Journal of Bioenergetics and Biomembranes, Vol. 21, No. 6, 1989

MINI-REVIEW

Sodium-Translocating Adenosine Triphosphatase in *Streptococcus faecalis*

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Received March 20, 1989

Abstract

Sodium-transloating ATPase in the fermentative bacterium *Streptococcus faecalis* exchanges sodium for potassium ions. Sodium ions stimulate its activity, but K^+ ions have no significant effect at present. Although the molecular nature of the sodium ATPase is not clear, the enzyme is distinct from other ion-motive ATPases (E_1E_2 type and F_1F_0 type) as judged by its resistance to vanadate as well as dicyclohexylcarbodiimde. The sodium ATPase is induced when cells are grown on media rich in sodium, particularly under conditions that limit the generation of a proton potential or block the constitutive sodium/proton antiporter, indicating that an increase in the cytoplasmic sodium level serves as the signal. The enzyme is not induced in response to K^+ deprivation. The sodium ATPase may have evolved to cope with a sodium-rich environment under conditions that limit the magnitude of the proton potential.

Key Words: Na^+-ATPase; inducibility; Na^+/H^+ antiporter; proton potential.

Introduction

The chemiosmotic proton circulation provides the unifying framework for bacterial energetics, but a sodium current plays a significant supporting role. Bacteria actively extrude sodium ions and maintain a sodium concentration gradient directed inward. The significance of sodium potential in bacteria is well recognized (Lanyi, 1979; Skulachev, 1988). The mechanism of sodium extrusion is generally thought to be a secondary antiport of sodium for

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0145-479X/89/1200-0679$06.00/0

protons energized by the protonmotive force; just as Mitchell envisaged (Mitchell, 1976; Harold and Altendorf, 1974; Krulwich, 1983). However, the activity of the Na^+/H^+ antiporter is supplemented by a variety of primary transport systems energized by ATP hydrolysis, redox potential or decarboxylation, and organisms differ from one another in their complement of transport systems (Harold, 1986; Skulachev, 1985, 1988). Thus, the movement of Na^+ ions illustrates the interplay between the primary and secondary modes of energy-linked transport.

This review is concerned with the sodium-translocating ATPase discovered in the fermentative bacterium *Streptococcus faecalis* (Heefner and Harold, 1982). We shall trace the development of the Na^+-ATPase model, which grew out of Mitchell's antiport hypothesis, and discuss the significance of the sodium circulation in streptococci.

The Trail to Na^+-ATPase

The Na^+/H^+ Antiport Model

Streptococci have several virtues as experimental organism: first they lack respiratory chains and anaerobic redox pathways, relying on glycolysis or arginine metabolism for the production of ATP. Second, streptococci are susceptible to ionophores and inhibitors that act on the cell membrane. Third, they are energy-depleted within minutes after withdrawal of the substrate because they generally lack reserve polymers. Taking advantage of these characteristics, Harold and his colleagues contributed most of the available information on Na^+ transport in *S. faecalis*. Interestingly, the early studies on Na^+ transport in *S. faecalis* were interpreted as support for a Na^+/H^+ antiporter driven by the proton potential (Fig. 1a). Sodium extrusion from the cells against the Na^+ gradient was blocked by DCCD,[3] an inhibitor of the proton-translocating ATPase, suggesting that sodium efflux requires a proton potential. Furthermore, H^+ influx accompanying Na^+ efflux was observed in alkalinized Na^+-loaded cells metabolizing in Na^+-free buffer. In this experiment the driving force for Na^+ efflux was presumably the Na^+ gradient directed outward since DCCD prevented establishment of the proton potential (Harold and Papineau, 1972).

These findings provided the first substantial support for a Na^+/H^+ antiporter in bacteria (West and Mitchell, 1974). Indeed, the observation that monensin, an antibiotic which exchanges Na^+ for H^+, could partially replace

[3]Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; TCS, tetrachlorosalicylanilide.

