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MINI-REVIEW

The Sodium Cycle: A Novel Type of Bacterial Energetics

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Abstract

The progress of bioenergetic studies on the role of Na^+ in bacteria is reviewed. Experiments performed over the past decade on several bacterial species of quite different taxonomic positions show that Na^+ can, under certain conditions, substitute for H^+ as the coupling ion. Various primary Na^+ pumps ($\Delta\bar{\mu}_{Na^+}$ generators) are described, i.e., Na^+-motive decarboxylases, NADH-quinone reductase, terminal oxidase, and ATPase. The $\Delta\bar{\mu}_{Na^+}$ formed is shown to be consumed by Na^+ driven ATP-synthase, Na^+ flagellar motor, numerous Na^+, solute symporters, and the methanogenesis-linked reverse electron transfer system. In *Vibrio alginolyticus*, it was found that $\Delta\bar{\mu}_{Na^+}$, generated by NADH-quinone reductase, can be utilized to support all three types of membrane-linked work, i.e., chemical (ATP synthesis), osmotic (Na^+, solute symports), and mechanical (rotation of the flagellum). In *Propionigenum modestum*, circulation of Na^+ proved to be the only mechanism of energy coupling. In other species studied, the Na^+ cycle seems to coexist with the H^+ cycle. For instance, in *V. alginolyticus* the initial and terminal steps of the respiratory chain are Na^+- and H^+-motive, respectively, whereas ATP hydrolysis is competent in the uphill transfer of Na^+ as well as of H^+. In the alkalo- and halotolerant *Bacillus* FTU, there are H^+- and Na^+-motive terminal oxidases. Sometimes, the Na^+-translocating enzyme strongly differs from its H^+-translocating homolog. So, the Na^+-motive and H^+-motive NADH-quinone reductases are composed of different subunits and prosthetic groups. The H^+-motive and Na^+-motive terminal oxidases differ in that the former is of aa_3-type and sensitive to micromolar cyanide whereas the latter is of another type and sensitive to millimolar cyanide. At the same time, both Na^+ and H^+ can be translocated by one and the same *P. modestum* ATPase which is of the F_0F_1-type and sensitive to DCCD. The sodium cycle, i.e., a system composed of primary $\Delta\bar{\mu}_{Na^+}$ generator(s) and $\Delta\bar{\mu}_{Na^+}$ consumer(s), is already described in many species of marine aerobic and anaerobic eubacteria and archaebacteria belonging to the following genera: *Vibrio, Bacillus, Alcaligenes, Alteromonas,*

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Salmonella, Klebsiella, Propionigenum, Clostridium, Veilonella, Acidaminococcus, Streptococcus, Peptococcus, Exiguobacterium, Fusobacterium, Methanobacterium, Methanococcus, Methanosarcina, etc. Thus, the "sodium world" seems to occupy a rather extensive area in the biosphere.

Key Words: Na^+ cycle; Na^+-NADH-quinone reductase; Na^+-motive oxidase; Na^+-ATP-synthase, Na^+ motor; H^+ cycle.

Introduction: Short Historical Review

Chronologically, the discovery of Na^+ solute symporters seems to be the first evidence showing that Na^+ can substitute for H^+ as the coupling ion in bacteria (Frank and Hopkins, 1969). In the past two decades many such mechanisms were described in the plasma membrane of bacterial as well as animal cells (for a review, see Krulwich, 1983; Skulachev, 1988).

It is well established that in animals the sodium potential ($\Delta\bar{\mu}_{Na^+}$),[2] utilized to support the uphill import of metabolites, is generated by the primary sodium pump, Na^+/K^+-ATPase.

In bacteria, $\Delta\bar{\mu}_{Na^+}$ was, for a fairly long time, believed to be produced only indirectly via the Na^+/H^+-antiporter consuming $\Delta\bar{\mu}_{H^+}$, which is generated by H^+ pumps, i.e., the H^+-motive respiratory and photoredox chain, bacteriorhodopsin or H^+-ATPase.

