THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 252, No. 24, Issue of December 25, pp. 9055-9064, 1977
Printed in U.S.A.

Resolution of the Multiplicity of the Glutamate and Aspartate Transport Systems of *Escherichia coli**

(Received for publication, March 1, 1977)

GERARD D. SCHELLENBERG AND CLEMENT E. FURLONG‡
From the Department of Biochemistry, University of California, Riverside, California 92502

Mutants of *Escherichia coli* D_2W with altered levels of glutamate and aspartate transport were used to resolve the following five separate transport systems for these two amino acids: (*a*) a binding protein-dependent, sodium-independent, cysteate-inhibitable glutamate-aspartate system; (*b*) a binding protein-independent, sodium-independent, glutamate-aspartate system which is inhibitable by β-hydroxyaspartate or cysteate; (*c*) a binding protein-independent, sodium-dependent, α-methylglutamate-inhibitable glutamate-specific system (Miner, K. M., and Frank, L. (1974) *J. Bacteriol.* 117, 1093-1098; Halpern, Y. S., Barash, H., Dover, S., and Druck, K. (1973) *J. Bacteriol.* 114, 53-58); (*d*) a binding protein-independent aspartate-specific system (Kay, W. W. (1971) *J. Biol. Chem.* 246, 7373-7382); and (*e*) a dicarboxylic acid transport (*dct*) system which transports succinate, malate, fumarate, and aspartate (Kay, W. W., and Kornberg, H. L. (1971) *Eur. J. Biochem.* 18, 274-281).

Mutants were generated which have specific alterations in the level of each system, and through the use of these mutants K_m values were obtained for three of these systems as well as estimates for the contribution of four of these systems to the total glutamate and aspartate transport in *E. coli* D_2W measured in the presence of 40 mM sodium ion and at substrate concentrations of 28 μM. The binding protein-dependent system has K_m values of 0.5 μM for glutamate and 0.5 μM for aspartate, and it is responsible for 15% of the glutamate and 25% of the aspartate transport. The β-hydroxyaspartate-inhibitable glutamate-aspartate transport system has K_m values of 5 μM for glutamate and 4 μM for aspartate, and it is responsible for 60% of the total glutamate and 52% of the total aspartate transport. The sodium-dependent glutamate-specific transport system has a K_m value of 1.5 μM for glutamate and accounts for 25% of the total glutamate transport. The aspartate-specific system is responsible for 23% of the total aspartate transport. The amount of aspartate transported through the dicarboxylic acid system was negligible under the conditions of these experiments.

The transport of glutamate and aspartate by *Escherichia coli* appears to be mediated by several overlapping transport systems. Kay and Kornberg (2) and Kay (3) have reported aspartate transport in whole cells to be mediated by two systems: a low affinity system shared by succinate, fumarate, malate, and aspartate, and a high affinity aspartate-specific system which is weakly inhibited by glutamate. The high affinity aspartate-specific system was found to be retained in membrane vesicles (3). Other investigators, working with mutants derived from *E. coli* B (4) and K_{12} (5) having derepressed levels of glutamate transport, have partially characterized a sodium-stimulated glutamate transport system which is also retained in membrane vesicles. Using this type of mutant the K_m of glutamate transport in whole cells and in membrane vesicles was found to be strongly dependent on sodium ion, decreasing 10-fold in the presence of 15 mM sodium ion (6, 7). This sodium-stimulated system appears to be specific for glutamate since aspartate does not inhibit glutamate transport in membrane vesicles prepared from *E. coli* K_{12} (8). However, Lombardi and Kaback (9), using membrane vesicles prepared from *E. coli* ML308-225, have shown that these two amino acids share a common transport system. Miner and Frank (4) have also reported the isolation of mutants of *E. coli* B which have elevated glutamate transport which is only partially stimulated by sodium in either whole cells or in membrane vesicles.

Willis and Furlong (10) have recently purified to homogeneity an osmotic shock-releasable protein from *E. coli* W which binds either glutamate (K_d = 0.7 μM) or aspartate (K_d = 1.2 μM). Glutamate-aspartate binding proteins have also been purified from *E. coli* K_{12} by Barash and Halpern (11) and from *Salmonella typhimurium* by Aksamit *et al.* (12). Similar proteins capable of binding other substrates have been shown to serve as recognition components of the specific transport systems (13-15) as well as the receptors for chemotactic responses toward the respective substrates (16, 17). Until now the role of the glutamate-aspartate binding protein in transport was not clearly established. Barash and Halpern have reported that mutants with elevated levels of the sodium-stimulated glutamate transport system also have elevated levels of the glutamate-aspartate binding protein (11). However, they have also found that the binding protein and this

* This work was supported by Research Grant GM-18311 from the United States Public Health Service. A preliminary account of this work has been presented in abstract form (1). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of United States Public Health Service Research Career Development Award 1 K04 GM 00001. To whom reprint requests should be sent. Present address, Department of Genetics, SK50, University of Washington, Seattle, Washington 98195.

transport system are not co-regulated under different culture conditions (18). In addition, sodium does not affect the *in vitro* binding of glutamate or aspartate to the binding protein (10). Thus, the binding protein does not appear to be the recognition component of a sodium-dependent transport system. Miner and Frank have suggested the presence of two glutamate transport systems, an osmotic shock-resistant, sodium-stimulated, membrane-bound system and an osmotic shock-sensitive system (4). Willis and Furlong postulated that the glutamate transport system of *E. coli* was conditionally dependent on the glutamate-aspartate binding protein (19).

In this paper we report the kinetic and physiological consequences of a series of specific mutations in the different transport systems for glutamate and aspartate. A model (Fig. 1) is presented for the multiplicity of transport of these two amino acids which is consistent with the data reported here as well as with observations of other workers. The glutamate-aspartate binding protein serves as the substrate recognition component for only one of five separate transport systems. These results also explain why so many conflicting observations regarding the mechanism of uptake for those two amino acids have appeared in the literature.

EXPERIMENTAL PROCEDURES

Materials

L-[*U*-^{14}C]Glutamate (238 mCi/mmol), L-[*U*-^{14}C]aspartate (207 mCi/mmol), L-[*U*-^{14}C]proline (261 mCi/mmol), L-[*U*-^{14}C]glutamine (252 mCi/mmol), L-[*U*-^{14}C]leucine (289 mCi/mmol), and [2,3-^{14}C]succinate (13 mCi/mmol) were from New England Nuclear. DL-α-Methylglutamic acid, *N,N'*-methylenebisacrylamide, DL-α-methylaspartic acid, and D(−)-tartrate were from Sigma Chemical Co. DL-*threo*-β-Hydroxyaspartate and L-cysteic acid were purchased from Calbiochem, and L-glutamate-γ-ethyl ester and Celu Ion phosphate were from Nutritional Biochemicals Corporation. Acrylamide was obtained from Eastman Chemical; and CM-23 (CM)-cellulose and DE-23 (DEAE)-cellulose ion exchange resins were obtained from Whatman. The acrylamide and *N,N'*-methylenebisacrylamide were recrystallized before use (20). Avicel-coated thin layer chromatography plates were obtained from Analtech Inc., Newark, Del. Nitrocellulose membrane filters (16-mm diameter) were punched from sheets of Schleicher and Schuell Co., Selectron B6, 0.45-μm membrane.

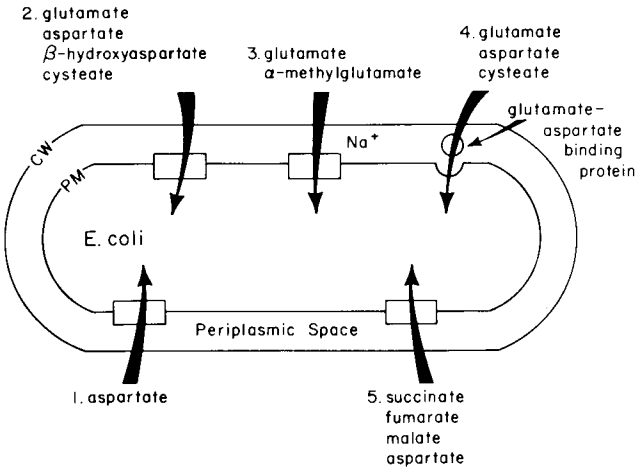

FIG. 1. A proposed model for the multiplicity of glutamate and aspartate transport in *Escherichia coli* W. The model presents the specificity of each of the five transport systems which recognize glutamate and/or aspartate. The transport systems are referred to in the text as follows: *1*, the aspartate-specific system; *2*, the β-hydroxyaspartate-inhibitable glutamate-aspartate system; *3*, the sodium-dependent glutamate-specific system; *4*, the glutamate-aspartate binding protein-dependent system; *5*, the dicarboxylic acid transport (*dct*) system. *CW*, cell wall; *PM*, plasma membrane.

