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Regulation of L-Cystine Transport in *Salmonella typhimurium*

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A kinetic analysis of L-cystine uptake in wild-type *Salmonella typhimurium* indicates the presence of at least two, and possibly three, separate transport systems. CTS-1 accounts for the majority of uptake at 20 μM L-cystine, with a V_{max} of 9.5 nmol/min per mg and a K_m of 2.0 μM; CTS-2 is a low-capacity, higher-affinity system with a V_{max} of 0.22 nmol/min per mg and a K_m of 0.05 μM; a third, nonsaturable process has been designated CTS-3. We find that wild-type CTS-1 levels are at least 11 times higher in sulfur-limited cells than in L-cystine-grown cells. Pleiotropic cysteine auxotrophs of the types *cysE* (lacking serine transacetylase) and *cysB*$^-$ (lacking a regulatory element of positive control) have very low levels of CTS-1 even when grown under conditions of sulfur limitation, which response is analogous to that previously observed for cysteine biosynthetic enzymes (N. M. Kredich, J. Biol. Chem. 246:3474-3484, 1971). CTS-1 is induced in *cysE* mutants by growth in the presence of *O*-acetyl-L-serine (the product of serine transacetylase), again paralleling the behavior of the cysteine biosynthetic pathway. Strain DW25, a prototrophic *cysB*^{c} mutant, which is constitutive for cysteine biosynthesis, is also derepressed for CTS-1 when grown on L-cystine. Since CTS-1 is regulated by sulfur limitation, *O*-acetyl-L-serine, and the *cysB* gene product, the same three conditions controlling cysteine biosynthesis, we propose that this transport system is a part of the cysteine regulon.

The biochemistry and genetics of amino acid transport in enteric bacteria have recently been subjects of considerable investigation, which has yielded valuable information on the specificity, kinetics, energy requirements, and binding proteins of various systems (11, 14, 22, 28). Some progress also has been made concerning the mechanisms by which transport activity is controlled (9, 14, 16); however, except for leucine (24-26) and glutamine (32) transport, the regulatory properties of most amino acid transport systems and their relationships, if any, to the biosynthesis of their respective amino acids remain obscure.

In *Salmonella typhimurium*, enzymes of the cysteine biosynthetic pathway are regulated by a genetic system of positive control. Derepression of this pathway occurs in the combined presence of sulfur starvation, the internal inducer *O*-acetyl-L-serine, and an intact *cysB* gene, which codes for an element of positive control (17). In this communication we describe an L-cystine transport system in *S. typhimurium* that is controlled by the same factors responsible for the regulation of cysteine biosynthesis.

MATERIALS AND METHODS

Materials. L-[G-^{14}C]proline, L-[G-^{14}C]histidine, and L-[^{35}S]cystine were obtained from Schwarz/Mann. The latter compound was purified by ion-exchange chromatography (1) and diluted before use with non-radiolabeled amino acid. L-Cystine from Sigma Chemical Co. was twice recrystallized from warm 5 N HCl before use. Bis-(α-methyl)-DL-cystine was synthesized by the method of Arnstein (4), and *O*-acetyl-L-serine was prepared as described previously (27). Lithium D-lactate and 1-*O*-methyl-α-D-glucopyranoside (α-methylglucoside) were purchased from Sigma. All other chemicals were obtained commercially and were of the highest purity generally available.

Bacterial strains. The mutants used in this work are all nonlysogenic derivatives of *S. typhimurium* LT2 (Table 1). The wild-type strain and cysteine auxotrophs came from the Demerec collection. Most strains and their construction have been previously described (17). The original *cysE2* strain from the Demerec collection has been shown to be a *cysE2 cysB1352* double mutant (10, 17), in which the *cysE2* allele results in cysteine auxotrophy owing to a lack of serine transacetylase and the *cysB1352* mutation leads to the constitutive expression of all the cysteine biosynthetic enzymes except serine transacetylase. DW18 and DW25 are "pure" *cysE2* and

