JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 7482–7485
0021-9193/92/227482-04$02.00/0

Vol. 174, No. 22

Properties of Recombinant Cells Capable of Growing on Serine without NhaB Na^+/H^+ Antiporter in *Escherichia coli*

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Received 19 February 1992/Accepted 27 August 1992

***Escherichia coli* HIT-1 has a mutation in the Na^+/H^+ antiporter gene, *nhaB* (P. Thelen, T. Tsuchiya, and E. B. Goldberg, J. Bacteriol. 173:6553–6557, 1991). This strain is not able to utilize serine as a carbon source (T. Ishikawa, H. Hama, M. Tsuda, and T. Tsuchiya, J. Biol. Chem. 262:7443–7446, 1987), because an active NhaB is required to maintain the electrochemical potential of Na^+, which drives serine transport via the Na^+/serine carrier, the major transport system for serine. We isolated recombinant cells from a cross between strains HIT-1 and Hfr, and these cells were able to grow on serine even though the NhaB Na^+/H^+ antiporter of the recombinant cells was still defective. We found that the activity of the H^+/serine cotransport system, one of the minor serine transport systems in *E. coli*, was elevated in the recombinant cells. H^+/serine cotransport activity was induced by leucine in the recombinant cells more strongly than in strain HIT-1. A kinetic analysis showed that the V_{max}, but not the K_m, of the transport system was much higher in the recombinant cells than in strain HIT-1 cells.**

Cells of *Escherichia coli* possess several transport systems for serine (1). The major system for serine uptake is a serine-threonine system (7) which is an Na^+-coupled cotransport system (3). The driving force for this transport system is an electrochemical potential of Na^+ across the cytoplasmic membrane, which is established by the Na^+/H^+ antiporter. An electrochemical potential of H^+ established by the respiratory chain is converted to an electrochemical potential of Na^+ by the Na^+/H^+ antiporter. A mutant defective in Na^+/H^+ antiporter activity is not able to take up enough serine, even though the Na^+/serine transport system is normal, so that the cells of the mutant are not able to use serine as a carbon source. We isolated mutant HIT-1, which was not able to grow on serine and which was defective in the Na^+/H^+ antiporter (5). Later, it became clear that *E. coli* cells possess at least two Na^+/H^+ antiporter genes which define the NhaA system and the NhaB system (10, 15). We mapped the mutation in strain HIT-1 to 25.6 min on the *E. coli* chromosomal map and found that cells of strain HIT-1 possess the NhaA system but do not possess the NhaB system (15). Thus, it seems that the NhaB system is needed to establish a large enough electrochemical potential of Na^+ to drive serine uptake so that there is sufficient serine for normal growth.

Cells of *E. coli* also possess a serine-specific transport system which is coupled to H^+ (4). This is not a major system for serine transport. At least two more minor serine transport systems are present in *E. coli*, the LIV system (2) and the glycine-alanine system (12).

During a study on serine utilization in strain HIT-1 and wild-type cells, we isolated recombinant cells (from a cross between strain Hfr [wild type] and strain HIT-1 cells) which grew on serine. We analyzed why the recombinant cells had the ability to grow on serine.

