ATP-driven Exchange of Na\(^+\) and K\(^+\) Ions by Streptococcus faecalis*

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We describe the characterization of KtrII, a novel potassium transport system of Streptococcus faecalis, first discovered by H. Kobayashi (1982) J. Bacteriol. 150, 506–511. KtrII requires sodium ions and mediates the stoichiometric exchange of internal Na\(^+\) for external K\(^+\). Potassium accumulation is not energized by the electrochemical potentials of either H\(^+\) or Na\(^+\); the energy source is probably ATP. Two lines of evidence indicate that KtrII is a manifestation of the sodium-stimulated ATPase reported earlier (Heefner, D. L., and Harold, F. M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2798–2802). (i) Mutants that lack the ATPase also lack KtrII, and revertants recover both in parallel. (ii) KtrII and the Na\(^+\)-ATPase are induced in parallel when cells are grown on media rich in sodium, particularly under conditions that limit the generation of a proton potential. KtrII is not induced in response to K\(^+\) deprivation. We propose that the Na\(^+\)-ATPase exchanges Na\(^+\) for K\(^+\) ions.

In bacteria, as in other cells and organisms, potassium is the chief intracellular cation. Cytoplasmic K\(^+\) ions activate a number of enzymes and are required for ribosomal protein synthesis. Beyond that, the movements of K\(^+\) and Na\(^+\) ions across the cytoplasmic membrane have been implicated in several homeostatic mechanisms, including the regulation of turgor and of the cytoplasmic pH; they may also help stabilize the proton potential (for reviews see Harold and Alten dorf, 1974; Epstein and Laimins, 1980; Padan et al., 1981; Helmer et al., 1982; Kruhwich, 1983; Booth and Kroll, 1983). Bacteria lack the ouabain-sensitive Na\(^+\),K\(^+\)-ATPase familiar from animal cells. Instead, the transport of K\(^+\) and Na\(^+\) ions is usually mediated by separate transport systems linked to the chemiosmotic proton circulation.

Two distinct potassium transport systems have been recognized in Streptococcus faecalis. The major one, here designated KtrI, is thought to be constitutive and resembles the Trk system of Escherichia coli (Bakker and Harold, 1980). Cells of S. faecalis accumulate K\(^+\) or Rb\(^+\) ions at a rate as high as 70 nEq/min·mg cells, with an approximate $K_0$ of 0.2 mM and an optimal pH near 7. The concentration gradient, $[K^+]_i/[K^+]_o$, can exceed 50,000. The mechanism of energy coupling to KtrI is mysterious. Potassium accumulation requires the cells to generate both ATP and a protonic potential;

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the same is true for the Trk system of E. coli (Rhoads and Epstein, 1977), but the mechanistic significance of this dual requirement is uncertain. The best guess at present is that both Trk and KtrI are secondary K\(^+\)/H\(^+\) symporters regulated by phosphorylation (Bekker and Harold, 1980; Stewart et al., 1985), but experimental support for this proposition is circumstantial.

This paper is concerned with KtrII, a second potassium transport system discovered by Kobayashi (1982), whose properties are quite different. KtrII has an alkaline pH optimum, prefers K\(^+\) to Rb\(^+\), and is markedly stimulated by the presence of Na\(^+\) ions; the energy donor appears to be ATP, and a proton potential is not required. The expression of KtrII depends strongly on growth conditions. Cells grown on the usual complex media have little or no activity, but the system is induced under a variety of conditions that limit K\(^+\) accumulation via KtrI. Such conditions were imposed either by the inclusion of proton-conducting ionophores in the growth medium or by use of mutant AS25 which has a defective $K_F$-ATPase. Kobayashi (1982) designated this new K\(^+\) transport system Kdp by analogy with a derepressible K\(^+\)-ATPase of E. coli (Epstein et al., 1978; Epstein and Laimins, 1980; Helmer et al., 1982). The analogy now appears dubious, and we prefer the noncommittal designation KtrII.

We report here that the accumulation of K\(^+\) ions via KtrII is obligatorily linked to the extrusion of Na\(^+\) ions by the sodium-stimulated ATPase described by Heefner and Harold (1982; Kinoshita et al., 1984). The uptake of K\(^+\) is independent of the membrane potential and of the electrochemical gradients of either Na\(^+\) or H\(^+\) ions, suggesting that the movements of both K\(^+\) and Na\(^+\) ions are directly coupled to ATP hydrolysis. The simplest interpretation of the results is that KtrII represents an activity of the Na\(^+\)-stimulated ATPase, which functions in the intact cell to expel Na\(^+\) ions by exchange for K\(^+\).

