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Na^+-coupled versus H^+-coupled energy transduction in bacteria

J.S. Lolkema, G. Speelmans, W.N. Konings *

Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

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1. Introduction

Protons and sodium ions are the major coupling ions in membrane associated bioenergetic processes in bacteria. Most bacteria maintain electrochemical gradients of both proton and Na^+ across the cytoplasmic membrane. The gradients exert a force on the cations to move from the extracellular medium across the membrane into the cell. From a thermodynamic point of view the protonmotive force and the sodium ion motive force are equivalent, and composed of both a chemical and an electrical component. The ion motive force (imf) expressed in electrical field units is described by

$$\Delta\tilde{\mu}_{X^+} = \frac{\Delta\mu_{X^+}}{F} = -\frac{2.3RT}{F}\Delta pX + \Delta\Psi$$

in which ΔpX represents the chemical concentration gradient of X^+ (either H^+ or Na^+) over the membrane and $\Delta\Psi$ the membrane potential. Specific systems in the membrane generate the cation gradients at the expense of an energy source. These systems are called primary pumps unless the source of energy is another electrochemical ion gradient across the membrane. A variety of membrane bound systems exist that use the energy stored in the cation gradients to drive energy requiring metabolic actions, e.g., the uptake of a solute, flagellar rotation. The combined action of cation gradient generating and consuming systems results in a continuously pumping of cations out and facilitated influx into the cell. In this way, H^+ and Na^+ cycles are created. It is striking that these energy converters use H^+ in one organism and Na^+ in another and even both H^+- and Na^+-dependent counterparts of one type of system have been found in one organism. Intriguing questions are how this diversity has come about and what the physiological advantage is for the use of H^+ or Na^+ as the coupling ion in energy-transducing processes. This difficult question can be answered only if many different aspects are taken into consideration. Here, we discuss (i) the presence of H^+ and/or Na^+ cycles in organisms from different natural habitats, (ii) the cation specificity of energy transducing enzymes, and (iii) the H^+ and Na^+ permeability of biological membranes.

2. Proton and Na^+ cycling in bacteria

A bioenergetic H^+ or Na^+ cycle may be defined as a continuous influx and efflux of the cation catalyzed by membrane bound enzymes. The organisms maintain an electrochemical gradient of the cation across the membrane that is used to drive energy requiring metabolic processes. The H^+ or Na^+ cycles are driven either by primary H^+ or Na^+ pumps or, indirectly, by secondary transport systems such as a Na^+/H^+ antiporter. Bacteria can be divided in three classes: those that have (1) an H^+ cycle but no Na^+ cycle, (2) both an H^+ and Na^+ cycle and (3) an Na^+ cycle but no H^+ cycle (Table 1).

Lactic acid bacteria are believed to use only a bioenergetic H^+ cycle (class 1). In *Lactococcus lactis* the pmf is generated by an H^+-ATPase and all studied secondary transport proteins are H^+ coupled [1]. All available information argues against a role for Na^+ in the chemiosmotic energy processes. Nevertheless, these bacteria maintain an electrochemical gradient of Na^+ across the membrane through the action of an Na^+/H^+

* Corresponding author. Fax: +31 50 635205.

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Table 1
Classification of some bacteria according to the use of bioenergetic cation cycles [a]

Class 1. H^+ cycle, no Na^+ cycle	
Lactococcus lactis	standard [b]
Bacillus acidocaldarius	high T, low pH
Class 2a. primary H^+ cycle, secondary Na^+ cycle	
Escherichia coli	standard
Klebsiella pneumoniae	aerobic
Vibrio alginolyticus	standard
Enterococcus hirae	standard
Bacillus firmus RAB	high pH
Bacillus stearothermophilus	high T
Halobacterium halobium	high [Na^+]
Class 2b. Primary H^+ cycle, conditional primary Na^+ cycle	
Escherichia coli	low pmf
Klebsiella pneumoniae	anaerobic, on citrate
Vibrio alginolyticus	high pH, high [Na^+]
Enterococcus hirae	high pH, high [Na^+]
Class 2c. Primary H^+ cycle, primary Na^+ cycle,	
Propionigenium modestum	anaerobic, on succinate
Methanococcus voltae	high [Na^+]
Methanosarcina strain Goe1	standard
Class 3. Na^+ cycle, no H^+ cycle	
Clostridium fervidus	anaerobic, high T

