The Nature of the Link Between Potassium Transport and Phosphate Transport in *Escherichia coli*

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A series of mutants of *Escherichia coli*, combining defects in either of the two phosphate transport systems with defects in one or more of the potassium transport systems, was used to study the nature of the previously observed obligatory requirement for each one of these ions in the transport of the other. The results show that no pair of systems is obligatorily linked, and that either ion can be transported by any one of its systems, provided that a means of entry for the other ion is available. Furthermore, in the total absence of P_i , K^+ entry accompanies the transport of other anions, such as aspartate, glutamate, *sn*-glycero-3-phosphate and glucose 6-phosphate. The results indicate that P_i and the other anions enter by symport with protons, and that a simultaneous K^+/H^+ exchange, which would serve to maintain the intracellular pH, is responsible for the observed K^+ 'symport' with these anions.

Inorganic phosphate (P_i) is transported into *Escherichia coli* K12 by two systems, designated Pit and Pst (Willsky *et al.*, 1973; Rosenberg *et al.*, 1977). These are energized by the protonmotive force and by 'phosphate-bond' energy respectively (Rosenberg *et al.*, 1977, 1978). As reported previously (Russell & Rosenberg, 1979), the uptake of P_i through both systems has an obligatory requirement for the presence of at least $50\mu M$ - K^+ in the external medium, and maximal rates of P_i uptake are not reached until the K^+ concentration is 1.0 mM. Protons also move into the cell in response to P_i uptake through both systems. Quantitative relationships have been shown to exist between the initial rates of uptake of K^+ and P_i , and of H^+ and P_i ; the value of these ratios depends upon the external pH.

A consideration of the possible nature of the link between K^+ and P_i uptakes (Russell & Rosenberg, 1979), advanced two alternative proposals: the first involved the possibility that a P_i carrier and a K^+ carrier may be associated in the membrane in a manner such that their transport activities are stringently linked. Alternatively, the uptakes of K^+ and P_i could be linked via the proton circulation, where the protons symported with P_i are promptly exchanged for K^+ , resulting in minimal disturbance of the cell pH and an overall electroneutral process.

Similarly, the stimulation of P_i uptake in *Streptococcus faecalis* by K^+ (Harold *et al.*, 1965) is now

Abbreviations used: Pit, P_i transport; Pst, phosphate-specific transport.

considered to be due to a K^+_{in}/H^+_{out} exchange process that maintains the intracellular pH that would otherwise be lowered by the symport process of $H^+/H_2PO_4^-$ uptake (Harold & Spitz, 1975).

In the present work we use a series of K^+ transport mutants carrying *pit* or *phoT* mutations to demonstrate that there is no stringent association of either of the two P_i transport systems with any of the K^+ transport systems. Further evidence is provided by the observed uptake of K^+ , in the absence of P_i , during the uptake of glutamate, aspartate, *sn*-glycero-3-phosphate and glucose 6-phosphate.

Materials and Methods

Chemicals

All chemicals used were of the highest purity available commercially. α -Glycerophosphate (DL-glycero-3-phosphate) and β -glycerophosphate (glycero-2-phosphate) were from Sigma.

Solutions of phosphate esters used as growth substrates or in uptake studies were freshly prepared and were sterilized by filtration to avoid hydrolysis.

Radioisotopes

^{42}KCl (0.5–1.0 GBq/mmol) and carrier-free [^{32}P]-orthophosphate (45 TBq/mmol) were from the Australian Atomic Energy Commission, Lucas Heights, NSW, Australia. L-[U- ^{14}C]Glutamic acid (8 GBq/mmol) was purchased from The Radio-

chemical Centre, Amersham, Bucks., U.K. The labelled compounds were diluted appropriately (with or without carrier) before use.

