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Properties of a Na^+-coupled serine-threonine transport system in *Escherichia coli*

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Based on the following experimental results we conclude that the serine-threonine transport system in *Escherichia coli* is a Na^+-coupled cotransport system. (1) Addition of serine to cell suspensions induced H^+ efflux in the presence of Na^+. (2) Addition of serine to cell suspensions induced Na^+ uptake by cells. (3) Imposition of an artificial electrochemical potential of Na^+ in starved cells induced serine uptake. Some of these phenomena were observed when threonine was added instead of serine or inhibited when cells were preincubated with threonine. The Na^+/serine (threonine) cotransport system was considerably repressed when cells were grown on a mixture of amino acids. Serine transport in cells grown in the absence of amino acids mixture was stimulated by Na^+. The half maximum concentration of Na^+ was 21 μM. Sodium ion increased the V_{max} of serine transport without affecting the K_m.

Introduction

Monovalent cation/nutrients cotransport systems are widely distributed in biological systems, and have been found both in prokaryotic and eukaryotic plasma membranes. It was originally thought that the coupling ion in microbial systems was H^+ and that in animal systems was Na^+ [1,2]. During the past decade, however, several transport systems which utilize Na^+ as a coupling ion for cotransport have been found or suggested in microbial cells [3]. In *Escherichia coli*, we have found that the melibiose transport system and the glutamate transport system are Na^+/substrate cotransport systems [4,5]. Recently, it was reported that the proline porter I is a cotransport system with Na^+ [6]. Thus some cotransport systems of *E. coli* have been shown to utilize Na^+ as a coupling ion, although most cotransport systems in *E. coli* seem to utilize exclusively H^+ as the coupling ion [7]. It should be noted that Na^+-coupled systems may utilize H^+ in addition to Na^+ as a coupling cation. During the course of the study on Na^+-coupled transport systems in *E. coli* we noticed that serine transport which has been reported to be a H^+-coupled cotransport [8] showed unexpected properties. So far three transport systems for serine are known in *E. coli*, one of which is a serine-threonine system [9,10]. This system is detectable in membrane vesicles [9]. The energy source for this system has been considered to be the protonmotive force. The second system is for alanine, serine, threonine and leucine [11]. A binding protein is involved in this system, and the energy source might be expected to be ATP. The third system, which has been found in ML strains,

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; NMe_4OH, tetramethylammonium hydroxide (TMAH); Mes, 4-morpholineethanesulfonic acid; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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transports alanine, glycine and serine. This system is a H^+ cotransporter [8]. Although the alanine-glycine-serine transport system is present in K-12 strain, L-serine is not a substrate of this system [11]. The serine-threonine transport system is the subject of this paper. Here we report the properties of this system and that it is a Na^+/serine(threonine) cotransport system.

Materials and Methods

Escherichia coli W3133-2 [12], a derivative of K-12, was used in this study. Cells were grown in a minimal medium [13] (Na^+ salts were replaced with K^+ salts) with 40 mM potassium lactate as the carbon source. Polypeptone (Daigo Eiyo Co.) (1%) was added when required. Cells were grown aerobically at 37°C and harvested at late-exponential phase of growth.

For measurement of H^+ movement the cells were washed twice with 120 mM choline chloride and suspended in the same solution to 25 mg of protein/ml. A portion (0.5 ml) of this suspension was diluted with 2.5 ml of a solution of 120 mM choline chloride. Monovalent cations (Cl^- salts) were added when required. Cells were incubated at 28°C in a plastic vessel with rapid stirring, and water-saturated N_2 gas was introduced continuously. A H^+-electrode was put into the vessel and, if necessary, pH was adjusted by addition of small amounts of dilute HCl or KOH. An anaerobic solution (3 μl) of 1 M L-serine was added, and pH changes were continuously recorded. Calibration was carried out by addition of known amounts of HCl.

