

D-Serine Transport System in *Escherichia coli* K-12¹

SHARON D. COSLOY²

Department of Microbiology, New York University School of Medicine, New York, New York 10016

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The D-serine transport system in *Escherichia coli* K-12 was studied by use of a mutant unable to form D-serine deaminase, yet resistant to D-serine. The mutant is greatly impaired in its ability to accumulate D-serine, D-alanine, and glycine. Transport of L-alanine is partially affected but transport of L-serine is unaffected. The mutant is also resistant to D-cycloserine, indicating that D-serine is transported by the system responsible for uptake of D-cycloserine. The D-serine transport system is not inducible, but appears to be formed constitutively, as are the transport systems of most amino acids. The transport mutation appears to be multistep and maps to the right of *malB* on the *E. coli* linkage map.

Piperno and Oxender suggested the presence of the following amino acid transport systems in *Escherichia coli* K-12: system I for alanine, serine, and glycine; system II for leucine, isoleucine, and valine; system III for tryptophan, tyrosine, and phenylalanine; and system IV for methionine (9). Those systems were shown to exist by means of competition between various amino acids. Use of permease mutants is another method of analyzing uptake systems.

Sensitivity to a toxic analogue affords an opportunity for direct selection of permease mutants. Schwartz and co-workers found that mutants of *E. coli* W selected for resistance to D-serine were deficient in the uptake of glycine, L-alanine, and D-serine (11). Curtiss and co-workers isolated mutants of *E. coli* K-12 resistant to D-cycloserine which were also permease mutants (5). Using these mutants, Wargel et al. found that the system which transports D-cycloserine, D-alanine, and glycine is different from the main transport system for L-alanine (14). These authors came to the same conclusions using competition studies (13).

Kessel and Lubin used a combination of (i) glycine transport mutants and (ii) competition studies to demonstrate that glycine, D-alanine,

and D-cycloserine are on the same transport system in *E. coli* W, and that L-alanine and D-serine appear to be transported by the glycine transport system but may have additional means of rapid access into the cell (6).

Isolation of a D-serine-resistant, D-serine permease-defective mutant of *E. coli* K-12 made possible the present study of D-serine uptake and analysis of its transport system.

MATERIALS AND METHODS

Materials. Uniformly labeled ¹⁴C-L-serine (135 Ci/mol), ¹⁴C-L-alanine (120 Ci/mol), and ¹⁴C-glycine (10 Ci/mol) were purchased from Nuclear-Chicago Corp. Uniformly labeled ¹⁴C-D-alanine (40.9 Ci/mol) and ¹⁴C-D-serine (18 Ci/mol) labeled in the third carbon were purchased from Amersham/Searle. Chloramphenicol was a gift from Parke, Davis & Co. D-Serine was obtained from Nutritional Biochemicals Corp., and D-cycloserine was obtained from K and K Laboratories. Membrane filters (0.45 μm pore size) were purchased from Millipore Corp.

Strains and media. All bacterial strains used in this study were derivatives of *E. coli* K-12. Table 1 lists the strains used and their relevant genetic markers and origins. The symbol *dag* is introduced to represent the genetic locus in the permease mutants: *d* for the D-amino acids transported, *a* for alanine, *g* for glycine.

All media utilized in this investigation have been described previously (8).

Isolation of D-serine resistant mutants. About 10⁸ cells of overnight cultures of D-serine deaminase-negative strains were spread on minimal agar plates supplemented with 500 μg of D-serine/ml. A small crystal of *N*-methyl-*N*-nitronitrosoguanidine was placed in the center of each plate, and the plates were

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² Present address: Department of Genetics, Public Health Research Institute of the City of New York, New York, N.Y. 10016.

TABLE 1. *Organisms used in this study*

Strain	Parental strain	Relevant genetic markers ^a	Laboratory of origin
W 3828		<i>dsdA</i> ⁺ , <i>dsdC</i> ⁺	P. H. A. Sneath
EM 1401	W 3828	<i>dsdCx</i> , <i>dsdA</i>	E. McFall
EM 1302	W 3828	<i>dsdA</i> , <i>dagA</i>	E. McFall
EM 1303	EM 1302	<i>dsdA</i> , <i>dagA</i> , <i>ilv</i>	E. McFall
EM 1304	EM 1303	<i>dsdA</i> , <i>dagA</i> , <i>met B</i>	E. McFall
EM 1002		<i>dsdA</i> , <i>dsdC</i> ⁺	E. McFall
K 140		<i>HfrC</i>	J. R. Scott
AB 312		<i>Hfr</i>	E. A. Adelberg
F14/AB 1206		<i>F'</i> (<i>argE-H</i> ⁺ , <i>met B</i> ⁺ , <i>ilv</i> ⁺)	B. Low
F10/JC 1553		<i>F'</i> (<i>malB</i> ⁺ , <i>arg E-H</i> ⁺ , <i>metB</i> ⁺)	B. Low