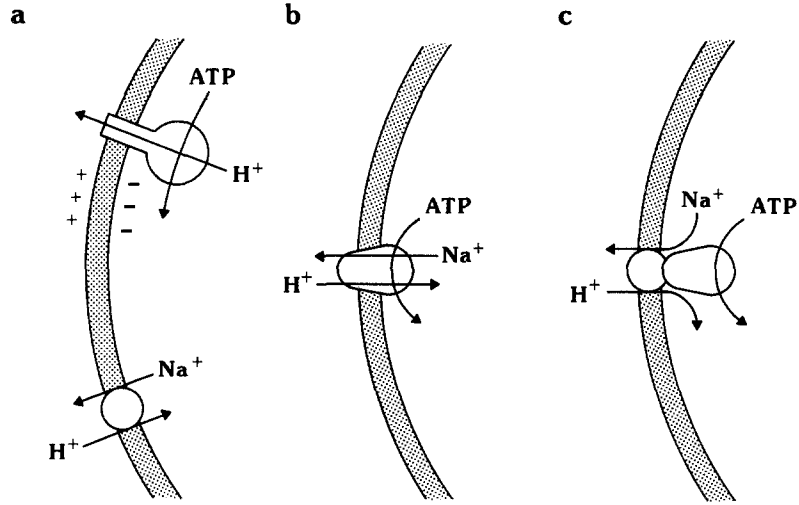

Fig. 1. Development of the Na^+ transport model. (a) The Na^+/H^+ antiporter; (b) the Na^+, H^+-ATPase; (c) the modular pump model.

the defective antiporter in mutant 7683 agreed well with the Na^+/H^+ antiporter model (Harold and Papineau, 1972).

Discovery of the Na^+-ATPase

Although most of the data on Na^+ extrusion fit the sodium/proton antiport model, there was one important observation which could not be easily explained by this model: net Na^+ movement and $^{22}Na^+/Na^+$ exchange were seen only in cells capable of generating ATP (Harold and Papineau, 1972). Why should Na^+ transport require ATP? The search for an answer led to the discovery of the sodium-translocating ATPase in *S. faecalis*.

In principle, the ATP requirement for sodium movement could have several explanations. One possibility is that the Na^+/H^+ antiporter requires the proton-translocating ATPase to expel protons, not only to generate the proton potential but also to alkalinize the cytoplasm. Alternatively, the sodium extrusion system may itself require the presence of ATP, either as an energy donor or in a regulatory capacity. To resolve these possibilities, Heefner and Harold examined whether sodium can be expelled against a concentration gradient, under conditions such that the proton circulation has been short-circuited. Potassium-loaded cells were suspended in a buffer, pH 7.4, containing 450 mM K^+ and 20 mM $^{22}Na^+$. The cell suspensions were treated with TCS and valinomycin, both to ensure that the cytoplasmic pH equalled that of the buffer and to prevent the establishment of any H^+ or K^+ gradient during the experiment. Upon addition of glucose or arginine, $^{22}Na^+$

was quickly expelled against a concentration gradient of about 100-fold. A variety of experiments confirmed that the proton potential was totally dissipated under these conditions. These observations ruled out the Na^+/H^+ antiporter mechanism, and indicated that ATP is the energy donor for active sodium extrusion (Heefner and Harold, 1980).

As described above, the contention that Na^+ extrusion is driven by the proton potential rested on earlier data showing that DCCD and protonophores blocked Na^+ efflux. What is the reason for the discrepancy between the earlier and the later work? Harold and Papineau (1972) worked under conditions such that there was a K^+ concentration gradient directed outward across the membrane. It was subsequently found (Bakker and Harold, 1980) that DCCD elicits K^+ efflux from metabolizing cells under these conditions, apparently by an electrogenic pathway. The primary sodium pump may have been inhibited by the cytoplasmic acidification expected as protons flow into the cells to compensate for the loss of K^+. No sodium extrusion in the presence of TCS and valinomycin was, in fact, observed at pH 6.0 (Heefner and Harold, 1980). Extrusion of sodium in the absence of the proton potential requires not only a high concentration of extracellular K^+ but also a relatively alkaline cytoplasmic pH.