Bacterial strains

The sources and derivation of the bacterial strains used, together with the relevant genetic information, are given in Table 1. Transductions of *pit* and *phoT* into the desired recipients were carried out using the method of Pittard (1965) with phage P1kc grown on strain C101a. Strains carrying *pit* were selected for resistance to arsenate on minimal medium plates containing 1 mM-P_i and 30 mM-arsenate. The use of arsenate to select P_i transport mutants is discussed by Rosenberg *et al.* (1977). Arsenate-resistant strains were further checked for the lack of constitutive alkaline phosphatase (Bracha & Yagil, 1969) and the effect of starvation on the P_i uptake rate (Medveczky & Rosenberg, 1971; Rosenberg *et al.*, 1977). Strains carrying *phoT* (and thus constitutive

for alkaline phosphatase) were selected for their ability to grow on minimal medium containing 100 mM-P_i with 10 mM-glycero-2-phosphate as the sole source of carbon (Torriani & Rothman, 1961). The ability of these strains to carry out exchange of internal for external P_i was also checked.

Media and buffers

All pH values are given at 37°C.

Cells were grown on a medium that contained K₂HPO₄ (61 mM), NaH₂PO₄ (39 mM), (NH₄)₂SO₄ (15 mM) and MgSO₄ (1 mM); the pH was adjusted to 6.8 with HCl. This medium was supplemented with carbon sources (usually 20 mM), and thiamin (3 µM). Arginine (1 mM), and 2,3-dihydroxybenzoate (10 µM) were added where required. Growth supplements were added as sterile solutions to the sterilized mineral salts base.

Strains carrying lesions in both the *Pit* and *Pst* systems were grown on 56LP medium (Sprague *et*

Table 1. *Strains of Escherichia coli* used
C.G.S.C. refers to the *E. coli* Genetic Stock Center (Yale University School of Medicine, New Haven, CT, U.S.A.)

Strain	Relevant genetic loci	Source and other information
AN710	<i>phoT101, argH, entA</i>	Rosenberg <i>et al.</i> , 1977
K10	<i>pit</i>	B. Bachmann, C.G.S.C. no. 5023 (derivative of Hfr Cavalli, Echols <i>et al.</i> , 1961)
AN259	<i>argH, entA</i>	G. Cox (Butlin <i>et al.</i> , 1973).
AN1088	<i>pit, argH, entA</i>	Isolated after transduction with strain K10 as donor and strain AN259 as recipient, followed by selection for arsenate resistance.
AN248	<i>ilvC, argH, entA</i>	G. Cox (Butlin <i>et al.</i> , 1973).
C101a	<i>pit, phoT101</i>	A. Garen (Garen & Otsuji, 1964).
C90	<i>phoT101</i>	A. Garen (Garen & Otsuji, 1964).
AN1574	<i>pit, ilvC, argH, entA</i>	R. Janki. Isolated after transduction with strain C101a as donor and strain AN248 as recipient, followed by selection for arsenate resistance.
AN1575	<i>pit, phoT101, argH, entA</i>	R. Janki. Isolated after transduction with strain C90 as donor and strain AN1574 as recipient, followed by selection on DL-glycero-3-phosphate and arsenate.
10B5	<i>pit-1, pst-2, glpR2, glpD3, phoA8, relA1, tonA22</i>	B. Bachmann, C.G.S.C. no. 5506 (Sprague <i>et al.</i> , 1975).
E15	<i>pit, phoA</i>	M. Schlesinger (derivative of K10 Cavalli, Fan <i>et al.</i> , 1966).
Frag5	<i>kdpABC5</i>	B. Bachmann, C.G.S.C. no. 4832 (Epstein & Davies, 1970).
2K133	<i>thi-1, kdpABC5, trkA133</i>	B. Bachmann, C.G.S.C. no. 4836 (Epstein & Kim, 1971).
2K142	<i>thi-1, kdpABC5, trkE142</i>	B. Bachmann, C.G.S.C. no. 4837 (Epstein & Kim, 1971).
2K401	<i>thi-1, kdpABC5, trkD, trkA401</i>	B. Bachmann, C.G.S.C. no. 4838 (Epstein & Kim, 1971).
TAD110	<i>thi-1, trkA, trkD</i>	K. Altendorf (TAD110 is probably identical with TK510, Rhoads & Epstein, 1978).
AN1576	<i>kdpABC5, pit</i>	Isolated after transduction with strain C101a as donor and strain Frag 5 as recipient, followed by selection for arsenate resistance.
AN1577	<i>kdpABC5, phoT101</i>	Isolated after transduction with strain C101a as donor and strain Frag 5 as recipient, followed by selection on glycero-2-phosphate.
AN1578	<i>thi-1, trkA, trkD, pit</i>	Derived as for AN1576 with strain TAD110 as recipient.
AN1579	<i>thi-1, trkA, trkD, phoT101</i>	Derived as for AN1577 with strain TAD110 as recipient.
AN1580	<i>thi-1, kdpABC5, trkD, trkA401, pit</i>	Derived as for AN1576 with strain 2K401 as recipient.
AN1581	<i>thi-1, kdpABC5, trkD, trkA401, phoT101</i>	Derived as for AN1577 with strain 2K401 as recipient.

