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Annual Review Letter

Bacterial Na^+ energetics

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Novel observations related to the Na^+-linked energy transduction in bacterial membranes are considered. It is concluded that, besides the well-known systems based on the circulation of protons, there are those based on the circulation of Na^+. In some cases, H^+ and Na^+ cycles co-exist in one and the same membrane. Representatives of the 'sodium world', i.e. cells possessing primary Na^+ pumps ($\Delta\tilde{\mu}Na$ generators and consumers) are found in many genera of bacteria. Among the $\Delta\tilde{\mu}Na$ generators, one should mention Na^+-NADH-quinone reductase and Na^+-terminal oxidase of the respiratory chain, Na^+-decarboxylases and Na^+-ATPases. For $\Delta\tilde{\mu}Na$ consumers, there are Na^+-ATP-synthases, Na^+-metabolite symporters and Na^+ motors. Sometimes, one and the same enzyme can transport H^+ or, alternatively, Na^+. For instance, an Na^+-ATP-synthase of the F_0F_1 type translocates H^+ when Na^+ is absent. Employment of the Na^+ cycle, apart from or instead of the H^+ cycle, increases the resistance of bacteria to alkaline or protonophore-containing media and, apparently, to some other unfavourable conditions.

Na^+ energetics; ATPase; Terminal oxidase; Methanogen; Decarboxylase

1. INTRODUCTION

The recent progress made in bioenergetic studies has stimulated interest in the role of Na^+. The dogma that had H^+ as the coupling ion in all the energy-transducing membranes, with the animal plasmalemma being the only exception, has been shaken. Besides $\Delta\tilde{\mu}H$ buffering [1–6], Na^+ has been shown to be directly involved in energy transduction in some bacteria, performing the functions previously ascribed to H^+ in other bacteria as well as in mitochondria and chloroplasts. The significant taxonomic variety of species, employing Na^+ as the primary coupling ion, points to the ubiquitous distribution of this novel type of membrane-linked energy transduction. The impression arises that there is a rather extensive area which may be defined as a 'sodium world', co-existing with the well-known 'protonic world'.

In this review, I shall summarize the most important recent observations on energy-linked functions of Na^+ and interrelations of H^+ and Na^+ energetics in bacteria.

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Abbreviations: $\Delta\tilde{\mu}H$ and $\Delta\tilde{\mu}Na$, electrochemical potential differences of H^+ and Na^+, respectively; ΔpH and ΔpNa, concentration differences of H^+ and Na^+; $\Delta\psi$, electric potential difference; DCCD, *N,N'*-dicyclohexylcarbodiimide

2. H^+ AND Na^+ CYCLES IN *VIBRIO ALGINOLYTICUS*

To date, the marine alkalotolerant bacterium *V. alginolyticus* is the system that has received most attention for studying the interplay of H^+- and Na^+-linked processes in energy transduction. It is in this microorganism that the Na^+-motive

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respiratory chain was discovered by Tokuda and Unemoto [7,8]. $\Delta\tilde{\mu}Na$, generated by this respiratory chain, was found to support the performance of the three main types of membrane-linked work, i.e. chemical, osmotic and mechanical. In studies on *V. alginolyticus* it was shown that amino acids and sugars are accumulated in the cell by means of symport with Na^+ [9,10]. Rotation of the flagellum is coupled to downhill Na^+ influx into the cell [11–13] and during the period 1986–1988, Dibrov et al. [14–18] in our group demonstrated Na^+-coupled respiratory phosphorylation in *V. alginolyticus*.