[a] For a more complete overview of proton and Na^+ cycles in bacteria and many references see Ref. [19].
[b] Standard refers to the normal growth conditions of the organism.

antiporter. This gradient is a consequence of the requirement to maintain a low internal concentration of the toxic Na^+.

Most bacteria fall into the second class, they have both a H^+ and an Na^+ cycle. Three subclasses may be discriminated: organisms that (2a) combine a primary proton pump and a secondary Na^+/H^+ antiporter for the generation of the gradients, (2b) use primary H^+ and under specific conditions also primary Na^+ pumps, (2c) normally use primary Na^+ pumps in combination with a proton cycle. *E. coli* falls into the first category. It generates a proton motive force by proton pumping catalyzed by electron transport systems or F_0F_1-ATPase. Though Na^+ dependent solute transport systems have been documented in *E. coli*, the organism does not seem to be strictly dependent on the sodium motive force (smf), since alternative, H^+-dependent, systems exist in parallel or can be induced [2]. An Na^+ cycle plays a more vital role in extremely alkaliphilic bacteria to keep the internal pH below the extracellular pH [3] and in aerobic thermophiles [4], rumen bacteria, e.g., [5], and extreme halophiles [6], since in these bacteria transport processes are obligatorily Na^+ coupled. Some bacteria that usually support an Na^+ cycle driven by a secondary Na^+/H^+ antiporter induce under special conditions a primary Na^+ pump (class 2b), e.g., [7]. An example of organisms in class 2c are the methanogens that use primary H^+ and Na^+ pumps at the same time [8].