al., 1975) containing Tris (100mM), KCl (10mM), (NH₄)₂SO₄ (15mM), MgCl₂ (10mM), NaH₂PO₄ (1mM), and casamino acids 0.1% (w/v); the pH was adjusted to 6.8 with HCl.

Phosphate-free medium used to deplete the cells of P_i and for P_i uptake studies, and the wash solution used for P_i uptake studies have been described previously (Rosenberg *et al.*, 1977).

The potassium-free buffer used for K⁺ uptake studies contained choline chloride (20mM), triethanolamine citrate (50mM with respect to triethanolamine) and MgSO₄ (1mM); the pH was adjusted as required with citric acid or LiOH. This medium was also used in place of the wash solution in ⁴²K uptake studies.

The lightly buffered medium used to suspend cells for H⁺ uptake studies has been described previously (Russell & Rosenberg, 1979).

Cell growth and preparation

The preparation of cells for uptake studies has been described elsewhere (Rosenberg *et al.*, 1975, 1977). The preparation of cells for studies using the ion-sensitive electrodes, and the measurement of the concurrent movements of labelled substrates with K⁺ and H⁺ were as described by Russell & Rosenberg (1979). A 4ml vol. of the bacterial suspension was used in all experiments. The K⁺-sensitive electrode used showed selectivity constants (K_{MN}) 10⁻⁴ and 10⁻⁵ for Na⁺ and Li⁺, respectively (Rosenberg, 1979).

When required, cells were depleted of internal K⁺ and P_i by shaking suspensions of washed cells at an A_{660} of 0.3 in potassium-free medium for 1 h in the absence of an energy source and in the presence of 1 mM-2,4-dinitrophenol.

Results

Uptake of phosphate and potassium in a series of K⁺/P_i transport mutants

We have previously shown that a series of mutants of *E. coli* defective in their ability to take up K⁺ were also affected to a similar degree in their ability to transport P_i (Russell & Rosenberg, 1979). Mutations in the *kdp* genes affect a repressible high-affinity system for K⁺ uptake while the *trkA* and *trkD* mutations each affect a constitutive, low-affinity K⁺ uptake system with a high and low velocity, respectively. In each of these K⁺ transport mutants, we have now eliminated one or the other P_i transport system to investigate whether individual K⁺ transport systems showed a preference for a particular P_i transport system. The rates of P_i uptake and K⁺ uptake in the strains lacking a P_i transport system were lower than in the parent strains carrying both P_i transport systems (Table 2).

The uptake characteristics of several different

Table 2. *Uptake of P_i and K⁺ by a series of K⁺/P_i transport mutants*

Uptake rates were measured at pH 6.5 with 20mM-glucose as energy source. The uptake of K⁺ was measured using the K⁺-sensitive electrode, and the simultaneous uptake of ³²P was measured following an addition of 500 nmol of [³²P]P_i (500 kBq/μmol) as detailed in the Materials and Methods section.

