Evidence for Two Na⁺/H⁺ Antiport Systems in *Escherichia coli.*

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Several insertion mutants of *Escherichia coli* in the *ant* gene, coding for Na⁻/H⁺ antiport activity, showed little, if any, reduction in the antiport activity. Na⁺-dependent transport activity also remained at wild type level. These facts led to the idea that *E. coli* has evolved at least two distinct systems for extrusion of Na⁺ The antiport activities were studied under various conditions to reveal different properties of these systems. For convenience these activities are referred to as major and minor activities. The distinguishing properties of the two systems include: kinetics (Km, Vm) at pH 7.8, competition pattern between Na⁺ and Li⁺, pH profiles, pattern of the change in kinetic parameters as a function of pH, and sensitivity to protease, chemicals, and heat.

KEY WORDS ▭ *E. coli*, Na⁺/H⁺ antiport, transport, *ant* gene

Many bacterial cells extrude Na⁺ using Na⁺/H⁺ antiport systems to maintain the transmembrane Na⁺ gradient (3, 4, 5, 7, 8, 25, 30, 35). This gradient can serve as a driving force for accumulation of nutrients (15, 22, 32, 34) or for motility (16). This Na⁺ gradient functions as a buffering system when the proton motive force tends to drop (31). This system also functions as a homeostasis mechanism in an alkaline environment (6, 17, 20, 23, 26, 36, 37, 38). In *E. coli*, the Na⁺/H⁺ antiporter system(s) appear to play a crucial role in metabolism of Na⁺ in this organism (9, 10, 19, 35).

Recently, Goldberg *et al.* (14) have characterized the *ant* gene of *E. coli*. Multicopy plasmids carrying the *ant* gene confer tolerance against inhibition by Li⁺ regarded as a toxic analogue of Na⁺. Membrane vesicles prepared from plasmid-carrying strains exhibit elevated Na⁺/H⁺ antiporter activity. DNA sequence analysis reveals that the *ant* gene product may be a membrane protein (18). In a previous paper we showed that *ant* is crucial for alkalinity tolerance as well as Na⁺ (Li⁺) tolerance under certain growth conditions and presented evidence in support of the idea that *ant* represents a structural gene coding for Na⁺/H⁺ antiport activity.

The first clue in our work that more than one activity might be present came when it was found that vesicles derived from insertion mutants of *ant* showed little, if any, reduction of overall activity. At this point, it was interesting to speculate that *E. coli* may have evolved at least two activities. Obviously the most convenient of these systems

to study is the *ant* system since this gene has been mapped and cloned, etc. While these studies were in progress Wilson *et al.* (23) reported on a probable point mutation displaying similar properties to our mutants. Interestingly, this mutaion does not result in total blockage of activity. Is the remaining activity due to the presence of a second Na⁺/H⁺ antiport system? These authors suggest that null mutations might help to clarify this point. Evidence presented below and based heavily on studies of membrane vesicles supports the idea of multiple Na⁺/H⁺ antiporters in *E. coli*.

MATERIALS AND METHODS

Bacterial strains

E. coli K12 strain, LS125, which was made by insertion of λ p*lac*MU53 in the minor antiporter gene *ant* of MC4100 [F⁻ *ara*D139 Δ(*arg*F *lac*)U 169 *rps*L150 *rel*A1 *flb*5301 *deo*C1 *pts*F25 *rbs*R], was used to study the major Na⁺/H⁺ antiport activity. Minor activity was studied in LS125(pNHA7), in which minor activity was amplified due to the high copy number of the *ant* gene. The construction and properties of these strains are described in Table 1.

Growth conditions

The minimal growth medium was M63 supplemented with 0.2% glucose or another carbon source at a concentration of 0.2% (24). The cells were grown aerobically at 37°C. The growth rates were determined in diauxic media. Diauxic culture media were M63 supplemented with 0.02% glucose, 0.2% of another carbon source (proline,

Table 1. *Bacterial strains, bateriophage, and plasmids.*

Strain, phage, or plasmid	Genotype
MC4100	F⁻ araD139(argF-lac)
	U169 rpsL150 relA1
	flbB5301 deoC1 ptsF25 rbsR
LS125	MC4100 ant1::λ placMu53
λ placMu53	immλ 'trp' lacZ⁺ lacA' 'uvrD
	Xho::kan Mu[cIts62 ner⁺A⁺ 'S]
pGM69	amp⁺ ant⁺
pNHA7	amp⁺ ant⁻

glutamate, serine, or melibiose), and 10 mM NaCl (13). Cultures exponentially growing in glucose minimal media were centrifuged (10,000×g, 5 min), the pellet was resuspended in the same volume as the original culture. Suspended cells (0.5 ml) were added to inoculate the fresh culture media (10 ml) in side-arm flasks. Growth was monitored by measuring A_{540} using a Klett-Summerson colorimeter.

