

## Characterization of a Novel L-Serine Transport System in *Escherichia coli*

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Received 14 October 1987/Accepted 13 January 1988

**A novel transport system for L-serine was found in *Escherichia coli* cells grown on medium containing amino acid mixture. This novel system is distinguishable from the known three transport systems for L-serine, namely, the serine-threonine system, one of the leucine-isoleucine-valine systems, and the glycine-alanine system. Uptake of L-serine via this novel system was inhibited by none of the amino acids tested, indicating that it is highly specific for L-serine. This system was induced by L-leucine, but not by L-serine. The  $K_m$  for L-serine was 50  $\mu$ M, and the  $V_{max}$  was 23 nmol/min per mg of cell protein. Transport of L-serine via this system was strongly inhibited by KCN, an inhibitor of the respiratory chain, or by carbonyl cyanide *m*-chlorophenylhydrazone, an  $H^+$  conductor. Uptake of  $H^+$  was induced by L-serine influx. These results indicate that an  $H^+$ -serine cotransport mechanism is operative in this novel L-serine transport system.**

There are many transport systems in the cytoplasmic membrane of *Escherichia coli*. Among them, about 30 systems for transport of amino acids are known. Each system has its own role and characteristics. It is sometimes difficult to characterize these systems because of their complicated substrate specificity and other properties.

So far, three transport systems for serine are known in *E. coli*. One is the serine-threonine system. Transport activity of this system is detectable in membrane vesicles (6) as well as in whole cells (14). The second system mediates transport of L-alanine, L-serine, L-threonine, and L-leucine. This system is sensitive to osmotic shock and is repressed by L-leucine. Therefore, it seems to be one of the leucine-isoleucine-valine (LIV) systems which require a binding protein for transport (12). It is not an ion-coupled cotransport system. The third system, reported in strain ML, mediates  $H^+$ -L-serine cotransport (2) and is termed the glycine-alanine system (2, 12). However, the glycine-alanine transport system of strain K-12 does not mediate the transport of L-serine (3, 12). Furthermore, L-serine does not inhibit the glycine-alanine transport system of strain ML (6). Therefore, two transport systems for L-serine are known in the K-12 strains.

Recently we have found that the serine-threonine transport system is an  $Na^+$ -coupled cotransport system (4a). Excess L-threonine inhibits L-serine transport via this system. During the course of the study we noticed that L-serine transport activity varied depending on the culture conditions. Our results suggest the presence of a third transport system for L-serine in *E. coli*.

Here we report the properties of a novel L-serine transport system which is highly specific for L-serine and is a cotransport system with  $H^+$ .

### MATERIALS AND METHODS

**Organism and growth.** *E. coli* W3133-2 (7), a derivative of K-12, was used. Cells were grown in a minimal medium (13) ( $Na^+$  salts were replaced with  $K^+$  salts) with 40 mM potassium lactate as the carbon source. Polypepton (1%) (Daigo Eiyō Co.) or amino acid (1 mM) was added when

required. Cells were grown aerobically at 37°C and harvested at the late exponential phase of growth.

**Transport assay.** Cells were washed twice with a buffer containing 0.1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) (pH adjusted to 7.0 with Tris) and 2 mM  $MgSO_4$  (washing buffer), and resuspended in the same buffer. Chloramphenicol was added at a concentration of 50  $\mu$ g/ml. The incubation medium for the transport assay consisted of 0.1 M MOPS-Tris buffer (pH 7.0), 2 mM  $MgSO_4$ , 10 mM Tris lactate, and 50  $\mu$ g of chloramphenicol per ml. L-Threonine (10 mM) was added 30 s before the addition of L-[ $^{14}C$ ]serine. After preincubation at 25°C for 3 min, L-[ $^{14}C$ ]serine (200  $\mu$ M) was added. Samples (180- $\mu$ l) were taken at intervals, filtered through membrane filters (0.45- $\mu$ m pore size; Toyo Roshi Co.), and washed with washing buffer. To examine the effect of inhibitors, preincubation was carried out for 5 min in the presence of each inhibitor.

