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New Method for Study of Peptide Transport in Bacteria[1]

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The transport system for glycylmethionine in *Escherichia coli* B and *Salmonella typhimurium* LT2 was examined by a new approach which may be applied to other types of exogenous materials. Physiological auxotrophs were prepared by growing wild strains in a methionine-containing medium to repress the methionine biosynthetic enzymes. Immediate protein synthesis was shown to take place in such physiological auxotrophs only in the presence of either exogenous methionine or a methionine peptide, e.g., glycylmethionine. Protein synthesis was dependent on glycylmethionine taken up by the cell and was indicated by assaying for the inducible enzyme lysine decarboxylase at 5- to 15-min intervals. Uptake was studied by using low concentrations of glycylmethionine, therefore making uptake by permease the limiting step in incorporation of methionine into protein, and by addition of competitor peptides to media containing saturating concentrations of glycylmethionine. Lysine decarboxylase activity in *S. typhimurium* LT2 was about 80 times that present in *E. coli* B. Glycylmethionine transport had a K_m of the order of 1 μM in *S. typhimurium*. Structural specificities observed for peptide transport by other workers were confirmed for *E. coli* B. Competitive inhibition of glycylmethionine uptake by dipeptides was observed in *E. coli*.

Uptake and utilization of peptides in *Escherichia coli* have been studied, and a few reports using other microorganisms have appeared (23, 30). Transport occurs via one or more systems for dipeptides and via one system for oligopeptides of three or more residues. It is followed by cleavage to amino acids by intracellular peptidases for subsequent use in protein synthesis. The relatively broad structural specificities of these transport systems have been used recently (1, 8) to transport normally impermeant molecules into cells as derivatives of peptides. Another area of current interest (9, 31) involves peptides which inhibit the growth of bacteria.

Past knowledge of peptide transport has been derived from studies relying on stimulation of growth of amino acid auxotrophs by peptides or on inhibition of growth of wild strains by peptides containing amino acid analogues. Both of these methods depend on growth as the criterion of transport and give at best semiquantitative data because they require time spans of 8 h or more. Use of radioactive peptides is hampered by the large numbers of possible structural combinations and their unavailability.

Transfer of *E. coli* or *Salmonella typhimurium* (13, 25) from a medium containing a complete complement of amino acids to a minimal medium creates a condition of general amino acid starvation. This effect is caused by repression of enzymes for amino acid biosynthesis in the complete medium and results in a state of "physiological auxotrophy" (6, 19, 24). Thus, the shift in environment creates a transient condition of amino acid starvation lasting 60 min or more during which protein synthesis stops and ribonucleic acid synthesis is reduced. We used wild cells in such a state of physiological auxotrophy to study peptide transport in *E. coli* B and *S. typhimurium* LT2. Evidence for the validity and potential of this approach is presented.

MATERIALS AND METHODS

Materials. All peptides were purchased from Cyclo Chemical Co., and amino acids were purchased from Nutritional Biochemicals Corp.

Microorganisms. *E. coli* B and *S. typhimurium* LT2 (wild type) and met used throughout this investigation were obtained from the Department of Microbiology, Pennsylvania State University. Stock cultures were carried on nutrient agar slants, transferred weekly, subcultured for experimental use by inoculation into 10 ml of glucose-salts medium (32) with cells from a slant and grown for 12 h with shaking at 37 C.

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Preparation of physiological auxotrophs. A 1-ml amount of stock glucose-salts culture of either *E. coli* or *S. typhimurium* LT2 was inoculated into 100 ml of medium containing the following components listed in grams per liter of distilled water: $Na_2HPO_4 \cdot 12H_2O$, 8.0; KH_2PO_4, 10.0; NH_4Cl, 1.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.41; glucose, 20.0, plus the following L-amino acids in milligrams per liter: threonine, 48; serine, 64; proline, 96; valine, 112; isoleucine, 80; leucine, 160; tryptophan, 64; arginine, 80; histidine, 48; phenylalanine, 32; glycine, 32; glutamine, 128; asparagine, 96; tyrosine, 80; cystine, 26; and methionine, 96. This amino acid mixture upon induction produced the maximum level of lysine decarboxylase as determined by earlier studies (5). Cells were grown at 37 C with shaking to 5×10^8 cells/ml as determined by turbidity at 650 nm. They were harvested by centrifuging at 5 C and 3000 $\times$ *g* for 10 min, were washed with an equal volume of cold 0.9% NaCl, and were recentrifuged as above. The washing procedure was repeated once.