ICR-191 (21, 22) was the kind gift of H. J. Creech of the Institute for Cancer Research, Philadelphia.

Methods

Bacterial Strains – All mutants used in this study were derived from *Escherichia coli* W strain D_2W which was obtained from Dr. L. Leive, National Institutes of Health, Bethesda, Md.

Media and Culture Conditions – The minimal salts media contained per liter: 7.35 g of K_2HPO_4, 3 g of KH_2PO_4, 0.55 g of Na_2SO_4, 0.2 g of $MgCl_2 \cdot 6\ H_2O$, and 1 ml of trace elements (23). Ammonium chloride (2.67 g/liter) was added as the nitrogen source and 1% sodium lactate as the carbon source unless otherwise indicated.

Cultures for transport assays and small scale osmotic shocks were grown at 37° in a rotary water-bath shaker.

Large scale cultures for protein purification were grown as previously described (24) using the above minimal salts media with ammonium chloride as the nitrogen source and 1% potassium succinate as the carbon source. Additional ammonium chloride was added as needed and the pH was maintained at 7.0 by the addition of 20% potassium succinate, pH 4.5. Cells were subcultured in a 10-liter New Brunswick Microfermentor, transferred to a 70-liter Fermentation Designs Inc. fermentor for growth, and harvested at early stationary phase with a continuous flow Cepa centrifuge.

Mutant Selection – Mutants resistant to β-hydroxyaspartate were isolated by placing 0.1 ml of a 0.23 M potassium β-hydroxyaspartate solution in a well (6 mm in diameter) punched in a minimal salts agar culture plate (containing 1% glucose as the carbon source) which had been spread with strain D_2W. Colonies from the zone of growth inhibition were picked, purified twice on nutrient agar plates, and screened for analogue sensitivity by the radial streak technique (25). Strains resistant to D-(−)tartrate were isolated by the same procedure using a 0.2 M solution of potassium tartrate in the well of a minimal salts culture plate containing 1% glycerol as the carbon source.

Mutants of *E. coli* selected for their ability to grow on glutamate as the sole carbon and nitrogen source were isolated by spreading approximately 10^7 cells (which had been washed twice with minimal salts media) on culture plates containing 10 mM potassium glutamate as the sole carbon and nitrogen source. After 5 days of incubation at 37°, small colonies were observed on a faint lawn. These colonies were picked under a dissecting microscope with a fine platinum wire and purified twice. The mutant with an elevated level of the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system was selected by the same procedure with the exception that the culture plate contained 1% glycerol and 1% potassium succinate as carbon sources and 5 mM potassium aspartate as the nitrogen source.

Mutants selected were either spontaneous or isolated from cultures mutagenized with the frameshift mutagen ICR-191 (26) as indicated.

Transport Assays – Transport in whole cells was assayed as previously described (19). Transport activity is expressed as nanomoles of substrate taken up/min/mg of cell protein. All buffers, substrates, and inhibitors used in the transport assays were prepared as potassium salts and sodium ion was added only where indicated.

Analogue Purity – Automatic amino acid analysis of the L-cysteic acid revealed no detectable glutamate or aspartate (less than 0.1%). L-Glutamate-γ-ethyl ester was found to contain approximately 1% glutamate by thin layer chromatography in an *n*-butyl alcohol/acetic acid/water (5:1:4, v/v/v) solvent system. The ester was recrystallized three times from ethanol which reduced the glutamate concentration to approximately 0.05%.

Electrophoresis – Polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate was performed as described by Ames (27) using a Studier type apparatus (28, 29) and the discontinuous buffer system of Laemmli (30). The gels were stained in 25% 2-propanol, 10% glacial acetic acid in water (v/v/v) with 0.1% Coomassie brilliant blue (w/v) for 1 h. Gels were destained by diffusion in the above solution without the dye.

Protein Purification – The initial steps in the purification of the glutamate-aspartate binding protein (osmotic shock, removal of material insoluble at pH 4.5, CM-cellulose chromatography, and DEAE-cellulose chromatography) were performed as previously described (10). The pass-through fraction from the DEAE-cellulose column which contained the glutamate-aspartate binding protein was dialyzed against deionized water and sodium acetate (pH 5.5) was added to a final concentration of 10 mM. Part of this material (74 mg) was loaded onto a phosphocellulose column with a resin bed

volume of 46 ml (30 cm in length, 1.4 cm in diameter) which had been equilibrated with 10 mM sodium acetate at pH 5.5. The column was washed with 5 column volumes of equilibration buffer followed by a linear sodium chloride gradient of 0 to 0.19 M salt in 20 column volumes of equilibration buffer. The gradient was controlled by an Ultragrad Gradient Maker (LKB) which held the salt concentration constant whenever the column effluent had an absorption at 280 nm greater than 0.3. These modifications resulted in a more efficient purification than previously reported (10–12). The glutamate-aspartate binding protein peak was eluted between 0.1 and 0.11 M sodium chloride. The specific activity of the pooled fractions was 25 nmol of glutamate bound/mg of protein. During the purification of this protein, the glutamate and aspartate binding activity co-chromatographed on CM-cellulose, DEAE-cellulose, and phosphocellulose ion exchange columns and no other glutamate or aspartate binding activities were detected.

Binding Assays – Ligand binding to the glutamate-aspartate and glutamine binding protein was measured as described previously (10, 31).

Immunodiffusion Assay – The relative levels of glutamate-aspartate binding protein present in osmotic shock fluids were determined by an Ouchterlony double diffusion immunoprecipitation assay in agar plates. Aliquots (5 μl) of serially diluted (1:2) concentrated osmotic shock fluid (10 mg/ml) were allowed to diffuse against antisera (5 μl) prepared against the glutamate-aspartate binding protein (10). The quantity of binding protein present was estimated from the lowest dilution that gave an observable immunoprecipitation reaction.

Protein Determinations – Protein concentrations were determined by the method of Lowry *et al.* (32) using bovine serum albumin (33) as a standard.

RESULTS

Transport Properties of Mutants with Altered Levels of Aspartate-specific and β-Hydroxyaspartate-inhibitable Glutamate-Aspartate Transport Systems – Mutants of *Escherichia coli* strain D_2W with decreased levels of transport through the aspartate-specific transport system and the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system (Fig. 1) were selected as colonies resistant to β-hydroxyaspartate as described by Kay (3) (Table I). The properties of 19 separately isolated mutants were examined and two classes of mutants were observed. Mutants such as HA9, isolated from small colonies appearing in the zone of growth inhibition (14 separate isolates), were found to be partially resistant to this analogue when examined by the radial streak method (Table II). Mutants isolated from large colonies (5 separate isolates such as mutant HA12) were fully resistant to growth inhibition by β-hydroxyaspartate (Table II). Both types of mutants were able to grow on succinate as sole carbon source and remained fully sensitive to D(−)-tartrate indicating that the dicarboxylic acid transport system (2) was still functional (Table II).

