Energy Coupling to K⁺ Uptake Via the Trk System in *Escherichia coli*: the Role of ATP

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The dual energy requirement (protonmotive force and ATP) of the *Escherichia coli* Trk potassium transport system has been investigated. Using inhibitors and *unc* mutants we show that Trk is not an ATPase but may be regulated by ATP. Possible mechanisms of energy coupling to Trk are discussed.

INTRODUCTION

The intracellular accumulation of K⁺ to high concentrations is ubiquitous in bacteria (Harold & Altendorf, 1974). This cation has roles in osmoregulation (Epstein & Schultz, 1965), in the activation of some enzymes (Suelter, 1970), in protein synthesis (Lubin & Ennis, 1964) and in the regulation of cytoplasmic pH (Plack & Rosen, 1970). The molecular mechanisms of K⁺ uptake into *Escherichia coli* are poorly understood, although genetic and biochemical studies have provided the foundation of an understanding (Helmer *et al.*, 1982). Thus there are known to be ten genetic loci coding for at least two separate K⁺ transport systems. The first of these, Kdp, is relatively well characterized as a K⁺-stimulated adenosinetriphosphatase, encoded by three structural genes *kdpABC* and a regulatory gene *kdpD* (Epstein *et al.*, 1978; Laimins *et al.*, 1978; Weiczorek & Altendorf, 1979). The second major system is Trk, which is encoded by six genetic loci, *trkA–E* and *G* (Helmer *et al.*, 1982). Of these genes, *trkA*, *trkE* and *trkG* appear to encode structural components of the transport system (Helmer & Epstein, 1981); the role of *trkD* is unclear. The role, if any, of *trkB* and *trkC* gene products in the uptake of K⁺ is unknown, but mutations in either lead to enhanced rates of K⁺ exit (Rhoads *et al.*, 1976). It seems possible that *trkB* and *trkC* may constitute one or more separate transport systems. A further K⁺ uptake system, TrkF, has been described but has not been genetically characterized. The energy coupling to the Trk system has only been partially resolved in that it appears to require both ATP and a protonmotive force (Rhoads & Epstein, 1978).

Our understanding of energy coupling to bacterial transport systems was greatly enhanced by the work on mutants deficient in adenosinetriphosphatase (*unc* mutants). These mutants were used to discriminate between those transport systems which require the generation of a protonmotive force and those which rely primarily on ATP (Berger & Heppel, 1974). Of the former class, proline and lactose transport systems are the archetypes, being energized by the electrochemical gradient of Na⁺ and H⁺, respectively (Stewart & Booth, 1983; West & Mitchell, 1973). The protonmotive force required to drive these two systems can be generated either by respiration or by hydrolysis of ATP by the ATPase. In *unc* mutants, therefore, these systems are only active in the presence of active respiration. The second type of system is energized directly either by ATP or by a metabolite derived from it (Berger & Heppel, 1974). The best examples of this type of system are the glutamine and histidine transport systems (Berger 1973; Rosen & Kashket, 1978). In an *unc* mutant these systems are only active when a glycolytic substrate is present.

Abbreviation: DCCD, dicyclohexylcarbodiimide.

This framework was used to investigate the energy coupling to the two K^+ transport systems (Rhoads & Epstein, 1977). The Kdp system appeared straightforward and was dependent upon ATP and not the protonmotive force. The Trk system on the other hand exhibited a more complex pattern of behaviour. In an *unc* mutant the Trk system was sensitive to uncouplers but was not energized by a protonmotive force unless a supply of ATP was also available. In the same mutant anaerobiosis inhibited uptake despite there being a ready supply of ATP. Thus it was concluded that Trk was either an ATPase regulated by the protonmotive force or a protonmotive-force-dependent system regulated by ATP (Rhoads & Epstein, 1977). A further complexity was added when it was discovered that K^+ exchange via Trk was dependent on ATP but not on the protonmotive force (Rhoads & Epstein, 1978). It has been proposed that the major K^+ transport system in *Streptococcus faecalis* is driven by the protonmotive force and regulated by ATP (Bakker & Harold, 1980).

