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Low-Affinity Potassium Uptake System in *Bacillus acidocaldarius*

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Cells of *Bacillus acidocaldarius* that were grown with 2.7 mM K^+ expressed a low-affinity K^+ uptake system. The following observations indicate that its properties closely resemble those of the *Escherichia coli* Trk and *Streptococcus faecalis* KtrI systems: (i) the *B. acidocaldarius* system took up K^+ with a K_m of 1 mM; (ii) it accepted Rb^+ (K_m of 6 mM; same V_{max} as for K^+); (iii) it was still active in the presence of low concentrations of sodium; (iv) the observed accumulation ratio of K^+ maintained by metabolizing cells was consistent with K^+ being taken up via a K^+-H^+ symporter; and (v) K^+ uptake did not occur in cells in which the ATP level was low. Under the latter conditions, the cells still took up methylammonium ions via a system that was derepressed by growth with low levels of ammonium ions, indicating that in the acidophile ammonium (methylammonium) uptake requires a high transmembrane proton motive force rather than ATP.

The transmembrane proton motive force (PMF) of acidophilic bacteria is composed of a pH difference (ΔpH) of up to 5 U (internally alkaline) and a small membrane potential, which is internally positive below pH_{out} 3 and internally negative above this pH (9, 12, 21, 23, 27, 39). The physiological function of such a composition of the PMF is clearly to maintain the cytoplasmic pH close to neutrality. Most likely, this situation is achieved by an effective electrogenic transport of ions other than protons across the cytoplasmic membrane. This process thereby abolishes the membrane potential generated by the activity of the primary electrogenic proton pumps linked to the respiratory chain of these organisms (7, 27). However, except for a few reports (34; D. McLaggan and A. Matin, Rep. 4th Eur. Bioenergetics Conf., 1986, p. 417), very little is known about the ion composition of and secondary ion movements in acidophiles. In neutrophilic bacteria, K^+ uptake leads to some interconversion of $\Delta\psi$ into ΔpH (6, 16, 19, 24). This led Booth (7) to propose that in acidophiles net K^+ uptake during growth may play an essential role in the conversion of $\Delta\psi$ into ΔpH and thereby in the development of the internally positive membrane potential at low external pH. To test this hypothesis, and because we intended to examine the consequences of a negligibly low membrane potential on bacterial cation transport mechanisms, we investigated the mechanism of K^+ uptake by the acidophilic, moderately thermophilic eubacterium *Bacillus acidocaldarius*. In this and the following communication (3), we report that the organism is able to express two K^+ uptake systems. The properties of the two systems closely resembled those of the K^+ uptake systems Trk and Kdp from *Escherichia coli*. In addition, we show here that *B. acidocaldarius* expresses an ammonium (methylammonium) uptake system when grown at low ammonium concentrations.

MATERIALS AND METHODS

Abbreviations. The abbreviations used in this paper are as follows: PMF, proton motive force; ΔpH, transmembrane pH difference; ΔpNa, transmembrane difference of the negative logarithm of the Na concentration; $\Delta\psi$, membrane potential. For these parameters, Δx is defined as $x_{out} - x_{in}$.

Bacterial strain and growth conditions. *B. acidocaldarius* ATCC 27009 was grown at 55°C in a minimal mineral medium consisting of 9.8 mM $(NH_4)_2SO_4$, 0.47 mM $CaCl_2$, 2.7 mM KH_2PO_4, 1 mM $MgSO_4$, trace elements detailed in reference 27, and 10 mM glucose, final pH 3.5 (10, 27). Cells were harvested by centrifugation after the optical density of the suspension had reached a value between 0.75 and 1.0 at 578 nm.

In experiments in which the effect of salts on cell growth and cell K^+ content was tested, growth medium was inoculated with cells from an overnight culture to an initial optical density at 578 nm of 0.05. The suspension was shaken at 59°C. The salts were added after the optical density of the suspension had reached a value of about 0.2. Samples for the determination of the K^+ content of the cells were withdrawn after the optical density of the control cells had reached a value of 0.8. The cells from these samples were centrifuged through a 1:1 mixture of silicone oils AR20 and AR200 (D = 1.025; Wacker Chemie, Munich, Federal Republic of Germany), and the K^+ content of the pellets was determined as described below.