^a Abbreviations: *dsdCx*, D-serine deaminase Cx (constitutive for D-serine deaminase); *dsdA*, D-serine deaminase A (unable to form D-serine deaminase); *dagA*, D-serine permease A; *ilv*, isoleucine-valine; *metB*, methionine B; *argE-H*, arginine E-H; *malB*, maltose B.

incubated at 37 C for 3 to 4 days. Colonies which appeared on the plates were picked and assayed for D-serine deaminase (8). Those strains which did not form D-serine deaminase but did grow on D-serine were designated as D-serine-resistant mutants.

Measurement of permease activity. Cells were grown in minimal medium at 37 C on a New Brunswick Gyrotary Shaker to a density of about 2×10^8 /ml. The cells were treated with 200 μ g of chloramphenicol/ml for 30 min at 37 C, followed by addition of radioactive amino acid. Samples of 1 ml were withdrawn at appropriate intervals and filtered on membrane filters. The filters were washed with 5 to 10 ml of minimal salts at room temperature. After drying under an infrared lamp, the filters were immersed in Liquifluor counting fluid and counted in a scintillation counter.

Bacterial matings. Mating procedures have been described previously (8).

RESULTS

Transport of D-serine. Strain EM 1302, which harbors the permease mutation *dagA*, was isolated as a D-serine-resistant derivative of strain EM 1301 (*dsdA*). It formed no detectable D-serine deaminase and did not utilize D-serine as a nitrogen source, yet it grew well in minimal medium in the presence of 500 μ g of D-serine/ml. It was therefore suspected of being defective in D-serine uptake.

The transport of D-serine in this mutant was compared with that in a strain wild type with respect to the permease, EM 1401 (Fig. 1). Uptake of ¹⁴C-D-serine was rapid within the first few minutes of addition, followed by leveling off to a steady state. The rate of uptake of 10 μ g of D-serine/ml in the mutant strain was 5 to 10% of that in the control. At 100 μ g of D-serine/ml, the

rate of uptake in the mutant was about one-third of the rate in the control.

Figure 2 demonstrates the kinetics of D-serine transport in the wild type, EM 1401. A Lineweaver-Burk plot (7) of D-serine transport was characterized by two intersecting line segments. Similar Lineweaver-Burk plots are shown by histidine (1) and, notably, D-alanine and glycine (13).

The K_m values, taken from a mean of two determinations, are $8.95 \pm 0.95 \times 10^{-6}$ and $1.2 \pm 0.2 \times 10^{-4}$ M for the apparent "high" affinity segment and "low" affinity segment, respectively.

Accumulation of other amino acids. This laboratory's results were correlated with those of Wargel and co-workers (14) by examining the uptake of D-alanine and glycine at the concentrations they used. As illustrated in Fig. 3, the rate of uptake of D-alanine was inhibited over 90% in EM 1302 as compared to the control. The accumulation of glycine in the mutant was similarly inhibited.

The rate of uptake of L-alanine was partially inhibited in strain EM 1302. At 10 μ g/ml, the uptake by the mutant attained only 40% of the rate in the control (Fig. 4). The rate of uptake of L-serine was unaffected by the mutation (Fig. 5).

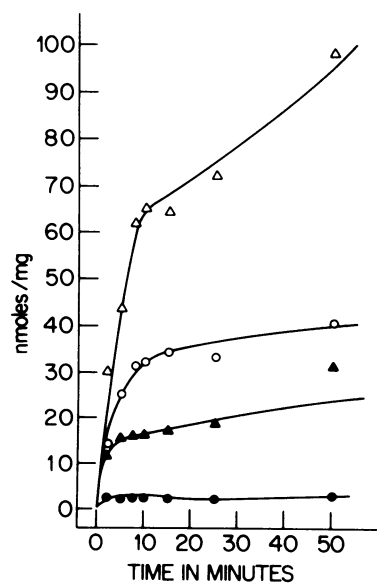


FIG. 1. Uptake of ¹⁴C-D-serine (specific activity, 0.1 μ Ci/ μ mol) in (●, ▲) EM 1302 (*dagA*) and in (○, Δ) EM 1401 (*dagA*⁺). Cells were incubated with ¹⁴C-D-serine at 37 C after 30-min treatment with 200 μ g of chloramphenicol/ml at 37 C. D-Serine concentrations: (○, ●) 10 μ g/ml, (Δ, ▲) 100 μ g/ml.

