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Cloning and Sequencing of a Gene Encoding a Glutamate and Aspartate Carrier of *Escherichia coli* K-12

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A gene encoding a carrier protein for glutamate and aspartate was cloned into *Escherichia coli* K-12 strain BK9MDG by using the high-copy-number plasmid pBR322. The gene (designated *gltP*) is probably identical to a gene recently cloned from *E. coli* B (Y. Deguchi, I. Yamato, and Y. Anraku, J. Bacteriol. 171:1314–1319). A 1.6-kilobase DNA fragment containing *gltP* was subcloned into the expression plasmids pT7-5 and pT7-6, and its product was identified by a phage T7 RNA polymerase-T7 promoter coupled system (S. Tabor and C. C. Richardson, Proc. Natl. Acad. Sci. USA 82:1074–1078) as a polypeptide with an apparent mass of 38 kilodaltons. A portion of the *gltP* polypeptide was associated with the cytoplasmic membrane. The nucleotide sequence of the 1.6-kilobase fragment was determined. It contained an open reading frame capable of encoding a highly hydrophobic polypeptide of 395 amino acids, containing four possible transmembrane segments. Uptake of glutamate and aspartate was increased 5.5- and 4.5-fold, respectively, in strains containing *gltP* plasmids. Glutamate uptake was insensitive to the concentration of Na^+ and was inhibited by L-cysteate and β-hydroxyaspartate. These results suggest that *gltP* is a structural gene for a carrier protein of the Na^+-independent, binding-protein-independent glutamate-aspartate transport system.

Escherichia coli K-12 strains generally do not grow well on L-glutamate as a sole source of carbon and energy, probably because the transport systems for glutamate are repressed in wild-type strains (11). Mutants with derepressed levels of glutamate uptake were isolated by selecting for sensitivity to toxic analogs of glutamate (6, 15). Studies with these and other strains identified five systems for dicarboxylic amino acid transport in *E. coli* DW2 (15): (i) a binding-protein-independent, Na^+-dependent, glutamate-specific system; (ii) a binding-protein-dependent, Na^+-independent system for transport of glutamate and aspartate; (iii) a binding-protein-independent, Na^+-independent glutamate-aspartate system; (iv) a binding-protein-independent, aspartate-specific system; and (v) a dicarboxylic acid transport system that carries aspartate in addition to malate, fumarate, and succinate. Cloning of the genes encoding the various components of the glutamate-aspartate transport systems would provide a method for studying regulation of expression of the genes as well as the nature and function of the gene products.

In a recent paper (4), the cloning of two *E. coli* B genes for glutamate and aspartate transport was described. One gene (*gltS*) specified a glutamate-specific, Na^+-dependent carrier and corresponds to the *E. coli* K-12 *gltS* gene mapped at 82 min on the *E. coli* chromosome (11). The other gene (*gltP*) specified a Na^+-independent, glutamate-aspartate carrier. In this paper, we describe the cloning, expression, sequencing, and function of a gene for glutamate-aspartate transport. Since there are significant similarities between the properties of the *gltP* gene cloned by Deguchi et al. (4) and the gene described here, we also used the gene designation *gltP*.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. *E. coli* K-12 strain BK9MDG [F^- *thi metC hsdS* (r^- m^-) *endB* Δ(*glnHPQ-chlE*)], constructed as described previously (13), was used throughout this study. Strain BJW1 was derived from BK9MDG by selecting for growth on glutamate. The plasmids used were pBR322, pT7-5, pT7-6, and pGPI-2. Nucleotide sequencing was performed by using phages M13mp18 and M13mp19.

Media. Strains were grown in either a rich medium (nutrient broth, medium 869, YT [containing, per liter of water, 8 g of Bacto-Tryptone {Difco Laboratories, Detroit, Mich.}, 5 g of yeast extract {Difco}, and 2.5 g of NaCl]) or medium E (17) supplemented with 40 μM thiamine HCl, 0.4 mM L-methionine, and 0.5% (wt/vol) D-glucose, L-glutamate, or glutamine. For uptake studies with whole cells and for the preparation of membrane vesicles, bacteria were grown in medium A (2) supplemented with 40 μM thiamine HCl, 0.4 mM L-methionine, 0.5% (wt/vol) L-glutamate, and 0.5% (wt/vol) sodium succinate. Antibiotics were used at the following concentrations: ampicillin, 35 μg/ml: tetracycline, 15 μg/ml; kanamycin, 40 μg/ml, and rifampin, 200 μg/ml. Solid medium contained 1.5% (wt/vol) Bacto-Agar (Difco).

Enzymes and chemicals. Restriction endonuclease and DNA enzymes were from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.), and New England BioLabs, Inc. (Beverly, Mass.). All chemicals of reagent grade were obtained from commercial sources.

Manipulations of DNA. Plasmid DNA was prepared from cleared lysates by CsCl-ethidium bromide centrifugation, as described by Davis et al. (3). The methods described by Maniatis et al. (10) were used for DNA manipulations.

Identification of gene products. The phage T7 RNA polymerase-T7 promoter coupled system of Tabor and Richardson (16) was used to identify gene products, with the exception that labeling with [^{35}S]methionine was for 10 rather than 5 min.