In 1968 Mitchell mentioned that ΔpNa produced by the Na^+/H^+-antiporter might be used to buffer pH (Mitchell, 1968). In 1978 I suggested that it is the K^+ influx that is responsible for the conversion of the $\Delta\bar{\mu}_{H^+}$ generator-produced $\Delta\Psi$ to ΔpH and ΔpK, so that ΔpK may serve as a $\Delta\Psi$ buffer. Thus, it was assumed that K^+ and Na^+ gradients may buffer both $\Delta\bar{\mu}_{H^+}$ constituents, i.e., $\Delta\Psi$ and ΔpH, respectively (Skulachev, 1978a, b). This suggestion was confirmed when it was shown that the K^+ and Na^+ gradients stabilize $\Delta\Psi$, ΔpH, [ATP], and the mortility rate in several bacterial species under conditions of energy deficiency (Wagner *et al.*, 1978; Brown *et al.*, 1979, 1983; Skulachev, 1979; Arshavsky *et al.*, 1981; Brown and Kim, 1982; Drachev *et al.*, 1985; Michels and Bakker, 1985).

The possibility that primary Na^+ pumps may exist in bacteria was never seriously considered before 1980. Devices of this kind such as Na^+/K^+-ATPase, were always regarded as an invention of the animal cell.

In 1980 Dimroth, however, described the Na^+-motive nonoxidative oxaloacetate decarboyxlase in the anaerobically grown bacterium *Klebsiella*

[2]Abbreviations: $\Delta\bar{\mu}_{H^+}$, electrochemical H^+ potential difference; $\Delta\bar{\mu}_{Na^+}$, electrochemical Na^+ potential difference; ΔpNa, H^+ concentration difference; ΔpNa, Na^+ concentration difference; $\Delta\Psi$, electric potential difference; DCCD, *N,N'*-dicyclohexylcarbodiimide; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; TMPD, tetramethyl-*p*-phenylenediamine.

aerogens (Dimroth, 1980). This was, in fact, the first primary Na^+ pump discovered in a nonanimal cell. Later the laboratories of Dimroth and Buckel succeeded in finding two other Na^+-decarboxylases in obligate and facultative anaerobic bacteria, including *Salmonella typhimurium*, some cocci, *Clostridium*, *Veilonella alcalensis*, and *Propionigenum modestum*. For a review, see Dimroth (1987) and Buckel (1986).

Initially the bioenergetic community qualified these observations as yet another curiosity of bacterial metabolism ("In bacteria one may find everything!"), especially since the authors failed to give a reasonable explanation why Na^+, not H^+, is pumped.

However, even at that time the possible advantage of Na^+ over H^+ under certain conditions was acknowledged. Thus, Guffanti *et al.* (1981) noted that the substitution of Na^+ for H^+ as the coupling ion might help explain how oxidative phosphorylation operates at alkaline pH. In fact, H^+ ions pumped from the cell to the outer medium fail to produce a ΔpH in the proper direction if pH_{in} is lower than pH_{out}, a situation that applies to alkalophilic and alkalotolerant bacteria (reviewed by Padan *et al.*, 1981; Skulachev, 1987, 1988, 1989; Grant, 1987). In such cases, $\Delta\Psi$ and ΔpH appear to be oppositely directed, thus preventing $\Delta\bar{\mu}_{H^+}$ from being effectively used to support any membrane-linked work. The problem may be solved if Na^+, instead of H^+, is extruded from the cell by the respiratory chain, with the downhill Na^+ influx to the cytoplasm being coupled to ATP synthesis.

In 1981 Dr. A. N. Glagolev called our attention to the above idea of Guffanti *et al.*, even though the authors failed to obtain any evidence in favor of this possibility in experiments on alkalophilic bacilli. Glagolev tried to find Na^+-coupled oxidative phosphorylation in *Vibrio harveyi*. But since preliminary experiments failed, he discontinued these investigations. Nevertheless, the problem of bioenergetic functions of Na^+ was still intriguing to me, since one could find observations in the literature that energy coupling in some cases seemed to be resistant to protonophorous uncouplers, which is at variance with the chemiosmotic theory. Assuming that Na^+ substitutes for H^+, one may hope to explain these findings.