Although these findings with intact cells suggested that ATP is required for Na^+ extrusion, it was a study with everted membrane vesicles that provided conclusive evidence indicating ATP as the direct energy donor. Heefner and Harold prepared everted membrane vesicles, which have the Na^+ pump activity, in the presence of various protease inhibitors to protect it (Heefner and Harold, 1982). These vesicles actively accumulated $^{22}Na^+$ ions when energized with ATP. As with intact cells, Na^+ uptake was not blocked by the combination of DCCD, TCS, and valinomycin, demonstrating once again that the proton potential was not required for Na^+ transport. When precautions were taken to reduce competing ATP-hydrolyzing reactions in everted vesicle preparations, Na^+-stimulated ATPase activity was found consistently in all vesicles showing Na^+ pump activity; no Na^+-ATPase was found in a mutant lacking the Na^+ extrusion system: and another mutant, which showed heightened Na^+ accumulation by everted vesicles, also showed a doubling of Na^+-ATPase activity. These results clearly demonstrate that *S. faecalis* contains sodium-translocating ATPase.

Heefner and Harold regarded the Na^+ pump as being electroneutral since no generation of membrane potential coupled with Na^+ movements could be detected in either intact cells or everted vesicles. Furthermore, both direct measurements of H^+ fluxes (Harold and Papineau, 1972) and studies with fluorescence dyes that monitor the intravesicular pH indicated that H^+ is the charge-neutralizing counterion (Heefner and Harold, 1982). Retaining the concept of a Na^+/H^+ antiporter, they proposed that the ATPase itself exchanges Na^+ for H^+ electroneutrally (Fig. 1b).

Machinery of the Na^+-ATPase

Catalytic Properties

As the Na^+-ATPase has not been purified, the molecular nature of the Na^+-ATPase is not clear. However, some important properties of this enzyme were elucidated with membrane vesicles; ATPase activity was maximal at pH 8.0–9.0, but not detectable at pH 6.0. It is saturated by Na^+ ions at the 20–25 mM level; the K_m value of the ATPase for Na^+ was 5–7 mM. Li^+ at 10 mM also enhanced ATPase activity, but K^+ and Ca^{2+} did not (Heefner and Harold, 1982; Kinoshita *et al.*, 1984; Kakinuma and Harold, 1985).

Two other ATPases have been identified in *S. faecalis*: the familiar F_1,F_0-ATPase and a vanadate-sensitive ATPase which Fürst and Solioz regard as the major K^+ uptake system (KtrI) (Fürst and Solioz, 1986). The Na^+-ATPase is quite distinct from these enzymes as judged by its resistance to inhibitors such as DCCD, efrapeptin, diethylstilbestrol, and vanadate. An antibody to purified F_1-ATPase did not inhibit Na^+-ATPase activity (Kobayashi and Kakinuma, 1985).

The Modular Pump Model

In a study with everted membrane vesicles prepared in the absence of protease inhibitors, Heefner *et al.* observed ATP-dependent $^{22}Na^+$ accumulation that was largely blocked by protonophores and DCCD, indicating that, in these vesicles, $^{22}Na^+$ uptake is energized by the proton potential (Heefner *et al.*, 1980). In addition, membrane vesicles prepared in the presence of protease inhibitors and incubated in a buffer without these protective agents showed a progressive increase in secondary antiporter activity (Heefner and Harold, 1982).

Mutant 7683 is totally deficient in Na^+ extrusion and does not grow on complex medium containing high levels of Na^+ (i.e., NaTY medium) at neutral pH (Harold *et al.*, 1970). Heefner and Harold isolated two distinct classes of spontaneous revertants capable of growing on NaTY medium and also able to extrude Na^+. One class, R-II, showed ATP-dependent Na^+ transport activity which was insensitive to DCCD and ionophores, in both intact cells and membrane vesicles; Na^+-ATPase activity was directly demonstrated in membrane vesicles. A second class of revertants, R-I, readily transported Na^+, but transport in both intact cells and membrane vesicles was blocked completely by reagents which dissipated the proton potential. Membrane vesicles from this revertant exhibited no Na^+-stimulated ATPase activity (Heefner *et al.*, 1980; Heefner and Harold, 1982). What is the source of this Na^+ transport activity which is energized by the proton potential? Heefner and Harold explained these findings by the modular pump

model (Fig. 1c). According to this model there is one Na^+ transport system composed of two subunits: a typical Na^+/H^+ antiporter and an attached catalytic subunit. Normally the association between the subunits is very tight and ATP hydrolysis by the catalytic subunit drives Na^+/H^+ exchange. Damage to the modular pump, either through protease action or through genetic manipulation, could alter the association between the two subunits, resulting in the expression of Na^+/H^+ antiport activity (Heefner and Harold, 1982; Heefner, 1982). The validity of this model will be reconsidered later.

