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ATP-driven sodium pump in *Streptococcus faecalis*

(ion transport/bacteria/antiporter/chemosmotic theory)

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ABSTRACT **Sodium extrusion by bacteria is generally attributed to secondary antiport of Na^+ for H^+ energized by the proton circulation. *Streptococcus faecalis* is an exception, in that sodium expulsion from intact cells requires the generation of ATP but does not depend on the protonmotive force. Unfortunately, studies with everted membrane vesicles failed to reveal the expected sodium pump; instead, the vesicles contained a conventional secondary Na^+/H^+ antiporter. We report here that everted membrane vesicles prepared in the presence of protease inhibitors retain an ATP-driven sodium transport system. The evidence includes the findings that (*i*) accumulation of $^{22}Na^+$ by these vesicles is resistant to reagents that dissipate the protonmotive force but requires ATP and (*ii*) the vesicles contain a sodium-stimulated ATPase that is distinct from F_1F_0 ATPase, and whose presence is correlated with sodium transport activity. Sodium movements appear to be electroneutral and are accompanied by movement of H^+ in the opposite direction. When membranes are incubated in the absence of protease inhibitors, a secondary Na^+/H^+ antiport activity emerges, possibly by degradation of the sodium pump. We suggest that *S. faecalis* expels Na^+ by means of an ATP-driven primary transport system that mediates exchange of Na^+ for H^+. The Na^+/H^+ antiporter seen in earlier membrane preparation is an artefact of proteolytic degradation.**

Virtually all cells expel sodium ions from the cytoplasm by active transport of one kind or another. In animal cells, this is the task of the Na^+-K^+ ATPase. In bacteria, and probably also in lower eukaryotes and in plants, sodium is extruded by exchange for protons. Since all these organisms maintain a substantial electrochemical proton gradient (interior alkaline and negative), antiport of Na^+ for H^+ can expel Na^+ against a substantial gradient and no direct coupling to ATP, or other energy donor, should be required. This hypothesis, first proposed by Mitchell (1) in the context of his chemosmotic theory, has received substantial support from bacterial physiologists (for review, see ref. 2), and it is generally accepted that *Escherichia coli*, *Azotobacter*, alkalophilic bacilli, and other bacteria extrude sodium by secondary Na^+/H^+ antiport (2–7). The discovery by MacDonald and his colleagues (8, 9) of a light-driven sodium pump in halobacteria was the first indication of diversity in bacterial sodium transport mechanisms. However, even in this case, the major pathway of sodium extrusion may be secondary Na^+/H^+ antiport; the light-energized pump appears to make but a minor contribution to the total Na^+ flux (10).

From the beginning, Na^+ extrusion by *Streptococcus faecalis* did not quite conform to the hypothesis of secondary Na^+/H^+ antiport, in that both net Na^+ movement and $^{22}Na^+/Na^+$ exchange were observed only in cells capable of generating ATP (11). In a detailed study with intact cells, we (12) demonstrated that glycolyzing cells could extrude Na^+ against a 100-fold concentration gradient in the presence of reagents that dissipated both the pH gradient and the membrane potential; evidently, the protonmotive force was not the energy source for sodium extrusion. Also, in concurrent studies with everted membrane vesicles of *S. faecalis*, we (13) found ATP-dependent accumulation of $^{22}Na^+$, but this was largely blocked by proton conductors and by inhibitors of the proton-translocating ATPase, evidence that, in vesicles, $^{22}Na^+$ uptake is energized by the proton circulation. Some of the observations reported did suggest that the secondary Na^+/H^+ antiport by the vesicles was an artefact (13), but the conflict between data from cells and from vesicles was not satisfactorily resolved.

We report here that membrane vesicles prepared in the presence of protease inhibitors accumulate $^{22}Na^+$ in the presence of ATP and are unaffected by dissipation of the protonmotive force. These vesicles also have a sodium-stimulated ATPase activity that is distinct from the proton-translocating F_1F_0 ATPase. We present evidence that the sodium-stimulated ATPase reflects a primary ATP-driven transport system that extrudes Na^+ by exchange for protons.

METHODS

Organisms and Growth Media. *S. faecalis* (faecium), ATCC 9790, and mutants R-I and R-II were grown on the complex medium NaTY as described (12). Mutant 7583, which does not grow on NaTY, was grown on the corresponding medium KTY.

