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Phenotypic Properties of a Unique *rpoA* Mutation (*phs*) of *Escherichia coli*

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The *phs* mutation of *Escherichia coli* has been suggested to affect the Na^+/H^+ antiport (D. Zilberstein, E. Padan, and S. Schuldiner, FEBS Lett. 168:327–330, 1980). We have recently shown that the mutation affects the *rpoA* gene and thus affects transcription. The extent of the pleiotropy of the *phs* mutation was investigated. In addition to the previously reported growth defect on L-glutamate and melibiose, the mutation also affects at least two other metabolic systems. The transport and metabolism of arabinose is impaired and the transport of sulfate is reduced. The extent to which the effects of the *phs* mutation on metabolism are due to a defect in the Na^+/H^+ antiport was investigated, and no causal role for this transport system in the metabolic defects was found.

The *phs* mutation of *Escherichia coli* causes a growth defect which has been associated with the loss of the Na^+/H^+ antiport function (31). Thus, growth on L-glutamate and melibiose is impaired, and the pH range for growth is restricted to values of the external pH (pH_o) below pH_o 8.3 (28–31). Using membrane vesicles derived from the mutant, it was demonstrated that Na^+-stimulated uptake and exit of L-glutamate and melibiose was severely impaired (30). Monensin failed to correct the coupling defect, and it was therefore suggested that the *phs* mutation affected the synthesis of a subunit involved in energy coupling common to the Na^+-driven systems and the Na^+/H^+ antiport (30). The possibility that *phs* caused a general membrane transport lesion was countered by showing that L-proline transport was unaffected by the mutation (28). However, it has subsequently been established that L-proline transport occurs via $Na^+(Li^+)$ symport (6, 6a, 26, 27). If the *phs* lesion affected Na^+ coupling directly then proline transport should be affected. Thus, the common thread of the *phs* phenotype was broken.

Provisional mapping of the *phs* mutation by conjugation placed it close to *metB* on the *E. coli* map (2, 30). Subsequently, we have shown that the *phs* mutation maps close to 72.5 min on the *E. coli* chromosome (21, 22). The error in the earlier work was due to a previously undiscovered aspect of the *phs* phenotype, namely a Cym auxotrophy (21). Cym auxotrophs can be satisfied by either cysteine or methionine, and the lesion is usually at the level of either transcription or translation of the *cys* regulon (19). As a result of fine mapping we have shown that *phs* is most probably an allele of *rpoA*, the gene for the α-subunit of RNA polymerase (22). Thus, it seems likely that the *phs* phenotype is the result of a transcription defect.

In view of the changed genetic basis of the *phs* phenotype we wished to reassess the growth properties of strains carrying this mutation. In particular, we wished to determine which metabolic systems are affected by the *phs* mutation and to what extent these effects can be attributed to a defect in the Na^+/H^+ antiport. Our data show that the metabolism of sulfur, arabinose, and possibly maltose is affected in addition to that of L-glutamate and melibiose. We found no evidence that impairment of the Na^+/H^+ antiport system plays a causal role in the metabolic defects caused by the *phs* mutation.

MATERIALS AND METHODS

Abbreviations. ACMA, 9-Amino-6-chloro-2-methoxyacridine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; TMG, thiomethylgalactoside.

Bacterial strains. A list of the bacterial strains used is given in Table 1.

Growth media. Complex medium (LBK) was used for routine growth of organisms for transport studies. Minimal medium S, a wholly K^+-based version of medium A (8), was used for testing auxotrophy, carbon source utilization, and growth rates. Medium K115 was used to grow cells at 37°C for the preparation of membrane vesicles and for experiments to measure Na^+ extrusion by cells. K115 contained (per liter): $K_2HPO_4 \cdot 3H_2O$, 10.5 g; KH_2PO_4, 3.1 g; $(NH_4)_2SO_4$, 1.05 g; $MgSO_4$, 0.098 g; glucose, 2 g. Required amino acids were present at 50 μg/ml and carbon sources at 0.2% (wt/vol) unless otherwise specified.

To carry out studies with Na^+-free media we devised an Na^+- and K^+-free medium which we refer to as K0Na0. This medium contains (per liter): $MgSO_4$, 0.098 g; $(NH_4)_2SO_4$ $FeSO_4$, 0.0023 g; $(NH_4)_2HPO_4$, 2.64 g; bistrispropane, 2.8 g; choline chloride, 16.75 g. The medium was adjusted to pH 7 with HCl and stored in plastic bottles. Growth of the cells was also carried out in plastic bottles to minimize Na^+ contamination arising from the walls of glass flasks. The potassium requirement of the cells was met by the addition to the medium of 10 mM KCl from a sterile stock solution.

