolism in *Bacteroides melaninogenicus*. J. Lipid Res. **13**:364–370.
9. **Lev, M., and A. F. Milford.** 1973. The 3-ketodihydrosphingosine synthetase of *Bacteroides melaninogenicus*: induction by vitamin K. Arch. Biochem. Biophys. **157**:500–508.
10. **Lev, M., and A. F. Milford.** 1977. Energy-dependent incorporation of sphingolipid precursors and fatty acids in *Bacteroides melaninogenicus*. J. Bacteriol. **130**:445–454.
11. **Lev, M., and A. F. Milford.** 1978. Role of nucleosides, 5-phosphoribosyl-1-pyrophosphate and ribose-1-phosphate in the biosynthesis of phosphosphingolipids in *Bacteroides melaninogenicus*. Arch. Biochem. Biophys. **185**:82–87.
12. **Lindley, R., and E. A. Delwiche.** 1969. Degradation of α-ketoglutarate by *Veillonella alcalescens*. J. Bacteriol. **98**:315–316.
13. **MacDonald, J. B., S. S. Socransky, and R. J. Gibbons.** 1963. Aspects of pathogenesis of mixed anaerobic infections of mucous membranes. J. Dent. Res. **42**:529–544.
14. **Mirelman, D., and Y. Nuchamowitz.** 1979. Biosynthesis of peptidoglycan in *Pseudomonas aeruginosa* 1. The incorporation of peptidoglycan into the cell wall. Eur. J. Biochem. **94**:541–548.
15. **Nathenson, S. G., and A. Shimada.** 1968. Papain solubilization of mouse H_2 isoantigens. An improved method of wide applicability. Transplantation **6**:662–663.
16. **Payne, J. W., and C. Gilvarg.** 1978. Transport of peptides in bacteria, p. 325–383. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
17. **Pittman, K. A., S. Lakshmanan, and M. P. Bryant.** 1967. Oligopeptide uptake by *Bacteroides ruminicola*. J. Bacteriol. **93**:1499–1508.
18. **Sewkow, S., H. L. Ko, and G. Pulverer.** 1974. Nahrstoffenspruche von *Bacteroides melaninogenicus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **228**:87–93.
19. **Vambutas, V. K., and M. R. J. Salton.** 1970. Incorporation of ^{14}C-glycine into *Micrococcus lysodeikticus* membrane protein and the effects of protein synthesis inhibitors. Biochim. Biophys. Acta **203**:83–93.