TABLE 1. *Bacterial strains*

Strain	Genotype
cysB403	*cysB403*
DW18[a]	*cysE2*
DW19	*cysE6*
DW23	*cysE30*
DW24	*cysE396*
DW25[a]	*cysB1352*
DW42	*cysB15 trpA160*
DW44	*cysB18 trpA160*
DW45	*cysB24 trpA160*
DW46	*cysB484 trpA160*
DW80	*cysB232 trpA160 ara-9*
DW387	Unknown; resistant to selenite

[a] Strain DW18 contains a wild-type *cysB* allele rather than the *cysB1352* mutation present in the original *cysE2* strain from the Demerec collection. Strain DW25 is a prototroph and was derived from the original *cysE2* strain by transduction to $cysE^+$ (17).

cysB1352 strains, respectively. DW387 was derived from the wild type by selecting for selenite resistance as described previously (15).

Growth and preparation of bacteria. All cultures were grown in the minimal medium E of Vogel and Bonner (31), which was supplemented with 5 g of glucose per liter and with 20 mg of L-tryptophan per liter for the growth of tryptophan auxotrophs. The methods used in substituting different sulfur sources for sulfate and for growth in the presence of *O*-acetyl-L-serine have been described previously (17).

Overnight cultures were diluted into fresh medium at a density of 0.5×10^8 to 1.0×10^8 cells per ml and incubated at 37°C with vigorous shaking. Growth was monitored by turbidity at 650 nm, using a spectrophotometer in which 10^9 cells per ml give an optical density of 1.0. After two to three doublings, bacteria were harvested by centrifugation, washed twice with sulfur-free minimal medium E containing 2 g of glucose and 160 mg of chloramphenicol per liter (solution A), and then resuspended in solution A at a density of 8×10^9 per ml.

For experiments in which the energy requirements of L-cystine uptake were studied, bacteria were deprived of energy stores in two different ways. In the first, cells in late log phase were collected by centrifugation, washed with 0.9% NaCl, and suspended to their original volume in glucose- and citrate-free medium E (8) containing 5 mM 2,4-dinitrophenol (5). After 12 h of shaking at 37°C, these bacteria were again harvested, washed four times, and suspended in a small volume of a phosphate- and sulfur-free solution, consisting of 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), 15 mM KCl, 15 mM NH_4Cl, 0.4 mM $MgCl_2$, and 160 mg of chloramphenicol per liter (7). This buffer was also used to make up the remainder of the amino acid uptake assay mixture. In the second method, cells were harvested in mid-log phase, washed as before, and then suspended in prewarmed glucose- and citrate-free medium E containing 40 mM sodium azide and 20 mM α-methylglucoside (23). After 2 h of shaking at 37°C, the bacteria were harvested, washed, and suspended as in the first procedure.

Osmotic shock was performed by the method of Berger and Heppel (6) with the addition of 4 mM ethylenediaminetetraacetate to the Tris-sucrose as described by Willis et al. (33). Cells in the mid-log phase were harvested and washed three times with 10 mM Tris-hydrochloride (pH 7.3)–30 mM NaCl. The pellet was taken up in 1/20 the original culture volume of 33 mM Tris-hydrochloride (pH 7.3) at 23°C, and an equal volume of 33 mM Tris-hydrochloride (pH 7.3)–40% sucrose–4 mM disodium ethylenediaminetetraacetate was added. This suspension was swirled at 23°C for 5 min, after which the cells were collected by centrifugation and rapidly suspended in 1/10 the original culture volume of 0.5 mM $MgCl_2$. After swirling at 4°C for 10 min, this preparation was again centrifuged, and the pellet was suspended in solution A for use in amino acid uptake studies.

Transport assays. All assays were performed at 23°C with cells and solutions previously equilibrated at this temperature. Reactions were initiated by mixing a portion of cell suspension with solution A containing approximately 0.1 μCi of radiolabeled amino acid in a final volume of 1.0 ml. Cell densities in reaction mixtures ranged from 2×10^8 to 8×10^8 cells per ml, and the standard assay contained 20 μM amino acid. Samples of 0.1 ml were taken at 15, 30, 60, 120, and 300 s after the start of the reaction and filtered through 25-mm nitrocellulose membrane filters (0.45-μm pore size). After a wash with 10 ml of a solution containing 0.1 M Tris-hydrochloride (pH 7.3), 0.15 M NaCl, and 0.5 mM $MgCl_2$ (6), the filters were dried, and radiolabel was measured in a liquid scintillation counter at 70% efficiency for either ^{35}S or ^{14}C.

