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Molecular Cloning of *gltS* and *gltP*, Which Encode Glutamate Carriers of *Escherichia coli* B

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Two genes encoding distinct glutamate carrier proteins of *Escherichia coli* B were cloned into an *E. coli* K-12 strain by using a cosmid vector, pHC79. One of them was the *gltS* gene coding for a glutamate carrier of an Na^+-dependent, binding protein-independent, and glutamate-specific transport system. The content of the glutamate carrier was amplified about 25-fold in the cytoplasmic membranes from a *gltS*-amplified strain. The *gltS* gene was located in a 3.2-kilobase *Eco*RI-*Mlu*I fragment, and the gene product was identified as a membrane protein with an apparent M_r of 35,000 in a minicell system. A gene designated *gltP* was also cloned. The transport activity of the *gltP* system in cytoplasmic membrane vesicles from a *gltP*-amplified strain was driven by respiratory substrates and was independent of the concentrations of Na^+, K^+, and Li^+. An uncoupler, carbonylcyanide *m*-chlorophenylhydrazone, completely inhibited the transport activities of both systems, whereas an ionophore, monensin, inhibited only that of the *gltS* system. The K_t value for glutamate was 11 μM in the *gltP* system and 3.5 μM in the *gltS* system. L-Aspartate inhibited the glutamate transport of the *gltP* system but not that of the *gltS* system. Aspartate was taken up actively by membrane vesicles from the *gltP*-amplified strain, although no aspartate uptake activity was detected in membrane vesicles from a wild-type *E. coli* strain. These results suggest that *gltP* is a structural gene for a carrier protein of an Na^+-independent, binding protein-independent glutamate-aspartate transport system.

Glutamate is important in the metabolism of bacterial cells, and especially in the assimilation of ammonia. Glutamine synthetase combines ammonia with glutamate to form glutamine when cells are grown with a low concentration of ammonia, whereas glutamate dehydrogenase mainly combines ammonia with 2-ketoglutarate to form glutamate at a high concentration of ammonia (21). Furthermore, glutamate provides nitrogen for the synthesis of most amino acids. It is well known that wild-type strains of *Escherichia coli* K-12 and B cannot grow with glutamate as the sole source of carbon and nitrogen. This inability seems to result from their low activity for glutamate uptake, and in fact, mutants that have acquired higher glutamate uptake activities can grow on glutamate (10).

The main transport system for glutamate in *E. coli* K-12 is mediated by a glutamate carrier, the *gltS* gene product, and is an Na^+-dependent transport system (16, 23). On the other hand, Frank and Hopkins (7) have found that glutamate transport in *E. coli* B is stimulated by Na^+ and that this stimulation is due to an increase in affinity for the substrate. As cytoplasmic membrane vesicles showed glutamate uptake activity, a periplasmic-binding protein was concluded not to be involved in the transport process (25). Later Tsuchiya et al. (31), Hasan and Tsuchiya (14), and MacDonald et al. (20) proposed a mechanism of Na^+/glutamate symport. Fujimura et al. (8, 9) further characterized the binding and transport reactions and proposed an Na^+/H^+/glutamate symport mechanism, based on the observation that a chemical gradient of H^+, Na^+, or both imposed directly on membrane vesicles caused the accumulation of glutamate. Schellenberg and Furlong (30) have reported that *E. coli* D_2W has multiple transport systems for glutamate, aspartate, or both and that these differ in Na^+ dependence, binding protein dependence, and substrate specificity.

Recently, results of molecular biological studies on several Na^+/substrate symport systems, including melibiose (32) and proline (5, 12, 13) transport systems in *E. coli*, have been reported. However, there are no reports of molecular biological studies on glutamate transport systems, although such studies are necessary for us to further understand not only the metabolic importance of glutamate but also that of Na^+/solute symport mechanisms.