Determination of cellular location of gene products. Cells were incubated with [^{35}S]methionine for either 2 or 20 min to label the plasmid-encoded proteins that were synthesized by the phage T7 RNA polymerase-T7 promoter coupled system. Membrane and supernatant fractions, prepared from

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the labeled cells as previously described (5), were examined by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis. For SDS-polyacrylamide gel electrophoresis, the stacking gel contained 3% acrylamide–0.08% bisacrylamide in 0.12 M Tris hydrochloride (pH 6.8), and the separation gel contained 12% acrylamide–0.32% bisacrylamide in 0.375 M Tris hydrochloride (pH 8.8). Both gels contained SDS at 0.1%. Electrophoresis was at 30 mA in buffer consisting of 0.1 M Tris hydrochloride (pH 8.3), 0.28 M glycine, and 0.4% SDS and was run until the tracking dye was approximately 1 cm from the bottom of the gel (about 4 h). Samples were boiled for 3 min prior to application. A series of cross-linked cytochrome *c* proteins were used as molecular weight standards. Gels were stained with Coomassie brilliant blue, treated with enhancer (Dupont, New England Nuclear Research Products, Boston, Mass.), dried, and exposed to X-ray film for autoradiography.

DNA sequencing. Suitable restriction fragments were cloned into the phage vectors M13mp18 and M13mp19, with *E. coli* JM107 as the host (19). DNA sequencing was by the chain termination method (14) with [^{35}S]dATP. Sequencing was begun with a universal primer suitable for both M13mp18 and M13mp19 DNAs. As nucleotide sequences became known, new primers (17- or 18-mers) were synthesized with a Cyclone DNA synthesizer and then used to sequence further into the insert DNA.

Uptake of glutamate and aspartate by whole cells. Cells from mid-exponential-phase cultures were harvested by centrifugation and then washed three times and suspended in 50 mM potassium phosphate buffer (pH 6.9) containing 0.5 mM $MgSO_4$ (buffer A). The assay for uptake of glutamate and aspartate was performed essentially as described by Willis and Furlong (18). The assay mixture contained cell suspension in buffer A (0.37 mg [dry weight] of bacteria per ml), 0.5% (wt/vol) D-glucose, 100 μg of chloramphenicol per ml, and 40 mM NaCl. Where applicable, inhibitors were added to the following concentrations; 24 mM L-cysteate, 34 mM methyl-DL-glutamate, 6.7 mM DL-threo-β-hydroxyaspartate. Reaction mixtures were preincubated for 15 min at 37°C prior to the addition of substrates. ^{14}C-labeled substrates (each at 10 μM) were added to the following specific activities: L-[U-^{14}C]glutamate (53 mCi/mmol) and L-[U-^{14}C]aspartate (55 mCi/mmol). Samples (200 μl) were removed at regular intervals, and the cells were collected by vacuum filtration on membrane filters (type HA, 0.45-μm pore size; Millipore Corp., Bedford, Mass.) that had been presoaked in buffer A containing 300 μg of chloramphenicol per ml. Each sample was rapidly washed with ice-cold buffer A (5 ml). The membranes were dried, and the radioactivity present was counted in toluene-based scintillation fluid (5 g of 2,5-diphenyloxazole [PPO] and 0.3 g of 1,4-bis[4-methyl-5-phenyl-2-oxazolyl]benzene [dimethyl POPOP] per liter of toluene).

Preparation of membrane vesicles. The cells from mid-exponential-phase cultures were harvested by centrifugation, washed twice with 10 mM Tris hydrochloride buffer (pH 8.0), and used to prepare membrane vesicles essentially as described by Kaback (7). Vesicles were suspended to a concentration of 5.5 mg of protein per ml in 0.1 M potassium phosphate buffer (pH 6.6) containing 10 mM EDTA and stored at −70°C in small portions. The protein concentration was determined by the method of Lowry et al. (9), with bovine serum albumin as the standard.

Glutamate and aspartate transport by membrane vesicles. Assays of glutamate and aspartate transport by membrane vesicles were performed the same way as whole-cell uptake assays, with the exception that the assay mixture contained vesicles (1 mg of protein per ml), 40 mM NaCl, 20 mM D-lactate, 25 mM $MgSO_4$, and 50 mM potassium phosphate buffer (pH 6.6). The filter membranes were washed with ice-cold potassium phosphate buffer (5 ml).

RESULTS

Isolation and characterization of a Glt$^+$ (suppressed) strain. The host used for gene cloning was *E. coli* K-12 strain BK9MDG, which contains a deletion, extending from *glnH* to *chlE* (13), that abolishes glutamine transport and growth