Inducibility of the Na^+-ATPase

Two years after the discovery of Na^+-ATPase, Kinoshita *et al.* reported Na^+-stimulated ATPase in *S. faecalis* mutant AS25 which is defective in H^+-ATPase and in the generation of the protonmotive force (Kinoshita *et al.*, 1984). They prepared everted membrane vesicles in the presence of phenylmethylsulfonyl fluoride as a protective reagent. The Na^+-stimulated ATPase activity characterized by Heefner and Harold was too low to account for the rate of sodium extrusion from intact cells (Heefner and Harold, 1982). By contrast, much higher specific activities of Na^+-stimulated ATPase and Na^+ transport were observed in the mutant. Another important observation was that expression of both Na^+-stimulated ATPase and Na^+ transport were observed in the mutant. Another important observation was that expression of both Na^+-ATPase and sodium transport was dependent on culture conditions. Mutant cells grown in media containing high concentrations of sodium (0.12 M) exhibited high activities, while those grown in low-sodium media (5–10 mM) exhibited much less enzymatic and transport activity. Sodium transport and Na^+-ATPase activity in the wild type strain were much lower than those in the mutant strain. When wild-type cells were grown in the presence of a protonophore, CCCP, both Na^+ transport and Na^+-ATPase activity were elevated. In addition, when cells were grown on media containing monensin, Na^+-ATPase activity was remarkably increased (Kakinuma, unpublished results). Thus, the level of Na^+-ATPase is affected both by the Na^+ concentration of the medium and by dissipation of proton potential, suggesting that induction of Na^+-ATPase is triggered by an increase in the cytoplasmic Na^+ concentration. The inducibility of Na^+-ATPase under various culture conditions was confirmed with the Na^+ ATPase defective mutant and its revertants (Kakinuma and Harold, 1985).

K^+ as a Counterion

Although Heefner and Harold regarded H^+ as the counterion for Na^+ efflux via the Na^+-ATPase (Figs. 1b and 1c), studies on K^+ uptake system by *S. faecalis* suggested that the true counterion may be potassium.

Table I. Sodium-Stimulated ATPase and KtrII[a]

Strain	Medium[b]	KtrII $[K^+]_i/[K^+]_o$	Na^+-ATPase (nmol/min/mg protein)
9790 (wild)	KTY	1	0.5
9790	KNaTY	180	10
9790	KNaTY plus CCCP	500	42
7683	KNaTY plus CCCP	1	0.6
R-I	KNaTY plus CCCP	1	5
R-II	KNaTY plus CCCP	400	26
AS25	NaTY	800	26

[a]Cells were grown on the media listed below. KtrII activity was assayed as described by Kakinuma and Harold (1985), in the presence of DCCD to block KtrI. Its activity is expressed here in terms of the potassium concentration gradient. Sodium-stimulated ATPase activity of membrane vesicles was assayed according to Kinoshita *et al.* (1984).
[b]All the media contained tryptone, yeast extract, and glucose. K^+ stands for 0.06 M K_2HPO_4, Na^+ for 0.06 M Na_2HPO_4. CCCP (20 μM).

Two distinct potassium uptake systems have been recognized in this organism. The major one, KtrI, is thought to be constitutive and resembles the Trk system of *Esherichia coli* (Bakker and Harold, 1980). The minor one, KtrII, is inducible, and is proposed to be analogous to the Kdp system of *E. coli* (Kobayashi, 1982). Kakinuma and Harold presented evidence that accumulation of K^+ ions via KtrII is obligatorily linked to the extrusion of Na^+ ions by the sodium-stimulated ATPaase. First, potassium uptake via KtrII required Na^+ ions to be present in the cytoplasm; overall, KtrII mediated equimolar exchange of Na^+ for K^+ ions. Second, the uptake of K^+ required the cells to generate ATP but did not depend on electrochemical gradients of either Na^+ or H^+ ions. Third, when the proton potential was dissipated, extrusion of Na^+ ions from intact cells was markedly stimulated by external K^+, but not by Rb^+ and tetraphenylphosphonium ion. These results suggest that the movements of both K^+ and Na^+ ions are directly coupled to ATP hydrolysis. Furthermore, growth conditions that lead to the expression of KtrII consistently induced enhanced levels of the sodium-stimulated ATPase (Table I). Mutant 7683 and R-I revertant, which lack Na^+-ATPase, lacked KtrII, and R-II revertant that recovered the former activity also possessed the latter. There is also a qualitative correlation between the levels of Na^+-ATPase and the initial rate of K^+ uptake. These results indicate that KtrII represents an activity of the Na^+-stimulated ATPase, which functions in the intact cells to expel the Na^+ ions by exchange for K^+. The Na^+-ATPase generates little or no membrane potential, which argues against Skulachev's proposal that KtrII is a K^+ uniporter analogous to valinomycin (Skulachev, 1988). In fact, the K^+ concentration gradient (in/out) of 800 established by KtrII was dissipated by addition of valinomycin