Previously, we characterized the glutamate carrier in *E. coli* B in detail (8, 9). In the present study we cloned the *gltS* gene for this carrier into *E. coli* K-12 for further study of the structure-function relationship of the Na^+/glutamate symport mechanism. We were also able to clone a new gene, designated *gltP*, which is a good candidate of the gene coding for a carrier that catalyzes active transport of glutamate and aspartate in membrane vesicles. In this report we describe the cloning of the *gltS* and *gltP* genes and the molecular properties of the two gene products.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains of *E. coli* K-12 used in this study are listed in Table 1. *E. coli* B (wild-type strain) and its derivative, strain 29-78, were obtained from T. Tsuchiya (Okayama University, Okayama, Japan). Strain 29-78 was selected for its ability to grow on glutamate at low concentrations (7). The plasmids used were pBR322 (2) and pHC79 (15).

Media. Luria broth (24) and B7 (7) medium were prepared as described previously.

Extraction of chromosomal DNA. Chromosomal DNA was extracted from strain 29-78 of *E. coli* B as described previously (28).

Construction of a chromosomal DNA library. Chromosomal DNA from *E. coli* B was partially digested with *Sau*3A, and DNA fragments of about 40 kilobases were obtained by sucrose density gradient centrifugation (22). These fragments were ligated to *Bam*HI-digested pHC79 with T4 DNA ligase, and the mixtures were subjected to in

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TABLE 1. *E. coli* K-12 strains used in this study

Strain	Relevant genotype	Source or reference
ST131	r_K^- m_K^+ *galK lacY met supE recA56 srlA*::Tn*10*	KH802 (33)
ST204	F$^-$ *thi-1 relA1 pyrE41 argG6 thyA25 rbs-1 gltS7 gadS1 gadR2 malA1 rpsL*	KL141 (18)
ST204 *recA*	Same as ST204, but *recA*	This study ST204 × NK6659
NK6659	Hfr *srlA1300*::Tn*10 recA56 ilv-318 thi-1 thr-300 relA1 spc-300*	K. Kutsukake
ST5101	F$^-$ *thr ara leu azi tonA lacY tsx minA gal minB rpsL malA xyl mtl thi sup hag*::Tn*10*	K. Ohnishi

vitro packaging (22). Infection of ST131 was carried out under standard conditions.

DNA manipulation. The standard procedures used for the manipulations of DNA, such as preparation of plasmid DNA on a small or large scale, digestion with restriction endonucleases, ligation with T4 DNA ligase, transformation with recombinant plasmids, and agarose gel electrophoresis, were done as described previously (22).

Southern blot hybridization. Plasmid DNA from the transformants was digested with restriction endonucleases, and the DNA fragments were separated by electrophoresis in 0.7% (wt/vol) agarose. Then they were transferred to a nitrocellulose filter for hybridization with a [^{32}P]DNA probe. Hybridization and autoradiography were performed as described previously (22).

Preparation of cytoplasmic membrane vesicles. Cytoplasmic membrane vesicles were prepared by the method of Yamato et al. (34), as modified by Hanada et al. (11).

Transport assay. The glutamate transport activity in intact cells was measured in B7 medium containing 20 mM glucose, 40 μg of chloramphenicol per ml, salts at the indicated concentrations, and 2 μM L-[U-^{14}C]glutamic acid (285 mCi/mmol) by a filtration method (27). The transport activity of cytoplasmic membrane vesicles was also measured by a filtration method. The standard assay medium (0.1 ml) contained 50 mM Tris-2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 8.0), 2 mM $MgSO_4$, salts at the indicated concentrations, and cytoplasmic membrane vesicles (40 to 50 μg of protein). After preincubation for 2.5 min at 25°C, L-[U-^{14}C]glutamic acid was added to a final concentration of 2 μM and incubation was continued for 30 s. The reaction was started by adding a respiratory substrate, that is, ascorbic acid-KOH plus phenazine methosulfate (PMS), at concentrations of 20 mM and 100 μM, respectively. The reaction was terminated by diluting the mixture with 5 ml of cold 0.1 M LiCl. The mixture was quickly filtered through a nitrocellulose filter (pore size, 0.45 μm; Toyo) by suction, and the filter was washed once with 5 ml of the same solution. The radioactivity that was trapped on the filter was measured. The aspartate transport activity was measured in the same way as that of glutamate transport, but 2 μM L-[U-^{14}C]aspartic acid (228 mCi/mmol) was used instead of L-[U-^{14}C]glutamic acid.