Strain	(Genotype)	Uptake (nmol/min per mg dry wt.) of	
		P _i	K ⁺
Frag 5	(<i>kdp</i>)	27.1	41.6
AN1576	(<i>kdp, pit</i>)	10.3	26.5
AN1577	(<i>kdp, phoT</i>)	9.1	25.0
TAD110	(<i>kdp⁺, trkA, trkD</i>)	16.8	32.0
AN1578	(<i>kdp⁺, trkA, trkD, pit</i>)	13.2	14.0
AN1579	(<i>kdp⁺, trkA, trkD, phoT</i>)	12.8	14.5
2K401	(<i>kdp, trkA, trkD</i>)	8.6	7.0
AN1580	(<i>kdp, trkA, trkD, pit</i>)	4.6	4.1
AN1581	(<i>kdp, trkA, trkD, phoT</i>)	2.6	4.0

Table 3. *P_i and K⁺ uptake by P_i-transport mutants*

The cells were grown on medium 56LP (see the Materials and Methods section) with DL-glycero-3-phosphate as a source of P_i and 20mM-glucose as carbon source. Glucose was also used as the energy source for ion uptakes, which were measured simultaneously at pH 6.5 (see the Materials and Methods section for details). For comparison, the ion uptakes measured in glucose-grown cells of strains AN710 and AN1088 are also included.

Strain	(Genotype)	Uptake (nmol/min per mg dry wt.) of	
		P _i	K ⁺
10B5	(<i>pit, pst</i>)	4.0	2.6
AN1575	(<i>pit, phoT</i>)	4.4	7.0
AN710	(<i>phoT</i>)	30.0	57.0
AN1088	(<i>pit</i>)	42.0	84.0

mutants lacking both P_i transport systems, but with all the K⁺ transport systems intact, were also examined (Table 3). These mutants required DL-glycero-3-phosphate as a source of phosphate for growth. This results in the induction of the GlpT system, which is probably responsible for the low rates of P_i uptake in such cells (Lin, 1976). Such cells show a correspondingly low rate of K⁺ uptake in the presence of P_i.

K⁺ movements with glutamate and aspartate

The uptake of glutamate and aspartate has been shown to have a K⁺ requirement in *E. coli* (Halpern

et al., 1973), *S. faecalis* (Harold & Baarda, 1967; Gale, 1971) and *Staphylococcus aureus* (Davies *et al.*, 1953; Gale & Llewellyn, 1972). The uptake of glutamate is also stimulated by Na^+ (Frank & Hopkins, 1969). These anions could be expected to enter the cell in symport with protons in a similar fashion to P_i , and their effect on K^+ transport was therefore investigated.

The addition of either of these anions to equilibrated cell suspensions in the presence of excess KCl and an energy source resulted in the uptake of K^+ (Fig. 1). A subsequent addition of P_i gave rise to an additional uptake of K^+ . The rate of uptake of K^+ in response to the addition of glutamate was less than that due to P_i addition, and reflects the difference in the rates of uptake of the two anions (see Table 5 below). The differences in the amounts of K^+ taken up may be due to the different internal pool sizes for P_i and glutamate.

At pH 6.5, the ratio of uptake of K^+ /uptake of glutamate, as determined by the procedure described in the Materials and Methods section, was approximately 2.0. At this pH, the two carboxyl groups of glutamic acid are fully dissociated. In the presence of excess KCl, protons are also taken up with glutamate (Fig. 2). Halpern & Even-Shoshan (1967) had reported that growth in the presence of glutamate enhanced the capacity of bacteria to take up glutamate. However, in these experiments, no significant differences were found between cells grown in the presence of glutamate (as nitrogen source) and cells grown on glucose with $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source (result not shown).

Fig. 2. Uptake of H^+ in response to the addition of glutamate

Cells (strain AN710) were prepared and suspended in the lightly buffered medium at pH 6.5 as detailed in the Materials and Methods section. Glucose ($40\mu\text{M}$; Glc) was added, followed by $1\mu\text{mol}$ of sodium glutamate (Glu).