E. coli W3133-2 (8) is a derivative of *E. coli* K-12. Strain HIT-1 (4) is an NhaB Na^+/H^+ antiporter-negative mutant (15) that was derived from strain W3133-2. Strain HIT-S, a derivative of strain HIT-1, was obtained from a cross between Tet^r Hfr strain CAG5055 (KL16 *zed-3069*::Tn*10* at 43 min [13]) and a Nal^r derivative of strain HIT-1. Exconjugants were selected on L agar plates (6) containing tetracycline (25 μg/ml) and nalidixic acid (40 μg/ml). These exconjugants were recipients which had incorporated DNA from the region around 43 min in the donor cell. We screened several hundred such Nal^r Tet^r exconjugants for the ability to grow on minimal medium plates (14) supplemented with 40 mM serine, 1 mM glycine, 1 mM isoleucine, 1 mM threonine, and 5 mM NaCl. Of the exconjugants tested, 40 to 50% grew on this medium. The ability of these cells to grow on serine was independent of NhaB since we found that some cells had normal antiporter activity, while others were NhaB negative. In all cases conjugal DNA transfer was able to proceed from 61 min (the origin) in a counterclockwise direction to 43 min (the site of the Tn*10* insertion). Only in some cases did the transfer continue past 25.6 min, the site of *nhaB*. Thus, strain HIT-S was capable of using serine as a carbon source, even though parental strain HIT-1 is not able to grow on serine (5). The growth media which we used were L broth (pH 7.1) (6) and modified Tanaka (minimal) medium (pH 7.0) (14) (the Na^+ salts in the original medium were replaced with K^+ salts) supplemented with either 40 mM potassium lactate, 40 mM glycerol or 40 mM serine, 1 mM glycine, 1 mM isoleucine, and 1 mM threonine (5). Cells were grown at 37°C under aerobic conditions. Growth was monitored turbidimetrically at 650 nm. Cells were harvested in the late exponential phase of growth.

The activities of serine transport systems were measured by two methods. One method involved measuring the uptake of radioactive serine, and the other involved measuring the uptake of H^+ elicited by a serine influx. In the first method, cells were washed twice with a buffer containing 0.1 M morpholinepropanesulfonic acid (MOPS)–Tris (pH 7.0) and

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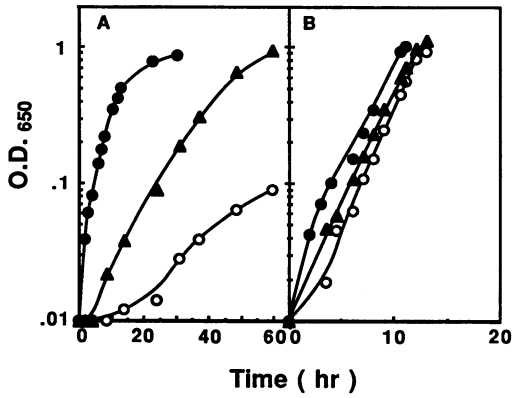

FIG. 1. Growth of the wild type and mutants on serine or glycerol. Cells of strains W3133-2 (wild type) (●), HIT-1 (○), and HIT-S (▲) were grown in modified Tanaka medium (pH 7.0) supplemented with 40 mM serine, 1 mM glycine, 1 mM isoleucine, and 1 mM threonine (A) or with 40 mM glycerol (B) at 37°C under aerobic conditions. Cell growth was monitored turbidimetrically at 650 nm. $O.D._{650}$, optical density at 650 nm.

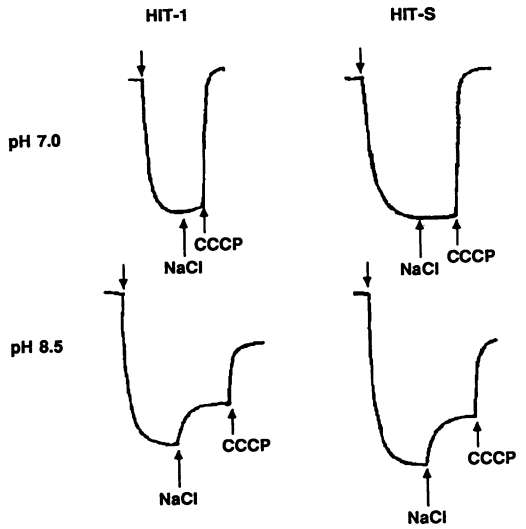

FIG. 2. Na^+/H^+ antiporter activity in membrane vesicles of strains HIT-1 and HIT-S. Cells of strain HIT-1 or HIT-S were grown in L broth. Membrane vesicles were prepared by the French press method. The activity of the Na^+/H^+ antiporter was measured by fluorescence quenching in an assay medium containing 10 mM Tricine-choline (pH 7.0 or 8.5), 1 μM quinacrine, and membrane vesicles (150 μg of protein in 2 ml of the assay mixture). Potassium lactate (5 mM) was added to initiate respiration (downward arrows). After a steady-state level of fluorescence quenching was attained, NaCl (5 mM) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (3 μM) were added (upward arrows).