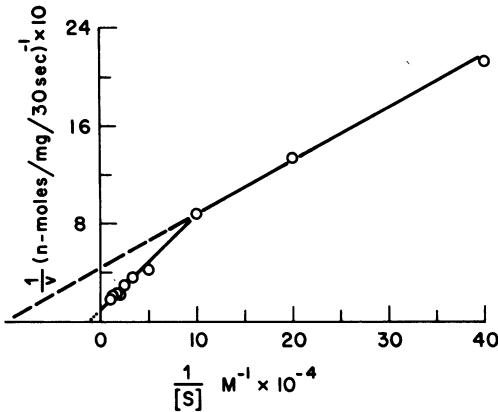


FIG. 2. Lineweaver-Burk plot of uptake of D-serine in EM 1401 (*dagA*⁺). Velocity is expressed in terms of nanomoles per milligram of cells per 30-s incubation. Cells were incubated with ¹⁴C-D-serine (specific activity, 1 μ Ci/ μ mol) for 30 s at 37 C after treatment for 30 min with 200 μ g of chloramphenicol/ml at 37 C. Controls consisted of incubation of various concentrations of ¹⁴C-D-serine without cells, and velocities were corrected for nonspecific sticking of radioactive amino acid to membrane filters.

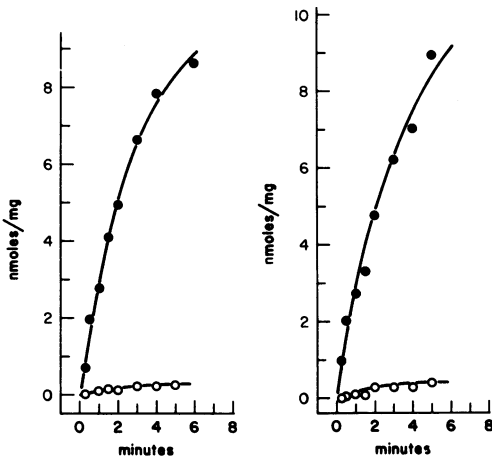


FIG. 3. (Left) Uptake of 0.025 μ mol of ¹⁴C-D-alanine/ml (specific activity, 0.5 μ Ci/ μ mol) in (O) EM 1302 (*dagA*) and (●) EM 1401 (*dagA*⁺). (Right) Uptake of 0.025 μ mol of ¹⁴C-glycine/ml (specific activity, 0.36 μ Ci/ μ mol) in (O) EM 1302 (*dagA*) and (●) E 1401 (*dagA*⁺). The cells were incubated with the ¹⁴C-amino acids at 37 C after 30-min treatment with 200 μ g of chloramphenicol/ml.

Regulation of D-serine permease. The experiments presented in Table 2 were performed to determine whether the synthesis of the D-serine uptake system was under the control of the D-serine deaminase regulatory gene *dsdC_x*, or independent of it. The strains employed in

the study were EM 1002, a *dsdA* mutant which still has the regulatory gene intact, and EM 1401, a *dsdA* mutant which is also mutant in *dsdC_x*. The *dsdA*⁺ parent of EM 1401 forms D-serine deaminase constitutively at approximately 400 times the rate of the inducible wild-type strain in the absence of inducer.

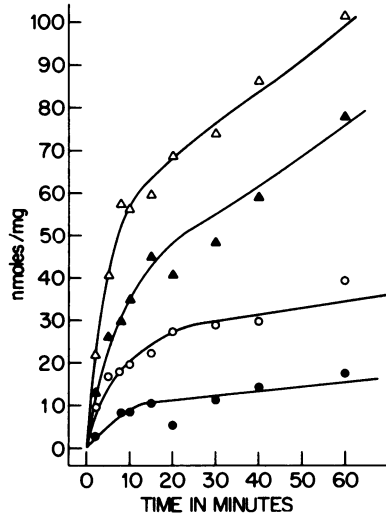


FIG. 4. Uptake of ¹⁴C-L-alanine (specific activity, 0.18 μ Ci/ μ mol) in (●, ▲) EM 1302 and (O, Δ) EM 1401. The cells were incubated with ¹⁴C-L-alanine at 37 C after 30-min treatment with 200 μ g of chloramphenicol/ml at 37 C. L-Alanine concentrations: (O, ●) 10 μ g/ml, (Δ, ▲) 100 μ g/ml.