Transport protocols. Cells were grown overnight in me-

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TABLE 1. Bacterial strains used

Strain	Genotype	Origin or reference
CS71	Hfr PO2A, *metB1 gltSo lacY*	S. Schuldiner
DZ3	CS71 *phs*	S. Schuldiner
GR303	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL thi* Δ(*his-gnd*) *zhc*::Tn*10*	21
GR302	GR303 *phs*	21
GR316	DZ3 *lac*⁺	This study
7	HfrC *phoA glpR*	B. P. Rosen
GR317[a]	GR316 *phs*⁺	This study
HfrC	Hfr PO2A, *metB*	P. Postma
JM2443	F⁻ *araAB lac* Δ(*his-gnd*) *araD rpsL araE*-λ *plac* Mu 1	P. J. Henderson
PG04	JM2443 *zhc*::Tn*10*	This study
PG05	JM2443 *phs zhc*::Tn*10*	This study

[a] Strain GR317 is a transductant of strain GR316 to growth on glutamate as sole carbon source (*phs*⁺), using strain 7 as donor.

dium S (8), containing 0.2% glycerol as carbon source plus essential supplements. After overnight growth at 30°C, the cells were diluted with fresh medium to an optical density at 650 of 0.2 and incubated at 30°C in an orbital shaker until the optical density at 650 nm equaled 0.8. The cells were then harvested by centrifugation at 5°C in a Sorvall RC-5 centrifuge (9,000 × *g* for 10 min) and washed twice by resuspension and recentrifugation with 20 mM bistris-propane (pH 7) containing 150 mM choline chloride as the wash buffer. For assays, the final cell suspension was approximately 0.5 mg (dry weight) per ml. When either melibiose or arabinose transport was to be assayed, 10 mM melibiose or 1 mM arabinose, respectively, was included in all growth media. To induce the arabinose transport system in arabinose-sensitive strains (*araD* strains), we added arabinose to the culture 2 h before harvesting for transport measurements. Transport assays were carried out at 30°C but otherwise were as described previously (5).

Measurement of potassium uptake and Na^+ efflux from Na^+-loaded cells was measured as described previously (4). The Na^+ loading of cells was carried out by the EDTA method (4).

Preparation of inverted membrane vesicles. Vesicles were made by a variant of the method described in reference 23 but with the specific aim of limiting the use of K^+ and Na^+ salts in the preparation. Strains DZ3 and CS71 were grown on K115 minimal medium with glucose as the carbon source and supplemented with methionine and were harvested in late log phase. The cells were harvested by centrifugation at 7,000 × *g* for 10 min at 5°C. The pellet was suspended in 25 mM K_2HPO_4–25 mM KH_2PO_4–10 mM $MgCl_2$ adjusted to pH 7 with KOH. After recentrifugation at 7,000 × *g* the pellet was suspended in 50 mM MOPS–10 mM $MgCl_2$–1 mM dithiothreitol–10% glycerol adjusted to pH 6.5 with Tris base. The cells were then collected by centrifugation at 7,000 × *g* for 5 min, and subsequently the pellet was suspended in 50 mM MOPS–10 mM $MgCl_2$–1 mM dithiothreitol adjusted to pH 6.5 with Tris base. The cell suspension (approximately 25 mg [dry weight] per ml) was then passed through a Ribi-cell fractionator (model RF-1; Ivan Sorvall, Inc., Norwalk, Conn.) at 68.9 mPa at 10 to 15°C. The nucleic acid in the lysate was destroyed by incubation with DNase and RNase for 10 min at 4°C. The suspension of lysed cells was then centrifuged at 40,000 × *g* for 10 min, and the supernatant was recentrifuged at 200,000 × *g* for 60 min. The pellet was suspended in 50 mM MOPS–10 mM $MgCl_2$–1 mM dithiothreitol–10% glycerol (pH 6.5), and the ultracentrifugation step was repeated. The final pellet of membranes was resuspended in 2 ml of the same buffer and stored in 500-μl aliquots in liquid nitrogen.

Assay of Na^+/H^+ antiport activity in membrane vesicles. A sample of membranes was thawed quickly and diluted with 100 mM Tris hydrochloride–10 mM $MgCl_2$ (pH 7.5) and centrifuged at 200,000 × *g* for 60 min. The pellet was resuspended in the same buffer and used the same day for antiport assays. Vesicles were incubated in a spectrofluorimeter cuvette with a fluorescent pH probe, ACMA, and the emission at 490 nm was recorded after excitation at 410 nm (14, 23). The reaction mixture contained ACMA (2 μM), vesicles at 50 μg of protein per ml, Tris hydrochloride (100 mM, pH 7.5), and $MgCl_2$ (10 mM). Respiration was initiated by the addition of 10 mM Tris succinate (pH 7.5), and after the quenching of ACMA fluorescence had reached a steady value further additions were made: KCl, 10 mM; NaCl, 10 mM; 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, 10 μM. The temperature of the reaction mixture was 23°C. Measurements were carried out on an Aminco SPF500 recording spectrofluorimeter.

