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THE EFFECTS OF WEAK ACIDS ON POTASSIUM UPTAKE BY *ESCHERICHIA COLI* K-12

INHIBITION BY LOW CYTOPLASMIC pH

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The activity of the *Escherichia coli* K^+ transport system TrkA was measured as a function of the cytoplasmic pH of the cell. For this purpose, pH_{in} was decreased by the addition of the weak acids acetic acid, benzoic acid or salicylic acid to K^+-depleted cells. Under these conditions, the initial rate of K^+ uptake decreased strongly with pH_{in}, and was almost independent of the acid used. This inhibition was due to a strong decrease in the V_{max} for K^+ uptake, which indicates that low cytoplasmic pH inactivates the TrkA K^+ uptake system. The relevance of this inhibition for growth and metabolism at low pH_{in} is discussed.

Introduction

Many intact bacteria maintain a constant cytoplasmic pH of about 7.5 (reviewed by Padan et al. [1]). The mechanism of this pH homeostasis is not yet known, but it has been proposed that an alkaline pH_{in} is established by the extrusion of H^+ in response to electrogenic uptake of K^+ [2–8], whereas a combination of or either one of the following processes may play a role in preventing pH_{in} to become too alkaline: (i) metabolic production of weak acids in the cytoplasm [9]; (ii) the activation of a Na^+–H^+ antiporter that allows protons to re-enter the cell [1,10–12]; (iii) a similar process that activates a K^+–H^+ antiporter [13,14].

The physiological role of pH homeostasis is probably to keep cytoplasmic enzymes optimally active. Indeed, some examples are known of key enzymatic processes that have a pH optimum at slightly alkaline pH: the ATPase activity of the H^+-translocating ATP-synthase from *Escherichia coli* has an optimum at alkaline pH [15,16]; the hexose transport system of the alga *Chlorella vulgaris* is inhibited by acidic pH_{in} [17], and for *E. coli* acidic cytoplasmic pH seems to be toxic, since this bacterium is repelled by agents that cause a decrease in pH_{in} [18,19].

Recently, we obtained results that could be explained by assuming that the main *E. coli* K^+ uptake system TrkA is inhibited by low pH_{in} [20]. In this communication we report on experiments that address this point directly For this purpose we lowered the cytoplasmic pH of K^+-depleted cells of *E. coli* by the addition of different weak acids and measured the initial rate of K^+ uptake by the cells under those conditions. The results indicate that the activity of the TrkA system is indeed a steep function of cytoplasmic pH.

Abbreviations: Aces, *N*-(2-acetamido)-2-aminoethanesulphonic acid; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; Mes, 2-(*N*-morpholino)ethanesulphonic acid; Mops, *N*-morpholinopropanesulphonic acid; $TPMP^+$, triphenylmethylphosphonium cation; TPP^+, tetraphenylmethylphosphonium cation; $\Delta\Psi$, membrane potential (out minus in); ΔpH, transmembrane pH gradient (pH_{out} minus pH_{in}); $\Delta\tilde{\mu}_{H^+}$, transmembrane electrochemical proton gradient (out minus in); pH_{in}, cytoplasmic pH.

Materials and Methods

Organism and growth conditions

Strain TK 1001 (*F⁻, trkD1, kdpABC5, lacZ, rha, thi, gal*) is derived from *E. coli* K-12 [21], and was grown on the minimal salt medium K5 in the presence of 1 μg/ml thiamine and 10 mM glucose [22]. This mutant contains the constitutive K^+-uptake system TrkA only. The outer membrane of the cells was disrupted by Tris-EDTA treatment [8,23]. Subsequently, the cells were washed twice with either one of the following buffers: 200 mM NaMes (pH 6.1), 200 mM NaAces (pH 6.8), 200 mM NaHepes (pH 7.5), or 100 mM triethanolamine phosphate (pH 6.8). Finally, the cells were resuspended at 10–20 mg dry weight/ml of the same buffer and shaken at 20°C at 220 rpm in a gyratory shaker bath. During the EDTA treatment and subsequent washing the cells had lost most of their K^+ and were therefore suitable for net K^+-uptake studies [8].