Cells with a derepressed ammonium uptake system were grown at 55°C in the medium described above, except that 10 mM arginine replaced ammonium sulfate as the nitrogen source.

K^+-depleted cells. Cells harvested from 1 liter of culture were suspended at a concentration of 0.5 mg of cell protein per ml of buffer containing 50 mM NaCl and 25 mM sodium citrate, pH 5.5. This suspension was shaken for 30 min at 40°C in a Gyrotory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) bath at 200 rpm. Subsequently, the cells were centrifuged and washed three times with the same buffer, but at pH 3.5 (suspension buffer). The cells were then suspended at a concentration of about 5 to 10 mg of cell protein per ml of suspension buffer and shaken at 40°C until the start of the experiment.

Cells that were depleted of Na^+ and K^+ were treated similarly, except that choline replaced the Na^+ in the incubation and suspension buffers. These cells are referred to as choline-loaded cells.

K^+ uptake. K^+-depleted cells or choline-loaded cells were suspended at a concentration of 0.5 mg of cell protein per ml of suspension buffer or suspension buffer in which Na^+ had

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been replaced by choline. After the addition of 10 mM of glucose, the suspension was shaken for 10 min at 40°C, and KCl was then added at the concentration indicated in the experiments. At different times, samples of 1.0 ml were withdrawn from the suspension and layered on top of 200 μl of silicone oil (D = 1.03 to 1.04; Rotatherm H oil; Carl Roth, Karlsruhe, Federal Republic of Germany; or AR200 oil) in a 1.5-ml polypropylene microcentrifuge tube (Carl Roth, Sarstedt, Numbrecht, Federal Republic of Germany). The cells were centrifuged through the oil in a Beckman B microcentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The cell pellet was suspended in 1.0 ml of 5% trichloroacetic acid, and this suspension was boiled for 5 to 10 min. After the addition of 3.0 ml of 6.7 mM CsCl, cell debris was removed by centrifugation and the K^+ (and often also the Na^+) content of the supernatant was determined by flame photometry (Eppendorf instrument, Hamburg, Federal Republic of Germany).

Uptake of other cations. The uptake of $^{86}Rb^+$ (final concentration from 20 to 100 μCi/liter at various RbCl concentrations) or that of [^{14}C]methylammonium (112 μCi/liter; 2 μM methylammonium) by the cells was determined at 40°C by a method similar to that described for K^+ uptake. The cell pellets obtained after centrifugation through oil were analyzed for their content of radioactivity by dissolving them in 0.75 ml of 0.4 M NaOH in a miniscintillation vial (Packard Instruments GmbH, Frankfurt, Federal Republic of Germany), subsequent mixing with 4.5 ml of Hydroluma (Baker, Gross Gerau, Federal Republic of Germany), and counting in a Packard 460C liquid scintillation counter at the appropriate setting.

Calculations. A cell suspension with an optical density of 1.0 at 578 nm was assumed to contain 0.205 g of cell protein per liter, and the volume of the cytoplasm was assumed to amount to 4.1 ml/g of cell protein (27). The concentration ratio of $[K^+]_{in}/[K^+]_{out}$ was calculated from the concentration of K^+ in the cytoplasm and supernatant. The latter was determined by flame photometry in a suspension in which 0.75 ml of supernatant obtained after the microcentrifugation step was mixed with an equal volume of a solution containing 2.5% trichloroacetic acid and 10 mM CsCl.

Other methods. Membrane potential and pH difference of nongrowing cells were determined at 40°C and corrected for probe binding as described previously (27). Protein was determined by the method of Lowry et al. (22), as modified by Hartree (15). Oxygen uptake by the cell suspension was determined with an oxygraph (6). The O_2 concentration of the assay buffer was taken to be 190 μM at 40°C. The osmolarity of the suspension of growing cells was measured in an OM801 osmometer (Vogel, Giessen, Federal Republic of Germany) by using a 200 mosM sorbitol solution as a standard.

Radiochemicals. [^{14}C]methylammonium (50 Ci/mol) and [^{14}C]acetylsalicylic acid (58 Ci/mol) were from Dupont/New England Nuclear, Dreieich, Federal Republic of Germany. KS^{14}CN (59 Ci/mol), ^3H_2O, and ^{86}RbCl were from Amersham-Buchler, Brunswick, Federal Republic of Germany, and [^{3}H]tetraphenylphosphonium bromide (2,400 Ci/mol) was from the Nuclear Research Center, Negev, Israel.

