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Effect of Growth Conditions on Glutamate Transport in the Wild-Type Strain and Glutamate-Utilizing Mutants of *Escherichia coli*

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The effects of growth conditions on the glutamate transport activity of intact cells and membrane vesicles and on the levels of glutamate-binding protein in wild-type *Escherichia coli* K-12 CS101 and in two glutamate-utilizing mutants, CS7 and CS2^{TC}, were studied. Growth of CS101 on aspartate as the sole source of carbon or nitrogen resulted in a severalfold increase in glutamate transport activity of intact cells and membrane preparations to levels characteristic of the operator-constitutive mutant CS7. The high glutamate transport activity of mutant CS7 was not derepressed further by growth on aspartate. Synthesis of glutamate-binding protein was not enhanced by aspartate in either strain. Mutant CS2^{TC} produces a heat-labile repressor of glutamate permease synthesis and is therefore able to grow on glutamate at 42 C but not at 30 C. CS2^{TC} cells grown in a glycerol-minimal medium at the restrictive temperature (30 C) exhibit low glutamate transport activity. Growth on aspartate at 30 C results in derepressed synthesis of glutamate permease. Cells grown on glycerol at 42 C have high glutamate transport activity. No further derepression is obtained upon growth on aspartate. Growth of CS101 and CS7 in "rich broth" greatly reduces the levels of glutamate-binding protein but does not appreciably affect glutamate transport by whole cells or membrane preparations. The identity of the carrier and the role of the binding protein in glutamate transport are discussed in the light of these findings.

A specific glutamate transport system of *Escherichia coli* K-12 has been studied in our laboratories (9, 10, 17). Mutants that acquired the ability to grow on glutamate as the major carbon source were shown to accumulate glutamate at faster rates and to higher concentrations than those observed with the wild-type parent strain (9, 17). Genetic and physiological analysis of the different mutants led us to the conclusion that the formation of the glutamate transport system in *E. coli* K-12 is controlled by repression (17, 18). However, the identity of the metabolite(s) that serves as the physiological regulatory signal in this system (as inducer or co-repressor [11]) remains unknown.

A glutamate-binding protein (GltBP) is released from the periplasmic space of *E. coli* K-12 by cold osmotic shock treatment according to Neu and Heppel (20) and during preparation of spheroplasts by the Repaske procedure (3, 24). The release of GltBP is accompanied by a decrease in glutamate transport activity. Glutamate transport in cold osmotic shock-treated cells and in spheroplasts is considerably stimulated and often fully restored by the addition of crude or purified GltBP (3, 4). However, the reproducibility of these restoration experiments is not very satisfactory. Kinetic studies of glutamate binding by partially purified GltBP supported the idea that GltBP is a component of the glutamate transport system of *E. coli* K-12 (3, 4). The involvement of specific binding proteins in the transport of solutes in gram-negative bacteria has been suggested by several authors (6, 23; D. L. Oxender and S. C. Quay, Ann. N.Y. Acad. Sci., in press) and has been rather convincingly documented for galactose (5), maltose (14), and histidine (2) transport. The participation of GltBP in glutamate transport by *E. coli* B has been considered in a recent paper by Miner and Frank (19). These authors conclude that GltBP is not an essential component of this transport system. Lombardi and Kaback described specific transport systems in membrane vesicles of *E. coli* ML 308-225, capable of rapid uptake and accumulation of a number of amino acids, including glutamate. Although these preparations did not con-

tain detectable amounts of binding proteins, they retained considerable fractions of the various transport activities of intact cells (16). However, recent results of Willis and Furlong (26) on glutamate uptake by *E. coli* D_2W suggest that, under certain conditions, the binding protein may function in transport.

In the present study we examine the effects of changes in the composition of the growth medium on the appearance of glutamate transport activity and on the synthesis of the membrane-bound glutamate carrier and the periplasmic GltBP. Our results indicate that the inducer of "glutamate permease" synthesis is metabolically derived from aspartate. Induction of glutamate transport is not accompanied by increased synthesis of GltBP. Furthermore, conditions that strongly repress GltBP synthesis do not reduce the glutamate transport capacity of the cell. These findings indicate that under certain conditions GltBP may not be essential for glutamate transport.