Membrane Vesicles. These were prepared as described (13), except that protease inhibitors were included at all stages of the preparation. Washed cells were converted to protoplasts by lysozyme treatment in 0.5 M glycylglycine/1 mM phenylmethylsulfonyl fluoride. The protoplasts were then suspended in ice-cold 0.2 M K maleate/0.05 M K Hepes/0.25 M sucrose/5 mM $MgSO_4$/ 10 mM mercaptoethanol/1 mM phenylmethylsulfonyl fluoride/1 mM *p*-toluenesulfonyl fluoride/1 mM *p*-toluenesulfonic acid, pH 7.4; DNase was then added and the protoplasts were disrupted in a French press. All subsequent steps were done as described (13).

Vesicles used in the ATPase assay were prepared in buffer containing 0.05 M Tris maleate instead of K maleate; the procedure was otherwise the same and protease inhibitors were present throughout.

Uptake of $^{22}Na^+$. The procedure was the same as before (13), except that the three protease inhibitors (1 mM each) were included in all assay mixtures. We draw attention to a source of

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; TCS, tetrachlorosalicylanilide.

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error in measuring the rate or extent of $^{22}Na^+$ uptake: the assay buffer contains 400 mM K^+, which may contribute as much as 80 μM contaminating Na^+ (from the manufacturer's specifications). We have ignored this and base our calculations only on the 10 μM $^{22}Na^+$ added to the assays; the conclusions of our study are not affected by the consequent underestimation of sodium uptake.

Other Procedures. ATPase was assayed as described (14); monensin (4 μg/ml) was included to allow Na^+ and H^+ to equilibrate across the vesicle membrane. Fluorescence of quinacrine and other dyes was monitored with an Aminco–Bowman spectrophotofluorimeter (15). Protein was measured by the Lowry procedure.

Reagents. Radioisotopes were purchased from New England Nuclear or from Amersham; other reagents were from Sigma. Monesin and efrapeptin were gifts from R. J. Hamill of the Lilly Research Laboratories.

RESULTS

Accumulation of $^{22}Na^+$ by Everted Membrane Vesicles Requires ATP but Not a Protonmotive Force. In a previous paper (13), we reported that everted membrane vesicles of *S. faecalis* accumulate $^{22}Na^+$ when incubated with ATP/Mg^{2+}; Na^+ accumulation was blocked by ionophores and by *N,N'*-dicyclohexylcarbodiimide (DCCD; an inhibitor of proton-translocating ATPase), suggesting that it is energized by the protonmotive force. Vesicles prepared in the presence of protease inhibitors differed strikingly from our earlier preparation. Uptake of $^{22}Na^+$ was strictly dependent on Mg^{2+} and either ATP or GTP, with maximal uptake pH 7 to 8. Sodium accumulation was not inhibited by the proton-conducting uncouplers tetrachlorosalicylanilide (TCS) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Fig. 1*A*) and was significantly stimulated by DCCD (Fig. 1*B*). Even the combinations DCCD/TCS, DCCD/CCCP, and DCCD/TCS/valinomycin did not prevent $^{22}Na^+$ uptake (Fig. 1*B*).

Three lines of evidence persuade us that these reagents effectively dissipate the electrochemical gradient of protons across the membrane of protease-protected vesicles. First, DCCD and ionophores blocked the enhancement of fluorescence seen when vesicles are incubated with MgATP and 1-anilino-8-naphthalene sulfonate; this fluorescence enhancement is generally taken as evidence for a membrane potential (interior positive). Second, protease-protected vesicles accumulate $S^{14}CN^-$ when incubated with MgATP, another indication of a positive potential; this uptake was again blocked by DCCD. Finally, DCCD blocked the quenching of quinacrine fluorescence by these vesicles in the presence of MgATP, indicating that it prevents establishment of a pH gradient (interior acid) (data not shown). Since neither DCCD nor proton conductors inhibit the uptake of $^{22}Na^+$ (Fig. 1), we conclude that sodium accumulation by protease-protected vesicles does not require a protonmotive force.