Incubation for transport studies. Harvested cells were equilibrated for 10 min at 37 C in the above complete medium except that methionine was omitted. Then 1% lysine (the inducer for lysine decarboxylase) was added. *S. typhimurium* LT2 was suspended to provide concentrations ranging from 0.024 to 0.240 mg dry weight/ml and *E. coli* was suspended to 1.3 mg dry weight/ml. Concentrations were determined from curves for turbidity (optical density) at 650 nm versus dry weight for each organism. Glycylmethionine or methionine was added to 5-ml portions (50 ml for the 0.024-mg/ml cell concentration) of these cell suspensions. They were incubated at 37 C for selected times, usually 5 to 15 min. At the end of the incubation period, chloramphenicol was added (50 μg/ml) to block any protein synthesis during handling of the experimental systems, and cells were centrifuged at 5 C and 3000 $\times$ *g* for 10 min. Cells were washed by resuspension in 20 ml of cold 0.9% NaCl solution and recentrifuged. Controls were run without methionine or methionine peptides. Chloramphenicol was added to one control before the incubation period. The other was treated with chloramphenicol as usual at the end of incubation.

Measurement of transport. Protein synthesis was taken as the indication of transport, and it was revealed by the closely related synthesis of the inducible enzyme, lysine decarboxylase. The enzyme was prepared for assay as follows. Washed cells were suspended in 6.0 ml of cold 0.05 M phosphate buffer (pH 7.0) in a cellulose nitrate tube, chilled in ice, and treated for five 30-s periods with a flat-tipped, 0.5-inch stephorn of a Branson sonifier. The mixture was cooled for 1 min between sonication periods. The resulting suspension was centrifuged at 5 C and 4000 $\times$ *g* for 25 min. The supernatant liquid was taken as the lysine decarboxylase extract. Relative protein concentration was determined by the method of Lowry et al. (16) and read at 500 nm by using bovine serum albumin as the reference protein.

Assay of lysine decarboxylase. The assay was modified from those described earlier (5, 17). It was based on reaction of lysine with ninhydrin and correlated with manometric measurement of CO_2 liberated during decarboxylation of lysine to cadaverine. A 2-ml portion of enzyme extract (0.1 to 0.5 mg of protein/ml) and a 0.5-ml portion of 0.010 M lysine (5 μmol) were combined. Assay times were adjusted between 30 and 90 min to allow for differences in enzyme concentration and to improve sensitivity for samples with low activity. Reaction was stopped by addition of 0.6 ml of 1 M HCl followed by 1.0 ml of 2.5% ninhydrin in methyl cellosolve (wt/vol). Other reagents and conditions were those described by Lu and Mallette (17). Absorbance was read at 515 nm in 1-cm cuvettes with a Beckman DU spectrophotometer. A set of lysine standards containing enzyme added after HCl was run in each experiment. Specific activity was defined as change in absorbance at 515 nm divided by absorbance at 500 nm from the protein determination. Any activity in the control containing chloramphenicol throughout incubation was subtracted from activity in other samples to correct them for low basal values. Transport rates are expressed as specific activities of lysine decarboxylase per gram dry weight per min. Assays were run in duplicate and experiments were repeated.

Peptide competition measurements. Competition between peptides for uptake was studied as follows. Lysine decarboxylase activity was measured by using glycylmethionine at permease-saturating level (0.2 mM) as the source of methionine for the physiological auxotrophs. A peptide not containing methionine was added to another sample of medium containing glycylmethionine, and enzyme synthesis was again measured for comparison. When the second peptide competed with glycylmethionine for uptake sites, a measurable decrease in enzyme synthesis would be expected. The size of the decrease should indicate the relative competitive power of the non-methionine peptide. Control experiments were included to show that non-methionine peptides did not compete with free methionine.