K^+ uptake and ΔpH

The EDTA-treated cells were diluted to 1 mg dry weight/ml of the resuspension buffer, and were preincubated for 20 min with the sodium salt of the weak acid employed, and for 10 min with 10 mM glucose. At zero time, 2 mM KCl was added. The incubations were carried out in 50 ml wide-neck Erlenmeyer flasks that were filled with maximally 10 ml of the cell suspension and shaken at 220 rpm at 20°C. Under these conditions the rate of oxygen uptake by the suspension is rather slow (i.e., 30–80 natoms oxygen per mg dry weight of cells per min [8]), and aeration is so efficient that anaerobiosis of the suspension does not occur during the incubation and subsequent centrifugation. At different time points the cells were separated from the medium by centrifugation through silicone oil (Rotitherm, Roth, Karlsruhe, F.R.G.) in a Beckman B minicentrifuge. The K^+ and Na^+ content of the pellet fractions was determined in an Eppendorf 700 Flame photometer (for details see Ref. 8). ΔpH was determined by [^{14}C]benzoic acid distribution [4,8]. These experiments were carried out in parallel to those for K^+ uptake, and the cell suspensions contained 2 μCi/ml ^3H_2O and 50–200 nCi/ml (2–8 μM) [^{14}C]benzoic acid as well.

Cells were separated from the medium at time-points identical to those for K^+ for K^+ uptake experiments, and ΔpH was calculated from the radioactivity of the isotopes in pellet and supernatant as described by Rottenberg [24].

Protonmotive force

Steady-state values of the membrane potential ($\Delta\Psi$) were determined by [^{3}H]TPP$^+$ distribution [8,25]. The total protonmotive force was calculated from the relationship:

$$\Delta\tilde{\mu}_{H^+}/F = \Delta\Psi - 58\Delta pH \quad (mV)$$

For this calculation the internal water space of the cells was taken to be 1.45 μl/mg dry weight of cells, and to be equal to 55% of the total water space of the cell pellet [8].

ATP content

The cells were incubated as described for the K^+-uptake studies. At different time-points after the addition of 2 mM KCl, samples of 1.0 ml were taken from the suspension and immediately mixed with 1.0 ml of an ice-cold solution that contained 12% $HClO_4$ and 5 mM Na_2HPO_4 [26]. The samples were incubated for 30 min at 4°C, and subsequently centrifuged at 5000 rpm in the cold, to remove denatured proteins. The supernatants were brought to pH 7.0 with a solution that contained 2 M KOH and 0.3 M Mops. The samples were frozen at −30°C, quickly thawed, and immediately centrifuged at 4°C to remove the crystals of $KClO_4$ formed. The supernatants were kept on ice and were used within a few hours for the ATP determination. For this purpose, 50 μl of supernatant were mixed with 400 μl of the assay buffer that contained 20 mM glycylglycine, 5 mM sodium arsenate and 4 mM $MgSO_4$, final pH 8.0 [26]. The determination was started by the addition of 50 μl of a crude luciferin-luciferase preparation that was isolated from firefly tails (Sigma Chemical Company, Taufkirchen, F.R.G.) according to method C in Ref. 27. The amount of light produced per minute was measured in a 9500 C type Lumostat (Berthold, F.R.G.). The amount of ATP present in the incubation was calculated from a calibration curve of the light produced by known amounts of ATP that were pretreated identically to the sam-

ples containing an unknown amount of ATP. The sodium salts of the weak acids employed did not affect the amount of light produced in the assay.

Materials

Hepes, Aces and Mes were from Sigma Chemical Company, Taufkirchen, F.R.G. Triethanolamine (p.A.) was from E. Merck, Darmstadt, F.R.G. TPP^+Br^- was a gift of Dr. Michael Eisenbach, The Weizmann Institute of Science, Rehovot, Israel. ^3H_2O was from Amersham/Büchler, Braunschweig, F.R.G.; [^{14}C]benzoic acid (22 Ci/mol) from New England Nuclear, Dreieich, F.R.G., and [^{3}H]TPP^+ (2400 Ci/mol) from the Nuclear Research Center, Negev, Israel. All other reagents were of the highest purity available commercially.