Fig. 1. Movement of K^+ in response to glutamate and aspartate

Cells (strain AN710) were prepared in K^+ -free buffer at pH 6.5 as described in Methods. After the addition of 500nmol of KCl, (K^+ standard), $1\mu\text{mol}$ of sodium aspartate (trace a; Asp) or $1\mu\text{mol}$ of sodium glutamate (trace b; Glu) was added. At an appropriate time interval, this was followed by the addition of 500nmol of P_i .

Dependence of [¹⁴C]glutamate uptake on the presence of potassium ions

The uptake of [¹⁴C]glutamate was studied in cells that had been treated with 2,4-dinitrophenol, and washed and resuspended in potassium-free buffer (Table 4). Maximal rates of uptake of [¹⁴C]glutamate were achieved in the complete absence of P_i, but the presence of K⁺ (1 mM) was required. The uptake of [¹⁴C]glutamate by a series of K⁺ transport mutants parallels their rate of uptake of P_i and reflects their impaired ability to transport K⁺ (Table 5).

Potassium movements with the uptake of certain phosphate esters

We examined the possibility that net K⁺ movement into the cell would follow the entry of anionic phosphate esters, such as *sn*-glycero-3-phosphate and glucose 6-phosphate. With these esters, spurious

effects might be observed if P_i were released by the action of the periplasmic alkaline phosphatase. This would apply particularly in the case of P_i-starved cells where the enzyme is fully induced. To avoid this, we used the phosphatase-negative strain E15 (*pit*, *phoA*). In suitably induced cells, the addition of DL-glycero-3-phosphate resulted in the uptake of K⁺ (Fig. 3a). Only a small response was elicited with glycero-2-phosphate and that was due to a 5% contamination with *sn*-glycero-3-phosphate. Similarly, glucose 6-phosphate also prompted K⁺ uptake in appropriately induced cells (Fig. 3b). The K⁺ uptake in response to the addition of glucose 6-phosphate was inhibited by arsenate (Figure 3c); the *sn*-glycero-3-phosphate-dependent uptake was also arsenate-sensitive (results not shown). Such results can be taken as an indication that the Uhp and GlpT transport systems respectively were induced. Subsequent additions of P_i after addition of DL-glycero-3-phosphate (Fig. 3a) and glucose 6-phosphate (results not shown) gave rise to a further uptake of K⁺ ions. This observation indicates that the internal P_i pool had not yet reached a maximum, and confirms the observation of Lin (1976) that *sn*-glycero-3-phosphate enters the metabolic pathways without hydrolysis.

A *phoA*, *pit*, *pst* mutant (10B5) which lacked both P_i transport systems, as well as alkaline phosphatase, and consequently required DL-glycero-3-phosphate for growth, showed negligible stimulation of K⁺ uptake by P_i, as expected, but DL-glycero-3-phosphate supported a substantial influx of K⁺ into the cells (Fig. 4).

Potassium exchange during 'steady-state' conditions

The K⁺/P_i uptake ratios obtained in the presence of excess KCl apply only to initial rates; the net uptake of K⁺ declines after 1–2 min, while P_i entry continues, although at a slower rate (Russell & Rosenberg, 1979). The use of ⁴²K in combination with the K⁺-sensitive electrode enabled a study to be made of the K⁺ movements across the cell membrane during this latter phase.

When an appropriate addition of ⁴²KCl was made to cells suspended in buffer containing 1 mM-K⁺ and an energy source, little entry of radioactivity was observed until the addition of P_i. An immediate and rapid increase in the rate of uptake of ⁴²K ensued, paralleled by a net uptake of K⁺ as shown by the K⁺-sensitive electrode (Fig. 5). At the point where the latter showed no further net uptake of K⁺, the rate of entry of ⁴²K had also virtually ceased. To ensure that this observed lack of exchange was not due to the fact that ⁴²K had equilibrated between the internal and external K⁺ pools, the experiment was repeated, but ⁴²K was now added at the point where net uptake of K⁺ had ceased (Fig. 6). Slow but significant entry of radioactivity into the cells could

Table 4. K⁺-ion dependence of [¹⁴C]glutamate uptake

Uptakes were measured in strain AN710 at pH 6.5 and with 20 mM-glucose as energy source. Cells were treated with 2,4-dinitrophenol as described in the Materials and Methods section and suspended in potassium-free buffer. All experiments were carried out in the presence of 1 mM-Na⁺. Uptake was initiated by the addition of 10⁵ μM-sodium [¹⁴C]-glutamate (750 kBq/μmol).