Determination of analog sensitivity. The sensitivity of different strains to toxic amino acid and sulfate analogs was determined by using sterile filter disks on solid media (1). Lawns of the appropriate strains were spread on minimal agar containing 0.2% glucose as the carbon source. A sterile filter disk was placed in the center of the lawn, and a sample of a sterile solution of the analog was placed aseptically onto the disk. The following compounds were tested: chromate, 200 nmol; DL-α-methylglutamate, 3.1 μmol; D-glutamate, 3.4 μmol; azetidinecarboxylate, 500 nmol; dihydroproline, 150 nmol; D-cycloserine, 500 nmol.

Chemicals. All complex media were purchased from Oxoid

TABLE 2. Sensitivity of *phs* mutants to chromate and amino acid analogs[a]

Toxic analog	Zone of inhibition (mm) with strain:		
	HfrC	CS71	DZ3
Chromate	36 ± 2	37 ± 1.5	24 ± 3
Chromate plus cysteine	26	23 ± 2	23 ± 1
DL-α-Methylglutamate	<6	22 ± 2	7
D-Glutamate	16 ± 2	34 ± 4	26.5 ± 1.5
D-Cycloserine	66 ± 6	65 ± 5	67
Azetidinecarboxylate	30	32.5 ± 0.5	32.5 ± 0.5
Dihydroproline	19.5	21 ± 1	19.5 ± 1.5

[a] Details as described in Materials and Methods.

TABLE 3. Carbon source utilization pattern of *phs* strains[a]

Second carbon source	Doubling time (h)		Ratio *phs*⁺/*phs*	Lag time (h)	
	phs⁺	*phs*		*phs*⁺	*phs*
Galactose	2.5	2.3	1.1	0	0
Succinate	2.5	2.8	0.89	1.0	2.0
Glycerol	1.7	2.0	0.85	0.8	0.8
Glucose	1.3	1.7	0.77		
Maltose	1.6	2.2	0.73	2.25	3.0
Arabinose	1.7	4.5	0.35	1.0	1.5
Melibiose	1.6	9.0	0.18	3	5
L-Glutamate	2.3	16	0.13	2	4

[a] See text and Fig. 1 for details.

TABLE 4. Arabinose transport in *phs* strains[a]

Transport system	Na^+ (mM)	Rate of transport (nmol/min per mg [dry wt])
AraE + AraF		
Strain GR303 (*phs*$^+$)	1	6.5
Strain GR303 (*phs*$^+$)		4.8
Strain GR302 (*phs*)		0.5
AraF only		
Strain PG04 (*phs*$^+$)	1	0.87
Strain PG04 (*phs*$^+$)		0.87
Strain PG05 (*phs*)		Nil

[a] Transport experiments were carried out as described previously (5). The final concentration of arabinose was 50 μM at a specific activity of 1.8 to 3.75 Ci/mol.

Ltd. (London, England) with the exception of MacConkey base, which was purchased from Difco Laboratories (Detroit, Mich.). Analar inorganic chemicals, glucose, and glycerol were obtained from BDH (Poole, England), and unless stated all organic chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Radiochemically labeled amino acids were purchased from Amersham International, [*methyl*-^{14}C]thiogalactoside was obtained from New England Nuclear Corp. (Boston, Mass.), and [^{14}C]arabinose was the generous gift of P. J. Henderson, Department of Biochemistry, University of Cambridge, Cambridge, England.

RESULTS

Cym auxotrophy of *phs* strains. We recently reported that the *phs* mutation causes a methionine-cysteine auxotrophy (21). In the absence of either cysteine or methionine the growth rate of strain GR302 (MJF1 *phs zhc*::Tn*10*) was reduced five- to sixfold compared with that of its isogenic parent, GR303 (MJF1 *zhc*::Tn*10*). Compared with methionine, cysteine resulted in slower growth rates in both the parent and the mutant. The ability of methionine to satisfy the auxotrophy suggests that incorporation of sulfate into amino acids is still occurring but at a much reduced rate. The provision of methionine spares the cysteine which would normally be used to synthesize this amino acid. Thus, the auxotrophy is leaky and indicates impaired gene expression rather than total loss of gene function in the *cys* regulon (21).

We previously reported that 1 mM thiosulfate would not complement the Cym lesion of *phs* mutants and suggested that the affected gene was *cysG* (21). However, we have now found that higher concentrations of thiosulfate (20 mM) do meet the sulfur requirements of these cells (data not shown). This suggested that *phs* might affect transport of sulfate and its analogs. The chromate sensitivity of the strains was tested to determine the activity of the sulfate transport system (16). The *phs* mutation caused a dramatic reduction in chromate sensitivity of *E. coli*, comparable to the repression of the sulfate transport system by cysteine (Table 2). Thus, sulfate transport is impaired in the *phs* strain. At this time we do not know if this is the only aspect of sulfur metabolism which is impaired.