RESULTS

Potassium content of growing cells. The growth medium of *B. acidocaldarius* (10) had an osmolarity of 50 mosM. Growing cells developed a turgor pressure, since the 100 mM of intracellular K^+ alone already exceeded medium osmolarity (Fig. 1B, open circle). In contrast to many other bacteria (25), growth of *B. acidocaldarius* was already inhibited at medium osmolarities above 200 to 300 mosM (Fig. 1A). Sodium citrate caused the strongest inhibition, whereas the smallest effect was caused by sodium chloride (Fig. 1A). The addition of increasing concentrations of these salts caused an increase of cell K^+, with the effect of the phosphate salt being much larger than that of the other salts (Fig. 1B). The relatively large variation in the K^+ content of cells growing in phosphate was related to the growth rate of the cells; slowly growing cells (values for the growth rate constant μ of control cells 0.4 h^{-1}) contained less K^+ than did rapidly growing cells (values of μ of control cells of 0.5 to 0.6 h^{-1}). At high osmolarities, the K^+ content of the cells could not be determined (Fig. 1B, right-hand part), since under these conditions the density of the medium was greater than that of the oil, and the cells could therefore not be pelleted through the oil anymore. We were unsuccessful in finding oils with higher densities through which the cells could still be centrifuged under these conditions. Remarkably, centrifugation without oil also gave unreliable results, since in this situation the pelleted cells lost up to half of the K^+ content between the centrifugation step and the addition of the pellet-containing part of the microcentrifuge tube to the trichloroacetic acid.

The observation (Fig. 1B) that cell K^+ increased with medium osmolarity indicates that in *B. acidocaldarius* K^+ plays a role in maintenance of turgor pressure too. However, the data of Fig. 1A and 1B did not correlate with each other, indicating that growth inhibition at higher osmolarities (Fig. 1A) was caused neither by failure of the cells to take up K^+ (i.e., with sodium citrate) nor by excessive K^+ uptake (sodium phosphate; Fig. 1B).

K^+ depletion. For the measurement of the kinetics of net K^+ uptake, it was necessary to create a condition at which the cells are able to increase their K^+ content. The method to deplete neutrophiles of K^+ by treatment with 2,4-dinitrophenol (4, 29) failed at pH 3.5 with *B. acidocaldarius*, since this led to irreversible damage. This was probably caused by the effect of the protonophore on internal pH (21, 27). Incubation of the cells in the sodium chloride-sodium citrate medium of pH 5.5 (see Materials and Methods), and subsequent washing of the cells with the same buffer but at the pH at which the experiments were done (i.e., pH 2.5 to 5), resulted in metabolically active cells with K^+ contents of 150 to 200 μmol/g of cell protein (40 to 50 mM). These cells increased their K^+ content fourfold, when the cation was added to glucose- or glycerol-metabolizing cells (data not shown).

Kinetic parameters. Initial rates of K^+ uptake by the cells followed Michaelis-Menten kinetics (Fig. 2, open symbols). The values for K_m and V_{max} were independent of pH between pH 2.5 and 4.5 and amounted to 1.0 to 1.1 mM and 50 to 80 $\mu mol \cdot min^{-1} \cdot g^{-1}$ of cell protein, respectively (results of three experiments [data not shown]). These cells also took up Rb^+ (Fig. 2, filled circles), with a K_m of 6 mM at a V_{max} equal to that for K^+ uptake, indicating that the system binds K^+ better than Rb^+ but is able to transport the two ions at the same rate.