It has been suggested that at its maximum rate of uptake the K^+ transport system, Trk, if functioning as an ATPase, could account for 75% of the energy generated by glucose oxidation (Rhoads & Epstein, 1977). On the basis of this suggestion we have investigated the role of protonmotive force and of ATP in the Trk system. We have combined two approaches. Firstly we have used an *unc* mutant to limit the rate of synthesis of ATP; and secondly we have used the inhibitors iodoacetate and arsenate to inhibit ATP synthesis. Additionally we have used well-characterized K^+ transport mutants treated with the ATPase inhibitor dicyclohexylcarbodiimide (DCCD) to complement studies of the *unc* mutant. These studies demonstrate unequivocally that the Trk system is not energized directly by ATP but may be regulated by ATP or a related compound.

METHODS

Bacterial strains. *Escherichia coli* strains Frag 1 (*thi-1 rha-4 lacZ-82 gal-33*), TK2242 (*kdp trkA trkD thi-1 rha-4 lacZ-82 gal-33*) were obtained from Dr W. Epstein, University of Chicago, USA. Strain NR71 (*unc*) was isolated by Rosen *et al.* (1978) as a partial revertant of an *uncA* strain, NR70 (Rosen 1973). The partial revertant *E. coli* NR71 was neomycin-sensitive, ATPase-negative and had a lower proton permeability than the parent strain (Rosen *et al.*, 1978).

Growth conditions and media. Cells were grown overnight at 30 °C on glucose minimal medium; *E. coli* Frag 1 and TK2242 were grown on K20 medium at pH 7 (Epstein & Kim, 1971) which contained (g l⁻¹): K_2HPO_4 , 1.53; KH_2PO_4 , 0.6; Na_2HPO_4 , 13.1; NaH_2PO_4 , 2.87; $(NH_4)_2SO_4$, 1.05; $MgSO_4$, 0.1; trisodium citrate, 0.3; thiamin. HCl, 0.001; $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$, 0.0023; glucose, 4. *E. coli* NR71 was grown on medium E at pH 7 which contained (g l⁻¹): $NaH_2PO_4 \cdot 2H_2O$, 5.3; K_2HPO_4 , 11.2; $(NH_4)_2SO_4$, 2.64; $MgSO_4$, 0.074; glucose, 4; and $FeSO_4$, 278 µg l⁻¹; $CaCl_2$, 1.47 µg l⁻¹; $ZnSO_4$, 1.61 µg l⁻¹. For induction of the lactose permease, *E. coli* NR71 was grown in medium E with glycerol as the carbon source and 1 mM-IPTG.

After overnight growth the cells were diluted with fresh medium of identical composition and grown from an initial $OD_{650} = 0.2$ to $OD_{650} = 0.8$. For induction of the lactose permease the medium was supplemented during the growth phase with 5 mM-cAMP to maximize induction of the *lac* operon. For induction of the Kdp transport system, *E. coli* NR71 was grown on medium K0, which is identical to medium K20 above except that the potassium salts were replaced by their sodium equivalents and the medium was supplemented with 100 µM- K^+ . After overnight growth the cells were diluted into fresh K0 medium to which no K^+ was added and were then incubated until growth had ceased. Such cells were not treated with EDTA since they were already K^+ -depleted (Kroll & Booth, 1981). The cells were prepared for transport experiments by washing twice in 200 mM-HEPES/NaOH pH 7.5 and stored on ice as a concentrated cell suspension.

All cells were grown in conical flasks with a ratio of medium volume to flask volume of 2.5 in a New Brunswick orbital shaker operating at 200 r.p.m.