Binding assay. Specific binding of glutamate to carriers in cytoplasmic membrane vesicles was measured by a centrifugation method described previously (26).

Analysis of gene products in minicells. Strain ST5101 was transformed with the recombinant plasmids, and transformants were selected for the ampicillin resistance phenotype. Minicells were purified by two sequential sucrose density gradient centrifugations as described previously (1) and suspended in methionine assay medium containing 1 mM concentrations of all amino acids except methionine. Then L-[^{35}S]methionine (1,215 Ci/mmol) was added at a final concentration of 100 μCi/ml to the minicell suspension at 37°C. After incubation for 30 min at 37°C, the minicell suspension was centrifuged and the precipitate was washed, suspended in 20 mM Tris hydrochloride (pH 7.5), and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and fluorography (3).

Other procedures. Protein was measured by the method of Lowry et al. (19), with bovine serum albumin used as a standard. SDS-PAGE (12.5% [wt/vol]) was performed as described previously (17). Samples for SDS-PAGE were dissolved in SDS buffer by incubation at 37°C for 30 min.

Chemicals. MES was purchased from Sigma Chemical Co. (St. Louis, Mo.). Restriction endonucleases were obtained from Takara Shuzo Co. (Kyoto, Japan). L-[U-^{14}C]glutamic acid (285 mCi/mmol), L-[U-^{14}C]aspartic acid (228 mCi/mmol), L-[^{35}S]methionine (1,215 Ci/mmol), nick-translation kit with [α-^{32}P]dATP (3000 Ci/mmol), and rainbow protein molecular weight markers were purchased from Amersham Japan (Tokyo, Japan). Other reagents were standard commercial products of analytical grade.

RESULTS

Cloning of the *gltS* and *gltP* genes. Wild-type strains of *E. coli* K-12 and B do not grow with glutamate as the sole source of carbon and nitrogen. On the other hand, mutants showing increased activity for glutamate uptake can grow on this substrate (10). Therefore, we thought that a glutamate carrier-overproducing strain could grow on glutamate. A chromosomal DNA library of an *E. coli* B derivative, strain 29-78, constructed with cosmid vector pHC79 was packaged in vitro and was infected into ST131, which is a restriction-deficient strain of K-12. Positive transformants were selected based on their ability to grow with 22 mM glutamate as the sole source of carbon and nitrogen.

We isolated two kinds of clones, ST131(pDES70) and ST131(pDEH17); they showed different dependences on Na^+ for growth on glutamate. ST131(pDES70) grew rapidly on glutamate only when Na^+ was present in the medium, and ST131(pDEH17) grew rapidly irrespective of the presence of Na^+. We prepared pDES70 and pDEH17 and transformed a *gltS*-deficient strain of K-12, ST204 *recA*, with them. Both plasmids conferred on the strain the ability to grow well on glutamate.

We determined the restriction enzyme sites of pDES70 and pDEH17 and constructed several deletion derivatives (Fig. 1). Intact cells of ST204 *recA* harboring pDES7001, pDES7002, and pDES7004 showed glutamate uptake activity, but those harboring pDES7003 did not (data not shown). These results suggest that the *gltS* gene is located in the 3.2-kilobase *Eco*RI-*Mlu*I fragment. pDEH17 did not have the same restriction enzyme sites as those of the *Eco*RI-*Mlu*I fragment; furthermore, the inserted DNA fragment of pDES70 did not hybridize to that of pDEH17 in a Southern blot hybridization test (data not shown). These results suggest that pDEH17 does not contain the *gltS* gene but contains a gene for another glutamate carrier. We named this gene *gltP*.