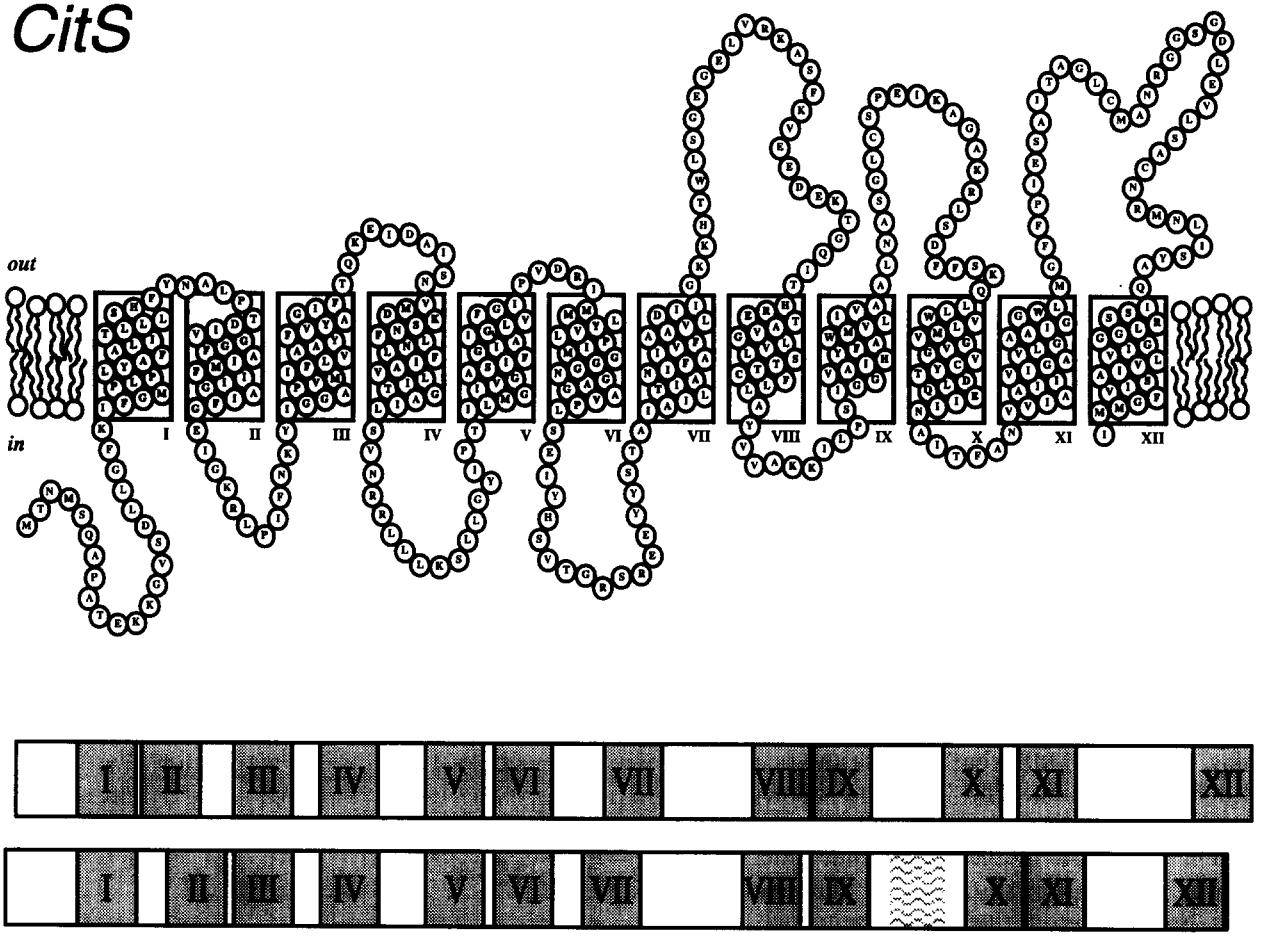
Recently, we have studied the chemiosmotic processes in the anaerobic thermophilic bacterium *Clostridium fervidus* [9–11]. It was demonstrated that this bacterium does not support a H^+ cycle (class 3). *C. fervidus* ferments peptides and amino acids and grows optimally at a temperature of 68°C, but only in a very narrow pH range (6.3–7.7). All amino acid uptake systems that have been tested use exclusively Na^+ as the coupling ion. The cells can generate a large electrochemical potential gradient of Na^+ (ΔpNa, $\Delta\Psi$), but no chemical gradient of protons (ΔpH). Also, the smf is generated by an ATPase that pumps exclusively Na^+ out of the cells at the expense of ATP hydrolysis. No Na^+/H^+ exchange could be demonstrated, indicating that this organism does not possess Na^+/H^+ antiporters.

3. Proton and Na^+ coupled secondary transporters

The number of different types of enzyme or enzyme system involved in the H^+ and Na^+ cycles in the different bacterial species is very large. By now, for most of these enzymes both the H^+- and Na^+-dependent counterparts have been described in the literature. Exceptions to this rule are the proton-pumping pyrophosphatases found in chromatophores of the phototrophic bacterium *Rhodospirillum rubrum*, the light-driven proton pumps in phototrophic bacteria and the Na^+ pumping decarboxylases in, for instance, *Klebsiella pneumoniae*. For the remainder of this discussion we will focus on the secondary solute transporters.

Secondary solute transporters use the free energy stored in the cation gradients to drive the uptake of a solute by coupling the translocation of solute and cation across the membrane. This coupling can occur in symport or antiport reactions. There is no relation between substrate specificity and coupling-ion specificity. A substrate may be coupled to H^+ in one organism and to