Effect of the *phs* mutation on carbon source utilization. Consequent upon the discovery of the complexity of the *phs* lesion (21) we screened the range of carbon sources utilized by the *phs* mutant. On solid media no differences in growth were observed between strains DZ3 and CS71 on the following carbon sources: glucose, fructose, maltose, sorbitol, L-rhamnose, galactose, xylose, mannitol, D-alanine, and

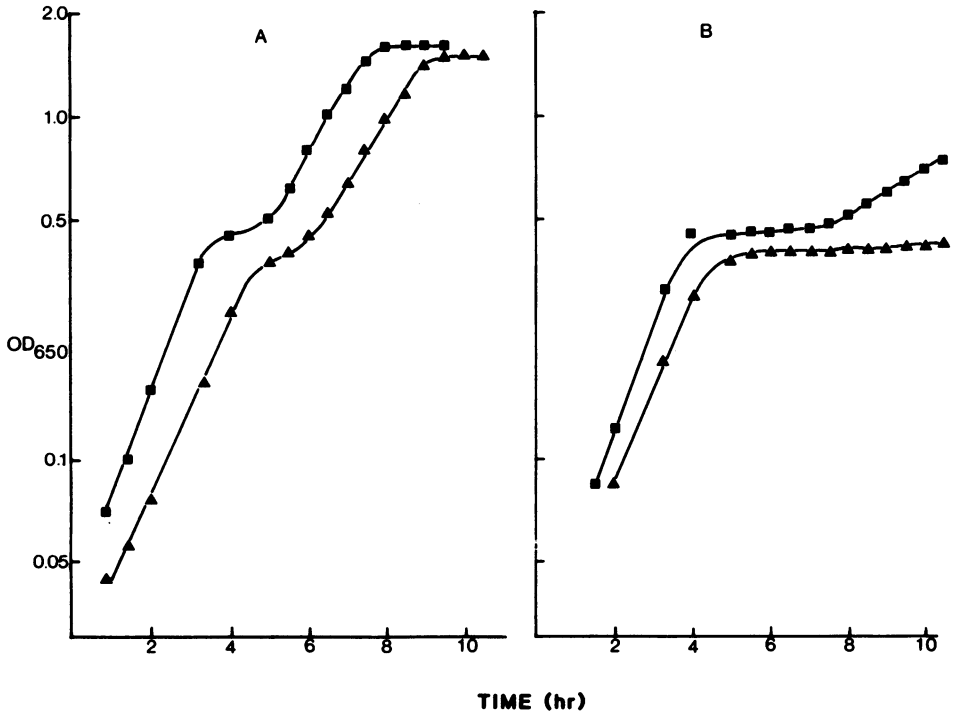

FIG. 1. Growth at 42°C on melibiose and lactose. Cells were grown in minimal medium with 0.02% glucose and either 0.2% lactose (A) or 0.2% melibiose (B) as the second carbon source. Symbols: ▲, *phs* strains; ■, *phs*$^+$ strains. The strains used were GR316 (*phs lacY*$^+$) and GR317 (*phs*$^+$ *lacY*$^+$). Growth was monitored by the increase in the optical density at 650 nm (OD_{650}).

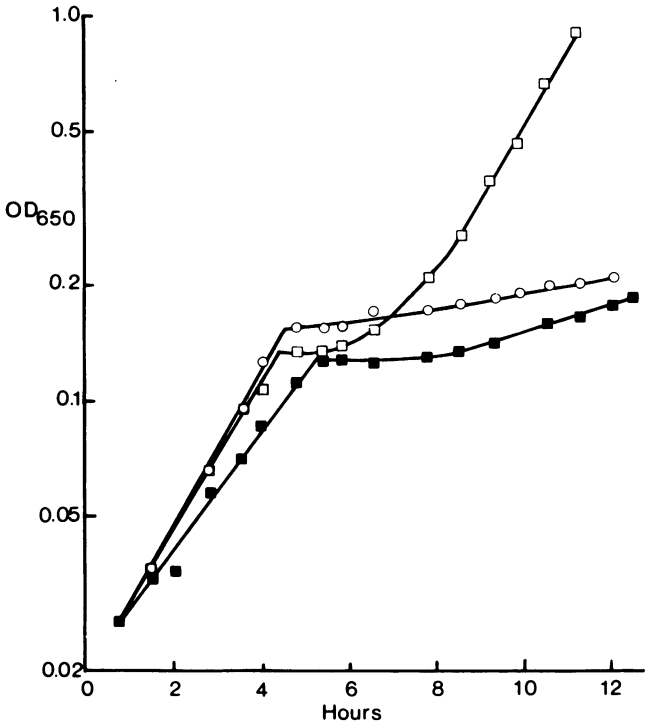

FIG. 2. Growth of *E. coli* on melibiose and L-glutamate in low-Na^+ medium. Strains CS71 (○, □) and DZ3 (■) were grown on K0Na0 medium containing 10 mM KCl and 0.02% glucose as the carbon source and with either 0.2% melibiose (□, ■) or 0.2% L-glutamate (○) as the second carbon source. The medium contained 2 μM Na^+ in the case of melibiose or 37 μM Na^+ when L-glutamate was the carbon source (in the latter case extra Na^+ was unavoidably introduced during the neutralization of glutamic acid with KOH which carried a low level of Na^+). Other details are as described in the legend to Fig. 1. OD_{650}, Optical density at 650 nm.