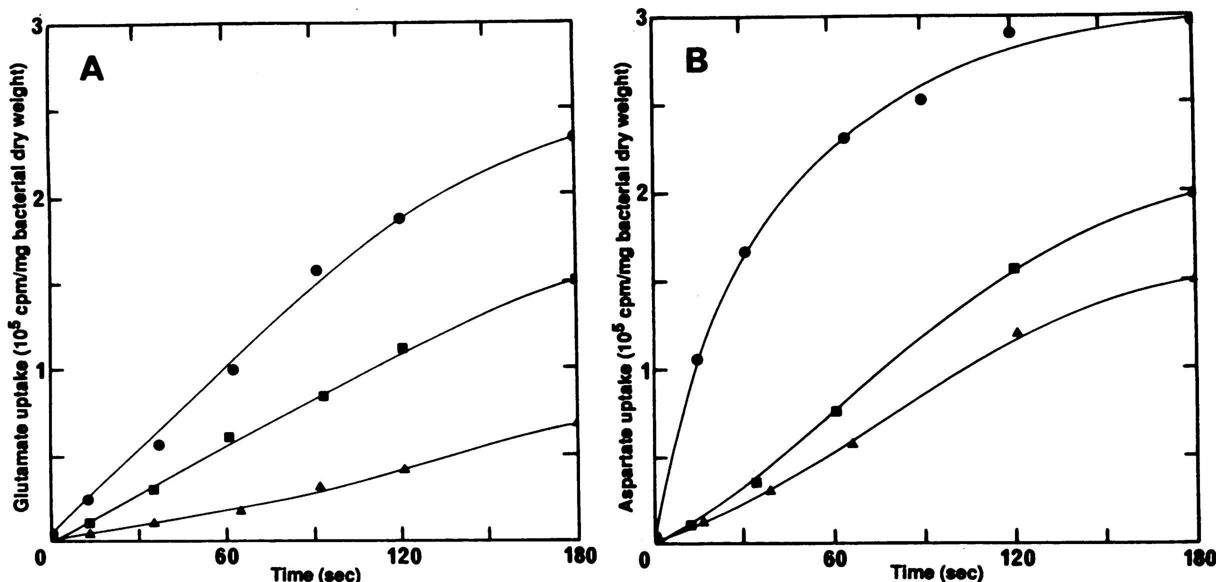

FIG. 1. Glutamate (A) and aspartate (B) uptake by whole cells of strains BK9MDG (▲), BJW1 (■), and DLH1 (●). Preparation of cell suspensions and conditions of uptake were as described in the text. Strain DLH1 was prepared by transforming BK9MDG with pBW8.

TABLE 1. Inhibition of glutamate uptake in strains BK9MDG, BJW1, and DLH1 by analogs of glutamate

Inhibitor	Concn (mM)	% Inhibition of glutamate uptake[a]		
		BK9MDG	BJW1	DLH1[b]
L-Cysteate	24	57	80	96
DL-Threo-β-hydroxyaspartate	6.7	27	70	93
α-Methyl-DL-glutamate	34	66	35	0

[a] Percent inhibition of glutamate uptake was determined by comparing initial uptake rates (calculated from both 1- and 2-min samples) in the presence and absence of inhibitors.
[b] Strain BK9MDG was transformed with pBW8.

on glutamine. Strain BK9MDG failed to grow, even slowly, on glutamate as a sole source of carbon and energy. The Glt^- phenotype was consistent with that of other strains of *E. coli* harboring mutations in the *glnHPQ* operon (12). If the inability of BK9MDG to grow on glutamate resulted from deficient transport of this substrate, then using BK9MDG as a host for cloning might allow for the selection of genes specifying glutamate carriers. Furthermore, investigation of the function of genes that reverse the Glt^- phenotype of BK9MDG may provide some insight into the relationship between mutations at *glnHPQ* and the ability to use glutamate as a carbon and energy source.

When strain BK9MDG was plated on mineral salts agar containing glutamate as the sole carbon source, clones arose with a frequency of 10^{-9} to 10^{-10}, confirming an earlier observation of Masters and Hong (12). These clones had not regained the ability to utilize glutamine and must have contained suppressor mutations that conferred the Glt^+ phenotype. One of the clones, BJW1, that grew well on glutamate was chosen for further investigation.

The rates of uptake of glutamate and aspartate by strain BJW1 were found to be 3- and 1.4-fold, respectively, those of strain BK9MDG (Fig. 1), suggesting that in the suppressed strain, a transport system for glutamate, and possibly for aspartate, was derepressed. The accumulation of glutamate was inhibited to a greater extent by L-cysteate and by β-hydroxyaspartate and to a lesser extent by α-methyl-DL-glutamate in strain BJW1 than in BK9MDG (Table 1).

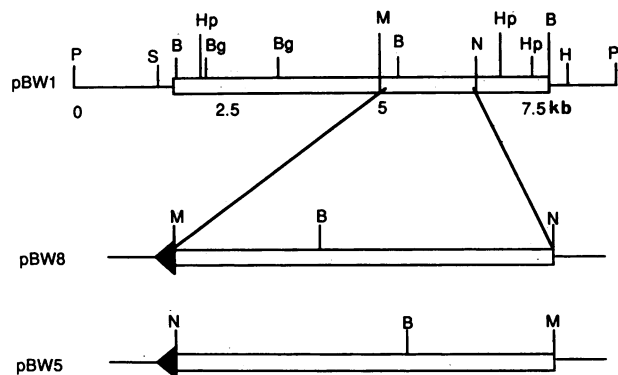

FIG. 2. Restriction map of insert DNA of pBW1 and of subclones pBW5 and pBW8. pBW1 was derived by ligating *Sau*3A fragments of DNA (from strain BJW1) into the *Bam*HI site of pBR322 (see text) and carries a DNA insert of 6.2 kb. Restriction sites were determined from an analysis of DNA fragments produced by cleavage on 0.7% agarose gels. ▭, Fragments contained within the recombinant plasmids; ——, vector DNA. pBW5 and pBW8 each carry the *Mlu*I-*Nsi*I fragment (1.6 kb) of pBW1, but in opposite orientations. Abbreviations: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Hp, *Hpa*I; M, *Mlu*I; N, *Nsi*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I.