Fig. 1. Models of the membrane topology of the Na^+ and H^+-dependent citrate carriers of *K. pneumoniae* (CitS) and *L. lactis* (CitP), respectively. The algorithm used in the predictions of the transmembrane spanning segments was according to Eisenberg et al. [20]. Some minor changes were made by hand to align the TMS's in the two proteins. The bars in the middle show an alignement of linear representations of the helix-loop motif in the two predicted structures. Shaded segments represent the helices. The length of each segment is proportional to the number of residues in the segment. In CitP an additional hydrophobic α-helix is predicted between TMS IX and X, but this helix has a comparatively low hydrophobicity (waved segment).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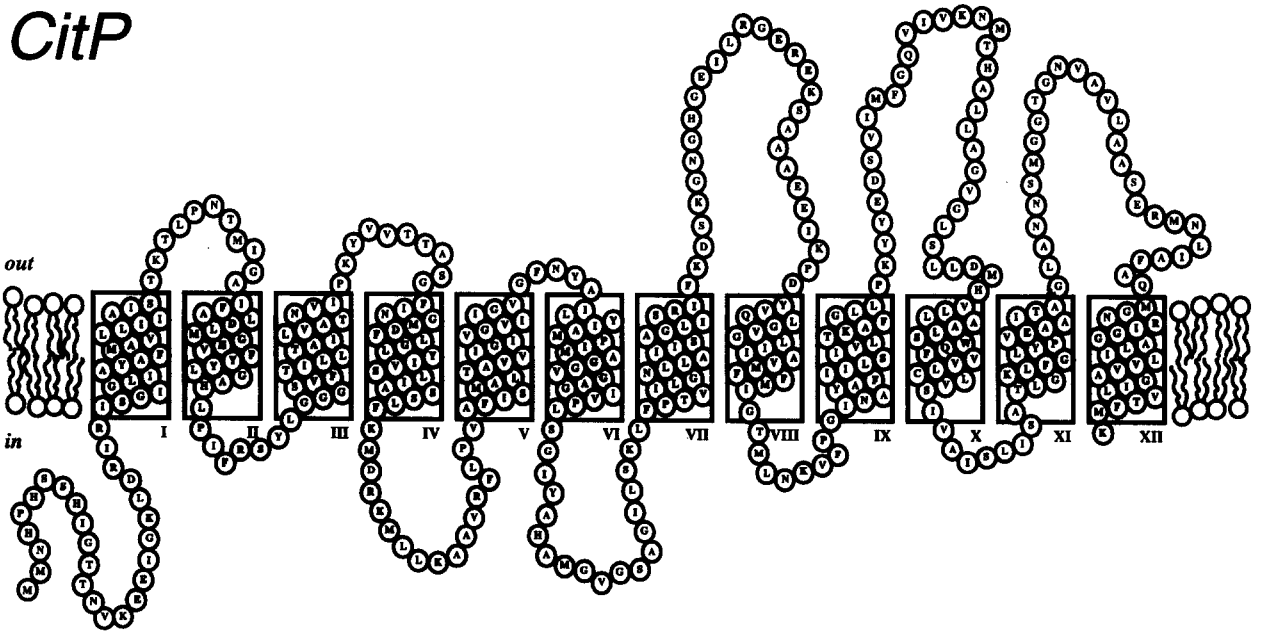
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Na^+ in another and in many instances both H^+ and Na^+-dependent systems exist in the same organism. With respect to cation usage, transporters can be classified in three classes: transporters that use (1) exclusively H^+, (2) both H^+ and Na^+, and (3) exclusively Na^+. Examples may be found abundantly in the literature. In view of the present discussion it is important to discriminate between carriers of the second class that use H^+ *or* Na^+ (class 2a) and carriers that use both cations at the same time and translocate more than one cation per catalytic cycle (class 2b). The melibiose carrier of *E. coli* (MelB) transports melibiose in symport with a single cation that may either be H^+ or Na^+ (or Li^+) [12]. On the other hand, the sodium-dependent citrate carrier of *K. pneumoniae* (CitS) obligatorily couples the translocation of citrate to the translocation of 2 Na^+ and 1 H^+. Na^+ cannot be exchanged for H^+ and vice versa [13]. These differences between the carriers must reflect differences in the structure of the cation binding sites of the proteins. With respect to cation specificity, the cation binding pockets of carriers in classes 1, 2b and 3 appear to be more stringent than those of carriers in class 2a.