The initial rates of glutamate and aspartate transport in the parent strain and in the transport-deficient mutant HA9 are shown in Table II. Mutant HA9 which is partially resistant to β-hydroxyaspartate has 75% of the wild type aspartate transport and no significant change in glutamate transport. Further, Fig. 2 shows that the glutamate-resistant component of aspartate uptake was lost in mutant HA9. This mutant therefore appears to be devoid of the aspartate-specific system described by Kay (3). Mutant HA9 still retains a component of glutamate and aspartate uptake which is inhibitable by β-hydroxyaspartate (Fig. 3). Mutant HA12, on the other hand, is fully resistant to β-hydroxyaspartate (Table II). In contrast to mutant HA9, glutamate transport in mutant HA12 has been reduced by 58% and aspartate transport by 75% compared with the parent strain *E. coli* D_2W (Table II). Further, since the remaining aspartate transport is effectively inhibited by glutamate (Fig. 4) and not by β-hydroxyaspartate (Fig. 3*A*), these results would suggest that this mutant lacks the aspartate-specific system as well as a system which transports both glutamate and aspartate, and which is also inhibited by β-hydroxyaspartate.

A mutant with increased levels of a β-hydroxyaspartate-inhibitable glutamate-aspartate transport system was isolated by a selection procedure based on the following rationale. Mutants with increased levels of this transport system should be able to utilize aspartate as a nitrogen source more efficiently than the parent strain and at the same time be more sensitive to growth inhibition by β-hydroxyaspartate. In order to avoid complications of aspartate entering through the

TABLE I

Glutamate and aspartate transport mutants of Escherichia coli D_2W

Strain or mutant	Parent	Selection method[a]	Transport system(s) elevated or missing[b]				
			Aspartate-specific	β-Hydroxyaspartate-inhibitable glutamate-aspartate	Glutamate-specific	Glutamate-aspartate binding protein-dependent	Dicarboxylic acid (dct)
D_2W			+	+	+	+	+
HA9	D_2W	Resistance to β-hydroxyaspartate	−	+	+	+	+
HA12[c]	D_2W	Resistance to β-hydroxyaspartate	−	−	+	+	+
HA12-MG1	HA12	Increased growth on glutamate	−	−	10X	+	+
HA12-ST51[c]	HA12	Resistance to D-(−)tartrate	−	−	+	+	−
HA12-GA7	HA12	Increased growth on glutamate	−	−	+	5–9X	+
IT52	D_2W	Resistance to D-(−)tartrate	+	+	+	+	−
IT52-SAsp6	IT52	Increased growth on aspartate	+	3–4X	+	+	−

[a] Details of the selection methods are outlined under "Methods."

[b] Symbols indicate either the presence (+) or absence (−) of the indicated transport system. Mutants with elevated levels of a transport system are indicated by a number reflecting the fold increase followed by an X.

[c] Mutants HA12 and IT52 were isolated from cultures which had been mutagenized with ICR-191 by the method described under "Methods."

TABLE II

Analogue sensitivities and specific activities of uptake of Escherichia coli D_2W and mutants used in this study

Strain or mutant	Analogue sensitivity[a]				Transport activity[b]			
	β-Hydroxyaspartate	α-Methylglutamate	D(−)-Tartrate	Growth on succinate[c]	Glutamate	Aspartate	Succinate[d]	Proline
					nmol/min/mg			
D_2W	S	R	S	+	21	22	10	12
HA9	P	R	S	+	22	17	9	12
HA12	R	R	S	+	8.1	5.6	11	11
HA12-MG1	R	S	S	+	53	5.6	13	11
HA12-ST51	R	R	R	−	N.D.[e]	5.0	0.5	N.D.
HA12-GA7	P	R	S	+	33	21	N.D.	12
IT52	S	R	R	−	20	19	1.5	12
IT52-SAsp6	SS	R	R	−	74	57	1.7	11

[a] Analogue sensitivities were determined by the radial streak method described under "Methods." Abbreviations are: S, sensitive; R, resistant; P, partially resistant; SS, more sensitive than the parent.

[b] Cells were grown and assayed as described under "Experimental Procedures." Glutamate, aspartate, and proline assays contained 40 mM sodium chloride and either 28 μM [^{14}C]aspartate, 28 μM [^{14}C]glutamate, or 28 μM [^{14}C]proline. Succinate assays contained 80 μM [^{14}C]succinate.

[c] Growth on succinate was determined by the radial streak method using minimal salts culture plates with 1% potassium succinate in a 6-mm well in the center of the plate as the sole carbon source.

[d] Cultures for succinate transport assays were grown on minimal salts media with 1% sodium lactate and 20 mM potassium succinate as carbon sources.

[e] N.D. indicates value not determined.

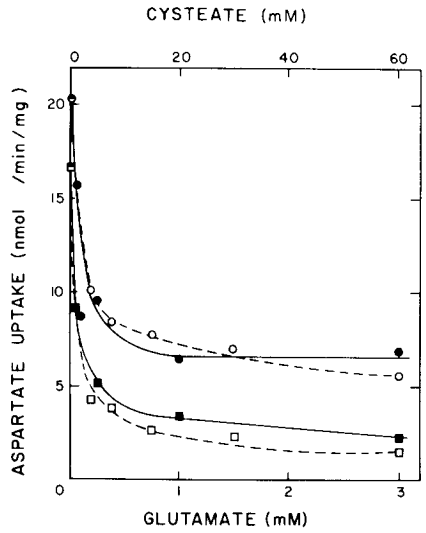

FIG. 2 (*left*). Inhibition of aspartate uptake by glutamate and cysteate in strain D_2W and in mutant HA9. Cells were grown and assayed as described under "Methods." Transport assays contained 40 mM sodium chloride, 28 μM [^{14}C]aspartate, and the potassium salts of the inhibitors at the concentrations indicated. ——, inhibition by glutamate; - - -, inhibition by cysteate; ○, ●, strain D_2W; □, ■, mutant HA9.

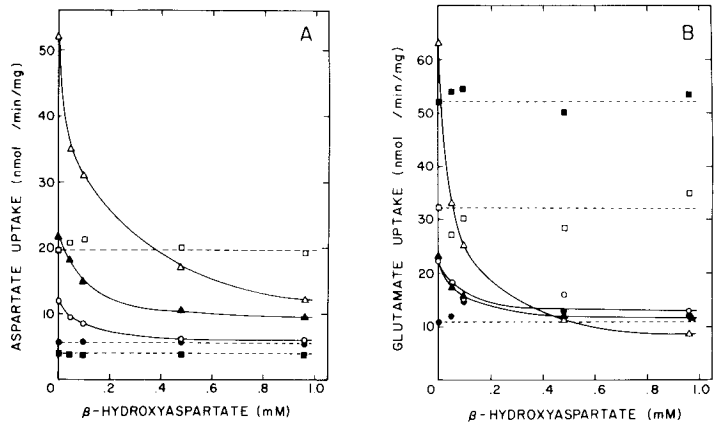

FIG. 3 (*center and right*). Inhibition of aspartate and glutamate uptake by β-hydroxyaspartate in strain D_2W and in mutants HA9, HA12, HA12-MG1, HA12-GA7, and IT52-SAsp6. Cells were grown and assayed as described under "Methods." Transport assays contained 40 mM sodium chloride, the potassium salt of β-hydroxyaspartate at the indicated concentrations, and either 28 μM [^{14}C]aspartate (*A*) or 28 μM [^{14}C]glutamate (*B*). ▲, strain D_2W; ○, mutant HA9; ●, mutant HA12; ■, mutant HA12-MG1; □, mutant HA12-GA7; △, mutant IT52-SAsp6.

dicarboxylic acid transport (*dct*) system, a *dct*-negative mutant (IT52) was used as the parent (Table I). The mutants were selected by plating a washed culture of mutant IT52 on a minimal media culture plate containing 1% glycerol as the carbon source and 5 mM aspartate as the sole source of nitrogen. Second step mutants appeared as small colonies on a faint lawn of the parent strain. Succinate was included in the culture media to prevent revertants of IT52 to a dicarboxylate transport positive phenotype. (Since the *dct* system is capable of transporting aspartate, *dct*-positive revertants would be able to efficiently utilize aspartate as a nitrogen source. However, the presence of excess succinate would keep the *dct* transport system saturated and thus prevent aspartate from entering the cell through this transport system.) Mutant IT52-SAsp6 was selected by this procedure and, as noted in Table II, is more sensitive to β-hydroxyaspartate than its parent IT52 or the parent of IT52 (*E. coli* D_2W).

