JOURNAL OF BACTERIOLOGY, Sept. 1975, p. 937-945

Vol. 123, No. 3
Printed in U.S.A.

L-Asparagine Uptake in *Escherichia coli*

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Received for publication 31 March 1975

The uptake of L-asparagine by *Escherichia coli* K-12 is characterized by two kinetic components with apparent K_m values of 3.5 μM and 80 μM. The 3.5 μM K_m system displays a maximum velocity of 1.1 nmol/min per mg of protein, which is a low value when compared with derepressed levels of other amino acid transport systems but is relatively specific for L-asparagine. Compounds providing effective competition for L-asparagine uptake were 4-carbon analogues of the L-isomer with alterations at the β-amide position, i.e., 5-diazo-4-oxo-L-norvaline (K_i = 4.6 μM), β-hydroxyamyl-L-aspartic acid (K_i = 10 μM), and L-aspartic acid (K_i = 50 μM). Asparagine uptake is energy dependent and is inhibited by a number of metabolic inhibitors. In a derived strain of *E. coli* deficient in cytoplasmic asparaginase activity asparagine can be accumulated several-fold above the apparent biosynthetic pool of the amino acid and 100-fold above the external medium. The high affinity system is repressed by culture of cells with L-asparagine supplements in excess of 1 mM and is suggested to be necessary for growth of *E. coli* asparagine auxotrophs with lower supplement concentrations.

There have been numerous investigations of the transport of various amino acids by *Escherichia coli* which have been the subject of recent reviews (12, 14). These studies have revealed a number of highly specific systems for individual amino acids as well as more general systems for groups of related amino acids. Several systems may occur simultaneously which are capable of transporting the same amino acid but with quite different apparent affinities and modes of genetic regulation.

Although the transport of all other natural amino acids found in protein has been investigated in *E. coli* (12), a transport system for L-asparagine has, as yet, not been described. The results of competition studies characterizing the specificity of transport systems for the closely related amino acids aspartate (6), glutamine (15), and glutamate (3) suggest that asparagine is not a substrate for these systems. Similar studies of other amino acid transport systems have not included asparagine. Such studies would be complicated by the presence of several asparaginase activities in this organism (1), but the existence of an asparagine auxotroph of *E. coli* K-12 (2) establishes that asparagine may enter this cell without destruction.

In a recent paper (16) we have described nutritional experiments with an asparagine auxotroph of *E. coli* K-12. The results suggested the presence of at least two systems mediating the entry of asparagine: a high-affinity system which appeared to be repressed by culture of cells with 1 mM asparagine and was required for growth of cultures of an asparagine auxotroph at supplement concentrations of 10 to 100 μM L-asparagine and a second, low-affinity system utilized by cells when asparagine was the sole nitrogen source of the culture. This paper characterizes a high-affinity kinetic component of asparagine uptake in *E. coli* K-12 strains which is relatively specific for L-asparagine and is repressed by growth of cultures with 1 mM L-asparagine. Transport systems have also been described for asparagine in the gram-positive bacteria *Lactobacillus plantarum* and *Streptococcus faecalis* where the specificity of the systems differ significantly from that reported here (4).

(A portion of this paper was submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree in Biological Sciences from the University of California at Irvine.)

MATERIALS AND METHODS

Chemicals. L-[U-^{14}C]asparagine (218 μCi/μmol), L-[U-^{14}C]aspartate (208 μCi/μmol), L-[U-^{14}C]glutamine (36.3 μCi/μmol), L-[U-^{14}C]glutamate (249 μCi/μmol), and L-[U-^{14}C]leucine (311 μCi/μmol) were purchased from Amersham-Searle. 5-Diazo-4-oxo-L-norvaline was a gift from R. E. Handschumacher,

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Department of Pharmacology, Yale University. L-β-Hydroxamyl aspartic acid, bovine serum albumin, *E. coli* B asparaginase, and natural amino acids were purchased from Sigma Chemical Co. Oxalacetic acid, β-ketoglutaric acid, fumaric acid (sodium salt), malonic acid, D-lactic acid (lithium salt), β-cyano-L-alanine, O-phospho-L-serine, and chloramphenicol were purchased from Calbiochem Co. L-Alanyl-L-asparagine, L-γ-aminobutyric acid, α-methyl-L-aspartic acid, N-methyl-DL-aspartic acid, and D-asparagine were purchased from Nutritional Biochemicals. Oxamic acid, oxalic acid dihydrate, and succinamide were purchased from Aldrich Chemical Co. All other materials were obtained from the usual commerical sources and were either reagent grade or the highest chemical purity available. Where required, solutions of basic and acidic materials were adjusted to neutrality with hydrochloric acid or potassium hydroxide, respectively.