The procedure of measurement of Na^+ movement was essentially similar to that of pH change measurement. Choline chloride (120 mM) was replaced with 0.1 M Mops, pH was adjusted to 7.0 with NMe_4OH. NaCl was added to a final concentration of 100 μM. A Na^+-electrode (Radiometer, Copenhagen) and a reference electrode were used instead of an H^+-electrode. Calibration was carried out by addition of known amounts of NaCl.

Transport was assayed as follows. Cells were washed twice with 0.1 M Mops buffer, pH was adjusted to 7.0 with Tris, containing 2 mM $MgSO_4$, and resuspended in the same buffer. Chloramphenicol was added to give final concentration at 50 μg/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, 0.25 mM leucine, and 50 μg/ml chloramphenicol. Monovalent cations (Cl^- salts) were added when required. After preincubation at 20°C for 5 min, [^{14}C]serine (50 μM) was added. Samples (200 μl) were taken at intervals, filtered on membrane filters (0.45 μm pore size; Toyo Roshi Co.), and washed with the Mops-Tris buffer containing 2 mM $MgSO_4$.

Energy-starved cells were used in an experiment where transport was driven by an artificial force. In the experiment cells were divided into four portions and harvested. One portion of cells (low Na^+, low pH) was washed twice with 20 mM Mes buffer, pH was adjusted to 6.0 with Tris, containing 5 mM $MgSO_4$ and 100 mM NH_4Cl. A second portion of cells (low Na^+) was washed twice with 20 mM Mes-Tris buffer (pH 8.0) containing 5 mM $MgSO_4$ and 100 mM NH_4Cl. A third portion of cells (low pH) was washed twice with 20 mM Mes-Tris buffer (pH 6.0) containing 5 mM $MgSO_4$ and 100 mM NaCl. The last portion of cells (control) was washed twice with 20 mM Mes-Tris buffer (pH 8.0) containing 5 mM $MgSO_4$ and 100 mM NaCl. Each portions were incubated with shaking in the same buffer containing 5 mM 2,4-dinitrophenol and 5 mM NaCN at 37°C for 1 h. After this energy starvation method, the cells were washed twice with the washing buffer and resuspended in the same buffer to 25 mg of protein per ml. CCCP, NaCN, and chloramphenicol were added to give final concentrations at 5 μM, 5 mM, and 50 μg/ml, respectively. The transport assay was initiated by the dilution of cells 100-fold into buffer containing 20 mM Mes-Tris (pH 8.0), 5 mM $MgSO_4$, 100 mM NaCl, 5 μM CCCP, 5 mM NaCN, 50 μg/ml chloramphenicol, 50 μM leucine (leucine was added to inhibit serine uptake via the alanine-serine-threonine-leucine system) and 1 μM [^{14}C]serine. Samples (180 μl) were taken at intervals, filtered, washed, and counted.

The protein concentration was determined by the method of Lowry et al. [14]. Na^+ concentration was determined by the atomic absorption method with a Hitachi 508 atomic absorption spectrophotometer.

L-[U-^{14}C]Serine, 150 mCi/mmol, was purchased from Commissariat a L'Energie Atomique, France. All other chemicals were reagent grade and obtained from commercial sources. L-Amino acids were used unless stated otherwise.

Results

Proton fluxes induced by serine

One of the most convincing evidence for H^+/substrate cotransport is the demonstration of H^+ uptake induced by substrate influx. L-Serine transport has been previously reported to be a H^+/substrate cotransport system in *E. coli* [8]. We confirmed that addition of serine to cell suspension induced H^+ uptake in a derivative of K-12 (Fig. 1). About 30 s after the addition of serine to the cell suspension, the pH of the medium started to fall, perhaps because of production of organic acid(s) due to metabolism of serine. In these experiments, we observed that H^+ uptake occurred immediately after addition of serine at pH 5.5, but that transient H^+ extrusion followed by H^+ uptake was observed when serine was added at pH 7.0 (Fig. 1). These observations suggest that H^+-serine cotransport predominates when the H^+ concentration is relatively high (pH 5.5), but when the H^+ concentration is relatively lower (pH 7.0), serine transport coupled to other cation.