Condition	[¹⁴ C]Glutamate uptake (nmol/min per mg dry wt.)
No K ⁺ , no P _i	2.5
No K ⁺ , 50 μM-P _i	3.1
1 mM-K ⁺ , no P _i	8.6
1 mM-K ⁺ , 50 μM-P _i	8.8

Table 5. Uptake of [¹⁴C]glutamate and [³²P]P_i by K⁺ transport mutants

Uptake rates were measured at pH 6.5 and with 20 mM-glucose as energy source. Uptake was initiated by the addition of 10⁵ μM-[¹⁴C]glutamate (750 kBq/μmol), or 50 μM-[³²P]P_i (500 kBq/μmol). The uptakes of [¹⁴C]glutamate were carried out in the presence of 1 mM-Na⁺.

Strain	(Genotype)	Uptake (nmol/min per mg dry wt.) of	
		[¹⁴ C]Glutamate	[³² P]P _i
Frag 5	(<i>kdp</i>)	13.3	23.9
TAD110	(<i>trkA</i> , <i>trkD</i>)	9.0	20.2
2K133	(<i>kdp</i> , <i>trkA</i>)	7.7	14.4
2K142	(<i>kdp</i> , <i>trkE</i>)	3.7	13.3
2K401	(<i>kdp</i> , <i>trkA</i> , <i>trkD</i>)	3.4	3.4

Fig. 3. Uptake of K^+ by a *phoA* strain in response to the addition of glycerol-2-phosphate, DL-glycerol-3-phosphate and glucose 6-phosphate

Cells of strain E15 (*pit, phoA*) were grown overnight in the presence of 20 mM-DL-glycerol-3-phosphate (for trace *a*) or 20 mM-glucose 6-phosphate (for traces *b* and *c*) and with 20 mM-succinate as carbon source. They were prepared for uptake experiments in K^+ -free buffer (pH 6.5) as detailed in the Materials and Methods section. Glycerol-2-phosphate and DL-glycerol-3-phosphate (1 μ mol) were added where indicated (Gro-2P and Gro-3P, respectively), followed by 500 nmol of P_i (trace *a*). Glucose 6-phosphate (1 μ mol) was added where indicated (Glc-6P, traces *b* and *c*). The addition of glucose 6-phosphate was followed by the addition of arsenate (As_i) (500 nmol) (trace *c*). At the point corresponding to the bottom of the trace, the K^+ concentration was 0.63 mM.

Fig. 4. Uptake of K^+ in a *pit, pst* strain in response to the addition of DL-glycerol-3-phosphate

Cells of strain 10B5 (*pit, pst, phoA*) were grown overnight on 56LP medium with 20 mM-succinate as carbon source and 20 mM-DL-glycerol-3-phosphate as P_i source. The cells were prepared in K^+ -free buffer (pH 6.5) as detailed in the Materials and Methods section. P_i (500 nmol) was added as indicated, followed by DL-glycerol-3-phosphate (1 μ mol; Gro-3P).

Fig. 5. Uptake of ^{42}K (●) in response to P_i
Cells (strain AN710) were prepared in K^+ -free buffer (pH 6.5) as described in the Materials and Methods section. The internal standard of 500 nmol of K^+ was added as ^{42}KCl (240 kBq/mmol), followed by 500 nmol of P_i . Samples (100 μ l) of the cell suspension were taken at 30 s intervals, filtered washed and counted for ^{42}K radioactivity (●) as described by Russell & Rosenberg (1979).

be measured, indicating some exchange of K^+ across the membrane. This result was obtained for both strains AN710 and AN1088, grown on both lactate and glucose.