EXPERIMENTAL PROCEDURES

Organisms and Growth Media—All the experiments were conducted with S. faecalis (faecium) ATCC 9790 or with mutants derived from this strain. These are strain 7683, a mutant defective in sodium extrusion, and its revertants R-I and R-II (Heefner and Harold, 1980) and mutant AS25, which is defective in the $K_F$-ATPase and in proton extrusion (Kobayashi and Unemoto, 1980; Kobayashi et al., 1982).

Organisms were grown on the complex media NaT (per liter: 10 g of Difco tryptone, 5 g of Difco yeast extract, 10 g of glucose, 8.5 g of Na$_2$HPO$_4$; pH adjusted to 7.5 with NaOH) or KTY (tryp tone, yeast extract, and glucose as above, plus 10 g of K$_2$HPO$_4$; pH adjusted to 7.5 with KOH). In some experiments media were modified further as described in context. Medium 2KTY contains 20 g of K$_2$HPO$_4$ plus yeast extract, tryptone, and glucose. Mutant AS25 tends to revert; to make sure that cell suspensions contained primarily mutant organ-
nisms they were diluted into 2KTY medium, pH 6.0, and those that grew were discarded.

Transport Experiments—Most of our experiments were done with sodium-loaded cells. Cells harvested from overnight cultures were washed twice with 2 mM MgSO4, resuspended in 50 mM sodium maleate (pH 8.0), and incubated at 37°C with the ionophore monactin (2 μg/ml, 15 min). The cells were collected by filtration and washed at least six times with sodium maleate buffer. They were then washed with the buffer to be used in the experiment and suspended in the latter at a density of 1 mg (dry weight)/ml.

A few experiments required cells containing neither Na+ nor K+ ions. These were prepared by incubating sodium-loaded cells in buffer containing 50 mM Tris chloride, 400 mM choline chloride, and 10 mM glucose, pH 8.5 (70 min, room temperature). It is likely that choline is the chief intracellular cation of these cells.

Analytical Procedures—Cell samples were collected by filtration on Millipore filters (pore size 0.45 μm) and were washed with 2 mM MgSO4, Sodium and potassium contents were determined by flame photometry after extraction of the cells with 5% trichloroacetic acid (Harold and Papineau, 1972; Heefner and Harold, 1980). The membrane potential was calculated from the uptake of 42K+ (Bakker and Harold, 1980). The cellular water space was taken to be 1.75 μl/mg, dry weight. Turnover of the potassium pool was examined by the use of 42K as described by Slayman and Tatum (1965).

Unless otherwise noted, membrane vesicles were prepared as described by Kinoshita et al. (1984) from cells harvested in the early stationary phase. The sodium-stimulated ATPase was assayed by the procedure of Kinoshita et al. (1984) in the presence of 0.5 mM DCCD.

Materials—[3H]TPP+ was a gift from Dr. H. R. Kaback, Roche Institute, Nutley, N.J. Other reagents were purchased from standard suppliers including Sigma and New England Nuclear.

RESULTS

Potassium Accumulation by Mutant AS25—According to Kobayashi and Unemoto (1980), mutant AS25 has a lesion in the F1F0-ATPase that impairs proton extrusion and the generation of an electrochemical proton potential. In consequence, Ktr should be inactive. Cells of this mutant nevertheless accumulate K+ ions under certain conditions; our experiments corroborate Kobayashi’s (1982) conclusion that K+ uptake by AS25 is the work of a novel potassium transport system distinct from Ktr.

Fig. 1A shows a typical experiment. Mutant AS25 was grown on medium NaTY; the cells were then loaded with sodium ions and allowed to glycolyze in Na+-Tricine buffer, pH 8.5. Upon addition of 0.5 mM KCl the cells exchanged part of their sodium complement for potassium, establishing a K+ concentration gradient, [K+]i/[K+]o, of approximately 5000. Potassium uptake by AS25 was partially inhibited by DCCD, a powerful inhibitor of the F1F0-ATPase (Fig. 1B) and also by the protonophore TCS (data not shown). However, neither reagent inhibited the initial rate of K+ uptake as measured with 42K+, suggesting that the site of inhibition is not the uptake of K+ into the cells (see below). Even in the presence of both DCCD and TCS the cells established a K+ concentration gradient of nearly 400 (Fig. 1C). Potassium uptake by AS25 was maximal at pH 8.5 to 9, and Rb+ ions were accumulated only to a small degree (data not shown).