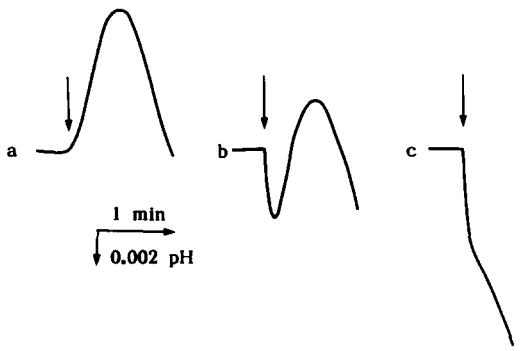

Fig. 1. Proton fluxes induced by the addition of serine to cell suspensions. Washed cells were incubated at 28° C in 120 mM choline chloride (a, b) or 120 mM choline chloride containing 10 mM NaCl (c) under anaerobic conditions. Small amount of 10 mM HCl or 10 mM KOH was added to adjust pH to 5.5 (a) or 7.0 (b, c). At the time indicated by arrows, an anaerobic solution of 1 M serine was added to a final concentration of 1 mM under rapid stirring, and pH changes of the assay mixture were monitored with a H^+-electrode. An upward deflection represents a rise in the pH of the medium.

We next tested the effect of monovalent cations on serine-induced H^+ fluxes. At pH 7.0, Na^+ drastically affected H^+ fluxes. Serine induced a large efflux of H^+ in the presence of Na^+ (Fig. 1). No uptake of H^+ was observed in this case. Li^+ lowered serine-induced H^+ uptake to some extent, K^+ gave no significant effect in H^+ uptake, and NH_4^+ slightly lowered H^+ uptake (data not shown). These observations are consistent with a view that a membrane potential is established by an electrogenic Na^+/serine cotransport producing secondary electrophoretic H^+ extrusion. A similar phenomenon was reported for Na^+/substrate cotransport via the melibiose transport system [15]. Other cations including Li^+ do not appear to serve as a coupling ion for serine transport because secondary H^+ extrusion induced by serine influx was not observed with those cations.

It should be noted that Na^+ was present as a contaminant in the assay mixture at about 20 μM even if Na^+ was not added. This Na^+ is believed to be derived from reagents and glassware (data not shown). Even the distilled and deionized water had some contaminating sodium. This concentration of Na^+ seems sufficient to support Na^+/serine cotransport at pH 7.0.

Although a large H^+ extrusion was observed in the presence of 10 mM Na^+ at pH 7.0, no H^+ extrusion was detected even in the presence of 10 mM Na^+ at pH 5.5. Rather, H^+ uptake was still induced by serine in the presence of 10 mM Na^+ at pH 5.5, although the level was very low. Serine induced H^+ uptake at pH 5.5 in the presence of any cation tested, although there were differences in extent.

Na^+ uptake induced by serine

To support the concept of Na^+/serine cotransport, we measured Na^+ uptake by cells induced by serine influx. Serine was added to cell suspensions under anaerobic conditions, and changes in Na^+ concentration in the assay medium were monitored with a Na^+ selective electrode. As shown in Fig. 2, an immediate fall in medium Na^+ concentration was observed when a small volume of anaerobic serine was added, suggesting Na^+/serine cotransport, as has shown previously for Na^+/melibiose cotransport [4] and Na^+/proline cotransport [6].

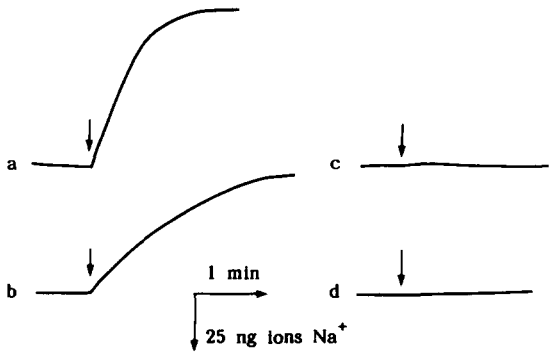

Fig. 2. Uptake of Na^+ induced by the addition of serine or threonine to cell suspensions. Washed cells were incubated at 28°C in 0.1 M Mops-TMAH buffer (pH 7.5) containing 100 μM NaCl under anaerobic conditions. An anaerobic solution of 1 M serine (a, c) or 1 M threonine (b, d) was added to a final concentration of 1 mM at the point indicated by the arrows. In two cases cells were preincubated with 10 mM threonine (c) or 10 mM serine (d) before the addition of serine or threonine. Changes in Na^+ concentration of the medium were monitored with a Na^+-electrode. An upward deflection indicates decrease in Na^+ concentration in the medium.

