Journal of Bioenergetics and Biomembranes, Vol. 21, No. 3, 1989

A Study on Na^+-Coupled Oxidative Phosphorylation: ATP Formation Supported by Artificially Imposed ΔpNa and ΔpK in *Vibrio alginolyticus* Cells[1]

P. A. Dibrov,[2] R. L. Lazarova,[2] V. P. Skulachev,[2,3] and M. L. Verkhovskaya[2]

Received August 31, 1988

Abstract

Addition of Na^+ to the K^+-loaded *Vibrio alginolyticus* cells, creating a 250-fold Na^+ gradient, is shown to induce a transient increase in the intracellular ATP concentration, which is abolished by the Na^+/H^+ antiporter, monensin. The ΔpNa-supported ATP synthesis requires an additional driving force supplied by endogenous respiration or, alternatively, by a K^+ gradient (high $[K^+]$ inside). In the former case, ATP formation is resistant to the protonophorous uncoupler. Dicyclohexylcarbodiimide and diethylstilbestrol, but not vanadate, completely inhibit Na^+ pulse-induced ATP formation. The data agree with the assumption that Na^+-ATP-synthase is involved in oxidative phosphorylation in *V. alginolyticus*. Interrelation of H^+ and Na^+ cycles in bacteria is discussed.

Key Words: Na^+-coupled phosphorylation; Na^+ cycle; Na^+ pulse; *Vibrio alginolyticus*.

Introduction

In bacteria living under alkaline conditions, intracellular pH is lower than extracellular pH (Padan *et al.*, 1981). This may create a problem for a cell energizing its membrane by means of $\Delta\bar{\mu}H^+$ generators that pump H^+ from

[1] Abbreviations: $\Delta\bar{\mu}H^+$ and $\Delta\bar{\mu}Na^+$, electrochemical gradients of H^+ and Na^+, respectively; $\Delta\psi$, transmembrane electric potential difference; ΔpH, ΔpNa, and ΔpK, concentration gradients of H^+, Na^+, and K^+, respectively; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; DES, diesthylstilbestrol; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; Tricine, *N*[2-hydroxy-1,1-*bis*(hydroxymethyl)ethyl]glycine.

[2] A. N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR.

[3] To whom correspondence should be addressed.

0145-479X/89/0600-0347$06.00/0

cytoplasm. $\Delta\psi$ formed by these generators is in fact counterbalanced by preexisting reverse ΔpH. Substitution of $\Delta\bar{\mu}Na^+$ generators for $\Delta\bar{\mu}H^+$ generators seems to be a way to solve the problem. This is the case for the alkalotolerant marine-respiring *Vibrio alginolyticus*. This bacterium (a) was shown to survive in the presence of protonophorous uncoupler discharging $\Delta\bar{\mu}H^+$ (Tokuda and Unemoto, 1983); (b) possesses a Na^+-motive NADH-quinone reductase exporting Na^+, not H^+, from the cell (Tokuda and Unemoto, 1982, 1984); and (c) utilizes $\Delta\bar{\mu}Na^+$ to import metabolites (Tokuda *et al.*, 1982; Kakinuma and Unemoto, 1985) and to rotate the flagellum (Chernyak *et al.*, 1983).

Our group demonstrated respiration-supported ATP synthesis in *V. alginolyticus* cells. The process was (a) stimulated by Na^+, (b) inhibited by the artificially imposed reverse ΔpNa ($[Na^+_{in}] > [Na^+_{out}]$). (c) uncoupler resistant, and (d) sensitive to the combination of monensin and the uncoupler (Dibrov *et al.*, 1986; Verkhovskaya *et al.*, 1987). It was assumed that *V. alginolyticus* possesses a Na^+-ATP-synthase that consumes the respiration-generated $\Delta\bar{\mu}Na^+$ and catalyzes a Na^+-coupled oxidative phosphorylation. Thus, in *V. alginolyticus*, the respiratory energy transduced to $\Delta\bar{\mu}Na^+$ can support all three types of the membrane-linked work, i.e., chemical, osmotic, and mechanical. Such a novel type of membrane energetics is called the Na^+ cycle (Skulachev, 1984, 1984a, b, 1986).