Results

The effect of weak acids on ΔpH and $\Delta\Psi$

The cytoplasmic pH of cells of *E. coli* can be decreased by the addition of weak acids [7,17,18]. We wished to investigate the effect of these acids on K^+ transport by the *E. coli* TrkA system as well. Since this system is known to require a high $\Delta\tilde{\mu}_{H^+}$ for activity [28], it was first examined whether the weak acids to be used affected the total $\Delta\tilde{\mu}_{H^+}$. For this purpose we measured $\Delta\Psi$ and ΔpH in the EDTA-treated cells in the absence and presence of acetic acid, benzoic acid or salicyclic acid. Despite the fact that $\Delta\Psi$ may be underestimated from [^{3}H]TPP^+ distribution [29], this method is still satisfactory for use as a qualitative indicator of the membrane potential. Fig. 1A shows that at pH_{out} 6.8 acetate decreased ΔpH (and therefore also pH_{in}, right-hand ordinate), and increased $\Delta\Psi$. Benzoate had a stronger affect on ΔpH, and still increased $\Delta\Psi$ to some extent (Fig. 1B). Salicylate abolished ΔpH completely, but had hardly any effect on $\Delta\Psi$ (Fig. 1C). The effect of acetate and benzoate on the total $\Delta\tilde{\mu}_{H^+}$ was relatively small. Thus, it seems unlikely that these acids directly affect the TrkA system due to a change in $\Delta\tilde{\mu}_{H^+}$. This may, however, not be the situation for salicylate, since 30 mM of this acid decreased $\Delta\tilde{\mu}_{H^+}$ from 160 to 110 mV. The effect of this acid on the components of $\Delta\tilde{\mu}_{H^+}$ is very similar to that of acetylsalicylate [30] in that both acids primarily decrease ΔpH. In this respect they differ from protonophores like FCCP that mainly affect the $\Delta\Psi$ component of $\Delta\tilde{\mu}_{H^+}$ (not shown).

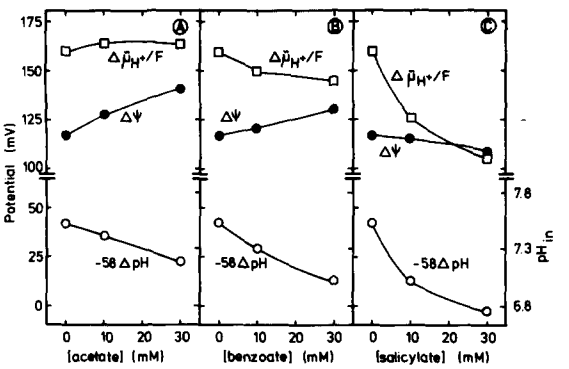

Fig. 1. Effect of weak acids on the components of $\Delta\tilde{\mu}_{H^+}$ in *E. coli*. EDTA-treated cells were resuspended at pH 6.8 in the NaAces medium as described under Materials and Methods in the presence of 2 mM KCl and the sodium salts of the weak acids at the concentrations indicated. Symbols: ○——○, $-58\,\Delta$pH; ●——●, $\Delta\Psi$; □——□, $\Delta\tilde{\mu}_{H^+}$.

Effect of weak acids on cation movements

Fig. 2 shows the effect of 30 mM benzoate on K^+ uptake, Na^+ extrusion (A) and pH_{in} (B) of *E. coli* cells at pH_{out} 6.8. After the addition of K^+ to the medium, control cells took up this cation rapidly. During the first 15 s this uptake was compensated for by the extrusion of almost equal amounts of Na^+ and H^+. As a consequence of the latter, the cytoplasmic pH rose immediately from 7.2 to 7.8. After 30 s, the rate of K^+ uptake decreased gradually, and K^+ exchanged almost exclusively against Na^+ (Fig. 2A). Meanwhile, pH_{in} slowly decreased to its equilibrium value of 7.5–7.6 (Fig. 2B). This exchange of K^+ against H^+ and Na^+ has been reported before [21,31], and the overshoot in pH_{in} after addition of K^+ has also been observed by others (Kroll, R.H. and Booth, I.R., personal communication).

By contrast, 30 mM benzoate prevented pH_{in} from rising above 7.0. Concomitantly, it caused a much lower rate of K^+ uptake that was now almost completely compensated for by Na^+ extrusion (Fig. 2, closed symbols).