Therefore I was not very much surprised by the discovery of Tokuda and Unemoto (1981, 1982) that the respiratory chain of marine alkalotolerant *Vibrio alginolyticus* possesses a Na^+-motive respiratory chain. Subsequently, the authors showed that the primary Na^+ pump is located in the initial segment of the respiratory chain, i.e., between NADH and quinone. Here a special FMN and FAD-containing, three-type subunit enzyme was found that differed much from the H^+-motive NADH-quinone reductase of other bacteria (Hayashi and Unemoto, 1986; Unemoto and Hayashi, 1989). Later a similar enzyme was shown in the halotolerant bacterium Ba_1 by Ken-Dror *et al.* (1984, 1986a, b). Quite recently Tokuda (1989) investigated

10 Gram-negative marine bacteria. Except for *Flavobacterium*, nine of the studies strains belonging to *Alcaligenes*, *Alteromonas*, and *Vibrio* genera were found to possess the Na^+-motive NADH-quinone reductase.

In 1982 we began a systematic study of *V. alginolyticus* energetics. The first series of experiments showed that the motility of this bacterium can be supported by $\Delta\bar{\mu}_{Na^+}$ produced by Na^+-motive respiration (Chernyak *et al.*, 1983; Glagolev *et al.*, 1984). Then a motility supported by artificially imposed ΔpNa was demonstrated (Dibrov *et al.*, 1986). In both cases, motility could be observed in the presence of high concentrations of protonophores. The device responsible for $\Delta\bar{\mu}_{Na^+}$-driven rotation of the flagellum, called a Na^+ motor, was isolated (Bakeeva *et al.*, 1986). These findings were in accord with those obtained by Imae's group who have published a series of papers since 1981 suggesting that alkalophilic bacilli possess a Na^+-driven motility mechanism (Hirota *et al.*, 1981; Hirota and Imae, 1983; Sugiyama *et al.*, 1985, 1986; Imae *et al.*, 1986; Imae and Atsumi, 1989). As to the mechanism of $\Delta\bar{\mu}_{Na^+}$ generation in these bacilli, it remains obscure. Guffanti and Krulwich still insist that primary Na^+ pumps are absent from *Bacillus alkalophilus* and *Bacillus firmus* (Guffanty and Krulwich, 1988; Krulwich and Guffanti, 1989).

Thus, $\Delta\bar{\mu}_{Na^+}$ produced by NADH-quinone reductase of *V. alginolyticus* powers the Na^+ motor, i.e., it performs mechanical work. Another type of membrane-linked work, namely osmotic, was likewise shown to occur at the expense of $\Delta\bar{\mu}_{Na^+}$ in the same bacterium. As was found by Unemoto's group (Tokuda *et al.*, 1982; Kakinuma and Unemoto, 1985), many amino acids and sucrose are accumulated by *V. alginolyticus* via corresponding Na^+ solute symporters. The crucial question was whether *V. alginolyticus* can use $\Delta\bar{\mu}_{Na^+}$ for the third and most important types of work, i.e., chemical. In other words, is it possible to support oxidative phosphorylation by means of the sodium potential? The answer was yes, as was shown by our group in 1985–1989 (Skulachev, 1985; Dibrov *et al.*, 1986, 1989; Verkhovskaya *et al.*, 1987).

The following observations appear to be the most important for verifying the idea of the Na^+-driven oxidative phosphorylation in *V. alginolyticus* cells:

(i) The protonophore failed to abolish ATP synthesis supported by lactate oxidation if ΔpNa of the proper direction ($[Na^+]_{in} < [Na^+]_{out}$) was present.

(ii) Oxidative phosphorylation could be sensitized to the same protonophore concentration when the medium was supplemented with the Na^+/H^+ antiporter, monensin, which, being added without protonophore, did not arrest ATP formation.

(iii) Reverse ΔpNa ($[Na^+]_{in} > [Na^+]_{out}$) inhibited oxidative phosphorylation.

(iv) In cells inhibited with HQNO, artificially imposed ΔpNa ($[Na^+]_{in} < [Na^+]_{out}$) and ΔpK ($[K^+]_{in} > [K^+]_{out}$) led to a transient increase in the ATP level, the process being abolished by monensin, not by the protonophore.