Some information on the properties of Na^+-ATP-synthase may be obtained by the study of ATP formation supported by artificially imposed ΔpNa since Na^+-ATP-synthase seems to be the only enzyme involved in such a process. Our group has shown that Na^+ ions added to the K^+-loaded *V. alginolyticus* cell incubated in a Na^+-free medium induce a transient increase in the ATP level (Dibrov *et al.*, 1986). This study of this phenomenon is described in the present article.

Materials and Methods

The *V. alginolyticus* 138-2 strain (a generous gift of Prof. H. Tokuda) was grown at 37°C under anaerobic conditions in the presence of 0.5% glucose, pH 8.6 [for other conditions, see Tokuda *et al.* (1984)]. The intracellular ATP concentration was measured with the luciferase method, as was described earlier (Arshavsky *et al.*, 1981).

To obtain K^+-loaded cells, the procedure described by Tokuda and Unemoto (Nakamura *et al.*, 1982) was used. The only modification was that diethylamine was substituted for methylamine. To increase the viability of the K^+-loaded cells, they were suspended in solutions supplemented with $MgCl_2$ (Nakamura *et al.*, 1984).

To impose a Na^+ gradient across the bacterial membrane, the K^+-loaded cells were incubated in a medium containing 10 mM $MgCl_2$, 25 mM Tricine (pH 8.6), and 500 mM KCl or LiCl (the K^+ or Li^+ media, respectively). After the 5 min incubation, the cell suspension was diluted twofold by a medium containing 10 mM $MgCl_2$, 25 mM Tricine (pH 8.6), and 500 mM NaCl (the Na^+ medium). Before the dilution, the protein concentration was 0.2–0.5 mg ml^{-1}. The concentrations of K^+ and Na^+ were measured with a PFM flame photometer.

Results

Usually, $[Na^+]_{in}$ in *V. alginolyticus* cells is 30–80 mM at 500 mM $[Na^+]_{out}$. Using the procedure suggested by Tokuda and Unemoto (Nakamura *et al.*, 1982), we replaced Na^+_{in} with K^+ so that $[Na^+]_{in}$ decreased to 1–2 mM. This also resulted in a very strong decrease in the content of ATP and endogenous respiratory substrates. In the K^+-loaded cells incubated without added substrates, the ATP concentration was maintained at the level of 1–10% of the normal, whereas the respiration rate was < 5% of the maximal. Addition of a respiratory substrate, say lactate, was shown strongly to increase both the respiration rate and the ATP level (Dibrov *et al.*, 1986).

In Fig. 1, the K^+-loaded cells were incubated in a K^+ medium and then diluted twofold with the Na^+ medium. It is seen that such a Na^+ pulse resulted in a fast, strong, but transient, increase in the ATP concentration. Subsequent addition of the respiratory substrate, lactate, caused a steady increase in the ATP level. The Na^+/H^+ antiporter, monensin, converting ΔpNa to ΔpH, vigorously inhibited the ATP synthesis supported by the Na^+ pulse and lowered somewhat that supported by lactate oxidation (Fig. 1A). Protonophore CCCP (5×10^{-5} M) failed to abolish the Na^+ pulse—as well as lactate oxidation-induced phosphorylations. At the same time, CCCP, added together with monensin, caused severe inhibition of both effects (Fig. 1B).

Further experiments showed, rather surprisingly, that the respiratory chain inhibitors HQNO and cyanide added at concentrations arresting endogenous respiration inhibit also the Na^+ pulse-stimulated ATP synthesis. To overcome the inhibition of the Na^+ pulse effect, it proved to be necessary to create a $[K^+]$ gradient across the bacterial membrane (high $[K^+]$ inside). As one can see in Fig. 2 (open circles), the Na^+ pulse increases the ATP level in the K^+-loaded cells incubated in the Li^+ medium supplemented with HONO, which arrests the effect of lactate. In the same experiment, it is shown that, in the Li^+ medium, twofold dilution of bacteria by the same medium instead of the Na^+ medium is almost without effect upon the ATP level (Fig. 2, closed circles).