Assay of the Trk system. (i) *EDTA-treatment of cells.* Exponential phase cultures were harvested by centrifugation at 16000 g at 4 °C for 5 min, and washed and resuspended in 120 mM-Tris/HCl buffer pH 8.0 (Leive, 1968). The washed cells were incubated at 10–15 mg dry wt ml⁻¹ at 37 °C for 2 min and sodium EDTA pH 7.0 was added to a final concentration of 1 mM. After an incubation period at 37 °C (2 min for NR71; 10 min for Frag 1 and TK2242) the cells were diluted fivefold with 120 mM-Tris/HCl pH 8.0 and centrifuged at 5000 g for 5 min at room temperature. The cells were then washed twice by centrifugation and resuspended in 200 mM-HEPES/NaOH pH 7.5 containing 50 µg chloramphenicol ml⁻¹. The washed EDTA-treated cells were stored on ice for up to 5 h at a density of 30 mg dry wt ml⁻¹.

(ii) *DCCD-treatment of cells.* EDTA-treated cells were incubated in 200 mM-HEPES/NaOH pH 7.5 at 1 mg dry wt ml⁻¹ in a New Brunswick shaker at 30 °C in the presence of 50 µM-DCCD. The cells were then harvested

and washed once with 200 mM-HEPES/NaOH buffer pH 7.5 by centrifugation and resuspension in the buffer.

(iii) *Measurement of K⁺ transport.* Cell suspensions (1 mg dry wt ml⁻¹) were preincubated in a stirred glass vessel at 25 °C in the presence of 1 mM-glucose for 10 min. When iodoacetate (5 mM, pH 7.5) or arsenate (10 mM) were to be present they were added at the end of this preincubation period. Potassium transport was initiated by the addition of 1 mM-KCl and 1 ml samples were taken at time intervals and rapidly transferred to Eppendorf centrifuge tubes. The tubes were centrifuged at 10000 g for 20 s. The supernatant was removed and the pellets resuspended in 1 ml of distilled water and assayed for K⁺ content by flame photometry (Corning 400 Flame Photometer).

When the Kdp system was assayed the K⁺ concentration was reduced to 50 µM (Rhoads & Epstein, 1978) and the decrease in the K⁺ content of the supernatant was assayed.

Measurement of ATP. This was carried out by the method of Kimmich *et al.* (1975). Suspensions (1 mg dry wt ml⁻¹) of EDTA-treated cells were preincubated in a stirred glass vessel at 25 °C in the presence of glucose for 10 min. At intervals 1 ml samples were transferred to conical centrifuge tubes containing 1 ml 12% (w/v) perchloric acid, 5 mM-phosphoric acid held at 4 °C in an ice/water bath. After 30 min incubation the samples were neutralized with a solution of 2 M-KOH containing 0.3 M-MOPS (approx. 1 ml). The samples were then sealed and frozen to assist precipitation of potassium perchlorate. The tubes were thawed and held at 4 °C during centrifugation at 1200 g for 15 min in a Sorvall refrigerated centrifuge. The supernatants were assayed for ATP content.

Samples (50 µl) of the supernatant were added to 925 µl of an assay buffer containing 20 mM-glycylglycine/NaOH pH 8.0, 5 mM-sodium arsenate and 4 mM-MgSO₄. Sigma firefly lantern extract, FLE-50 (25 µl), was added, the sample was vortex-mixed and after a standard 20 s delay was counted for 12 s in a Packard 3255 liquid scintillation counter set at 90% gain, level 50–∞ with the coincidence switch on. In this assay system the square root of the scintillation count is proportional to the ATP content. The system was calibrated for each assay using a concentration range of ATP [1–5 nmol (ml buffer)⁻¹]. The standards were treated identically to the cell samples.

Measurement of thiomethylgalactoside transport. EDTA-treated (Na⁺-loaded) cells of *E. coli* NR71 were incubated at 25 °C for 10 min in the presence of 4 mM-glycerol, and iodoacetate was added to a final concentration of 5 mM. At various time intervals 1 mM-K⁺ was added, followed after a further 30 s incubation by thiomethylgalactoside (3 mM; sp.act. 0.21 µCi µmol⁻¹; 7.7 kBq µmol⁻¹). Samples (100 µl) were taken at timed intervals and filtered under vacuum through Whatman GFF glass fibre filters (Zilberstein *et al.*, 1982). The filters were washed with two samples (2 ml) of the incubation buffer and dried at 60 °C overnight. The samples were then counted by liquid scintillation counting as previously described (Kroll & Booth, 1981).