The transport properties of mutant IT52-SAsp6 are shown in Table III. Both glutamate and aspartate transport activities are increased in this mutant compared to its parent IT52 or the parent of IT52, *E. coli* D_2W (Table II), while no change in the transport of succinate, proline (Table II), leucine, or glutamine, was observed (data not shown). Further, the increased activity is inhibited by β-hydroxyaspartate (Fig. 3). Using the portion of uptake that is inhibitable by β-hydroxyaspartate as a measure of the level of the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system, this mutant has a 3.8-fold increase in glutamate transport and a 3.1-

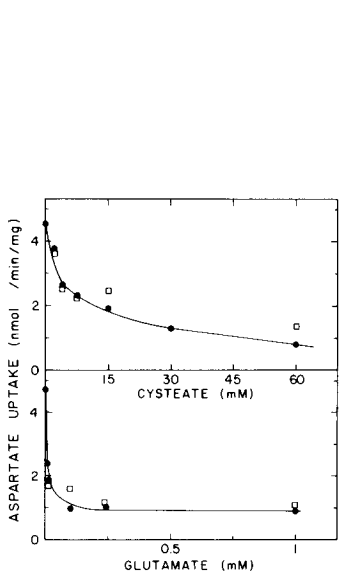

FIG. 4 (*left*). Inhibition of aspartate uptake by glutamate and cysteate in mutants HA12 and HA12-MG1. Cells were grown and assayed as described under "Methods." Transport assays contained 28 μM [^{14}C]aspartate, 40 mM sodium chloride, and potassium salts of the inhibitors at the concentrations indicated. ●, mutant HA12; □, mutant HA12-MG1.

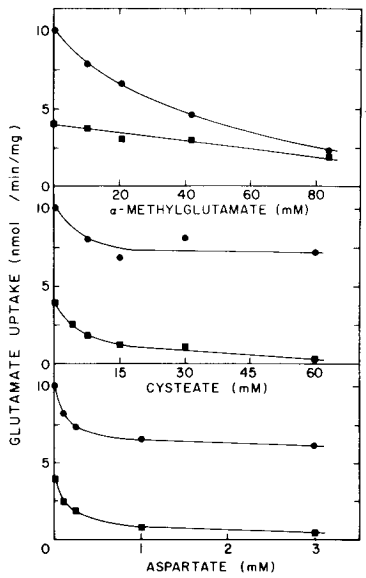

FIG. 5 (*center*). Inhibition of glutamate uptake by α-methylglutamate, cysteate, and aspartate in mutant HA12. Cells were grown and assayed as described under "Methods." Transport assays contained 28 μM [^{14}C]glutamate, potassium salts of the inhibitors at the concentrations indicated, and either sodium (●) or potassium (■) chloride at a final concentration of 40 mM.

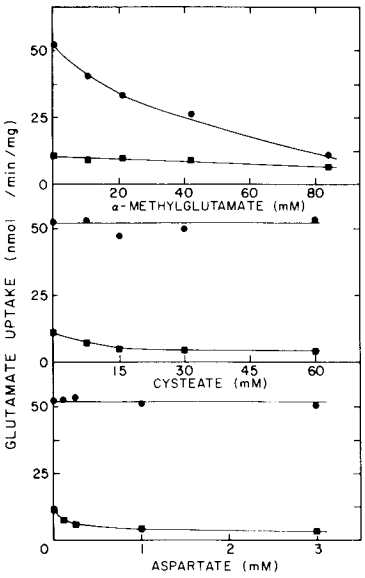

FIG. 6 (*right*). Inhibition of glutamate uptake by α-methylglutamate, cysteate, and aspartate in mutant HA12-MG1. Cells were grown and assayed as described under "Methods." Transport assays contained 28 μM [^{14}C]glutamate, potassium salts of the inhibitors at the concentrations indicated, and either sodium (●) or potassium (■) chloride at a final concentration of 40 mM.

TABLE III

Inhibition of glutamate and aspartate uptake in mutants IT52 and IT52-SAsp6

Transport assays contained 40 mM potassium chloride and either 28 μM [^{14}C]aspartate or [^{14}C]glutamate. Cells were grown and assayed as described under "Experimental Procedures."

Inhibitor	Inhibitor concentration	Mutant IT52		Mutant IT52-SAsp6	
		[^{14}C]Glutamate uptake	[^{14}C]Aspartate uptake	[^{14}C]Glutamate uptake	[^{14}C]Aspartate uptake
	mM	*nmol/min/mg*		*nmol/min/mg*	
None		21	25	61	51
β-Hydroxyaspartate	6.7	6.4	12	6.1	11
α-Methylglutamate	84	4.3	12	13.8	30
Aspartate	3	0.6	1.7	0.8	1.7
Cysteate	60	0.5	7.3	0.6	4.8
Glutamate	3	0.5	10	1.0	6.7

fold increase in aspartate transport through this system. The increased sensitivity to β-hydroxyaspartate is also consistent with the observed increase in activity of this system (Table II). Cysteate also effectively inhibits the elevated glutamate and aspartate uptake in this mutant (Table III).

The specificity of the glutamate and aspartate transport activity in mutant HA12 was examined. Aspartate uptake is effectively inhibited by either glutamate or cysteate (Fig. 4). Moreover, the inhibition pattern of glutamate uptake is altered by the presence of sodium ion (Fig. 5). In the absence of sodium ion (replaced by 40 mM potassium chloride) either aspartate or cysteate are effective inhibitors of glutamate transport while α-methylglutamate only weakly inhibits glutamate transport. However, in the presence of 40 mM sodium ion the glutamate transport activity increases 2.5-fold and the increased increment of transport is more sensitive to inhibition by α-methylglutamate. Aspartate and cysteate inhibit approximately the same absolute amount of glutamate transport in the presence or absence of sodium ion. However, since the total glutamate uptake is increased by sodium ion, inhibition by aspartate or cysteate plateaus after inhibiting only about 40% of the total glutamate transport. Various combinations of the three inhibitors were used to inhibit glutamate uptake. As seen in Table IV, cysteate and aspartate appear to inhibit the same component of glutamate transport. Either analogue alone inhibits 35 to 40% of the glutamate transport while both analogues present together produce no further inhibition. The inhibition by either aspartate or cysteate, on the other hand, is additive with the α-methylglutamate inhibition. The presence of α-methylglutamate with either aspartate or cysteate inhibits approximately 90% of glutamate transport. The same results were obtained with mutant HA12-ST51, a double mutant missing the *dct* system, the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system, and the aspartate-specific transport system (data not shown).

These results indicate in mutant HA12 at least two glutamate transport systems remain, a sodium-independent system which is inhibitable by either aspartate or cysteate, and a sodium-dependent system which is inhibitable by α-methylglutamate (Fig. 1).

Selection of Mutants with Increased Levels of Glutamate-specific and Glutamate-Aspartate Binding Protein-dependent Systems – Mutants with specific increases in the glutamate-specific transport system and the glutamate-aspartate binding protein-dependent system were isolated by plating washed cultures of mutant HA12 (which is devoid of the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system and the aspartate-specific system) on minimal media plates containing 10 mM potassium glutamate as the sole carbon and

TABLE IV

Inhibition of glutamate uptake in mutant HA12 in presence of sodium chloride

Cells were grown and assayed as described under "Experimental Procedures." Transport assays contained 40 mM sodium chloride, 25 μM [^{14}C]glutamate, and inhibitors at the concentrations indicated.

Addition	Inhibitor concentration	Glutamate transport
	mM	*% control*
None		100
Aspartate	3	61
Cysteate	60	67
α-Methylglutamate	84	30
Aspartate+ Cysteate	3 + 60	66
Aspartate+ α-Methylglutamate	3 + 84	12
Cysteate+ α-Methylglutamate	60 + 84	7

nitrogen source. Mutant colonies growing on a faint lawn of the parent strain appeared after incubation at 37° for 5 days. Two mutants with differing phenotypes were isolated by this procedure. One mutant (HA12-MG1), unlike its parent HA12, is sensitive to α-methylglutamate while the second mutant (HA12-GA7) retains the parental phenotype of α-methylglutamate resistance (Table I).

