Characterization of the Respiration-dependent Na$^+$ Pump in the Marine Bacterium Vibrio alginolyticus*

Hajime Tokuda$^1$ and Tsutomu Unemoto

From the Department of Membrane Biochemistry, Research Institute for Chemobiodynamics, Chiba University, 1-8-1 Inohana, Chiba, Japan

The respiration-dependent Na$^+$ pump (Tokuda, H. and Unemoto, T. (1981) Biochem. Biophys. Res. Commun. 102, 265-271) is examined in detail under various conditions using the cation-loaded Vibrio alginolyticus. The Na$^+$ pump can extrude Na$^+$ against its electrochemical gradient and generate a membrane potential (inside negative) in the presence of a proton conductor, carbonylcyanide m-chlorophenylhydrazone (CCCP). As a result, a passive uptake of H$^+$ occurs that leads to the generation of ΔpH (acidic inside) of similar magnitude to that of the membrane potential. Anoxia or a respiratory inhibitor, 2-heptyl-4-hydroxyquinoline-N-oxide, inhibits the Na$^+$ extrusion, membrane potential generation, and H$^+$ uptake in the presence of CCCP while these activities resume immediately when oxygen or an artificial electron donor, N,N,N',N'-tetramethyl-p-phenylenediamine, is supplied. The Na$^+$ pump is independent of ATP since arsenate drastically decreases the level of intracellular ATP but has no effects on the Na$^+$ extrusion and membrane potential generation. The Na$^+$ pump has a pH optimum at about 8.5 to 9.0 and the generation of membrane potential, extrusion of Na$^+$, and uptake of H$^+$ in the presence of CCCP are not observed at acidic pH. At alkaline pH, Na$^+$ markedly stimulates the generation of membrane potential and rates of oxygen consumption by K$^+$-loaded cells. Such results strongly indicate that the Na$^+$ pump is indispensable to energetics of V. alginolyticus under alkaline conditions. We conclude that V. alginolyticus is able to pump out not only H$^+$ but also Na$^+$ as an immediate result of electron transport at alkaline pH. We also discuss the possible roles of the primary Na$^+$ pump under Na$^+$-rich environments.

A number of papers have been presented concerning the generation of an electrochemical potential of H$^+$ (a proton motive force) across bacterial membranes and its roles in energy-dependent reactions (1–4). From these reports, it is no doubt that the Na$^+$ pump is unique and may provoke the question about the fundamental concepts of energetics in halophilic bacteria, it is necessary to characterize the Na$^+$ pump in detail. In this paper, the activities of Na$^+$ pump were examined under various conditions and it was concluded that Na$^+$ is pumped out by V. alginolyticus as a direct result of electron transport.

EXPERIMENTAL PROCEDURES

Growth of Cells—The marine bacterium V. alginolyticus 138-2 was grown aerobically at 37 °C on a synthetic medium (18) containing 0.3 M NaCl and 1% glycerol as a sole source of carbon. The cells were harvested at the late logarithmic phase of growth by centrifugation at 4 °C.

Preparation of cells loaded with various monovalent cations was performed as described (16) using DEA-HCl, pH 8.5, containing 0.4 M desired cation as a chloride salt. After the second treatment with DEA buffer, the cells were washed with and resuspended in a specified buffer containing 0.4 M salt and kept on ice until use.

The abbreviations used are: AIB, α-aminoisobutyric acid; Δψ, membrane potential; Δp, the electrochemical potential of H$^+$; CCCP, carbonylcyanide m-chlorophenylhydrazone; HQNO, 2-heptyl-4-hydroxyquinoline-N-oxide; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine; TPP$, tetraphenyl phosphonium ion; DEA, diethanolamine; MES, 2-(N-morpholino)ethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tricine, N-[tris(hydroxymethyl)methyl]glycine; Tris, tris(hydroxymethyl)methylamine.

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2 H. Tokuda, M. Sugasawa, and T. Unemoto, unpublished results.