The alkaline pH optimum of K+ uptake, the discrimination against Rb+, and the relative indifference to DCCD and to proton conductors distinguish K+ uptake by AS25 from that by the parent strain, 9790 (Bakker and Harold, 1980) and warrant its attribution to a second transport system. Operationally, we recognize KtrII by its capacity for DCCD-resistant uptake of K+ ions (but not of Rb+ ions) at pH 8.5. A second criterion, an absolute dependence on sodium ions, will be described below. In our experience, K+ uptake by AS25 must be attributed entirely to KtrII.

Conditions for the Expression of KtrII—As a matter of convenience most of our experiments were done with mutant AS25 which effectively lacks KtrI. In the parent strain, 9790, significant activity of KtrII was observed only when the cells were grown under special conditions (Kobayashi, 1982). Table I summarizes a series of experiments in which AS25 and 9790 were grown on various media; the activity of KtrII was then assayed by the accumulation of K+ under a standard set of conditions.
conditions specified in the legend. We interpret the results to mean that KtrII is induced when the cells were grown in media rich in sodium and under conditions that reduce the proton motive force, whether by mutation or for other reasons. Cells of 9790 grown on NaTY medium had low but significant KtrII activity. Note that growth on potassium-deficient medium was neither necessary nor sufficient to elicit expression of KtrII and that the presence of high K+ concentrations did not repress it.

**System KtrII Mediates Exchange of Intracellular Na+ for Extracellular K+**—We mentioned above that KtrII was induced only when the cells were grown on sodium-rich media, and Kobayashi (1982) reported that Na+ ions were required for its operation. Fig. 2 documents that K+ uptake by KtrII specifically requires intracellular Na+ ions. Mutant AS25 was grown on NaTY medium, and the cells were then depleted of both K+ and Na+ ions as described under "Experimental Procedures." Such cells glycolyzed well (30-40 nmol of H+/mg cells-min) but took up K+ only when the medium was supplemented with Na+ (Fig. 2A). By contrast, sodium-loaded cells took up K+ in the absence of external Na+ (Fig. 2B). We would emphasize that the total amount of Na+ present in the latter cells if released into the medium would bring the external Na+ concentration to 1 mM; this level of Na+ supported limited K+ uptake and at a reduced rate. We infer that K+ uptake via KtrII requires intracellular Na+ ions.

Fig. 3 documents the complementary conclusion that expulsion of Na+ ions from AS25 is markedly stimulated by external K+. This is an important point. Under the conditions of the present experiments (50 mM Na+-Tricine, 2 mM KCl or RbCl, pH 8.2, DCCD) the wild-type strain 9790 extruded only a small part of its sodium complement by exchange for K+ or Rb+ (Fig. 3A). The reason, we believe, is that inhibition of the F1F0-ATPase by DCCD abolished the activity of KtrI, while KtrII activity is minimal (Table I). Cells grown on KTY did not extrude Na+ ions at all (data not shown); this suggests that the minimal sodium extrusion seen in Fig. 3A is due to KtrII rather than to residual KtrI activity. By contrast with the parent strain, AS25 slowly but steadily exchanged internal Na+ for external K+; Rb+ was a poor substitute (Fig. 3B). We attribute this sodium extrusion to the capacity of KtrII to exchange Na+ for K+ ions. Cells of AS25 grown on 2KTY medium, which does not induce KtrII, were unable to expel Na+ ions (Fig. 3C).

**The Sodium-ATPase Is Part of KtrII**—We know from earlier work that sodium extrusion by S. faecalis 9790 is a primary process, probably mediated by a sodium-translocating ATPase (Heefner and Harold 1980, 1982). Very recently, Kinoshita et al. (1984) discovered that mutant AS25 produces elevated levels of the Na+-stimulated ATPase when grown on NaTY medium and so does the parent strain 9790 when grown on NaTY medium plus CCCP. Since these conditions also induce KtrII which, moreover, requires internal Na+ ions, we asked whether the Na+-stimulated ATPase plays a role in that potassium transport system.