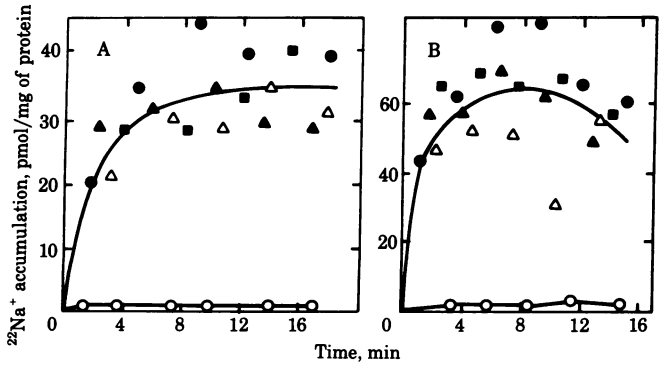

FIG. 1. Accumulation of $^{22}Na^+$ by protease-protected vesicles. Vesicles were prepared and assayed in K maleate/Hepes/sucrose/$MgSO_4$, pH 7.4, containing protease inhibitors as described above. (*A*) Uptake was initiated by adding vesicles (1.0 mg/ml) to aliquots of the same buffer containing 10 μM $^{22}NaCl$ and various supplements. (*B*) The vesicles were incubated with DCCD (0.27 μmol/mg of protein) for 10 min before use as in *A* and assayed in the presence of 0.1 mM DCCD. ○, No ATP; ●, 4.7 mM ATP; △, ATP/20 μM CCCP; ▲, ATP/20 μM TCS/4 μM valinomycin; ■, ATP/20 μM TCS.

A Sodium-Stimulated ATPase in Protease-Protected Vesicles. The findings described above suggest that sodium accumulation is due to a primary ATP-driven sodium pump, which ought to be detectable as a Na^+-dependent ATPase. To demonstrate this activity, it was first necessary to block the powerful F_1F_0 ATPase with DCCD; this reduced the total ATPase activity of the membranes by 80%. ATPase activity was routinely assayed in the presence of 3 mM EGTA, so as to exclude any contribution from a possible calcium-activated ATPase (15); further reduction of background ATPase activity was achieved by addition of 50 μM NH_4VO_3 (at this concentration, neither meta- nor orthovanadate inhibited $^{22}Na^+$ accumulation by the vesicles). With these precautions, a small stimulation of ATP hydrolysis by Na^+ was consistently observed in protease-protected vesicles (Fig. 2). The activity was maximal at ≈pH 7.5 and saturated at ≈1 mM Na^+ (because of the high background of Na^+-independent ATPase, we were not able to measure a proper K_m). Li^+ at 10 mM also enhanced ATPase activity but K^+ did not, either in the presence of Na^+ or in its absence.

Sodium-stimulated ATPase activity is not conspicuous but its presence is clearly correlated with ATP-driven sodium transport (Fig. 2). No Na^+ ATPase activity was seen in vesicles prepared in the absence of protease inhibitors, nor was it found in membranes of mutant 7683, which is defective in sodium transport (11, 12, 13). Previous work (12) had led to the isolation of two classes of revertants that had recovered the capacity to extrude Na^+. In class R-I, sodium transport in both intact cells and membrane vesicles was extremely sensitive to inhibition by ionophores and by DCCD (this sensitivity was confirmed with protease-protected vesicles; data not shown). We believe that sodium transport in R-I revertants is energized by the protonmotive force, and the absence of sodium-stimulated ATP hydrolysis (Fig. 2) is consistent with this view. Revertants of class R-II had recovered ionophore-resistant $^{22}Na^+$ transport in both intact cells and protease-protected vesicles; (in the latter, DCCD stimulated $^{22}Na^+$ accumulation to a level about twice that of DCCD-treated vesicles from wild-type cells). The Na^+ ATPase activity of R-II membranes (Fig. 2) was about twice that of the parent strain. We therefore conclude that the Na^+-stimulated ATPase corresponds to the ATP-driven sodium transport system shown in Fig. 1.

Sodium-stimulated ATPase is quite distinct from the familiar F_1F_0 ATPase, as judged by its sensitivity to inhibitors. In addition to DCCD, both efrapeptin and diethylstilbestrol inhibit F_1F_0 ATPase and proton transport into the vesicles; however, they had little effect on $^{22}Na^+$ accumulation or on the Na^+-dependent ATPase (Fig. 3). Efrapeptin, at least, is known to act on the catalytic site of F_1F_0 ATPase (16), suggesting that the catalytic unit of the Na^+ ATPase is distinct from F_1.