Bacteria. All strains used in this study were derivatives of *E. coli* K-12. Strain W3110 was obtained from D. L. Wulff, Department of Biochemistry, University of California, Irvine. Other strains used were ATCC 25287 (*asn*$^-$, *thi*$^-$), originally isolated by E. Reich, Rockefeller University, and 47, a cytoplasmic asparaginase-deficient mutant (16) of strain 25287.

Culture. The maintenance and culture of bacterial strains has been described previously (16). Changes in the media composition are noted. Carbon-starved cells were prepared from cultures originally supplemented with 0.04% glucose and harvested for 4 h after growth ceased.

Cell suspensions. Cells used for transport assays were harvested in the mid-exponential phase of growth. The cells were harvested from cultures and collected from wash solutions by centrifugation for 10 min and 10,000 × *g* at room temperature. The cells were washed twice by resuspension into 0.5 original volume buffer A (10 mM potassium phosphate, pH 6.9, 1.0 μM magnesium chloride, 100 μg. of chloramphenicol per ml). After the last wash, the cell pellets were resuspended at 6 to 8 mg of cellular protein/ml in buffer A. The washed cells were stored at either room temperature or 4 C until used for assay. Cells stored at 4 C were incubated for 15 min at 37 C before dilution for assay. Transport activity remained constant for at least 4 h under either condition of storage.

Transport assays. All operations, except where noted, were performed at room temperature and materials were prepared in buffer A. Transport was assayed in 60-μl reaction mixtures containing, in order of addition: 20 μl of appropriately diluted cell suspension; 20 μl of energy source, routinely 75 mM glucose; and after a 10-min incubation period 20 μl of ^{14}C-labeled substrate (20 to 70 μCi/μmol) and, where indicated, unlabeled competitor. Assays were terminated by transferring 50 μl of assay mixture to the center of a wetted Millipore filter (0.45 μm, HAWP) under vacuum and the collected sample was washed by vacuum filtration with 5 ml of buffer A. The backside of the filter containing the washed sample was blotted and the filter was rapidly transferred to a scintillation vial containing 2 ml of Aquasol (New England Nuclear). Radioactivity was determined by scintillation counting. Standardization of counts per minute to microcuries was performed by adding a known amount of radioactivity to a sample counted previously, recounting the sample, and determining the difference in counts per minute. All data is reported in nanomoles of asparagine taken up per minute per milligram of protein. Protein was determined by the method of Lowry et al. (9).

Routinely, assay mixtures contained 2 to 3 mg of cellular protein/ml, i.e., 0.10 to 0.15 mg of protein/filter, assay times were 30 s, and the specific activity of the isotope was 20 to 70 μCi/μmol. The cell concentrations used in all assays were predetermined such that less than 10% of the substrate was removed during a 30-s incubation. In experiments requiring many samples to be taken with time, the assay mixtures were increased proportionally in volume and a 28-mm outer diameter scintillation vial was used in place of a tube (13 by 100 mm) as the reaction vessel. The vial was used to allow an increase in the surface area-volume ratio of the reaction mixture and thereby prevent oxygen starvation of cells during long-term incubation.

Extraction and identification of accumulated materials. Immediately after the collection and wash of a sample by membrane filtration, the filter was transferred to a 28-mm outer diameter scintillation vial containing 5 ml of 50% ethanol equilibrated to 95 C. The vial was recapped and sample was extracted by continued incubation at 95 C for 10 min. The vial was then vigorously vortexed and the solution was transferred to a centrifuge tube. The extraction was repeated a second time and the solutions were combined. The extract solutions were clarified by centrifugation at 16,000 × *g* for 15 min and the volume of the clarified extract solution was adjusted to 10 ml with water. The solution was divided and evaporated to dryness at 60 C with a rotary Evapomix (Buchler Instruments). One fraction was resuspended in 50 μl of 50% methanol. The other fraction was incubated for 120 min at 37 C with 4 to 5 units of *E. coli* B asparaginase in a total volume of 100 μl of 50 mM ammonium bicarbonate, pH 7.6. The solution was evaporated to dryness and resuspended in 50 μl of 50% methanol. Equal volumes of both fractions were spotted on adjacent lanes of a 250-μm surface depth Silica Gel G thin-layer chromatography plate (Brinkmann) and chromatographed with a chloroform-methanol-ammonium hydroxide-water (8:8:3:1) solvent system. Developed plates were dried and autoradiograms were prepared (16). The location of asparagine on the chromatograms was determined by the absence or loss of radioactivity in samples treated with asparaginase. Similarly, the position of aspartate was detected by the increase in radioactivity in samples treated with asparaginase. The areas of interest were removed from the chromatogram by scraping, and the radioactivity of the scraped sample was determined by scintillation counting in 5 ml of toluene scintillation solution (16).