Zero-time values were established either by using toluene-treated cells (7) or by extrapolation when uptake was linear during the first minute of assay. Activities are expressed as initial velocities in terms of nanomoles per minute per milligram of cell protein, using uptake values comprising less than 10% of total input. Cell protein concentrations were estimated by using a previously reported value for *S. typhimurium* of 271 mg of protein per 10^{12} cells (18). An intracellular volume of 5.4 μl/mg of protein was calculated from the value of 2.7 μl/mg (dry weight) reported for *Escherichia coli* (34), with the assumption that 50% of the dry weight is protein.

Other methods. The methods used for the preparation of cell extracts and their assay for sulfite reductase and *O*-acetylserine sulfhydrylase have been described previously (17). Cysteine desulfhydrase was assayed by measuring the rate of sulfide production, with correction for product inhibition (19). Protein in crude extracts was determined by the method of Lowry et al. (21), using bovine serum albumin as a standard.

RESULTS

General properties of L-cystine transport in *S. typhimurium*. Sulfate-grown cells of wild-

type S. typhimurium were found to take up L-[35S]cystine rapidly, concentrating radiolabel 17-fold in 15 s and 28-fold in 30 s at an external L-cystine concentration of 20 μM (Fig. 1). Initial rates of uptake varied between 6 and 8 nmol/min per mg and were increased by approximately 0 and 25% by preloading cells with 20 μM non-radiolabeled L-cystine for 1 and 5 min, respectively, prior to the addition of L-[35S]cystine. Harvesting and washing bacteria at 23°C rather than the usual 4°C had essentially no effect on initial rates of L-cystine uptake. Bacteria harvested during the stationary phase of growth transported L-cystine at about 75% the rate observed in log-phase cells, and uptake activities in harvested cells of both types were stable for at least 3 h of storage at 23°C.

Although we have not investigated the immediate fate of intracellular radiolabel, Berger and Heppel noted rapid metabolism of L-cystine transported by E. coli W (6), and the same is probably true in our system as well. Theoretically, L-cystine catabolism could create special problems in evaluating kinetic data in cells grown on this amino acid and, hence, induced for cysteine desulfhydrase (19). Nonetheless, we have assumed that initial rates of uptake represent a reasonable measure of transport activity, and later in this communication we present evidence indicating that cysteine desulfhydrase does not seriously interfere with our assay.

L-Cystine transport activity is reduced to low levels in sulfate-grown S. typhimurium depleted of energy stores by growth in the presence of either 2,4-dinitrophenol or α-methylglucoside plus sodium azide (Table 2). The latter treatment was employed after we noted significant lysis of cells during 12 h of incubation with 2,4-dinitrophenol. Both treatments also markedly lowered rates of uptake for L-histidine and L-proline, amino acids whose transport systems have been previously characterized as, respectively, shock sensitive and shock resistant (7). Preincubation with glucose increased uptake rates for all three amino acids, but only L-proline transport was stimulated by preincubation with D-lactate (Table 2).

Osmotic shock is known to decrease the activities of amino acid transport systems in which periplasmic binding proteins participate (7). Subjecting S. typhimurium cells to such treatment produced a 34% decrease in the rate of L-cystine transport, an effect comparable to that observed for L-histidine uptake (Table 3), which is known to be mediated by a shock-sensitive transport system (2). L-Proline transport, a shock-resistant system, was slightly stimulated