2 mM $MgSO_4$ and were resuspended in the same buffer. Serine uptake was measured as described previously (4). Unless indicated otherwise, the concentration of serine in the assay mixture was 0.1 mM. When necessary, 5 mM threonine or another amino acid was added to the assay mixture 30 s before initiation of the transport assay. In the second method, cells were washed twice with a solution containing 120 mM choline chloride and 2 mM $MgSO_4$ and then resuspended in the same solution. The uptake of H^+ elicited by serine influx was measured as described previously by using an H^+ electrode (4).

To measure Na^+/H^+ antiporter activity, everted membrane vesicles were prepared, and quinacrine fluorescence quenching resulting from respiration and dequenching caused by addition of NaCl (as a result of Na^+/H^+ antiport) were measured (5).

We measured the growth of strains W3133-2, HIT-1, and HIT-S in a liquid medium containing serine as the carbon source (Fig. 1). Cells of strain W3133-2 (the wild type) grew well, and cells of strain HIT-1 (the mutant) grew very poorly; the growth of strain HIT-S (the recombinant) was intermediate (Fig. 1A). Thus, it seems that cells of strain HIT-S utilize serine fairly well, although not as well as the wild type. Cells of strains W3133-2, HIT-1, and HIT-S grew almost equally well when glycerol was the carbon source (Fig. 1B).

Since cells of strain HIT-1 are not able to grow on serine because of the lack of NhaB, a major Na^+/H^+ antiporter, it seemed likely that in recombinant strain HIT-S the Na^+/H^+ antiporter activity was restored. A number of exconjugants which grew on serine were tested for Nha activity in vivo. Some of these, including strain HIT-S, still lacked NhaB activity (data not shown). To verify this result in vitro and to determine explicitly that *nhaB* was still mutant, we measured the antiporter activity in everted membrane vesicles by the fluorescence quenching method. It is known that NhaA Na^+/H^+ antiporter activity is negligible at pH 7.0 and is high at pH 8.5 and that NhaB Na^+/H^+ antiporter activity is high regardless of the pH of the assay medium (10, 15). Since mutant HIT-1 lacks the NhaB Na^+/H^+ antiporter and possesses the NhaA Na^+/H^+ antiporter (15), it was anticipated that the vesicles of strain HIT-1 would show no antiporter activity at pH 7.0 and some activity at pH 8.5. As expected, we observed no activity at pH 7.0 and some activity at pH 8.5 with vesicles of strain HIT-1 (Fig. 2). Vesicles prepared from cells of strain HIT-S gave the same results (Fig. 2); that is, we observed no Na^+/H^+ antiporter activity with vesicles of strain HIT-S at pH 7.0 and some activity at pH 8.5. Therefore, we concluded that there is no difference in Na^+/H^+ antiporter activity between strains HIT-S and HIT-1.

Since cells of strain HIT-S are able to use serine as a carbon source, it is anticipated that they transport sufficient serine. Thus, we tested serine transport in strains HIT-S and HIT-1. When cells were grown in L broth (rich medium containing an amino acid mixture), we observed that the level of transport activity for serine in strain HIT-S cells was severalfold higher than that observed in strain HIT-1 cells (Fig. 3A). On the other hand, when cells were grown in a minimal salt medium containing lactate as a carbon source, the level of serine transport activity was low in both strains, although the level of activity in strain HIT-S was about two times higher than that in strain HIT-1 (Fig. 3B). The Na^+/serine cotransport system seems to predominate in cells grown in minimal medium containing lactate (3, 4). It is known that the H^+/serine cotransport system, a minor system for serine transport in *E. coli* K-12 strains, is induced by leucine (4). When cells were grown in minimal medium supplemented with lactate plus 1 mM leucine, we observed much higher levels of serine transport activity than we observed in cells grown in the absence of leucine both with strain HIT-S and with strain HIT-1 (Fig. 3C). The level of serine transport was higher in strain HIT-S than in strain HIT-1. Thus, the higher level of serine transport activity in cells of strain HIT-S grown in L broth might be due to the H^+/serine system.