Peptidase assays. Peptidase activity of crude extracts from both *E. coli* B and *S. typhimurium* on glycylmethionine alone was determined by the method outlined by Payne (22) for glycylglycine and other dipeptides. Crude extracts were the same as those taken for lysine decarboxylase assay. Peptidase assays were performed in triplicate and averaged. Activity on glycylmethionine in the presence of a second peptide, leucylleucine, was measured in the following manner. Peptidase extracts were prepared by the method of Payne (21). To 1.0 ml of enzyme extract (approximately 0.2 mg of protein) in 0.05 M phosphate buffer (pH 7.0) were added glycylmethionine (0.2 μmol) and leucylleucine (2.0 μmol). The mixture was incubated at 37 C with shaking for 60 min. Reaction was stopped by adding 0.2 ml of 6 M HCl. Repeated stirring with a Vortex mixer at room temperature for 15 min coagulated protein. After centrifuging at 7000 $\times$ *g* for 20 min, the supernatant was saved and HCl was removed by repeatedly drying down the sample with a stream of N_2 after redissolving the successive residues in water. The final residue was taken up in 0.2 ml of water, and 0.025 ml was spotted on sheets of 3 MM Whatman paper (46 by 57

cm). The sample plus reference amino acids and peptides was run 17 h ascending in 1-butanol:4 M NH_3:ethanol (11:8:4 vol/vol/vol). The chromatogram was dried 4 h at 35 C. Areas corresponding to amino acids hydrolyzed from test peptides were located by spraying with ninhydrin and cut out, wicks of Whatman no. 1 paper were attached, and the spots were eluted with 70% ethanol in water (vol/vol) for 48 h. The solvent was evaporated under N_2, and the residues were assayed by the method of Moore and Stein (18) for amino acids at 570 nm. Amino acid released is reported as absorbancy units per hour.

RESULTS

Protein synthesis in physiological auxotrophs. As shown by the second line in Table 1, protein synthesis ceased when either *E. coli* B or *S. typhimurium* LT2 was shifted from a complete medium containing methionine to a medium lacking it. The protein level after incubation in the medium lacking methionine or glycylmethionine was the same as that of the control in which protein synthesis was blocked by addition of chloramphenicol before incubation. This observation confirms earlier reports (6, 13, 24) that shift from complex to minimal media blocked protein synthesis in wild strains of *E. coli* and *S. typhimurium*. Lawrence et al. (14) showed that growth of *S. typhimurium* in the presence of methionine repressed methionine biosynthetic enzymes. Therefore, such organisms were in a state of physiological auxotrophy.

Appreciable levels of lysine decarboxylase activity indicate protein synthesis, and the rate of protein synthesis in both organisms was the same in the presence of equivalent amounts of methionine or glycylmethionine (Table 1). Data on *S. typhimurium* met$^-$ blocked for methionine biosynthesis were included to show that results were comparable in our system whether "physiological" or "true" amino acid auxotrophs were used. Note that specific activity of lysine decarboxylase in *S. typhimurium* LT2 was approximately 80 times that in *E. coli* B. The low activity in one control on *S. typhimurium* indicated synthesis or turnover of a small amount of protein in the absence of exogenous methionine or glycylmethionine. This level of enzyme may occur because of: (i) accumulation of methionine taken into the cell from the complete medium prior to starvation for methionine, (ii) breakdown of protein within the cell to yield methionine, and/or (iii) traces of medium methionine left during the washing procedure. Small amounts of protein synthesis have been reported (24) in *E. coli* and *S. typhimurium* when using amino acid mutants incubated under deficiency conditions rather than the physiological auxotrophs primarily employed here. A longer period of equilibration prior to addition of methionine might have helped to exhaust endogenous methionine supplied but might have affected cellular physiology.