Counter ion. Cells that were depleted of K^+ in Na^+-based buffers contained high concentrations of the latter cation (data not shown). During K^+ uptake these cells extruded a stoichiometric amount of Na^+ (Fig. 3, open symbols). The movement of these two ions was not obligatorily coupled, since in choline-loaded cells, which were depleted of most of their K^+ and Na^+ (see Materials and Methods), K^+ uptake

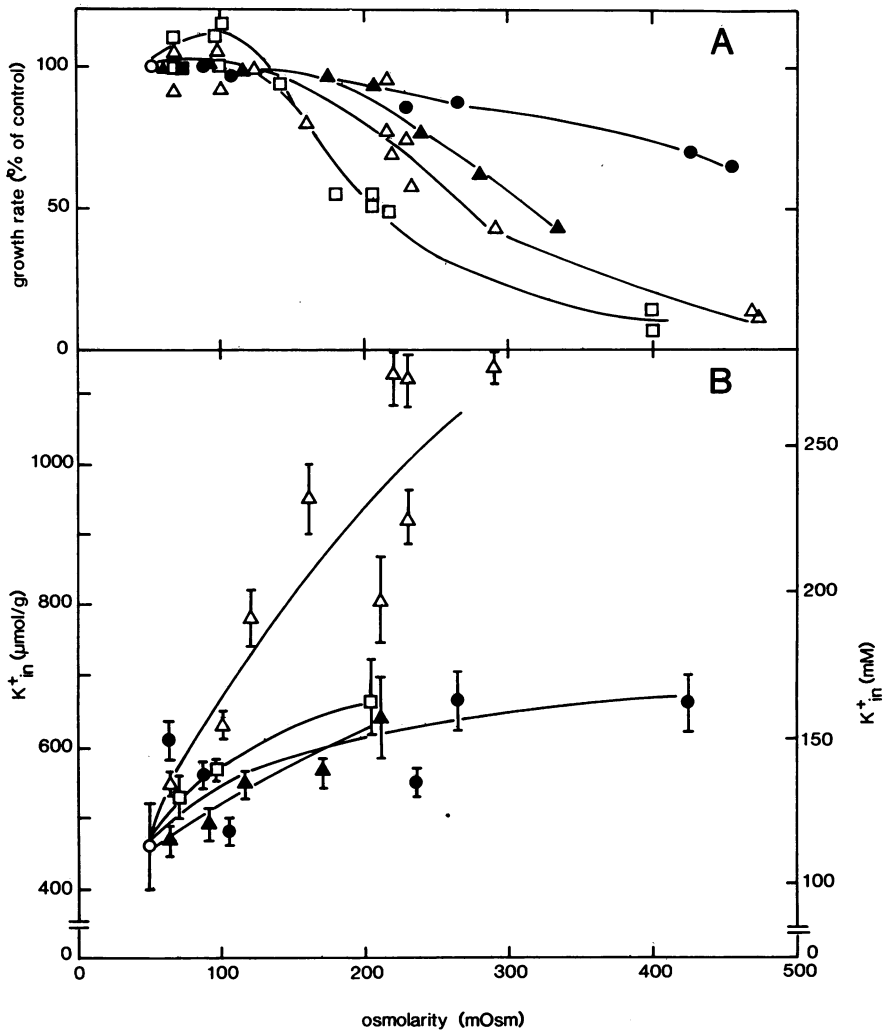

FIG. 1. Growth (A) and K^+ content (B) of *B. acidocaldarius* cells as a function of medium osmolarity. Cells were grown at 59°C in the absence (○) or presence of the following inorganic sodium salts: △, phosphate (present up to 150 mM); □, citrate (present up to 150 mM); ●, chloride (present up to 300 mM); ▲, sulfate (present up to 150 mM). The K^+ content of the cells growing at high osmolarities is not given, because under these conditions the density of the medium was greater than that of the oil, and the cells therefore could not be centrifuged through the oil anymore.

became strongly biphasic (Fig. 3 and 4B, filled symbols). During the initial, rapid phase, K^+ uptake was mainly accompanied by proton extrusion, as indicated by the rapid rise of internal pH (Fig. 4A), and was coupled to only a minor extent with sodium extrusion (Fig. 3, filled symbols). The rapid phase of K^+ uptake ceased after the internal pH of the cells suspended in the choline-based medium had reached a value close to that of Na^+-washed cells (Fig. 4). We assume that under these conditions the process of pH homeostasis of the cells prevented further alkalinization of the cytoplasm and thereby limited exchange between protons and K^+ across the cytoplasmic membrane. The subsequent slow phase of K^+ uptake could then either be coupled to the extrusion of protons derived from metabolically produced weak acids (33) or be coupled to the movement of unidentified other ions. This point was not investigated further. The membrane potential did not change during K^+ uptake by cells loaded with either sodium or choline (results not shown).