Respiration studies. These were done using a Clark oxygen electrode as previously described (Kroll & Booth, 1981).

RESULTS

Effect of iodoacetate and arsenate on the energy status of E. coli cells

Both iodoacetate and arsenate inhibit ATP generation and consequently decrease both the ATP pool and for the total flux of carbon through the cell. As a consequence it was to be expected that both of the inhibitors would affect respiration and hence the capacity to restore the protonmotive force subsequent to its partial dissipation by an energy consuming process, e.g. K⁺ transport. Thus the effect of the inhibitors on the energy status was investigated.

In *E. coli* NR71 (*unc*) the ATP pool declined by almost 75% in a 5 min period following addition of either inhibitor (Fig. 1). In a wild-type strain (Frag 1 – *unc*⁺) the effect of iodoacetate was dependent upon the presence of DCCD (Fig. 2*a, b*). In the absence of DCCD the ATP pool was stable in the presence of iodoacetate (Fig. 2*b*). However, if oxidative phosphorylation was inhibited with DCCD then the ATP pool declined in the presence of iodoacetate at a similar rate to that in the *unc* mutant (Fig. 2*a*). The effect of arsenate on Frag 1 was not investigated.

Glucose utilization by *E. coli* NR71 was inhibited by iodoacetate. Control incubations exhibited rates of glucose consumption in the range 15–20 nmol glucose min⁻¹ (mg cells)⁻¹ in the presence of K⁺. Addition of iodoacetate reduced consumption to close to zero within 30 s (data not shown). Similar studies with arsenate as the inhibitor were not undertaken, but the continued high rate of respiration (see below) indicates that some glucose consumption continues in these cells.

Arsenate and iodoacetate inhibited glucose-stimulated respiration of *E. coli* NR71 to different extents. In both cases there was a lag of approximately 2 min prior to inhibition of respiration becoming apparent. The initial rate of respiration was 42–45 nmol O₂ min⁻¹ (mg cells)⁻¹ and subsequently decayed to 50% of the original rate in the presence of arsenate [22.5 nmol O₂ min⁻¹

Fig. 1

Fig. 2

Fig. 1. Effect of iodoacetate and arsenate on the ATP pool of *E. coli* NR71 (*unc*). EDTA-treated cells were prepared as described in Methods and incubated in 200 mM-Na/HEPES pH 7.5 at a density of 1 mg dry wt cells ml⁻¹ at 25 °C. After 5 min preincubation with glucose (1 mM) either (a) iodoacetate or (b) arsenate was added, to 5 mM and 10 mM, respectively. After addition of the inhibitor, 1 mM-KCl (closed symbols) was added (arrow). Samples were taken for ATP analysis at intervals. Open symbols – samples incubated in the absence of K⁺.

Fig. 2. Effect of DCCD-treatment on the sensitivity to iodoacetate and K⁺ of the ATP pool of *E. coli* Frag 1. EDTA-treated cells were prepared and incubated for 30 min in the presence (a) or absence (b) of 50 μM-DCCD. Subsequently the cells were prepared for transport assays as described in Methods. Other experimental conditions were as in Fig. 1. ○, No KCl added; ●, 1 mM-KCl added at arrow.

(mg cells)⁻¹] and 13% in the presence of iodoacetate [5.6 nmol O₂ min⁻¹ (mg cells)⁻¹]. The new rates were established at 4 min and 7 min, respectively, after addition of the inhibitor. This inhibition of respiration did not begin until ATP concentrations had already fallen significantly.

Is Trk an ATPase?