Addition of threonine also induced Na^+ uptake (Fig. 2). Thus, Na^+/threonine cotransport also appears to exist in *E. coli*. The initial velocity of Na^+ uptake induced by serine and threonine were 3.4 and 1.4 ng ions $\cdot$ $min^{-1} \cdot$ (mg of protein)$^{-1}$, respectively, under the described conditions (Fig. 2). Since *E. coli* possesses a serine-threonine transport system [9,10], it was possible that both Na^+/serine and Na^+/threonine cotransport are mediated via this transport system. To test this possibility, we added serine to cell suspensions in the presence of threonine, and vice versa. As shown in Fig. 2, Na^+ uptake was no longer induced by serine when cells were preincubated with excess threonine, nor by threonine when preincubated with excess serine. Thus serine and threonine compete with each other, suggesting that Na^+/serine and Na^+/threonine cotransport are mediated via the same system, the serine-threonine system. D-Serine, which is a poor substrate of this system [16], induced only a slight Na^+ uptake (data not shown).

Since Li^+ slightly lowered the H^+ uptake induced by serine, we tested the possibility of Li^+/serine cotransport. We examined whether changes in the Li^+ concentration of cell suspensions occurred when serine was added using a Li^+-electrode [17]. No detectable Li^+ uptake was observed when serine was added to cell suspensions (data not shown). Thus, Li^+/serine cotransport seems unlikely in *E. coli*.

Effect of growth conditions on ion/serine cotransport

It was not clear whether Na^+/serine cotransport and H^+/serine cotransport took place via the same transport system. Some properties of Na^+/serine cotransport and H^+/serine cotransport appeared different. When cells were grown on lactate plus polypeptone (mixture of amino acids), both Na^+/serine cotransport and H^+/serine cotransport were observed (Fig. 3a). On the other hand, serine-induced Na^+ uptake was greatly enhanced in cells grown on lactate, and serine-induced H^+ transport was not detected (Fig. 3b). Thus, it is likely that Na^+/serine cotransport and H^+/serine cotransport occur via different transport systems.

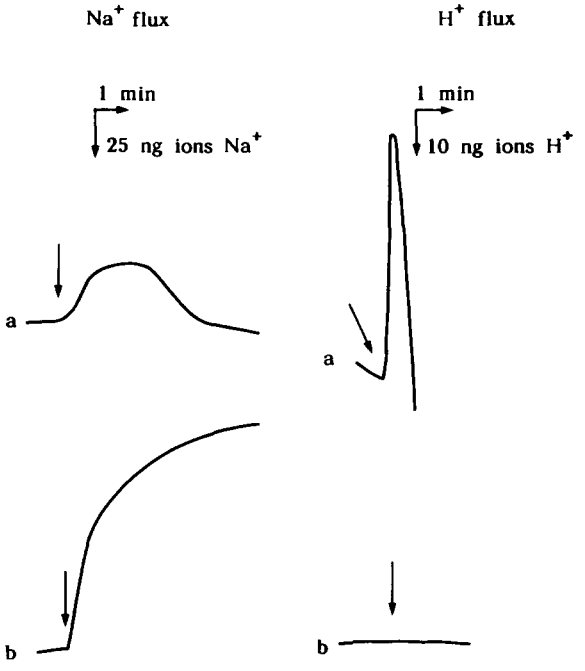

Fig. 3. Repression and induction of serine transport systems. Cells were grown in a minimal medium supplemented with 40 mM potassium lactate plus 1% polypeptone (a) or potassium lactate (b). Changes in Na^+ and H^+ concentration induced by the addition of serine (final 1 mM) to cell suspensions were monitored with either Na^+-electrode (Na^+ flux) or H^+-electrode (H^+ flux).