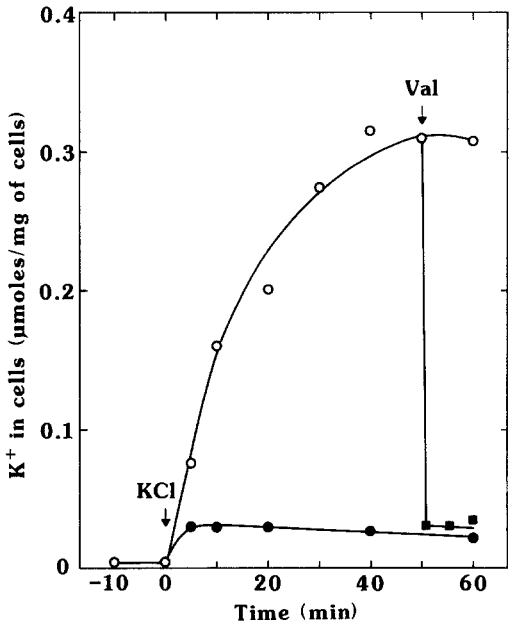

Fig. 2. Effect of valinomycin on KtrII activity. Mutant AS25 was grown on NaTY medium, loaded with Na^+, and resuspended in 50 mM Na^+-Tricine buffer, pH 8.5. The suspension was supplemented with 10 mM glucose and 0.2 mM DCCD (− 10 min); uptake was initiated by the addition of 0.5 mM KCl (0 min). Subsequent treatments were as follows: ○, control; ●, plus 3 μM valinomycin at − 5 min; ■, plus valinomycin (Val) at 50 min.

(Fig. 2). The conditions that induce KtrII expression suggest that a rise in the cytoplasmic Na^+ level, rather a lowered level of K^+, is the effective signal (Kakinuma and Harold, 1985; Harold and Kakinuma, 1985).

Heefner and Harold suggested that Na^+-ATPase mediates exchange of Na^+ for H^+, but the evidence bearing on the identity of the counterion was never strong, and it was confused by the presence of another Na^+ transport system—a true Na^+/H^+ antiporter, as described later.

If the Na^+-stimulated ATPase catalyzes exchange of Na^+ for K^+, one should expect cells grown under appropriate conditions to contain a membrane-bound ATPase that is stimulated synergistically by Na^+ and K^+ ions. We have observed little or no stimulation of its activity by K^+ ions. This must be counted as an objection in our hypothesis, but not necessarily a fatal one. Under the assay condition 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 in the ouabain-sensitive ATPase of animal cells but will refrain from pursuing this speculation.

The Refutation of the Modular Pump Model

Observations on the sodium ATPase as described above led to questioning the premise that *S. faecalis* has only a single system for sodium extrusion and that the Na^+/H^+ antiport activity arises by proteolytic damage to the Na^+,H^+-ATPase (Heefner and Harold, 1982; Heefner, 1982). First, the sodium ATPase apparently exchanges Na^+ for K^+ rather than for H^+. It seems unlikely, therefore, that proteolytic degradation of this enzyme would generate Na^+/H^+ antiport activity. Second, the sodium ATPase of *S. faecalis* is an inducible enzyme that is produced when the cells are grown in medium rich in Na^+. Cells grown in medium lacking sodium ion should then be unable to expel sodium, which again seemed implausible.