**Measurement of  $H^+$  movement.** Cells were washed twice with 120 mM choline chloride with 2 mM  $MgSO_4$  and resuspended in the same solution at the concentration of 20 mg of protein per ml. A portion (0.5 ml) of this suspension was diluted with 2.5 ml of choline chloride plus 2 mM  $MgSO_4$ . Cells were incubated at 28°C in a plastic vessel in which an  $H^+$  electrode was placed. Water-saturated  $N_2$  gas was introduced continuously into the vessel with vigorous stirring. An anaerobic solution (6  $\mu$ l) of 0.5 M L-serine was added, and the pH of the assay medium was recorded. Calibration was carried out by the addition of known amounts of HCl.

**Protein assay.** Protein concentration was determined by the method of Lowry et al. (8) with bovine serum albumin as a standard.

**Chemicals.** L-[U- $^{14}C$ ]serine (150 mCi/mmol) was purchased from the Commissariat à l'Énergie Atomique, France. All other chemicals were reagent grade and were purchased from the commercial sources.

### RESULTS

**A novel L-serine transport system.** As mentioned above, two transport systems for L-serine (the serine-threonine system and one of the LIV systems) are known in the cell membrane of *E. coli* K-12. L-Threonine is a substrate for the serine-threonine system, and L-leucine is a substrate for the

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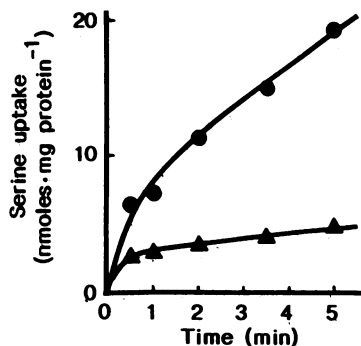


FIG. 1. Change of L-threonine-affected L-serine uptake. Cells were grown on lactate-minimal medium, either unsupplemented (▲) or supplemented with 1% polypepton (●). Cells were washed and assayed for the transport of L-[<sup>14</sup>C]serine in the presence of 50-fold excess L-threonine.

LIV system. We noticed that *E. coli* cells grown under certain conditions possess L-serine transport activity even in the presence of excess L-threonine and L-leucine. Such a result suggests the presence of another transport system for L-serine.

Transport of L-serine in cells grown in a minimal medium containing lactate as a sole carbon source was very low in the presence of excess L-threonine, whereas that in cells grown with lactate in the presence of amino acid mixture was considerably higher (Fig. 1). This suggests that there is another L-serine transport system which is induced by a certain amino acid(s). This possibility could be tested by investigating the substrate specificity of such a system. We tested the effect of all naturally occurring L-amino acids (except tyrosine), D-serine, D-alanine,  $\alpha$ -methyl-DL-serine, and O-methyl-DL-serine on L-serine transport. None of them showed an inhibitory effect at 50-fold higher concentration than L-serine (data not shown). Thus, it became clear that this system is very specific for L-serine and is different from the serine-threonine system and from the LIV system. Since glycine and D- and L-alanine did not inhibit L-serine transport, this system is also different from the glycine-alanine system.

We also tested whether or not this novel system is present in strain ML. We observed uptake of L-serine which was not inhibited by L-threonine, L-alanine, or L-leucine in cells of strain ML308-225 grown on lactate plus amino acid mixture (polypepton) (data not shown). We conclude that the novel L-serine-specific transport system is present not only in strain K-12 but also in the ML strain.

The activity of the L-serine-specific transport system was relatively constant at pH levels ranging from 6.0 to 7.5; at higher pH values the accumulation was reduced to some extent (data not shown).