L-proline. Only growth on L-glutamate, melibiose, and arabinose was visibly affected.

Diauxic growth from limiting glucose in liquid media was used to quantitate growth rate effects since this established a common state from which the adaptation to a new carbon source can be monitored. During our initial characterization of the *phs* mutant DZ3, we observed that it grew more slowly on minimal media than did its isogenic parent CS71 (Table 3) and that this effect was more marked at 30°C than at 37°C (data not shown). The differential in growth rates between DZ3 and CS71 was affected by the identity of the carbon source (Table 3). In terms of this growth rate differential a hierarchy of carbon source utilization was observed: galactose > glycerol > succinate > glucose > maltose > arabinose > melibiose > glutamate. This hierarchy does not correlate with the growth rate on the individual carbon sources or with the adaptation times (Table 3).

The arabinose growth defect could be used in genetic analysis of the *phs* allele. On arabinose-MacConkey plates replica colonies of *phs* strains remained white for 7 to 8 h after phs^+ colonies had turned red, and they did not develop the full color intensity even upon prolonged incubation. The *phs* mutation caused arabinose resistance in strains carrying an *araD* lesion and thus could be scored in this way. Arabinose sensitivity in *araD* strains arises due to a block in the metabolism of ribulose 5-phosphate (10), and resistance can arise from failure either to transport or to metabolize arabinose. We have previously shown that the transcription of the metabolic operon *araBAD* is impaired in *phs* strains (22), and thus, we sought to measure the transport of the sugar. There are two transport systems for arabinose, encoded by the *araE* and *araFG* genes, respectively (7, 13). The *phs* mutation reduced the activity of the *araE*-encoded system 20-fold and abolished transport via the *araFG* system (Table 4). Sodium had no significant stimulatory effect on either system (Table 4).

Role of the Na^+/H^+ antiport in the melibiose growth defect. Melibiose transport can occur via either the *melB*-encoded α-galactoside permease or via the *lacY*-encoded β-galactoside permease (18). The *melB*-encoded system is unusual in that solute uptake can be driven by gradients of either H^+ or Na^+ (15), and it is inactive at 42°C (20). These properties allow growth on melibiose to be used to provide a stringent test of the role of the Na^+/H^+ antiport in the *phs* phenotype. We constructed lac^+ derivatives of *phs* and phs^+ strains (GR316 and GR317, respectively) and tested their ability to grow on lactose and melibiose at 42°C (Fig. 1). Growth on lactose was essentially identical in the two strains, indicating that *phs* does not impair functioning of the *lac* operon (22), but growth on melibiose (via the activities of the *lacY* and *melA* gene products) was eightfold slower in the *phs* strain (Fig. 1).

Similarly, in the absence of Na^+, transport of melibiose via the *melB*-encoded system occurs principally by H^+ symport (15), a condition which would be expected to relieve a melibiose growth defect caused by a deficiency in the Na^+/H^+ antiport. In the absence of added Na^+, CS71 (phs^+) is able to grow on melibiose but not on L-glutamate, which exhibits close coupling to Na^+. However, under these conditions DZ3 (*phs*) was unable to grow on melibiose (Fig. 2).

These two sets of data demonstrate that the mechanism of the *phs*-induced growth defect on melibiose cannot be ascribed simply to an Na^+/H^+ antiport defect. Rather, they indicate that the synthesis of α-galactosidase is impaired and thus expression of the *melAB* genes must be directly affected.

Effect of the *phs* mutation on Na^+-dependent transport systems. Impairment of the transport system for a carbon source can be detected in three ways: loss of growth on that carbon source; failure to transport radioactively labeled substrates; and insensitivity to toxic analogs of the carbon source. The growth studies reported above were complemented with the other two approaches.

The Na^+-dependent transport of L-glutamate, L-proline, and TMG (a melibiose analog) was assayed in strains DZ3 and CS71. As reported previously (28), L-proline transport was unaffected by the *phs* mutation but was Na^+ dependent. Uptake of both TMG and L-glutamate was reduced fourfold (Fig. 3). The residual transport of L-glutamate in strain DZ3 was not Na^+ dependent, indicating that this activity is due to one of the other glutamate transport systems (24). The low level of TMG transport in the *phs* strain was still Na^+ dependent, indicating that it occurred via the *melB*-encoded permease (15). This is consistent with the growth data, which showed a more severe impairment of L-glutamate utilization than of melibiose (Table 3). It is noteworthy that the rate of transport of L-proline in both strains exceeds that of L-glutamate and TMG. Since all three systems require the Na^+/H^+ antiport to be active, it seems unlikely that a primary defect in the antiport can be invoked to explain the loss of transport activity.