Membrane vesicles prepared in the presence of protease inhibitors and incubated in a buffer without these protective agents showed a progressive increase in secondary antiporter activity, but no decrease of Na^+ pump activity (Heefner and Harold, 1982). Proteolytic damage to Na^+-ATPase did not correspond with expression of Na^+/H^+ antiporter activity. Mutant 7683 is undoubtedly defective in Na^+-ATPase activity, but its properties suggest a more complex phenotype than would be expected for a defect in the structural gene for the Na^+-ATPase. First, the cells are also very leaky to K^+ ions (Harold *et al.*, 1970). Second several membrane proteins were shown to be missing in the mutant by SDS gel electrophoresis (Kakinuma, unpublished results). Third and most important, mutant 7683 was isolated after mutagenesis with 25 μg/ml NTG for 4 h (Harold *et al.*, 1970), probably too harsh a treatment to induce a single-gene mutation. Our attempts to isolate further mutants of this type by gentle treatment with NTG were unsuccessful. We believe 7683 to be pleiotropic, or at least a double mutant.

As an alternative explanation of these data, we propose a model consisting of two independent Na^+ transport systems (Fig. 3) (Kakinuma, 1987a). One system is the $Na^+,(K^+)$-ATPase, while the other would be an ATP-regulated, secondary Na^+/H^+ antiporter. There is no simple Na^+/H^+ antiporter because Na^+ transport always requires ATP (Harold and Papineau, 1972). According to this model, membrane vesicles prepared in the absence of protease inhibitors exhibit chiefly the activity corresponding to the ATP-regulated Na^+/H^+ antiporter, because the sodium ATPase is damaged by proteolysis. A mixture of multiple protease inhibitors did protect the sodium ATPase, but also inhibited the Na^+/H^+ antiporter. Antiporter activity was recovered by dilution of the mixture. We tentatively regard 7683 as a double mutant, having lost both Na^+ transport systems; revertant R-I would

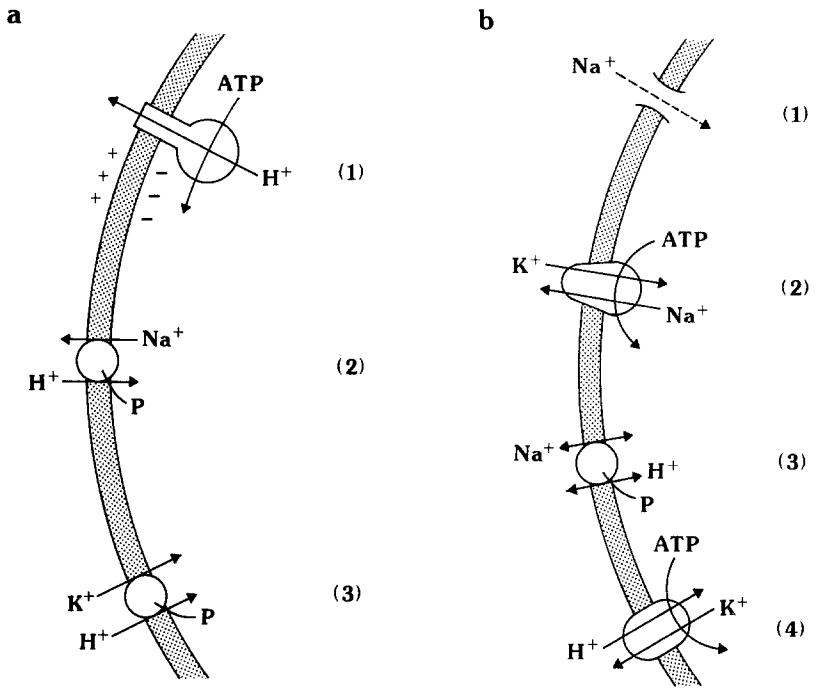

Fig. 3. Mechanisms of sodium circulation in *Streptococcus faecalis*: a hypotheses. Na^+ fluxes are attributed to the interaction of other systems. (a) In media poor in Na^+ and at low pH: (1) the proton-translocating F_1F_0-ATPase; (2) a regulated Na^+/H^+ antiporter; (3) KtrI, a regulated K^+/H^+ symporter. (b) In media rich in Na^+ and at high pH (F_1F_0-ATPase and KtrI were omitted because of their smaller contribution): (1) the nature of this Na^+ pathway is unknown; (2) KtrII, Na^+,(K^+)-ATPase; (3) a regulated Na^+/H^+ antiporter; (4) the K^+/H^+ antiport pump (K^+,H^+-ATPase?).

have regained only the Na^+/H^+ antiporter, and revertant R-II would have recovered the sodium ATPase but not the antiporter.