It was concluded that $\Delta\bar{\mu}_{Na^+}$ generated by the Na^+-motive NADH-quinone reductase was utilized by a Na^+-driven ATP-synthase to form ATP in a protonophore-resistant fashion. In agreement with this conclusion, it was found that inside-out subcellular vesicles of *V. alginolyticus* are competent in the ATP-dependent Na^+ uptake which was inhibited by monensin and was strongly increased by agents discharging $\Delta\Psi$, i.e., by valinomycin or the protonophore. The very fact that the protonophore proved to be necessary for fast Na^+ import by the vesicles if no other $\Delta\Psi$-discharging agents were added, completely excluded any involvement of $\Delta\bar{\mu}_{H^+}$ in the ATP-driven Na^+ transport (Fig. 1B).[3] It was assumed that the transport was catalyzed by a Na^+-motive ATPase. Such activity may well be due to reversal of the above-postulated Na^+-driven ATP-synthase reaction (Dibrov *et al.*, 1988).

The Na^+-driven ATP-synthase was first described by Dimroth and coworkers (Dimroth and Hilpert, 1984). The authors showed that marine anaerobic bacteria *Propionigenum modestum* employ only one reaction to obtain all the biologically useful energy, namely, Na^+-motive decarboxylation of methylmalonyl-CoA to propionyl-CoA. The former is obtained from succinate, whereas the latter is converted to propionate, the end product of this fermentation process. Decarboxylase-produced $\Delta\bar{\mu}_{Na^+}$ was shown to support ATP formation catalyzed by Na^+-ATP-synthase. The same enzyme operating in the opposite direction carried out an uphill transport of Na^+ ions, which was stimulated by the protonophore or valinomycin.

$\Delta\bar{\mu}_{Na^+}$-Driven ATP synthesis was also demonstrated in other anaerobic bacteria, namely, methanogens *Methanococcus voltae* and *Methanobacterium thermoantotrophicum*. This was done by Lancaster and associates in 1985–1986 (Crider *et al.*, 1985; Lancaster *et al.*, 1986; Al-Mahrouq *et al.*, 1986; Carper and Lancaster, 1986). The Na^+-dependent ATP formation was shown to utilize $\Delta\Psi$ generated by H^+ flux under the conditions of the artificially imposed ΔpH. To convert ΔpH to $\Delta\Psi$, the protonophore was

[3] In the same figure, two other examples of the protonophore-stimulated Na^+ uptake in subcellular vesicles of bacteria are shown. The transport was catalyzed by *Bacillus* FTU NADH-quinone reductase (A) or terminal oxidase (C, D), respectively. These experiments, carried out out recently in our group by Dr. A. L. Semeykina, preclude explanation of the protonophore-resistant energy coupling processes in alkalotolerant bacteria by inefficiency of classical uncouplers as protonophores at alkaline pH (see, e.g., MacLeod *et al.*, 1988). One can see that in all three cases, protonophores were quite effective in *stimulating*, not inhibiting, the uphill Na^+ transport.