MATERIALS AND METHODS

Bacterial strains. The following *E. coli* K-12 strains were used: CS101, a methionine auxotroph unable to grow on glutamate as the source of carbon (supplemented with L-methionine, 25 μg/ml); CS7, a glutamate-utilizing mutant of CS101, constitutive for glutamate transport (17); $CS2^{TC}$, a mutant of CS101 capable of utilizing glutamate as a carbon source at 42 C but not at 30 C, producing a thermolabile repressor of "glutamate permease" synthesis (18).

Chemicals. L-[U-^{14}C]glutamic acid (237 mCi/mmol) was purchased from New England Nuclear Corp., Boston, Mass.; L-[U-^{14}C]proline (290 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, England. All other chemicals were of reagent grade, obtained from commercial sources.

Growth media. The basal medium of Davis and Mingioli (7), from which citrate was omitted, was supplemented with L-methionine (25 μg/ml) and with glucose (0.5%), glycerol (0.5%), sodium succinate (1%), sodium L-glutamate (0.5%), or sodium L-aspartate (0.5%) as indicated (minimal medium [MM]). "Rich broth" contained peptone (2%), yeast extract (1%), NaCl (0.5%), and glucose (2%) (all from Difco).

Transport assays with intact cells. The cultures were grown with aeration and harvested in the exponential phase (turbidity of 80 Klett units, filter no. 54). The bacteria were washed twice, suspended in uptake buffer (basal medium plus glucose [0.5%], NaCl [15 mM], and chloramphenicol [200 μg/ml]) to a concentration of 88 μg of protein per ml, and incubated at 30 C for 30 min. The uptake reaction was started by the addition of 0.5 ml of the cell suspension to 1.5 ml of prewarmed uptake buffer containing the desired concentration of the ^{14}C-labeled amino acid (L-[^{14}C]glutamate, 1 mCi/mmol; L-[^{14}C]proline, 26.3 mCi/mmol). After incubation at 30 C with aeration for 6 min, the entire reaction mixture was filtered and the filter was washed twice with 5 ml of uptake buffer. Other experimental details were as described previously (10).

Transport assays with membrane vesicles. Membrane vesicles were prepared by the method of Kaback (12), except that lysozyme was used at a concentration of 100 μg/ml during spheroplast formation. L-[^{14}C]glutamate (237 mCi/mmol) uptake was determined by the method of Lombardi and Kaback (16), with D-lactate (20 mM) or with sodium ascorbate (20 mM) plus phenazine methosulfate (0.1 mM) as the electron donor, as indicated.

Preparation of sonic extracts. Sonic extracts were prepared by the method of Barash and Halpern (4).

Double-diffusion immunoassay of GltBP. The double-diffusion immunoassay used was a modification of the Ouchterlony method (22). The antiserum was prepared by injecting into a rabbit intradermally 1 ml of purified homogeneous GltBP (4) (356 μg/ml) diluted with an equal volume of Freund adjuvant. A second injection (0.5 ml) of the protein-adjuvant mixture was administered after 2 weeks. A booster dose of 100 μg of GltBP was given a week later. One week after the third injection the rabbit was bled, and the serum was partially purified by precipitation with a 50% saturated solution of ammonium sulfate and by dialysis against veronal buffer (2.5×10^{-2} M, pH 8.2). The serum was stored at −20 C. Double-diffusion assays were carried out on glass slides covered with a layer (2 mm thick) of 2% Noble agar in veronal buffer. Holes were cut in the agar with a guide block drilled with three sets of six holes 5 mm apart. The center hole was filled with 20 μl of antiserum. Aliquots (20 μl each) from five different dilutions of the bacterial sonic extracts tested were placed in the outer wells. The slides were incubated in a moist chamber at 25 C for 24 h. The antiserum was calibrated against purified homogeneous GltBP. The minimum amount of GltBP giving a detectable reaction was 0.24 to 0.32 μg.

Chemotaxis assays. Chemotaxis assays were carried out by the method of Adler (1), except that 10-μl Vitrex microcapillary pipettes (4.0 cm long, internal diameter of 0.56 mm; manufactured by Modulohm I/s, DK-2730, Denmark) were used. A capillary was filled with attractant to about 2 cm and inserted into a well of a tissue culture flat-bottom microtiter plate (A/S Nunc, Denmark) covered with Parafilm, which contained the bacterial suspension. The experiments were carried out at 30 C for 1 h.