We take the results of Fig. 3 and 4 to support two conclusions. (i) Neither Na^+ nor proton extrusion is obligatorily coupled to the uptake of K^+ via the K^+ uptake system investigated. (The alternative that K^+ uptake is coupled to the extrusion of Na^+ or H^+ at the molecular level is rejected in the Discussion section as a less likely possibility.) (ii) According to the criteria developed for *E. coli* (19, 20), *B. acidocaldarius* exerts pH homeostasis towards alkaline pH_{in}.

Energy coupling. We determined the maximal K^+ concentration ratio maintained across the cytoplasmic membrane of the cells. This ratio was compared at different values of pH_{out} with the parameters possibly involved in driving K^+ uptake (Fig. 5). The K^+ accumulation ratio ($\Delta\mu_{K^+} = RT/F \ln([K^+]_{in}/[K^+]_{out})$; Fig. 5, filled circles) was independent of external pH and amounted to values of 1,000 to 1,500. As in neutrophiles (1, 4, 36), K^+ accumulation was apparently not driven by $\Delta\psi$ alone (uniport mechanism), since the membrane potential was much too small, and below pH 3 it possessed the wrong sign to be able to generate the high K^+ accumulation ratios observed (Fig. 5, open and filled circles,

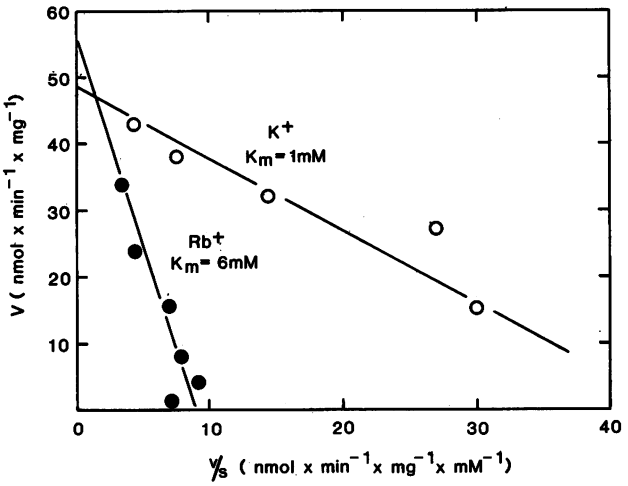

FIG. 2. Kinetics of K^+ and Rb^+ uptake by *B. acidocaldarius* cells. The initial rates of uptake after the addition of KCl or $^{86}RbCl$ to K^+-depleted cells are plotted according to the method of Woolf, Augustinsson, and Hofstee (35).

respectively). The data are, however, consistent with K^+ uptake taking place via a K^+-H^+ symport mechanism. According to this mode of coupling, the driving force for uptake is equal to $2\Delta\psi - 62\Delta pH$ (4), and the value of this parameter was always somewhat larger than the K^+ accumulation ratio and independent of external pH too (Fig. 5, filled squares and filled circles, respectively).

Role of ATP. The constitutive K^+ uptake systems of several bacteria require for activity both a high PMF and a high cytoplasmic ATP level (4, 28). It was therefore investigated whether the system described here also requires ATP. We could not answer this question unequivocally since it turned out that conventional inhibitors of energy coupling in neutrophilic bacteria had unexpected effects on *B. acidocaldarius*. The inhibitor of proton translocation via the F_o part of the ATP-synthase, dicyclohexylcarbodiimide (11), was for instance without any specific effect on energy coupling at pH values up to 5.5. Incubation with the inhibitor at higher external pH caused irreversible inhibition of metabolism. Moreover, weak acid inhibitors like arsenate or

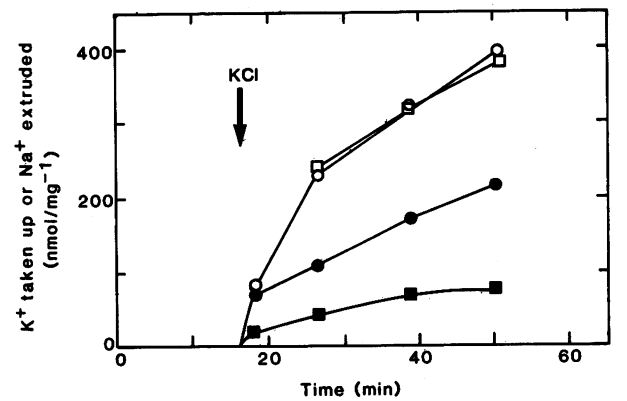

FIG. 3. K^+ uptake and Na^+ extrusion by K^+-depleted cells. The experiment was done either in suspension buffer (○, □) or with choline-loaded cells in cholate-based suspension buffer (●, ■). ○, ●, K^+ uptake; □, ■, sodium extrusion. KCl (1 mM) was added at the indicated time.