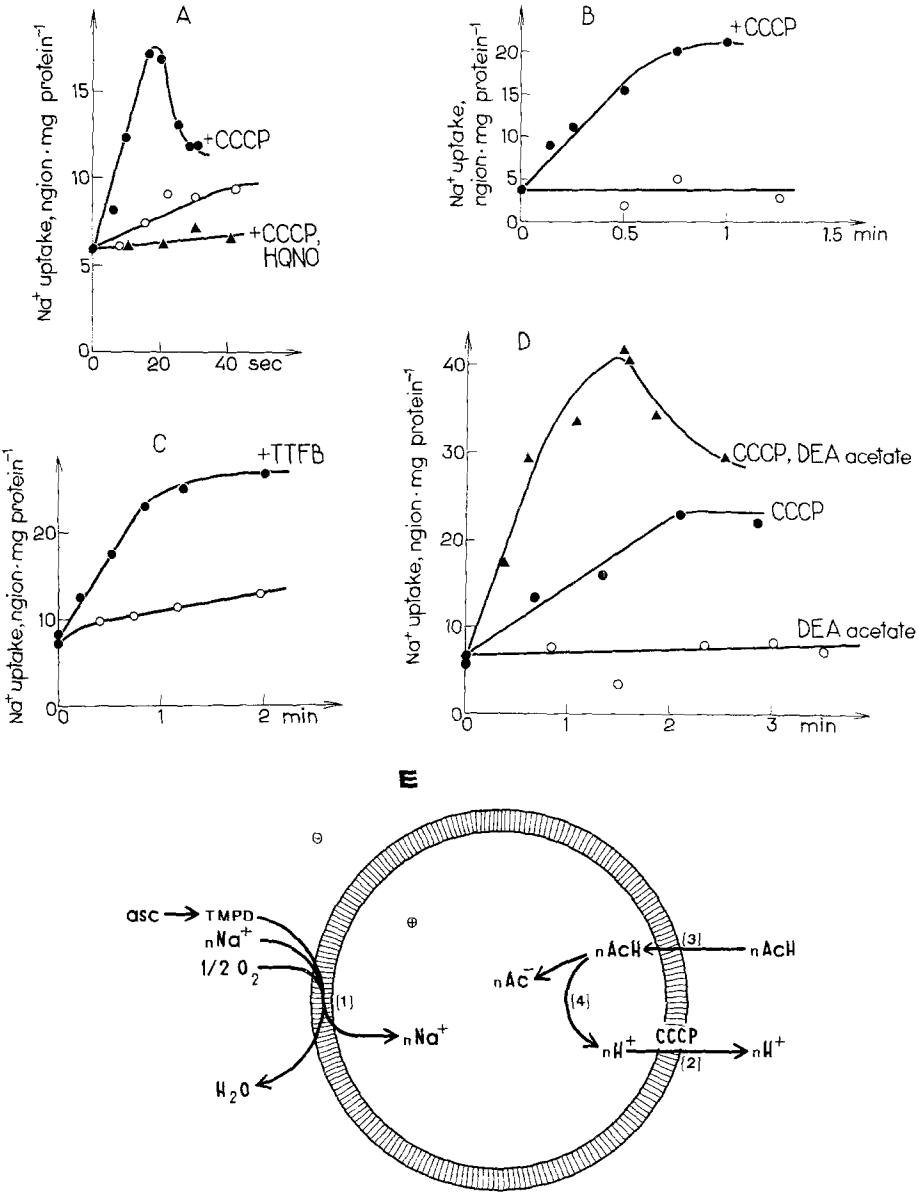

Fig. 1. A simple probe for the primary Na^+ pump: protonophores stimulate the uphill Na^+ uptake by inside-out subcellular vesicles oxidizing a substrate or hydrolyzing ATP. (A, C, D) *Bacillus* FTU vesicles; (B) *V. alginolyticus* vesicles. Protonophores—trichlorocarbonylcyanide phenylhyrazone (CCCP) and tetrachlorotrifluoromethylbenzimidazole (TTFB). At zero time, the following additions were made: (A) NADH; (B) ATP; (C) diaminodurene (DAD); (D) *N,N'*-tetramethyl-*p*-phenylenediamine (TMPD). DEA, diethylamine. In (A), 10 mM cyanide and

added. The protonophore addition proved to be absolutely necessary for the ΔpH-supported ATP synthesis.

In *Methanosarcina barkeri* (Blaut *et al.*, 1985; Gottschalk, 1987) Gottschalk and coworkers recently described a Na^+-dependent redox reaction involved in methanogenesis from methanol. This reaction represents, in fact, reverse electron transfer in the direction of more negative redox potentials. The required energy was shown to be supplied by $\Delta\bar{\mu}_{Na^+}$. This reaction proceeding in the opposite (i.e., energy-releasing) direction was found to produce $\Delta\bar{\mu}_{Na^+}$ (Müller *et al.*, 1988).

Experiments recently completed in our group (Verkhovskaya *et al.*, 1988; Semeykina *et al.*, 1989) have shown that an alkalo- and halotolerant *Bacillus* FTU possesses a Na^+-motive terminal oxidase. In the exhausted cells the addition of ascorbate and TMPD (or diaminodurene) resulted in an uphill extrusion of Na^+ which was stimulated by the protonophore. In inside-out *Bacillus* FTU vesicles, ascorbate oxidation gave rise to an uphill influx of Na^+, also strongly stimulated by the protonophore (Fig. 1C). Valinomycin could substitute for the protonophore in this system. The protonophore effect was potentiated with the salt of a penetrated weak base (diethylammonium) and acid (acetate) (Fig. 1D, E). Na^+ transport was completely inhibited by monensin and a high concentration of cyanide (half-maximal effect, at 6 mM). Low cyanide and HQNO concentrations had no effect.[4]