Sensitivity to analogues

The sensitivity to toxic amino acid analogues was determined as described (13) with slight modifications. A sterile filter disk containing an analogue was placed in the center of the lawn of the strain spread on minimal plates supplemented with 10 mM NaCl and 0.2% glycerol as the carbon and energy source. The analogue levels used were: 500 nmol (2-Azetidinecarboxylic acid), 150 nmol (3,4-dehydroproline), 3.4 μmol(D-glutamate), 3.1 μmol (DL-α-methylglutamate), and 500 nmol (D-cycloserine).

Preparation of everted membrane vesicles

Everted membrane vesicles were prepared as described by Rosen (29) with slight modifications. Cells were grown to late log phase or to early stationary phase in M63 minimal medium supplemented with 0.5% glycerol as the carbon and energy source. Cells were harvested by centrifugation (12,000×g, 10 min) and washed once in Buffer A (10 mM Tris-HCl, pH 7.5, 0.25 M sucrose, 140 mM choline chloride, and 0.5 mM dithiothreitol) and resuspended (5 ml to 10 ml/g of wet weight) in Buffer A. The cells were broken by a single passage through an American Instrument Company French pressure cell at 8,000 psi. After treatment of lysed cells with 5 mg of DNase/ml and 5 mM MgCl₂ for 10 min at room temperature, unbroken cells were removed by centrifugation (12,000×g, 10 min). Everted membrane vesicles were pelleted by centrifugation (100,000×g, 60 min), washed once, and resuspended (2 mg of protein/ml) in Buffer A. Aliquots of small amounts were stored at −70°C after freezing with liquid nitrogen.

Fluorescence assay

ΔpH was estimated from the quenching of acridine orange fluorescence as previously decribed (29). Reactions were assayed in Buffer B (10 mM Trichine-HCl, pH 7.8, 140 mM KCl, and 1 μM acridine orange), and 100 μg of membrane protein were added to 2 ml of Buffer B. Quenching was initiated by addition of DL-lactate adjusted to pH 7.8 with KOH to the final concentration of 10 mM. In some cases, the pH of assay buffer and lactate were adjusted to the indicated values with KOH. The initial rates of antiport activities were calculated from the rapid enhancement in fluorescence from the steady state value upon the addition of cations. The major activity was determined in LS125. The values for the minor activity were determined as the difference in activity between LS125(pNHA7) and LS125. An Aminco-Bowman spectrometer was used to measure fluorescence with excitation at 470 nm and emission at 530 nm.

Na⁺/H⁺ antiport assay with pH meter

The change in extracellular pH of anaerobic suspension of *E. coli* upon adding NaCl solution was monitored as described with slight modifications (35). A culture of *E. coli* was grown to stationary phase in M6 medium supplemented with 0.2% glucose. An inoculum of 10 ml of this culture ws added to 200 ml of M63 medium supplemented with 0.2% succinic acid. The culture was grown for 10 h at 37°C aerobically. The cells were harvested by centrifugation (10,000×g, 4°C, 5 min) 1 h after the stationary phase of growth was reached. The cells were washed twice Buffer C (150 mM KCl and 3.0 mM glycylglycine, pH 7.0 adjusted with KOH). The bacteria were suspended to a density of 4 mg of protein/ml in the Buffer C, stored at 4°C, and used for assay within 5 h. The closed electrode vessel was filled with 8 ml of bacterial suspension. The suspension was equilibrated anaerobically by bubbling nitrogen gas through the suspension at 22°C for 60 min. Anaerobic solutions of KOH and HCl were occasionally added to the suspension to compensate the slow pH drift. The outflow of H⁺ was effected by the addition of anaerobic solution of NaCl to 5 mM. All anaerobic solutions were made by bubbling nitrogen gas through the solutions for 30 min. The time-course of change in extracellular pH was monitored using a pH meter (Orion Research, Model 701A/digital Ionalyzer).