The following observations should be mentioned (i) ATP synthesis coupled to oxidation of added lactate was found to be protonophore-resistant, provided that a high ΔpNa in the proper direction was maintained ($[Na^+]_{out} > [Na^+]_{in}$). However, a decrease of ΔpNa caused by addition of monensin sensitized the ATP synthesis to protonophores [14–16]. (ii) Reversal of the ΔpNa ($[Na^+]_{out} < [Na^+]_{in}$) inhibited oxidative phosphorylation [14–16]. (iii) Artificially imposed Na^+/K^+ gradients (high $[Na^+]_{out}$ vs high $[K^+]_{in}$) induced ATP synthesis in the absence of respiration, the process being protonophore-resistant and monensin-sensitive [15–18]. ATP synthesis supported by respiration or an Na^+ pulse, was inhibited by DCCD [15,17,18]. (iv) Inside-out subcellular particles of *V. alginolyticus* accumulated Na^+ in an ATP-dependent fashion. Na^+ uptake was stimulated by the protonophore or valinomycin + K^+ as if it were catalyzed by an electrogenic Na^+-ATPase. Monensin was inhibitory. Low concentrations of DCCD, which slightly inhibited the ATPase activity of the vesicles, were found to increase the ATPase-supported Na^+ transport [19,20]. The latter effect resembles the so-called 'coupling' action of the F_0 inhibitors on submitochondrial vesicles partially depleted of factor F_1 [21]. Higher levels of DCCD arrested both ATPase activity and Na^+ uptake [19,20]. Under identical conditions, solubilized *V. alginolyticus* ATPase, according to Dmitriev and Chernyak [22], was resistant to the DCCD concentrations used. This 360 kDa ATPase contained two major subunits (58 and 55 kDa) and at least two minor subunits. The ATPase, which was found to be inhibited by NBD chloride and azide, was essentially unaffected by vanadate, fluoride and *N*-ethylmaleimide. The enzyme required Mg^{2+}, which could be partially replaced by Ca^{2+}. Rates of hydrolysis decreased in the following order: ATP > ITP > GTP ⋙ UTP > CTP.

According to the data of our group [27,37,38], *V. alginolyticus* vesicles can pump not only Na^+, but also H^+ in an ATP-dependent fashion. At neutral pH, ATP addition resulted in acidification of the intravesicular space. This effect was stimulated by valinomycin + K^+ and inhibited by the protonophore and high [DCCD]. Again, low [DCCD] stimulated the ATP effect. These facts may indicate that one and the same DCCD-sensitive ATPase is competent in the transport of either Na^+ or H^+.

As shown by Dimroth and Laubinger [39], the DCCD-sensitive Na^+-ATPase from *Propionigenum modestum* transports H^+ when Na^+ is absent (see section 5). Similarly, animal Na^+/K^+-ATPase performs H^+/K^+ antiport at acidic pH in an Na^+-free medium [40]. The Na^+ channel in the animal cell outer membrane has been shown to be 2.5×10^2-times more permeable for H^+ than for Na^+ [41]. Nevertheless, under physiological conditions, the channel transports Na^+ rather than H^+, since $[Na^+]$ is about 10^6-times greater than $[H^+]$. An increase in $[H^+]$ was shown to decrease the Na^+ conductance of the channel [42]. A few examples are known in which ion/metabolite symporters recognize both H^+ and Na^+ [43–50].

Such developments, as stated by Boyer [51]: "suggest that one should look for similar structural arrangements that might with modest but important rearrangement bind either Na^+ or H^+". According to Boyer [51,52], substitution of Na^+ for H^+ can be explained assuming that the cation-binding sites are organized like crown ethers coordinating Na^+ or H_3O^+. In such compounds, complexation of Na^+, quite analogous to that of H_3O^+, can be achieved by appropriate three-dimensional arrangement of coordinating groups [51].

In *V. alginolyticus* subcellular vesicles, Na^+ uptake could also be supported by NADH oxidation [19,20,24,25]. At the same time, succinate oxidation was coupled to H^+ uptake [26,27]. Similar relationships were revealed in *Vibrio costicola* vesicles where the initial and terminal steps of the respiratory chain proved to be Na^+- and H^+-motive, respectively [28]. It was shown by

Unemoto and co-workers [25,28] that the respiratory chain-driven Na^+ pumping in *V. alginolyticus* is catalyzed by the Na^+-motive NADH-quinone oxidoreductase. The enzyme contrasts strongly with the more familiar H^+-motive NADH-ubiquinone oxidoreductase in that it is composed of three subunits (52, 46 and 32 kDa). FMN and FAD serve as prosthetic groups [25,28–31]. Tokuda et al. [33–36] isolated a *V. alginolyticus* strain having a point mutation in the β-subunit of the Na^+-motive NADH-quinone reductase. The mutant enzyme proved to be ineffective as an Na^+ pump. The mutant, in contrast to the wild strain, was unable to use respiration to support motility and uphill amino acid transport in the presence of a protonophore [36]. Apparently, Na^+-coupled respiratory phosphorylation was also inoperative in the mutant, since the cells could not grow at an alkaline pH on acetate the metabolism of which does not cause ATP synthesis at the substrate level. As concerns H^+-driven respiratory chain phosphorylation, this was inhibited, under alkaline conditions, by reverse ΔpH ($pH_{in} > pH_{out}$). In agreement with this reasoning, the mutant could grow on glucose (substrate-level phosphorylation being involved). The wild strain and the revertant were shown to grow on acetate at both neutral and alkaline pH [36].