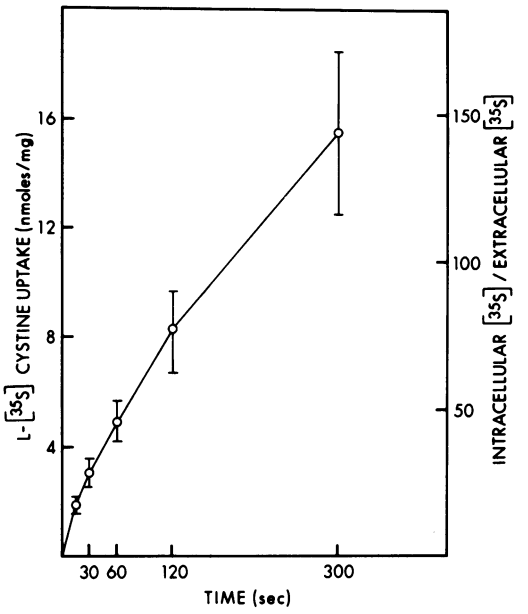

FIG. 1. *Time course of L-[35S]cystine uptake by wild-type cells grown on sulfate. The preparation of cells and the uptake assay are described in the text. The standard deviation determined for each time point is based on assays of 20 different cultures. The right-hand ordinate indicates the fold concentration of radiolabel and is expressed as the ratio of intracellular to extracellular 35S.*

TABLE 2. *Effects of energy source on amino acid transport*[a]

Amino acid	Energy source (mM)	Amino acid uptake (nmol/min per mg)	
		Dinitrophenol treated	α-Methylglucoside and azide treated
L-[35S]cystine	None	1.16	0.88
	Glucose, 11	3.56	7.00
	D-Lactate, 20	0.44	0.72
L-[14C]histidine	None	1.28	1.08
	Glucose, 11	3.48	2.76
	D-Lactate, 20	1.36	0.20
L-[14C]proline	None	0.28	0.28
	Glucose, 11	9.44	7.64
	D-Lactate, 20	2.12	2.88

[a] Sulfate-grown, wild-type cells were energy deprived by growth on either 2,4-dinitrophenol or α-methylglucoside plus sodium azide as described in the text. Cells were preincubated with the indicated energy source for 10 min at 23°C before addition of the labeled amino acid at a concentration of 20 μM.

TABLE 3. *Effect of osmotic shock on amino acid transport*[a]

Amino acid	Amino acid uptake (nmol/min per mg)		Activity retained in shocked cells (%)
	Control cells	Shocked cells	
L-[^{35}S]cystine	5.56 (±0.74)	3.66 (±0.36)	66
L-[^{14}C]histidine	3.76 (±0.48)	2.12 (±0.20)	56
L-[^{14}C]proline	5.30 (±0.78)	6.36 (± 0.48)	120

[a] Sulfate-grown, wild-type cells were osmotically shocked as described in the text. All amino acids were at a final concentration of 20 μM. The standard deviation for each value is based on assays of eight different cultures.

in osmotically shocked cells.

Kinetic studies demonstrated that the rate of L-cystine uptake is a saturable process. For sulfate-grown, wild-type cells, a plot of (velocity)$^{-1}$ versus (L-cystine concentration)$^{-1}$ gave a line with a slight, but distinct, upward convexity, consistent with the presence of at least two separate transport systems (Fig. 2). This plot was resolved, by the iterative technique of Winter and Christensen (35), into the sum of two different transport systems. The first of these, designated CTS-1, had a K_m of 2.0 μM and, with its V_{max} of 9.5 nmol/min per mg, accounted for most of the transport activity of sulfate-grown cells at our standard L-cystine concentration of 20 μM. The second system, CTS-2, showed a much greater affinity for L-cystine, with a K_m of 0.05 μM, but had a V_{max} of only 0.22 nmol/min per mg. Technical problems prevented the accurate measurement of uptake activity of L-cystine concentrations higher than 20 μM, but the data (not shown) suggested the presence of a relatively low-activity, nonsaturable system, designated CTS-3, with a rate constant of approximately 0.04 nmol/min per mg per μM L-cystine.

The selenite-resistant mutant DW387 proved to be very useful in confirming the conclusions reached from kinetic studies on wild-type cells. The rate of L-cystine uptake in sulfate-grown cells of this strain was found to be only about 10% of that measured in the wild type at 20 μM L-cystine. Kinetic analysis showed what appeared to be the usual amount of CTS-3 activity, no CTS-1, and the presence of CTS-2 with a K_m of 0.11 μM and a V_{max} of 0.29 nmol/min per mg.