Mutant 7683 lacks the capacity to extrude Na+ ions and also lacks the Na+-stimulated ATPase (Heefner and Harold, 1982). When grown on medium NaKTY plus CCCP, conditions that induce KtrII in the parent strain, no DCCD-resistant potassium uptake was observed (Table II). Two revertants of this mutant were also examined. R-I, which has recovered only limited capacity for sodium extrusion (possibly attributable to a Na+/H+ antiporter) and does not contain Na+-stimulated ATPase, again lacked KtrII activity. By contrast R-II, which has recovered the sodium-stimulated ATPase, contained KtrII activity as well (Table II). These observations suggest that KtrII is a manifestation of the sodium-stimulated ATPase.

The Steady-State K+ Level—Our observations, like those of Kobayashi (1982), indicate that KtrII is a primary transport process whose energy source is ATP. We must then account for the partial inhibition of K+ accumulation by DCCD and TCS, which do not inhibit the Na+-ATPase (Heefner and Harold, 1982; Heefner et al., 1980). The following experiments suggest that the K+ concentration gradient attained by KtrII is a steady state in which rapid influx is balanced by rapid efflux and that the inhibitors selectively accelerate efflux.

Fig. 4 depicts net potassium efflux from cells of AS25. The suspension was first allowed to accumulate K+ ions; at the steady state [K+] was 257 mM, [K+]i was 0.05 mM, and the concentration gradient about 5000. The suspension was then diluted 10-fold with fresh buffer; in the course of the next hour [K+] fell to 114 mM, [K+]o rose back to 0.03 mM, and the gradient stabilized near 4000. Cells incubated with 0.05 mM K+ from the beginning attained the same steady-state K+ content. Addition of DCCD to cells that had first accumulated K+ also elicited net efflux. [K+] fell to 257 to 200 mM, and the concentration gradient was only 1400.

Given that the potassium pool represents a steady state, does DCCD reduce entry of K+ or stimulate exit? Fig. 5 shows that the turnover of the K+ pool was more rapid in the presence of DCCD than in its absence; the rate constant for influx was almost unaffected, but that for efflux was elevated 3-fold (legend). Separate experiments confirmed that the initial rate of net K+ uptake was almost unaffected by DCCD or by TCS: 25 nmol/min/mg cells in the controls, 20 nmol in the presence of DCCD or TCS. We conclude that the inhibition of K+ accumulation by DCCD (and also by TCS; data not shown) is primarily due to enhanced K+ efflux.

We do not know how DCCD and TCS enhance K+ efflux from AS25, but it may be pertinent that the inhibitors reduced the electrical potential difference across the plasma membrane. AS25 is thought to have a defective F1F0-ATPase, and its capacity to extrude protons is limited (Kobayashi and Unemoto, 1980). Nevertheless, cells of AS25 (grown on NaTY) glycolyzing in Na+-Tricine buffer maintained a membrane potential of approximately −50 mV as judged by [H] TPP* uptake. In the presence of DCCD (0.2 mM) or of TCS (10 μM) this was reduced to approximately −30 mV. A number of other reagents that lower the steady-state K+ level, including the lipophilic cations triphenylmethyolphosphonium ion (1 mM) and dibenzyldimethylammonium ion (5 mM), may also exert their effects by modulating the membrane potential.
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FIG. 3. Potassium ions are required for Na⁺ extrusion.  *S. faecalis* 9790, or mutant AS25, were grown on the media listed below; the cells were loaded with Na⁺ and resuspended in 50 mM Na⁺-Tricine buffer, pH 8.2.  All suspensions received DCCD (0.2 mM) at -15 min.  The sodium content of the initial cells was designated as 100%; absolute Na⁺ levels are listed below.  A, parent strain 9790 grown on NaTY medium, 1.05 pmol of Na⁺/mg of cells; B, AS25 grown on NaTY, 0.85 µmol of Na⁺/mg of cells; C, AS25 grown 2KTY medium, 0.95 µmol of Na⁺/mg of cells.  All-quant of each suspension received the following additions: 0, none; O, plus 16 mM glucose at -5 min.  ■, plus glucose, followed by 2 mM RbCl at 0 min (arrow).  ▲, plus glucose, followed by 2 mM KCl at 0 min (arrow).