Some misgivings about the physiological significance of the sodium-stimulated ATPase do, nonetheless, stem from its low activity. In the parent strain, Na^+ stimulated ATP hydrolysis by 1.7 ± 0.3 nmol·min^{-1}·mg^{-1} of protein (the activity of F_1F_0 ATPase is 10-fold higher). From our previous studies (12), we estimate the rate of sodium extrusion from intact cells to be at

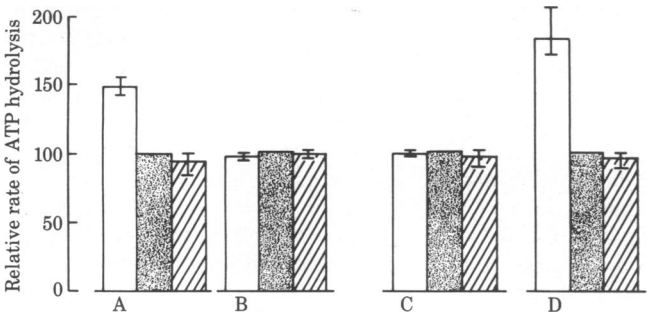

FIG. 2. Sodium ion stimulates ATP hydrolysis. Vesicles were prepared from wild type (*A*), mutant 7683 (*B*), and revertants R-I (*C*), and R-II (*D*) cells in Tris maleate/Hepes/sucrose/$MgSO_4$, pH 7.4, containing protease inhibitors as described above. Vesicles (4 mg protein) were suspended in 3.15 ml of buffer containing 0.76 mM DCCD/3 μM monensin/3 mM EGTA/50 μM NH_4VO_3 and incubated at room temperature for 10 min. Aliquots (150 μl) were then treated with small volumes of buffer containing NaCl (□; final concentration, 10 mM) or KCl (▨; final concentration, 10 mM). ▩, Control (no additions). Reaction was started by addition of 5 mM MgATP; release of P_i was monitored at intervals over 120 min. The plot, a composite of four experiments, shows relative rates of ATP hydrolysis, taking the rate in unsupplemented buffer as 100% (the absolute rate is ≈4 nmol of $P_i \cdot min^{-1} \cdot mg^{-1}$ of protein for both parent and mutant strains). Results are mean ± range.

least 50 $nmol \cdot min^{-1} \cdot mg^{-1}$ of protein. Clearly, assuming any reasonable stoichiometry of Na^+ to ATP, the activity of the Na^+ ATPase is too low by an order of magnitude to account for the rate of sodium extrusion from intact cells (there is no such glaring discrepancy in vesicles in which the measured Na^+ ATPase activity appears to be roughly sufficient to account for the rate of $^{22}Na^+$ uptake). Part of the discrepancy may be due to the fact that no more than half the vesicles in our preparation are everted, but we do not believe that the problem is so easily dismissed. Observations presented below suggest that, despite the presence of protease inhibitors, a portion of the ATPase is damaged in the course of vesicle preparation. It is also possible that our assay conditions are suboptimal; a cursory search for activation by intermediates of glycolysis was not productive. We have tried to find Na^+-stimulated ATPase activity with cells permeabilized with toluene but so far these attempts have been unsuccessful.