The effects of various analogues on L-cystine transport were assayed by using sulfate-grown cells of the wild type to estimate CTS-1 activity and sulfate-grown DW387 for measurements of CTS-2 activity. These studies showed that, at analogue concentrations 40 to 100 times greater than that of L-cystine, CTS-1 is more than 50% inhibited by DL-selenocystine, L-cystine hydroxamate, D-cystine, and *meso*-cystine (Table 4). It seems likely that inhibition by the latter three compounds resulted, at least in part, from the

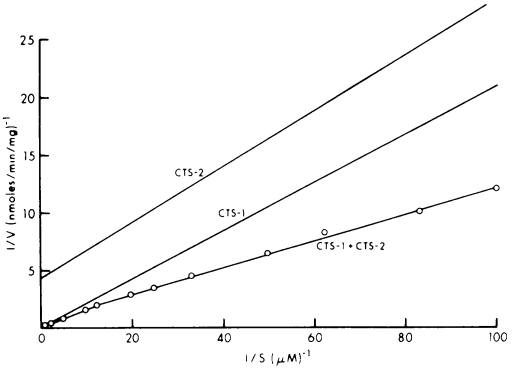

FIG. 2. *Double-reciprocal plot of L-[^{35}S]cystine uptake by wild-type cells grown on sulfate. Lines labeled CTS-1 and CTS-2 are the theoretical plots calculated from the K_m and V_{max} values derived by iteration as described in Results. The sum of these two lines (CTS-1 + CTS-2) is compared with the actual experimental values* (○).

presence of small contaminants of L-cystine. The inhibition pattern observed for CTS-2 was similar to that of CTS-1. DL-Diaminopimelate, L-cystathionine, DL-lanthionine, and L-cystine dimethyl ester, which are potent inhibitors of one component of L-cystine transport in *E. coli* W (6), had lesser or negligible effects on CTS-1 and CTS-2.

CTS-1 activity after growth on different sulfur sources. Wild-type cells were grown on several different sulfur compounds and assayed for transport activity at the standard L-cystine concentration of 20 μM. Growth on L-djenkolate, a poor sulfur source, resulted in the highest rate of uptake, which was 11 times greater than that noted in cells grown on L-cystine itself. Bacteria grown on either sulfite, sulfate, or reduced glutathione exhibited activities intermediate to these two extremes (Table 5). Thus, the expression of L-cystine transport activity varies with the source of sulfur used for growth in the same fashion as that known to occur for sulfite reductase, *O*-acetylserine sulfhydrylase A (Table 5), and other enzymes of the cysteine biosynthetic pathway (17). Since at 20 μM L-cystine, CTS-2 and CTS-3 are only

minor contributors to the total cystine transport activity of sulfate-grown cells, most, if not all, of the differences noted must have been due to changes in CTS-1.

L-Cystine transport in *cys* regulatory mutants. The coincident expression of CTS-1 and cysteine biosynthetic enzymes, observed during growth on different sulfur sources, prompted us to examine whether both processes might be under the control of the same regulon. Our approach consisted of measuring L-cystine transport activity in strains carrying mutations in the two genes known to be involved in regulating the biosynthetic pathway, *cysE* and *cysB* (17).

TABLE 4. *Effects of L-cystine analogues on CTS-1 and CTS-2 activities*

Inhibitor[a]	Activity (% control)	
	CTS-1[b]	CTS-2[b]
None	100	100
DL-Selenocystine	5	23
L-Cystine hydroxamate	35	17
D-Cystine	34	72
meso-Cystine	47	24
DL-Lanthionine	72	41
Cystamine	72	129
Bis-(α-methyl)-DL-cystine	86	115
L-Cystathionine	88	58
L-Cystine dimethyl ester	99	88
L-Djenkolate	128	96
DL-Diaminopimelate	134	89

[a] Each inhibitor was present at a concentration 100 times greater than that of L-[^{35}S]cystine: 2 mM inhibitor for CTS-1 and 0.05 mM inhibitor for CTS-2, except that DL-selenocystine and L-cystine hydroxamate were used at 0.4 mM in CTS-1 assays.

[b] CTS-1 was assayed at 20 μM L-[^{35}S]cystine (10 times the K_m value) with sulfate-grown wild-type cells, and CTS-2 was assayed at 0.5 μM L-[^{35}S]cystine (10 times the K_m value) with DW387 cells grown on sulfate. Control values were 6.68 and 0.15 nmol/min per mg for CTS-1 and CTS-2, respectively.