The involvement of ATP in Trk activity could be either in energy coupling or as a regulator. Inhibition of glucose-metabolizing *E. coli* cells with either iodoacetate or arsenate leads to a severe impairment of ATP production and allowed us to discriminate between the two possible roles of ATP in Trk activity. The recent characterization of Kdp as a K⁺-ATPase (Epstein *et al.*, 1978, Wieczorek & Altendorf, 1979) offered a control for the role of ATP in energy coupling to Trk. Iodoacetate caused 92% inhibition of the Kdp system within 3 min of its addition to cells of *E. coli* NR71. Although both Kdp and Trk systems are present in such cells the Trk system is inactive due to rapid depletion of the ATP pool by the Kdp system and inhibition by intracellular K⁺ which is retained to a higher initial concentration (approx. 80 mM compared with 10–20 mM in cells not induced for Kdp). Cells of *E. coli* TK2240, a mutant lacking Trk, show a similar inhibition profile when incubated with iodoacetate and DCCD. This suggests that the loss of Kdp activity was due to depletion of the ATP pool and that Trk does not interfere with the assay of Kdp. The Trk system was assayed in cells not induced for Kdp and was only inhibited by 23% after 3 min incubation with iodoacetate (Fig. 3). This insensitivity of the Trk system to depletion of the ATP pool occurred despite the Trk system having a greater maximal velocity than Kdp. Since Kdp has been characterized as an ATPase, the relative insensitivity of the Trk system to inhibition of ATP generation suggests that ATP is not the energy source for K⁺ uptake via the Trk system.

Does Trk require ATP for activity?

Clearly Trk is not a conventional ATPase (Fig. 3) and thus the question arises does Trk require ATP for activity? We therefore investigated the activity of Trk during the period follow-

Fig. 3. Effect of iodoacetate on the Kdp and Trk K⁺ transport systems. Cells of *E. coli* NR71 were prepared such that either Trk or Kdp-plus-Trk K⁺ uptake systems were present. Assays were done as described in Methods except that 2 mM-KCl was used to assay the Trk system. Although non-EDTA-treated cells were used for assays of the Kdp system, similar results have been obtained in EDTA- and DCCD-treated cells of TK 2240, a strain lacking Trk (Rhoads *et al.*, 1976). The initial rate values (●) apply to uptake over the first 30 s, and steady state values (○) apply to the plateau values of intracellular potassium content. (a) Trk activity; (b) Kdp activity.

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Fig. 4. Time course of the inhibition of Trk activity by iodoacetate in *E. coli* NR71. Cells were prepared and incubated as described in Fig. 1. At various times (●, No inhibitor; ○, 30 s; □, 90 s; ■, 4 min; △, 6 min) after the addition of iodoacetate, 1 mM-KCl was added to initiate K⁺ uptake and transport was assayed as described in Methods. Similar data were obtained with arsenate.

ing inhibition by either iodoacetate or arsenate. In iodoacetate-treated cells of the *unc* strain, NR71, both the initial rate and the steady state accumulation of K⁺ declined as a function of the time of incubation with iodoacetate (Fig. 4). Similar data were obtained with arsenate-inhibited cells (data not shown). Comparison with Fig. 1 shows that this reduction in activity followed a similar time course to the decline both of the ATP pool *per se* and of the capacity to regenerate the ATP pool. The latter was assessed from the observation that in control incubations K⁺ uptake did not perturb the ATP pool. However, in cells inhibited by either iodoacetate or arsenate, K⁺ uptake was associated with an accelerated decline of the ATP pool, indicating that the capacity to regenerate the ATP pool was impaired. Arsenate addition to K⁺-replete cells did not cause significant leakage (data not shown). Thus the lowered steady state accumulation in the presence of the inhibitor represents an inhibition of uptake.

Fig. 5

Fig. 5. Effect of inhibitors on thiomethylgalactoside uptake by *E. coli* NR71. Cell suspensions were induced for the *lac* operon and were prepared for transport assays as described in Methods. Cells were incubated with glycerol (10 mM) for 5 min and either iodoacetate or arsenate was added. Thiomethylgalactoside (3 mM; sp. act. $0.21 \mu\text{Ci } \mu\text{mol}^{-1}$) was added at intervals to initiate uptake, which was assayed in the presence (○) or absence (●) of KCl (1 mM), which was added 30 s prior to the β -galactoside. Data are expressed as a percentage of uptake (either initial rate or steady state) of a control suspension incubated without inhibitor. (a) Arsenate, initial rate; (b) arsenate, steady state; (c) iodoacetate, initial rate; (d) iodoacetate, steady state. The use of glycerol as energy source did not significantly affect the inhibition of Trk activity by either inhibitor (data not shown).