RESULTS

Optimum conditions for measurement of asparagine uptake. The standard assay condi-

tions for measurement of asparagine uptake were determined by studies of the pH-buffer optimum and cation dependence. The initial rate of asparagine uptake displays a pH optimum over the range of 6 to 7 with 50 mM dibasic potassium phosphate-monobasic potassium phosphate buffer. Uptake activity is greatly diminished below pH 5.0 with 50 mM sodium acetate-acetic acid buffer, and above pH 7.5 with 50 mM trizma base-hydrochloric acid buffer. At similar pH both sodium acetate and tris(hydroxymethyl)aminomethane buffers were inhibitory in comparison to uptakes measured in the presence of a respective potassium phosphate buffer. The optimum potassium phosphate buffer concentration was in the range of 10 to 50 mM. In studies with 10 mM potassium phosphate buffer, the pH of assay mixtures containing cell densities equivalent to 2 mg of cellular protein per milliliter was maintained for 20 min at 37 C. The uptake of asparagine was not dependent on added sodium ion in studies utilizing concentrations of sodium chloride from 0.01 to 100 mM. At concentrations in excess of 100 mM both sodium and potassium chloride inhibited the apparent initial rate of asparagine uptake. Magnesium chloride at concentrations from 1 to 10 μM provided the maximum uptake activity. Calcium chloride had little effect.

The uptake of asparagine was dependent on temperature and the initial rate was linear with temperature from 10 to 50 C. Incubation of cells for longer than 2 min at temperatures greater than 50 C destroyed the asparagine uptake capacity of cells.

Metabolism of [^{14}C]asparagine during uptake assays. Asparagine is rapidly metabolized by *E. coli* K-12 cells with cytoplasmic asparaginase activity (16). To show that metabolism of the transported asparagine does not interfere with initial rate measurements, uptake was measured in an isogenic pair of strains, 25287 and 47; strain 47 is deficient in cytoplasmic asparaginase activity. The results of these measurements (Fig. 1) indicate that the initial rates

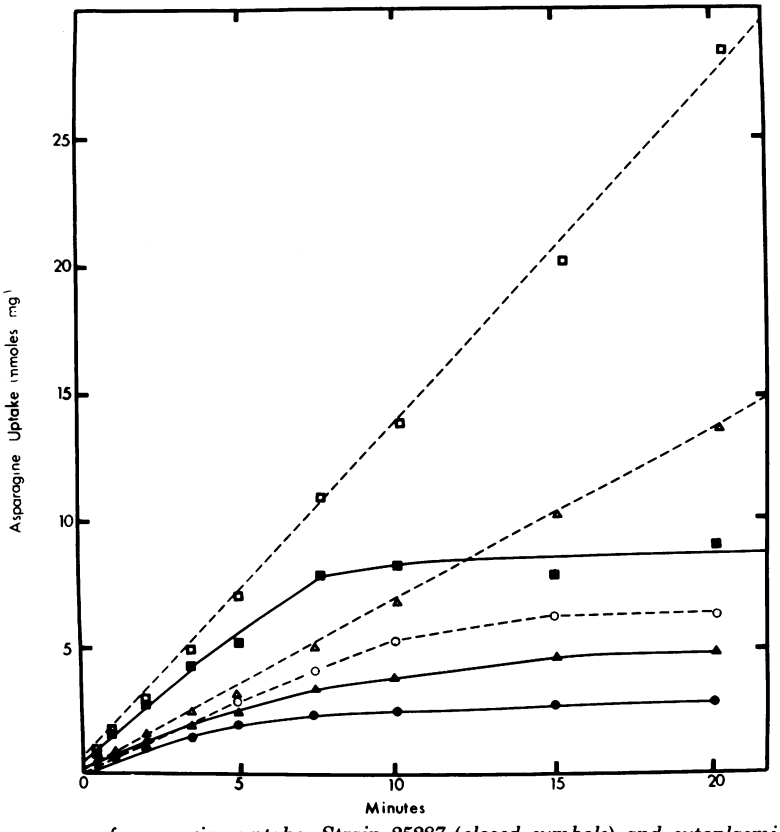


FIG. 1. *Time course of asparagine uptake. Strain 25287 (closed symbols) and cytoplasmic asparaginase-deficient strain 47 (open symbols) were incubated in assay mixtures initially containing [^{14}C]asparagine at concentrations of 7.5 μM (circles), 27.5 μM (triangles), and 207 μM (squares). Incubation mixtures were samples at the times indicated and radioactivity accumulated by the cells was determined as described in Materials and Methods.*

of asparagine uptake, 30-s determinations, are nearly identical whether metabolism is blocked or normal. The measurement of radioactivity accumulated during 30-s incubations of cells with [^{14}C]asparagine is therefore a relatively valid estimation of the initial rate of asparagine uptake.