The transport system for Na^+-serine seems to be repressed by certain amino acid(s). We investigated which amino acids contained in polypeptone repressed the Na^+/serine cotransport system and which ones induced the H^+/serine cotransport system. Preliminary results suggest that tryptophan represses the Na^+/serine cotransport system to some extent, while leucine or methionine induce the H^+/serine cotransport system (unpublished observation). Furthermore threonine, which is a substrate of the Na^+/serine cotransport system, did not inhibit the uptake of H^+ elicited by serine, and threonine did not elicit H^+ uptake. Therefore we conclude that Na^+/serine cotransport and H^+/serine cotransport took place via different systems.

It should be pointed out that in cells grown on lactate only Na^+/serine cotransport but not H^+/serine cotransport was observed. Thus, it seems suitable to use cells grown on lactate to analyze the Na^+/serine cotransport system. Furthermore, in cells possessing the H^+/serine cotransport system rapid acidification of the assay medium was observed, which suggests rapid metabolism of serine in the cell. On the other hand, no acidification was observed with cells possessing only the Na^+/serine cotransport system, which suggests very slow metabolism of serine in such cells. Therefore, we used cells grown on lactate, which possess the Na^+/serine cotransport system but not the H^+-serine system, for further analysis.

TABLE I

EFFECT OF MONOVALENT CATIONS ON SERINE TRANSPORT

Cells were grown in a minimal medium supplemented with 40 mM lactate. Cells were preincubated in assay mixture containing various salts (0.5 mM) at 25 °C for 5 min. To determine the initial velocity, samples were taken at 30 s after the addition of [^{14}C]serine.

Salts added	Serine uptake (nmol·min^{-1}·(mg protein)$^{-1}$)
none	4.2
NaCl	16.2
KCl	3.2
LiCl	4.4
NH_4Cl	4.6
Choline Cl	4.4

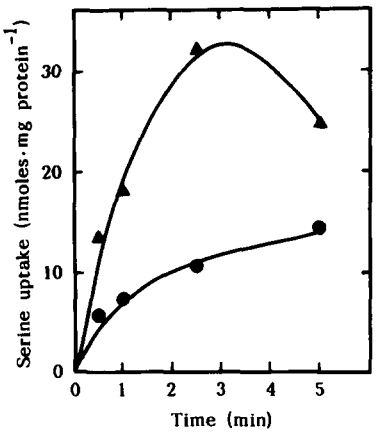

Fig. 4. Na^+ stimulation of the serine uptake. Cells were grown in a minimal medium supplemented with 40 mM potassium lactate. Cells were washed and assayed for transport of serine in the presence (▲) or absence (●) of 1 mM NaCl.

Effect of Na^+ on serine transport

Stimulation of serine transport by Na^+ has never been reported in *E. coli*. Rather an inhibitory effect has been reported in membrane vesicles [9]. If Na^+/serine cotransport is real, then we should be able to observe stimulation of serine transport by Na^+ under appropriate conditions. Thus we tested the effect of Na^+ on serine transport in cells and observed a 2–3-fold stimulation by Na^+ (Fig. 4). We also observed considerable uptake of serine in the absence of added Na^+, possibly due to contaminating Na^+ present in the assay mixture. In contrast, Li^+, K^+, NH_4^+ and choline$^+$ did not stimulate serine uptake (Table I). In fact, potassium ion inhibited slightly. Harmaline, an inhibitor of Na^+-coupled processes in animal cells [18] and *E. coli* [6], was also inhibitory (data not shown).

The relationship of Na^+ concentration to serine transport was investigated. Although a large stimulation was observed at considerably low concentrations of Na^+ (a few mM), the stimulation decreased with increasing concentrations of Na^+ (Fig. 5). At concentrations higher than 40 mM some inhibition was observed, consistent with the results reported with membrane vesicles [9].