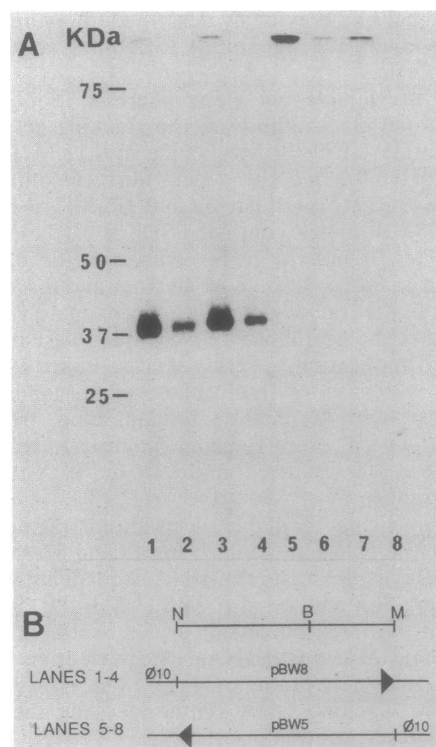

FIG. 3. Identification of the *gltP* gene product and the direction of transcription of *gltP*. (A) Radioautogram from SDS-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled, plasmid-mediated proteins synthesized in the T7 RNA polymerase-T7 promoter coupled system (16). Lanes 1 through 4, pT7-6 plasmids containing the 1.6-kb DNA fragment derived by cleavage of pBW1 with *Mlu*I and *Nsi*I. Lanes 1 and 3 (duplicates) were loaded with sonic extracts containing 6×10^5 and 8×10^5 cpm, respectively; lanes 2 and 4 (duplicates) contained 2×10^5 and 3×10^5 cpm, respectively. Lanes 5 through 8, pT7-5 plasmids containing the 1.6-kb fragment. Lanes 5 and 7 (duplicates) were loaded with sonic extracts containing 2×10^4 and 8×10^3 cpm, respectively; lanes 6 and 8 (duplicates) contained half as many counts per minute as lanes 5 and 7. The molecular mass markers are given in kilodaltons. (B) Plasmids were constructed as described in the text by inserting a 1.6-kb fragment of DNA (▶), derived by digestion of pBW1 with *Mlu*I and *Nsi*I, into either pT7-6 (yielding pBW8) or pT7-5 (yielding pBW5). The orientation of the fragment in pBW8 is the reverse of that in pBW5. φ10, Phage T7 promoter (arrows indicate direction of transcription).

A puzzling feature of the behavior of strain BK9MDG was that although uptake by BK9MDG occurred at approximately one-third the rate of the uptake by BJW1, strain BJW1 grew well on glutamate plates, whereas BK9MDG did not grow on this medium even after several days of incubation. Both strains grew well on L-aspartate as the sole source of carbon and energy.

Cloning of a glutamate-aspartate transport gene. The results of the uptake experiments suggested that increased expression of glutamate and aspartate transport genes reversed the Glt^- phenotype of strain BK9MDG. We therefore used a multi-copy-number plasmid to clone glutamate transport genes using strain BK9MDG as a host. DNA from strain BJW1 was isolated and partially restricted with *Sau*3A.

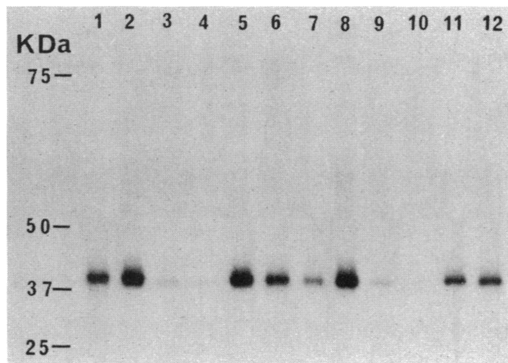

FIG. 4. Cellular location of *gltP*-encoded protein. Membrane and supernatant fractions from sonicated cells with [^{35}S]methionine-labeled, plasmid pBW8-encoded proteins synthesized in the T7 RNA polymerase-T7 promoter coupled system (16) were prepared as described previously (5) and analyzed by SDS-polyacrylamide gel electrophoresis. Samples of membrane and supernatant fractions containing approximately 20,000 cpm were used. Lane 1, Whole cells lysed in buffer (60 mM Tris hydrochloride [pH 6.8], 1% SDS, 1% 2-mercaptoethanol, 10% glycerol); lane 2, supernatant after sonication; lane 3, supernatant from the first membrane washing; lane 4, supernatant from the second membrane washing; lane 5, membrane fraction after sonication; lane 6, membranes after two washings with 0.4 M NaCl. Lanes 7 through 12 are duplicates of lanes 1 through 6, except that the fractions contained 10,000 to 12,000 cpm and labeling with [^{35}S]methionine was for 20 min rather than 2 min.