Similar results were obtained using toxic analogs to assay the activity of the L-glutamate and L-proline transport systems. The *phs* mutation caused increased resistance to

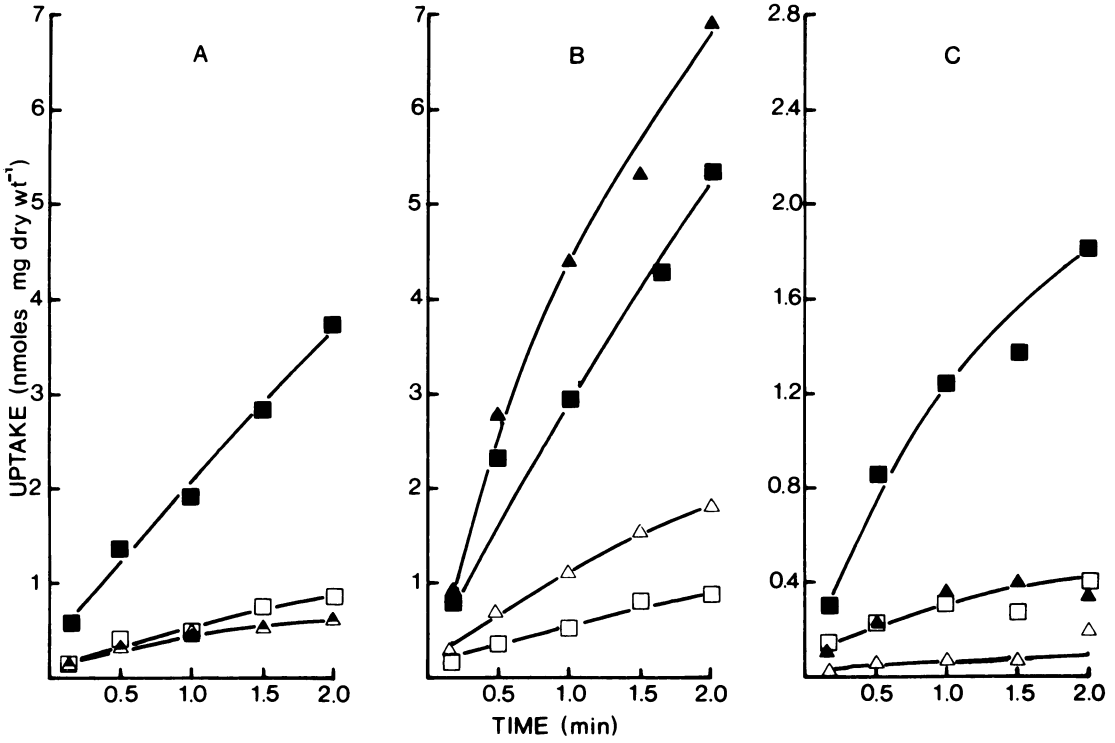

FIG. 3. Transport of L-glutamate (A), L-proline (B), and TMG (C). Cells of strains DZ3 (▲, △) and CS71 (■, □) were prepared and assayed as described previously (5, 6, 29). The transport buffer was 10 mM bistrispropane–150 mM choline chloride–1 mM KCl (pH 7). Cells were incubated with 1 mM glucose in either the presence (closed symbols) or absence (open symbols) of 1 mM NaCl. Glucose was omitted from the assay of TMG transport to avoid inducer exclusion effects.

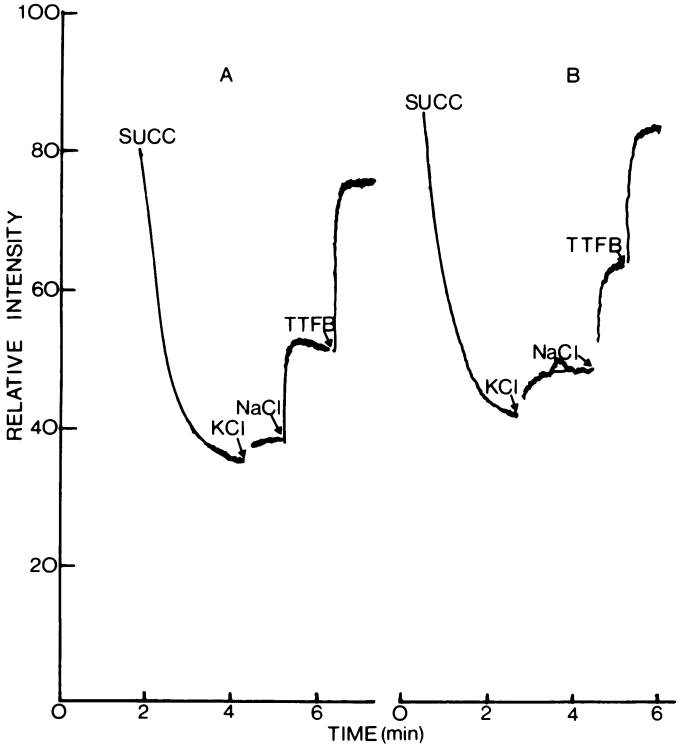

FIG. 4. Qualitative assessment of Na^+/H^+ antiport activity in inverted *E. coli* membrane vesicles. Inverted membrane vesicles were prepared as described in the text and were assayed for antiport activity by the Na^+-dependent reversal of quenching of ACMA fluorescence. Respiration of succinate (SUCC) was used to quench the fluorescence of ACMA. Addition of an uncoupler, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB), was used to demonstrate the complete reversibility of the fluorescence quenching.