TABLE 5. *L-Cystine transport and cysteine biosynthetic activities in the wild type grown on different sulfur sources*[a]

Sulfur source (mM)	nmol/min per mg		
	L-Cystine transport	Sulfite reductase	O-Acetylserine sulfhydrylase
L-Cystine, 0.5	0.92	0	170
Sulfite, 1.0	4.36	15	1,560
Sulfate, 0.8	7.32	33	6,000
Reduced glutathione, 0.5	5.40	60	11,600
L-Djenkolate, 0.5	9.85	86	14,100

[a] L-[^{35}S]cystine uptake was assayed with intact cells at a concentration of 20 μM, whereas sulfite reductase and O-acetylserine sulfhydrylase were assayed in crude extracts of separately grown cultures.

The *cysE* locus codes for serine transacetylase (20), which catalyzes the synthesis of the cysteine precursor and inducer of the biosynthetic pathway, O-acetyl-L-serine. Therefore, sulfur-limited cultures of *cysE* mutants are not derepressed for cysteine biosynthetic enzymes unless grown in the presence of exogenous O-acetyl-L-serine (17). L-Cystine transport activities are also very low in sulfur-limited cultures of the *cysE* mutants DW18, DW19, DW23, and DW24, but increase two- to sixfold upon addition of O-acetyl-L-serine to the growth medium (Table 6). This effect was noted only after 10 min of growth in the presence of inducer and was prevented by prior addition of chloramphenicol. Control studies showed that L-cystine transport is not significantly affected by the inclusion of 1.0 mM O-acetyl-L-serine in the assay itself.

The *cysB* locus codes for an element of positive control, most likely a protein (29), which is necessary for derepression of all the cysteine biosynthetic enzymes except serine transacetylase (17). Mutants of the type *cysB*$^-$ are cysteine auxotrophs, owing to low or absent levels

TABLE 6. *L-Cystine transport activities of cysE mutants*

Strain	O-acetyl-L-serine[a]	L-Cystine uptake (nmol/min per mg) when grown on:	
		Reduced glutathione[b]	L-Cystine[b]
Wild type	−	5.40	0.92
	+	5.50	
DW18	−	1.10	0.58
	+	4.92	
DW19	−	1.08	1.18
	+	6.26	
DW23	−	1.12	0.94
	+	5.48	
DW24	−	1.79	0.74
	+	3.67	

[a] Cultures were grown for two generations in the presence (+) or absence (−) of 0.5 mM O-acetyl-L-serine.

[b] Sulfur sources were 0.5 mM in the growth medium. Reduced glutathione was used as a limiting sulfur source, since *cysE* mutants grow very poorly on L-djenkolate. L-[^{35}S]cystine uptake was measured at a concentration of 20 μM.

of these enzymes, even during sulfur starvation with an excess of exogenous *O*-acetyl-L-serine. L-Cystine uptake was found to be very slow in sulfur-limited cells of all six *cysB*$^-$ strains studied (Table 7) and was not enhanced by growth on *O*-acetyl-L-serine. Kinetic analyses of two mutants showed depressed levels of CTS-1 activity with small changes in CTS-2.

The prototrophic mutant DW25 carries a *cysB*^{c} mutation, which results in the constitutive expression of the cysteine biosynthetic pathway even during growth on L-cystine and in the absence of *O*-acetyl-L-serine (10, 17). L-Cystine-grown cells of this strain were found to be almost fully derepressed for CTS-1 as well, with a low level of CTS-2 (Table 7).

Physiological consequences of defective control of CTS-1 activity. The growth of wild-type *S. typhimurium* is inhibited by high concentrations of L-cysteine (12), and we find, to a lesser extent, the same is true for L-cystine as well. The exact mechanisms involved are not well understood but probably include inhibition of one or more vital enzymes by L-cysteine (13). The addition of 2 mM L-cystine to a sulfur-limited culture of the wild type causes a lag phase (Fig. 3), followed by exponential growth with a doubling time of 80 min (versus 50 min on sulfate). In contrast to the wild type, similarly treated *cysB403* shows only a negligible lag phase and grows with a doubling time of 54 min. Each of the five other *cysB*$^-$ strains tested behaved exactly like *cysB403*. Of particular interest is the response of the *cysB*^{c} strain, DW25, which exhibited a marked increase in sensitivity to growth inhibition by L-cystine (Fig. 3). Sensitivity to L-cysteine was similar to that of the wild type in all *cysB*$^-$ strains studied, but DW25 was even more markedly inhibited by this amino acid as well as by its derivative L-cystine.