Treatment with proteases and chemicals

All treatments were carried out at room temerature in 2 ml of Buffer B. Each reaction mixture contained 100 μg of membrane protein. Trypsin treatment was started by addition of typsin at 5 mg/ml and terminated at the indicated time by addition of 25 mg of trypsin inhibitor/ml. α-Chymotrypsin treatment was performed with 2 mg of α-chymotrypsin/ml. Modification with
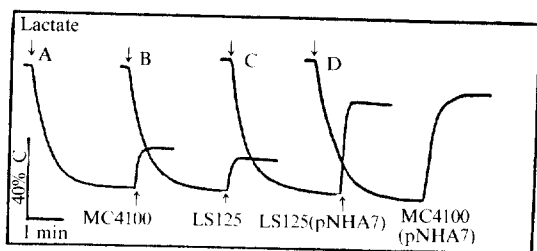
Fig. 1. Effect of ant mutations on Na$^+$/H$^+$ antiport activity. Antiport activities were assayed in Buffer B containing 140 mM KCl as described under "Experimental Procedures". Everted membrane vesicles prepared from differnt strains were added to 50 μg/ml. The initial rates of antiport activities were determined upon addition of 12 mM LiCl (indicated by arrow). Curve A, MC4100; Curve B, LS125; Curve C, LS125(pNHA7); Curve D, mC4100 (pNHA7)

Fig. 2. Effect of ant mutations on H$^+$ extrusion effected by Na$^+$ inflow in anaerobic suspensions of E. coli. The electrode vessel was filled with 8 ml of Buffer C (150 mM KCl and 3.0 mM glycylglycine, pH 7.0) containing bacteria (32 mg of protein). After 60 min equilibration under anaerobic conditions, 40 μl of 1 M NaCl were added to initiate the efflux of H$^+$ iors. Curve A, MC4100; Curve B, LS125.

dicyclohexylcarbodiimide (DCCD) was carried out with 0.05 mM DCCD.

Protein determinations

Protein concentrations were determined by the Lowry et al. (21) using bovine serum albumin as standard.

Chemicals

Pancreatic trypsin (1,530 BAEE units/mg protein), soy bean trypsin inhibitor (10,000 BAEE units/mg protein), α-Chymotrypsin (500 BAEE units/mg protein), and acridine orange were purchased from Sigma Chemical Co. All other chemicals were of reagent grade and were obtained from commercial sources.

RESULTS

Effect of ant mutations on Na$^+$/H$^+$ antiport activity

The first experiment summarized in Fig. 1 is concerned with the levels of Na$^+$/H$^+$ antiporter activities of vesicles prepared from various mutant strains (since all mutants showed similar activities, the result from a mutant strain LS125 is shown as a representative of insertion mutants). Note that hosts transformed with plasmid pNHA7 (Fig. 1C), which carries the structural with previous studies (14, companion paper). However, studies of the comparative activities of insertional mutants such as LS125 and its parental strain led to the unexpected result shown in Fig. 1A, B. Note that little difference of activities are observed (140% Fluorescence Increase(FI) min^{-1} for MC 4100 compared to 120% FI min^{-1} for LS125). Several insertion mutants did not show significant reduction in the antiport activity compared to parental strain. It should be noted that MC4100
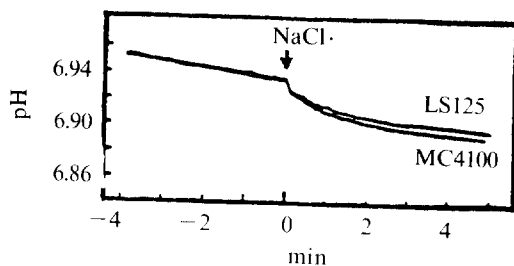
(pNHA7) showed activities similar to LS125 (pNHA7), indicating the antiport activities are increased in both strains due to the high copy number of ant gene on the plasmid. To verify that ant mutations do not completely block the antiport activity, another method was used to assay Na$^+$/H$^+$ antiporter activity (35). Cells were incubated anaerobically for 60 min to equilibrate the intracellular and extracellular media. Extrusion of Na$^+$ ions was initiated by the addition of anaerobic solutions of NaCl to a final concentration of 5 mM (Fig. 2). A rapid initial decrease in pH is followed by a slow, but, extensive decrease in pH. The rapid initial decrease is due to the effect of Na$^+$ on the electrode, as it is seen in the absence of cells. The subsequent slower decrease is from Na$^+$/H$^+$ antiport activity. Once again, there is little difference in antiport activity between mutant (LS 125) and the parent (MC4100). Membrane vesicles made from several different insertion mutants showed a level of Na$^+$/H$^+$ antiport activity similar to those made from wild type. These studies lead to the idea of multiple Na$^+$/H$^+$ antiport systems in E. coli.