In the case of succinate oxidation by *V. alginolyticus*, the mechanism responsible for H^+ pumping requires elucidation. This may be H^+-motive ubiquinol-cytochrome *c* oxidoreductase and/or terminal oxidase(s).

Thus, in summary, work with *V. alginolyticus* has shown the coexistence of Na^+- and H^+-motive ATPase activities, as well as the operation of initial and terminal steps of the respiratory chain that are Na^+- and H^+-motive, respectively. This allows us to organize both Na^+ and H^+ cycles in the same coupling membrane as shown in fig.1. The scheme assumes that $\Delta\tilde{\mu}Na$ produced by the Na^+-motive NADH-quinone reductase is consumed by Na^+-ATP-synthase, whereas $\Delta\tilde{\mu}H$, generated in terminal steps of the respiratory chain, drives ATP synthesis with H^+-ATP-synthase involved. While it is possible that the Na^+- and H^+-ATP-synthetases represent separate enzymes, as suggested by Tsuchiya and co-workers [53] for *V. parahaemolyticus*, an attractive alternative possibility suggested by Dibrov et al. [19] has the *same* ATP-synthase

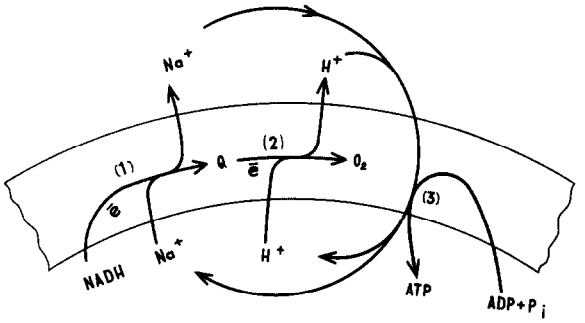

Fig.1. Na^+ and H^+ cycles in *Vibrio alginolyticus*: a 'minimal' scheme.

operating with both ions. Thus, the ATPase will catalyze the influx of H^+ when $[H^+]_{out}$ is high but when $[H^+]_{out}$ is low Na^+ will be used instead. Such regulation seems to be of great physiological importance for *V. alginolyticus*, a symbiont of photosynthetic algae which strongly alkalinize the medium in the light. In algal mats, the pH value is neutral in the morning, but shifts strongly to the alkaline region during daytime. A decrease in $[H^+]_{out}$ results in formation of a reverse ΔpH that inhibits H^+-coupled oxidative phosphorylation. A switch to Na^+-coupled oxidative phosphorylation solves the problem. Thus, we may say that bacteria like *V. alginolyticus* and similar forms "use H^+ for breakfast and Na^+ for dinner".

3. THE Na^+-MOTIVE TERMINAL OXIDASE OF AN ALKALO- AND HALOTOLERANT *BACILLUS*

Na^+-motive NADH-quinone oxidoreductase activity, discovered in *V. alginolyticus*, was also recently described in *V. costicola* [28,54], *V. parahaemolyticus* [55], the halotolerant bacterium Ba_1 [56–59], and some other marine bacteria [60]. The terminal steps of the respiratory chain in each of these organisms have until recently been assumed to be H^+-motive or non-coupled to energy conservation.

The first indication that the terminal oxidase can operate in an Na^+-motive fashion was obtained recently in our group by Verkhovskaya, Semeykina and co-workers [61,62]. The authors studied the alkalo- and halotolerant *Bacillus FTU* which could grow in the presence of a pro-

tonophore, provided the Na^+ concentration was high.