A double-reciprocal plot of Na^+ concentration (at low concentrations) versus initial velocity of serine transport revealed that the half maximum concentration of Na^+ was 21 μM (Fig. 6). In these assays the Na^+ concentration in each assay mix-

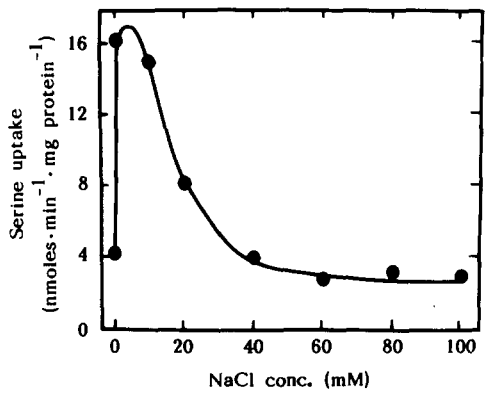

Fig. 5. Effect of NaCl concentration on serine transport. Various concentrations (up to 100 mM) of NaCl were added to the assay mixtures, and preincubated for 5 min. Samples were taken at 30 s after the addition of [^{14}C]serine.

ture was determined by the atomic absorption method.

We tested the effect of Na^+ concentration on kinetic parameters of serine transport. As shown in Fig. 7, Na^+ increased V_{max} of serine transport without giving large effect on K_m (about 8 μM). By adding 100 μM of Na^+ to the assay mixture, about 5-fold increase of V_{max} (2.8 nmol · min^{-1} · (mg protein)$^{-1}$ to 15.1 nmol · min^{-1} · (mg protein)$^{-1}$) was observed.

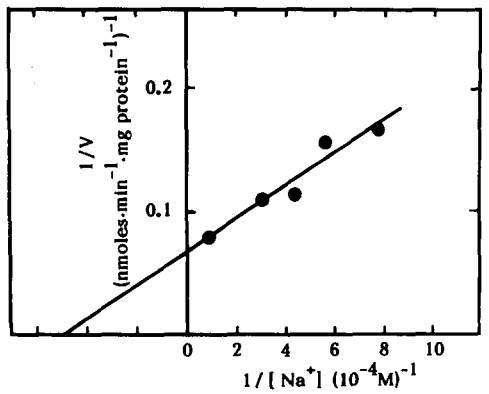

Fig. 6. Determination of the affinity of Na^+ with serine transport carrier. Various concentrations (less than 100 μM) of NaCl were added to the assay mixtures. To determine the initial velocity, samples were taken at 20 s after the addition of [^{14}C]serine. The concentration of Na^+ in each assay mixture was determined, and the actual concentration of Na^+ was plotted. The contaminative Na^+ concentration in the assay mixture was 13 μM.

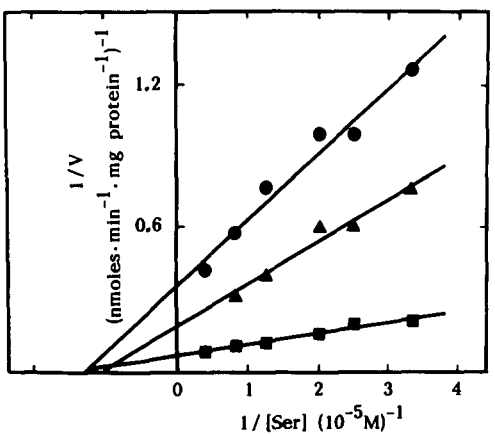

Fig. 7. Determination of kinetic parameters of serine transport. Various concentrations of [^{14}C]serine and NaCl were added to the assay mixtures. To determine the initial velocity, samples were taken at 20 s after the addition of [^{14}C]serine. The data are expressed as a double-reciprocal plot of the initial velocity and serine concentration. The contaminative Na^+ concentration in the assay mixture was 3 μM. Concentrations of added NaCl were: 0, ●; 20 μM, ▲; 100 μM, ■.