TABLE II

Correlation between KtrII activity and the Na⁺-stimulated ATPase

Cells grown on the media listed below were harvested in the early stationary phase.  KtrII activity was assayed with sodium-loaded cells suspended in 50 mM Na⁺-Tricine buffer, pH 8.5, in the presence of glucose, 0.2 mM DCCD, and 0.5 mM KCl.  Na⁺-ATPase activity was assayed as described under “Experimental Procedures,” with and without 25 mM NaCl.  The number in parentheses is the basal activity in the absence of Na⁺.  Please see legend to Table I for explanation of media.

| Strain | Medium          | Initial rate of K⁺ uptake | [K⁺]/[K⁺] in steady state | Na⁺-ATPase |
|--------|-----------------|---------------------------|---------------------------|------------|
|        |                 | [K⁺] nmol/mg of cells     | [K⁺] nmol/mg protein      |            |
| 9790   | KTY             | 0.0                       | 1                         | 0.5 (18)   |
| 9790   | KNaTY           | 1.6                       | 180                       | 19 (46)    |
| 9790   | KNaTY plus 20 µM CCCP | 5.3         | 490                       | 42 (117)   |
| 7083   | KNaTY plus CCCP | 0.0                       | 1                         | 0.6 (73)   |
| R-I    | KNaTY plus CCCP | 0.0                       | 1                         | 5 (75)     |
| R-II   | KNaTY plus CCCP | 3.8                       | 390                       | 26 (117)   |
| AS25   | NaTY            | 15                        | 800                       | 26 (44)    |

*These membrane vesicles were prepared as described by Heefner and Harold (1982).

DISCUSSION

On the basis of Kobayashi’s paper (1982) and of the present study, the characteristics of KtrII can be summarized as follows. (i) KtrII selects K⁺ over Rb⁺ but has a relatively low affinity (Kₐ approximately 0.5 mM); the optimal pH is about 8.5. (ii) Potassium uptake requires Na⁺ ions to be present in the cytoplasm; overall, KtrII mediates equimolar exchange of Na⁺ for K⁺ ions. (iii) Potassium uptake by KtrII requires the cells to generate ATP but does not depend on a proton potential. (iv) KtrII is expressed by cells growing in sodium-rich media, particularly under conditions that limit the generation of a normal proton potential; it is not produced in response to potassium deprivation or is repressed by excess potassium.  We infer from these characteristics that KtrII is quite unlike the Kdp system of *E. coli*, functionally as well as mechanistically.  KtrII cannot scavenge traces of K⁺ from a deficient medium; its function is to permit growth under conditions that render KtrI inoperative.  The conditions that induce KtrII expression suggest that a rise in the cytoplasmic Na⁺ level, rather than a lowered level of K⁺, is the effective signal.

We began these studies in the expectation that KtrII was a porter of some kind, possibly a Na⁺/K⁺ symporter that allows the cells to accumulate K⁺ in response to a sodium gradient established by the sodium pump.  The data presented above are clearly incompatible with this notion.  Fig. 1A shows that the cells maintain a gradient of potassium concentration, [K⁺]/[K⁺]₀ of 5000, while [Na⁺]/[Na⁺]₀ is about 10; the sodium gradient is in the wrong direction to contribute to the driving force upon a symporter.  A Na⁺/K⁺ antiport mechanism might be consistent with Fig. 1 but would not explain K⁺ accumulation under the conditions of Fig. 2A in which [Na⁺] is lower than [Na⁺]₀ (data not shown).  The electrical potential is too small to rescue the hypothesis that KtrII is a
At this time (0 min), "K+ of high specific activity was added to both pH 8.5, plus 10 mM glucose and 1 mM KCI. To account even for the reduced K+ accumulation shown in the experiment, the level of the Na+-ATPase is marked elevated in AS25 but observed little or no stimulation of its activity by K+ ions. This must be counted as an objection to our hypothesis, but not necessarily a fatal one. Under the assay conditions presently employed, even Na+ ions stimulate the rate of ATP hydrolysis by less than 2-fold, and there is a high basal activity that requires neither Na+ nor K+. Whether the in vitro activity of the Na+-ATPase is representative of that in the intact cells remains open to doubt. We suspect that the coupling between K+ and Na+ ions is less direct than it is in the ouabain-sensitive ATPase of animal cells but will refrain from pursuing this speculation.