Fig. 6. Potassium exchange during 'steady-state'. A standard K⁺-uptake experiment was carried out at pH 6.5 with strain AN710, as detailed in the Materials and Methods section. At the point of equilibration of net K⁺ uptake, 250 nmol of ⁴²KCl (240 kBq/mmol) was added. At this point of the trace, the recorder pen was reset to compensate for the resulting change in K⁺ concentration. Samples (100 μ l) were withdrawn at 30 s intervals, filtered, washed and counted for ⁴²K radioactivity. ●, ⁴²K uptake, calculated on the same scale as net K⁺ movement (—), using appropriate standards.

Movements of potassium, protons and P_i following a sudden increase in osmolarity

Potassium ion movements in response to a sudden increase in osmolarity ('upshock') have been studied previously by other workers (Epstein & Schultz, 1965; Rhoads & Epstein, 1978; Durgaryan & Martirosov, 1978a,b,c) under conditions significantly different from those generally used in this study. In all these cases, P_i was included in the media in concentrations from 50–250 mM. The movements of P_i and H⁺ with the uptake of K⁺ following 'upshock' were of particular interest.

A rapid uptake of K⁺ was observed in response to an increase in the osmolarity of the medium (Fig. 7). The uptake was most rapid (110 nmol of K⁺/min per mg dry wt. at an external K⁺ concentration of 1 mM) in the first 30 s following 'upshock', and was completed within 2–3 min. Within this period some 80 nmol of K⁺/mg dry wt. of cells was taken up. This represented 12.5% of the available K⁺ in the electrode vessel. It would be expected from previous work (Epstein & Schultz, 1965; Rhoads & Epstein, 1978) that the amount of K⁺ taken up, and the rate of this uptake, would vary with the external K⁺ concentration. The rate of K⁺ uptake obtained is

Fig. 7. K⁺ and H⁺ movements in response to 'upshock' in the presence and absence of P_i.

Glucose-grown cells of strain AN710 were prepared in potassium-free buffer (pH 6.5) as described in the Materials and Methods section. Portions (4 ml) of the cell suspension were placed in the electrode vessel and supplemented with 1 mM-K⁺ and 5 mM-glucose. When net movements of K⁺ and H⁺ ceased (white arrow) 1 ml of 1 M-sucrose (in the same buffer as that of the cell suspension) was added. P_i was either present from the start at 0.25 mM (traces a and b) or was added 3 min after 'upshock' (black arrow, 500 nmol, traces c and d). The movements of K⁺ (traces a and c) and H⁺ (traces b and d) were monitored with specific electrodes. The traces have been copied from the original recordings and arranged in time synchrony to compensate for the slight time shift on the original recording caused by the dual pen arrangement.

comparable with that found for the TrkA system by Rhoads & Epstein (1978) (77 nmol of K⁺/min per mg dry wt.), and with the normal rate of P_i-dependent K⁺ uptake obtained in our studies (about 50 nmol of K⁺/min per mg dry wt.)

When 'upshock' was carried out in the absence of P_i, K⁺ uptake still occurred, but H⁺ efflux was not observed (Fig. 7d). Upon addition of P_i, proton efflux, as well as a further K⁺ uptake, took place. The movement of H⁺ has been postulated to be responsible for the preservation of electroneutrality during K⁺ uptake in response to 'upshock' (Epstein & Schultz, 1965; Durgaryan & Martirosov, 1978c).

The K⁺ movement in response to 'upshock' was completely inhibited by 100 μ M-arsenate (result not shown). The apparent small uptake of protons that was always measured in the initial 30 s after

'upshock' (Fig. 7) occurred in the absence of cells (results not shown) and is thus a physical phenomenon of the pH electrode.