L-glutamate analogs (D-glutamate and DL-α-methylglutamate) but did not affect the sensitivity to toxic analogs of L-proline and D-alanine (Table 2). The resistance of strain DZ3 (*phs*) to L-glutamate analogs was comparable to that of a strain (HfrC) which does not express the Na^+-linked L-glutamate permease.

In view of the above results we tried to assay the Na^+/H^+ antiport. Inverted membrane vesicles have been frequently used to assay antiport activity (B. P. Rosen, Methods Enzymol., in press). When inverted membrane vesicles were assayed for Na^+/H^+ antiport activity, no difference was found between vesicles prepared from DZ3 and from CS71 (Fig. 4). Thus, addition of Na^+ reversed fluorescence quenching to the same extent and with similar kinetics. Similar results have been observed by other (Rosen, in press; G. Leblanc, personal communication). However, it is difficult to quantitate such measurements. We therefore determined K^+-Na^+ exchange in whole cells to obtain a quantitative indication of the antiport activity. Experiments were carried out at both pH 7.5 and pH 8.7, since at the latter pH value growth is thought to be impaired due to reduced antiport activity (28, 29). We have previously established that the rate of K^+ uptake into K^+-depleted cells is dependent upon the presence of a mobile cation inside the cell (3). If Na^+ extrusion capacity is impaired in *phs* strains the rate of potassium uptake into Na^+-loaded cells would be simi-

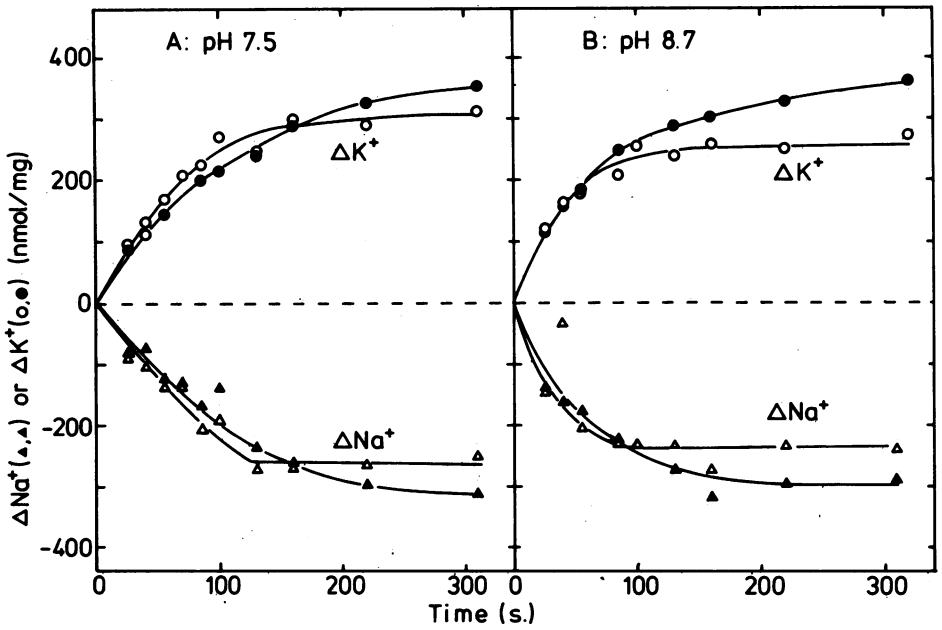

FIG. 5. K^+-Na^+ exchange of Na^+-loaded cells. EDTA-treated cells (3, 4) were washed twice with and finally suspended in either 200 mM Na^+-HEPES buffer (pH 7.5) (90 mM Na^+) or 150 mM Na^+-tris(hydroxymethyl)methylaminopropanesulfonic acid buffer (pH 8.7) (100 mM Na^+). These cells were preincubated with glucose (10 mM) for 20 min at 30°C at a concentration of 0.5 mg (dry weight) of cells per ml. Potassium uptake and sodium extrusion were initiated by the addition of KCl (3 mM) at zero time, and the Na^+ and K^+ content of the cells was assayed as a function of time. At zero time the cells contained 15 (strain CS71) and 25 (strain DZ3) nmol of K^+ per mg (dry weight) and 575 and 800 nmol of Na^+ per mg (dry weight) (strain DZ3) and 450 and 625 nmol of Na^+ per mg (dry weight) (strain CS71) at pH 7.5 and 8.7, respectively. Symbols: △, ▲, Na^+ extrusion; ○, ●, K^+ uptake; closed symbols, strain DZ3; open symbols, strain CS71.