Distribution of radioactivity accumulated. Figure 2 shows the distribution of radioactivity accumulated by cells with normal and blocked asparagine metabolism when incubated for 10 min in the presence of 10 μM [^{14}C]asparagine. Greater than 80% of the radioactivity accumulated by the asparaginase-deficient strain 47 was recovered as asparagine, whereas only 20% of the radioactivity accumulated by strain 25287 with normal asparaginase activity was recovered as asparagine. When experiments were similarly performed with 1-min incubations, greater than 90% of radioactivity accumulated by 47 cells and approximately 60% of the radioactivity accumulated by 25287 was recovered as asparagine. In the experiment shown in Fig. 2, strain 47 accumulated 5 nmol of asparagine/mg of cell protein during the 10-min incubation period. It can be estimated on the basis of cell volume-protein ratios (10) that the cellular concentration of extractable asparagine is 0.9 mM. This value represents nearly a 100-fold accumulation or maintenance of the intracellular asparagine pool above the initial external level. The accumulated pool is rapidly lost after dilution of cells into medium containing 1 mM 2,4-dinitrophenol. Either asparagine uptake or the maintenance of the internal asparagine pool would then appear to involve an active process (7).

Dependence of asparagine uptake on metabolic energy. Cells prepared from either carbon-starved or normal cultures retain considerable endogenous energy for the transport of asparagine (Table 1). As expected, cells from carbon-starved cultures have less capacity for uptake than cells from normal cultures. The initial rate of transport in both cell preparations can be stimulated above the endogenous levels by incubation with the carbon sources glucose, D-lactate, or succinate. No preference for a particular energy source is observed with the exception of succinate. At concentrations above 25 mM, succinate inhibited the transport of asparagine as well as aspartate, glutamine, and glutamate. This effect may be due to the high concentrations of cation associated with dicarboxylic acid.

The initial rate of asparagine uptake is inhibited by a variety of metabolic inhibitors routinely used to establish the dependence of transport on energy including the succinate dehydrogenase inhibitor, malonate, and lactate dehydrogenase inhibitors, oxamate and oxalate (Table 1). The glucose-stimulated asparagine uptake is not as sensitive to inhibition by malonate, oxamate, or oxalate as the succinate-

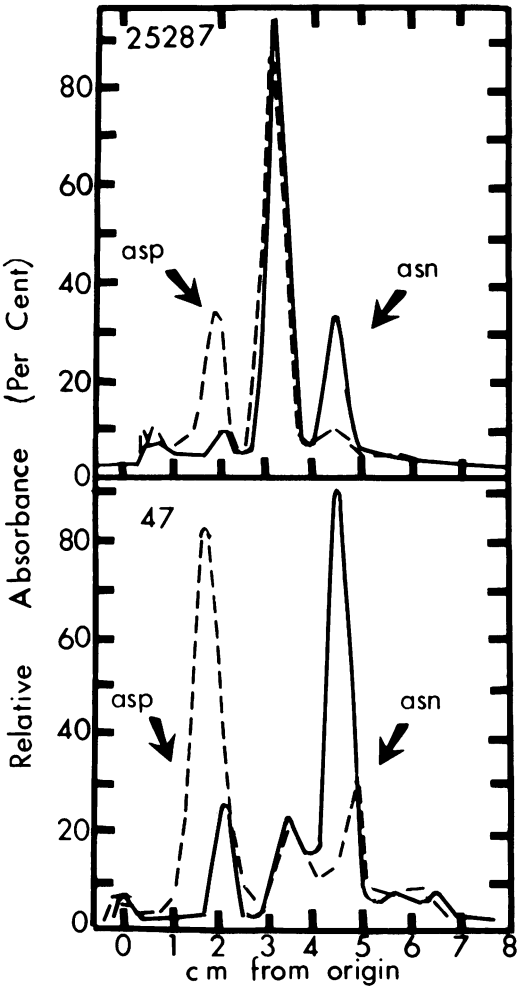


FIG. 2. *The distribution of the radioactivity accumulated during incubation of strains 25287 and 47 with* [^{14}C]*asparagine. Shown are the densitometric tracings of autoradiograms (16) of the thin-layer-chromatographed cell extracts. Cells were incubated for 10 min with 10 μM* [^{14}C]*asparagine (100 μCi/μmol). Assays were terminated and extracts were prepared and chromatographed as described in Materials and Methods. The* [^{14}C]*asparagine accumulated by the cells is identified by the loss of radioactivity in areas of chromatographed untreated sample (solid lines) when compared to an equivalent fraction of asparaginase B-treated sample (broken lines).*

stimulated uptake with malonate or D-lactate-stimulated uptake with oxamate or oxalate. These results suggest that the energy provided for the transport of asparagine is not exclusive to any one particular dehydrogenase (8), but may be provided by several different metabolic reactions leading to an energized state of the cytoplasmic membrane.