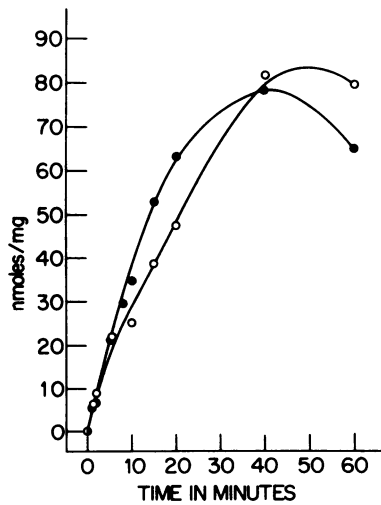


FIG. 5. Uptake of 10 μ g of ¹⁴C-L-serine/ml (specific activity 0.17 μ Ci/ μ mol) in (●) EM 1302 and in (O) EM 1401. The cells were incubated with ¹⁴C-L-serine at 37 C after treatment for 30 min with 200 μ g of chloramphenicol/ml at 37 C.

TABLE 2. Noninducibility of D-serine permease^a

Additions	Time of incubation	EM 1002	EM 1401
I. D-Ser* ^b	30 s	9.5	7.4
	10 min	24.4	15.9
II. CM; D-ser*	30 s	7.1	5.2
	10 min	17.5	13.3
III. D-Ser; CM; D-ser*	30 s	9.0	11.5
	10 min	21.9	22.0
IV. CM; D-ser; CM; D-ser*	30 s	3.2	—
	10 min	23.6	—

^a The results are presented as nanomoles of ¹⁴C-D-serine taken up per milligram of cells. The cells were incubated with the reagents listed in the first column, in the order presented.

^b D-ser*, ¹⁴C-D-serine, specific activity 0.1 μ Ci/ μ mol (cells incubated with 10 μ g/ml for the times indicated); CM, chloramphenicol (cells incubated with 200 μ g/ml for 30 min); D-ser, unlabeled D-serine (cells incubated with 150 μ g/ml for 90 min after which they were washed and resuspended in fresh minimal medium).

In experiment I, ¹⁴C-D-serine was added to both strains, and at 30 s and 10 min samples were withdrawn. Measurements of uptake at 30 s gave initial rates, whereas 10 min gave steady-state values. In experiment II, the same procedure was followed, except that the cells were pretreated with 200 μ g of chloramphenicol/ml. The uptake was similar in both strains and the results of experiment II were similar to those of experiment I, demonstrating that the rate of synthesis of the D-serine transport system is not under the control of *dsdCx*.

Experiments III and IV were performed to determine whether permease synthesis is induced by D-serine. The maximal rate of induction of D-serine deaminase in *dsdA*⁺ *dsdC*⁺ strains growing in minimal medium was found to occur after 90 min of incubation with 150 μ g of D-serine/ml (McFall, unpublished data). In experiment III, unlabeled D-serine was incubated with the cells for 90 min. The cells were then washed with minimal medium and treated with 200 μ g of chloramphenicol/ml for 30 min. ¹⁴C-D-serine was added, and samples were taken as indicated. Experiment IV was the same as III, except that the cells were pretreated with 200 μ g of chloramphenicol/ml for 30 min before induction. Uptake for both strains in experiment III was similar and the results of experiment IV paralleled those of experiment III, thus showing that the synthesis of the D-serine uptake system is unresponsive to D-serine.

Mapping of the D-serine permease mutation. The *dagA* mutation in strain EM 1302 is

similar to a D-cycloserine resistance mutation described by Curtiss and Wargel and their co-workers (5, 14). EM 1302 appears to correspond to their most resistant multistep mutant which had also lost 90% of the transport activity for D-alanine and glycine. EM 1302 grew after 24 h of incubation at 37 C on minimal agar medium supplemented with 1.2×10^{-4} M, but not with 3.0×10^{-4} M, D-cycloserine. It was growth at this concentration by which Curtiss et al. (4) defined their most resistant multistep mutant.

D-Cycloserine resistance is located near *metB* on the K-12 linkage map (12). The location of *dagA* was determined by the following experiments (Fig. 6). EM 1303, an *ilv* derivative of EM 1302, was used as a recipient in a cross with K 140 (*HfrC metB ura*), and *ilv*⁺ recombinants were selected. Among the recombinants, 42% were completely sensitive to D-serine, whereas 58% retained full or partial resistance. Of the completely sensitive recombinants, 92% were *metB*. One of the partially resistant recombinants was tested and was found to have an intermediate permease activity. However, introduction of *HfrC* genetic material appeared to alter the expression of D-serine resistance, since one of the apparently partially resistant recombinants was found to have a completely defective transport system.