The protonophore was found to stimulate uphill Na^+ efflux from the cells oxidizing ascorbate via tetramethyl-*p*-phenylenediamine or diaminodurol. The Na^+ efflux was found to be electrogenic. $\Delta\psi$ formation was inhibited only slightly by the protonophore. The degree of inhibition strongly increased if a penetrating weak base, e.g. diethylamine, was also added. Inside-out subcellular vesicles of *Bacillus FTU* were found to accumulate Na^+ when oxidizing ascorbate in the presence of tetramethyl-*p*-phenylenediamine; Na^+ transport was markedly stimulated by the protonophore or K^+ + valinomycin and completely inhibited by millimolar cyanide or monensin. The stimulatory effect of protonophore was potentiated by diethylammonium acetate. In the presence of valinomycin, H^+ was also taken up, the process being inhibited by micromolar cyanide, protonophore or diethylammonium acetate. It was concluded that there exist two terminal oxidases in *Bacillus FTU* strongly differing in cyanide sensitivity. One of them exports Na^+ whereas the other exports H^+ [61,62].

An indication was also obtained that $\Delta\tilde{\mu}Na$ formed by this oxidase is employed in forming ATP in a protonophore-resistant manner. In intact cells, ascorbate oxidation was shown to result in ATP formation, the process being protonophore-resistant in the presence of Na^+.

4. Na^+-REDOX PUMP AND Na^+-ATPase IN METHANOGENIC BACTERIA

Gottschalk and co-authors [63,64] have found that there occurs an Na^+-dependent step during methanogenesis in *Methanosarcina barkeri*, namely, the oxidation of methanol to the redox level of formaldehyde. This partial reaction of methane formation from methanol ($4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$) is thermodynamically unfavourable, and therefore resembles reverse electron transfer in the respiratory chain, proceeding from the components of more positive redox potentials to those with more negative values. The energy is supplied by other (thermodynamically favourable) electron-transfer steps of methanogenesis, which are proton-motive and form $\Delta\tilde{\mu}H$. An Na^+/H^+ antiporter [65] seems to transduce $\Delta\tilde{\mu}H$ to $\Delta\tilde{\mu}Na$ which is utilized to support the energy-consuming step during methanol oxidation [66]. Recently, the same group succeeded in demonstrating that the above reaction proceeding in the opposite direction (i.e. reduction of formaldehyde to the redox level of methanol) is electrogenic and Na^+-motive [67].

Schönheit and Perski [68] have reported that valinomycin-mediated K^+ efflux from *Methanobacterium thermoautotrophicum* results in an increase in the ATP level. The effect was greatly stimulated by Na^+ [68] or Li^+ [69]. One of the explanations of this phenomenon lies in the reversal of hypothetical Na^+-ATPase by means of $\Delta\psi$ generated by the K^+ efflux. However, Na^+ fails to stimulate the ATPase activity of *M. thermoautotrophicum* membrane vesicles [68]. This inability might be due to (i) substitution of H^+ for Na^+ when the latter is absent, assuming that the ATPase in question can transport both Na^+ and H^+, or to (ii) a very low K_m for Na^+; for example, in another methanogenic bacterium (*Methanococcus voltae*) it has been shown that the K_m for the Na^+ in the Na^+-isoleucine-symporter was as low as 4.5×10^{-6} M [70].

Recently, the observation reported by Schönheit and Perski was extended by Lancaster and coworkers [71–74] to *Mc. voltae*. It was found that ATP synthesis in *Mc. voltae* cells could be supported by a diffusion potential of K^+ (+ valinomycin) or H^+ (+ protonophore). The protonophore-mediated ATP synthesis was shown to require Na^+. It was inhibited by K^+ + valinomycin as if Na^+-ATP-synthase utilized the H^+ flux-generated $\Delta\psi$. An artificially imposed ΔpNa (Na^+ pulse) was also shown to be competent in ATP synthesis, monensin and diethylstilbestrol being inhibitory.

Similar relationships were also observed by the same group in *M. thermoautotrophicum*. The ΔpNa-supported formation of ATP was arrested by harmaline, an inhibitor of some Na^+-dependent systems [73]. The use of Na^+ pulses proved to be effective only if at least 5 mM Na^+ was present in the growth medium. This may indicate that Na^+-ATPase is inducible by Na^+ [75]. In the same bacterium, it was found that protonophore failed to inhibit completely methanogenesis-supported $\Delta\psi$ formation. In the presence of protonophore, ATP synthesis coupled to methanogenesis was

shown to occur at $\Delta\tilde{\mu}H < 100$ mV [76]. Thus, it may be suggested that the Na^+ cycle is involved in ATP synthesis in *M. thermoautotrophicum*. This possibility should also be taken into account when dealing with *Ms. barkeri* maintaining, under certain conditions, a reverse ΔpH ($pH_{in} < pH_{out}$) [77].