Serine transport driven by artificial Na^+ gradient

An attempt was made to drive accumulation of serine with an artificially-imposed electrochemical potential of Na^+. To minimize endogenous uptake of serine, energy-depleted cells were prepared. Several methods of energy starvation previously reported [5,6,15,19] were employed. Serine transport was inactivated by these procedures unless 5 mM Mg^{2+} was added to the starvation medium and the incubation time at 37 °C was shortened to 1 h, as described in Materials and Methods. Energy-starved cells were divided into four portions, and three types of driving force were given. No artificial driving force was given to the first portion of cells. An inwardly-directed concentration gradient of Na^+ (ΔpNa) was imposed on the second portion. An outwardly-directed diffusion potential of H^+ in the presence of CCCP ($\Delta\psi$, inside negative) was imposed on the third portion. Both of the Na^+ gradient (ΔpNa) and the diffusion potential of H^+ ($\Delta\psi$) were imposed on the fourth portion. In the absence of an artificial driving force little accumulation of serine was observed (Fig. 8). Imposition of a Na^+ gradient increased the initial velocity of serine transport about 20-fold. Imposition of a membrane potential alone increased transport about 10-fold. More

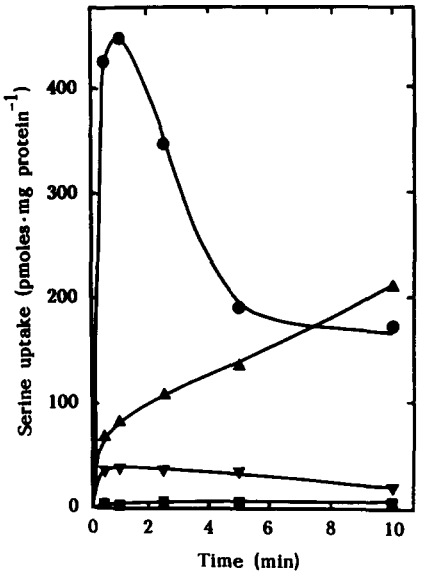

Fig. 8. Serine transport induced by artificial driving forces in energy-depleted cells. A membrane potential ($\Delta\psi$), interior negative, was imposed as a diffusion potential of H^+ in the presence of 5 μM CCCP. An inwardly directed chemical gradient of Na^+ (ΔpNa) was imposed by addition of NaCl to the external medium. Details are described in Materials and Methods. Symbols are: control (no driving force), ■; $\Delta\psi$, ▼; ΔpNa, ▲; $\Delta\psi$ and ΔpNa, ●.

than 100-fold stimulation of serine transport was observed when both a Na^+ gradient and a membrane potential were imposed together. These results suggest that the Na^+ influx driving serine uptake took place rapidly when both Na^+ gradient and membrane potential were imposed together, and that the driving force (ΔpNa and $\Delta\psi$) dissipated quickly. These results are consistent with those obtained with other Na^+/substrate cotransport systems [5,6,15].

Discussion

The results presented in this paper indicate that the serine-threonine transport system in *E. coli* is a Na^+-coupled cotransport system. The following data support this conclusion. (1) Serine transport was stimulated by Na^+, but not by other cations. (2) Na^+ influx was induced by adding serine to cell suspension. (3) An electrochemical potential of Na^+ induced serine uptake. Thus far, three transport systems have been reported to be Na^+/substrate cotransport systems in *E. coli*: the melibiose system [4], the glutamate system [5], and the proline porter I [6]. In this paper we extended the list to include the serine-threonine system.