DNA fragments in the size range of 4 to 8 kilobases (kb) were isolated from low-melting-point agarose gels and purified by passing the samples through NACS.52 PREPAC columns (Bethesda Research Laboratories). A chromosomal gene library was formed by ligating fragments of DNA with pBR322 DNA that had been restricted with *Bam*HI and dephosphorylated. Following transformation of strain BK9MDG with samples of the ligation mixture, 20 clones that were resistant to ampicillin and capable of growth on glutamate mineral salts medium were isolated. Several clones contained plasmids that were larger than pBR322. One clone (containing plasmid pBW1) was selected for further study.

Digestion of pBW1 with one of several restriction enzymes (*Cla*I, *Eco*RI, *Hin*dIII, *Pst*I, and *Sal*I) yielded linear DNA approximately 10.5 kb in size, suggesting that pBW1 contained a DNA insert of approximately 6.2 kb. The restriction enzyme sites within this insert are shown in Fig. 2. An *Ava*I site that is situated 553 base pairs to the right of the *Bam*HI site at 5.5 kb is not shown in Fig. 2 but was revealed at position 230 in the nucleotide sequence (see Fig. 6). The most significant sites for our purpose were the *Mlu*I and *Nsi*I sites that flanked a 1.6-kb DNA fragment. The *Mlu*I-*Nsi*I fragment was removed from pBW1, treated with S1 nuclease to produce blunt ends, and introduced into the *Sma*I site of pT7-6. The ligation mixture produced positive complementation (i.e., a Glt$^+$ phenotype) when transformed into BK9MDG, suggesting that the gene responsible, *gltP*, was contained within the 1.6-kb DNA fragment. The Glt$^+$ clones contained plasmids that were 3.8 kb in size. The insert DNA (1.6 kb) was removed from one of the plasmids (pBW8) by using the *Pst*I and *Eco*RI sites of pT7-6 and, by using the same restriction sites, was introduced into pT7-5 in the orientation opposite to that of pBW8 to form pBW5.

Characterization of the gene product of *gltP*. To determine the nature of the polypeptide product encoded by *gltP*, pBW8 and pBW5 were transformed (separately) into strain BK9MDG. pGPI-2 was then transformed into the strains containing either pBW8 or pBW5 by selecting the kanamycin resistance phenotype conferred by pGPI-2. The gene product(s) encoded by pBW8 and pBW5 was then identified by the phage T7 RNA polymerase-T7 promoter coupled system of Tabor and Richardson (16). Under these conditions, pBW8 expressed a single polypeptide product with a molecular mass of approximately 38 kilodaltons (kDa) (Fig. 3). No polypeptide products were formed from expression of pBW5, suggesting that *gltP* was transcribed in the direction *Nsi*I to *Mlu*I. The 38-kDa polypeptide of pBW8 occurred in

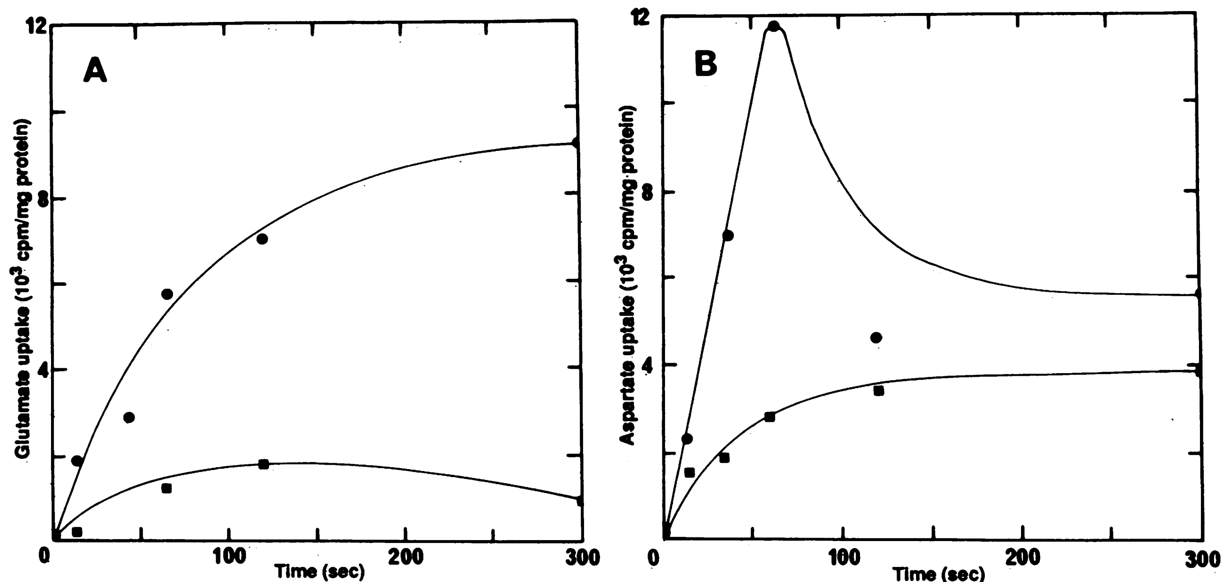

FIG. 5. Glutamate (A) and aspartate (B) uptake by cytoplasmic membrane vesicles from strains BK9MDG (■) and DLH1 (●). Preparation of membrane vesicles and conditions for uptake were as described in the text.