**Induction of the L-serine-specific transport system.** Fairly high L-serine-specific transport activity was observed in cells grown in the presence of amino acid mixture and not in cells grown in its absence. This means that the L-serine-specific transport system is induced by a certain amino acid(s). We compared the L-serine transport activity of various types of cells grown under various conditions (Fig. 2). Cells grown in the absence of amino acid mixture showed very low L-serine transport activity when measured in the absence of Na<sup>+</sup> and the presence of excess L-threonine, whereas they showed high activity when measured in the presence of Na<sup>+</sup> and the absence of L-threonine (Fig. 2A). The latter activity should be due to the serine-threonine system, which requires Na<sup>+</sup>

(4a). On the other hand, cells grown in the presence of amino acid mixture showed L-serine transport activity in the absence of Na<sup>+</sup> and the presence of excess L-threonine (Fig. 2B). Slightly higher activity was observed when Na<sup>+</sup> was added and L-threonine was omitted. Thus, cells of this type possess high activity of the L-serine-specific transport system and low activity of the serine-threonine system. This indicates that a certain amino acid(s) contained in polypepton induces the L-serine-specific system. We then tested the effect of the various amino acids on the induction of the L-serine-specific system. High L-serine-specific transport activity was observed in the cells grown in the presence of L-leucine (Fig. 2C). We added a small amount of L-isoleucine together with L-leucine to the culture medium to release growth inhibition by L-leucine. Cells grown in the presence of L-isoleucine and in the presence of L-leucine did not show any activity of the L-serine-specific transport system. Also, cells grown in the presence of L-valine plus L-isoleucine, L-serine, L-threonine, glycine, or L-alanine did not show significant activity of the L-serine-specific system (data not shown). Thus, we conclude that L-leucine induces the L-serine-specific transport system. It should be noted that the activity of the L-serine-specific system in cells grown with L-leucine (plus L-isoleucine) was slightly lower than that in cells grown with amino acid mixture (Fig. 2B and C). This suggests that another amino acid(s) than L-serine, perhaps together with L-leucine, is necessary for the full induction of the L-serine-specific transport system. The cells grown in the presence of L-leucine and L-isoleucine also showed Na<sup>+</sup>-stimulated L-serine uptake (Fig. 2C). On the other hand, cells grown in the presence of the amino acid mixture showed very low levels of Na<sup>+</sup>-stimulated L-serine uptake. At present, it is not clear which amino acid(s) repressed the Na<sup>+</sup>-coupled serine-threonine system. Our preliminary result, however, indicated that tryptophan repressed the serine-threonine system to some extent (data not shown).

**Kinetics of L-serine transport.** Since we observed the highest activity of L-serine transport via the L-serine-specific system in cells grown in the presence of amino acid mixture, we used these cells for kinetic analysis. Such cells possess very low activity of the serine-threonine system. The initial rate of L-serine transport was measured in the presence of 10 mM L-threonine and in the absence of Na<sup>+</sup> to reduce the residual uptake of L-serine via the serine-threonine system. Although at early stages of this work we had added L-leucine

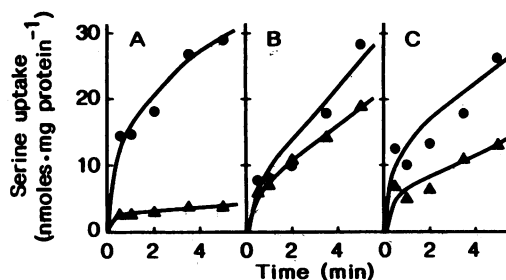


FIG. 2. Induction and derepression of L-serine transport systems. Cells were grown on lactate-minimal medium alone (A) or on lactate-minimal medium supplemented with 1% polypepton (B) or 10 mM L-leucine plus 0.1 mM L-isoleucine (C). Cells were washed and assayed for the transport of L-[<sup>14</sup>C]serine in two distinct conditions. To measure the activity of the two L-serine transport systems, 5 mM NaCl was added (●). To measure L-serine uptake via the L-serine-specific system, NaCl was omitted and L-threonine (10 mM) was added (▲).