Concentration dependence of asparagine uptake. The effect of asparagine concentration on the initial rates of asparagine uptake is shown in Fig. 3. The reciprocal plot can be

TABLE 1. *Dependence of asparagine uptake by strain W3110 on metabolic energy*[a]

Culture conditions[b]	Additions[c]		% Uptake[d]
	Energy source	Inhibitor	
Normal	None	None	100
	0.025 M glucose	None	180
	0.025 M glucose	0.050 M malonate	51
	0.025 M glucose	0.050 M oxalate	25
	0.025 M glucose	0.050 M oxamate	18
	0.025 M glucose	0.002 M 2,4-dinitrophenol	5
	0.025 M glucose	0.010 M potassium cyanide	51
	0.025 M glucose	0.001 M iodoacetate	64
	0.025 M glucose	0.020 M sodium azide	56
	0.050 M succinate	None	60
	0.025 M succinate	None	114
	0.025 M succinate	0.050 M malonate	19
	0.050 M D-lactate	None	190
	0.025 M D-lactate	None	186
	0.025 M D-lactate	0.050 M oxalate	6
	0.025 M D-lactate	0.050 M oxamate	3
Carbon starvation	None	None	59
	0.025 M D-lactate	None	144
	0.025 M glucose	None	135

[a] Uptake was measured during a 60-s incubation period with 20 μM [^{14}C]asparagine.

[b] The preparation of normal and carbon-starved cells is described under Materials and Methods.

[c] The inhibitors were added to the cell suspensions 20 min before initiation of the assay and energy source 10 min before initiation of the assay. Basic and acidic compounds were neutralized with hydrochloric acid and potassium hydroxide, respectively.

[d] One hundred percent uptake was 0.71 nmol/min per mg.

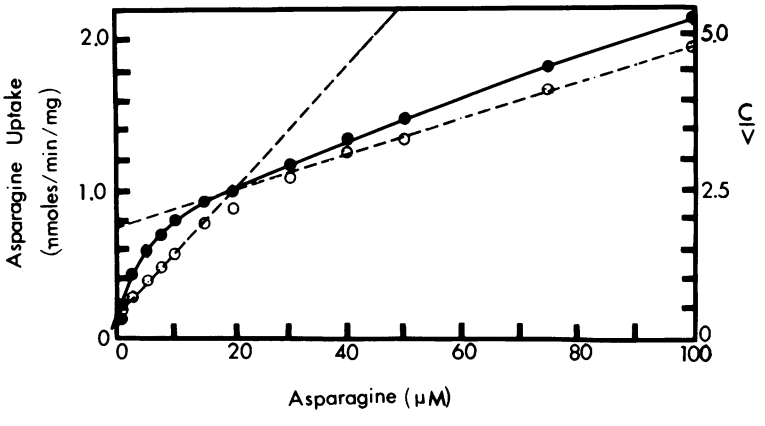


FIG. 3. *Concentration dependence of asparagine uptake. Asparagine uptake was measured with strain 47 at the initial* [^{14}C]*asparagine concentration indicated. The incubation mixtures were sampled at 30-s intervals for 2 min. Closed symbols represent the initial velocity of asparagine uptake. Open symbols represent C/V transformations of the initial rate data. C is in units of μM and V is in nmol/min per mg.*

represented by two linear segments and suggests that uptake of asparagine may be mediated by two systems. The apparent K_m and V_{max} values determined are 3.5 μM and 1.1 nmol/min per mg for the high-affinity component and 80 μM and 33 nmol/min per mg for the low-affinity component, respectively.

Regulation of the high-affinity component of asparagine uptake. When the K-12 strains used in this study were cultured in minimal medium supplemented with less than 100 μM asparagine, the low-affinity component of asparagine uptake was not apparent in the C/V transformations of initial velocity (v) measurements at substrate concentrations (c) below 20 μM. However, the supplementation of cultures with 1 mM asparagine repressed the high-affinity component of asparagine uptake. The repression is observed in Fig. 4 as the appearance of the low-affinity component in the transformed initial velocity data at substrate concentrations below 20 μM.

The growth of the asparagine-requiring auxotroph, 47, in nutrient broth is limited due to insufficient asparagine. Supplementation of nutrient broth with 100 μM L-asparagine overcomes this deficiency and 47 cultures establish doubling times and achieve cell densities equivalent to 3110 cultures. The high-affinity component of asparagine transport is derepressed under these conditions. As observed with the minimal medium cultures supplementation of nutrient broth with 2 mM L-asparagine represses the high-affinity component of asparagine transport. These results suggest that the repression is specific to asparagine.

Asparagine uptake does not appear to be subject to catabolite repression. Culture of the K-12 strains in minimal medium with 1% succinate replacing glucose did not significantly change the kinetic parameter of uptake from those indicated for glucose-grown cultures in Fig. 3.