Effect of ant mutations on sodium mediated transport systems

The effect of the ant mutations on Na$^+$-dependent transport systems was studied by two different methods (13): ability to grown on the substrates, which are known to be cotransported with Na$^+$; and sensitivity to toxic analogues of the substrates. As summarized in Table 2 the mutant strain and its parent showed similar growth rates on substrates, whose transport is dependent on Na$^+$. For example, the growth rates on proline, on serine, or on melibiose were 109, 100, 90 min for the mutant compared to 109, 98, 105 min for its parent. Sensitivity to toxic

Table 2. *Effect of the ant mutation on growth on different carbon sources.*[a].

Second Carbon Source	Doubling Time (min)		Lag Time (h)	
	MC4100	LS125	MC4100	LS125
Glucose	61	61	0	0
Melibiose	105	90	4	4
Proline	109	109	5	5
Glutamate	624	621	6	6
Serine[b]	98	100	4	4

[a] 0.02% glucose and 0.2% of a second carbon source were used for diauxic growth. NaCl was added to 10 mM.

[b] When serine (40) mM is used as a carbon and energy source, glycine, isoleucine, and threonine were also added to 1 mM, respectively.

Table 3. *Effect of the ant mutation on sensitivity to toxic amino acid analogues.*[a]

Toxic Analogue[b]	Inhibition Zone (mm)	
	MC4100	LS125
Azetidinecarboxylic acid	23	24
3,4-dehydroproline	17	16
D-glutamate	16	16
DL-α-methylglutamate	16	17
D-cycloserine	15	16

[a] see "MATERIALS AND METHODS" for details.
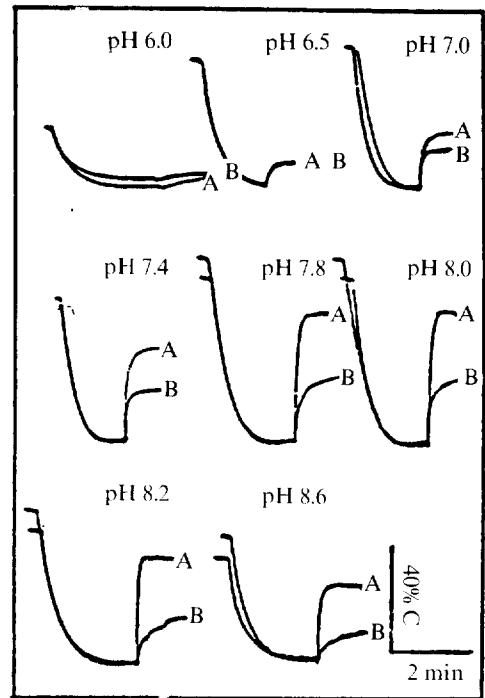[b] Size of inhibition zone (mm) was determined after 12 h incubation at 37°C.

Fig. 3. *Effect of pH on Na$^+$/H$^+$ antiport activities from different strains. To study the pH effect on antiport activities, assay buffer and lactate were adjusted with KOH to the indicated pH values. Antiport activities were determined upon addition of 12 mM LiCl (indicated by arrow). Curve A, LS125; Curve B, LS125 (pNHA7).*

analogues, does not reveal any difference with mutants and parental strains (Table 3). All these data support the idea that the mutations in the *ant* gene do not change greatly the activity of the sodium dependent transport systems.

Effect of pH

To prove this idea, the Na$^+$/H$^+$ antiporter activities were tested under various conditions with an eye towards finding different properties of antiport activities. The first experiment to biochemically differentiate activities was done by studying the effect of pH on antiport activity (Fig. 3). As seen in this figure, membrane vesicles from LS125 showed a constant level of activity from pH 7.0 to 8.2 and reduced activity at pH 6.5 and 8.6. Activity at pH 6.0 was vary small with a small proton gradient (10% Fl min^{-1}). This behavior pattern is similar to results from previous studies (3-5, 30). Activity from membranes from LS125 (pNHA7) showed clear dependence on pH values. While LS125(pNHA7) showed the same activity as LS125 at pH 6.5 or below, LS125(pNHA7) showed much higher activity than LS125 at pH