5. THE Na^+ CYCLE IN *PROPIONIGENUM MODESTUM*

In some anaerobic bacteria, non-oxidative decarboxylation of certain carboxylic acids is coupled to Na^+ extrusion from the cell, as first revealed by Dimroth [78]. Such an effect was described for decarboxylation of (i) oxaloacetate to pyruvate (*Klebsiella pneumoniae*, and *Salmonella typhimurium*; fermentation of citrate to acetate); (ii) glutaconyl-CoA to crotonyl-CoA (*Acidaminococcus fermentans, Streptococcus asaccharolyticus, Clostridium symbiosum, Peptococcus aerogenes* and *Fusiobacterium nucleatum*, fermentation of glutamate to butyrate) and (iii) methylmalonyl-CoA to propionyl-CoA (*Veilonella alcalensis*, fermentation of lactate to propionate; *Propionigenum modestum*, fermentation of succinate to propionate). For reviews of these studies, see [79–83].

Of these systems, the Na^+-motive oxaloacetate decarboxylase from *K. pneumoniae* has received considerable attention. It was found that this biotin-containing enzyme is composed of α, β and γ (63.6, 34 and 12 kDa) subunits, and can be reconstituted from isolated subunits and incorporated into proteoliposomes which then can be shown to accumulate Na^+ in an electrogenic fashion. It appears that Na^+ translocation is coupled to release of free CO_2 from the carboxylated biotin intermediate of the decarboxylation process [78,79,82,84]. The sequence of the biotin-containing α-subunit showed an astonishing degree of homology with other biotin enzymes [85]. In *P. modestum*, the fermentation of succinate to propionate proved to be the only energy-conserving reaction, Na^+-motive methylmalonyl-CoA decarboxylase being the only energy-conserving enzyme in the cell. The $\Delta\tilde{\mu}Na$ so generated appears to be consumed by an Na^+-ATP-synthase to form ATP [79,86]. The Na^+-ATP-synthase incorporated into proteoliposomes has also been shown to be competent in electrogenic transfer of Na^+ [79,86–88] or, in the absence of Na^+, of H^+ [39] coupled to ATP hydrolysis.

A striking degree of structural analogy has been revealed between the Na^+-ATP-synthase of *P. modestum* and the H^+-ATP-synthase of *E. coli*. The Na^+-ATP-synthase is composed of (i) a membrane-linked DCCD-sensitive sector (F_0) and (ii) a detachable catalytic sector (F_1) resistant to low DCCD concentrations. F_0 contains subunits a, b and c of 26, 23 and 7.5 kDa, respectively, as demonstrated by SDS electrophoresis. In *E. coli*, a similar method resulted in the values 24, 19 and 8.5 kDa. Subunit c binds DCCD. It forms a supramolecular complex which probably contains 6 copies. This complex is so stable that it resists dissociation by SDS at 100°C; a temperature of 121°C is required for its dissociation. Like DCCD-binding F_0 subunits of H^+-ATP-synthase from other sources, subunit c of *P. modestum* contains a small amount of polar amino acids and no histidine and tryptophan. The content of hydrophobic amino acids such as leucine, isoleucine and phenylalanine was lower compared to other subunits c (18% vs 28–36%) [87].

The F_1 part was composed of five subunits, i.e. α, β, γ, δ and ϵ. Their respective molecular masses are 58, 56, 37.6, 22.7 and 14 kDa for *P. modestum* vs 55, 50, 31.5, 19.5 and 15 kDa for *E. coli* F_1. The ATPase activity of the F_0F_1 complex was stimulated 10-fold by Na^+, whereas that of isolated F_1 was not [89]. The ATPase of F_0F_1 was inhibited by venturicidin, tributyltin and azide, but was resistant to vanadate. The ATP-dependent Na^+ accumulation in F_0F_1 proteoliposomes was stimulated 4–5-fold in the presence of a $\Delta\psi$-discharging agent, i.e. K^+ + valinomycin or protonophore [87].