Characterization of glutamate carriers encoded by the *gltS* and *gltP* genes. We prepared cytoplasmic membrane vesicles from ST204 *recA*(pDES70) and ST204 *recA*(pDEH17) and

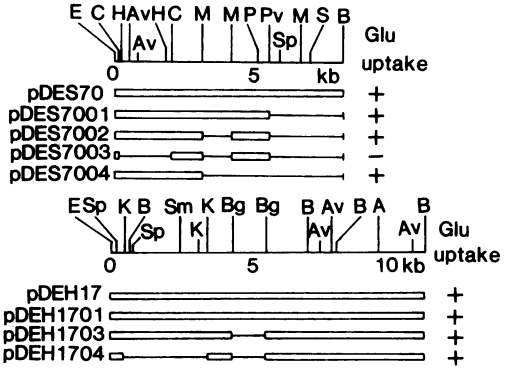

FIG. 1. Restriction maps of insert DNA fragments of pDES70, pDEH17, and their derivatives. Restriction sites were determined by 0.7% agarose gel electrophoresis. Fragments harbored by recombinant plasmids are indicated with open bars. Thin lines represent the extents of deletions. pDES70 carries the *Eco*RI-*Bam*HI fragment, with pBR322 used as a vector. pDES7001 is a *Pvu*II deletion derivative of pDES70. pDES7002 and pDES7003 are *Mlu*I, and *Mlu*I and *Cla*I deletion derivatives, respectively, of pDES70. pDES7004 contains the *Eco*RI-*Mlu*I DNA fragment derived from the insert DNA fragment of pDES70. pDEH17 carries the *Eco*RI-*Bam*HI fragment, with pHC79 used as a vector. pDEH1701 carries the same fragment, with pBR322 used as a vector. pDEH1703 and pDEH1704 are *Bgl*II, and *Bgl*II and *Kpn*I deletion derivatives, respectively, of pDEH1701. Restriction endonuclease abbreviations: A, *Acc*I; Av, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sm, *Sma*I; Sp, *Sph*I. Glu uptake + indicates that ST204*recA* cells harboring the indicated plasmid showed increased glutamate uptake activity, and Glu uptake − indicates that the cells had low uptake activity. kb, Kilobases.

measured their glutamate uptake activities (Fig. 2). The glutamate uptake activity of membrane vesicles from ST204 *recA*(pDES70) depended on the Na^+ concentration, which was maximal with 10 mM Na^+. On the other hand, the activity of ST204 *recA*(pDEH17) did not depend on the Na^+ (Fig. 2B) or K^+ or Li^+ (data not shown) concentrations.

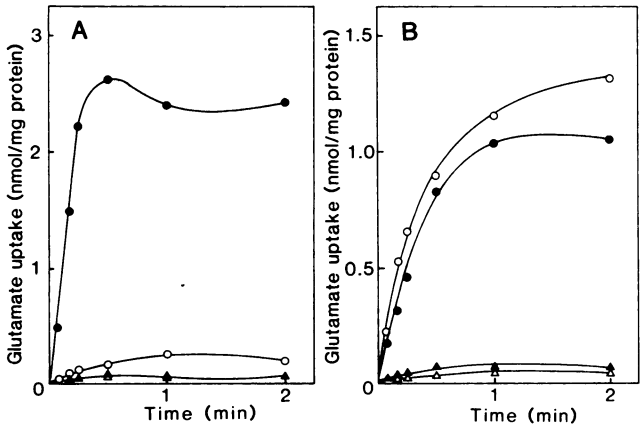

FIG. 2. Glutamate uptake by cytoplasmic membrane vesicles from ST204 *recA*(pDES70), ST204 *recA*(pDEH17), and ST204 *recA* driven by 20 mM ascorbic acid–KOH plus 100 μM PMS. Membrane vesicles were prepared as described in the text. (A) Glutamate uptake by membrane vesicles from ST204 *recA*(pDES70) (●, ○) and ST204 *recA* (▲, △) in the presence of 10 mM NaCl (●, ▲) or 10 mM KCl (○, △). (B) Glutamate uptake by membrane vesicles from ST204 *recA*(pDEH17) (●, ○) and ST204 *recA* (▲, △) in the presence of 10 mM NaCl (●, ▲) or 10 mM KCl (○, △).