Specificity of the high-affinity component of asparagine uptake. Several compounds, including amino acids, asparagine analogues, and keto acids, were tested for inhibition of asparagine uptake (Table 2). The system mediating asparagine uptake at low concentrations (5 μM asparagine) appears to be stereospecific for L-asparagine and recognizes only the L-isomers of analogues of asparagine with substitution at the β-amide position. Compounds providing greater than 20% inhibition of uptake

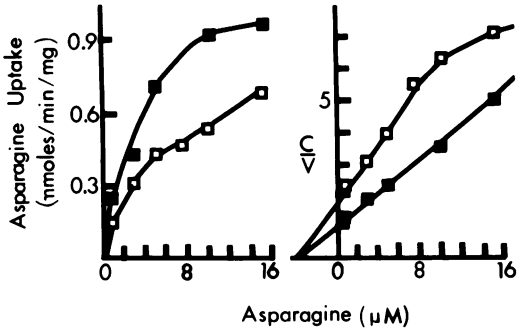


FIG. 4. *Repression of asparagine uptake. Strain W3110 was cultured under* (■) *standard conditions and* (□) *with a 1 mM* L-*asparagine supplement. Asparagine uptake was measured by a 30-s incubation with the concentrations of* [^{14}C]*asparagine indicated.*

TABLE 2. *Specificity of the high-affinity component of asparagine uptake by strain W3110*[a]

Unlabeled compound[b]	% Maximum[c] uptake
Asparagine	5
Aspartate	60
Glutamine	80
Glutamate	102
Leucine	100
Proline	90
Serine	85
Phenylalanine	92
Methionine	96
Lysine	94
D-Asparagine	80
Alanyl asparagine	80
β-Cyanoalanine	70
β-Aspartyl hydroxamate	10
Diazo-oxo-norvaline	5
α-Methyl aspartate	97
N-methyl-DL-aspartate	98
Succinamide	101
Pyrazole-DL-alanine	90
Diamino propyl phosphonic acid	94
O-phospho serine	105
O-phospho threonine	109
L-γ-amino butyrate	85
Succinate	99
Fumarate	97
Malate	102
Oxaloacetate	100
NH_4Cl (pH = 6.9)	95

[a] Uptake was measured with a 60-s incubation period and assay mixtures contained 5 μM L-[^{14}C]asparagine.

[b] Five hundred μM L-isomer unless indicated otherwise. Acidic compounds were neutralized with potassium hydroxide. Basic compounds were neutralized with hydrochloric acid.

[c] One hundred percent uptake was 1.2 nmol/min per mg.

when present at concentrations 100-fold in excess of L-[^{14}C]asparagine were 5-diazo-4-oxo-L-norvaline, β-hydroxyamyl-L-aspartic acid, L-aspartic acid, and L-β-cyanoalanine. Compounds providing greater than 10% inhibition of asparagine uptake were, in addition to those listed above, L-glutamine, D-asparagine, L-alanyl-L-asparagine, L-γ-amino butyrate, and L-serine. The apparent K_i values determined as shown in Fig. 5 of compounds providing greater than 20% inhibition of L-asparagine uptake are summarized in Table 3.

The inhibition of asparagine uptake by diazo-oxo-norvaline is noteworthy. Effects by the analogue are observed on both the slope and intercept of double reciprocal plots of experiments when the analogue and asparagine are added simultaneously (Fig. 6). If cells are first pretreated with the diazo derivative, washed to remove the compound, and subsequently tested for inhibition by the analogue, the intercept effect is no longer observed and the inhibition appears to be exclusively of the competitive type (Fig. 7). Comparison of the two experiments (Fig. 6 and 7) suggest that the action of the inhibitor may in part be explained by an irreversible inhibition of a portion of the transport activity. Direct evidence that the velocity is affected in an irreversible manner is provided by the experiment of Fig. 8. The K_m for asparagine uptake in the absence of the inhibitor does not seem to have been appreciably affected by the preincubation. The effect appears to be related to asparagine uptake since kinetic parameters of aspartate and glutamine transport are not altered in the pretreated cells.