Fig. 4. Net K\textsuperscript+ efflux from AS25. Organisms were grown on NaTY, loaded with Na\textsuperscript+, and resuspended in 50 mM Na-Tricine buffer, pH 8.5, at 1 mg of cells/ml. Glucose (10 mM) was added at -20 min, 0.5 mM KCI at 0 min. Subsequent treatments were as follows: O, control, no additions; 1, plus 0.2 mM DCCD at 50 min; 2, at 50 min, diluted the cell suspension 10-fold with Na-Tricine buffer plus glucose; 3, a parallel cell suspension in Na+-Tricine buffer plus glucose and 0.05 mM KCI, at a density of 0.1 mg of cells/ml.

Fig. 5. DCCD increases the steady-state turnover of the potassium pool. AS25 was grown on NaTY medium; the cells were loaded with Na\textsuperscript+ and incubated for 1 h in 50 mM Na+-Tricine buffer, pH 8.5, plus 10 mM glucose and 1 mM KCI, O, control; [K\textsuperscript+], 464 mM, [K\textsuperscript-], 0.2 mM; 2, plus 0.2 mM DCCD; [K\textsuperscript+], 273 mM, [K\textsuperscript-], 0.5 mM. At this time (0 min), "K" of high specific activity was added to both suspensions, and turnover of the pool was monitored; the cells' K\textsuperscript+ content remained constant for the duration of the experiment. The following rate constants were calculated: control, influx 5.4 x 10\textsuperscript{-9} min\textsuperscript{-1}, efflux 2 x 10\textsuperscript{-8} min\textsuperscript{-1}; plus DCCD, influx 8.6 x 10\textsuperscript{-9} min\textsuperscript{-1}, efflux 6 x 10\textsuperscript{-8} min\textsuperscript{-1}.

porter. Glycolyzing cells of AS25 grown on NaTY do generate a membrane potential of about -50 mV under certain conditions, by an unknown mechanism. Addition of DCCD and/or TCS reduced this to -30 mV, a potential gradient too small to account even for the reduced K\textsuperscript+ accumulation shown in Fig. 1, B and C. Our experiments gave no hint of the participation of chloride or of other ions. If K\textsuperscript+ accumulation is to be attributed to either symport or antiport with Na\textsuperscript+ ions or protons one must postulate either a strange stoichiometry or else a localized ion current. On balance, we conclude that KtrII is not a porter but a primary transport system in which a chemical reaction supplies the driving force to exchange Na\textsuperscript+ for K\textsuperscript+.

The obvious candidate for such a reaction is the sodium-stimulated ATPase discovered by Heefner and Harold (1982). Two lines of argument support the identification of KtrII with this sodium pump. (i) Growth conditions that lead to the expression of KtrII consistently induced enhanced levels of the sodium-stimulated ATPase (Table I; Kinoshita et al. (1984). (ii) Mutant 7683, which lacks the sodium-stimulated ATPase, lacks KtrII; and revertants that recover the former also possess the latter. There is also a qualitative correlation between the level of Na\textsuperscript+-ATPase and the initial rate of K\textsuperscript+ uptake (Table II). Previous papers from this laboratory suggested that the sodium-stimulated ATPase mediates exchange of Na\textsuperscript+ for H\textsuperscript+ (Heefner et al., 1980; Heefner and Harold, 1982), but the evidence bearing on the identity of the counterion was never strong. The present results suggest that, at least in AS25, the enzyme exchanges Na\textsuperscript+ for K\textsuperscript+. We cannot distinguish between two possible formulations. (i) The Na\textsuperscript+-ATPase has relatively low specificity with respect to its counterion, accepting H\textsuperscript+ in place of K\textsuperscript+ under some conditions. For all we know, even Tris may be a low-affinity substrate. On this view, what we call KtrII is simply the expression of high levels of the Na\textsuperscript+-ATPase. (ii) It remains possible that KtrII is a modified form of the Na\textsuperscript+-ATPase that exchanges Na\textsuperscript+ for K\textsuperscript+ rather than for H\textsuperscript+. We prefer the former hypothesis because it is simpler.