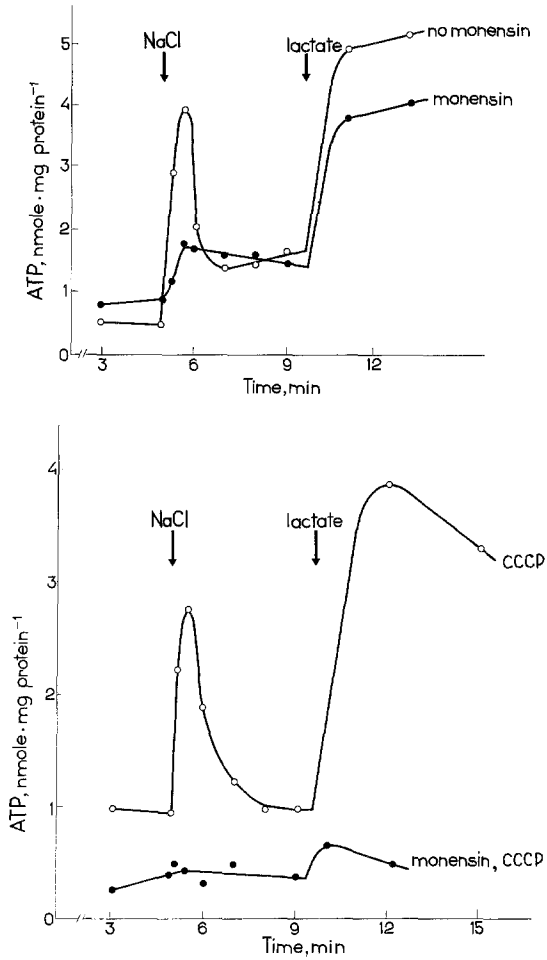

Fig. 1. ATP synthesis in K^+-loaded *V. alginolyticus* cells incubated in the K^+ medium. Incubation mixture (0.25 mM Tricine, pH 8.6, 10 mM $MoCl_2$, and 500 mM KCl) was diluted twofold with similar solution, but containing NaCl instead of KCl. The dilution is indicated as addition of Na^+. Additions: 75 mM D,L-lactate, 5×10^{-5} M monensin, and 5×10^{-5} M CCCP.

In Fig. 2, valinomycin was added to increase the K^+ permeability of the *V. alginolyticus* membrane. Later it was found that a qualitatively similar ATP response occurs also without valinomycin, apparently due to endogenous K^+ conductance (see the *Discussion*) so that, in further experiments, valinomycin was excluded from the mixture.

Figure 3 demonstrates the inhibiting effect of low K^+ concentrations on the Na^+ pulse-stimulated ATP-formation in cyanide-treated K^+-loaded

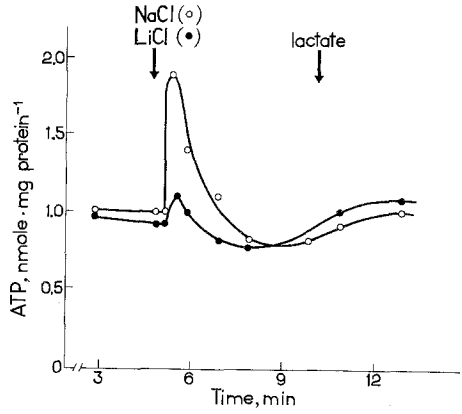

Fig. 2. Na^+ pulse-induced ATP synthesis in the K^+-loaded *V. alginolyticus* cells incubated in the Li^+ medium supplemented with HQNO. Incubation mixture (25 mM Tricine, pH 8.6, 10 mM $MgCl_2$, 500 mM LiCl, 5×10^{-6} M valinomycin, and 1×10^{-4} NQNO) was diluted twofold with the same solution (Li^+, closed circles) or by solution containing 500 mM NaCl instead of LiCl (Na^+, open circles). Addition: 75 mM D,L-lactate.