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-150                                        Bcl1                       -100
CAAGGGTTGC  GCAACATACC  GCGCAAATGA  TACTGATCAT  AAGCGTTAAA  AAAATCTACA

                                    -50
AACCAACGCA  ACACAATTCA  TGCCCTGGCA  GTATGTCACG  TTCTCGCGTT  TCTGAACGGG

   Hae2                       -1 Nde1
GAACGGCGCT  CCATTGAGGA  AGTCATTCAT  ATG AAA AAT ATA AAA TTC AGC CTG GCC
                                    Met Lys Asn Ile Lys Phe Ser Leu Ala

                                50
TGG CAG ATT CTG TTT GCT ATG GTG CTG GGC ATT CTC CTG GGA AGC TAC CTG CAC
Trp Gln Ile Leu Phe Ala Met Val Leu Gly Ile Leu Leu Gly Ser Tyr Leu His

                        100
TAC CAT AGC GAC AGC CGC GAC TGG CTG GTC GTC AAT TTG CTC TCT CCG GCG GGT
Tyr His Ser Asp Ser Arg Asp Trp Leu Val Val Asn Leu Leu Ser Pro Ala Gly

                150
GAT ATC TTC ATC CAT CTG ATT AAA ATG ATT GTT GTG CCG ATT GTG ATC TCC ACG
Asp Ile Phe Ile His Leu Ile Lys Met Ile Val Val Pro Ile Val Ile Ser Thr

            200                                         Ava1
CTG GTG GTG GGT ATC GCG GGT GTT GGT GAT GCC AAA CAG CTC GGG CGT ATT GGC
Leu Val Val Gly Ile Ala Gly Val Gly Asp Ala Lys Gln Leu Gly Arg Ile Gly

        250                         Bcl1
GCG AAA ACC ATT ATC TAC TTC GAG GTG ATC ACC ACC GTC GCC ATC ATT TTG GGA
Ala Lys Thr Ile Ile Tyr Phe Glu Val Ile Thr Thr Val Ala Ile Ile Leu Gly

300                                                                 350
TCA CTC TGG CGA ACC GTC TTC CAG CCC GGT GCC GGG GTG GAT ATG TCG CAG TTG
Ser Leu Trp Arg Thr Val Phe Gln Pro Gly Ala Gly Val Asp Met Ser Gln Leu

                                                                400
GCG ACC GTC GAT ATC TCG AAA TAT CAG AGC ACT ACG GAA GCG GTA CAA AGC AGT
Ala Thr Val Asp Ile Ser Lys Tyr Gln Ser Thr Thr Glu Ala Val Gln Ser Ser

                                                        450
TCC CAC GGC ATT ATG GGC ACG ATT TTG TCG CTG GTG CCG ACG AAC ATT GTG GCG
Ser His Gly Ile Met Gly Thr Ile Leu Ser Leu Val Pro Thr Asn Ile Val Ala

                                                    500
TCG ATG GCG AAA GGC GAA ATG CTG CCG ATC ATC TTT TTC TCG GTG CTG TTT GGT
Ser Met Ala Lys Gly Glu Met Leu Pro Ile Ile Phe Phe Ser Val Leu Phe Gly

                                                550
CTG GGG CTT TCT TCC CTG CCC GCG ACG CAT CGT GAA CCG CTG GTG ACC GTG TTC
Leu Gly Leu Ser Ser Leu Pro Ala Thr His Arg Glu Pro Leu Val Thr Val Phe

                            Dra1                    600
CGC TCC ATC TCT GAA ACC ATG TTT AAA GTG ACT CAC ATG GTG ATG CGT TAT GCA
Arg Ser Ile Ser Glu Thr Met Phe Lys Val Thr His Met Val Met Arg Tyr Ala

                                    650
CCG GTG GGT GTG TTT GCG CTG ATT GCG GTG ACG GTG GCT AAC TTT GGT TTC TCG
Pro Val Gly Val Phe Ala Leu Ile Ala Val Thr Val Ala Asn Phe Gly Phe Ser

          Bal1                  700
TCT CTG TGG CCA CTG GCG AAA CTG GTG CTG CTG GTG CAT TTC GCC ATT CTG TTC
Ser Leu Trp Pro Leu Ala Lys Leu Val Leu Leu Val His Phe Ala Ile Leu Phe

                            750
TTC GCG CTG GTA GTG CTG GGA ATT GTG GCG CGC CTG TGC GGG TTA AGC GTC TGG
Phe Ala Leu Val Val Leu Gly Ile Val Ala Arg Leu Cys Gly Leu Ser Val Trp

BamH1                   800
ATC CTG ATT CGT ATT CTG AAA GAT GAG CTG ATT CTG GCG TAC TCC ACT GCC AGC
Ile Leu Ile Arg Ile Leu Lys Asp Glu Leu Ile Leu Ala Tyr Ser Thr Ala Ser

                850          Nde1    Mbo2
TCT GAA AGC GTC GTG CCG CGA CAT ATG AGA AGA TGG AAG CCT ACG GAG CAC CGG
Ser Glu Ser Val Val Pro Arg His Met Arg Arg Trp Lys Pro Thr Glu His Arg

        900
TGT CGA TCA CCA GTT TCG TGG TGC CGA CCG GTT AAC CTC TTT TAC CTT GAT GGT
Cys Arg Ser Pro Val Ser Trp Cys Arg Pro Val Asn Leu Phe Tyr Leu Asp Gly