RESULTS

Effect of growth medium on glutamate transport activity. *E. coli* CS7 was grown in MM with glycerol, glucose, glutamate, or aspartate as the major carbon source and in rich broth. Accumulation of [^{14}C]glutamate by intact washed cells from each of these cultures was measured at different concentrations of substrate under equilibrium conditions. The values of K_m and Y (capacity [15]) given in Table 1 have been obtained from Lineweaver-Burk plots. The glutamate-utilizing mutant

TABLE 1. *Effect of growth medium on glutamate transport by intact cells of E. coli K-12 CS7*[a]

Medium	K_m (μM)	Capacity (L-[^{14}C]glutamate, nmol/mg of protein)
MM + glycerol	10.0	121.4
MM + glucose	10.0	121.4
MM + glutamate	13.3	145.7
MM + aspartate	10.0	144.1
Rich broth	10.0	121.4

[a] The cultures were grown at 37 C.

CS7 exhibited a high capacity for glutamate uptake when grown on any one of the above-mentioned carbon sources. Growth in the presence of glutamate or aspartate as the source of carbon resulted in a small increase in the capacity for glutamate uptake (<20%). In contrast to the glutamate transport-constitutive strain CS7, its wild-type CS101 parent exhibited low glutamate uptake capacity after growth in glycerol-MM or in rich broth. However, growth of CS101 on aspartate as the major carbon source resulted in derepressed synthesis of "glutamate permease." Similarly, strain CS2^{TC}, which produces a thermolabile repressor (18), is derepressed for "glutamate permease" when grown on aspartate as the carbon source at the restrictive temperature (30 C). Interestingly, strain CS2^{TC}, but not the wild-type CS101 parent, is also derepressed when grown in rich broth at 30 C (Table 2). Proline uptake was not affected, in any of the three strains tested, by growth on different carbon sources (data not shown). Further information on the effect of aspartate on the formation of the glutamate transport system in strain CS101 is given in Fig. 1. Two following points should be emphasized. (i) Growth on aspartate, although greatly enhancing the cells' capacity for glutamate uptake, does not affect at all the affinity of the transport system for glutamate. (ii) Addition of aspartate to a glycerol-ammonia medium (or to succinate-ammonia, data not shown), i.e., under conditions in which aspartate does not serve as a major carbon or nitrogen source, does not result in any derepression of "glutamate permease" synthesis in *E. coli* CS101. Although not shown here, derepression is obtained when aspartate serves as the major nitrogen source (in the presence of glycerol).

Effect of growth medium on the synthesis of GltBP. Table 3 summarizes a series of experiments in which we compared the amounts of GltBP present in wild-type and mutant cultures grown under various conditions. The amount of GltBP was estimated by the double-diffusion technique on agar slides, with a constant amount of specific antiserum to pure homogeneous GltBP (4) and various dilutions of sonic extracts from the different cultures. Pure homogeneous GltBP served as a standard for comparison. No significant differences in the amounts of GltBP in cultures of strains CS101, CS7, and CS2^{TC} grown in MM with glycerol or glutamate as the major carbon source were found. Furthermore, no derepression of GltBP synthesis was observed in strain CS101 or in strain CS2^{TC} grown at the restrictive temperature in a medium in which aspartate was the carbon source. Moreover, aspartate even seemed to reduce the amounts of GltBP in all three strains to 33 to 67% of that formed in a glycerol-ammonia medium. A dramatic decrease in GltBP synthesis (to less than 5 to 7%) was obtained upon growth of either strain in rich broth. It should be pointed out that the formation of the glutamate transport system was not repressed by growth in rich broth (Tables 1 and 3). A double-diffusion immunoassay of GltBP in strain CS7 grown on different media is presented in Fig. 2.

Glutamate chemotaxis in E. coli CS7 grown on different carbon sources. Ordal and Adler (21) have recently shown that the galactose-binding protein, which is an essential component of the galactose transport system in *E. coli* (5), is also essential for galactose chemotaxis. It was of interest, therefore, to see whether growth conditions that resulted in repression of GltBP synthesis would affect chemotaxis of *E. coli* K-12 towards glutamate. A concentration response curve (1) of *E. coli* CS7 grown in glycerol-MM is shown in Fig. 3. Similar curves were obtained with cells grown in media with glutamate or aspartate as the major carbon source and with rich broth cultures. The nature of the carbon source for growth did not affect the concentration of glutamate required for maximum chemotaxis. Table 4 shows that the

TABLE 2. *Glutamate transport in cells of wild-type and mutant E. coli K-12 strains grown on different carbon sources*