Concluding this section, we may say that the list of primary Na^+ pumps now includes several Na^+-motive energy-releasing enzymes, i.e., at least two of three respiratory chain coupling sites (NADH-quinone reductase and terminal oxidase), some nonoxidative decarboyxlases, and ATPase. The energy produced by these $\Delta\bar{\mu}_{Na^+}$ generators is utilized for performing all of the three types of membrane-linked work, i.e., chemical (by Na^+-ATP-synthase), osmotic (by Na^+ metabolite symporters), and mechanical (by the Na^+ motor). It is *V. alginolyticus* that proved to be the first example of a cell

[4] In the high cyanide-treated *Bacillus* FTU subcellular veicles, NADH oxidation by fumarate was found to be also Na^+-motive, the process being sensitive to the submicromolar HQNO (Fig. 1A). Most probably, this reaction is catalyzed by Na^+-NADH-quinone reductase (A. L. Semeykina, unpublished results).

5 mM fumarate are present. (E) Scheme illustrating the CCCP- and acetate-induced activation of the Na^+ uptake by the inside-out *Bacillus* FTU vesicles oxidizing ascorbate + TMPD. (1) Na^+-motive terminal oxidase catalyzes the electrogenic Na^+ import into vesicles, resulting in the formation of $\Delta\Psi$ which prevents large-scale Na^+ uptake. (2) CCCP-mediated H^+ efflux decreases $\Delta\Psi$ and, as a result, stimulates the Na^+ uptake. However, $\Delta\Psi$ is converted to ΔpH which restricts the CCCP stimulation. (3) and (4) Acetic acid moves into the vesicles and dissociates inside to acetate and H^+, discharging ΔpH (P. A. Dibrov and M. V. Sokolov, unpublished results; Semeykina *et al.*, 1989; A. L. Semeykina, unpublished results).

Table I. Chronology of Main Ideas and Observations on the Bioenergetic Functions of Na^+ in Bacteria

Idea or finding	Reference
Ideas of Na^+/H^+ antiport and of ΔpNa as possible ΔpH buffer	Mitchell, 1968
Bacterial Na^+, soluble symport	Frank and Hopkins, 1969
Discovery of Na^+/H^+ antiport	Harold and Papineau, 1972; West and Mitchell, 1974
Concept of Na^+/K^+ gradients as a $\Delta\bar{\mu}_{H^+}$ buffer and its verification	Skulachev, 1978a, b; Wagner *et al.*, 1978; Arshavsky *et al.*, 1981; Brown *et al.*, 1983
Na^+-motive oxaloacetate decarboxylase in *Klebsiella*	Dimroth, 1980
Na^+ motor in an alkalophilic *Bacillus*	Hirota *et al.*, 1981
Suggestion that respiration and phosphorylation may be Na^+-coupled	Guffanti *et al.*, 1981
Na^+-motive NADH-quinone reductase in *Vibrio alginolyticus*	Tokuda and Unemoto, 1981, 1982
Na^+-motive glutaconyl-CoA-decarboxylase in *Acidaminococcus fermentans*	Buckel and Semmler, 1982
Na^+-ATPase of *Streptococcus faecalis*	Heefner and Harold, 1982
Na^+-motive methylmalonyl-CoA-decarboxylase in *Veilonella alcalencens*	Hilpert and Dimroth, 1983
Na^+ motor in *V. alginolyticus*	Chernyak *et al.*, 1983
Na^+-motive NADH-quinone reductase in a halotolerant bacterium, Ba_1	Ken-Dror *et al.*, 1984
Na^+-ATPase and the Na^+ cycle in *Propionigenum modestum*	Hilpert *et al.*, 1984
Na^+ oxidative phosphorylation and the Na^+ cycle in *V. alginolyticus*	Skulachev, 1985; Dibrov *et al.*, 1986
Na^+-ATPase in *Methanococcus voltae*	Carper and Lancaster, 1986
Na^+-ATPase of *P. modestum* is of the F_0F_1 type and can transport H^+ in a Na^+-free medium	Dimroth, 1987; Dimroth and Laubinger, 1987; Laubinger and Dimroth, 1988
Na^+-driven reverse electron transfer step in methanogenesis of *Methanosarcina barkeri*	Müller *et al.*, 1987, 1988
Na^+-motive terminal oxidase in halo- and alkalotolerant *Bacilles* FTU	Verkhovskaya *et al.*, 1988; Semeykina *et al.*, 1989
Na^+-motive NADH-quinone reductase in nine species of marine Gram-negative bacteria (*Alcaligenes*, *Alteromonas*, and *Vibrio*)	Tokuda, 1989

employing all these $\Delta\bar{\mu}_{Na^+}$ consumers. The Na^+ pumps were found to be widely distributed among bacteria including such typical respirers as *V. alginolyticus* or *Bacillus* FTU, and some anaerobes. In one case (*Propionigenum modestum*), the Na^+ cycle is shown to be the only mechanism of energy coupling.