Fig. 6

Fig. 6. Effect of arsenate on K^+ -replete cells of *E. coli* NR71. Cells were harvested and washed with 20 mM-HEPES/140 mM-choline chloride/50 μg chloramphenicol ml^{-1} pH 7.5. The cells were incubated at 25°C with 1 mM-glucose. After 5 min the cells were diluted into an equal volume of the same buffer containing 0.8 M-sorbitol to increase the osmotic pressure from 300 mOsm to 700 mOsm. The cells were incubated in the presence (●, ○, □) or absence (■) of 2 mM-KCl. Samples were taken at intervals for K^+ uptake (a) or ATP analysis (b). Incubations were carried out in the presence (■, □, ○) or absence (●) of arsenate (10 mM) added 1 min prior to the osmotic shock.

Since both inhibitors of ATP production also affect the respiratory rate, we wished to verify that the capacity to regenerate a protonmotive force during K^+ uptake was not limiting the uptake of this cation. The lactose transport system is pmf-dependent and has a similar V_{max} and capacity to that of the Trk uptake system. A saturating concentration of thiomethylgalactoside (3 mM) was used in order to obtain both a rate and a total net uptake of a similar order of magnitude of K^+ uptake. Iodoacetate-inhibited or arsenate-inhibited cells showed no decline in the accumulation of thiomethylgalactoside over the first 8 min of treatment with iodoacetate (Fig. 5), although the rate of thiomethylgalactoside uptake decreased by approximately 30%. However, these effects were qualitatively and quantitatively different to the effects of iodoacetate on K^+ uptake. Further, prior addition of K^+ to such cells did not diminish the uptake of thiomethylgalactoside (Fig. 5). Thus, the limitation of K^+ uptake cannot result from a loss of capacity to regenerate the protonmotive force.

Effect of K^+ uptake via Trk on the ATP pool

If the Trk system were an ATPase regulated by the protonmotive force, K^+ uptake would be expected to be a significant drain on the ATP pool. To examine this we sought to inhibit resynthesis of ATP once it has been utilized. Iodoacetate treatment of NR71 (*unc*), Frag 1 (*unc*⁺) and DCCD-treated Frag 1 was expected to limit the capacity to synthesize ATP and thus ATP consumption by Trk should be evident. This was found to be the case. In all cases K^+ addition to iodoacetate-inhibited cells stimulated the consumption of the ATP pool (Figs 1a and 2a, b). However, comparison of the net K^+ uptake with the net decrease in ATP suggested that the relationship was not of a constant stoichiometry (a range of 44–96 nmol K^+ taken up per nmol decrease in the ATP pool) and that Trk was not an ATPase. In a mutant lacking the Trk uptake system (TK2242) there was no effect of K^+ on the ATP pool. Identical results were obtained using arsenate as the inhibitor of the *unc* mutant (data not shown).

The studies reported above utilized K^+ uptake into K^+ -depleted, Na^+ -loaded cells. To rule out artefactual effects on the ATP pool due to this unusual cytoplasmic constitution we investigated K^+ uptake into K^+ -replete cells. An increase in external osmotic pressure has been shown to stimulate net K^+ uptake (Rhoads & Epstein, 1978). Arsenate-inhibited K^+ -replete cells of *E. coli* NR71 were subjected to an increase in osmotic pressure from 300 mOsm to 600 mOsm and K^+ uptake and the ATP pool were monitored. Net K^+ uptake was partially inhibited by arsenate but ATP consumption was still evident (Fig. 6).