The transport properties of the α-methylglutamate-sensitive mutant, HA12-MG1, are shown in Tables II and VI and in Figs. 3 and 6. Glutamate uptake in the presence of 40 mM sodium chloride in mutant HA12-MG1 is 6.5-fold higher than in the parent strain, HA12 (Table II), whereas in the presence of 40 mM potassium chloride, glutamate transport in this mutant is only slightly higher than in strain HA12 (Fig. 6). The sodium-stimulated glutamate transport in mutant HA12-MG1 is inhibited by α-methylglutamate but not by β-hydroxyaspartate, aspartate, cysteate (Figs. 3*B* and 6), or α-methylaspartate (data not shown). Aspartate transport is not altered in this mutant (Table II) and remains inhibitable by glutamate and cysteate (Fig. 4) but not by β-hydroxyaspartate (Fig. 3*A*) or α-methylaspartate (data not shown).

These results indicate that a sodium-stimulated glutamate transport system which does not recognize aspartate has been elevated in mutant HA12-MG1 (Fig. 1). The specific activity of succinate, proline (Table II), leucine, or glutamine uptake was not changed by this mutation (data not shown).

A second mutant, HA12-GA7, grows well on glutamate but, unlike HA12-MG1, is not sensitive to growth inhibition by α-methylglutamate (Table II). The transport properties of this mutant are summarized in Table V. The total level of glutamate and aspartate uptake in the absence of sodium ion increased 9-fold and 5-fold, respectively, in the mutant relative to the parent strain (Table V) while the uptake of proline (Table II), leucine, or glutamine was not altered (data not shown). The transport system elevated in this mutant does not correspond to the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system since β-hydroxyaspartate does not inhibit either the elevated glutamate or aspartate transport (Fig. 3). Nor does this elevated transport correspond to the sodium-stimulated glutamate-specific system since the glutamate transport in this mutant is inhibited by cysteate and aspartate and is not dependent on the presence of sodium

TABLE V

Inhibition of glutamate and aspartate uptake in mutants HA12 and HA12-GA7

The cells were grown and assayed as described under "Experimental Procedures." Transport assays contained either 28 μM [^{14}C]glutamate or 28 μM [^{14}C]aspartate and 40 mM potassium chloride.

Inhibitor	Inhibitor concentration	Mutant HA12		Mutant HA12-GA7	
		[^{14}C]Glutamate uptake	[^{14}C]Aspartate uptake	[^{14}C]Glutamate uptake	[^{14}C]Aspartate uptake
	mM	*nmol/min/mg*		*nmol/min/mg*	
None	0	3.0	4.3	28	21
Aspartate	3	0.2	1.0	1.8	1.5
Glutamate	3	0.2	1.0	1.0	1.2
Cysteate	60	0.3	0.4	1.9	2.2
α-Methylglutamate	84	0.9	2.0	16	18

ion (Tables V and VI). In addition, aspartate, which is not a substrate of the glutamate-specific system, is transported by the system elevated in this mutant. The specificity of the glutamate and aspartate transport elevated in HA12-GA7 does correspond to cysteate-inhibitable sodium-independent glutamate and aspartate transport observed in the parent HA12 (Figs. 4 and 5) which is attributed to the glutamate-aspartate binding protein-dependent transport system (Fig. 1).

Comparison of Binding Protein Levels in Parent and Mutants—The level of the glutamate-aspartate binding protein in osmotic shock fluids derived from *E. coli* strain D_2W and mutants HA12 and HA12-GA7 was assayed by immunodiffusion as described under "Experimental Procedures." Shock fluids derived from mutant HA12-GA7 contain approximately eight times more binding protein than either its parent, HA12, or the parent of HA12, *E. coli* D_2W. Slab gel electrophoresis of osmotic shock fluid proteins in the presence of sodium dodecyl sulfate (Fig. 7) shows that of the shock-releasable proteins, only the glutamate-aspartate binding protein appears to be elevated in mutant HA12-GA7, and further, the binding protein level is not significantly altered in any of the other mutants shown in this figure.

Specificity of Glutamate-Aspartate Binding Protein—Discrepancies in the reported specificity of the glutamate-aspartate binding protein led us to reinvestigate the kinetic properties of this protein. Willis and Furlong (10) and Barash and Halpern (11) reported that the γ-ethyl esters of glutamate were inhibitors of the glutamate-aspartate binding protein, however, there was a significant difference in the reported K_i values. Further Aksamit, *et al.* reported that cysteate inhibited aspartate binding to a glutamate-aspartate binding protein purified from *Salmonella typhimurium* (12), an observation that was interesting in light of our studies on the specificity of glutamate and aspartate transport in *E. coli*.

The glutamate-aspartate binding protein and the amino acid analogues were purified as described under "Experimental Procedures." L-Cysteate inhibits glutamate binding to the glutamate-aspartate binding protein with a K_i of 25 μM (Fig. 8). The same data plotted by the method of Cornish-Bowden (34) (data not shown) indicate that the inhibition is competitive. Cysteate also inhibits aspartate binding (data not shown). Glutamate binding was not significantly inhibited by a 300-fold excess of DL-α-methylglutamate, an inhibitor of the sodium-dependent glutamate transport system (Fig. 1), or by a 100-fold excess of β-hydroxyaspartate, an inhibitor of the β-

TABLE VI

Kinetic parameters of glutamate and aspartate transport

Transport system	K_m[a]				V_m[b]				Strain used for determination of K_m and/or V_m	Contribution to the total wild type transport velocity[c]	
	Aspartate		Glutamate		Aspartate		Glutamate			Aspartate	Glutamate
	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl			
	μM				*nmol/min/mg*					%	
Glutamate-aspartate binding protein-dependent system	0.5	0.5	0.5	0.6	21	20	31	29	HA12-GA7[d]	25[e]	15[e]
β-Hydroxyaspartate-inhibitable glutamate-aspartate system	4	5	5	6	59	57	67	68	IT52-SAsp6[f]	52[g]	60[g]
Glutamate-specific system (sodium-dependent)			1.5	N.D.[h]	0	0	50	N.D.	HA12-MG1[i]	0	25[j]
Aspartate-specific system	N.D.	N.D.			5	5	0	0	D_2W, HA9	23[k]	0

[a] Cells for K_m determinations were grown and assayed as described under "Methods." Transport assays contained either 40 mM sodium or potassium chloride and either [^{14}C]aspartate or [^{14}C]glutamate at concentrations between 0.1 μM and 50 μM. The K_m values were determined from reciprocal linear transformation plots (*s/v versus s*) of the velocity *versus* substrate data (35).

[b] The V_m values were determined from linear transformation of the velocity *versus* substrate data and reflect the sum of all the transport systems present in the indicated mutant.

[c] The percentage of contribution of each system to the total is that determined in the presence of 40 mM sodium chloride.

[d] In mutant HA12-GA7 in the presence of potassium chloride, the binding protein-dependent glutamate-aspartate transport system accounts for 93% of the glutamate transport and 90% of the aspartate transport as indicated by the portion of the glutamate or aspartate transport in this mutant which is inhibitable by cysteate (Table V). In the presence of sodium ion, this system accounts for 88% of the glutamate and 95% of the aspartate transport as indicated by the portion of the glutamate or aspartate transport activity in this mutant which is inhibitable by cysteate in the presence of 40 mM sodium chloride (data not shown).

[e] The contribution of the glutamate-aspartate binding protein-dependent system to the wild type uptake of glutamate is estimated to be the portion of the glutamate transport in mutant HA12 inhibitable by aspartate or cysteate (3.2 nmol/min/mg) (Fig. 5). The contribution of this system to the total aspartate uptake is estimated to be the aspartate transport remaining in mutant HA12 (5.6 nmol/min/mg) (Table II) after genetic loss of the aspartate-specific system and the β-hydroxyaspartate-inhibitable glutamate-aspartate system.