7.8 or above. In LS125(pNHA7) there are at least two different antiport activities, which show different behavior patterns with pH. For convenience the activity from LS125 is referred to as the major one. Amplified minor activity was determined as the difference in activities between LS125 and LS125(pNHA7). It should be mentioned that the antiport activity from MC4100 (pNHA7) showed the same pH-dependent pattern as that of LS125, indicating this pH dependence is not due to the mutation in LS125. Another point is that LS125(pGM69) showed similar activity, lowering the probability that mutation on *ant* gene on the plasmid pNHA7 cause the gene product from this mutated gene to have properties different from wild type antiporter. That both activities were obtained with constant driving force (ΔpH) after adjusting ΔpH with various amounts of NH$_4$Cl and plotted as a function of extravesicular (ie. intracelluar) pH (Fig. 4). The major system shows a constant level of activity between pH 7.0 and 8.0. In contrast, amplified minor system shows very small activity at pH 7.0
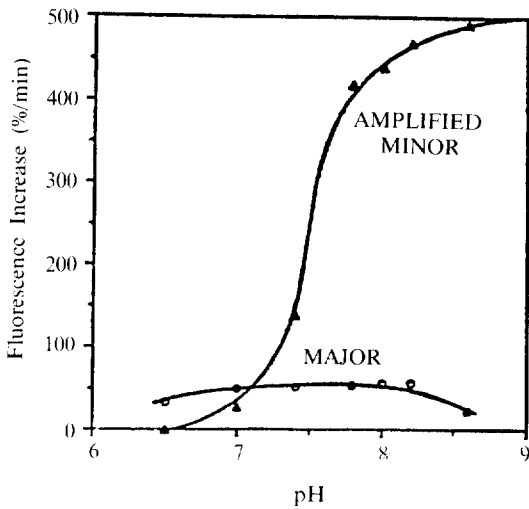
Fig. 4. pH profiles of two antiport systems. Assay buffer and lactate were adjusted with KOH to the indicated pH values. After steady state Δ pH was reached, different amounts of NH$_4$Cl were added to adjust fluorescence quenching to the same level prior to addition of 12 mM LiCl. Curve A, LS125; Curve B, difference between LS125(pNHA7) and Curve A. The levels of NH$_4$Cl added were (mM); 0.25(pH 6.5), 0.45(pH 7.0), 0.45(pH 7.4), 0.35(pH 7.8), 0.30(pH 8.0), 0.20(pH 8.2), 0.20(pH 8.6).

Fig. 5. Substrate specificity of two antiport systems. Specificity of the two systems was studied using Buffer B containing of the two systems was studied using Buffer B containing 140 mM KCl. H$^+$ efflux was initiated by adding cations to 10 mM at the indicated time; A, 10 mM NaCl; B, 10 mM LiCl. Competition between the two ions(Na$^+$ and Li$^+$) was studied (C,D). Membrane vesicles were preincubated with 10 mM NaCl(C) or LiCl(D) prior to the addition of lactate. LiCl(C) or NaCl(D) was added to 10 mM at the indicated time. Curve a, LS125; Curve b, LS125(pNHA7).

but large activity above pH 7.8.

Substrate specificity

The ability of ions to dissipate Δ pH and their ability to prevent other ions from doing so were considered to determine the substrate specificity of the Na$^+$/H$^+$ antiport activities (5, 8) (Fig. 5). The minor system as well as the major system shows activity specific only with Li$^+$ or Na$^+$ (Fig. 5A, B). Competition between Li$^+$ and Na$^+$ ions was tested. Preincubation of membrane vesicles from LS125 with Na$^+$ (10 mM) prevented the dissipation of Δ pH by Li$^+$ (10 mM) (Fig. 5). In the reciprocal experiment, Li$^+$ also prevents the dissipation of Δ pH by Na$^+$. With membranes of LS125 (pNHA7), the competition pattern is dependent on the order of addition (Fig. 5C, D). While Li$^+$ completely inhibits the Δ pH dissipation by Na$^+$, Na$^+$ does not completely prevent the dissipation of Δ pH by Li$^+$. This data suggests that saturation of the major system is achieved by either 10 mM Li$^+$ or Na$^+$ while that of the minor system is not achieved by 10 mM Na$^+$.