Lysine decarboxylase production in E. coli and S. typhimurium. Figure 1 shows the appearance of lysine decarboxylase as a function of time for *E. coli* B and *S. typhimurium* after addition of methionine or glycylmethionine to methionine-deficient medium. The lag time is approximately 3 to 4 min. Pardee and Prestidge (20) found that other inducible enzymes of *E. coli*, including β-galactosidase, tryptophanase and D-serine deaminase, begin to appear about

TABLE 1. *Protein and enzyme synthesis in E. coli B and S. typhimurium LT2*

Medium[a]	*E. coli*		*S. typhimurium*			
			Wild		met$^-$	
	Protein[b] (relative %)	Lysine decarboxylase[c] sp act	Protein (relative %)	Lysine decarboxylase[c] sp act	Protein (relative %)	Lysine decarboxylase sp act
Control	100	0.000	100	0.00	100	0.00
+0	100	0.000	100	0.03	100	0.20
+ Methionine (0.1 mM)	118	0.037	119	3.17	123	3.11
+ Glycylmethionine (0.1 mM)	119	0.037	120	3.19	120	3.30

[a] Data are for equivalent weights of cells. The control medium contained chloramphenicol (50 μg/ml), 1% lysine, and the complete medium listed in Materials and Methods, but without methionine. Chloramphenicol was added to the other three systems after incubation for 15 min. Other additions are listed.

[b] Protein was determined on sonic extracts of cells by the method of Lowry et al. (16), read at 500 nm and reported as percentage of protein from the control cells in which protein synthesis was blocked. Values were reproducible to about ±2%.

[c] Specific activity was obtained by dividing lysine decarboxylase in activity per milliliter of enzyme extract by absorbance at 500 nm due to protein and was reproducible to within ±7%.

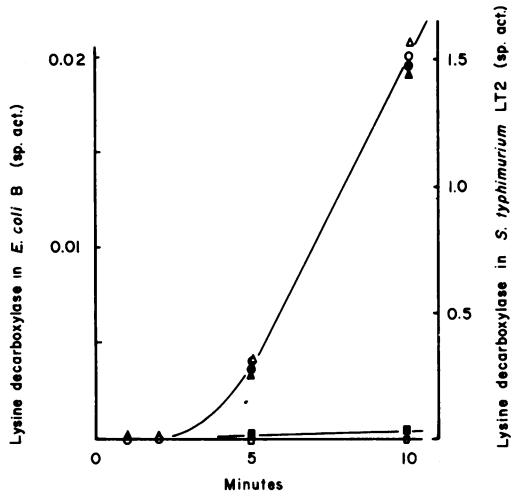

FIG. 1. *Lysine decarboxylase formation in E. coli B and S. typhimurium LT2. Samples were withdrawn at the indicated times, and chloramphenicol (50 μg/ml) was added to prevent further synthesis. Lysine decarboxylase was assayed as described in Materials and Methods. E. coli B is represented by open symbols with circles (methionine 0.7 mM), triangles (glycylmethionine 0.7 mM), and squares (control). S. typhimurium LT2 is represented by closed symbols for the same concentrations. All six systems read zero at 1 and 2 min. Synthesis of lysine decarboxylase was linear for at least 5 more min than shown by the data of this plot.*

3 min after addition of inducer. This lag was attributed (11) to the time required for de novo enzyme synthesis and other factors. It is important that there was no significant difference between curves obtained by using glycylmethionine or methionine for lysine decarboxylase formation. If peptide hydrolysis were rate limiting, one would expect a difference between the curves for free amino acid and peptide.