Strain	Growth temp (C)	Capacity (L-[^{14}C]glutamate, nmol/mg of protein) of cells grown on:		
		MM + glycerol	MM + aspartate	Rich broth
CS101	37	28.0	149.3	28.0
CS7	37	121.4	144.1	121.4
CS2^{TC}	30	19.7	153.4	121.4
CS2^{TC}	42	121.4	153.4	121.4

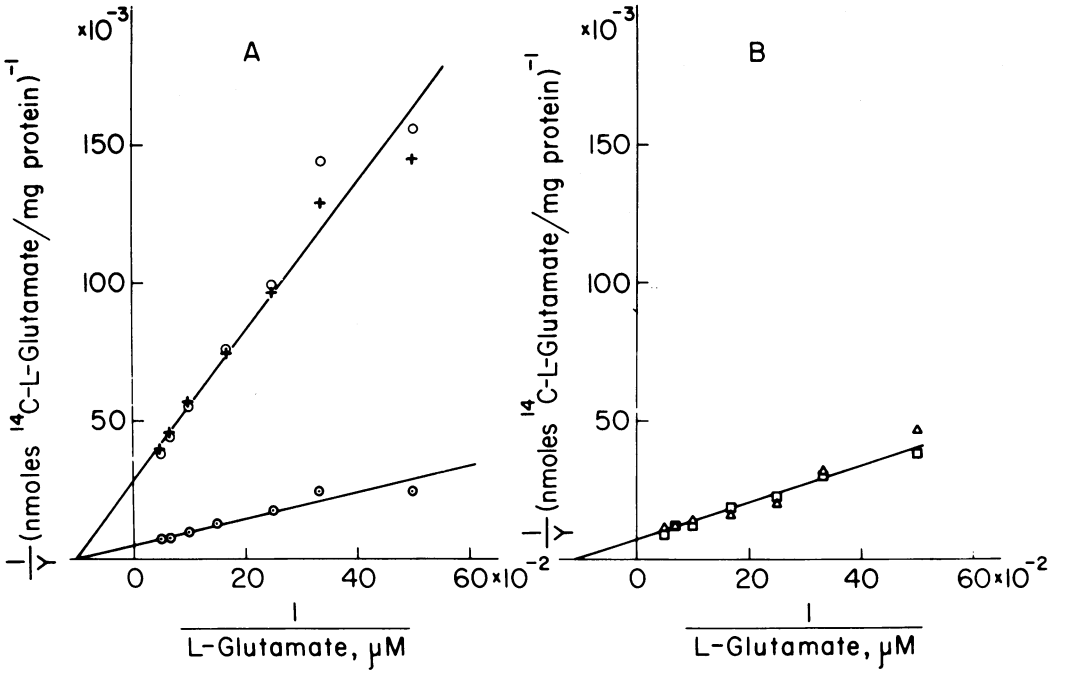

FIG. 1. *Effect of aspartate on the synthesis of "glutamate permease" in wild-type and mutant E. coli K-12 strains. The cultures were grown at 37 C. (A) Strain CS101 grown on glycerol-ammonia (○); strain CS101 grown on glycerol-ammonia supplemented with* L-*aspartate (0.5%) (+); strain CS7 grown on glycerol-ammonia (⊙). (B) Strain CS101 grown on aspartate-ammonia (△); strain CS7 grown on aspartate-ammonia (□).*

TABLE 3. *Effect of growth medium on the synthesis of GltBP by E. coli K-12*[a]

Strain	Concn GltBP (μg/mg of total cell protein)			
	MM + glycerol[b]	MM + glutamate	MM + aspartate	Rich broth
CS101	3.0–4.0	No growth	2.0–2.7	<0.3
CS7	3.0–4.0	3.0–4.0	2.0–2.7	<0.3
CS2^{Tc}, 30C	3.0–4.0	No growth	1.5–2.0	<0.3
CS2^{Tc}, 42C	3.0–4.0	3.0–4.0	1.5–2.0	<0.3

[a] GltBP concentration was estimated by a double-diffusion immunoassay.
[b] Growth medium.

magnitude of the chemotactic response to glutamate at optimum concentration was practically the same with glycerol-, glutamate-, aspartate-, and rich broth-grown cultures, although the amount of GltBP formed in rich broth was less than 7% of that found in glycerol-grown cells. Similar results were obtained in regard to aspartate taxis.