TABLE 2. Parameters of glutamate and aspartate transport and binding of cytoplasmic membrane vesicles[a]

Strain	Amino acid	Transport		Binding	
		K_t	V_{max}	K_d	BS_{max}
ST204 *recA*(pDES70)	Glu	3.5	5.2	6.9	1.8
ST204 *recA*(pDEH17)	Glu	11	2.6	ND[b]	ND
	Asp	3.2	2.3	7.2	0.14

[a] The initial rates of transport activities were measured as described in the text in the presence of 20 mM NaCl for 10 s. The K_t and V_{max} values are expressed in micromolar and nanomoles per milligrams of protein per minute, respectively. Binding activities were measured at pH 6.1 for glutamate binding and pH 5.0 for aspartate binding in the presence of 40 mM NaCl. The K_d and the maximum number of binding sites (BS_{max}) are expressed in micromolar and nanomoles per milligram of protein, respectively. At a concentration of 1 μM, glutamate bound to the cytoplasmic membrane vesicles from ST204 *recA*(pDEH17) was less than 5 pmol/mg of protein, and the K_d and BS_{max} values were not determined.

[b] ND, Not determined.

Membrane vesicles from ST204 *recA*(pDES70) bound glutamate in an Na^+-dependent manner (data not shown). The Michaelis constant of transport (K_t), the maximum rate of transport (V_{max}) values for glutamate transport, the dissociation constant (K_d), and the maximum number of binding sites (BS_{max}) of glutamate binding are summarized in Table 2. The K_t for transport and K_d for binding of ST204 *recA*(pDES70) agreed well with those of a wild-type strain of *E. coli* B which Fujimura et al. (8, 9) examined previously. Therefore, we concluded that pDES70 carries the *gltS* gene of *E. coli* B. The BS_{max} value of ST204 *recA*(pDES70) (1.8 nmol/mg of protein) was about 25 times that of the wild-type strain of *E. coli* B (70 pmol/mg of protein), indicating that the content of glutamate carrier is amplified about 25-fold in ST204 *recA*(pDES70).

The substrate specificities and energy-coupling properties of the glutamate carriers were examined. The uptake activities were measured in the presence of several amino acids that are structural analogs of L-glutamate (Table 3). The glutamate uptake by the *gltP* carrier was completely inhibited by 5 mM L-aspartate and partially inhibited by 5 mM L-asparagine. On the other hand, L-aspartate and L-asparagine at the same concentration did not inhibit the glutamate uptake by the *gltS* carrier. These results suggest that the substrate specificities of the two glutamate carriers are different. The effects of an uncoupler, carbonylcyanide *m*-chlorophenylhydrazone (CCCP), and an ionophore, monensin, on glutamate uptake are shown in Fig. 3. CCCP (10 μM) completely inhibited glutamate uptake by both carriers, whereas monensin (3 μM) inhibited only that by the *gltS* carrier.

TABLE 3. Inhibition of glutamate uptake by amino acids[a]

Amino acid	% Inhibition in:	
	ST204 *recA*(pDES70)	ST204 *recA*(pDEH17)
None	0	0
L-Glutamate	99	97
D-Glutamate	98	63
L-Aspartate	0	97
L-Glutamine	88	76
L-Asparagine	0	82

[a] Initial rates of glutamate uptake by membrane vesicles from ST204 *recA*(pDES70) and ST204*recA*(pDEH17) was measured as described in the text in the presence of a 5 mM concentration of the indicated amino acids for 10 s.

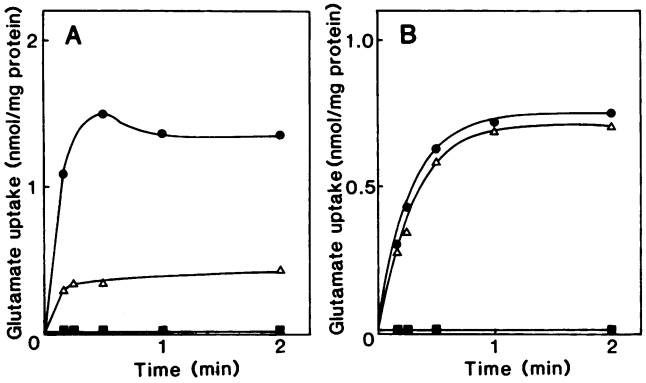

FIG. 3. Effects of inhibitors on glutamate uptake by membrane vesicles. Glutamate uptake by membrane vesicles form ST204 *recA*(pDES70) (A) and ST204 *recA*(pDEH17) (B) driven by 20 mM ascorbic acid–KOH plus 100 μM PMS in the presence of 5 mM NaCl was measured with no addition (●) or in the presence of 10 μM CCCP (■) or 3 μM monensin (△).