[f] In the presence of sodium ion, 85% of the glutamate transport and 75% of the aspartate transport in mutant IT52-SAsp6 is mediated by the β-hydroxyaspartate-inhibitable glutamate-aspartate system as determined by the portion of the glutamate and aspartate uptake inhibitable by β-hydroxyaspartate (Fig. 3). In the absence of sodium, 90% of the glutamate uptake and 78% of the aspartate uptake is inhibitable by β-hydroxyaspartate (Table III).

[g] The difference in aspartate transport between mutants HA9 (17 nmol/min/mg) and HA12 (5.6 nmol/min/mg) (Table II) is estimated to be the contribution of the β-hydroxyaspartate-inhibitable glutamate-aspartate system to the total aspartate transport. The contribution of this system to the total glutamate transport is taken as the portion of the glutamate uptake in the wild type strain D_2W inhibitable by β-hydroxyaspartate (12.6 nmol/min/mg) (Fig. 3*B*).

[h] N.D. indicates value not determined.

[i] The glutamate-specific transport system accounts for 85% of the glutamate uptake in the presence of sodium ion in mutant HA12-MG1 with the remaining uptake being through the glutamate-aspartate binding protein-dependent system. The velocity contribution of the glutamate-specific system in this mutant was determined by subtracting the velocity of the glutamate-aspartate binding protein-dependent system (8 nmol/min/mg) from the total glutamate transport activity of mutant HA12-MG1 (53 nmol/min/mg, Table III). The activity of the binding protein-dependent system is estimated as the portion of the glutamate transport (in the absence of sodium ion) inhibitable by aspartate (Fig. 6).

[j] The glutamate-specific system velocity is estimated from the portion of the sodium-stimulated glutamate transport in mutant HA12 resistant to inhibition by aspartate or cysteate (5.3 nmol/min/mg) (Fig. 5).

[k] The contribution of the aspartate-specific system to the total aspartate transport is taken as the amount of aspartate transport lost when this system is genetically removed in mutant HA9 (5 nmol/min/mg) (Table II).

hydroxyaspartate-inhibitable glutamate-aspartate transport system. Three times recrystallized L-glutamate-γ-ethyl ester inhibited binding with a K_i of 500 μM, a value compatible with the inhibition that would be caused by the residual 0.05% glutamate still present in this preparation. The K_i appears to increase in proportion with the purity of the ester.

Kinetic Parameters of Glutamate and Aspartate Transport – The K_m values of transport were determined by using three mutants (IT52-SAsp6, HA12-MG1, and HA12-GA7) which have elevated levels of the β-hydroxyaspartate-inhibitable glutamate-aspartate system, the glutamate-specific system and the binding protein-dependent glutamate-aspartate system, respectively. In each of these three mutants, the transport system which has been specifically elevated accounts for 75% or more of the total glutamate and/or aspartate transport (Table VI). This allows for the kinetic characterization of these three systems in the absence of significant velocity contributions by the other glutamate and aspartate transport systems. The reciprocal plots used to determine K_m values (35) presented in Table VI were linear in all cases.

As shown in Figs. 5 and 6, the glutamate-specific transport system is stimulated by sodium and has a K_m of 1.5 μM in the presence of sodium ion (Table VI). It was not possible to characterize this system kinetically in the absence of sodium ion since under these conditions glutamate transport is effectively inhibited by aspartate or cysteate indicating that most of the glutamate transport is mediated by the binding protein-dependent system (Fig. 6). The presence of sodium ion does not affect the K_m or V_m values of either the glutamate-aspartate binding protein-dependent system or the β-hydrox-

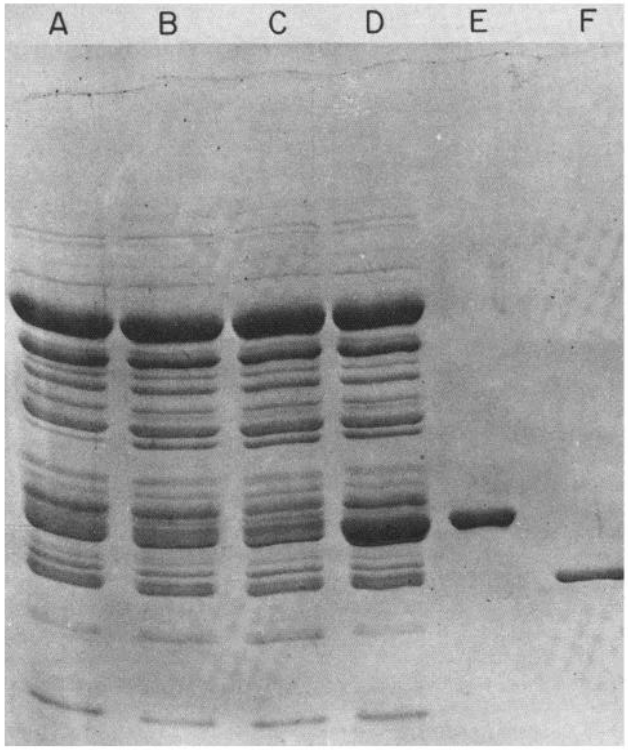

FIG. 7. Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of osmotic shock-releasable proteins from strain D_2W and mutants HA12, HA12-MG1, and HA12-GA7. The resolving gel contained 11% acrylamide and 0.29% *N,N'*-methylenebisacrylamide. The osmotic shock fluids were concentrated 20-fold by lyophilization. *A* to *D*, osmotic shock proteins (20 μg/lane) from strain D_2W and mutants HA12, HA12-MG1, and HA12-GA7, respectively; *E*, purified glutamate-aspartate binding protein (1.3 μg); *F*, purified glutamine-binding protein (1.3 μg).

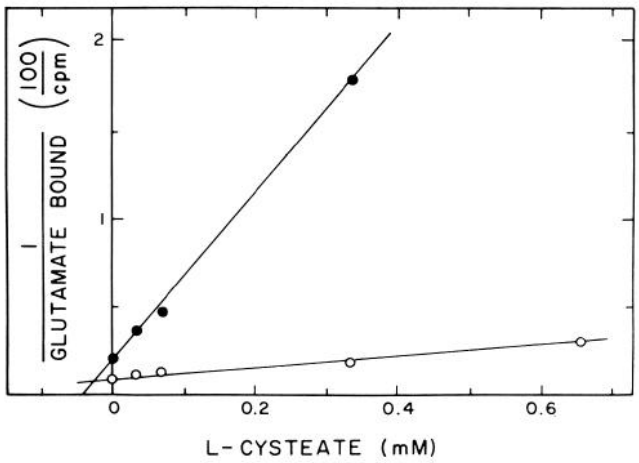

FIG. 8. Dixon plot of cysteate inhibition of glutamate binding to the glutamate-aspartate binding protein. The assay mixture contained 40 mM potassium phosphate buffer (pH 6.9), 20 mM magnesium chloride, cysteate at the indicated concentrations, 0.8 μg of binding protein, and either 6.6 μM (○) or 0.8 μM (●) glutamate in 40 μl. Of this volume, 30 μl were applied to a nitrocellulose filter and washed with 250 μl of 0.2 M magnesium chloride.

yaspartate-inhibitable glutamate-aspartate system.

The combination of the inhibition data and the specific activities of transport in the mutants allows for the estimation of the contributions of four of the transport systems to the total glutamate and aspartate transport in the wild type strain D_2W (Table VI). Under the conditions of these assays, the *dct* system does not appear to catalyze a significant percentage of the total aspartate transport since the genetic removal of this system (mutant HA12-ST51) does not result in a significant change in the level of aspartate transport (Table II). Under the assay conditions used, 60% of the glutamate transport is mediated through the β-hydroxyaspartate-inhibitable glutamate-aspartate transport system, 25% through the sodium-dependent glutamate-specific system, and 15% through the binding protein-dependent system. For aspartate, 52% of the uptake is mediated by the β-hydroxyaspartate-inhibitable glutamate-aspartate system, 23% by the aspartate-specific system, and 25% by the binding protein-dependent system.