Glutamate transport in membrane vesicles of glycerol-grown and aspartate-grown E. coli CS101. In the accompanying paper (13), it is shown that membrane vesicles of the wild-type strain CS101 exhibit low D-lactate- and ascorbate-phenazine methosulfate-stimulated transport (16) of glutamate. However, vesicles prepared from cultures of the glutamate-utilizing mutant CS7 transport glutamate at very fast rates in the presence of D-lactate or the artificial electron donor system. As shown in Fig. 4, CS101 vesicles prepared from cells grown on aspartate as the major carbon source take up glutamate in the presence of ascorbate-phenazine methosulfate at a rate 17 times as high as that observed with vesicles from glycerol-grown cells and very similar to the rate of glutamate transport in vesicles of strain CS7 (see Fig. 2 in reference 13).

Comparison of glutamate transport in membrane vesicles of strain CS7 grown in glycerol-MM and in rich broth. Reciprocal plots of glutamate uptake by membrane preparations from CS7 cultures grown on glycerol-ammonia medium and in rich broth are presented in Fig. 5. The two preparations gave identical K_m values for glutamate. The rates of uptake were slightly lower with the preparation from rich broth; the difference found was within the range of fluctuations observed with different preparations from cultures grown in the same medium. These results are similar to those obtained for glutamate transport by whole cells (Table 1).

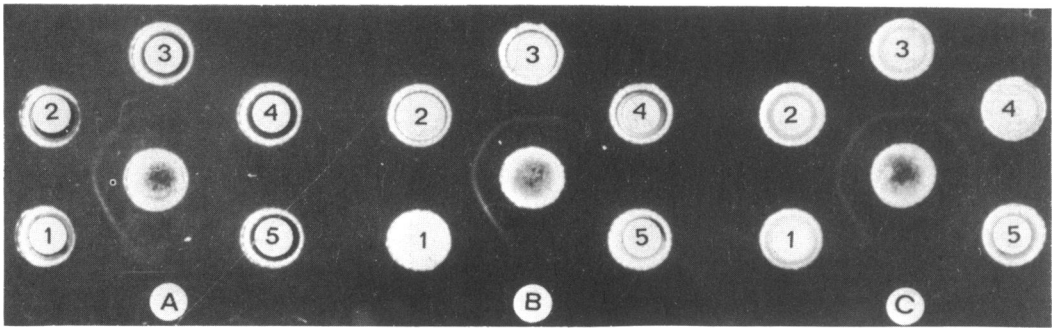

FIG. 2. *Double-diffusion immunoassay of GltBP in E. coli K-12 CS7 grown in different media. The cultures were grown at 37 C. (A) Culture grown in MM plus glycerol. Protein concentrations (milligrams per milliliter): 1, 8.0; 2, 6.0; 3, 4.0; 4, 3.0; 5, 2.0. (B) Culture grown in MM plus aspartate. Protein concentrations (milligrams per milliliter): 1, 40.0; 2, 20.0; 3, 12.0; 4, 6.0; 5, 4.0. (C) Culture grown in rich broth. Protein concentrations (milligrams per milliliter): 1, 100.0; 2, 80.0; 3, 70.0; 4, 60.0; 5, 50.0.*

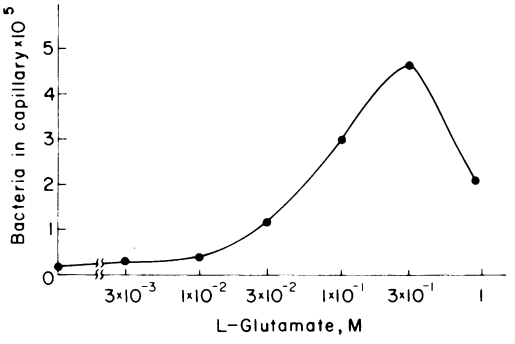

FIG. 3. *Concentration response curve in L-glutamate taxis by E. coli K-12 CS7. The cells were grown in MM plus glycerol with aeration at 37 C. The initial concentration of bacteria outside the capillary was 8.5 × 10⁷ cells/ml.*

TABLE 4. *Effect of growth medium on chemotaxis of E. coli K-12 strain CS7 to glutamate*[a]

Growth medium	No. of bacteria in capillary (×10⁻⁵)
MM + glycerol	5.46
MM + glutamate	5.88
MM + aspartate	5.20
Rich broth	5.86

[a] The initial concentration of bacteria outside the capillary was 10^8 cells per ml. The cultures were grown at 37 C.