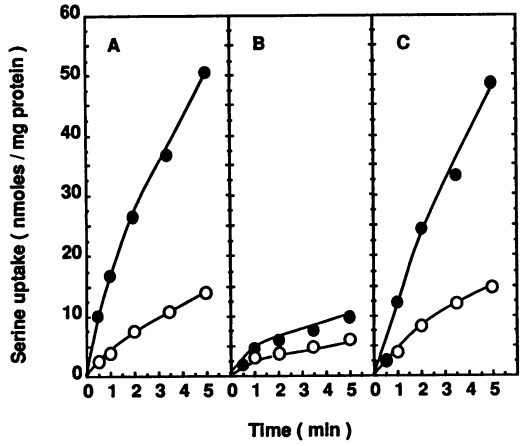

FIG. 3. Serine transport activity in cells grown under various conditions. Cells of strain HIT-1 or HIT-S were grown in L broth (A), in modified Tanaka medium supplemented with 40 mM potassium lactate (B), or in modified Tanaka medium supplemented with 40 mM potassium lactate plus 1 mM leucine (C) at 37°C under aerobic conditions. The cells were harvested in the late exponential phase of growth. Serine transport was measured in an assay mixture containing 0.1 M MOPS–Tris (pH 7.0), 2 mM $MgSO_4$, 10 mM Tris-lactate, chloramphenicol (50 μg/ml), and cells (30 μg of protein per ml). The transport reaction was initiated by adding [^{14}C]serine (0.1 mM). Symbols: ○, strain HIT-1; ●, strain HIT-S.

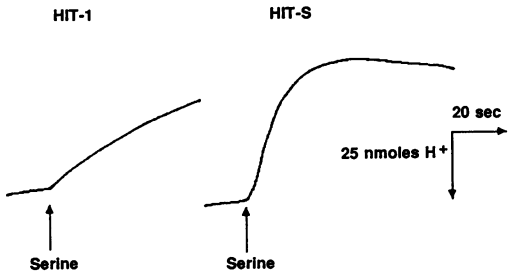

FIG. 4. H^+ uptake elicited by serine influx. Cells of strain HIT-1 or HIT-S were grown in L broth, harvested, washed with a solution containing 120 mM choline chloride and 2 mM $MgSO_4$, and resuspended in the same solution. Each cell suspension (4 mg of cell protein) was incubated at 28°C under anaerobic conditions. An anaerobic solution of serine was added to the cell suspensions to give a final concentration of 0.1 mM (arrows). The concentration of H^+ in the medium (pH) was monitored with an H^+ electrode.

We then sought to confirm that the higher level of serine uptake in strain HIT-S is mediated by the H^+/serine cotransport system. First, we tested the effects of energy inhibitors on serine transport in strain HIT-S. Cells were grown in L broth, and serine transport was measured. An H^+ conductor, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and an inhibitor of the respiratory chain, KCN, strongly inhibited serine transport (data not shown). Addition of NaCl to the assay mixture (NaCl is known to stimulate serine transport via the Na^+/serine system) did not have any significant effect on serine transport (data not shown). Second, we tested the effects of 19 other amino acids on serine transport (each amino acid was added at a concentration of 5 mM, 50 times the concentration of serine) and observed no significant inhibition of serine transport (data not shown). Only threonine, which is a substrate of the Na^+/serine transport system (3, 7), and cysteine produced slight inhibition. Third, we measured H^+ uptake because of serine influx into cells of strains HIT-S and HIT-1. As Fig. 4 shows, we observed a severalfold-higher rate of uptake of H^+ elicited by serine influx into cells of strain HIT-S than into cells of strain HIT-1. We also measured H^+ uptake with cells of strain CAG5055 and observed a low rate of H^+ uptake elicited by serine similar to that observed with HIT-1 cells (data not shown). Thus, it is clear that the elevated serine transport activity in strain HIT-S is due to the serine-specific H^+/serine cotransport system.