3 Kakinuma, Y., and Unemoto, T., manuscript in preparation.

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† To whom all correspondence should be addressed.
The intracellular concentration of loaded cation was about 0.4 mM and that of Na+ in K+ - or Li+-loaded cells was negligible (16).

Determination of δψ and δpH—Generations of δψ (negative inside) and δpH (acidic inside) were examined at room temperature from the equilibrium distribution of [3H]TPP+ and [14C]methylamine, respectively. The distribution of these probes was determined by flow dialysis (3) and filtration.

Flow dialysis was performed as described (17, 18) with a sample flow rate of 1 ml/min. Radioactivity was continuously monitored by a Flow-One radioactivity monitor (Radiometric Instruments and Chemical Co., Tampa, FL) using liquid scintillator (flow rate, 4 ml/min) for H+ or solid scintillator for C +. Counts accumulated for 1 min were collected and plotted after the correction for background. Oxygenated buffer was pumped from the lower chamber of the flow dialysis cell and the upper chamber was kept under a stream of oxygen. Where cited, nitrogen gas was employed instead of oxygen.

In some experiments, δψ and δpH were determined by filtration. Cells were preincubated at 25°C for 6 min in 50 μl of 50 mM specified buffer containing 0.4 mM NaCl and 20 mM glycerol. The assay was started by the addition of radioactive probe. At time intervals, the uptake was terminated by addition of 2 ml of 0.4 mM NaCl at room temperature and by filtration with cellulose acetate filters (Schleicher and Schuell, pore size 0.45 μm). The filters were washed once with 2 ml of 0.1 M NaCl to obtain all activities and δpH values were determined.

Steady state concentration gradients of TPPI and methylamine were calculated by using a value of 3.3 μl of internal water space/mg of cell protein (18). δψ was determined from the Nernst equation, δψ = 29 log[TPP+] [TPPP-] ... Internal pH was calculated from the distribution of methylamines (pKα = 10.60) as described (19), pH = -log[H+] in units, the chemical potential of H+ (59ApH, in mV), and δpH (δψ = 59ApH, in mV) were then calculated. Flow dialysis and filtration gave essentially the same values of δψ and δpH.

Determination of ATP—Intracellular ATP was determined by the luciferin/luciferase method as described (18).

Results

Effects of Membrane-permeable Amines and CCCP on the Generation of δψ—The first indication of the presence of a primary ion pump that extrudes ion(s) other than H+ was obtained when the generations of δψ (negative inside) and δpH (acidic inside) by Na+-loaded V. alginolyticus were monitored from the distribution of [3H]TPP+ and [14C]methylamine, respectively, by flow dialysis (Fig. 1). Although CCCP almost completely collapsed δψ generated in 50 mM DEA- HCl, pH 8.5, containing 0.4 mM NaCl (Fig. 1A, closed circles), δψ generated in 50 mM Tricine-NaOH, pH 8.5, containing 0.4 mM NaCl was only transiently collapsed by 10 μM CCCP as shown by open circles in Fig. 1A. The δψ generated in DEA buffer was calculated to be ~152 mV and Δψ values of ~167 and ~153 mV were detected in Tricine buffer before and 15 min after the addition of CCCP, respectively. While δψ generated at pH 8.5 in 0.4 mM NaCl with buffers like sodium phosphate and Tris as well as Tricine was insensitive to CCCP, the addition of DEAE (Fig. 5), ethanamine (Fig. 1A), or methylamine (not shown) at a final concentration of 50 mM subsequent to the addition of CCCP led to near complete collapse of δψ. Since a large δψ was generated before the addition of CCCP in DEA buffer (Fig. 1A) and the addition of ethanamine or methylamine in the absence of CCCP did not collapse this δψ, it was obvious that the addition of these amines and CCCP in combination was necessary for the collapse of δψ. The reason for such a strange effect of amines on the sensitivity to CCCP was not clear until the generation of reversed δpH was monitored under the same conditions. Although the accumulation of [14C]methylamine by the cells was small in DEA buffer whether CCCP was present or not (Fig. 1B, closed circles), the cells assayed in Tricine buffer showed significant stimulation of the accumulation upon the addition of CCCP (open circles). δpH of ~2.3 units (acidic inside, correspond to ~136 mV of chemical potential) was calculated 15 min after the addition of CCCP. Thus, if at this point was only ~17 mV. Such a result clearly indicated that the cytoplasmic membrane of the Na+-loaded cells in the presence of CCCP was permeable to H+. The CCCP-dependent δpH was diminished when DEAE (Fig. 5), methylamine (not shown), or ethanamine (Fig. 1B, open circles) was added at a final concentration of 50 mM. Such effects of amines were expected since these amines were thought to permeate membranes in their unprotonated form and alkalinize the cytoplasm by their protonation. These results forced us to draw the unexpected conclusion that the δψ observed at pH 8.5 in the presence of CCCP is generated by a pump which is extruding ion(s) other than H+. When membranes become permeable to H+ by CCCP, such a δψ causes the passive accumulation of H+ until the magnitude of δpH reaches the magnitude of δψ and then the net influx of H+ ceases. On the other hand, H+ influx mediated by CCCP is continuous in the presence of membrane-permeable amines since these amines collapse δpH. This is the reason why the combined addition of CCCP and amines is necessary for the depolarization of δψ. These results also indicated that the ion which is responsible for the generation of CCCP-insensitive δψ should be the one present in much higher concentration than H+.