This result may indicate that pH_{in} directly influences the rate of K^+ uptake by the TrkA system. Before we considered this possibility, the

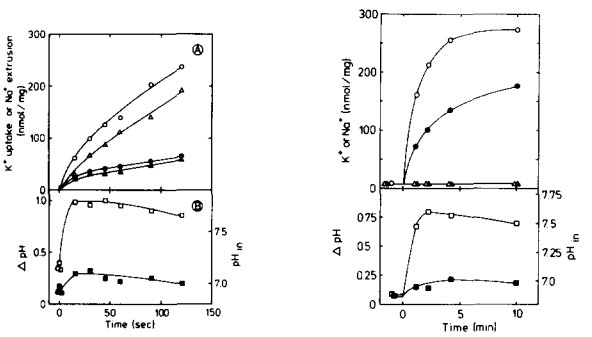

Fig. 2. Effect of benzoate on cation movement in *E. coli* K-12. Cells were preincubated with or without 30 mM sodium benzoate in the NaAces medium at pH 6.8. At zero time, 2 mM KCl was added. A: K^+ uptake (○,●), Na^+ extrusion (△,▲); B: ΔpH (□,■). Open symbols: control cells; closed symbols, benzoate-treated cells.

Fig. 3. Effect of benzoate on cation movements in the absence of Na^+. Cells were washed and resuspended in the triethanolamine phosphate medium of pH 6.8. Otherwise, the experiment was identical to that of Fig. 2, except that samples were taken at different time-points, and that the triethanolamine salt of benzoate was employed. Symbols: see legend to Fig. 2.

nature of the inhibitory effect of benzoate needed to be characterized further. The first important point was to establish that pH_{in} did not have a direct and rate-limiting effect on the extrusion of H^+ or Na^+ that compensated for K^+ uptake (Fig. 2). Fig. 3 shows that this is probably not the situation for Na^+ extrusion, since cells loaded with triethanolamine instead of Na^+ [32] still showed a rapid K^+ uptake that was inhibited by benzoate (Fig. 3A). Concomitantly, this acid also prevented pH_{in} from reaching a value of 7.6 (Fig. 3B). It is also unlikely that the rate of protein extrusion is rate-limiting for K^+ uptake in the benzoate-treated cells, since Fig. 2 shows that the rapid initial uptake of K^+ by control cells was for 50% against protons.

Kinetic parameters

Secondly, we investigated the effect of benzoate on the kinetics of initial K^+ uptake (Fig. 4). It shows that this compound strongly decreased V_{max}, and had little effect on K_m. The same was observed for acetate (Fig. 4). This result may indicate that benzoate and acetate decrease the number of active TrkA molecules or the turnover number of the carrier. The results of Fig. 4 also indicate that at the K^+ concentration of 2 mM used in the experiments presented here, the activity of the TrkA system was assayed under V_{max} conditions.

pH_{in} affects the TrkA system directly

We then examined whether acidic pH_{in} inhibits K^+ uptake directly. To collect more data on this point, the experiment of Fig. 2 was repeated at different concentrations of benzoate, acetate or salicylate. Fig. 5 shows that all three acids progressively decreased both pH_{in} and the rate of K^+ uptake. Similar experiments were carried out at pH_{out} 6.1 and pH_{out} 7.5. In the former case, the results were comparable to that of Fig. 5, except that less acid was required to reduce both pH_{in} and the rate of K^+ uptake (not shown). At pH 7.5, however, where ΔpH is negligible [8,23,27,33,34], even 100 mM of benzoate or acetate did not affect pH_{in} or the rate of K^+ uptake (not shown). This

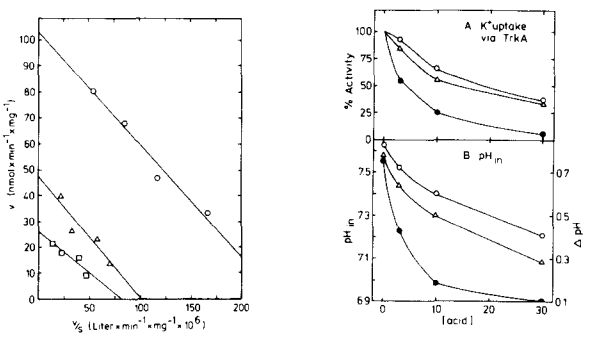

Fig. 4. Effect of benzoate and acetate on the kinetic parameters of K^+ uptake. Cells were preincubated in the NaAces medium at pH 6.8. in the presence or absence of 30 mM sodium benzoate or 30 mM sodium acetate. At zero time, different concentrations of K^+ were added. The initial rate of K^+ uptake was taken to be equal to the uptake during the first 90 s after addition of K^+. The data are plotted according to Woolf, Augustinsson and Hofstee [49]. Symbols: ○———○ control cells; △———△, 30 mM acetate; □———□, 30 mM benzoate. The values of K_m and V_{max} were 440, 310 and 480 μM and 103, 48 an 26 nmol/min per mg dry weight, respectively.