We also measured kinetic parameters of serine transport. Cells of strains HIT-S and HIT-1 were grown in L broth, and serine transport was measured in the presence of a great excess of threonine to eliminate the contribution of the Na^+/serine (threonine) system. It was clear that the V_{max} of serine transport in strain HIT-S cells (25 nmol · min^{-1} · mg of $protein^{-1}$) was about 2.3-fold higher than that in strain HIT-1 cells (11 nmol · min^{-1} · mg of $protein^{-1}$) (Fig. 5). On the other hand, the K_m values in strain HIT-S (6.1 μM) were almost the same as those in strain HIT-1 (5.3 μM). Thus, it is very likely that the amount of H^+/serine transport protein is greater in strain HIT-S than in strain HIT-1.

We measured the activity of serine deaminase, which is an important enzyme for serine metabolism (9), in strains HIT-S and HIT-1. No significant difference in activity was detected (data not shown).

Our results indicate that strain HIT-S can grow on serine because of the elevated activity (perhaps because of the amount) of the H^+/serine carrier. The H^+/serine cotransport system is induced by leucine (4), and the induction was stronger in strain HIT-S than in strain HIT-1. There are two possible explanations for the stronger induction of the serine transport system by leucine. First, the recombination in strain HIT-S took place in the regulatory region of the gene encoding the H^+/serine transport system and resulted in elevated expression of the gene or in an elevated response of the gene to leucine. Second, the recombination took place in the leucine-responsive regulatory protein (Lrp) system (11), which may affect the induction of the H^+/serine transport

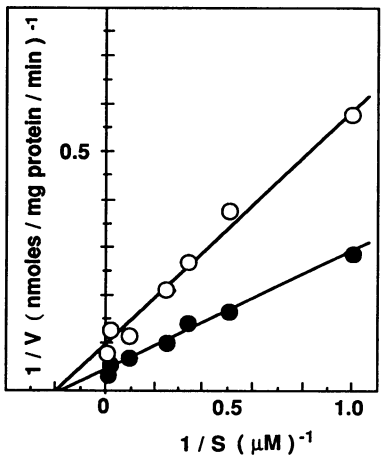

FIG. 5. Kinetic parameters of serine transport in strains HIT-1 and HIT-S. Cells of strain HIT-1 or HIT-S were grown in L broth. The initial velocity of serine transport was measured as described in the legend to Fig. 3 at various concentrations of serine. The data are expressed as a double-reciprocal plot of serine concentration versus initial velocity. Symbols: ○, strain HIT-1; ●, strain HIT-S.

system by leucine, and resulted in elevated induction of the H^+/serine system. As we observed higher levels of serine transport activity in strain HIT-S even when it was grown in the absence of leucine than in strain HIT-1 (Fig. 3B), the first possibility seems more likely.

The Na^+/serine cotransport system is the major system for the uptake of serine in *E. coli*. Since cells of strain HIT-1 are not able to establish a sufficient Na^+-motive force because of the defect in the NhaB Na^+/H^+ antiporter, the Na^+/serine system cannot function efficiently. This is the reason why strain HIT-1 is not able to use serine as a carbon source. Cells of strain HIT-S have elevated H^+/serine cotransport system activity. Sufficient H^+-motive force is established by the respiratory chain so that sufficient serine can support cell growth via the elevated activity of the H^+/serine system in strain HIT-S. Thus, a sort of switching to the H^+/serine system from the Na^+/serine system as a functional and efficient serine transport system enabled strain HIT-S to grow on serine.

We are trying to clone the gene encoding the H^+/serine carrier. Strain HIT-S is a convenient strain for this purpose. Also, this strain should be useful in analyzing the mechanism of leucine induction of the H^+/serine transport system.

We thank L. Grinius for helpful discussions.

This research was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan to T.T. and in part by Public Health Service grant GM13511 from the National Institutes of Health to E.B.G.

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