As further support for the claim that peptide hydrolysis was not rate limiting, peptidase activity on glycylmethionine was measured in both *E. coli* B and *S. typhimurium*. These organisms hydrolyzed 6.58 and 4.31 μmol of glycylmethionine per min per mg of protein, respectively, and the values are comparable to those reported (22) for glycylglycine. This activity is far in excess of that needed by these cells to supply methionine for synthesis of lysine decarboxylase and all other cellular proteins according to the rationale of Payne (22) applied as follows. A 1-mg portion of dry cells of *S. typhimurium* contains about 500 μg of protein. Methionine (MW 149) at as much as 5% of the total protein would amount to 0.17 μmol/mg of cells. This amount would be required for the culture to double in mass. For a generation time of 30 min, specific peptidase activity toward glycylmethionine required to provide this amount would be 0.011 μmol of peptide per min per mg of protein. Average actual specific activity in vitro of peptidase activity on glycylmethyionine was 4.31 μmol per min per mg of protein, at least a 400-fold excess over that needed to provide all methionine necessary for one generation. Growth in this study was always less than one generation.

Glycylmethionine and methionine uptake by S. typhimurium. The approach outlined below is similar to that used by Ames (2) to measure K_m values for permeases for histidine and aromatic amino acids in *S. typhimurium*. As the measure of uptake, she employed incorporation of radioactive amino acids of high specific radioactivity and low concentration into total protein at low cell populations in minimal medium. Our system used production of a single protein, lysine decarboxylase, as the measure of uptake. Table 2 reveals that the amount of lysine decarboxylase formed is constant over the concentration range of 5 to 100 μM. Hence, protein synthesis operated at capacity at these concentrations. Ames (2) attributed a similar independence of incorporation of histidine and phenylalanine into cellular protein at relatively high concentrations to saturation of the protein synthesizing system. The concentration (5 μM) at which lysine decarboxylase synthesis became saturated was the same for either glycylmethionine or methionine although they use different uptake systems. Likewise, Ames (2) observed that saturating concentrations were similar for histidine and phenylalanine, although histidine used a specific per-

TABLE 2. *Lysine decarboxylase production by S. typhimurium as a function of methionine concentration*

Methionine or glycylmethionine (μM)	Lysine decarboxylase[a]	
	Methionine	Glycylmethionine
100	0.314	0.316
10.0	0.311	0.297
7.5	0.318	0.313
5.0	0.320	0.311
2.5	0.245	0.276

[a] Lysine decarboxylase activity per milligram dry weight of cells per minute as described in Materials and Methods. Measurements were made by using cells at 0.24 mg dry weight/ml in a state of physiological auxotrophy for methionine. Incubation time was 10 min with added methionine or glycylmethionine.

mease (K_m = 0.08 μM) whereas phenylalanine used an aromatic permease (K_m = 0.59 μM). At 2.5 μM (below the saturating level) uptake was limiting in the present studies, and a difference was observed between glycylmethionine and methionine, reflecting differences in efficiency of uptake for the two systems. This observation is analogous to that for histidine and phenylalanine (2).

At sufficiently low concentrations of glycylmethionine, permease for this peptide should not be saturated, and therefore transport would limit incorporation of methionine into lysine decarboxylase. As a result, determination of the rate of lysine decarboxylase formation at low peptide concentrations might be used to assay permease activity. However, lower cell concentrations (of the order of 0.024 mg dry weight/ml) and a shorter incubation must be used to limit concentration changes during such assay.

A Lineweaver-Burk (15) double reciprocal plot of rate of lysine decarboxylase formation versus glycylmethionine concentration is shown in Fig. 2 for one such experiment. K_m was about 1 μM for glycylmethionine uptake by *S. typhimurium* LT2. This value is indicative only, because possible concentration changes during incubation were not measured, the 5 min incubation was relatively long, and dilution of cells to such a low concentration may have affected cellular physiology. In computing K_m for the process, the assumption was made that peptide uptake and not peptide hydrolysis was the rate limiting step in utilization of methionine from the peptide for lysine decarboxylase synthesis.

Norleucine has been reported (4) to inhibit methionine uptake into *S. typhimurium* by 41% when radioactive L-methionine (0.2 μM) was present with DL-norleucine (800 μM). Under our conditions, lysine decarboxylase production was inhibited by 19% in medium containing 2.5 μM L-methionine and 400 μM L-norleucine when compared with a control without norleucine. Although this result suggests competition for transport sites, the effect is reduced, perhaps by the higher methionine concentration, perhaps by omission of D-norleucine. On the other hand, it is possible that norleucine may have interfered with a process unrelated to transport, such as protein synthesis.