Discussion

Results presented previously have demonstrated that P_i is transported adequately by *E. coli* strains provided that at least one K^+ transport system is functional (Russell & Rosenberg, 1979). The present experiments with strains lacking K^+ transport systems and in which *pit* or *phoT* mutations have been inserted, indicate that the K^+ transport systems exhibit a similar lack of selectivity for the P_i transport system that provides the flux of the anion. These results lead to the conclusion that the combination of any of the two P_i transport systems with any of the K^+ transport systems assures adequate entry of both ions.

We have proposed (Russell & Rosenberg, 1979) that the apparent symport of K^+ with P_i into *E. coli* could be the net result of a process in which protons, symported into the cell with anions such as P_i , efflux in exchange for K^+ via a K^+/H^+ antiport system. Indeed, such a proposal was considered by Gale & Llewellyn (1972) to account for the uptake of both K^+ and H^+ with aspartate and glutamate in *S. aureus*. The uptake of these anions by *S. faecalis* is also accompanied by the movement of K^+ (Harold *et al.*, 1970), but no proton uptake has been observed (Gale & Llewellyn, 1972).

In *E. coli* the situation is somewhat more complicated, as the nature of the K^+ requirement for glutamate uptake (Davies *et al.*, 1953) is not clear, and it is possible that in *E. coli* this process requires a high internal K^+ concentration (Halpern *et al.*, 1973). Our present results show that the transport of glutamate has a requirement for external K^+ , at least in cells whose internal K^+ has been depleted, and that protons move into the cell with glutamate. It appears that glutamate, like P_i (Russell & Rosenberg, 1979), is taken up with an overall positive charge. We did not measure proton movements with *sn*-glycero-3-phosphate and glucose 6-phosphate, but Ramos & Kaback (1977) demonstrated that the active transport of glucose 6-phosphate into *E. coli* membrane vesicles occurred in response to the ΔpH at pH 5.5, and that protons were symported with this substrate. (It should be noted that DL-glycero-3-phosphate was used in all experiments. Statements referring to the movement of *sn*-glycero-3-phosphate rest on the unproven assumption that only this enantiomorph is taken up.)

Potassium ions accompany the movement of P_i into the cell only during the initial rapid phase of uptake (Russell & Rosenberg, 1979), which represents the time taken for the internal P_i pool to reach capacity (Medveczky & Rosenberg, 1971). The

suggested connection between K^+ movements and the filling of the P_i pool is further reinforced by the fact that K^+ movements are only observed in cells in which the P_i pool is depleted (Russell, 1980). Once the P_i pool is filled, P_i entry into the cell is limited by the rate of its esterification. Since this process results in a decrease of acid equivalents in the cytoplasm, continuing P_i uptake would no longer be associated with significant inward movement of K^+ . The ^{42}K experiments show that, at the point where the P_i pool is filled and net K^+ movement ceases, a slow exchange is taking place between internal and external K^+ . The significance of this exchange is not clear.

Our previous results (Russell & Rosenberg 1979) indicated that the uptake of K^+ showed an obligatory requirement for the presence of P_i , which was assumed to be unique since a number of other anions failed to show this effect. The present finding that the uptakes of aspartate, glutamate, *sn*-glycero-3-phosphate and glucose 6-phosphate also resulted in the concomitant uptake of K^+ , means that K^+ uptake can be independent of P_i . Furthermore, the rate and extent of uptake of K^+ following 'upshock' is not dependent upon the presence of P_i in the medium. Epstein & Schultz (1965) have shown that the intracellular P_i content does not increase after 'upshock'.

The proposal that the link between K^+ uptake and P_i uptake is an indirect one via the proton circulation is given good support by the experimental evidence presented in this paper. This interpretation of the observed cation movements with phosphate entry provides a better understanding of the variation in H^+/P_i and K^+/P_i ratios over the pH range 5.5–7.5 reported previously (Russell & Rosenberg, 1979). It should be stressed that the rates of proton entry used in our calculations represent the difference between an inward movement and an outward movement which is linked to K^+ entry. This latter movement is related to the external pH, in that it is faster at higher external pH values. This explains the observed low H^+/P_i and high K^+/P_i ratios at the more alkaline pH values.

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