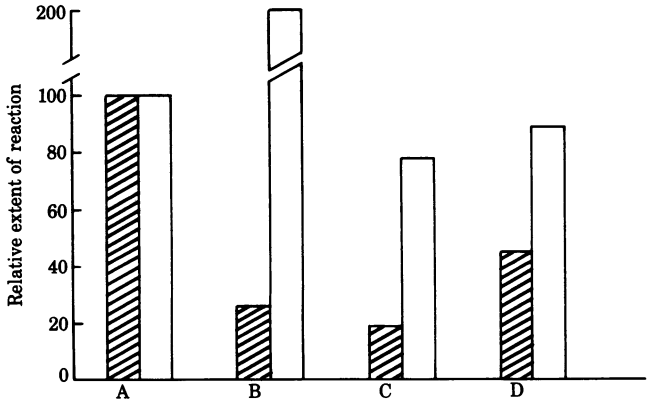

FIG. 3. Differential effects of inhibitors on ATPase activity and $^{22}Na^+$ accumulation. Vesicles were prepared in K^+ maleate/Hepes/sucrose/$MgSO_4$, pH 7.4, containing protease inhibitors. Sodium uptake (□) and ATPase activity (▨) were assayed in 0.05 M Tris maleate containing protease inhibitors. The concentration of $^{22}Na^+$ was 10 μM in the transport assay and 10 mM in the ATPase assay. Inhibitors were added and reaction was initiated 15 min later by addition of 5 mM ATP. Both ATP hydrolysis and $^{22}Na^+$ accumulation were measured over a 15-min period. (*A*) Control (no inhibitor). (*B*) DCCD at 0.7 mM. (*C*) Efrapeptin at 18 μM. (*D*) Diethylstilbestrol at 2 mM. Data are relative to results in the absence of inhibitors (taken as 100%).

The Sodium Pump Mediates Na^+/H^+ Exchange. A decade ago, Harold and Papineau (11) proposed that extrusion of Na^+ from intact cells of *S. faecalis* involved the electroneutral exchange of Na^+ for H^+; later findings, summarized by Heefner and Harold (12) are consistent with this hypothesis. It now appears that ATP-driven $^{22}Na^+$ uptake by membrane vesicles likewise involves the exchange of Na^+ for H^+.

If the sodium pump were electrogenic, vesicles that are taking up Na^+ should develop a membrane potential (interior positive), even in the presence of reagents that block the proton pump. In fact, when vesicles were incubated with DCCD, MgATP, and 1 mM Na^+, there was no enhancement of 1-anilino-8-naphthalenesulfonate fluorescence; there was also no measurable accumulation of $S^{14}CN^-$. An alternative possibility, *a priori* implausible, is that a negative potential develops during Na^+ uptake. This was ruled out by parallel experiments with 3,3′-dihexyl-2,2′-oxacarbocyanine iodide: no fluorescence quenching was seen (data not shown). As far as they go, these results suggest that sodium accumulation by vesicles is probably electroneutral.

An electroneutral sodium pump implies the exchange of Na^+ for another cation, since no anion dependence was noted. Exchange of Na^+ for Ca^{2+} is excluded by the observation that $^{22}Na^+$ uptake required no added Ca^{2+} and was not inhibited by 3 mM EGTA. The ATP-driven system for Ca^{2+} transport, reported in *S. faecalis* vesicles by Kobayashi *et al.* (15), is quite distinct from the sodium pump discussed here: the Ca^{2+} pump is seen even in the absence of protease inhibitors, is energized solely by ATP, and is present in mutant 7683 (data not shown). Since the presence of Mg^{2+} is required for the Na^+ ATPase, we could not exclude Mg^{2+} as a possible counterion.

To ensure that Na^+ accumulation by the vesicles does not reflect obligatory exchange of Na^+ for K^+, we prepared membrane vesicles from cells whose K^+ complement had been replaced with Na^+ by the monactin procedure (17). Such vesicle preparations took up $^{22}Na^+$ almost as well as those shown in Fig. 1 (20 pmol/mg of protein at the steady state) and were resistant to DCCD. We must mention that both monactin-treated cells and the vesicles made from them retain ≈2% of their original K^+ complement; nevertheless, these findings make it unlikely that Na^+ uptake requires exchange for K^+.

In contrast to the above series of negative results, positive evidence was obtained that Na^+ uptake is accompanied by H^+ efflux. The intravesicular pH was monitored by quinacrine fluorescence, as was done by Rosen and co-workers (18). As shown in Fig. 4*A*, when protease-protected vesicles were incubated with quinacrine, ATP, and Mg^{2+}, there was a dramatic quenching of quinacrine fluorescence, evidence that the vesicle lumen has become acidic. Quenching was partially reversed by Na^+, indicating efflux of H^+; Li^+ could substitute for Na^+ but K^+ did not. It is noteworthy that alkalinization was not abolished by valinomycin (Fig. 4*B*): since valinomycin renders the membrane permeable to K^+, we can infer that movements of Na^+ and H^+ are coupled directly rather than through preservation of electroneutrality. Alkalinization by Na^+ was not seen in vesicles from mutant 7683 but was evident in both R-I and R-II revertants (Fig. 4 *B–D*). Taken together, the data suggest that Na^+ is accumulated by exchange for H^+ in an electroneutral manner.