In a subsequent experiment, recombinants were challenged with 0.1×10^{-5} , 4.0×10^{-5} , 1.2×10^{-4} , and 3.0×10^{-4} M D-cycloserine to determine the true proportion of intermediate and fully resistant types. In these experiments, it appeared that 0.1×10^{-5} M D-cycloserine was the maximal concentration that would not cause inhibition, and growth at 3×10^{-4} M but not at 6×10^{-4} M defined the fully resistant type of mutation. Full or partial resistance was retained by 46% of the recombinants; i.e., 46% grew on minimal medium supplemented with 4×10^{-5} M D-cycloserine. Of these, 70% were fully resistant, 15% retained second level resistance

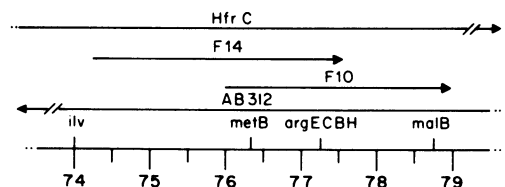


FIG. 6. Partial chromosome map of *E. coli* K-12, showing map positions in minutes for relevant markers and the origins of transfer for the donor strains F' F14, F' F10, HfrC, and HfrAB312 according to the revised Taylor-Trotter map (12).

(i.e., grew at 4×10^{-5} and 1.2×10^{-4} M, but not at 3×10^{-4} M, D-cycloserine), and 6.5% were resistant to 4×10^{-5} M only. These results indicated that there are, indeed, a minimum of three cistrons involved in the transport of D-serine, D-alanine, glycine, and D-cycloserine.

When the same recipient was mated with AB 312, whose direction of transfer is opposite to that of *HfrC*, 10% of the *ilv*⁺ recombinants were completely sensitive to D-serine, thus indicating that the *dagA* locus is to the right of *ilv*.

In an attempt to localize the permease genes, various F factors harboring genetic regions near *ilv* were introduced into EM 1302. The permease activity is apparently not carried on F14, which contains genetic material from *argE* to *ilv*, since not one of 100 *ilv*⁺ recombinants resulting from a cross between F14/AB1206 and EM1303 was D-serine sensitive. Similarly, a cross between EM 1304 and F10/JC1553 yielded no *metB*⁺ recombinants which were D-serine sensitive. Thus, unless *dagA* is dominant in *dagA/dagA*⁺ merodiploids, it is not carried on F10 or F14 and therefore lies just to the right of *malB*.

DISCUSSION

The permease system responsible for the transport of D-serine in *E. coli* K-12 also transports D-alanine and glycine. L-Serine is not transported by the system. Kaback (personal communication) has confirmed this independence of the D- and L-serine transport systems in *E. coli* ML by competition studies. The *dagA* system also accounts for part of the transport of L-alanine. This permease is apparently the same as the one described by Wargel et al. for D-cycloserine (13, 14).

Curtiss et al. found that D-cycloserine resistance occurred in step mutations (5), the most resistant multistep mutation being the one with the most defective permease. Other D-serine-resistant mutants isolated in this laboratory were of the less resistant, intermediate permease activity types.

EM 1302 appears to map to the right of *metB*, just outside the limits of F10, or in other words just to the right of *malB*.

D-Serine does not induce the synthesis of the D-serine transport system. In this respect, the D-serine permease is like that of most other amino acid transport systems, with exception of tryptophan which can also serve as a catabolite (2, 10).

Ames found that histidine was taken up in *Salmonella typhimurium* by both specific histidine and general aromatic transport systems,

and that a Lineweaver-Burk plot for histidine was characterized by two-line segments (1). Brown found that a general aromatic transport system with high affinity exists in *E. coli* in addition to lower affinity specific transport systems for tyrosine, tryptophan, and phenylalanine (3). Uptake of D-serine at low D-serine concentration (10^{-4} M) in EM 1302 was inhibited by 90%, whereas uptake at higher levels (10^{-3} M) was inhibited by only 70%. This could indicate a second low affinity specific transport system for D-serine, but it is more likely the result of a higher concentration of D-serine saturating the permease. Wargel et al. suggested that the phenomenon of a double-component Lineweaver-Burk plot, which they also found for glycine and D-alanine uptake, need not be considered as an indication of two components, but only as a characterization of the particular transport system (14). More work, along the line of competition studies as performed by Brown (4) will have to be carried out before this problem is satisfactorily answered.

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