    950
TCG ACG CTG TAT CAA AGT ATT GCC GCT ATC TTC ATC GCG CAG TTG TAT GGC ATT
Ser Thr Leu Tyr Gln Ser Ile Ala Ala Ile Phe Ile Ala Gln Leu Tyr Gly Ile

1000                                                                1050
GAC CTG TCC ATC TGG CAG GAA ATC ATT CTG GTC GTG ACG CTG ATG GTG ACC TCG
Asp Leu Ser Ile Trp Gln Glu Ile Ile Leu Val Val Thr Leu Met Val Thr Ser

                                                            1100
AAA GGG ATT GCT GGC GTG CCT GGC GTG TCG TTT GTG GTG TTG CTG GCA ACG CTG
Lys Gly Ile Ala Gly Val Pro Gly Val Ser Phe Val Val Leu Leu Ala Thr Leu

                                                        1150
GGT AGC GTA GGT ATC CGC TGG AAG GTC TGG CGT TTA TTG CTG GTG TTG ACC GTA
Gly Ser Val Gly Ile Arg Trp Lys Val Trp Arg Leu Leu Leu Val Leu Thr Val

                                                1200
TCC TCG ACA TGG CGC GTA CTG CCG TGA AC GTGGTGGGTA ATGCGCTGGC GGTGCTGGTG
Ser Ser Thr Trp Arg Val Leu Pro  *

                                   1250    Hae2
ATTGCCAAGT  GGGAACACAA  ATTTGACCGT  AAGAAAGCGC  TGGCTTATGA  GCGTGAAGTG

                       1300             Mbo2
CTGGGCAAAT  TTGATAAAAC  TGCGGATCAA  TAATTGAAGA  TTGCCGGGGA  TATCCACCCG

          1350                                    Stu1              1400
GCAATGTGTG  AATGCCTGAT  GCGACGCTTG  CCGCGTCTTA  TCAGGCCTAC  GCCAGACAGC

                                                         1450
GCAATAGCCT  GATTTAGCGT  GATTTTGTAG  GTCGGATAAG  GCGTTTATGC  CGCATCCGAC

ATCAACGCCT  GATGCGACGC
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FIG. 6. The nucleotide sequence of the 1.6-kb *Mlu*I-*Nsi*I fragment containing *gltP* and the deduced amino acid sequence encoded by this gene (GenBank accession no. M32488). The antisense (mRNA-like) strand is shown. The nucleotide sequence was determined by the M13 dideoxynucleotide-chain termination method (14). Complementary 15- to 18-base-pair oligonucleotides (including the 15-base-pair universal oligonucleotide) were used as primers (see text), and [^{35}S]dATP was used to label the products. Fractionation of the single-stranded DNA products of the primer elongation reactions was performed on 8% polyacrylamide gels. Sites for restriction enzymes and possible canonical −35 and −10 sequences in the promoter are indicated by bars either above or below each sequence.

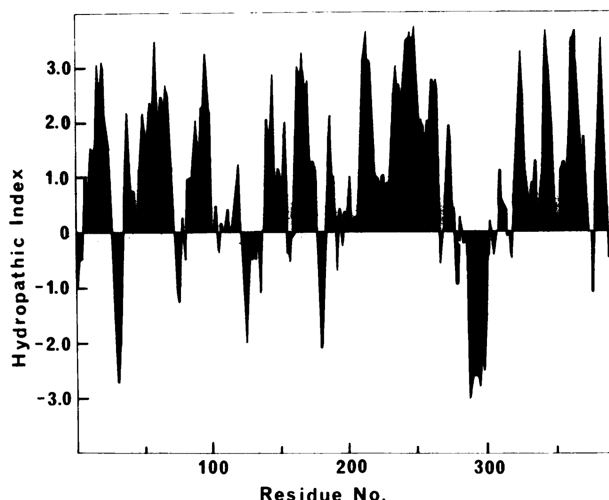

FIG. 7. Hydropathy index of the *gltP* polypeptide, determined by the method of Kyte and Doolittle (8) with a window of 19 amino acids. The hydrophobic regions lie above the horizontal zero line, whereas relatively hydrophilic regions lie below the zero line. The amino acid numbers are the same as those in Fig. 6.

substantial amounts in both membrane and supernatant cell fractions from cells labeled for either 2 or 20 min with [^{35}S]methionine (Fig. 4). Washing the membrane twice with 0.4 M NaCl did not remove the 38-kDa polypeptide.

Biochemical function of GltP. The rates of uptake of glutamate and aspartate by whole cells of strain BK9MDG containing the plasmid pBW8 (strain DLH1) were 5.5- and 4.5-fold, respectively, those of strain BK9MDG (Fig. 1). In contrast to strain BK9MDG, the uptake of glutamate by strain DLH1 was almost completely inhibited by L-cysteate and by β-hydroxyaspartate but was insensitive to α-methylglutamate (Table 1) and was not inhibited by omission of Na^+ from the reaction mixture (result not shown). When tested in transport assays, membrane vesicles prepared from strains DLH1 and BK9MDG gave results similar to those of the whole cells (Fig. 5). Although the initial rate of aspartate uptake (to 90 s) by vesicles of DLH1 was severalfold that of BK9MDG vesicles, leakage of aspartate from vesicles of the former strain appeared to occur (Fig. 5B). The reason for the leakage of aspartate, but not glutamate, is unclear and should be investigated further. Uptake of glutamate in vesicles of DHL1 was inhibited only slightly when Na^+ was omitted from the reaction mixture (result not shown).