Characterization of aspartate transport by the *gltP* carrier. As glutamate uptake by membrane vesicles from ST204 *recA*(pDEH17) was completely inhibited by 5 mM L-aspartate, we measured the aspartate uptake activity directly. Aspartate was actively taken up by the membrane vesicles, and the uptake was driven by a respiratory substrate and was not dependent on the Na^+ concentration (Fig. 4). Furthermore, the uptake activity was completely inhibited by 5 mM L-glutamate (data not shown). On the other hand, the membrane vesicles from ST204 *recA*(pDES70) and ST204 *recA* did not take up aspartate, irrespective of the presence or absence of Na^+. These results were strongly suggestive that the *gltP* gene carried on pDEH17 is a structural gene for a carrier protein that is responsible for Na^+-independent, binding protein-independent, glutamate-aspartate transport. The kinetic parameters of the aspartate transport by the membrane vesicles are summarized in Table 2. The K_t for aspartate was one-fourth of that for glutamate.

Biochemical identification of the *gltS* gene product. The SDS-PAGE profile of cytoplasmic membrane vesicles from ST204 *recA*(pDES70) showed that the density of one band corresponding to an apparent molecular weight of 35,000 was greatly increased, whereas the densities of other bands

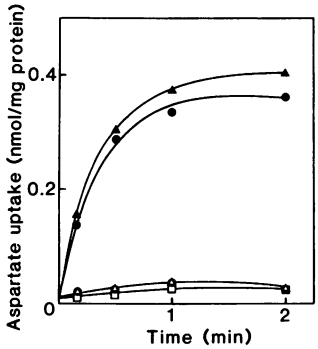

FIG. 4. Aspartate uptake by cytoplasmic membrane vesicles from ST204 *recA*(pDEH17), ST204 *recA*(pDES70), and ST204 *recA*. Aspartate uptake was measured in the same way as glutamate uptake, but 2 μM aspartate was used instead of 2 μM glutamate. The strains and NaCl concentrations added to the uptake mixture were as follows: ST204 *recA*(pDEH17), 0 mM (●) and 100 mM (▲); ST204 *recA*(pDES70), 20 mM (□); ST204 *recA*, 0 mM (△) and 100 mM (○).