Collins et al. [8] reported H^+/serine cotransport in a derivative of ML strain, and we have confirmed this observation. However, this cotransport took place via a transport system different from the Na^+-coupled serine-threonine system. It seems that the H^+/serine cotransport in K-12 strain is mediated by a different and so far unreported transport system. Since the LIV system, which also transports serine, is a binding protein-related system [11], H^+/serine cotransport would not be expected to occur via this transport system. In the ML strain H^+/serine cotransport seems to take place via the same system as for glycine and D- and L-alanine [8]. In the K-12 strain, however, a transport system for glycine, D- and L-alanine and D-serine (glycine-alanine system) does not mediate transport of L-serine [11]. Since glycine and alanine did not compete with the H^+/serine cotransport (data not shown), this cotransport system is distinguishable from the glycine-alanine system. Although little is known about the H^+/serine cotransport system, our result demonstrate that the cotransport system and enzyme(s) responsible for serine metabolism are induced simultaneously. It may be that genes for H^+/serine cotransport carrier and enzyme(s) involved in serine metabolism consist of one operon or one regulon. A comparison of the structures of the Na^+-serine and the H^+-serine carriers would give insight into the structure-mechanism relationship of the carriers. Furthermore, a comparison between the two carriers might yield insight into the evolution of H^+-coupled transport and Na^+-coupled systems. Cloning and sequencing of their structural genes would be of value for those studies.

As in the case of Na^+/proline cotransport [6], there were several factors for previous failures to recognize Na^+/serine(threonine) cotransport. The presence of low levels of Na^+ in many reagents as a contaminant and the leaching of Na^+ from glassware made it difficult to control Na^+ concentration in the assay mixtures. We used plastic containers for buffers and solutions, and plastic vessels for transport assays and determined final concentration of Na^+ in the assay mixture. Usu-

ally we detected about 20 μM of Na^+ as contaminant. This concentration of Na^+ is very close to the half maximum concentration of Na^+ for serine transport. When we used glass vessels for transport assays we usually detected 50 to 100 μM of Na^+ as contaminant. Therefore it is difficult to observe a stimulation of serine transport by added Na^+ if glass is used. A similar situation was observed in the study of Na^+-coupled proline transport system, in which the half maximum concentration of Na^+ was 37 μM [6].

A high concentration of Na^+ was inhibitory for serine transport. Under most conditions Na^+ stimulates Na^+-substrate cotransport systems [5,6,15]. However, under certain conditions inhibition by Na^+ has been observed, for example inhibition of proline transport by Na^+ has been observed [9], even though that system is a Na^+-proline cotransport system. Therefore, care should be taken in the interpretation of the effects of Na^+ on transport. It is perhaps more important that Na^+ has an effect, whether stimulatory or inhibitory. In either case, the possibility of Na^+/substrate cotransport should be examined. In the case of serine transport, some inhibition by Na^+ had been reported previously [9], but now it is clear that a Na^+/serine cotransport mechanism exists in *E. coli*. Transport of glycine, aspartic acid, phenylalanine and cysteine have also been reported to be inhibited by Na^+ to some extent [9], and these systems should be re-examined. This raises the possibility that Na^+ coupling is a major mechanism of cotransport system in *E. coli* and in other microorganisms.

The serine-threonine transport system possesses unique properties among Na^+-coupled transport systems in *E. coli*. First, the serine-threonine system utilizes only Na^+ as coupling cation, although other systems utilize H^+ (the melibiose system [4] and the glutamate system [20]) or Li^+ (the melibiose system [17] and the proline system [21]) in addition to Na^+. Thus, if the idea that cotransport system evolved from H^+-coupled type to Na^+-coupled type [2] is correct, then the serine-threonine system is at a more evolved stage than others in *E. coli*. If the situation is reverse [22], then the serine-threonine system is at more primitive stage. Second, Na^+ increases the V_{max} of transport in the serine-threonine system without significantly affecting the K_m, even though Na^+ lower the K_m without affecting the V_{max} in the melibiose [23] and the glutamate [20] systems. This suggests that the mechanism of Na^+/serine (threonine) cotransport may be different from Na^+/melibiose and Na^+/glutamate cotransport.

Na^+-coupled cotransport has also been reported in other microorganisms [3]. Investigations on Na^+-coupled transport systems in prokaryotes are relevant to our understanding of the evolution of cation/substrate cotransport. Especially, of value would be cloning [24] and sequencing [25] of the structural gene of Na^+/substrate cotransport systems.

Acknowledgments

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