Production of the Na^+/H^+ Antiporter by Proteolysis. Membrane vesicles prepared as described above, in the presence of protease inhibitors, exhibit ATP-driven Na^+ uptake and Na^+-

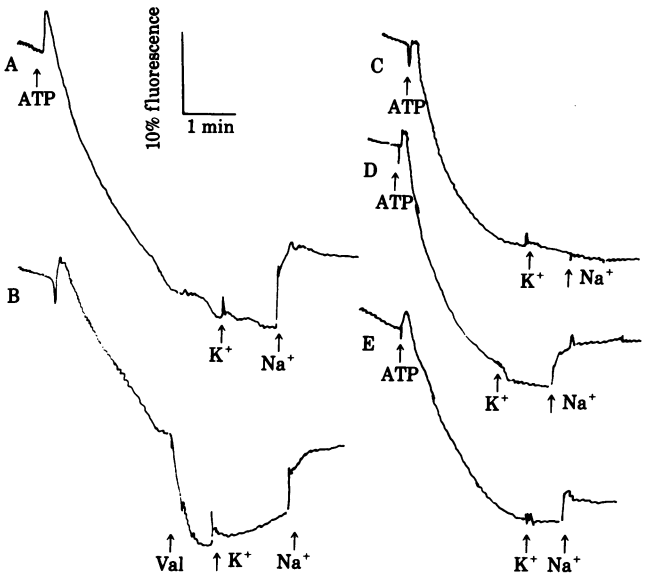

FIG. 4. Fluorimetric evidence for $^{22}Na^+/H^+$ exchange. Membrane vesicles from parent and mutant strains were suspended (0.1 mg of protein/ml) in K maleate/sucrose/Hepes/$MgSO_4$, pH 7.4, containing protease inhibitors. Quinacrine (1.2 μM) was added, followed by 1.3 mM ATP and then by K_2SO_4 and Na_2SO_4 (final concentration of Na^+, 6.6 mM). (*A*) Parent strain; note partial reversal of fluorescence quenching by Na^+. (*B*) Parent strain; 4 μM valinomycin (Val) did not prevent reversal of quenching by Na^+. (*C*) Mutant 7683; no response to Na^+. (*D* and *E*) Revertants R-I and R-II; both respond to Na^+.

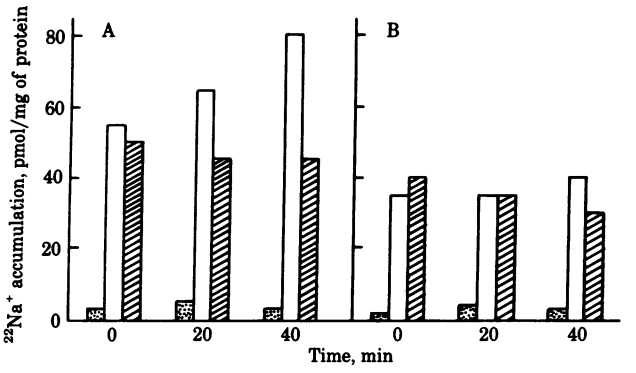

FIG. 5. Na^+/H^+ antiporter activity emerges when vesicles are incubated in buffer. Vesicles were prepared in K maleate/sucrose/Hepes/$MgSO_4$ containing protease inhibitors, collected by centrifugation, and resuspended in the same buffer; half of each batch was then treated with the usual supplement of protease inhibitors (*B*) while the other half was not (*A*), and both were then incubated at room temperature. At intervals, $^{22}Na^+$ uptake was assayed as in Fig. 1. ▨, No ATP; □, ATP; ▨, ATP/DCCD/TCS/valinomycin. In each case, the full time course of $^{22}Na^+$ uptake was determined; the figure shows the extent of uptake at 13 min, by which time a steady state had been reached.

stimulated ATPase. Vesicles prepared in the absence of protease inhibitors (13) lack Na^+ ATPase, and Na^+ uptake is energized by the protonmotive force. The simplest interpretation is that, in the latter vesicles, the Na^+/H^+ antiporter is produced by an endogenous protease.