DISCUSSION

It was originally proposed that ATP played a role in Trk K^+ transport activity (Rhoads & Epstein, 1977). On the basis of our studies we wish to propose that ATP is not the energy source for uptake, but rather that it, or a related compound, is a regulator of activity of the Trk K^+ transport system. This proposal is based upon the loss of activity of the Trk system over the same time course as the decline of the ATP pool. It is unlikely that the loss of Trk activity reflects side effects of the inhibitors themselves, since they are different in structure and mode of action. Similarly, the loss of Trk activity cannot be attributed to coupling to the respiratory chain (Elferinck *et al.*, 1984) since transport activity declines more rapidly than respiration. On the other hand, K^+ transport via Trk was inhibited whenever the supply of ATP was compromised. Thus we wish to propose a model for the Trk system which could explain the results of this and earlier studies (Rhoads & Epstein, 1977; 1978).

Two basic types of models may be envisaged in which ATP plays a regulatory role in the activity of the Trk system. In the first model ATP would phosphorylate the Trk proteins to give an active form of the protein complex. Phosphorylation would be reversed at a rate dependent upon either the internal K^+ concentration or the turgor pressure. If the phosphate intermediate were of the ester type a phosphorylation-dephosphorylation cycle would consume energy which might explain the observed stimulation of ATP consumption during K^+ uptake (Figs 1 and 2). Alternatively, if phosphorylation were by anhydride-bond formation then the phosphorylation-dephosphorylation cycle need not be energy consuming. In this case the observed ATP-consumption would arise from an indirect stimulation of ATP-consuming metabolism.

In the second model, K^+ transport activity would depend upon the binding of ATP to an allosteric site on the transport complex. One argument against such a model is that transport activity does not correlate with the ATP pool *per se* but rather with the capacity to synthesize ATP. Thus even cells in which the ATP pool is very low still exhibit an initial burst of activity consistent with residual active complex. The following sequence of events is thus envisaged to occur during K^+ uptake. In cells replete with ATP but depleted of K^+ the Trk complex is in an active state and K^+ uptake occurs driven by the proton motive force. As K^+ accumulates either the rate of phosphorylation decreases and/or the rate of dephosphorylation increases (model one) or the affinity for ATP decreases (model two). Further cycles of K^+ uptake would require phosphorylation of the carrier proteins or ATP binding to restore activity of the complex. If the pool of ATP is small and the capacity to regenerate ATP is low, as in the presence of arsenate or iodoacetate, then phosphorylation or ATP binding cannot occur and the Trk system will remain inactive. All the data we have presented are consistent with such a hypothesis. Further the data could also explain the requirement of K^+ exchange via the Trk system for ATP alone (Rhoads & Epstein, 1978). In the above models activation of the transport system to allow energetically silent K^+ - K^+ exchange would require ATP.

It is clear from earlier studies that the activity of the Trk complex is regulated by osmotic pressure (Rhoads & Epstein, 1978). In our studies arsenate inhibited the stimulation of Trk activity by an increase in the external osmotic pressure which suggests that again the control of Trk by turgor pressure is via either phosphorylation or ATP binding.

Since we have proposed ATP as a regulator it is pertinent to discuss how the system can be energized by the protonmotive force. It has been demonstrated that the K^+ gradient maintained by Trk exceeds the magnitude of the membrane potential (Bakker, 1980, 1981). At present the simplest explanation of this observation is that of K^+/H^+ symport (Bakker & Harold 1980). The

advantage of such a system is that the driving force ($2\Delta\psi - Z\Delta\text{pH}$) will be considerable at all values of external pH thus allowing the possibility of a constant K^+ concentration in the cytoplasm independent of the environmental conditions. Such a system would have to be regulated to avoid the overloading of the cytoplasm with K^+ . At least two possible models for regulation present themselves. In the first, the excess K^+ could be pumped out by a second system such as the K^+/H^+ antiport (Plack & Rosen, 1980). This mechanism is wasteful of energy. Alternatively the activity of Trk itself could be regulated by either the internal K^+ concentration or the turgor pressure to prevent the system reaching equilibrium with the components of the proton motive force. The phosphorylation mechanism proposed above would meet this requirement. Furthermore it would be consistent with the observed feedback regulation of Trk activity (Rhoads & Epstein, 1978).

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