Cation-specificity in the Generation of CCCP-insensitive δψ—V. alginolyticus loaded with Li+ or K+ was prepared and assayed for the generation of δψ in 50 mM Tricine buffer (pH 8.5) containing 0.4 mM LiCl, KCl, or NaCl. Li+-loaded cells assayed in the presence of 0.4 mM LiCl generated a large δψ (~155 mV) which was diminished to ~103 mV by the addition of CCCP (open circles in upper patterns of Fig. 2), while K+-loaded cells in the presence of 0.4 mM KCl generated a small
were initiated by the addition of the radioactive probe at 0 time to the equilibrium distribution of $[^3]HTPP^+$ (19 μCi, 404 μCi/μmol) and $[^4]C$-methylamine (21 μCi, 44 μCi/μmol), respectively. Experiments were initiated by the addition of the radioactive probe at 0 time to 800 μl of 0.4 M NaCl containing 20 mM glycerol and either 50 mM DEA-HCl, pH 8.5, (●) or 50 mM Tricine-NaOH, pH 8.5, (□). Cells of $Na^+$-loaded V. alginolyticus prepared as described under "Experimental Procedures" were added at 15 min to give a final concentration of 2.2 mg of protein/ml. Additions of CCCP at 30 min and of ethanolamine-HCl (EtAm), pH 8.5, at 45 min were made to give final concentrations of 10 μM and 50 mM, respectively. Radioactivity in dialysate was continuously monitored and counts accumulated for 1 min were plotted in log scale after the correction for background. Counts at 5 min in each curve were 1400 cpm in A and 4500 cpm in B.

Δψ (−107 mV) which was completely collapsed by CCCP (open circles in lower patterns). When both cation-loaded cells were assayed in the presence of 0.4 M NaCl, the generation of Δψ, especially in the presence of CCCP, was significantly stimulated (closed circles). Δψ values of −152 and −131 mV were generated in the presence of NaCl and CCCP by $Li^+$- and $K^+$-loaded cells, respectively. Furthermore, $K^+$-loaded cells assayed in NaCl without CCCP generated Δψ of −144 mV which was considerably larger than that (−107 mV) generated by the cells in KCl without CCCP. These results indicated that $Na^+$ is essential for the generation of a maximum magnitude of Δψ whether CCCP is present or not and that the $Na^+$ pump is functioning to generate Δψ at pH 8.5. As discussed in a later section (Fig. 6) and in a previous paper (17), the examination of $Na^+$ extrusion at pH 8.5 clearly demonstrated the presence of the primary $Na^+$ pump. The fact that $Li^+$-loaded cells generated a considerable magnitude of Δψ even in the presence of CCCP may indicate that $Li^+$ can partly substitute for $Na^+$ in the generation of Δψ.