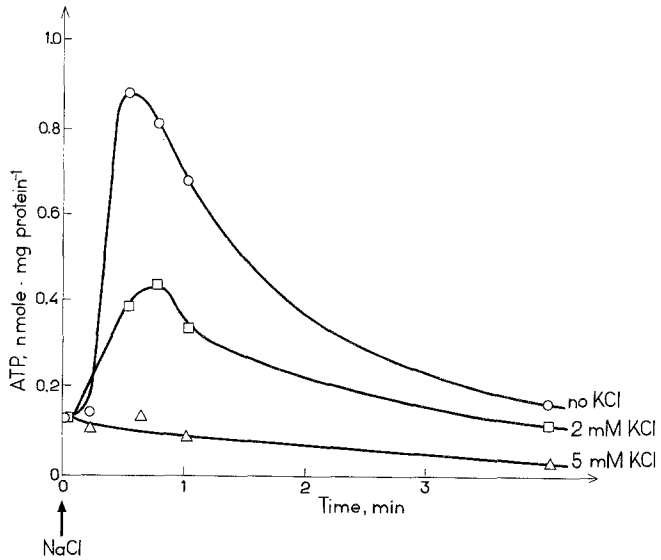

Fig. 3. Effect of external K^+ upon the Na^+ pulse-supported ATP formation in the Li^+ medium. Incubation mixture contained 25 mM Tricine, pH 8.6, 10 mM $MoCl_2$, 2×10^{-4} M KCN, 500 mM LiCl and, if indicated, 2 mM or 5 mM KCl. At zero time, a sample was taken to measure ATP and, in 5 sec, the mixture was diluted with similar solution, but containing Na^+ instead of Li^6.

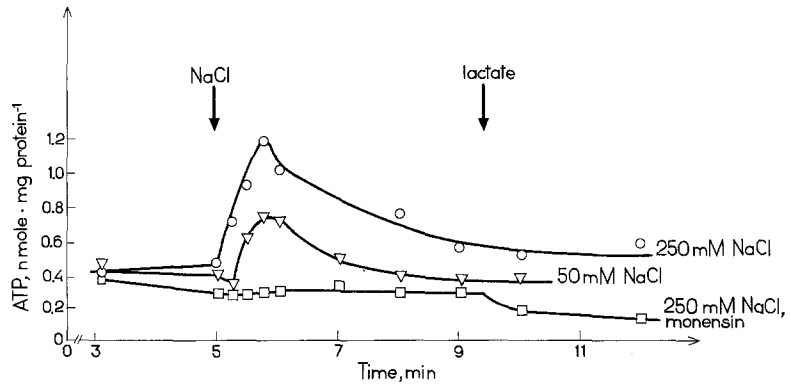

Fig. 4. Effect of Na^+ concentration and monensin upon the magnitude of the Na^+ pulse-induced ATP response. The mixture was as in Fig. 3, but 1.5×10^{-4} M HQNO was substituted for KCN. Addition; 5×10^{-5} M monensin and 75 mM D,L-lactate.

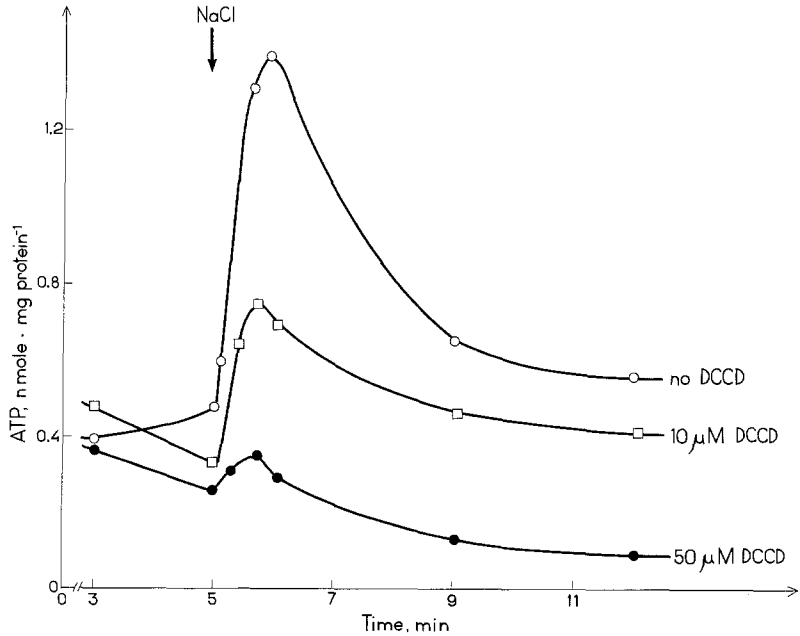

Fig. 5. Dicyclohexylcarbodiimide inhibition of the Na^+ pulse-induced ATP synthesis. Conditions were as in Fig. 3, but 0.5 mM NaCN was added instead of KCN.