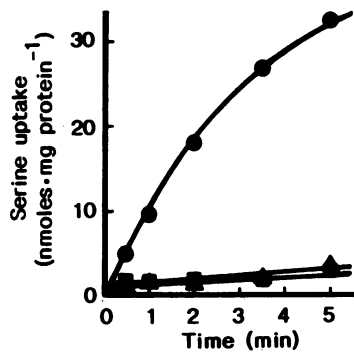


FIG. 3. Effects of energy transduction inhibitors on L-serine uptake. Cells were grown on lactate-minimal medium containing 1% polypepton, washed, and assayed in the presence of 50-fold excess L-threonine. Preincubation was carried out for 5 min with no addition (●), 5 mM KCN (■), or 20  $\mu$ M CCCP (▲).

to the assay mixture to inhibit L-serine transport via the LIV system, it became clear that the omission of L-leucine did not have any significant effect on L-serine transport in the strain used, W3133-2. Therefore we did not add L-leucine to the assay mixture in this experiment. The L-serine transport showed saturation kinetics. The double-reciprocal plot gave a  $K_m$  of 50  $\mu$ M and a  $V_{max}$  of 23 nmol/min per mg of cell protein (data not shown).

**Energy coupling to L-serine transport.** We tested the effect of several energy coupling inhibitors on L-serine transport via the L-serine-specific system to understand the type of energy coupling in this system. Serine transport was strongly inhibited by 5 mM KCN, an inhibitor of the respiratory chain, or by 20  $\mu$ M carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a potent  $H^+$  conductor (Fig. 3). These results support the idea that an electrochemical potential of  $H^+$  is the driving force, directly or indirectly. We then tested the possibility of  $H^+$ -serine cotransport. Since the  $V_{max}$  of this transport system is large enough, we would be able to observe  $H^+$  uptake elicited by serine influx by using an  $H^+$  electrode, if  $H^+$ -serine cotransport exists. The addition of L-serine (final concentration, 1 mM) to the cell suspension caused the uptake of  $H^+$  when cells grown in the presence of amino acid mixture were used (Fig. 4). No uptake of  $H^+$ , however, was induced by the addition of L-serine in cells grown in the absence of amino acid mixture. The  $H^+$  uptake elicited by L-serine was not affected by preincubation of the cells with an excess amount of either L-threonine, glycine, L-alanine, L-leucine, L-isoleucine, or L-valine. Furthermore, the addition of these amino acids did not cause the detectable uptake of  $H^+$ . Addition of CCCP to the assay mixture completely abolished the  $H^+$  uptake induced by L-serine (data not shown). Thus, it became clear that L-serine is cotransported with  $H^+$  via the L-serine-specific system. We could not detect  $Na^+$  uptake induced by L-serine in the presence of an excess amount of threonine in cells grown in the presence of amino acid mixture (data not shown). This means that the L-serine-specific system does not utilize  $Na^+$  as a coupling cation.

## DISCUSSION

We found a novel transport system for L-serine in *E. coli*. This system is very specific for L-serine taken up by a mechanism of  $H^+$ -serine cotransport, and it is induced by L-leucine. One or more other amino acids may also be

involved in full induction of this system. Most of our experiments were performed using cells grown in the presence of an amino acid mixture. Fortunately, these cells possess high L-serine-specific transport activity, very low serine-threonine system activity, and little activity of the LIV system, which transports L-serine.

Transport activity of the  $H^+$ -L-serine cotransport system should be detected in membrane vesicles because this type of system does not require a binding protein. Lombardi and Kaback reported just one transport system for L-serine, the serine-threonine system, in membrane vesicles prepared from ML308-225 (6). They did not detect an L-serine-specific system. This is probably because they prepared the membrane vesicles from cells grown in a medium containing glucose as a sole source of carbon. Activity of the L-serine-specific transport system in such cells should be very low because of the absence of the inducer for this system.

Collins et al. reported  $H^+$ -serine cotransport via the glycine-alanine system in strain ML308-225 and its derivatives grown in medium containing succinate alone (2). However, glycine transport and alanine transport in the membrane vesicles of strain ML308-225 cells have been reported not to be inhibited by a great excess of L-serine (6). We observed  $H^+$ -L-serine cotransport which was not inhibited by excess L-alanine or L-threonine in ML308-225 cells grown in medium containing lactate plus amino acid mixture. Thus, we believe that  $H^+$ -serine cotransport in ML308-225 cells grown in the presence of amino acid mixture also takes place via the L-serine-specific system.