Fig. 5. Dependence of the rate of K^+ uptake (A) and of pH_{in} (B) on the concentration of different weak acids. EDTA-treated cells were resuspended in the NaAces medium (pH 6.8) in the presence of different concentrations of the sodium salts of acetic acid (○), benzoic acid (△) and salicylic acid (□). The initial rate of K^+ uptake was taken to be equal to the uptake during the first 2 min after the addition of 2 mM KCl.

result indicates that it is pH_{in}, rather than the concentration of the acid anion in the cytoplasm, that inhibits K^+ uptake. Support for this notion comes from Fig. 6, in which the data at the three values of pH_{out} were combined. For this purpose it had to be taken into account that V_{max} (but not K_m) of the TrkA system also increases with pH_{out} [34]. Therefore, the rate of K^+ transport at pH_{in} 7.5 at a particular value of pH_{out} was taken as a 100% value. Fig. 6 shows that for acetate and for benzoate there existed a single relationship between pH_{in} and the rate of K^+ uptake. At the highest concentrations of salicylate K^+ uptake was inhibited more strongly than expected on the basis of the value of pH_{in} (Fig. 6, open squares). This may be related to the decrease in $\Delta\tilde{\mu}_{H^+}$ by high concentrations of salicylate (Fig. 1C, see Discussion).

Experiments failed to establish how the curve in Fig. 6 continued above pH 7.6. First, we tried the effect of the weak base methylamine at pH_{out} 8.5, where it is supposed to decrease ΔpH, and therefore to increase pH_{in}. However, methylamine

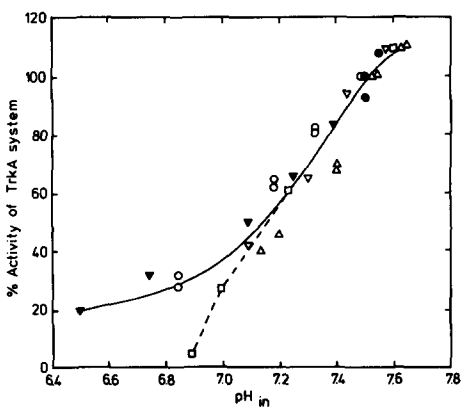

Fig. 6. Activity of the TrkA system as a function of pH_{in}. EDTA-treated cells were washed and resuspended in NaMes buffer (pH 6.1), NaAces buffer (pH 6.8) or NaHepes buffer (pH 7.5) in the absence and presence of difference concentrations of the sodium salts of the weak acids. The initial rate of K^+ uptake was determined as indicated in the legend to Fig. 5. In a parallel experiment, pH_{in} was also determined. K^+ uptake rates at a certain value of pH_{in} and pH_{out} were normalized by taking the rate of K^+ uptake at pH_{in} 7.5 as a 100% value (see text). Symbols: ▼———▼, benzoate, pH_{out} 6.1; ▽———▽, benzoate, pH_{out} 6.8; □———□, salicylate, pH_{out} 6.8; ○———○, acetate, pH_{out} 6.1; △———△, acetate, pH_{out} 6.8; ●———●, acetate, pH_{out} 7.5.

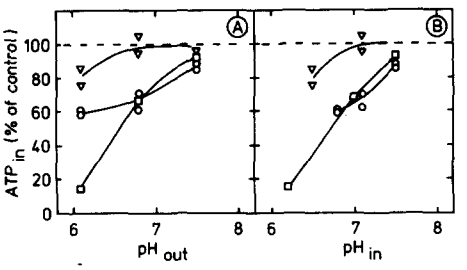

Fig. 7. The effect of weak acids on the cytoplasmic ATP content of the cells. Cells were washed with and resuspended in the buffers described in the legend to Fig. 6 in the presence or absence of 30 mM benzoate (▽———▽), 30 mM acetate (○———○) or 30 mM salicylate (□———□) A: ATP content of the cells at different values of pH_{out}. The control value varied between 3.0 and 4.5 nmol ATP/mg dry weight of cells, and was independent of pH_{out}. B: ATP content of the cells at different values of pH_{in}. For this purpose, the data were combined of Fig. 7A (ATP content) with those of Fig. 6 (internal pH).

strongly decreased the amount of K^+ taken up by the cells, rather than the rate of K^+ uptake. Addition of the lipid-soluble cation $TPMP^+$ was also not successful. Unlike in *Halobacterium halobium* [36], addition of 1 mM of this cation did not increase pH_{in} (not shown).