Sodium Circulation of *S. faecalis*

Sodium/Proton Antiporter

Na^+-ATPase is induced when the cells are grown in media rich in Na^+. Is it true that cell grown in media lacking sodium ion will be unable to expel Na^+? Kakinuma found that the wild type cells grown under conditions that do not induce production of the sodium ATPase were still capable of energy-dependent sodium extrusion. Sodium extrusion from these cells was observed at pH 7.4 but not at pH 8.5, and it was inhibited by DCCD, CCCP, or

valinomycin, indicating that it requires the cells to generate a proton potential. Sodium extrusion was not observed by mutant AS25, which is defective in H^+-ATPase but not in Na^+-ATPase, grown in media lacking Na^+. An artificially imposed pH gradient drove active sodium extrusion. In response to a pH gradient (with exterior acid), energy-depleted cells exhibited a transient sodium extrusion which was unaffected by treatments that dissipated the membrane potential and which was blocked by proton conductors. Sodium movement in response to a pH gradient was also observed in the cells in which Na^+-ATPase has been induced. These results indicate that *S. faecalis* contains a constitutive sodium/proton antiporter (Fig. 3) (Kakinuma, 1987a).

Pathway of Na^+ Entry

The existence of at least one such route is necessary if K^+ uptake via KtrII is to support cell growth; and uptake of Na^+ ions is, in any event, attested by observation. The nature of this pathway is presently under investigation. The limited data at hand suggest that the Na^+/H^+ antiporter mentioned above does allow Na^+ ions to enter, but only in glycolyzing cells whose membrane potential has been collapsed (Fig. 3b). A different pathway was observed by Heefner and Harold (1980) who showed that when a membrane potential (inside negative) was imposed by K^+ efflux, the cells took up Na^+ ions by exchange for K^+. The process involved has low affinity but high capacity ($K_m > 20\,\text{mM}$; $V_{\max} > 50\,\text{nmol/min/mg}$ of cells) and apparently responds to both the concentration gradient and the electrical potential. Because of its low affinity, we suspect that this pathway is relatively nonspecific and reflects some kind of leakage down the electrochemical potential gradient.

Na^+ Circulation and Environmental Adaptation

Expression of the sodium ATPase in *S. faecalis* is closely related to its tolerance for salts and alkaline pH; *S. faecalis* grows well in the presence of 6.5% NaCl and at pH 10 (Deibel and Seeley, 1974; Kakinuma, 1987b). In media poor in Na^+ and at acid pH, the protonmotive force generated by the H^+-ATPase, although smaller than that in aerobes, suffices to drive a Na^+/H^+ antiporter and other proton-linked transport systems (Fig. 3a). High sodium concentration, however, pose a problem for a cell dependent on a proton-translocating ATPase. Sodium ions leak into the cytoplasm, collapsing the membrane potential as reported in the related organism, *S. lactis* (Kashket and Barker, 1977). Under these conditions, electrogenic H^+ expulsion by the H^+-ATPase accelerates, but as the cytoplasmic pH rises above the enzyme's pH optimum (pH 6.5) the proton potential will diminish.

Na^+ extrusion via the antiporter is insufficient to suppress the increase in cytoplasmic Na^+, and K^+ uptake via KtrI (which depends on the proton potential) comes to a halt. For all these reasons, continued growth of the cells depends on the induction of the Na^+-ATPase which exchanges Na^+ for K^+ (Fig. 3b).