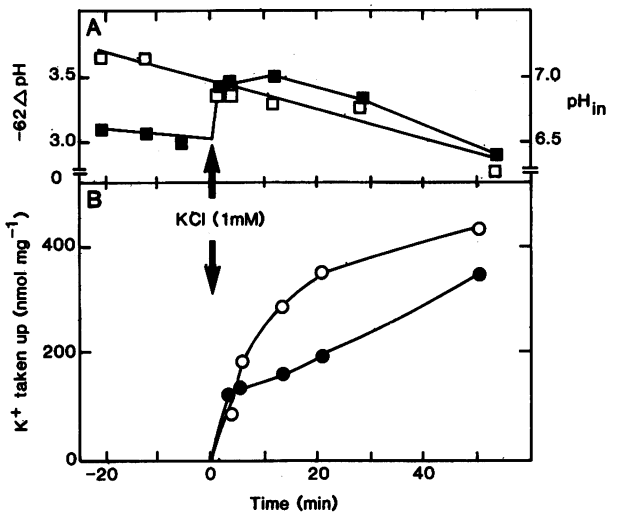

FIG. 4. ΔpH (A) (□, ■) during K^+ uptake (B) (○, ●) by *B. acidocaldarius*. The experiment was done as described in the legend to Fig. 3. The open and filled symbols indicate the same conditions as in Fig. 3. KCl (1 mM) was added at the time indicated.

iodoacetic acid both exerted very strong effects on internal pH, which are unlikely to be the direct result of their interference with glycolysis only (data not shown). We observed, however, (i) that between pH 2.5 and 4.5, when the cells maintained steep gradients of K^+ (Fig. 5), the cells also contained between 7 and 11 μmol of ATP per g of cell protein and (ii) that the addition of 30 mM of acetate

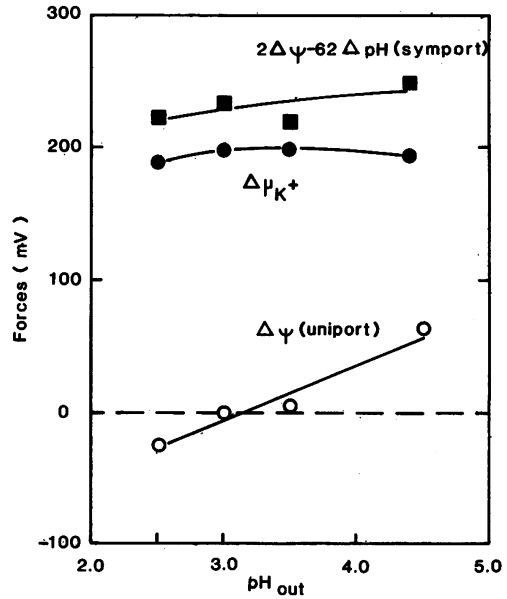

FIG. 5. K^+ accumulation according to a K^+-H^+ symport mechanism. K^+-depleted cells were shaken at 0.5 mg/ml of suspension buffer at 40°C. Glycerol (50 mM) and KCl (200 μM) were added at zero time, and the K^+ concentration in cells and medium was determined as a function of time. The steady-state value of the K^+ accumulation ratio given as $\Delta\mu_{K^+}$ (= RT/F ln $([K^+]_{in}/[K^+]_{out})$ (●) was compared with the membrane potential ($\Delta\psi$) (○) and the parameter $2\Delta\psi - 62\Delta pH$ (■) that is the driving force for K^+ uptake if the system is a K^+-H^+ symporter. See the text for further details.