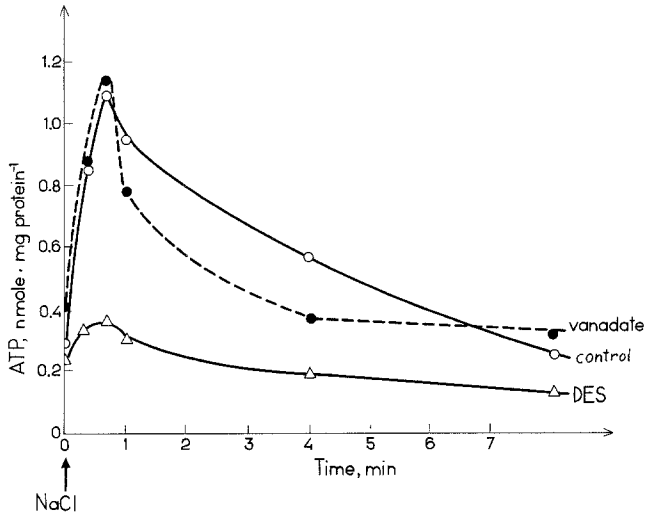

Fig. 6. Effect of DES and vanadate upon the Na^+ pulse-induced ATP synthesis. Conditions were as in Fig. 2. Additions: 2×10^{-4} M orthovanadate and 1×10^{-4} M DES.

cells: 2 mM K^+ induced half-maximal inhibition. In Fig. 4, we varied concentration of the added Na^+. One can see that a decrease in the Na^+ concentration from 250 mM to 50 mM drastically lowers the magnitude of the pulse-induced ATP response. Most probably, even a 250-mM concentration of Na^+ was too low to support the maximal rate of ATP synthesis under these conditions, since the highest ATP level caused by the Na^+ pulse in the presence of respiratory inhibitors was two- to fourfold lower than that supported by oxidative phosphorylation.

Inhibitor analysis of the Na^+ pulse-induced phosphorylation revealed that it is sensitive to DCCD (the half-maximal inhibition at concentration slightly below 1×10^{-5} M), sensitive to 1×10^{-4} M DES, and resistant to 2×10^{-4} M vanadate (Figs. 5 and 6). Oxidative phosphorylation has similar inhibitor sensitivity (not shown in Figures).

Discussion

The above data showed that a sudden increase in the outer Na^+ concentration results in a rise of the ATP level in the *V. alginolyticus* cells. To observe this effect, it proved to be necessary (a) to decrease $[Na^+]_{in}$ beforehand by substituting K^+ for Na^+ in the cytoplasm and (b) to retain some respiration or, alternatively, to induce a K^+ diffusion potential ($[K^+]_{in} \gg [K^+]_{out}$). The measurements showed that, even in K^+-loaded cells, $[Na^+]_{in}$ is

never lower than 1 mM. This means that addition of 250 mM Na^+ to the Na^+-free medium generates, at the first moment, $\Delta pNa \leqslant 2.3$. It is clear that ΔpNa must decrease in time due to the rise of $[Na^+]_{in}$, resulting from the downhill Na^+ influx. To maintain the formed ΔpNa long enough to obtain a measurable [ATP] increase, one may use the Na^+-motive respiratory chain that pumps Na^+ ions, coming to the cytoplasm, back to the outer medium. Apparently just this role was performed by respiration utilizing traces of endogenous substrates that still are retained in the cell after K^+-for-Na^+ exchange. The rate of this respiration was more than 20-fold lower than the maximal one. It was not sufficient to maintain the high ATP level in the living cell where many ATP-consuming processes are operative. In this way, we may explain why the Na^+ pulse-supported ATP synthesis in the first series of experiments was sensitive to the respiratory chain inhibitors.

To verify this suggestion, we tried to replace endogenous respiration with the K^+ diffusion potential as a driving force additional to the artificially imposed ΔpNa. To this end, we substituted Li^+ for K^+ in the incubation mixture with K^+-loaded cells. Under these conditions, a K^+ concentration gradient (ΔpK) persisted across the bacterial membrane. As was shown by Tokuda *et al.* (1984), a K^+ import system, driven by $\Delta\psi$, is activated in *V. alginolyticus* at pH $\geqslant$ 8.0. The downhill efflux of K^+ via this system should be electrogenic.

Apparently, the $\Delta\psi$ formed by the K^+ efflux was too low to support the high steady-state level of ATP. However, it proved to be sufficient to make a detectable contribution to $\Delta\tilde{\mu}Na$ in the Na^+-pulse experiments, thereby replacing endogenous respiration. As it was found in the experimental series shown in Figs. 2–6, the Na^+ pulse was effective even in the presence of cyanide or HQNO, provided there was a large ΔpK between the cytoplasm and the medium.