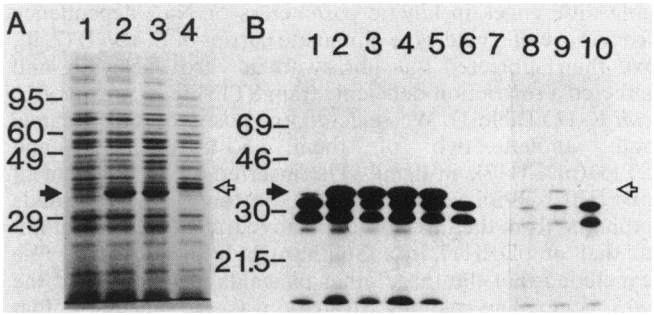

FIG. 5. (A) SDS-PAGE of membrane vesicles. Membrane vesicles were prepared as described in the text and analyzed by SDS–12.5% PAGE. Lanes: 1, ST204 *recA*; 2, ST204 *recA* (pDES70); 3, ST131(pDES70); 4, ST204 *recA*(pDEH17). The solid arrow indicates a protein with an M_r of 35,000 that was amplified in membrane vesicles from ST204 *recA*(pDES70) and ST131(pDES70). The open arrow indicates a protein with an M of 38,000 that was slightly amplified in membrane vesicles from ST204 *recA*(pDEH17). Molecular weight markers were as follows: phosphorylase *b*, 95,000; catalase, 60,000; fumarase, 49,000; bovine carbonic anhydrase B, 29,000. (B) Proteins synthesized in minicells containing recombinant plasmids. Proteins were labeled with L-[^{35}S]methionine in minicells containing recombinant plasmids and then analyzed by SDS–12.5% PAGE followed by fluorography. The solid arrow indicates the *gltS* gene product. The open arrow indicates a protein with an M_r of 38,000 that is likely to be the *gltP* gene product. The plasmids used were as follows: Lane 1, pBR322; land 2, pDES70; and 3, pDES7001; lane 4, pDES7002; lane 5, pDES7004; lane 6, pDES7003; lane 7, no plasmid; lane 8, pDEH1701; lane 9, pDEH1704; lane 10, pBR322. Molecular weight markers are rainbow protein molecular weight markers (Amersham).

were rather similar to those of ST204 *recA* (Fig. 5A, lanes 1 and 2). This suggests that the protein with an M_r of 35,000 might be the *gltS* gene product.

The products encoded by pDES70 and its derivatives are labeled with L-[^{35}S]methionine in a minicell system and analyzed by SDS-PAGE and fluorography. As shown in Fig. 5B (lanes 2 to 6), a polypeptide with an M_r of 35,000 was found in the products of pDES70, pDES7001, pDES7002, and pDES7004 but not in those of pDES7003. The three polypeptides with molecular weights of 32,000, 29,000, and 26,000 that were also present in the products of pBR322 (Fig. 5B, lane 1) were from β-lactamase encoded by the Ap^r gene (6). The polypeptide with an M_r of 34,000 (Fig. 5B, lane 1) was probably a product of the tetracycline resistance gene. As mentioned above, intact cells of ST204 *recA* harboring any of the deletion derivatives of pDES70 except pDES7003 showed increased glutamate uptake activity, indicating that the protein with an M_r of 35,000 is the *gltS* gene product.

We also analyzed the products encoded by derivatives of pDEH17 in a minicell system (Fig. 5B, lanes 8 and 9). The products of both pDEH1701 and pDEH1704 gave a band corresponding to an apparent M_r of 38,000. Close inspection of the SDS-PAGE profile of membrane vesicles from ST204 *recA*(pDEH17) showed that this protein was also slightly amplified (Fig. 5A, lane 4). These results suggest that the protein with an M_r of 38,000 is the product of the *gltP* gene.

DISCUSSION

To clone a putative *gltS* gene of *E. coli* B, we first prepared a chromosomal DNA library of the organism, which was strain 29-78 of *E. coli* B. This strain was shown to have enhanced glutamate transport activity, with no remark-

able differences in kinetic parameters or Na^+ dependence from those of a wild-type glutamate carrier of *E. coli* B (7, 9). We then subjected this library to in vitro packaging and infected a restriction-deficient strain ST131 (r_K^-, m_K^+) of *E. coli* K-12 (Table 1). We selected five positive transformants and studied two of them, ST131(pDES70) and ST131(pDEH17), in detail. The inserted DNA fragment of pDES70 hybridized to those of the three other plasmids prepared from the three other positive transformants but not to that of pDEH17 in a Southern hybridization test. We concluded that the three other plasmids also contained the *gltS* gene. This cloning strategy was so successful that expression of the *gltS* gene on pDES70 in the ST204 *recA* strain was 25-fold more than that in wild-type *E. coli* B and complemented the *gltS* deficiency of the host strain. Through this experimental operation, we could, fortunately, also clone a new gene that was responsible for glutamate utilization by the cells. We named this gene *gltP* and showed that it is a good candidate for the gene encoding a glutamate-aspartate carrier.