TABLE 1. Effect of acetate on cation uptake and energetics of *B. acidocaldarius*[a]

[Acetate] (mM)	ATP level[b]	ΔpH[b]	Internal pH	Oxygen uptake[b]	K^+ uptake[b]	Methylammonium uptake[b]
0	100	100	6.9	100	100	100
10	20	92	6.6	60	40	60
30	3	80	6.2	20	2	55

[a] Cells were grown on arginine and depleted of K^+ in Na^+-containing buffers as described in Materials and Methods. The experiment was done at pH 3.5.

[b] Expressed as a percentage of the control value. These values were: ATP level, 11 μmol g^{-1}; ΔpH, 3.4 U; rate of oxygen uptake, 420 μatoms of O min^{-1} g^{-1}; extent of K^+ uptake, 520 μmol · g^{-1}; extent of methylammonium uptake, 2.1 μmol g^{-1}.

specifically reduced the ATP level of the cells to a very low level. Under these conditions, ΔpH was reduced by 20% (internal pH 6.2) and the rate of oxygen uptake was reduced by 80%. However, K^+ uptake was completely abolished (Table 1), suggesting a correlation between ATP level and the occurrence of K^+ uptake. The observation that in the presence of 30 mM acetate these cells still took up methylammonium ions (Table 1) served as a positive control. The V_{max} value and extent of K^+ uptake were, however, 1 to 2 orders of magnitude larger than those of methylammonium uptake. Since it is also possible that the process of K^+ uptake is more sensitive to low internal pH than is methylammonium uptake, we consider the data of Table 1 only to give preliminary evidence for a requirement of ATP for K^+ uptake via the low-affinity system.

Ammonium (methylammonium) uptake. Like many other bacteria (17, 18), *B. acidocaldarius* expressed an ammonium (methylammonium) uptake system when an amino acid like arginine replaced ammonium ions as the nitrogen source in the growth medium of the organism (M. Michels, Ph.D. thesis, University of Osnabrück, Osnabrück, Federal Republic of Germany, 1985). Cells grown on arginine took up methylammonium with a V_{max} of 5 μmol · min^{-1} · g^{-1} of cell protein and a K_m of 500 μM. The cells accumulated methylammonium approximately 90-fold. Ammonium was probably the natural substrate of the carrier, since these ions inhibited methylammonium uptake with high affinity (K_i = 8 μM). In neutrophiles, methylammonium (ammonium) uptake is believed to be coupled to the PMF alone (17, 18). Our data (Table 1) are consistent with the view that this is also the situation in *B. acidocaldarius*.

DISCUSSION

Despite the differences in habitat between acidophilic and neutrophilic bacteria, *B. acidocaldarius* contained a low-affinity K^+ uptake system with properties that closely resemble those of the systems KtrI of *Streptococcus faecalis* and Trk of *E. coli* (for a review, see M. O. Walderhaug, D. C. Dosch, and W. Epstein, *in* B. P. Rosen, ed., *Ion Transport in Bacteria*, in press). First, these systems have similar affinities for K^+ with K_m values around 1 mM (4, 29; Fig. 2). Second, they possess similar K^+/Rb^+ specificities with discrimination factors between 6 and 10 (2, 30; Fig. 2). Third, these systems have no specific requirement for Na^+, although in the presence of Na^+, K^+ uptake activity is linked to an almost stoichiometric extrusion of Na^+ (5, 14; Fig. 3 and 4). The data of Fig. 3 and 4 indicate either that at the molecular level K^+ uptake is coupled to either Na^+ or H^+ extrusion or that the observed Na^+ or H^+ extrusion serves a role in maintaining electroneutrality during electrogenic K^+ uptake. The latter explanation is compatible with the result that the distribution ratio of K^+ across the cytoplasmic membrane indicates that K^+ is taken up by K^+-H^+ symport (Fig. 5). The movement of charge has to be compensated during this electrogenic uptake process, and, in the absence of other permeant ions, the cell achieves this apparently by extruding protons or sodium ions (Fig. 3 and 4).