DISCUSSION

The studies presented in this paper were undertaken to characterize the glutamate and aspartate transport systems of *Escherichia coli* and to assess the role of the glutamate-aspartate binding protein in transport. Previous studies in our laboratory (10, 19) as well as those by Barash and Halpern (11) and Kahane *et al.* (18) have suggested that the glutamate-aspartate binding protein is probably involved in the transport of glutamate and aspartate; however, the exact role of this protein had remained elusive. Our attempts to resolve this question have resulted in the work reported here where we have found that the binding protein appears to serve as the substrate recognition component for one of five separate transport systems which operate by at least three different mechanisms (*i.e.* sodium-independent membrane-bound, sodium-dependent membrane-bound, and binding protein-dependent). Earlier work resulted in the partial characterization of three of the five systems (2, 3, 5, 7); however, in many cases the data were difficult to interpret due to the unresolved multiplicity of the transport systems which accumulate these two amino acids. The five separate transport systems for these two amino acids are: the membrane-bound aspartate-specific system (3), a previously uncharacterized binding protein-independent β-hydroxyaspartate-inhibitable system shared by glutamate and aspartate, a sodium-dependent, membrane-bound glutamate-specific system (7), a binding protein-dependent glutamate-aspartate system, and the dicarboxylic acid transport system (2, 3) which recognizes succinate, fumarate, malate, and aspartate (Fig. 1).

Genetic removal of the aspartate-specific system (mutant HA9) results in the loss of a component of aspartate transport which is resistant to inhibition by glutamate or cysteate (Fig. 2) while glutamate transport is not altered (Table II). The system lost in this mutant appears to be the same aspartate-specific system previously described by Kay (3). In addition to aspartate, this system probably also recognizes β-hydroxyaspartate since mutant HA9 was isolated by virtue of its resistance to this analogue (Table I). Kay has also reported that β-hydroxyaspartate inhibits aspartate transport through the aspartate-specific system (3), although the ratio of inhibitor to substrate needed to demonstrate significant inhibition was much higher than the ratio used in this study (Fig. 3). Kay's studies indicate that in whole cells the aspartate-specific system has a K_m for aspartate transport of 3.7 μM and that this system is retained in membrane vesicles. Willis and Furlong (19) have also reported aspartate transport to be retained in membrane vesicles prepared from cultures of *E. coli* D_2W grown on succinate as the sole carbon source. The

aspartate transport in these preparations had a K_m of 2 μM and was not inhibited by glutamate, suggesting that the activity that they observed probably corresponds to the aspartate-specific system described in this paper and by Kay.

The β-hydroxyaspartate-inhibitable glutamate-aspartate transport system was characterized by generating a mutant (IT52-SAsp6) with a specific 3- to 4-fold increase in the level of this system (Fig. 3, Table III). The aspartate-specific system does not appear to be elevated in this mutant since the glutamate-resistant component of aspartate transport remains essentially the same in the mutant IT52-SAsp6 as in its parent. The K_m values for glutamate and aspartate uptake were 5 μM and 4 μM, respectively (Table VI). Neither the maximal velocities nor the K_m values of this system were affected by sodium ion. Preliminary data indicate that membrane vesicles prepared from *E. coli* D_2W cultured as described in this paper retain a shared glutamate-aspartate transport system which is inhibitable by β-hydroxyaspartate and cysteate.[1] Lombardi and Kaback have also observed a shared glutamate-aspartate transport system in membrane vesicles prepared from glucose-cultured *E. coli* ML 308-225 (9).

Mutant HA12 is devoid of the aspartate-specific system since all of the aspartate transport remaining is inhibitable by glutamate (Fig. 4). This mutant also lacks the β-hydroxyaspartate-inhibitable glutamate-aspartate system since β-hydroxyaspartate does not inhibit either the glutamate or aspartate transport in this mutant (Fig. 3). The fact that mutant HA12 was derived from a single step selection using mutagenized cells suggests the possibility that these two systems are genetically linked or perhaps share a common component, although a double mutation has not yet been ruled out. Kinetic analysis of glutamate and aspartate transport in this mutant indicates that in the presence of sodium ion, there are at least two remaining components of glutamate transport (Fig. 5, Table IV) and one for aspartate transport (Fig. 4). The amount of glutamate transport lost in mutant HA12 is the same as that inhibited by β-hydroxyaspartate in the parent strain *E. coli* D_2W (assayed in the presence of sodium ion), indicating that the mutation did not affect the level of the two remaining glutamate transport systems.

The transport systems remaining in mutant HA12 were characterized by examining the properties of two different types of second step mutants which were isolated on the basis of their ability to utilize glutamate as a sole carbon and nitrogen source more efficiently than the parent, mutant HA12. The first type of mutant (HA12-MG1) has an increase in only the sodium-dependent glutamate-specific transport system. This increase results in sensitivity to the analogue α-methylglutamate (Table I) as reported earlier by Halpern and Umbarger (36). In agreement with Frank and Hopkins (6) we have also found that mutant HA12-MG1 is more sensitive to growth inhibition by L-*allo*-γ-hydroxyglutamate than HA12 indicating that the glutamate-specific system probably recognizes this analogue as well. The maximal velocity of glutamate transport (45 nmol/min/mg) through this system in the derepressed mutant HA12-MG1 accounts for 85% of the glutamate transport in the presence of sodium ion; the remaining 15% goes through the glutamate-aspartate binding protein-dependent transport system (Table VI). In the presence of sodium ion, this glutamate-specific system is a high affinity system with a K_m of 1.5 μM for glutamate transport (Table VI). The recent work by Kahane *et al.* indicates that the sodium-dependent glutamate-specific system is active in membrane vesicle preparations (37). The reported noncompetitive inhibition of glutamate transport by aspartate in whole cells (19, 38) is more than likely due to aspartate inhibition of the binding protein-dependent system and/or the β-hydroxyaspartate-inhibitable glutamate-aspartate system since there is little or no inhibition by aspartate of the sodium-dependent glutamate-specific system (Fig. 6). In cases where multiple transport systems are operative, it is difficult to assess the nature of inhibition by a substrate analogue since the inhibitor may be affecting several systems with differing inhibition constants while having no effect on the activity of other systems.

The other type of second step mutant (HA12-GA7) capable of utilizing glutamate as a carbon and nitrogen source exhibits an increase in the glutamate-aspartate binding protein-dependent transport system. The 5- to 9-fold increase in the cysteate-inhibitable glutamate-aspartate transport (Table V) corresponds well with the approximate 8-fold increase in the glutamate-aspartate binding protein. More than 90% of the glutamate and aspartate transport in this mutant (measured in the absence of sodium ion) is mediated by the binding protein-dependent system since the glutamate-specific system, which is also present in this mutant, is strongly dependent on sodium ion (Table VI). Mutant HA12-GA7, like its parent, mutant HA12, is missing the aspartate-specific system and the β-hydroxyaspartate-inhibitable glutamate-aspartate system (Fig. 3 and Table V). The binding protein-dependent system has the highest substrate affinity of all the observed glutamate and aspartate transport systems with K_m values of 0.5 μM for both glutamate and aspartate (Table VI). The K_m value determined for glutamate transport in mutant HA12 in the absence of sodium ion was also 0.5 μM (data not shown) indicating that the mutation to HA12-GA7 resulted only in an increase in the maximal velocity of the binding protein-dependent system. The affinity of the transport system correlates well with the reported K_D values of 0.7 μM for glutamate and 1.2 μM for aspartate binding to the isolated glutamate-aspartate binding protein (10). The K_m values of whole cell glutamate and aspartate transport through the binding protein-dependent system (Table VI) and the K_D values of binding to the purified glutamate-aspartate binding protein are not affected by the presence of sodium ion (10).

The disc gel electrophoretic analysis of the osmotic shock-releasable proteins (Fig. 7) shows that only one of this class of proteins, the glutamate-aspartate binding protein, is derepressed in mutant HA12-GA7. An important point with respect to the disc gel electrophoretic analysis is that the change in protein profiles is sufficiently dramatic that in a similar situation where the gene product had not previously been identified and purified, it would be possible to determine which protein is derepressed simply by observing the gel profile.