We concluded that pDES70 and all its derivatives except pDES7003 contain the *gltS* gene for the following reasons. (i) The plasmids pDES70, pDES7001, pDES7002, and pDES7004 conferred a *gltS*-deficient strain, ST204 *recA*, with the ability to utilize glutamate as a sole source of carbon and to take up glutamate. (ii) Cytoplasmic membrane vesicles prepared from ST204 *recA*(pDES70) retained glutamate uptake activity driven by a respiratory substrate, such as ascorbate-PMS. (iii) Glutamate transport and binding activities were Na^+ dependent, and the parameters of these reactions coincided with those of a wild-type strain of *E. coli* B which Fujimura et al. (8, 9) reported previously.

Several lines of evidence indicated that pDEH17 and its derivatives contain another gene, *gltP*, that was distinct from *gltS*. (i) pDEH17 and its derivatives conferred ST204 *recA* with the ability to utilize glutamate as the sole source of carbon. (ii) Cytoplasmic membrane vesicles from ST204 *recA*(pDEH17) showed that the uptake activity for glutamate and aspartate is driven by a respiratory substrate but is not dependent on the Na^+ concentration. (iii) The restriction sites of pDEH17 were different from those of pDES70, and the inserted DNA fragment of pDES70 did not hybridize to that of pDEH17. (iv) The substrate specificity and the energy dependence of glutamate transport of membrane vesicles of ST204 *recA*(pDEH17) were different from those of ST204 *recA*(pDES70). Based on following reasons, we suggest that the *gltP* gene is likely to be a structural gene for this glutamate-aspartate carrier. *E. coli* uses ammonia preferably as a source of nitrogen under ammonia-rich conditions, and under ammonia-limited conditions several genes which are relevant to nitrogen assimilation are derepressed (21). We selected a positive clone, ST204 *recA*(pDEH17), for its ability to grow on glutamate, and under this condition nitrogen control is thought to be derepressed because of the absence of ammonia (21). ST204 *recA*, however, could not grow on glutamate under these conditions, indicating that there was no uptake activity of glutamate. Furthermore, there has been no evidence reported to show the uptake activity of glutamate in *E. coli* K-12 with the same properties as those of the *gltP* transport system. Therefore, we suggest that *E. coli* K-12 does not have the *gltP* transport system and that we cloned a structural gene for a *gltP* carrier of *E. coli* B. However, there is another possibility that the structural gene for the *gltP* transport system exists in *E. coli* K-12, but that its gene expression is severely suppressed, whereas the fact that the *gltP* gene is a putative gene for a positive regulator of transcription of this cryptic carrier gene cannot be excluded.

Schellenberg and Furlong (30) have reported that *E. coli* D_2W has five separate transport systems for glutamate, aspartate, or both: (i) a binding protein-dependent, Na^+-independent glutamate-aspartate system; (ii) a binding protein-independent, Na^+-independent glutamate-aspartate system; (iii) a binding protein-independent, Na^+-dependent glutamate-specific system; (iv) a binding protein-independent, aspartate-specific system; or (v) a dicarboxylic acid transport system. Judging from the substrate specificity of the *gltP* carrier and the effects of the inhibitors CCCP and monensin, this carrier seems to correspond to the binding protein-independent, Na^+-independent glutamate-aspartate transport system.

A product of the *gltS* gene was identified as a cytoplasmic membrane protein with an apparent M_r of 35,000 (Fig. 5B). In many cases, the molecular weights of membrane carriers estimated by SDS-PAGE are rather smaller than those deduced from their predicted amino acid sequences (4, 29, 35). Thus, the actual molecular weight of the *gltS* gene product may be more than 35,000.

The *gltP* gene product was also analyzed in a minicell system. One protein band corresponding to an apparent M_r of 38,000 was encoded by pDEH1701 and pDEH1704, as well as Ap^r gene products, but no other bands were detected (Fig. 5B, lanes 8 and 9). Therefore, the protein with an M_r of 38,000 may be the *gltP* gene product. This is still uncertain, however, because pDEH1704 has an inserted DNA fragment of about 6 kilobases. Therefore, it probably contains other open reading frames in addition to that of the *gltP* gene.

In this study we cloned genes coding for an Na^+-dependent glutamate carrier and an Na^+-independent glutamate-aspartate carrier. Comparison of the primary structures of the two glutamate carriers would provide important information on substrate recognition sites and cation-binding sites.

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