Nucleotide sequence of the 1.6-kb DNA fragment containing *gltP*. The nucleotide sequence of the 1.6-kb *Nsi*I-*Mlu*I fragment containing *gltP* was determined in both directions by the M13-dideoxynucleotide chain termination method (Fig. 6). Inspection of the sequence (1,630 base pairs) suggested that the *gltP* gene was contained within an open reading frame extending from positions 1 to 1185 and encoded a gene product of 395 amino acids. A putative Shine-Dalgarno sequence, AGGAAG, at positions −14 to −9 precedes the

coding frame. We did not determine the transcription start point, but the sequences ATGATAC (−124 to −118) and AAAAAT (−101 to −96) are suggested as possible canonical −35 and −10 promoter regions.

Amino acid sequence and properties of GltP. Examination of the amino acid sequence of GltP inferred from the nucleotide sequence indicated that the polypeptide was extremely hydrophobic, since 63% of the total amino acid residues contained nonpolar side chains. GltP contains a total of 30 lysine-plus-arginine residues and 18 glutamic acid-plus-aspartic acid residues, suggesting a protein with an overall positive charge. The calculated molecular mass of 43.3 kDa for GltP is larger than its apparent molecular mass of 38 kDa determined by SDS-polyacrylamide gel electrophoresis. Such anomalous behavior can be attributed to the high degree of hydrophobicity of the protein and is characteristic of membrane transport proteins (1). Examination of GltP for potential transmembrane regions by the method of Kyte and Doolittle (8) indicated the presence of four such regions (Fig. 7). The hydrophobic nature of GltP is consistent with its location in the cell membrane and its function as a transport protein.

DISCUSSION

The observation that a suppressor clone (strain BJW1), isolated by plating strain BK9MDG (Δ [*glnHPQ*]) to glutamate, contained elevated levels of glutamate and aspartate uptake compared with those of the parent strain (Fig. 1) indicates that the Glt^- phenotype of BK9MDG can be reversed by increasing the level of glutamate transport. This was confirmed by the isolation of a clone of BK9MDG (strain DLH1) that contained a high-copy-number plasmid harboring a gene (*gltP*) encoding a glutamate-aspartate carrier protein. The reason for the Glt^- phenotype of strain BK9MDG is unclear. The results might suggest that there is an interaction between the glutamine transport system and one or more of the transport systems for glutamate. Perhaps strain BK9MDG (and other *glnH* strains) fails to grow on glutamate because this interaction is destroyed by mutation, resulting in rates of transport that are insufficient for growth. It is difficult, however, to reconcile this interpretation with the observation that although BK9MDG has one-third the uptake rate for glutamate compared with BJW1, the latter strain grows well on glutamate, whereas the former does not. It is quite possible that the inability of *glnHPQ* mutants to grow on glutamate is only indirectly related to uptake.

The properties of the transport gene contained in plasmid pBW1 and of its gene product are sufficiently similar to those of a gene, *gltP*, cloned recently from *E. coli* B by Deguchi et al. (4) to lead us to believe that we cloned the same gene from *E. coli* K-12. The restriction map of the insert of pDEH17 (4) contains restriction sites for *Bam*HI, *Bgl*II, and *Ava*I in almost the same positions as those of the insert of pBW1 (Fig. 2 and 6). The molecular mass of the gene product expressed by pBW8 was 38 kDa, the same as that found for the *gltP* gene product of *E. coli* B (4). The gene product specified by pBW8 stimulated both glutamate and aspartate uptake in whole cells (Fig. 1) and membranous vesicles (Fig. 5). The stimulated uptake was not Na^+ dependent and was sensitive to inhibition by L-cysteate and β-hydroxyaspartate but not α-methylglutamate. These properties suggest that the gene described in this paper encodes a carrier that is part of the Na^+-independent, binding-protein-independent glutamate-aspartate transport system. The facts that this carrier is a hydrophobic protein with four possible transmembrane regions and that a considerable proportion of it was firmly bound in the membrane are consistent with a transport function. The observation (Fig. 4) that not all of it was found within the membrane fraction in strain DLH1 may be due to saturation of a limited number of membrane sites by GltP.

There is some evidence to suggest that expression of *gltP* is repressed in *E. coli* K-12. When present in a high-copy-number plasmid, *gltP* complemented strain BK9MDG. However, in the mini-F plasmid (pMFC17) (13), *gltP* did not confer a Glt^+ phenotype on strain BK9DMG (B. Wallace, unpublished observation). Furthermore, it was not possible to clone a gene from strain BJW1 that complemented BK9MDG by using the mini-F plasmid (Y.-J. Yang, unpublished result). These observations suggest that the suppressor gene of strain BJW1 encodes a regulatory component (possibly a repressor) that controls expression of *gltP*. Inactivation of the repressor by mutation would permit derepression of *gltP* and lead to the higher levels of glutamate and aspartate transport observed in BJW1. It would not be possible to clone such a repressor gene directly, but the same effect could be achieved by cloning the regulated gene (*gltP*) on a high-copy-number plasmid, the effect of which would be to titrate repressor molecules present in BK9MDG and permit expression of *gltP*.

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