Glycylmethionine uptake by E. coli B. Competition between glycylmethionine and non-methionine peptides for uptake was established in *E. coli* B as follows. Lysine decarboxylase was measured after incubation of cells for 15 min with a saturating level of glycylmethionine (0.2 mM). This system was the positive control. Non-methionine peptides or peptide derivatives were added to matching systems together with glycylmethionine. Competition decreased lysine decarboxylase according to Table 3. Equivalent concentrations of methionine and glycylmethionine gave the same amounts of enzyme. "Competing" peptides had no effect on lysine decarboxylase levels when methionine was present.

Table 3 substantiates the structural specifici-

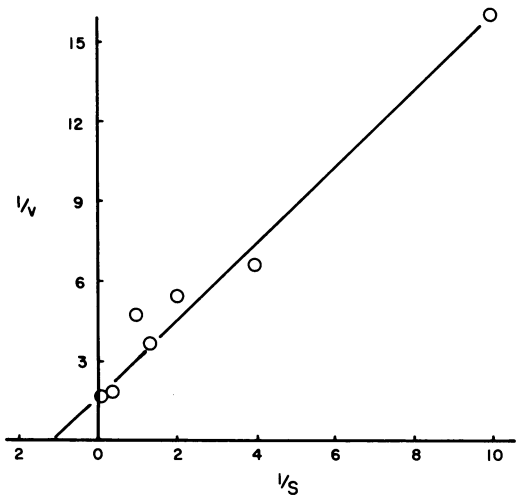

FIG. 2. *Glycylmethionine uptake by S. typhimurium LT2. V is lysine decarboxylase activity per milligram dry weight of cells per minute. S is glycylmethionine concentration (μM). Cellular concentration was 0.024 mg dry weight/ml, and incubation was 5 min.*

TABLE 3. *Competition between peptides for transport into E. coli B*

Peptide	Relative lysine decarboxylase act[a]	
	Glycylmethionine (0.2 mM)	Methionine (0.2 mM)
Control[b]	100	100
L-Leu-L-Tyr	79	102
D-Leu-L-Tyr	94	100
CBZ-L-Leu-L-Leu[c]	99	101
L-Leu-L-Leu	46	98

[a] Lysine decarboxylase is reported as percentage of lysine decarboxylase relative to that formed by the control containing 0.2 mM methionine. Results were reproducible to less than ±7%.

[b] Control contained either 0.2 mM glycylmethionine or 0.2 mM methionine as indicated. All other samples contained either 0.2 mM glycylmethionine or 0.2 mM methionine plus 2 mM of a second peptide. Cells (5 ml at 1.3 mg dry weight/ml in a state of physiological auxotrophy) were incubated for 15 min, centrifuged, washed, sonically treated, and assayed.

[c] CBZ, carbobenzyloxy.

ties established for dipeptide transport by other workers (23). These specificities include the following. A protonatable terminal amino group is required as shown by failure of carbobenzyloxy-L-Leu-Leu to compete while L-Leu-L-Leu did. Dipeptides containing a D-amino acid as in D-Leu-L-Tyr did not compete while L-Leu-L-Tyr did. Peptide specificity is relatively general rather than strictly individual, inasmuch as both L-Leu-L-Leu and L-Leu-L-Tyr competed with glycylmethionine although to different extents. It could be argued that this competitive effect took place at the level of peptide hydrolysis and not peptide uptake. However, assay of peptidase activity (described in Materials and Methods) showed that hydrolysis of glycylmethionine (0.2 mM) to glycine and methionine was the same in the presence (0.11 ± 0.02 absorbancy unit/h) or absence (0.12 ± 0.02 absorbancy unit/h) of leucylleucine (2.0 mM). Therefore, competition takes place at the level of peptide uptake, not peptide hydrolysis. Peptidase activity toward leucylleucine was also detected.