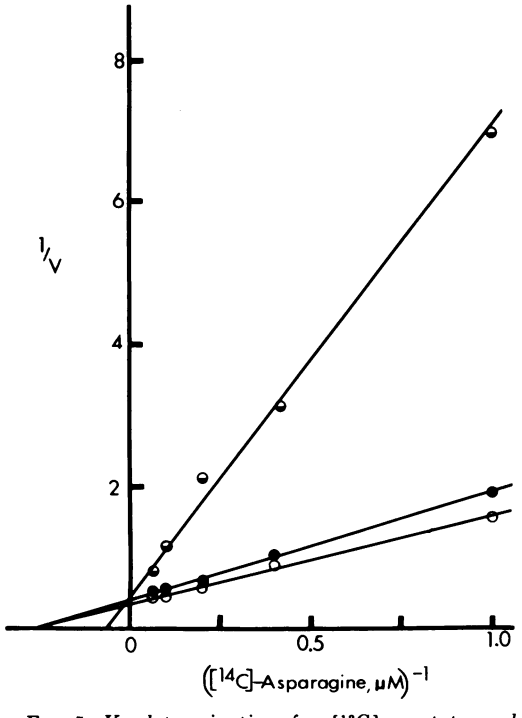


FIG. 5. *K_i determination for [^{12}C]aspartate and [^{12}C]asparagine inhibition of [^{14}C]asparagine uptake. Strain W3110 was incubated 30 s with the indicated concentrations of [^{14}C]asparagine and 25 μM [^{12}C]-aspartate or 25 μM [^{12}C]asparagine. V is in units of nmoles of asparagine/min per mg. Symbols represent: (○) no inhibitor; (●) 25 μM L-aspartate; (○) 25 μM L-asparagine.*

TABLE 3. *K_i values determined for inhibitors of asparagine uptake by strain W3110*[a]

Unlabeled competitor	K_i (μM)
L-asparagine	3.0
L-5-diazo-4-oxo-norvaline	4.6[b]
L-β-hydroxamyl aspartate	10
L-aspartate	50
L-β-cyanoalanine	275

[a] K_i values were determined as shown in Fig. 5 of $1/V_i$ versus $1/c$ plots where V_i is velocity in presence of an inhibitor and c is the initial concentration of L-[^{14}C]asparagine.

[b] The K_i value was determined with cells pretreated with 5 μM L-5-diazo-4-oxo-norvaline as described in the text.

DISCUSSION

Earlier studies suggested that *E. coli* may not actively accumulate asparagine (1, 2). Our results indicate that asparagine uptake is mediated by two transport systems which are distinguishable on the basis of specificity and regulation. The high-affinity component of asparagine uptake (K_m = 3.5 μM) does provide an energy-dependent accumulation of asparagine and most likely represents the system which maintains the endogenous asparagine pool required for protein synthesis. This system is repressed by culture of cells in medium containing asparagine supplements of 2 mM.

In a previous paper (16) we reported lags in the establishement of characteristic growth rates when cultures of the asparagine auxotroph 25287 growing with 1 mM asparagine supplements were subcultured into medium containing 0.01 to 0.1 mM asparagine supplements. The lag periods were not observed if subcultures were initiated from cultures grown with 0.05 to 0.07 mM asparagine supplements. A repression of the high-affinity asparagine transport system and, subsequently, the time required for depression of the system with the shift of cells into

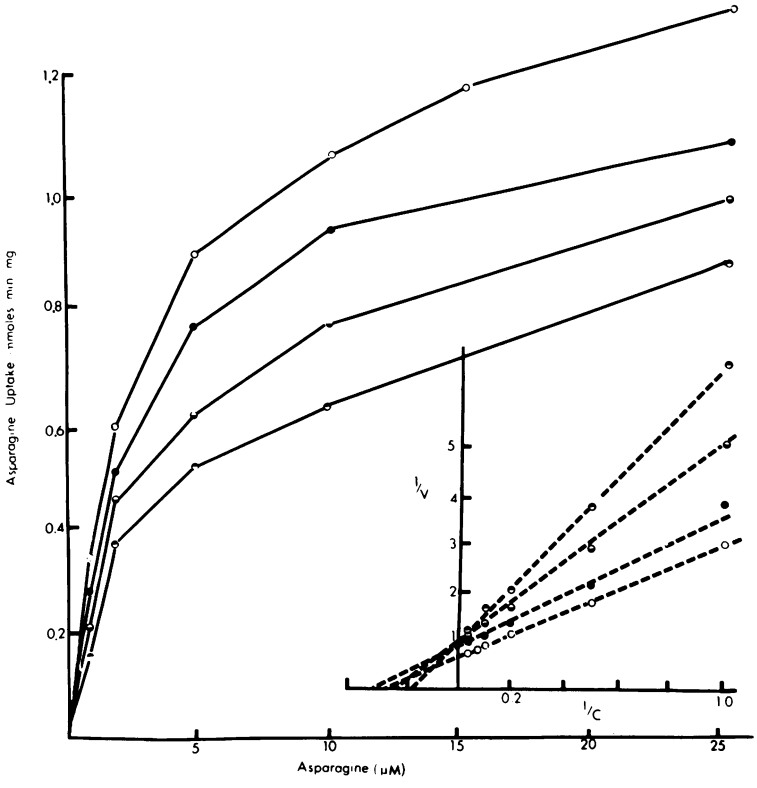
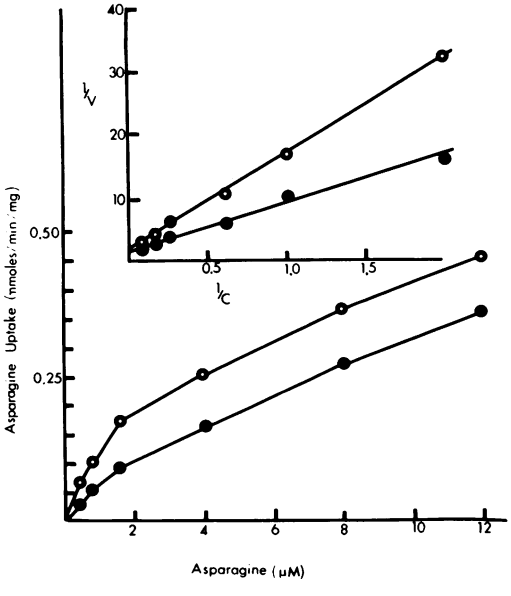