It should be mentioned that, in "protonic" bacteria, ATP formation induced by H^+ pulse can be demonstrated without any additional driving force. In these cases, however, the artificially imposed ΔpH was always much higher than ΔpNa in the above Na^+-pulse experiments [cf., e.g., $\Delta pH = 4.5$ in the article by Maloney (1978), and $\Delta pNa = 2.3$ in this work). A higher ΔpNa cannot be formed since it was impossible to reduce $[Na^+]_{in}$ below 1 mM without irreversible damage to the cells.

In the first experimental series, the Na^+ pulse-induced [ATP] increase cannot be explained by the activating effect of Na^+ upon Na^+-motive respiration since (a) this increase is transient, (b) it requires 250 mM Na^+, whereas the activation of respiration is already maximal at 1 mM Na^+, and (c) it is abolished by monensin. Similarly, the effect of Na^+ pulse cannot be accounted for by the operation of H^+-ATP-synthase consuming ΔpH that would arise if an endogenous Na^+/H^+ antiporter were operative. If this were

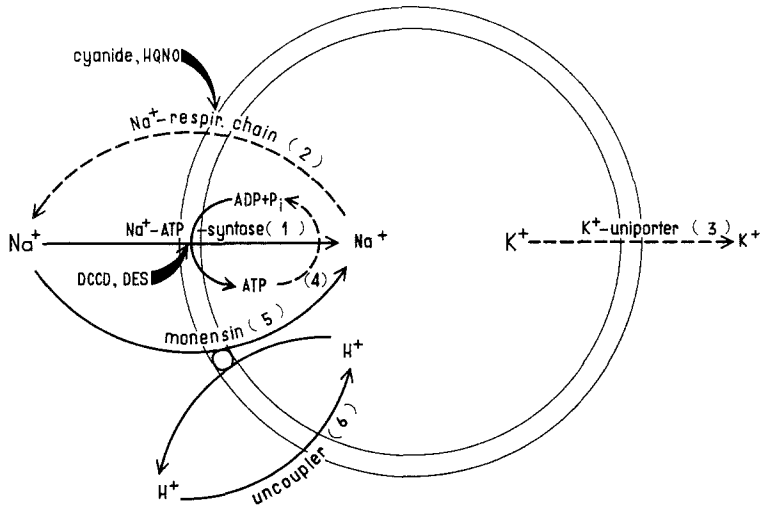

Fig. 7. Interplay of Na^+, K^+, and H^+ transports in the Na^+-pulse experiments: (1) downhill import of Na^+ coupled to ATP formation by Na^+-ATP-synthase, (2) uphill export of Na^+ by the Na^+-motive respiratory chain oxidizing endogenous substrates, (3) downhill K^+ export via endogenous K^+ uniporter (4) ATP utilization by the cell (5) monensin-mediated Na^+_{out}/H^+_{in} antiport and (6) uncoupler-mediated H^+ import. Heavy arrows show effects of inhibitors. Broken lines represent slower processes.

the case, (a) monensin would be an activator rather than an inhibitor and (b) the effect would be sensitive to the protonophorous uncoupler, 5×10^{-5} M CCCP, which, at pH 8.6, completely dissipates $\Delta\bar{\mu}H^+$ across the *V. alginolyticus* membrane (Tokuda and Unemoto, 1982). It should be stressed that CCCP greatly potentiated the inhibiting effect of monensin on oxidative phosphorylation (Fig. 1B), this fact indicating that CCCP was operative under the conditions used. This inhibition is apparently due to the discharge by CCCP of the $\Delta\psi$ constituent of the respiration-produced $\Delta\bar{\mu}Na^+$, whereas monensin discharged its ΔpNa constituent. K^+ uniport was apparently too slow to compete effectively with Na^+-ATP-synthase for the $\Delta\psi$ generated by active respiration. Therefore, monensin, without the uncoupler, inhibited oxidative phosphorylation only partially (Fig. 1A). The fact that oxidative phosphorylation occurs in the presence of monensin excluded involvement of an electrogenic Na^+/nH^+ antiporter, since a combination of this antiporter and electroneutral Na^+/H^+ exchanger monensin should result in uncoupling.

The interplay of Na^+-ATP-synthase and some natural and artificial porters under the conditions of the Na^+-pulse experiment is illustrated in Fig. 7, where the slower processes are shown by broken lines.