According to Dimroth [79], Na^+-decarboxylase of *P. modestum* transports 2 Na^+ per molecule of decarboxylated methylmalonyl-CoA. The energy yield of this reaction is as low as 27 $kJ \cdot mol^{-1}$, i.e. less than the energy requirement of ADP phosphorylation (about 43 $kJ \cdot mol^{-1}$ under physiological conditions). Therefore, Na^+-ATP-synthase is assumed to transport more than 2 Na^+ (probably 4) per molecule of ATP formed (fig.2).

Currently, there is no evidence that an H^+ cycle is operative in *P. modestum*. Thus, this microorganism seems to be the first example of a living cell employing Na^+ as the sole coupling ion.

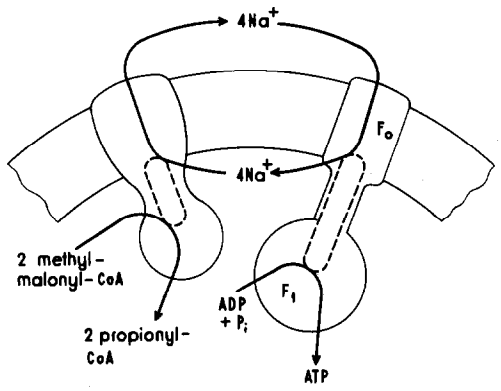

Fig.2. Na^+ cycle in *Propionigenum modestum* [79]. Conformational coupling of chemical reactions and Na^+ translocation is represented by dashed lines. It is assumed that Na^+-decarboxylase and Na^+-ATP-synthase translocate 2 and 4 Na^+ per turnover, respectively.

6. SOME OTHER BACTERIA

Low Na^+-stimulated ATPase activity has been described in anaerobic *Streptococcus faecalis* [90]. A strong increase in the level of this enzyme was revealed under conditions when energization of the membrane by H^+-ATPase proved impossible. This occurred due to either (i) mutation in H^+-ATPase, or (ii) growth in the presence of a protonophore and Na^+. The pH optimum of the enzyme proved to be located within an alkaline pH region (between pH 8 and 9). Na^+-stimulated ATPase of *S. faecalis* clearly differs, both structurally and functionally, from Na^+-ATP-synthase of *P. modestum* [91]. The authors assumed that it catalyzes, in fact, Na^+/K^+ antiport rather than Na^+ uniport [92]. It was also suggested that the Na^+/H^+ antiporter of *S. faecalis* is a product of partial proteolysis of this ATPase [90]. However, an indication was recently obtained that Na^+/H^+ antiport is inherent in intact *S. faecalis* cells [93].

There is some evidence indicating that the facultative alkalophile *Exiguobacterium aurantiacum* possesses an Na^+-ATPase utilizing glycolytic ATP without $\Delta\tilde{\mu}H$ being involved [94].

Components of the Na^+ cycle were found in alkalophilic bacilli, i.e. *B. firmus* and *B. alcalophilus*. Here, osmotic and mechanical work is carried out by means of Na^+-metabolite symporters and an Na^+ motor, respectively (review [95]). When the bacilli were grown at pH 11.5, $\Delta\tilde{\mu}H$ was found to be close to the zero level [50] so that it could not be the driving force for oxidative phosphorylation which, according to Krulwich et al. [95], still occurs under these conditions. The simplest explanation is that ATP synthesis, like accumulation of substrates and motility, is Na^+-coupled and catalyzed by Na^+-ATP-synthase. If this is true, then this enzyme must be of the F_0F_1 type, as in *P. modestum*, since F_0F_1 was shown to be responsible for the ATPase activity of subcellular vesicles of alkalophilic bacilli [96]. The facts that Na^+ is not necessary for ATP synthesis by these vesicles and that Na^+ pulses fail to induce ATP formation [97] seem to argue against the idea of Na^+-coupled phosphorylation. Nevertheless, such negative results are hardly conclusive if one assumes that (i) F_0F_1 of the bacilli can operate alternatively as an H^+ and Na^+ pump and (ii) the conditions used were more favourable for H^+ pumping, rather than for Na^+.

Accumulation of proline supported by succinate oxidation in a protonophore-resistant *E. coli* strain [98] might be due to the operation of a primary respiratory Na^+ pump, since in *E. coli* proline is known to be transported by the Na^+-proline symporter [50].