The rate of K^+ uptake showed a correlation with pH_{in} only (Fig. 6) and not with any of the following parameters: the change in $\Delta\Psi$ or ΔpH induced by the acids, the concentration of the free acid or of that of the acid anion in the cells (not shown), or the ATP level in the cells. The latter contention is based on the results shown in Fig. 7. It shows that, particularly at low external pH, the presence of 30 mM salicylate or 30 mM acetate decreased the level of ATP_{in}, whereas 30 mM benzoate had only a small effect on the ATP level at $pH_{out} = 6.1$ (Fig. 7A). Since at a certain value of pH_{in} acetate and benzoate had almost identical effects on K^+ uptake (Fig. 6), but the ATP level was different under those conditions (Fig. 7B), it is concluded that the results of Fig. 6 cannot be explained by differences caused by the presence of the acids on the ATP level of the cells (see Discussion).

Discussion

The constitutive K^+ transport system TrkA of *E. coli* and the similar system in *Streptococcus*

faecalis are unusual, in that they require for activity both a high $\Delta\tilde{\mu}_{H^+}$ and a high level of ATP in the cytoplasm [28,37]. Identification of the role of these parameters in K^+ transport has been hampered by the fact that these transport systems lose activity in vesicles derived from intact cells. Therefore, this identification has to be carried out in intact cells, in which $\Delta\tilde{\mu}_{H^+}$ and the ATP level can be manipulated to only a limited extent. For some constitutive bacterial K^+ uptake systems, it has been proposed that the magnitude of $\Delta\tilde{\mu}_{H^+}$ regulates the activity, and that ATP or another high-energy intermediate drives the system [38–40], whereas we have proposed the opposite [37,41]. However, the evidence in support of either one of these two models is still inconclusive. In this communication we report that the cytoplasmic pH is also an important factor for the rate of K^+ uptake by the TrkA system. This contention is based on the results of Fig. 6 that show that K^+ uptake strongly decreased with pH_{in}, regardless of whether pH_{in} was decreased by acetate, benzoate or low concentrations of salicylate. Apparently, this inhibition is either caused by an inactivation of part of the carriers or by a decrease of the turnover number of the carrier at acidic cytoplasmic pH.

Since, then, ATP, $\Delta\tilde{\mu}_{H^+}$ and cytoplasmic pH may all have an effect on the TrkA system, the question can be asked, whether it is pH_{in} alone, and not a concomitant change in $\Delta\tilde{\mu}_{H^+}$ or the ATP level in the cells that determines the results of Fig. 6. For high concentrations of salicylate this seems indeed to be the situation, since under those conditions this acid both decreases $\Delta\tilde{\mu}_{H^+}$ (Fig. 1) and the ATP level in the cells (Fig. 7), and inhibited K^+ uptake at a certain value of pH_{in} more strongly than expected on the basis of the results obtained with the other two acids (Fig. 6). By contrast, benzoate and acetate hardly affected $\Delta\tilde{\mu}_{H^+}$ (Fig. 1), and induced an almost similar relationship between pH_{in} and the rate of K^+ uptake (Fig. 6), despite the fact that acetate decreased the ATP level in the cells much more that did benzoate (Fig. 7). This may indicate that $\Delta\tilde{\mu}_{H^+}$ and pH_{in} directly determine the rate of K^+ uptake via the TrkA system, and that ATP plays a secondary (i.e., regulatory) role on K^+ transport.