**Effect of External pH on the Generation of CCCP-insensitive Δψ and CCCP-dependent ΔpH**—The generation of Δψ by $Na^+$-loaded cells was examined at pH 6.0 and 8.5 in the presence of various concentrations of CCCP (Fig. 3). Although Δψ at pH 8.5 was hardly changed by up to near 50 μM CCCP, Δψ at pH 6.0 was almost completely collapsed by 2 μM CCCP. When the respiration-dependent $H^+$ extrusion was examined at pH 8.5, CCCP added at 2 μM completely abolished the $H^+$ extrusion. Hence, the sensitivity of membranes to CCCP was not altered by external pH. Δψ and ΔpH in the presence of 10 μM CCCP were determined over the pH range of 6.0 to 9.0 (Fig. 4). As shown by closed circles, the generation of CCCP-insensitive Δψ was pH-dependent and had a pH optimum at
The Intracellular Level of ATP and the Generation of Δψ—Although it seemed unlikely from the results described above that ATP was required for the generation of CCCP-insensitive Δψ, such a possibility was examined by determining ATP level and comparing it with Δψ under various conditions (Table I). CCCP added alone had effects on neither the ATP level nor Δψ determined in the presence of 20 mM glycerol. It may be noteworthy that in the absence of glycerol, the ATP level was decreased by CCCP to about half of control level. Although arsenate did not collapse the CCCP-insensitive Δψ at all, ATP level was significantly reduced and only about 20% of the level was found after the addition of arsenate. Furthermore, the level of ATP under the presence of CCCP and HQNO was not affected by TMPD and remained in a high level, on the contrary, TMPD was necessary to the generation of Δψ under such conditions. These results demonstrate that the intracellular ATP is not essential for the generation of Δψ by the Na⁺ pump.

Energetics of Na⁺ Extrusion at Alkaline pH—If the Na⁺ pump is truly responsible for the generation of CCCP-insensitive Δψ, the extrusion of Na⁺ must be shown under the conditions where the CCCP-insensitive Δψ was generated. In our previous paper (17), it was shown that Na⁺ extrusion at pH 8.5 in the presence of glycerol required K⁺ as a counter ion and was not inhibited by CCCP, while the strong inhibition of Na⁺ extrusion by CCCP was observed at pH 6.5. These results let us propose that the extrusion of Na⁺ at pH 8.5 was a primary process and responsible for the generation of CCCP-insensitive Δψ. Energetics of Na⁺ extrusion was further examined to confirm and extend the proposal (Fig. 6).

When respiration was inhibited by HQNO, CCCP-insensitive Na⁺ extrusion did not take place even in the presence of K⁺ (Fig. 6A, open circles). While TMPD added to the cells treated with HQNO failed to induce the CCCP-insensitive generation of Na⁺ at pH 6.5 (Fig. 6A, triangles), the extrusion of Na⁺ at pH 8.5 in the presence of CCCP immediately took place upon the addition of TMPD (Fig. 6A, closed circles). As expected from the result that membrane-permeable amines in combination with CCCP collapsed Δψ, the addition of Δψ, switching of the gas to oxygen immediately caused the generation of Δψ (−154 mV) in the presence of CCCP. On the other hand, Δψ, shown by open circles, was small, if any, before the switching of gas and was not affected by CCCP. When Δψ was generated by aerobicism, the concomitant generation of ΔpH (−123 mV) was also observed.

As shown by closed circles in Fig. 5B, Δψ detected after additions of cell suspensions and HQNO was rather stable and calculated to be about −160 mV. This may be due to the fact that about 10% of oxygen consumption is insensitive to HQNO and, moreover, may indicate that HQNO does not have an ionophoric activity. In any event, Δψ generated under such conditions was collapsed by CCCP. The inhibition of oxygen consumption in V. alginolyticus by HQNO occurs at the site before cytochrome c and can be overcome by the addition of artificial electron donor TMPD, which probably donates electrons to cytochrome c (22). As expected from such data, the addition of TMPD led to the generation of Δψ of about −145 mV in the presence of HQNO and CCCP, whereas a large ΔpH (−130 mV) was not generated until the reduced TMPD was added to the cells treated with HQNO and CCCP (open circles). Although results were omitted from the figures, if CCCP was not added, oxygen or TMPD did not lead to the generation of ΔpH.