Studies using growth of amino acid auxotrophs on peptides as criteria have also demonstrated (21, 23) such competitive effects. In some cases it was shown that competition took place at the level of peptide uptake. Because of the relatively low lysine decarboxylase activity of *E. coli* B, the present work employed high cell densities and saturating levels of glycylmethionine. Development of a more sensitive assay for lysine decarboxylase or use of a different, more active enzyme would permit lower cell and peptide concentrations.

DISCUSSION

Advantages of the approach described for study of peptide transport include measurement of transport over a period of minutes rather than hours. Thus, a large number of peptides may be screened. In addition, the method is applicable to wild strains of many different microorganisms. Synthesis of radioactive peptides is not required, and the method may possibly be applied to osmotically shocked cells.

Although we have used "physiological" auxotrophs in most of our studies, there is no reason why "true" amino acid auxotrophs cannot be handled in the same way. Physiological auxotrophy provides the potential advantage of deriving readily a series of different auxotrophs by omitting different amino acids from the preinduction medium. Other types of studies involving states of physiological auxotrophy have been conducted with *Aerobacter aerogenes* and *Bacillus subtilis* (24).

We chose lysine decarboxylase as the indicator of protein synthesis because: it is produced in relatively large quantities by these species (27), it is not subject to catabolite repression (7) as is β-galactosidase, its induction does not involve biosynthesis of a permease as for β-galactosidase, it is produced in non-dividing as well as dividing cells (26), and it is widely distributed among microorganisms including *B. subtilis*, *Klebsiella pneumoniae*, *Pseudomonas maltophilia*, *A. aerogenes*, and *Serratia marcescens* (10). Other inducible enzyme systems should be evaluated for other organisms when investigating transport problems in order to select the system with the greatest and most consistent response.

The large "excess" peptidase activity reported in this paper and by others (22, 28), provides evidence that peptide hydrolysis is not the rate-limiting step for use of glycylmethionine. Formation of lysine decarboxylase at the same rates for equal amounts of methionine and glycylmethionine provides additional evidence on this point. Studies (29) based on growth rates of amino acid auxotrophs showed that responses to amino acid in free and dipeptide forms are identical. Therefore, peptide uptake, not peptide hydrolysis, was the rate-limiting step for utilization of glycylmethionine. Because peptide uptake can be made the limiting step, we were able to estimate a K_m for glycylmethionine uptake in *S. typhimurium* LT2 by using our new method based on physiological auxotrophs. Active transport has been established (12, 23) as a mechanism for dipeptide uptake. Yet facilitated diffusion may also play a role, and dipeptides could make some use both of dipeptide permeases and oligopeptide permease (23) to enter the cell. Thus, the significance of K_m from a Lineweaver-Burk plot is questionable. One would expect a more complex, possibly biphasic plot. Because it was possible to look at glycylmethionine transport only at low concentrations due to saturation of the protein-synthesizing system of the cell at higher concentrations, a change in slope of the curve at higher concentrations may have been obscured. Ames (2) was unable to observe the biphasic plot reported for histidine uptake. Her method also encountered saturation of the protein synthesizing system of the cell at higher concentrations.

Extensive production of lysine decarboxylase by *S. typhimurium* LT2 compared with *E. coli* B indicates that surveys of this and other inducible enzymes in different microorganisms may reveal richer sources of such enzymes. Application of this approach to uptake of a wide variety of exogenous materials is conceivable.

The various dipeptide specificities previously established (23) for *E. coli* and verified in this report acted as checks of the new method.

Anraku and Heppel (3) demonstrated induction of β-galactosidase by isopropylthiogalactoside in osmotically shocked but metabolically active cells. They found that the differential rate of β-galactosidase synthesis was the same for shocked and unshocked cells. Preliminary experiments show that this observation applies equally well to lysine decarboxylase in osmotically shocked *E. coli* B and should make the method useful for study of peptide transport in spheroplasts.

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