The results presented here indicate that, like other microbial enzymes [16,17], the TrkA system functions much better at the cytoplasmic pH maintained during growth [12,32,42] than at a more acidic cytoplasmic pH. Since growth of *E. coli* and other bacteria is also inhibited by low pH_{in} (Refs. 42–44 and our unpublished observations), the question arises whether under those conditions the rate of K^+ uptake via the TrkA system becomes limiting for growth. The answer is probably no, since (i) the TrkA system has a large overcapacity, and (ii) wild-type cells of *E. coli* respond to low K^+ concentrations in the cytosol by the induction of the high-affinity K^+-transporting ATPase [45]. It is more likely that one or a few as yet unidentified key processes limit the rate of growth at low pH_{in}. Why, then, do more bacterial enzymes have a strongly diminished activity at low pH_{in}? The explanation may be that under those conditions the cells have a mechanism not only to reduce the rate of growth, but also that of metabolism. Thus, low pH_{in} may slow down metabolism in both stationary cells of acid-producing bacteria and in microbial spores, which are both known to have a low internal pH [2,46,47].

From the results presented here that acidic cytoplasmic pH inhibits K^+ uptake, the question arises whether K^+ uptake is required for establishing an alkaline cytoplasmic pH, as has been proposed for several bacteria [3–8]. The results of Fig. 2 (control value) and of Ref. 8 indicate that this is indeed the situation. Upon addition of K^+, the internal pH rose rapidly from 7.2 to 7.8. However, this experiment was carried out in the presence of Na^+ and under those conditions the internal pH of K^+-depleted cells of *E. coli* never drops below 6.9 [8]. Apparently, K^+ transport is then still fast enough to increase pH_{in} rapidly. Cells depleted of K^+ and Na^+ lose pH control altogether (Ref. 7 and Kroll, R.H., Booth, I.R. and Bakker, E.P., unpublished observations). At low pH_{out} and in the absence of other permeant ions the uptake of K^+ by these cells is so slow that the internal pH increases very gradually, and the cells never reach their normal cytoplasmic pH of 7.5.

Our results also indicate that Na^+ is not a substrate of the TrkA system. In the absence of this cation, cells resupended in the triethanolamine phosphate buffer took up K^+ at a rate comparable to cells resuspended in a NaHepes medium (Figs. 2 and 3). Moreover, in the presence of Na^+ the

initial K^+ uptake was half balanced by H^+ rather than Na^+ extrusion (Fig. 2 and Kroll, R.H. and Booth, I.R., personal communication). Apparently, Na^+ extrusion is a secondary phenomenon, and occurs against protons [10–12] that had previously exchanged against K^+. This notion is similar to that given for the K^+ transport system of *S. faecalis* [3], but contradicts the conclusions from Ref. 48 for *E. coli* and Refs. 39, 40 for *Mycoplasma*, where it is proposed that the stimulatory effect of Na^+ on K^+ uptake is due to Na^+ transport by the respective constitutive K^+ uptake system itself.

At present it is not yet clear, whether the primary K^+–H^+ exchange (Figs. 2, 3) is catalyzed by the TrkA system alone or whether the cellular proton pumps respond to K^+ uptake. via TrKA by the extrusion of extra protons [2,3]. Experiments to clear this point are in progress.