From these results and the result that cyanide inhibits the generation of CCCP-dependent Δψ (17), it is clear that the generation of CCCP-insensitive Δψ with a concomitant generation of ΔpH requires continuous respiration.
of DEA and CCCP in combination could serve as a counter ion and induced a bulk extrusion of Na⁺ (Fig. 6B, closed circles). Compared to control (Fig. 6B, open circles), more than half of intracellular Na⁺ was extruded for 5 min after the addition of DEA and CCCP. Although results were omitted from the figure, a single addition of DEA or CCCP caused less than 10% of Na⁺ extrusion for 1 min and the levels of intracellular Na⁺ at 5 min were about 80% in both cases. Effects of arsenate on the CCCP-insensitive Na⁺ extrusion were examined in such systems. Arsenate affected neither the rate nor the extent of Na⁺ extrusion induced by the addition of DEA and CCCP (Fig. 6B, triangles). These results show complete agreement with the results of Δψ generation in the presence of 10 μM CCCP and 50 μM HQNO determined by filtration. Other values of Δψ were calculated from the experiments shown in Figs. 1 and 5.

Effects of inhibitors on the intracellular level of ATP and Δψ in Na⁺-loaded V. alginolyticus

Cells of Na⁺-loaded V. alginolyticus were resuspended at a concentration of 1.5 mg of protein/ml in 2 ml of 50 mM Tricine-NaOH, pH 8.5, containing 0.4 M NaCl and 20 mM glycerol and incubated at 25 °C. Additions of CCCP, sodium arsenate, HQNO, and TMPD were made as indicated by arrows at final concentrations of 10 μM, 10 μM, 50 μM, and 0.14 mM, respectively. At time intervals, aliquots (50 μl) of cell suspensions were treated for exactly 5 min with 2.5 ml of boiling 20 mM glycolline, pH 8.0, containing 4 mM MgSO₄ and 5 mM sodium arsenate for the extraction of ATP. ATP content was determined by the luciferin/luciferase method as described (19). The values of ATP shown were determined at 15 min after the addition of inhibitor(s). The Δψ in the presence of 10 μM CCCP and 10 μM arsenate was determined from the distribution of [³²H]TPP⁺ by flow dialysis. [³²H]TPP⁺ uptake in cells was treated and plotted as mentioned in Fig. 1.

Additions | [ATP]₅₀ | Δψ
---|---|---
None | 17.2 | -167
CCCP | 16.1 | -153
CCCP + arsenate | 2.9 | -165
CCCP + HQNO | 14.2 | -50
CCCP + HQNO + TMPD | 13.5 | -145

Effects of arsenate on the CCCP-insensitive Na⁺ extrusion

Cells were resuspended at a concentration of 1.7 mg of protein/ml at 15 min was made with 50 μM HQNO, CCCP (10 μM), TMPD (0.14 mM) and DEA-HCl, pH 8.5, (50 μM) were added as indicated. Radioactivity obtained in dialysate was treated and plotted as mentioned in Fig. 1.

Table I

| Additions | [ATP]₅₀ | Δψ |
|---|---|---|
| None | 17.2 | -167|
| CCCP | 16.1 | -153|
| CCCP + arsenate | 2.9 | -165|
| CCCP + HQNO | 14.2 | -50|
| CCCP + HQNO + TMPD | 13.5 | -145|

Effects of inhibitors on the intracellular level of ATP and Δψ in Na⁺-loaded V. alginolyticus