Of the mutants described in this paper, only those selected by resistance to D(−)-tartrate (HA12-ST51 and IT52 and its derivative IT52-SAsp6) have altered succinate transport and thus have reduced levels of or completely lack the *dct* system. Further, the loss of the *dct* system in mutant HA12-ST51 does not affect glutamate or aspartate transport as expressed in lactate grown cells at the substrate concentrations used in this study (28 μM) (Table II). These results demonstrate that the *dct* system is distinct from the four other transport systems described in this paper (Fig. 1).

The possibility of the involvement in chemotaxis of the

[1] G. D. Schellenberg, unpublished results.

soluble glutamate-aspartate binding protein and membrane-bound aspartate binding activity was recently investigated by Aksamit *et al.* in *Salmonella typhimurium* (12). These workers concluded that the specificity of neither the binding protein nor a membrane-bound aspartate binding activity agreed with the inhibition of aspartate chemotaxis by glutamate, malate, α-methylaspartate, β-methylaspartate, and D(−)-tartrate. However, considering the extensive multiplicity of glutamate and aspartate transport systems reported here, it is quite probable that the multiplicity of receptors for glutamate and aspartate chemotaxis is just as complicated; and that some or all of the substrate recognition components of the individual glutamate and aspartate transport systems may also serve as attractant receptors for chemotaxis. Consistent with this possibility of shared components is the recognition of several glutamate and aspartate analogues by both the transport system reported here and the glutamate and aspartate chemoreceptor activities which have been reported by others. For example, cysteate, which is recognized by both the β-hydroxyaspartate-inhibitable glutamate-aspartate system and the glutamate-aspartate binding protein-dependent system (Fig. 1), has been shown by Mesibov and Adler to be an attractant for *E. coli* (39). The aspartate analogue β-hydroxyaspartate which is recognized by both the aspartate-specific system and the β-hydroxyaspartate-inhibitable glutamate-aspartate system is also an attractant for *E. coli* (39). Similarly, succinate, malate, and fumarate, substrates of the dicarboxylic acid transport system, are attractants of *E. coli* (39), and D(−)-tartrate, an analogue recognized by this transport system, has been shown to be an attractant of *S. typhimurium* (12). Preliminary experiments indicate that chemotaxis toward α-methylglutamate is increased in mutant HA12-MG1, a mutant which has an elevated level of the glutamate-specific transport system (1). In addition, Mesibov and Adler have isolated an aspartate chemotaxis negative mutant of *E. coli* which they suggest lacks a common component for several aspartate chemoreceptors (39). These mutants had reduced chemotaxis toward aspartate, glutamate, β-hydroxyaspartate, succinate, malate, and fumarate whereas aspartate transport in this organism was unaffected.

It is not surprising that conflicting reports concerning glutamate and aspartate transport have appeared in the literature considering that there are five separate transport systems for these two amino acids which are probably individually regulated with differing responses to catabolite repression (3, 18, 19) and with one system having a requirement for sodium ion. Since the data presented here appear to explain observations made in a number of different strains of *E. coli* as well as preliminary observations of aspartate transport made in *S. typhimurium* (12), it is probably not strain differences which have contributed to the confusion in the literature so much as the overlapping specificities of the five independent transport systems as well as the differential expression of these systems under different culture conditions.

Acknowledgments – We express our appreciation to Dr. R. C. Willis for his comments and for checking the cysteic acid by automatic amino acid analysis; to Dr. D. W. Woodward, Dr. P. K. Kury, Dr. W. Parce, and Theresa Canfield for helpful comments during the preparation of this manuscript; and to John Leonard for generating mutants IT52 and HA12-ST51 as well as for helpful suggestions.

REFERENCES

1. Schellenberg, G. D. (1976) *Fed. Proc.* **35**, 1617
2. Kay, W. W., and Kornberg, H. L. (1971) *Eur. J. Biochem.* **18**, 274-281
3. Kay, W. W. (1971) *J. Biol. Chem.* **246**, 7373-7382
4. Miner, K. M., and Frank, L. (1974) *J. Bacteriol.* **117**, 1093-1098
5. Halpern, Y. S., and Lupo, M. (1965) *J. Bacteriol.* **90**, 1288-1295
6. Frank, L., and Hopkins, I. (1969) *J. Bacteriol.* **100**, 329-336
7. Halpern, Y. S., Barash, H., Dover, S., and Druck, K. (1973) *J. Bacteriol.* **114**, 53-58.
8. Kahane, S., Metzer, E., and Halpern, Y. S. (1976) *Eur. J. Biochem.* **66**, 583-589
9. Lombardi, F. J., and Kaback, H. R. (1972) *J. Biol. Chem.* **247**, 7844-7857
10. Willis, R. C., and Furlong, C. E. (1975) *J. Biol. Chem.* **250**, 2574-2580
11. Barash, H., and Halpern, Y. S. (1975) *Biochim. Biophys. Acta* **386**, 168-180
12. Aksamit, R. R., Howlett, B. J., and Koshland, D. E., Jr. (1975) *J. Bacteriol.* **123**, 1000-1005
13. Ames, G. F.-L., and Lever, J. E. (1972) *J. Biol. Chem.* **247**, 4309-4316
14. Boos, W. (1972) *J. Biol. Chem.* **247**, 5414-5424
15. Kellermann, O., and Szmelcman, S. (1974) *Eur. J. Biochem.* **47**, 139-149
16. Ordal, G. W., and Adler, J. (1974) *J. Bacteriol.* **117**, 517-526
17. Hazelbauer, G. L. (1975) *J. Bacteriol.* **122**, 206-214
18. Kahane, S., Marcus, M., Metzer, E., and Halpern, Y. S. (1976) *J. Bacteriol.* **125**, 762-769
19. Willis, R. C., and Furlong, C. E. (1975) *J. Biol. Chem.* **250**, 2581-2586
20. Shuster, L. (1971) *Methods Enzymol.* **22**, 412-433
21. Creech, H. J., Breuninger, E., Hankwitz, R. F., Jr., Polsky, G., and Wilson, M. L. (1960) *Cancer Res.* **20**, 471-494
22. Peck, R. M., Preston, R. K., and Creech, H. J. (1961) *J. Org. Chem.* **26**, 3409-3414
23. Kelmers, A. D., Hancher, C. W., Phares, E. F., and Novelli, G. D. (1971) *Methods Enzymol.* **20**, 3-9
24. Willis, R. C., Morris, R. G., Cirakoglu, C., Schellenberg, G. D., Gerber, N. H., and Furlong, C. E. (1974) *Arch. Biochem. Biophys.* **161**, 64-75
25. Roth, J. R. (1970) *Methods Enzymol.* **17(A)**, 3-35
26. Miller, J. H. (1972) *Experiments in Molecular Genetics*, pp. 130-134, Cold Spring Harbor Laboratory, New York
27. Ames, G. F.-L. (1974) *J. Biol. Chem.* **249**, 634-644
28. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 227-248
29. Reid, M. S., and Bieleski, R. L. (1968) *Anal. Biochem.* **22**, 374-381
30. Laemmli, U. K. (1970) *Nature* **227**, 680-685
31. Weiner, J. H., and Heppel, L. A. (1971) *J. Biol. Chem.* **246**, 6933-6941
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
33. Webster, G. C. (1970) *Biochim. Biophys. Acta* **207**, 371-373
34. Cornish-Bowden, A. (1974) *Biochem. J.* **137**, 143-144
35. Dowd, J. E., and Riggs, D. S. (1965) *J. Biol. Chem.* **240**, 863-869
36. Halpern, Y. S., and Umbarger, H. E. (1961) *J. Gen. Microbiol.* **26**, 175-183
37. Kahane, M., Marcus, H. B., Halpern, Y. S., and Kaback, H. R. (1975) *FEBS Lett.* **56**, 235-239
38. Halpern, Y. S., and Even-Shosan, A. (1967) *J. Bacteriol.* **93**, 1009-1016
39. Mesibov, R., and Adler, J. (1972) *J. Bacteriol.* **112**, 315-326

Resolution of the multiplicity of the glutamate and aspartate transport systems of Escherichia coli.

G D Schellenberg and C E Furlong

J. Biol. Chem. 1977, 252:9055-9064.