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References

1 Padan, E., Zilberstein, D. and Schuldiner, S. (1981) Biochim. Biophys. Acta 650, 151–166
2 Harold, F.M., Pavlasova, E. and Baarda, J.R. (1970) Biochim. Biophys. Acta 196, 235–244
3 Harold, F.M. and Papineau, D. (1972) J. Membrane Biol. 8, 45–62
4 Kashket, E.R. and Barker, S.L. (1977) J. Bacteriol. 130, 1017–1023
5 McCarthy, J.E.G., Ferguson, S.J. and Kell, D.B. (1981) Biochem. J. 196, 311–321
6 Tokuda, H., Nakamura, T. and Unemoto, T. (1981) Biochemistry 20, 4198–4203
7 Kroll, R.G. and Booth, I.R. (1981) Biochem. J. 198, 691–698
8 Bakker, E.P. and Mangerich, W. (1981) J. Bacteriol. 147, 820–826
9 Sanders, D. and Slayman, C.L. (1982) J. Gen. Physiol. 80, 377–402
10 West, I.C. and Mitchell, P. (1974) Biochem. J. 114, 87–90
11 Schuldiner, S. and Fishkes, H. (1978) Biochemistry 17, 706–711
12 Zilberstein, D., Agmon, V., Schuldiner, S. and Padan, E. (1982) J. Biol. Chem. 257, 3687–3691
13 Plack, R.H. and Rosen, B.P. (1980) J. Biol. Chem. 255, 3824–3825
14 Mandel, K.G., Guffanti, A.A. and Krulwich, T.A. (1980) J. Biol. Chem. 255, 7391–7396
15 Evans, D.J. (1970) J. Bacteriol. 100, 914–922
16 Davies, P.L. and Bragg, P.D., Biochim. Biophys. Acta 266 (1972) 273–284
17 Komor, E., Schwab, W.G.W. and Tanner, W. (1979) Biochim. Biophys. Acta 555, 524–530
18 Repaske, D.R. and Adler, J. (1981) J. Bacteriol. 145, 1196–1208
19 Kihara, M. and MacNab, R.M. (1981) J. Bacteriol. 145, 1209–1221
20 Bakker, E.P. and Mangerich, W.E. (1982) FEBS Lett. 140, 177–180
21 Rhoads, D.B., Waters, F.B. and Epstein, W. (1976) J. Gen. Physiol. 67, 325–341
22 Epstein, W. and Kim, B.S. (1971) J. Bacteriol. 108, 639–644
23 Padan, E., Zilberstein, D. and Rottenberg, H. (1976) Eur. J. Biochem. 40, 431–437
24 Rottenberg, H. (1979) Methods Enzymol. 55, 547–569
25 Zilberstein, D., Schuldiner, S. and Padan, E. (1979) Biochemistry 18, 669–673
26 Kimmich, G.A., Randles, J. and Brand, J.S. (1975) Anal. Biochem. 69, 187–206
27 Bergmeyer, H.U. (ed.) (1974) Methoden der Enzymatischen Analyse, p. 2177, Verlag Chemie, Weinheim
28 Rhoads, D.B. and Epstein, W. (1977) J. Biol. Chem. 252, 1394–1401
29 Bakker, E.P. (1982) Biochim. Biophys. Acta 681, 474–483
30 Ahmed, S. and Booth, I.R. (1981) Biochem. J. 200, 573–581
31 Schulz, S.G., Epstein, W. and Solomon, A.K. (1963) J. Gen. Physiol. 47, 329–346
32 Russel, L.M. and Rosenberg, H. (1980) Biochem. J. 188, 715–723
33 Booth, I.R., Mitchell, W.J. and Hamilton, W.A. (1979) Biochem. J. 182, 687–696
34 Ugurbil, K., Rottenberg, H., Glynn, P. and Shulman, R.G. (1982) Biochemistry, 21, 1068–1075
35 Bakker, E.P. (1983) FEMS Microbiol. Lett. 16, 229–233
36 Bakker, E.P. Rottenberg, H. and Caplan, S.R. (1976) Biochim. Biophys. Acta 440, 557–572
37 Bakker, E.P. and Harold, F.M. (1980) J. Biol. Chem. 255, 433–440
38 Hellingwerf, K., Friedberg, I., Lolkema, J.S., Michels, P.A.M. and Konings, W.N. (1982) J. Bacteriol. 150, 1183–1191
39 Benyoucef, M., Rigaud, J.L. and LeBlanc, G. (1982) Biochem. J., 208, 529–538
40 Benyoucef, M., Rigaud, J.L. and LeBlanc, G. (1982) Biochem. J., 208, 539–547
41 Bakker, E.P. (1981) in Vectorial Reactions in Electron and Ion Transport (Palmieri, F., Quagliariello, E., Siliprandi, N. and Slater, E.C., eds.), pp. 315–318, Elsevier/North-Holland, Amsterdam
42 Harold, F.M. and Van Brunt, J. (1977) Science 197, 372–373
43 Kobayashi, H. and Unemoto, T. (1980) J. Bacteriol. 143, 1187–1193

44 Ahmed, S. and Booth, I.R. (1982) J. Gen. Microbiol., in the press
45 Epstein, W. and Laimins, L. (1980) Trends Biochem. Sci. 5, 21–23
46 Barton, J.K., Den Hollander, J.A., Lee, T.M. MacLaughlin, A., Shulman, R.G. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2470–2473
47 Setlow, B. and Setlow, P. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2474–2476
48 Sörensen, E.M., and Rosen, B.P. (1980) Biochemistry, 19, 1458–1462
49 Segel, I.H. (1976) Biochemical Calculations, pp. 236–237, John Wiley & Sons, New York