Some properties of hypothetical Na^+-ATP-synthase may be inferred from the inhibitor analysis of Na^+ pulse-driven phophorylation. According

to Figs. 5 and 6, the process is DCCD sensitive, DES sensitive, and vanadate resistant. The same characteristics are inherent in oxidative phosphorylation in the *V. alginolyticus* cell, which suggests that both Na^+ pulse- and respiration-supported phosphorylations are catalyzed by the same enzyme.

DCCD is shown to inhibit all of the known H^+-ATPases and H^+-ATP-synthase [for reviews, see Skulachev (1988) and Salloz (1984)], Ca^{2+}-ATPase of the sarcoplasmic reticulum (Murphy, 1981) and Na^+/K^+-ATPase (Robinson, 1974). DES inhibits H^+-ATP-synthase (McEnery and Pedersen, 1986), plasmalemmal H^+-ATPase from plants and fungi (Skulachev, 1988), and some ion-transfer ATPases (Hinkel and Lathe, 1970).

Significantly, the Na^+ pulse-supported ATP synthesis in *Methanococcus voltae* is DES sensitive (Lancaster *et al.*, 1986), whereas Na^+-ATP-ase from *Propionigenum modestum* is inhibited by DCCD (Dimroth and Hilpert, 1984; Dimroth, 1987). The latter enzyme proved to be composed of the membranous and peripheral parts that, being detached from the membrane, was shown to lose the DCCD sensitivity as well as ability to be activated by Na^+. Its subunit composition was similar to that of the F_1 part of H^+-ATP-synthase (Laubinger and Dimroth, 1987). The impression is that Na^+-ATP-synthase involved in the Δ-μNa^+-driven ATP formation in bacteria is a derivative of H^+-ATP-synthase competent in Na^+ translocation. it is not excluded that the same enzyme, under other conditions, can transport H^+, operating as an H^+-ATP-synthase. In fact, Na^+-ATPase of *P. modestum* pumps protons into proteoliposomes when Na^+ is absent (Dimroth and Laubinger, 1987). According to our data, subcellular vesicles from *V. alginolyticus* are capable of transporting H^+ as well as Na^+ in an ATP-dependent, DCCD-sensitive fashion. ΔpH formation was completely inhibited whereas ΔpNa formation was strongly stimulated by a protonophorous uncoupler (Smirnova *et al.*, 1987; Dibrov *et al.*, 1988).

It should be stressed that *V. alginolyticus* possesses both Na^+-motive and H^+-motive respiratory chains (Tokuda and Unemoto, 1982; Smirnova and Kostyrko, 1989). The same seems to be true for *Vibrio costicola* (Udagawa *et al.*, 1986). Indications were obtained that NADH-quinone step of the respiratory chain is Na^+ motive (Tokuda and Unemoto, 1982; Smirnova and Kostyrko, 1988), whereas the rest is H^+ motive (Smirnova and Kostyrko, 1988). One may speculate that both $\Delta\bar{\mu}Na^+$ and $\Delta\bar{\mu}H^+$ are consumed by one and the same $H^+(Na^+$-ATP-synthase). Perhaps marine alkalotolerant vibriones, being typical respirers, employ H^+-coupled and Na^+-coupled oxidative phosphorylations to survive at neutral pH and alkaline pH, respectively.

In this connection, we may mention that in *M. voltae*, ATP synthesis can be supported by artificially imposed $\Delta\bar{\mu}Na^+$ or $\Delta\bar{\mu}H^+$ in the presence or in the absence of the uncoupler, respectively (Lancaster *et al.*, 1986; Crider *et al.*, 1985; Carper and Lancaster, 1986). It is also known that H^+ is the only ion

1985; Carper and Lancaster, 1986). It is also known that H^+ is the only ion that can be effectively substituted for Na^+ in animal Na^+/K^+-ATPase. The affinity of this enzyme for H^+ is, in fact, at least 2 orders of magnitude higher than for Na^+. The only reason for Na^+/K^+-ATPase to translocate Na^+ rather than H^+ is that $[Na^+]$ is $\sim 10^5$-fold higher than $[H^+]$ at physiological pH. However, at slightly acidic conditions (pH 5.7), some ATPase activity and H^+/K^+ antiport were reported in Na^+/K^+-ATPase proteoliposomes incubated in a Na^+-free medium (Hara *et al.*, 1986).