The chronology of the most important concepts and findings in the field of bacterial Na^+ energetics is given in Table I.

Interrelationships of H^+ and Na^+ Cycles

In several bacteria, the Na^+-cycle enzymes were found to coexist with the H^+-cycle enzymes in one and the same membrane. This is the case in *V. alginolyticus* and *V. costicola* where the initial respiratory chain segment is Na^+-motive, middle and/or terminal segment(s) being H^+-motive (Tokuda and Unemoto, 1982; Udagawa *et al.*, 1986; Smirnova and Kostyrko, 1988; Vaghina and Kostyrko, 1989). In our group, it was established that inside-out vesicles of *V. alginolyticus* are competent in the ATP-dependent uphill transport of not only Na^+ (Dibrov *et al.*, 1988; Sokolov *et al.*, 1988) but also of H^+ (Smirnova *et al.*, 1987a, b; Vaghina and Kostyrko, 1989). The latter process does not require Na^+ and so involvement of the Na^+/H^+ antiporter seems to be excluded (Fig. 2).

In *Bacillus* FTU, besides the Na^+-motive terminal oxidase resistant to micromolar cyanide, we found an H^+-motive terminal oxidase of aa_3 type sensitive to low cyanide concentrations (Kostyrko and Vaghina, unpublished results).

Thus, Na^+-motive oxidase seems to differ from H^+-motive oxidase just as Na^+- and H^+-motive NADH-quinone reductase activities are inherent in two quite different enzymes.

The principle "two ions–two enzymes" is hardly true for Na^+- and H^+-ATP-synthases. According to Dimroth (1987, 1988), Na^+-ATP-synthase of *P. modestum* is very similar to the H^+-ATP-synthase of *E. coli* in both

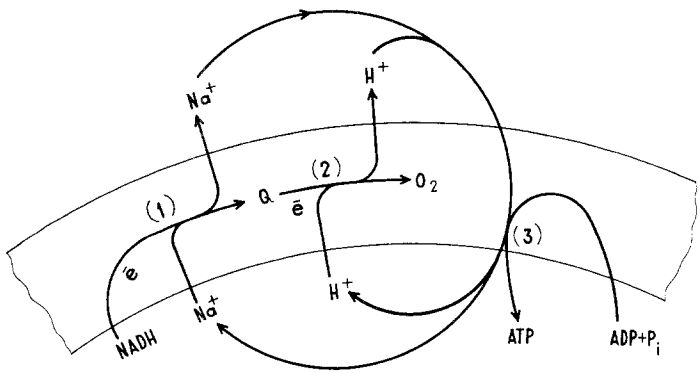

Fig. 2. Na^+- and H^+-motive respiratory chain phosphorylations in *Vibrio alginolyticus* (a "minimal" scheme). (1) Na^+-motive NADH-quinone reductase. (2) H^+-motive redox reaction(s) in the middle and/or terminal segments of the respiratory chain. (3) $Na^+(H^+)$-driven ATP-synthase which translocates Na^+ or H^+ at high or low $[Na^+]_0/[H^+]_0$ ratios, respectively (Skulachev, 1989).

subunit composition (F_0F_1 complex) and sensitivity to DCCD.[5] Even more importantly, the *P. modestum* enzyme, being incorporated into proteoliposomes, catalyzes an ATP-dependent H^+ transfer if Na^+ is absent (Dimroth and Laubinger, 1987).