Most of the transport systems for amino acids are constitutive or repressible (9). The L-serine-specific transport system is an inducible system, L-leucine being the inducer. It has been reported that L-leucine has various effects on transport systems and metabolic pathways (11). L-Leucine is also an inducer of L-serine deaminase (10). Therefore, the structural gene for the L-serine-specific transport system and the gene encoding L-serine deaminase might be regulated in the same operon. It should be noted that the activity of the L-serine-specific transport system was not affected by glucose (unpublished data). This means that the expression of this system is not under the control of catabolite repression.

Although the activity of each serine transport system varies depending on the growth condition, the total activity of L-serine transport remains fairly constant at a high level (Fig. 2). L-Serine is an important amino acid because it is one of the key compounds for the synthesis of other amino acids (4). It is known, however, that serine causes growth inhibition in *E. coli* (1). This growth inhibition is released by the

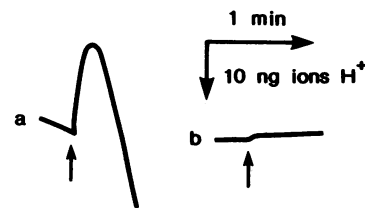


FIG. 4. Proton fluxes induced by the addition of L-serine to the cell suspensions. Cells were grown on lactate-minimal medium supplemented with (a) or without (b) 1% polypepton. Washed cells were incubated at 28°C in 120 mM choline chloride plus 2 mM  $MgSO_4$  under anaerobic conditions. At the time indicated by arrows, an anaerobic solution of 0.5 M L-serine was added to give a final concentration of 1 mM, and pH changes of the assay mixture were recorded. An upward deflection represents a rise in the pH of the medium.

addition of a small amount of L-isoleucine, L-threonine, 2-keto-butyric acid, or homoserine to the culture medium (unpublished data). When L-serine is the sole source of carbon, addition of a small amount of glycine, L-isoleucine, and L-threonine (or L-leucine) is required for the growth of *E. coli* W3133-2 (5). Thus, L-serine or its metabolite(s) seems to regulate the synthesis of other amino acids. On the other hand, gene expression of the transport system(s) and enzyme(s) involved in L-serine utilization is regulated by other amino acids (L-leucine and so on). Thus, the network of the regulation of amino acid metabolism is very complicated. We are interested in elucidating the properties of the L-serine utilization system(s) and the mechanism of regulation of L-serine-related processes. Further investigations in this direction are under way.

*E. coli* cells possess at least two ion-coupled transport systems for L-serine. One system utilizes H<sup>+</sup> as a coupling cation, and the other utilizes Na<sup>+</sup>. This is advantageous for the cells: when an electrochemical potential of H<sup>+</sup> is large, cells are able to take up more serine via the H<sup>+</sup>-coupled system, and when an electrochemical potential of Na<sup>+</sup> is large, cells can take up more serine via the Na<sup>+</sup>-coupled system. Since the driving forces and the transport systems are under appropriate and complicated controls in the cells, this idea may be too simplified.

It is interesting to compare the H<sup>+</sup>-serine cotransport carrier and the Na<sup>+</sup>-serine (and threonine) cotransport carrier at the molecular level. Especially, information on the structural domains involved in substrate recognition or cation recognition might be obtained from comparison of the structure of the two carriers. Furthermore, the evolutionary relationship between the two transport systems may be clarified by comparing the primary structures of the two carriers and the nucleotide sequences of the two structural genes. Thus we are trying to clone the genes and to determine the nucleotide sequence.

#### ACKNOWLEDGMENTS

This research was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas of "Bioenergetics" to T.T. from the Ministry of Education, Science and Culture of Japan and by a grant from The Naito Foundation.

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