FIG. 6. *Inhibition of asparagine uptake by diazo-oxo-norvaline. Strain W3110 was incubated for 30 s in assay mixtures containing diazo-oxo-norvaline and [^{14}C]asparagine at the concentrations indicated. Symbols:* (○) *no diazo-oxo-norvaline;* (●) *2 μM diazo-oxo-norvaline;* (◒) *5 μM diazo-oxo-norvaline; and* (◓) *10 μM diazo-oxo-norvaline. V is in units of nanomoles of asparagine per minute per milligram.*



medium containing the low-asparagine supplements provide an explanation for these previous results. The lags in growth rate observed with the asparagine auxotroph represent the time required for return of the high-affinity transport system to a level of activity which is not growth rate limiting.

The low-affinity system (K_m > 80 μM) has not been studied in any detail but appears to be necessary for *E. coli* to use asparagine as a nitrogen source (16). Preliminary experiments indicate that both aspartate and ammonia are potent inhibitors of asparagine degradation by whole cells, whereas neither provides significant inhibition of asparagine degradation by toluene-

FIG. 7. *Uptake of asparagine in the presence* (●) *and absence* (○) *of 5 μM diazo-oxo-norvaline by cells pretreated with 5 μM diazo-oxo-norvaline for 30 min at 37 C in buffer A followed by centrifugation and resuspension in buffer A. The cells were then used to measure uptake in the standard manner.*

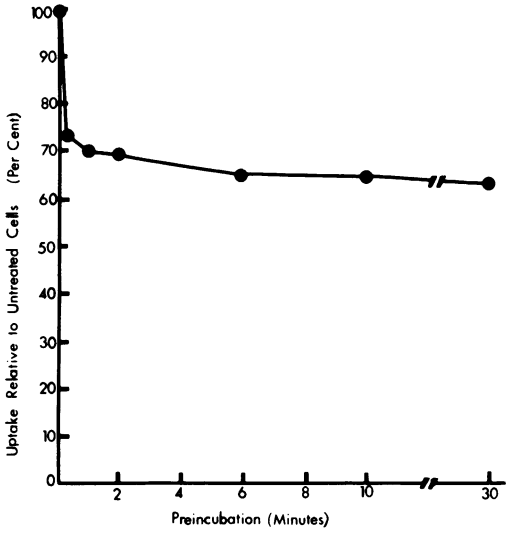


FIG. 8. *Effect of preincubation of cells with diazo-oxo-norvaline on asparagine uptake. Cells were preincubated with 5 μM diazo-oxo-norvaline in buffer A at 37 C for the times indicated and washed to remove diazo-oxo-norvaline as indicated for Fig. 7, and the uptake of asparagine by the washed cells was measured in the standard manner. The activity of the cells is expressed in percent relative to the uptake of cells similarly manipulated without the exposure to diazo-oxo-norvaline.*

treated cells or cell extracts. These preliminary results suggest that aspartate and ammonia may be preferred substrates for the transport system(s) represented by the low-affinity component of asparagine uptake. A similar conclusion is suggested from the results of [^{15}N]-ammonia sparing studies of Roberts et al. (13). However, other workers have found that asparagine does not serve as a competitor for the transport of aspartate (6), glutamine (15), or glutamate (3) by those systems believed to be primarily responsible for the uptake of the latter amino acids by *E. coli*. In this regard, unpublished experiments in this laboratory have confirmed these observations of the previous workers with the strain of *E. coli* used in this investigation. The general finding that excess asparagine does not inhibit the transport of these related amino acids together with the finding here that the related amino acids are not effective inhibitors of asparagine transport (Table 2) argue strongly for the individuality of the high-affinity asparagine transport system reported here.

ACKNOWLEDGMENTS

This research was supported by Public Health Service Predoctoral Research Fellowship award GM 42291 from the National Institute of General Medical Sciences awarded the senior author and by Public Health Service grant CA-08390 from the National Cancer Institute.

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