Strains carrying mutations in *cysE* also were found to be resistant to L-cystine but regained wild-type sensitivity when pregrown in the presence of *O*-acetyl-L-serine (data not shown). This inducer did not affect the response of *cysB*$^-$ mutants to L-cystine.

In addition to their resistance to growth inhibition by L-cystine, several *cysB*$^-$ strains were poorly induced by this amino acid for cysteine desulfhydrase. Levels of this enzyme during growth on 2 mM L-cystine ranged from normal to barely detectable in the six *cysB*$^-$ mutants tested, whereas each of these strains was well induced during growth on 0.25 mM L-cysteine (Table 8). The latter response indicates that the poor induction by L-cystine was not secondary to effects of *cysB* mutations on the regulation of cysteine desulfhydrase itself. Induction of cysteine desulfhydrase by L-cystine was normal in *cysE* mutants, even in the absence of exogenous *O*-acetyl-L-serine.

TABLE 7. *L-Cystine transport activity in cysB mutants*

Strain	L-Cystine uptake (nmol/min per mg) when grown on:			
	0.5 mM L-cystine	0.5 mM L-djenkolate		
	Total[a]	Total[a]	CTS-1[b]	CTS-2[b]
Wild type	0.92	9.85	9.5	0.22
cysB403	1.36	1.36	0.7	0.10
DW42	0.71	0.75		
DW44	0.68	1.52		
DW45	0.94	2.26	2.5	0.16
DW46	1.03	1.74		
DW80	0.41	1.35		
DW25	8.40	10.7	7.0[c]	0.04[c]

[a] Total activity is that measured at 20 μM L-cystine. Discrepancies between this value and the sum of CTS-1 and CTS-2 are due to the presence of CTS-3 and the fact that assays were performed on different cell preparations.

[b] The values given for CTS-1 and CTS-2 are estimates for V_{max} as determined by kinetic analyses. Values for K_m varied from 1.0 to 2.2 μM for CTS-1 and from 0.05 to 0.22 μM for CTS-2.

[c] These values are from L-cystine-grown cells of DW25.

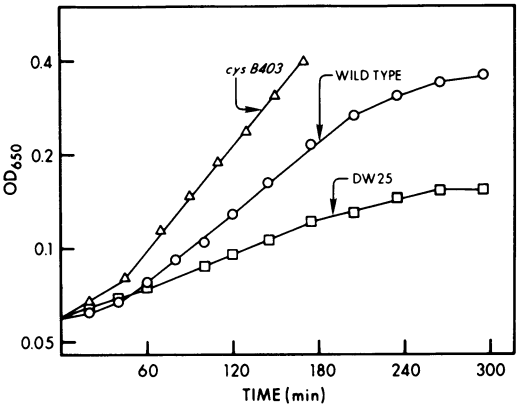

FIG. 3. *Growth on 2 mM L-cystine of wild type (○), cysB403 (△), and DW25 (□). Overnight cultures grown on 0.5 mM L-djenkolate were diluted at zero time in fresh medium containing 2 mM L-cystine, and optical density at 650 nm (OD_{650}) was followed with time.*

DISCUSSION

The ease of substrate saturation and the energy requirements of L-cystine uptake in *S. typhimurium* establish this process as a form of mediated transport. The extremely early and

marked concentration of radiolabel observed in sulfate-grown, wild-type cells further suggests that transport is active, although rapid metabolism of L-cystine, such as has been observed in *E. coli* (6), admittedly could account for our results without a significant intracellular accumulation of unaltered, free amino acid. CTS-1 resembles the transport system of L-histidine more closely than that of L-proline, as evidenced by the failure of D-lactate to serve as an energy source and by the partial decrease in activity after osmotic shock. From these observations one might predict the existence of *S. typhimurium* of a shock-sensitive L-cystine binding protein similar to that described in *E. coli* (6), and preliminary experiments are consistant with this prediction (E. W. Baptist, unpublished data).