If it were true that the Na\textsuperscript+-stimulated ATPase of AS25 or 9790 catalyzes exchange of Na\textsuperscript+ for K\textsuperscript+, one should expect cells grown under the appropriate conditions (Table I) to contain a membrane-bound ATPase that is stimulated synergistically by Na\textsuperscript+ and K\textsuperscript+ ions. We have confirmed the finding of Kinoshita et al. (1984) that the level of the ATPase is markedly elevated in AS25 but observed little or no stimulation of its activity by K\textsuperscript+ ions. This must be counted as an objection to our hypothesis, but not necessarily a fatal one. Under the assay conditions presently employed, even Na\textsuperscript+ ions stimulate the rate of ATP hydrolysis by less than 2-fold, and there is a high basal activity that requires neither Na\textsuperscript+ nor K\textsuperscript+. Whether the in vitro activity of the Na\textsuperscript+-ATPase is representative of that in the intact cells remains open to doubt. We suspect that the coupling between K\textsuperscript+ and Na\textsuperscript+ ions is less direct than it is in the ouabain-sensitive ATPase of animal cells but will refrain from pursuing this speculation.

We note in passing that our conclusions concerning the nature of KtrII are virtually identical to those drawn by Benyoucef et al. (1982a, 1982b) regarding the uptake of K\textsuperscript+ ions by Mycoplasma mycoides; in these organisms, also, exchange of Na\textsuperscript+ for K\textsuperscript+ appears to be mediated by an ATPase that is stimulated only by Na\textsuperscript+ ions.

Fig. 6. Potassium transport in AS25: an interpretation. This mutant has a defective F,F\textsubscript{0}-ATPase (1). KtrI (2) is assumed to be a potassium/proton symporter that functions only when it is phosphorylated. In the parent strain, 9790, the F,F\textsubscript{0}-ATPase generates the protein potential that supports K\textsuperscript+ accumulation via KtrII. AS25 cannot sustain a large proton potential; potassium uptake by this strain depends on KtrI (9), the Na\textsuperscript+-stimulated ATPase that exchanges cytoplasmic Na\textsuperscript+ for external K\textsuperscript+. If it is not clear whether the Na\textsuperscript+-ATPase is specific for K\textsuperscript+ or can also accept H\textsuperscript+ and other ions, KtrI functions chiefly in the direction of K\textsuperscript+ efflux, setting a limit to the potassium concentration gradient. There must be at least one route for Na\textsuperscript+ ions to enter the cell (4); the nature of this pathway is not known.
Another doubtful matter is the relationship between the KtrII, the vanadate-insensitive Na\(^+\)-ATPase, and the exquisitely sensitive K\(^+\)-stimulated ATPase isolated by Hugentobler et al. (1983). When intact streptococci were loaded with vanadate by incubation with 0.1 mM vanadate plus glucose, KtrI and KtrII activities were both unaffected, but calcium extrusion was blocked. In our view, the ATPase of Hugentobler et al. (1983) corresponds to neither of the two potassium transport systems known to exist in \(S.\) \textit{faecalis}.

Returning now to the physiology of the cell, what is the nature of the K\(^+\) efflux pathway that sets a limit to the K\(^+\) concentration gradient attained by KtrII? The existence of an efflux system was reported by Bakker and Harold (1980), who noted the rapid loss of K\(^+\) from cells glycolyzing in the presence of DCCD or of proton-conducting ionophores. Much earlier, Harold et al. (1967) isolated a mutant of \(S.\) \textit{faecalis} that was leaky to K\(^+\); yet the only known defect in that mutant is a reduced ability to extrude protons (Harold and Kroll, 1983); they seem likely to play a major role in the homeostatic functions of ion transport.

To account for K\(^+\) accumulation by KtrII we must invoke an additional element, a pathway for Na\(^+\) ions to enter the cells. The existence of at least one such pathway in \(S.\) \textit{faecalis} is established (Heefner and Harold, 1980), but its nature is unclear; Fig. 6 shows a dashed arrow to acknowledge this fact. Ill-defined pathways for Na\(^+\) entry have also been postulated in other bacteria (Padan et al., 1981; Krulwich, 1983; Booth and Kroll, 1983); they seem likely to play a major role in the homeostatic functions of ion transport.

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\(^2\) Y. Kakinuma, unpublished data.