Cells of Na⁺-loaded V. alginolyticus were resuspended at a concentration of 1.3 mg of protein/ml in 2 ml of 50 mM Tricine-NaOH, pH 8.5, containing 0.4 M NaCl and 20 mM glycerol. A, the experiments were performed under the stream of nitrogen instead of oxygen and the buffer saturated with nitrogen was pumped from the lower chamber. Additions of cells, CCCP, and DEA-HCl, pH 8.5, were made as indicated by arrows at final concentrations of 1.7 mg of protein/ml, 10 μM and 50 mM, respectively, and the gas was switched to oxygen at 45 min. B, additions of cells (1.7 mg of protein/ml) at 15 min was made with 50 μM HQNO, CCCP (10 μM), TMPD (0.14 mM) and DEA-HCl, pH 8.5, (50 μM) were added as indicated. Radioactivity obtained in dialysate was treated and plotted as mentioned in Fig. 1.
presence of CCCP and confirm that the extrusion of Na" is a primary process driven solely by electron transport. It should be pointed out that K"-dependent extrusion of Na" was significantly inhibited by arsenate (results not shown). This presumably represents the requirement of ATP for K" transport system, but not for the Na" pump, which is absolutely necessary to the K"-dependent Na" extrusion (16). It is also notable that the extrusion of Na" at pH 6.5 in the presence of CCCP was not induced by the addition of TMPD (Fig. 6A) or DEA (not shown). The Na"/H" antiport system, driven by the proton motive force, seems to be the only way to extrude Na" at acidic pH.

**Respiration-dependent Flux of Protons across the Membrane**—Anaerobic suspensions of V. alginolyticus were pulsed with oxygen under various conditions and the external pH was monitored to examine the flux of H" (Fig. 7). When Na"-loaded cells were assayed at pH 6.5, the pulse caused a transient acidification of external medium (A) which indicated that H" was extruded on respiration. If the cells were treated with CCCP prior to the pulse, such an acidification was completely inhibited (B). When the assay was performed at pH 8.5 (C), a slight alkalinization occurred after the pulse and then it was followed by the acidification which was slower than that at pH 6.5. On the other hand, the assay performed at pH 8.5 with the CCCP-treated cells (D) gave the result which was strikingly different from that at pH 6.5. The oxygen pulse to such cells led to the marked alkalinization of external medium which indicated that H" was taken up on respiration. In order to characterize the electrogenic feature of H" flux at pH 8.5 in the presence and absence of CCCP, a membrane-permeable cation TPP" was included in the assay medium. The acidification by the control cells in the presence of TPP" (E) was significantly faster than that in the absence of TPP" (C). This indicates that the extrusion of H" occurs against its electrical potential and that TPP", as a counter ion, stimulates the extrusion. On the other hand, TPP" almost completely abolished the alkalinization by the CCCP-treated cells (F).

![Fig. 7 Extrusion and uptake of H" induced by oxygen pulse.](image)

The results presented in this paper confirmed and extended our previous proposal that V. alginolyticus possesses a unique system to generate the proton conductor-insensitive Δψ. The generation of such a Δψ was dependent on Na" (Fig. 2). The examination of Na" extrusion revealed that the process at pH 8.5 was a primary transport system and was dependent on respiration (Fig. 6). From the results shown in Figs. 5 and 7, where the generation of Δψ and uptake of H" in the presence of CCCP were demonstrated by the supply of oxygen to
Therefore, the generation of such a $\Delta\psi$ cannot be ascribed to the presence of CCCP showed maximum activity at alkaline pH. A possible involvement of ATP in the phenomena discussed above was excluded since the fluctuation of ATP level was not related to $\Delta\psi$ generation (Table I) and arsenate did nothing to the extrusion of Na$^+$ in the presence of CCCP (Fig. 6).