Kinetics of two Na$^+$/H$^+$ antiport systems

The initial rates of both Na$^+$/H$^+$ antiport activities were plotted as a function of concentrations of cations (Fig. 6). The major system
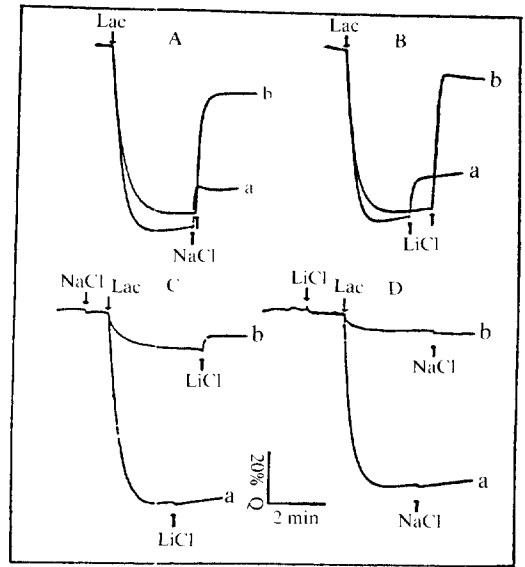
shows hyperbolic kinetics with Li$^+$ and Na$^+$ in agreement with reports by Beck and Rosen (5). The Km of the major antiport activity is 2.2 mM for Li$^+$ and 0.8 mM for Na$^+$. The Vm is greater with Na$^+$ than with Li$^+$ (130% FI min^{-1} with Na$^+$ compared to 50% FI min^{-1} with Li$^+$). All values of kinetic parameters for the major system are consistent with the previously obtained values (3, 4, 5, 25, 28). The hyperbolic curve was also observed for the minor Na$^+$/H$^+$ antiport activity with both ions (Fig. 6B). As predicted from the competition experiment, the Km for Li$^+$ (2.8 mM) is much lower than that for Na$^+$ (12 mM). The major system shows higher activity with Na$^+$ than with Li$^+$ in contrast to the minor system.

Effect of pH on kinetics

Kinetic parameters of the two Na$^+$/H$^+$ antiport systems were monitored as a function of the pH of the assay buffer (Fig. 7). The patterns of change in kinetic parameters as a function of pH are quite different for the two systems. For the major system Vm is relatively constant between pH 7.0 to 8.2 (slightly lower at pH 6.5 and 8.6); Km is
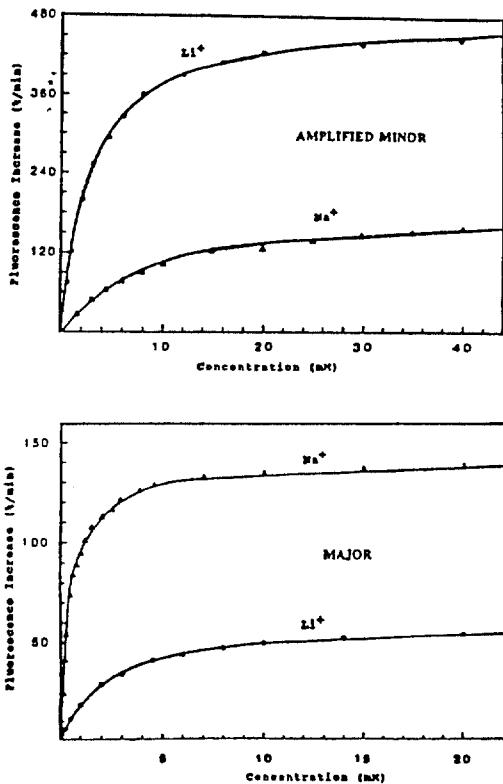
Fig. 7. *Kinetics as a function of pH. Kinetic parameters were determined at indicated pH values as described in Fig. 6 and plotted as a function of pH. Initial ΔpH was adjusted to the same value as described in Fig. 4 prior to the addition of various amounts of LiCl. A, Vm; B, Km. In each panel Curve a, amplified minor system; Curve b, major system.*

Fig. 6. *Kinetics of two Na$^+$/H$^+$ antiporter systems. Initial rates of antiport activities were plotted as a function of cation concentrations. Antiport activities were measured in Buffer B at pH 7.8 after initial ΔpH was adjusted to constant value used in Fig. 4 with NH$_4$Cl. Km and Vm were determined by the direct linear plot of Eisenthal and Cornish-Bowden. Km and Vm were determined as median values after plotting lines at 10 different concentrations. A, major system; B, amplified minor system.*