In the *V. alginolyticus* vesicles, we found only one membrane-linked DCCD-sensitive ATPase (Dmitriev and Chernyak, 1988). Both Na^+- and H^+-transports supported by ATP hydrolysis were DCCD-sensitive. Small DCCD concentrations produced a coupling effect (Dibrov *et al.*, 1988; Sokolov *et al.*, 1988). According to Dibrov *et al.*, (1988), Na^+ may be transported via the F_0 complex assuming that the ion selectivity of F_0 is decreased in such a fashion that it cannot well discriminate between H^+ and Na^+. If such is the case, Na^+ should have an advantage over H^+ when $[H^+]$ is low. This means that one and the same ATP-synthase may be involved in the H^+ cycle at low pH and in the Na^+-cycle at high pH. Further investigations are necessary to test this attractive speculation.

In any case, the very fact that DCCD-sensitive F_0F_1 ATPase can transport Na^+ seems to be of crucial significance for understanding the mechanism of this key bioenergetic enzyme. It seems obvious that the H^+ ions released when ATP is hydrolyzed ($ATP^{4-} + H_2O \rightarrow ADP^{3-} + PO_4^{3-} + 2H^+$) are not identical with H^+ ions transported across the membrane as a result of this reaction since the same mechanism is competent in translocation of Na^+ instead of H^+. Most probably, ATP hydrolysis by F_1 is coupled to ion translocation via F_0 in some indirect manner, say, by means of conformational change and/or rotation of some components of the F_0F_1 complex (Skulachev, 1988). As to the interaction of the transported ion with F_0, it must be organized in such a way that either H^+ or Na^+ can enter the gate or combine with a cation-acceptor group. Here it is noteworthy that the Na^+ channel of the animal cell plasma membrane is H^+-permeable and the only reason why it transports Na^+ rather than H^+ *in vivo* is that $[Na^+]$ is very much higher than $[H^+]$ (Mozhayeva and Naumov, 1983). The Na^+/K^+-ATPase was found to catalyze the H^+/K^+ antiport at slightly acidic pH and low $[Na^+]$ (Hara *et al.*, 1986; Polvani and Blostein, 1988). Correspondingly, it was found that the H^+/K^+-ATPase of the gastric mucosa plasma membrane carries out Na^+/K^+ antiport when $[Na^+]$ and pH are high (S. Karlish, personal communication). A few examples are described when an ion metabolite symporter recognizes both H^+ and Na^+ (for references, see Skulachev, 1989).

[5] It should be noted, however, that another bacterial Na^+ ATPase found by Heefner and Harold (1982) in anaerobic *Streptococcus faecalis* differs significantly from the F_0F_1 type in inhibitor sensitivity, being resistant to DCCD, diethylstilbestrol, and efrapeptin. The authors suggested that it catalyzes the electroneutral Na^+/K^+-antiport rather than the electrogenic Na^+ uniport (Kakinuma and Harold, 1985; Kakinuma and Igarashi, 1989). Thus, there are at least two types of Na^+-transported ATPases in bacteria.

Boyer (1988) recently indicated that crown ethers of Na^+ and H_3O^+ are structurally very similar. One may speculate that H_3O^+, rather than H^+, interacts with ATPases, porters, or channels.

The existence of H^+ and Na^+ cycles in one and the same bacterial cell may stabilize the membrane energetics under unfavorable conditions. Alkalotolerant bacteria like *V. alginolyticus* seem to be the most impressive example of this kind. *V. alginolyticus* living in mats of algae is always exposed to strong pH fluctuations occurring due to photosynthetic activity of the algae (i.e., pH which is neutral in the morning) shifts to high values in the evening. One may suppose that such a shift is accompanied by energetics switchover from the H^+ to the Na^+ cycle.

It is known that many types of membrane damage result first of all in an increase of H^+ conductance which makes the H^+ cycle inefficient. The existence of an alternative mechanism, the Na^+ cycle, may allow the cell to survive in spite of protonic energetics uncoupling. Such an effect should be taken into account in at least two cases:

(i) Some representatives of the "sodium world" are pathogenic. Among them one may mention *Vibrio parahaemolyticus* (Tsuchiya and Shinoda, 1985) and *V. cholerae* (Bakeeva *et al.*, 1986). It is clear therefore that antibacterial drugs which affect such bacteria due to their protonophore action will be ineffective if the Na^+ cycle is operative. Combined protonophore and monensin treatment might be recommended in such cases.

(ii) One may hope that useful bacteria employed in the microbiological industry will survive in aggressive media due to co-operation of H^+ and Na^+ cycles. Perhaps, these bacteria will be productive within a wider range of conditions than those possessing the H^+ cycle only.

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