It is our conclusion that V. alginolyticus is able to pump out Na$^+$ as well as H$^+$ by its respiratory chain (Fig. 9). At alkaline pH, the electrogenic extrusion of Na$^+$ in addition to H$^+$ occurs to generate $\Delta\psi$. When the membrane becomes permeable to H$^+$ by the action of CCCP, $\Delta\psi$ due to H$^+$ collapses but that due to Na$^+$ exists since the concentration of Na$^+$ is several orders of magnitude higher than that of H$^+$. Therefore, the addition of CCCP causes the accumulation of H$^+$ which continues until the magnitude of $\Delta\psi$ becomes similar to that of $\Delta\phi$. The membrane-permeable amines added in high concentration scavenge H$^+$ accumulated inside the cells and make the continuous influx of H$^+$ possible. Thus, CCCP with a help of such amines is able to collapse $\Delta\psi$ generated by the Na$^+$ pump. Although questions still remain whether or not the extrusion of H$^+$ at acidic pH and the extrusion of H$^+$ plus Na$^+$ at alkaline pH are performed by the same respiratory chain and whether or not the participation of the additional components at alkaline pH is necessary for the extrusion of Na$^+$, it is likely that at least one “Na$^+$ site” exists after cytochrome c of the respiratory chain since TMPD causes the extrusion of Na$^+$ (Fig. 6) with concomitant generation of $\Delta\phi$ (Fig. 5) in the presence of CCCP. Detailed molecular mechanisms of the respiratory-dependent Na$^+$ pump, including the possibility of other Na$^+$ sites, are currently under examinations.

It is of interest that both H. halobium (11, 12) and V. alginolyticus live in Na$^+$-rich environments and have developed devices by which energy is directly converted to the Na$^+$ electrochemical potential across the membranes. Furthermore, it has recently been demonstrated that Klebsiella aerogenes that requires Na$^+$ for the anaerobic growth on citrate also possesses an inducible Na$^+$ pump (23-25). However, the mechanisms involved are totally different among these bacteria. H. halobium conserves light energy as the Na$^+$ electrochemical potential by means of the light-dependent Na$^+$ pump halorhodopsin. K. aerogenes obtains the energy from the decarboxylation of oxaloacetate and V. alginolyticus utilizes redox energy for the generation of Na$^+$ electrochemical potential by the respiratory-dependent Na$^+$ pump. Although, so far reported, the proton motive force plays a central role in energetics of nonhalophilic bacteria, the results presented here and those with H. halobium seem to indicate that it is the Na$^+$ electrochemical potential, or the sodium motive force, that plays a central role in energetics of halophilic bacteria. Consistent with this idea, transport of solutes in these bacteria are all driven by the Na$^+$ electrochemical potential $\Delta\psi$ generated by the Na$^+$ pump may be able to drive ATP synthesis since a red mutant of H. halobium lacking bacteriorhodopsin synthesizes ATP upon illumination (26, 27) although the ion which actually passes through the ATPase complex is likely to be H$. It should be pointed out that the proton motive force in V. alginolyticus also plays an indispensable role in energetics, especially at acidic pH. Since the respiratory-dependent Na$^+$ pump has a low activity at acidic pH, it must be the proton motive force that drives the generation of the Na$^+$ motive force via the Na$^+/H^+$ antiport system and the synthesis of ATP at acidic pH.

The Na$^+$ pump seems to give advantages to V. alginolyticus living in Na$^+$-rich environments. First of all, under the conditions where a passive influx of Na$^+$ is significant, the extrusion of Na$^+$ as an immediate result of electron transport may be more economical than the extrusion of Na$^+$ by the Na$^+/H^+$ antiport system energized by the proton motive force. Furthermore, the driving force for solutes uptake can be obtained directly from an oxidation of substrate as in the case of nonhalophilic bacteria which generate the proton motive force and take up many solutes by H$^+$-symport systems. There may be an additional advantage in having the primary Na$^+$ pump at alkaline pH. As far as reported, the cytoplasmic pH in microorganisms is regulated near neutral and becomes even more acidic than external pH under alkaline conditions. The proposed mechanism concerning the regulation of cytoplasmic pH involves the Na$^+/H^+$ antiport system which enables cells to generate $\Delta\psi$ and $\Delta\phi$ in opposite directions (28). However, since such a system may consume more energy at alkaline pH than it does at acidic pH (29), the generation of $\Delta\psi$ by the Na$^+$ pump, which does not cause the alkalization of cytoplasm, seems to be economically superior under alkaline and Na$^+$-rich environments to the generation of $\Delta\phi$ by the proton pump, which inevitably causes the alkalization.

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