DISCUSSION

In an earlier communication (18) we suggested that the specific glutamate transport system of *E. coli* K-12 (9, 10, 17) is controlled by a genetically determined mechanism of repression. This was based on genetic and physiological analysis of mutants selected for the acquisition or loss of the ability to grow on glutamate as the sole carbon source. Three genetic loci were defined and mapped. The first, *gltS*, is located at min 73 on the Taylor map of the *E. coli* K-12 chromosome (25). Mutants with lesions at this locus exhibited a 3-fold decrease in the rate of glutamate transport and a 10- to 20-fold reduction in the affinity of the uptake system for glutamate. *gltS* was thus defined as a structural gene of "glutamate permease." The second, *gltC*, is very closely linked to *gltS*. Mutations at the *gltC* locus resulted in a four- to sixfold increase in glutamate transport activity, but the K_m for glutamate remained the same as in the parent strain. The third, *gltR*, not linked to the *gltS-gltC* cluster, mapped at min 81. This gene was mapped in mutants able to grow on glutamate at 42 C but not at 30 C. These mutants, when grown at 42 C, exhibited four to five times higher rates of glutamate uptake than did cultures grown at 30 C. However, the K_m for glutamate was not affected by growth temperature. The kinetics of "glutamate permease" synthesis after temperature shifts and after brief heat treatment in the absence of growth suggested that *gltR* specifies the formation of a repressor protein. The repressor probably acts at the *gltC* locus, which was tentatively defined as the operator gene (18). From the behavior of known repressible systems (11), one would expect the glutamate transport regulatory system to respond to a metabolic effector(s) signalling a need for glutamate uptake (or a lack of it). The data presented in Tables 1 and 2 indeed show that the presence of aspartate in the growth medium as a source of carbon or nitrogen enhances "glutamate permease" synthesis in the repressible CS101 strain more than fivefold. The fact that a similar induction by aspartate is also obtained with the temperature-conditional *gltR* mutant $CS2^{TC}$ grown at the restrictive temperature (30 C) but that no significant effect is seen with

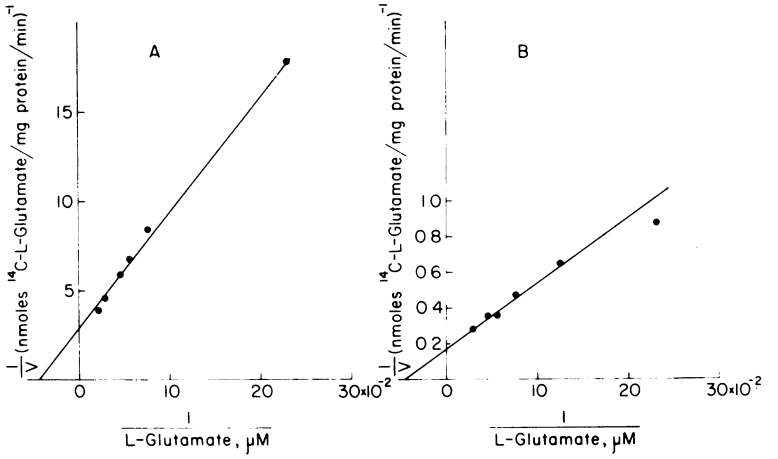

FIG. 4. *Comparison of glutamate transport by membrane vesicles of E. coli K-12 strain CS101 grown on glycerol and on aspartate as the source of carbon. Ascorbate-phenazine methosulfate served as the electron donor for transport. The bacteria were grown with aeration at 37 C. (A) Culture grown in MM plus glycerol. (B) Culture grown in MM plus* L-*aspartate.*