Fig. 8. *Sensitivity of two systems to α-chymotrypsin. Membrane vesicles (50 μg/ml) were treated for 4 min with 2 mg of α-chymotrypsin/mg of membrane protein. The reaction was carried out in fluorescence assay buffer (Buffer B) at room temperature. Initial levels of δpH of untreated vesicles (Curve b) were adjusted to that of digested vesicles (Curve a) by adding 0.08 mM NH$_4$Cl. NaCl (10 mM) was added to initiate the fluorescence recovery. A, LS125; B, LS125 (pNHA7).*

higher at lower pH. This pattern is consistent with previous studies (3, 4, 5, 30). Larger variations are seen in Vm and Km of the minor antiporter system. Vm increases sharply with higher pH until it plateaus at pH 8.2(at pH 6.5 0% FI min $^{-1}$, at pH 7.8 500% FI min $^{-1}$). The Km decreases sharply with higher pH (from 10 mM at pH 7.0 to 0.3 mM at pH 8.6). In conclusion, changes in pH affects both kinetic properties of the minor system while altering only the Km of the major system.

Effect of proteases, chemicals and heat

The sensitivity of the two antiport systems to proteases was studied by using trypsin and α-chymotrypsin. Activities of both antiporters are
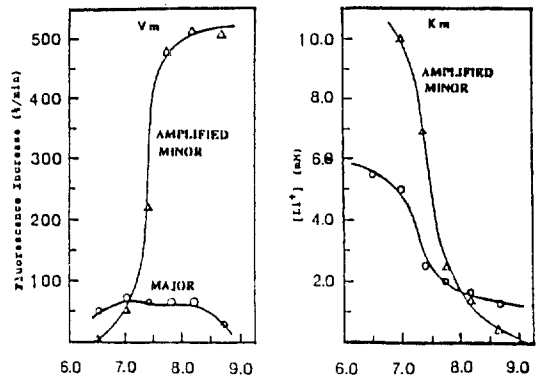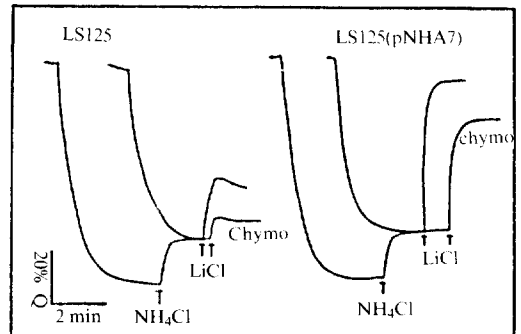
resistant to treatment with trypsin as long as the ΔpH is maintained (data not shown). The difference in sensitivity to treatment with α-chymotrypsin is shown in Fig. 8. The major system is sensitive to α-chymotrypsin in contrast to the minor system. The major antiport lost 53% of its activity in contrast to a 9% with the minor system after treatment with 2 mg of α-chymotrypsin per mg of membrane protein for 4 min.
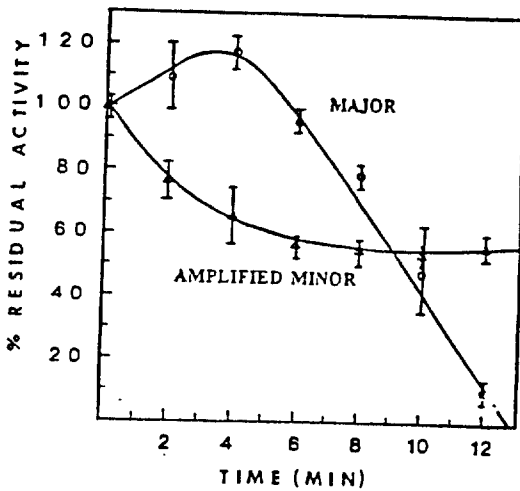
Fig. 9. *Sensitivity of two systems to heat. Membrane vesicles (2 mg/ml of Buffer A) wer treated at 55°C for various times. An aliquot (100 µg of membrane protein) of treated vesicles was added to Buffer B containing 140 mM KCl. Fluorescence recovery (as %) after addition of 10 mM NaCl was determined and plotted as a function of time for heat treatment. Curve a, LS125(pNHA7); Curve b, LS125.*

The effect of chemical modification was studied by using DCCD, which reacts preferentially with glutamic or aspartic acid residues (12). The difference in sensitivity to this inhibitor is also evident. The minor system is more resistant than the major system (20% loss of activity with the major system compared to no damage with the minor system after treatment with 0.01 M DCCD for 3 min).