Support for this view is presented in Fig. 5. Membrane vesicles were prepared in the presence of protease inhibitors, collected by centrifugation, and resuspended in fresh buffer with or without protease inhibitors; uptake of $^{22}Na^+$ was assayed at intervals thereafter. Uptake sensitive to inhibition by DCCD/ionophores is taken as a measure of antiporter activity; the remaining fraction is a measure of pump activity. The results show that antiporter activity develops over time of incubation when vesicles are incubated in the absence of the protease inhibitors but is delayed in their presence. By contrast, pump activity remained almost unchanged for the duration of the incubation.

DISCUSSION

We suggest the thesis that everted membrane vesicles of *S. faecalis*, appropriately prepared, accumulate Na^+ by a transport system that uses the free energy of ATP (or GTP) hydrolysis but does not require the protonmotive force. These vesicles also exhibit Na^+-stimulated ATPase activity, which we propose represents the biochemical basis of sodium transport in this organism. The secondary Na^+/H^+ antiporter previously described in vesicle preparations (13) is apparently an artefact that arises by proteolytic degradation of some other transport system, most probably the sodium pump.

The case rests on the following findings. In studies with intact cells (12), it has been shown that, under certain conditions, glycolyzing cells expel sodium against a large concentration gradient even though they are unable to establish either a membrane potential or a pH gradient, indicating direct coupling of sodium transport to metabolic energy at the level of ATP. We report here that everted membrane vesicles, prepared in the presence of protease inhibitors, accumulated Na^+ when incubated with MgATP (or MgGTP); this accumulation was unaffected or even stimulated by reagents that collapse the protonmotive force across the vesicle membrane (Fig. 1). These vesicles also exhibited Na^+-stimulated ATPase activity; the presence of this enzyme was correlated with sodium transport activity by the use of mutants (Fig. 2). Na^+-stimulated ATPase is distinguishable from F_1F_0 ATPase by its differential response to mutations and to inhibitors (Fig. 3). This point is of some importance, for one can imagine that a Na^+/H^+ antiporter may be so closely apposed to a proton-translocating ATPase as to give the appearance of direct energy coupling (19). The observation that the Na^+-ATPase is refractory to inhibitors of F_1 ATPase suggests that the former represents a distinct enzyme, exclusively concerned with sodium translocation.

In intact cells, and also in membrane vesicles, sodium transport appears to be an electroneutral process. Fig. 4 presents our chief reasons for believing that the counterion for Na^+ is H^+; neither K^+ nor Ca^{2+} seem to be involved. If this is correct, we can describe the sodium pump of *S. faecalis* as an ATP-driven Na^+/H^+ antiporter, analogous to the ATP-driven K^+/H^+ antiporter of gastric mucosa (20, 21) and to other ATP-driven cation pumps of animal cells. The energy source for sodium extrusion from intact cells is normally ATP. The observation that, under certain conditions, sodium extrusion requires the cells to generate a protonmotive force as well (11) is best explained by the high pH optimum of the sodium pump: maintenance of a high cytoplasmic pH requires proton extrusion and, generally, K^+ uptake as well (22). In consequence, sodium extrusion is functionally linked to K^+ uptake even though the two fluxes are mechanistically quite independent. Revertants of class R-I differ from the wild type with respect to the mechanism of energy coupling: ATP is not involved, and sodium extrusion is mediated by a free Na^+/H^+ antiporter energized by the protonmotive force.

The secondary Na^+/H^+ antiporter makes its appearance under two conditions: as the result of a genetic lesion in R-I and also in membrane vesicles of the parent strain that have been prepared or incubated in the absence of protease inhibitors (Fig. 5). It is reasonable to infer that the antiporter arises by proteolytic degradation of some other system present in the membrane

and is therefore an artefact. But what is its source? Fig. 5 shows that development of the Na^+/H^+ antiporter is not accompanied by diminution of the sodium pump; nevertheless, we suspect that the Na^+/H^+ arises by proteolytic cleavage of the ATP-driven exchange carrier. The Na^+ ATPase activity that we measure is too low by an order of magnitude to account for the rate of sodium extrusion from intact cells. Perhaps our assay procedures miss a fraction of the ATPase that is susceptible to degradation. A more positive argument derives from the finding that revertants of mutant 7683 (devoid of Na^+ transport) recover either the Na^+ pump or the Na^+/H^+ antiporter. This seems to us a strong hint that the ATP-driven Na^+/H^+ pump is of modular construction, subject to both genetic and biochemical cleavage that liberates the antiporter module from the ATPase unit.