The result shown in Fig. 5 is, however, more difficult to interpret if one assumes that at the molecular level K^+ extrusion is coupled to extrusion of either Na^+ or H^+. If such a carrier were to catalyze an electroneutral exchange of cations, one would expect K^+ to distribute with ΔpH or ΔpNa (31), which was not observed (Fig. 5; unpublished data on ΔpNa). If the carrier were driven by a chemical reaction, high accumulation ratios of K^+ would indeed be possible, but in this situation one would not a priori expect the pH profile of the accumulation of K^+ to follow the pattern as given in Fig. 5. We, therefore, prefer the former mechanism, which is similar to that proposed for neutrophiles (4, 5, 14, 36), namely, that K^+ is taken up in symport with protons and that the observed extrusion of other cations during K^+ uptake serves the maintenance of electroneutrality.

ATP probably activates the low-affinity K^+ uptake systems of *E. coli* and *S. faecalis* (4, 37). With *B. acidocaldarius*, we obtained only preliminary evidence that the low-affinity K^+ uptake system also requires ATP (Results; Table 1). The difficulties encountered were that it was impossible to vary the PMF and the cellular ATP level independently of each other. Lowering of the ΔpH always led to irreversible damage to the cells because internal pH became too low (see for instance the effect of 2,4-dinitrophenol, described in Results). Similarly, compounds effective in diminishing the ATP level of neutrophiles had unusual effects on the acidophile (see Results). We suspect that, because of the similar and special mode of energy coupling of acidophilic bacteria (for reviews, see references 7 and 8), it might turn out to be difficult to vary the PMF or ATP level of other acidophiles too.

It is unclear why treatment with 30 mM acetate caused the cytoplasmic ATP level to diminish to a very low level (Table 1). One possibility is that acetic acid is freely permeant and that the ensuing accumulation of the acetate anion caused swelling of the cells, leading to the effects observed in Table 1. However, in that situation the cells also have to take up large amounts of cations in exchange for the protons that entered the cells during acetic acid uptake and subsequently dissociated in the cytoplasm. Such a mechanism applies to the combined K^+, H^+, and phosphate uptake observed in *E. coli* (32, 38) and might explain the excessive K^+ uptake observed at high phosphate concentrations in *B. acidocaldarius* as well (Fig. 1). However, the addition of acetate led to inhibition, rather than stimulation, of K^+ and NH_4^+ uptake (Table 1). It is also unclear why swelling would exert a specific effect on the ATP level of the cells but leave ΔpH and pH_{in} at relatively high values (Table 1). We propose, therefore, as an alternative that the high cytoplasmic acetate concentration reverses the acetate-kinase reaction (26) and thereby depletes the cells of ATP.

The maximal rate of K^+ transport via the low-affinity system was 50 to 80 μmol min^{-1} g^{-1}, about three times lower than that of oxygen uptake by the cells under similar conditions (results not shown), and must therefore be much lower than the rate of proton extrusion by the cells. This observation, together with the observation that the uptake of K^+ by Na^+-loaded cells does not lead to interconversion of

the components of the PMF (Fig. 3, 4A, and the membrane potential measurements [data not shown]), argues against the proposal (7) that the net uptake of K^+ by the cells makes a major contribution to the formation and maintenance of the large ΔpH and the low or internally positive membrane potential of the acidophile. We cannot, however, exclude the possibility that in cells derepressed for the high-affinity K^+ uptake system, which show faster K^+ uptake rates (3), the uptake of the K^+ cation and the extrusion of anions like chloride, for which a transport system in an acidophile has been described recently (McLaggan and Matin, Rep. 4th Eur. Bioenergetics Conf.), are involved in the development of this potential.

Like many other bacteria (17, 18), *B. acidocaldarius* expresses a high-affinity ammonium (methylammonium) uptake system. The function of the system is probably to counteract the loss of NH_3 from cells growing at low NH_4^+ (17). In neutrophiles, ammonium uptake via this system is generally considered to be driven by $\Delta\psi$ (18). The data of Table 1 indicate that in *B. acidocaldarius* the uptake of methylammonium, which was used to probe the properties of the system, was indeed not linked to ATP. However, the membrane potential alone was also not driving the uptake, since the cells still accumulated methylammonium under conditions at which $\Delta\psi$ was zero or internally positive (Michels, Ph.D. thesis). One possibility is that the carrier functions as an NH_4^+-H^+ symporter. This could be tested by measuring the characteristics of methylammonium uptake by the membrane vesicles described for *B. acidocaldarius* (13). Unfortunately, we have not yet been able to produce such vesicles from arginine-grown cells.

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