As shown in Fig. 9, the two activities display different inactivation profiles when incubated at 55°C. Note that the major activity showed an increase during the first few minutes of incubation followed by a sharp decline with only 10% activity remaining after 12 min. In contrast the minor Na$^+$/H$^+$ antiport activity decreases rapidly during the first few minutes reaching a plateau with 60% activity remaining after 12 min.

DISCUSSION

Energized *E. coli* cells maintain a constant Na$^+$ gradient over a large range of extracellular sodium concentrations (9, 10) by extruding Na$^+$ using their Na$^+$/H$^+$ antiporter system(s) (3-5, 7, 8, 25, 28, 30, 33, 35). Na$^+$/H$^+$ antiporters mediate Na$^+$ extrusion by coupling upward Na$^+$ movement against the Na$^-$ electrochemical potential gradient to downward H$^+$ movement along the H$^+$

electrochemical potential gradient (7, 35). In this paper, evidence is presented for at least two different Na$^+$/H$^+$ antiport systems in *E. coli*.

Evidence for two Na$^+$/H$^+$ antiporters

The first experimental clue was the finding that little, if any, difference in Na$^+$/H$^+$ antiport activity is observed in vesicles from insertional (null) mutants and their parents (see previous paper for characterization of mutants). The Na$^+$/H$^+$ antiport activities were studied under various conditions with an eye towards differentiating between these activities. The distinguishing properties of the two systems include: different pH profiles (Fig. 3, 4), differences in kinetic parameters as a function of pH (Fig. 5), differenti sensitivity to protease (Fig. 8), chemicals (Table 3), or heat (Fig. 9).

In conclusion, *E. coli* seems to have evolved at least two Na$^-$ extrusion systems. This multiplicity of metabolic systems is not surprising because there are many known examples for amino acids and sugars of multiple transport systems which may function under different environmental conditions (1, 2). For example, in the ion transport area there are at least three different systems for extrusion of K$^-$ (2) and two calcium export systems (1).

Role of two Na$^+$/H$^+$ antiporters

Because null mutations in the *ant* gene reduce Na$^+$/H$^+$ antiport activity and Na$^-$-dependent transport little, if any, the *ant* gene seems to represent a minor activity at least under these growth conditions. A mutant reported by Ishikawa *et al.* (17), seems to have a mutation in the major system since the mutant showed little Na$^+$/H$^+$ antiport activity. Since no genetic information about this mutant has been reported direct comparison of *ant* mutants with this mutant cannot be made. Since the mutant could not grow at alkaline pH in minimal medium, the major Na$^-$/H$^+$ system seems to play a major role in pH regulation in alkaline medium. Mutations in the *ant* gene also prevent bacterial growth under certain conditions (in LB plus 0.45 M NaCl and in LB at pH 8.6). It should be recalled that K$^+$ restores the growth defect of *ant* mutants under the above conditions and no growth defect in minimal medium was demonstrated at alkaline pH. The *ant* system seems to fulfill a fairly specialized role mainly in maintaining pH as well as extruding Na in an alkaline environment when the environmental Na$^-$/K$^+$ ratio becomes high. Under normal laboratory conditions this gene is dispensable becoming essential during conditions of stress. The fact that at high pH this system shows high affinity for Li$^+$ (see Fig. 6), enabling cell to lower intracellular pH to the fullest in an alkaline environment, supports this idea.

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초 록: 대장균에서 두가지 Na⁺/H⁺ Antiport 체계에 대한 증거

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Na⁺/H⁺ artiport 유전자를 여러가지 돌연변이 방법으로 변화시켜 얻은 대장균 변이주들이 모체와 같은 정도의 Na⁺/H⁺ antiport 활성도와 Na⁺에 의존하는 운반활성도를 가졌다. Kinetics (Km, Vm), Na⁺과 Li⁻ 사이의 경쟁패턴, PH 변화에 따른 Kinetics의 변화, Protease, 화학물질, 열들의 예민도 등을 비교 연구한 결과 대장균이 적어도 두가지 이상의 서로 다른 체계를 가지고 있을 것이라는 사실이 증명되었다.