larly reduced. No difference in the rates of K^+ uptake and Na^+ extrusion was observed between DZ3 and CS71 at either pH (Fig. 5). The transport of these two cations occurred with an approximate stoichiometry of 0.7 Na^+:1 K^+ (3, 25), and the minimum estimate of the velocity of the Na^+/H^+ antiport in both strains was 200 nmol of Na^+ expelled per min per mg (dry weight) of cells. This far exceeds the requirement for amino acid and sugar transport (Fig. 3) and suggests that there is no significant antiport defect in the *phs* strain.

DISCUSSION

The data presented here indicate that the metabolic defects caused by the *phs* mutation are not exerted via impairment of the Na^+/H^+ antiport function. Several strands of evidence lead to this conclusion. First, growth on melibiose is impaired independent of whether Na^+ is the coupling ion for α-galactoside transport. It could be argued that this reflects the absence of an Na^+-coupling subunit which is essential to the transport system even in the absence of Na^+ (30). However, substitution of the *lacY* gene product as the route of substrate entry does not relieve the melibiose growth defect. Since this system is not Na^+ coupled the absence of the Na^+/H^+ antiport or of a putative Na^+-coupling subunit should not interfere with melibiose entry via this route. These experiments suggest that the expression of both *melA* and *melB* genes is impaired by the *phs* mutation.

Second, at least two metabolic systems which are not coupled to Na^+, those for arabinose utilization and cysteine biosynthesis, are reduced in activity by the *phs* mutation. Cysteine synthesis is reduced due to a sulfate transport defect, and the activities of both of the arabinose transport systems are affected by the *phs* mutation (Tables 2 and 4). Sulfate uptake occurs via a binding protein-dependent system (9, 12, 17), and arabinose can be transported either via an H^+-linked system (*araE* [7, 13]) or via a binding protein-dependent system (*araFG* [7, 13]). The activity of all three systems is affected by the *phs* mutation. Further, we have previously shown that the transcription of the *araBAD* operon, which forms part of the arabinose regulon under the control of the AraC protein, is very much reduced in *phs* mutants (22).

Third, L-proline transport which is Na^+ dependent (6, 6a, 26, 27) is unimpaired in the *phs* mutant. Indeed, the activity of this system can be poised to exceed that of the L-glutamate and melibiose systems, indicating that an Na^+ current in excess of that required for the latter can be sustained by the cells (Fig. 3). Finally, we could find no evidence for a decreased capacity to extrude Na^+ in the *phs* mutant (Fig. 4 and 5). Thus, the evidence suggests that a defect in the Na^+/H^+ antiport is not responsible for the phenotype of the *phs* mutant. Rather, we propose that the phenotype arises from defective transcription of several operons. In this light it can be seen that transport defects arise due to reduced synthesis of the carrier proteins rather than defective energy coupling. In particular, the observation that L-glutamate efflux from membrane vesicles of the *phs* mutant is not stimulated by Na^+, which led to the proposal of an Na^+-coupling subunit (30), can be seen to be due to the failure to synthesize the Na^+-coupled system.

We have proposed that the phenotype of the *phs* mutation is not mediated through the action of the Na^+/H^+ antiport. Since in *E. coli* this mutation has been central to establishing a role for Na^+ in pH regulation at alkaline pH it is necessary to comment on the status of this hypothesis in the light of our observations (22, 28, 29). There are two possible explanations for the pH sensitivity reported for the *phs* mutant. In

the first the level of transcription of the gene for some enzyme or transport system is impaired to a degree which does not affect growth at neutral pH but which is insufficient to support growth at alkaline pH (clearly the gene for an antiport could be such a system [31; Rosen, in press]). Alternatively, growth at alkaline pH may require the synthesis of new proteins, and the transcription of these genes is impaired in the *phs* mutant. At this time we cannot discriminate between these two possibilities.

In conclusion, we showed that the phenotypic changes caused by the *phs* mutation (Mel^-, Glt^-, Cym auxotrophy, and slow growth on arabinose) are not exerted via a defect in the Na^+/H^+ antiport. The phenotype may be more extensive than has so far been uncovered since only those defects which are obvious have been studied. The relationship between gene expression and the cell phenotype is a complex one (11), and further studies are necessary to determine the extent of the pleiotropy of the *phs* mutation. However, as a result of our studies on the phenotype of the original mutant it may be possible to use positive selection to isolate further mutants of the same class. The *phs* mutation was isolated by penicillin enrichment during growth on melibiose and L-glutamate (31). Resistance to chromate, arabinose in *araD* strains, or DL-α-methylglutamate obtained by localized mutagenesis of the *rpoA* locus might provide the basis for isolation of additional mutations affecting the α-subunit of RNA polymerase.

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