The protonmotive force is drastically decreased at pHs above 8. At pH 10, *S. faecalis* still grows well, but the proton potential across the plasma membrane goes to zero, and in some cases turns positive by 1 unit or more (Kakinuma, 1987b). In alkaline media, the Na^+/H^+ antiporter cannot extrude Na^+ ions and is more likely to mediate Na^+ accumulation. The KtrI system also does not function at alkaline pH. Consequently, in low-K^+ media at alkaline pH, the only means by which the cells can accumulate K^+ is the sodium ATPase. Growth of *S. faecalis* under these conditions does, in fact, depend on the inclusion of Na^+ ions (Kakinuma, unpublished results). Furthermore, at alkaline pH, accumulation of K^+ is essential for regulation of the cytoplasmic pH via the K^+, H^+ antiport pump (possibly a K^+, H^+-ATPase) (Kakinuma and Igarashi, 1988). Induction of the sodium ATPase is important for cell growth at alkaline pH (Fig. 3b). Even in Na^+-limited media, the Na^+-ATPase level and the rate of Na^+ transport were much increased by alkalinization of the medium (Kakinuma and Igarashi, manuscript in preparation). Induction of the Na^+-ATPase should be also enhanced by a defect of the Na^+/H^+ antiporter; this is consistent with the observation that Na^+-ATPase level is elevated in revertant R-II (Heefner and Harold, 1982). In sum, the existence of the two systems for sodium extrusion allows the organism to cope with an environment subject to fluctuations in both ionic composition and pH.

Significance of a Na^+ Potential in *S. faecalis*

In bacteria, especially in marine bacteria, a sodium potential is widely utilized for transport of amino acids and sugars, pH regulation via the antiporter, flageller rotation, and ATP synthesis (Lanyi, 1979; Skulachev, 1988). In *S. faecalis*, there is no evidence for any essential functions of these kinds. Why, then, must Na^+ be extruded from *S. faecalis*? Is Na^+ pumped out of the cells simply as a counterion for K^+ uptake via the Na^+-ATPase? In *S. faecalis*, a Na^+ electrochemical gradient of as much as -100 mV can be generated under some conditions (Kakinuma, unpublished results). We doubt that organisms disdain to utilize such a powerful energy source. Na^+-dependent K^+-uptake is reported in *S. lactis* (Kashket, 1979), and Na^+-dependent amino acid uptake has recently been found in *S. bovis* (Russell *et al.*, 1988). To understand the significance of a Na^+ potential in *S. faecalis*, it is important to find similar sodium-linked processes.

Comparison with Other Na^+-ATPases

The occurrence of DCCD-resistant Na^+-ATPase in other streptococci is expected, but has not been reported so far. Based on their tolerance to salts and alkaline pH, some related streptococci appear to have evolved a Na^+-ATPase although it may be less prominent than that of *S. faecalis*. Interestingly, the Na^+-ATPase in *Mycoplasma mycoides*, a glycolytic organism, is virtually identical to that of *S. faecalis*; in these organisms, also, exchange of Na^+ for K^+ appears to be mediated by an ATPase that is stimulated only by Na^+ ions (Benyoucef *et al.*, 1982). Furthermore, Jinks *et al.* have reported a Na^+-stimulated ATPase activity in membrane preparations of *Acholeplasma laidlawii*. This ATPase may be of the F_1F_0 type judging by its subunit composition and DCCD sensitivity (Jinks *et al.*, 1978; Lewis and McElhaney, 1983).

An electrogenic Na^+-ATPase has also been reported in *Vibrio alginolyticus* and some methanogenic bacteria (Dibrov *et al.*, 1988; Carper and Lancaster, 1986). In these bacteria, it is proposed that the ATPase mediates ATP synthesis energized by the Na^+ potential. These enzymes are DCCD-sensitive and belong to the F_1F_0 class. Another peculiar Na^+-translocating ATPase reported in *Propionigenium modestum* is also DCCD sensitive and of F_1F_0 type. Recently, it has been purified and reconstituted into proteoliposome (Laubinger and Dimroth, 1987, 1988).

Conclusion

There should be a relationship between the kinds of transport systems found in any given organism and its habitat or metabolic economy. In this context the characteristics of the Na^+-ATPase may be relevant to the fact that growing *S. faecalis* maintains a significantly lower proton potential than do growing aerobic bacteria under some conditions. This may be one reason why this organism evolved a primary pump and a proton-linked porter to extrude Na^+.

Acknowledgments

We thank F. M. Harold for invaluable support of our work and critical reading of the manuscript. We also thank V. P. Skulachev for helpful discussion.

Work from our laboratory described in this review was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

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