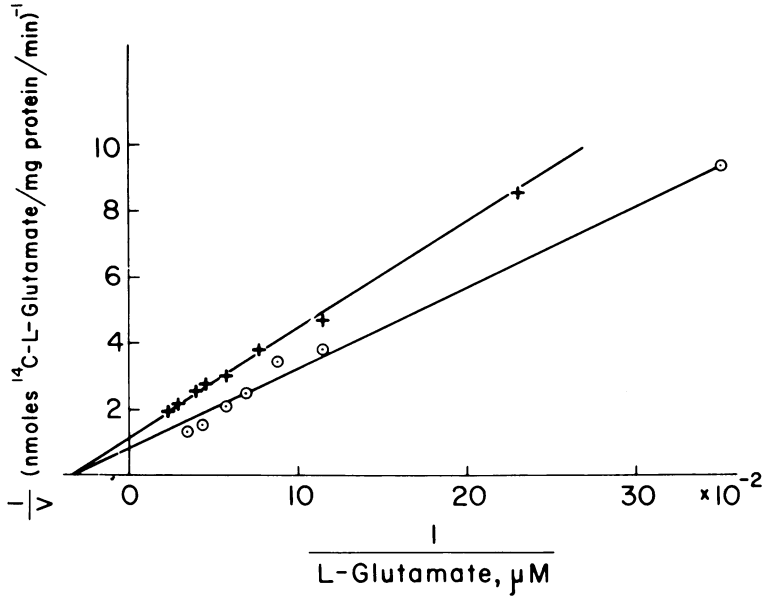

FIG. 5. *Comparison of glutamate transport by membrane vesicles of E. coli K-12 strain CS7 grown in glycerol-MM and in rich broth.* D-*Lactate served as the electron donor for transport. The bacteria were grown with aeration at 37 C. Culture grown in MM plus glycerol (⊙); culture grown in rich broth (+).*

the "operator-constitutive" *gltC* mutant CS7 or with strain CS2^{TC} grown at 42 C provides further support for our model. We think that aspartate does not participate directly in the induction process. It seems more likely that some metabolic derivative of aspartate is the actual inducer. This is based on the observation that CS101 grown in media containing other readily available carbon and nitrogen sources, together with aspartate (MM-glycerol-aspartate, rich broth), has low repressed levels of "glutamate permease." It is perhaps significant that, unlike CS101, strain CS2^{TC} grown in rich broth at the restrictive temperature shows derepressed levels of glutamate transport activity. The mutant repressor of CS2^{TC} may have a higher affinity for the inducer than the wild-type repressor of CS101 and can, therefore, be induced in

media in which the concentration of inducer molecules does not suffice to induce the wild-type strain.

We have recently shown that glutamate transport in membrane vesicles of *E. coli* K-12 strain CS7 is dependent upon Na^+ ions (S. Kahane, M. Marcus, H. Barash, Y. S. Halpern, H. R. Kaback, FEBS Lett., in press), as is transport of glutamate by intact cells (10). Furthermore, the accompanying paper (13) clearly demonstrates that the phenotypes of the wild-type CS101 and mutant CS7 strains in respect to glutamate utilization and transport, as observed with intact cells, are faithfully reflected in the glutamate transport behavior of the corresponding preparations of membrane vesicles. These data indicate that the glutamate carrier resides in the cytoplasmic membrane, as Miner and Frank recently suggested for *E. coli* B (19). Our present finding—that one can induce ascorbate-phenazine methosulfate-driven glutamate transport in membrane vesicles of CS101 at rates as high as those obtained with CS7 vesicles by growing the cells on aspartate (Fig. 4)—furnishes strong evidence for the membrane location of the glutamate carrier in *E. coli* K-12 and for its control by the *glt* regulatory system.

The role of the periplasmic GltBP in transport of glutamate in *E. coli* K-12, as emerges from the present study, is less clear and probably more complicated. On the one hand, the kinetic data and inhibitor studies on in vitro glutamate binding (3, 4) and on transport in intact cells (9) and membrane vesicles (13), as well as the data on glutamate transport in "shocked cells" and spheroplasts and its restoration by preparations with GltBP activity, seem to suggest that GltBP does participate in physiological transport of glutamate in *E. coli* K-12. On the other hand, we have reported here that the synthesis of GltBP in strain CS101, unlike transport of glutamate in intact cells and in membrane vesicles, is not induced by growth on aspartate. This indicates that GltBP synthesis is not under the control of the *glt* regulatory system. Moreover, GltBP synthesis is strongly repressed upon growth in rich broth (Table 3), a condition not affecting glutamate transport in whole cells and in vesicles, or glutamate chemotaxis (Tables 1 and 4, Fig. 5). In a recent study with *E. coli* D_2W, Willis and Furlong (26) noted changes in the apparent dependency of glutamate uptake on binding protein levels with changes in culture conditions (4% glucose-grown versus 0.4% succinate-grown cultures). They suggest that the observed differences "may be related to a modulation in the expression and organization of components of the same transport system which in turn are dependent on the physiological state of the cell." Isolation and analysis of GltBP mutants should be instrumental in elucidating the role of this binding protein.

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