Kadner has reported that, in *E. coli*, prior loading of cells with L-methionine for several minutes markedly diminishes the rate of uptake of that amino acid (16). We find that such inhibition does not occur during L-cystine transport in *S. typhimurium* and that preloading actually stimulates uptake activity slightly. Presently we have no good explanation for this unusual effect.

The fortuitous finding of a total lack of CTS-1 in the selenite-resistant mutant DW387 affirms and lends a genetic basis to our kinetically derived conclusion that at least two, and possibly three, separate processes are involved in L-cystine transport in *S. typhimurium*. We have found that approximately 10% of the selenite-resistant mutants have obvious defects in CTS-1, but currently have no satisfactory explanation for the association between these two phenomena.

In contrast to our success in obtaining reproducible kinetic data for DW387 and other CTS-1-deficient mutants, similar analyses of the low activities found in the L-cystine-grown wild type have been technically unsatisfactory. If we assume, however, that CTS-2 and CTS-3 in the wild type are unaffected by growth on different sulfur sources, the activities of these two systems at 20 μM L-cystine should be 0.22 and 0.8 nmol/min per mg, respectively. The sum of these two is very close to the value of 0.92 nmol/min per mg observed in the L-cystine-grown wild type, suggesting that CTS-1 may be almost completely absent in such cells. A consideration of CTS-3 activity also offers a plausible explanation for the ability of CTS-1-deficient cells to grow normally on 0.5 mM L-cystine as the sole sulfur source. Previous studies have established the sulfur requirement of wild-type *S. typhimurium*, growing with a doubling time of 50 min, to be approximately 3.6 nmol/min per mg (18), a value that can be achieved by CTS-3 at an external L-cystine concentration of only 0.09 mM.

TABLE 8. *Induction of cysteine desulfhydrase in cysB strains*

Strain	Cysteine desulfhydrase activity (nmol/min per mg) when grown on:	
	2 mM L-cystine	0.25 mM L-cysteine
Wild type	346 (±59)[a]	408 (±49)[a]
cysB403	6	342
DW42	23	289
DW44	132	309
DW45	310	342
DW46	400	348
DW80	228	277
DW25	277	383

[a] Mean (± standard deviation) for seven separate experiments for L-cystine-grown cells and eight separate experiments for L-cysteine-grown cells.

In addition to its negative control by growth on L-cystine, CTS-1 is dramatically affected by mutations in *cysB*, the regulatory gene for cysteine biosynthesis. Thus, *cysB*$^-$ mutants cannot be fully derepressed for CTS-1, whereas the prototrophic *cysB*^{c} strain DW25 is constitutive for both cysteine biosyntehsis and CTS-1 during growth on L-cystine. The behavior of DW25 is of additional importance, since it indicates that the low levels of CTS-1 in the L-cystine-grown wild type are secondary neither to feedback inhibition (16) nor to artifactual interference with our assay.

O-acetyl-L-serine is also necessary for the expression of both CTS-1 and the biosynthetic pathway. The failure of an exogenous source of this inducer to increase CTS-1 levels in nongrowing or chloramphenicol-treated *cysE* mutants implies that its effect is mediated through a process requiring new protein synthesis, rather than by modification of an existing transport system.

Substrate-mediated, negative control of amino acid transport activity is a well-recognized phenomenon in bacteria and appears often to involve repression of genes coding for amino acid-specific transport proteins. Little is known, however, about the genetic elements involved in the regulation of such systems. Studies in *E. coli* have demonstrated that leucyl-transfer ribonucleic acid plays a role in the repression of both the biosynthesis (30) and transport (24) of branched-chain amino acids. It is clear, however, that these two processes are regulated by genetic systems that are otherwise independent from one another (3, 26). Glutamine synthetase and glutamine transport in both S. typhimurium and *E. coli* are also coor-

dinately expressed according to the availability of readily utilizable nitrogen (9, 32). It is interesting to speculate on whether this system will prove to be analogous to CTS-1 and cysteine biosynthesis, since both are concerned with the economy of a major elemental nutrient. Thus far, however, CTS-1 and the cysteine biosynthetic pathway appear to be unique in that they are both controlled by the same regulon.

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