Why should *S. faecalis* expel sodium by an ATP-driven primary pump, while most other bacteria apparently rely on secondary Na^+/H^+ antiporters energized by proton circulation? It is possible that some, or even all, of these secondary antiporters are experimental artefacts of vesicle preparation, but we think this unlikely. A more appealing hypothesis is that *S. faecalis*, physiologically an anaerobe, has a restricted capacity to generate a protonmotive force (23, 24) and must therefore depend on primary transport systems for purposes that aerobic organisms can accomplish with secondary ones. We should also bear in mind that two additional systems have been reported to couple metabolic energy to sodium transport: a light-driven sodium pump is well-documented in halobacteria (8, 9), and there is evidence that oxaloacetate decarboxylase serves as a sodium translocase in *Klebsiella* (25). Sodium extrusion must be an important matter that warrants a diversity of mechanisms, appropriate to all seasons; yet we would be hard put to explain just what makes it so indispensible.

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1. Mitchell, P. (1966) *Biol. Rev. Cambridge Philos. Soc.* **41**, 445–502.
2. Lanyi, J. K. (1979) *Biochim. Biophys. Acta* **559**, 377–398.
3. Schuldiner, S. & Fishkes, H. (1978) *Biochemistry* **17**, 707–710.
4. Bhattacharyya, P. & Barnes, E. M. (1978) *J. Biol. Chem.* **253**, 3848–3851.
5. Beck, J. C. & Rosen, B. P. (1979) *Arch. Biochem. Biophys.* **194**, 208–214.
6. Mandel, K. A., Guffanti, A. A. & Krulwich, T. A. (1980) *J. Biol. Chem.* **255**, 7391–7396.
7. Guffanti, A. A., Blanco, R., Beneson, R. A. & Krulwich, T. A. (1980) *J. Gen. Microbiol.* **119**, 79–86.
8. MacDonald, R. E., Greene, R. V., Clark, R. D. & Lindley, E. V. (1979) *J. Biol. Chem.* **254**, 11831–11838.
9. Greene, R. V. & Lanyi, J. K. (1979) *J. Biol. Chem.* **254**, 10896–10994.
10. Luisi, B. F., Lanyi, J. K. & Weber, H. J. (1980) *FEBS Lett.* **117**, 354–358.
11. Harold, F. M. & Papineau, D. (1972) *J. Membr. Biol.* **8**, 45–62.
12. Heefner, D. L. & Harold, F. M. (1980) *J. Biol. Chem.* **255**, 11396–11402.
13. Heefner, D. L., Kobayashi, H. K. & Harold, F. M. (1980) *J. Biol. Chem.* **255**, 11403–11407.
14. Harold, F. M., Baarda, J. R., Baron, C. & Abrams, A. (1969) *J. Biol. Chem.* **244**, 2261–2268.
15. Kobayashi, H., Van Brunt, J. & Harold, F. M. (1978) *J. Biol. Chem.* **253**, 2085–2092.
16. Cross, R. L. & Kohlbrenner, W. E. (1978) *J. Biol. Chem.* **253**, 4865–4873.
17. Harold, F. M. & Baarda, J. R. (1968) *J. Bacteriol.* **95**, 816–823.
18. Brey, R. N., Beck, J. C. & Rosen, B. P. (1978) *Biochem. Biophys. Res. Commun.* **83**, 1588–1594.
19. Mitchell, P. (1979) *Eur. J. Biochem.* **95**, 1–20.
20. Shackman, R., Schwartz, A., Saccomani, G. & Sachs, G. (1977) *J. Membr. Biol.* **32**, 361–381.
21. Chang, H., Saccomani, G., Rabon, E., Shackman, R. & Sachs, G. (1977) *Biochim. Biophys. Acta* **464**, 313–327.
22. Harold, F. M., Pavlasova, E. & Baarda, J. R. (1970) *Biochim. Biophys. Acta* **196**, 235–244.
23. Kashket, E. R., Blanchard, A. G. & Metzger, W. A. (1980) *J. Bacteriol.* **143**, 128–134.
24. Kashket, E. R. (1981) *J. Bacteriol.* **146**, 369–376.
25. Dimroth, P. (1980) *FEBS Lett.* **122**, 234–236.