Motility and $\Delta\psi$ generation in the alkalo- and halotolerant cyanobacterium *Oscillatoria brevis* have been found to be Na^+-dependent and protonophore-resistant, again consistent with the idea that a primary Na^+ pump is present in its cytoplasmic membrane [50,99]. This might be Na^+-ATPase, the Na^+-motive respiratory chain or the Na^+-motive photosynthetic chain. In the latter case, one may mention the observation that Na^+ is necessary for an electron-transfer step localized near photosystem II [100] (see also [50,101–104]).

7. CONCLUSION

There are already at least two firmly established examples of the energy produced by a primary Na^+ pump being utilized by $\Delta\tilde{\mu}Na$ consumers. In *V. alginolyticus*, $\Delta\tilde{\mu}Na$, generated by Na^+-motive NADH-quinone reductase, can be utilized to drive the synthesis of ATP, import metabolites and rotate the flagellum. In *P. modestum*, Na^+-motive methylmalonyl-CoA decarboxylase forms $\Delta\tilde{\mu}Na$ which is used by Na^+-ATP-synthase to produce ATP. In the former case, the quinol oxidation is

H^+-motive. The $\Delta\tilde{\mu}H$ so generated is utilized by H^+-ATP-synthase which may, or may not, be identical to Na^+-ATP-synthase. *P. modestum* appears to have no special $\Delta\tilde{\mu}H$ generators. Nevertheless, Na^+-ATP-synthase can transport H^+ under artificial conditions in the absence of Na^+.

In contrast to *V. alginolyticus*, the halo- and alkalotolerant *Bacillus FTU* possesses a primary Na^+ pump in the terminal step of the respiratory chain (Na^+-motive terminal oxidase).

In some methanogenic bacteria, a $\Delta\tilde{\mu}Na$-driven reverse electron-transfer step is involved in the formation of CH_4 from CH_3OH. In the same bacteria, as well as in some other anaerobes, Na^+-ATPase has been described.

A number of anaerobic bacteria employ decarboxylation of oxaloacetate or glutaconyl-CoA to pump Na^+ from the cell.

Among the bacteria possessing primary Na^+ pumps, one may find vibriones, cocci, bacilli, cyanobacteria, methanogens, *Salmonella, Klebsiella, Clostridium, Fusiobacterium, Propionigenum, Exiguobacterium,* etc. This indicates that the sodium world occupies an extensive area of the biosphere and that Na^+ energetics arose during a rather early stage of biological evolution.

There is an obvious reason for the use of Na^+ instead of H^+ as the coupling ion by marine bacteria. This concerns adaptation to alkaline conditions when $[H^+]_{out}$ is low (less than $[H^+]_{in}$) so that $\Delta\psi$, negative inside the cell, and ΔpH prove to be in opposite directions. In this case, H^+, if pumped from the cell by, say, the respiratory chain, cannot support work when returning to the cytoplasm, since the concentration of H^+ in the cytoplasm is greater than that outside the cell. This problem can be resolved if Na^+ is pumped by the respiratory chain instead of the more familiar H^+.

However, adaptation to high pH does not constitute the only reason for employing the Na^+ cycle. Among the representatives of the sodium world, we can find not only alkalotolerant bacteria but also some neutrophiles. Obviously, the combination of H^+ and Na^+ cycles may stabilize the system against various damaging effects of the outer medium. This can be demonstrated by adaptation of *S. faecalis* to growth in the presence of a protonophore, which involves the induction of Na^+-ATPase. It is worthy of consideration that this is important for the use of bacteria in biotechnology, since those which possess the Na^+ cycle in addition to the H^+ cycle could survive under less favourable conditions than for representatives of the 'protonic world'. Indications in this direction have been recently obtained by our group in a study of cyanobacteria [105]. (For a discussion of the potential advantages of Na^+ over H^+, see [50,79,80].)

It should be stressed that a comparative study of H^+- and Na^+-linked systems appears to be very promising for gaining a better understanding of the 'classical' protonic energy-transducing mechanisms. For instance, the very fact that Na^+-ATP-synthase may be organized as an F_0F_1 complex, similar to H^+-ATP-synthase, constitutes evidence against the idea that merely the two protons, released from H_2O when ATP is hydrolyzed, are pumped across the membrane. Instead, we should focus our attention on more indirect mechanisms of H^+ pumping, which are conformationally coupled to ATP hydrolysis.

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