References

Arshavsky, V. Yu., Baryshev, V. A., Brown, I. I., Glagolev, A. N., and Skulachev, V. P. (1981). *FEBS Lett.* **133**, 22–26.

Carper, S. W., and Lancaster, J. P. (1986). *FEBS Lett.* **200**, 177–180.

Chernyak, B. V., Dibrov, P. A., Glagolev, A. N., Sherman, M. Yu, and Skulachev, V. P. (1983). *FEBS Lett.* **164**, 38–42.

Crider, B. P., Carper, S. W., and Lancaster, J. P. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 6793–6796.

Dibrov, P. A., Lazarova, R. L., Skulachev, V. P., and Verkhovskaya, M. L. (1986). *Biochim. Biophys. Acta* **850**, 458–466.

Dibrov, P. A., Skulachev, V. P., Sokolov, M. V., and Verkhovskaya, M. L. (1988). *FEBS Lett.* **233**, 355–358.

Dimroth, P. (1987). *Microbiol. Rev.* **51**, 320–340.

Dimroth, P., and Hilpert, W. (1984). In *16th FEBS Meeting*, Abstr. 71.

Dimroth, P., and Laubinger, W. (1987). *Hoppe Sevlers Z. Physiol. Chem.* **368**, 547–548.

Hara, Y., Yamada, J., and Nakao, M. (1986). *J. Biochem.* **99**, 531–539.

Hinkel, T. A. J., and Lathe, G. H. (1970). *Biochem. J.* **118**, 187–189.

Kakinuma, J., and Unemoto, T. (1985). *J. Bacteriol.* **163**, 1293–1295.

Lancaster, J. P., Rogers, K. R., Crider, B. P., Carper, S. W., and Al-Mahrouq, H. A. (1986). *EBEC* **4**, 69.

Laubinger, W., and Dimroth, P. (1987). *Eur. J. Biochem.* **168**, 475–480.

Maloney, P. C. (1978). *Biochem. Biophys. Res. Commun.* **83**, 1496–1503.

McEnery, M. W., and Pedersen, P. L. (1986). *J. Biol. Chem.* **261**, 1745–1752.

Murphy, A. J. (1981). *J. Biol. Chem.* **266**, 12,040–12,050.

Nakamura, T., Tokuda, H., and Unemoto, T. (1982). *Biochim. Biophys. Acta* **692**, 389–396.

Nakamura, T., Tokuda, H., and Unemoto, T. (1984). *Biochim. Biophys. Acta* **776**, 330–336.

Padan, E., Zilberstein, D., and Schuldiner, S. (1981). *Biochim. Biophys. Acta* **650**, 151–166.

Robinson, J. D. (1974). *FEBS Lett.* **38**, 325–328.

Salioz, M. (1984). *Trends Biochem. Sci.* **9**, 309–312.

Skulachev, V. P. (1984). *Trends Biochem. Sci.* **9**, 483–485.

Skulachev, V. P. (1985a). *Biokhimiya* **50**, 179–183 [in Russian].

Skulachev, V. P. (1985b). *Eur. J. Biochem.* **155**, 199–208.

Skulachev, V. P. (1986). *Biol. Membr.* **3**, 5–25 [in Russian].

Skulachev, V. P. (1988). *Membrane Bioenergetics*, Springer-Verlag, Berlin.

Smirnova, I. A., and Kostryakov, V. A. (1988). *Biokhimiya* [in Russian] (in press).

Smirnova, I. A., Vulfson, E. N., and Kostryko, V. A. (1987). *FEBS Lett.* **214**, 343–346.

Tokuda, H., and Unemoto, T. (1982). *J. Biol. Chem.* **257**, 10,007–10,014.

Tokuda, H., and Unemoto, T. (1983). *J. Bacteriol.* **156**, 636–643.

Tokuda, H., and Unemoto, T. (1984). *J. Biol. Chem.* **259**, 7785–7790.

Tokuda, H., Sugasawa, M., and Unemoto, T. (1982). *J. Biol. Chem.* **257**, 788–794.

Tokuda, H., Nakamura, T., and Unemoto, T. (1984). *Biochemistry* **20**, 4198–4203.

Udagawa, T., Unemoto, T., and Tokuda, H. (1986). *J. Biol. Chem.* **261**, 2616–2622.

Verkhovskaya, M. L., Dibrov, P. A., Lazarova, R. L., and Skulachev, V. P. (1987). *Biokhimiya* **52**, 883–889 [in Russian].