The global secondary structure of the bacterial secondary solute transporters is believed to be similar (e.g. see Ref. [14]). The proteins consist of 12 membrane spanning segments (TMS) that traverse the membrane in an α-helical fashion. The TMS are connected by hydrophilic loops that are usually shorter at the periplasmic side than at the cytoplasmic side of the membrane. Fig. 1 shows models of the membrane topology, predicted from the primary sequence, of the Na^+-dependent citrate carrier of *K. pneumoniae* (CitS) [15] and the H^+-dependent citrate carrier of *L. lactis* (CitP) [16]. CitS and CitP are obligatorily coupled to Na^+ and H^+, respectively. The genes coding for the two transporters share 29% identical residues and an additional 20% similar residues. The models show that the position of the predicted TMS and loops are even more conserved in the two proteins. In both predicted structures, the C-terminal halves are characterized by large periplasmic and short cytoplasmic loops, which is just opposite from what is observed for, by now, a large number of secondary carriers. Also, in this part of the molecules von Heynes' 'positive inside' inside rule is not very apparent [17]. The correctness of the model has to be substantiated by experimental evidence. Nevertheless, the shared 'deviations' in the secondary structure models, together with the primary sequence similarity, suggest that within the 12-TMS family of bacterial secondary transporters CitS and CitP form a subfamily that share some detailed features in the three-dimensional structure. However, one of the two enzymes is Na^+ coupled, the other H^+ coupled. Clearly, the determinants of Na^+ and H^+ specificity of secondary transporters, most likely, reflect minor details in the structure. A single amino acid side chain may make the difference.

4. Proton and Na^+ permeability of biological membranes

An organism that maintains an electrochemical gradient of either H^+ and/or Na^+ across its cell membrane will lose metabolic energy because of passive leaks of the cations through the membrane. The proton permeability of liposomes prepared from the lipids of the mesophilic organism *E. coli* or from the thermophilic organisms *B. stearothermophilus* and *C. fervidus* do not differ significantly when assayed at the same temperature (unpublished data and Ref. [18]). However, since the proton permeability increases rapidly with temperature, at their optimal growth temperature, thermophilic organisms, will be confronted with much higher rates of passive proton leakage across the membrane than mesophiles. *B. stearothermophilus*

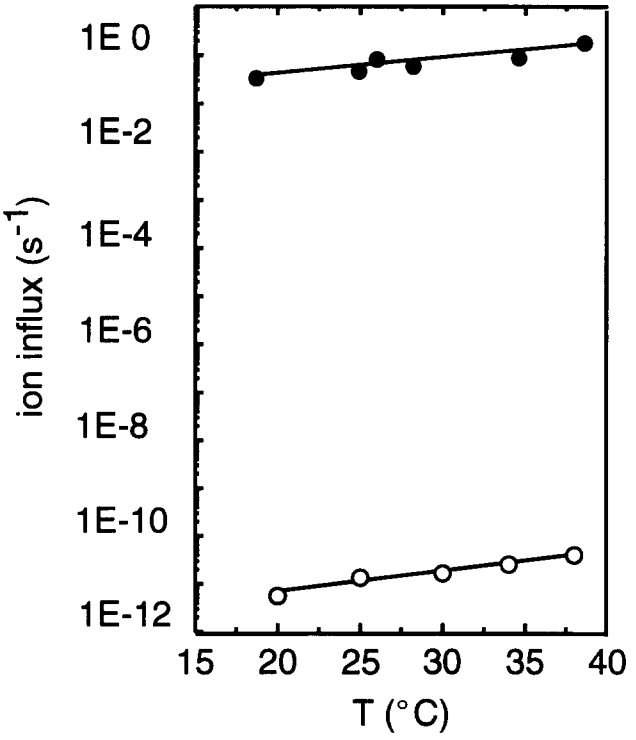

Fig. 2. Permeability of phospholipid membranes to H^+ and Na^+ at different temperatures. Liposomes were prepared from a mixture of *E. coli* phosphatidylethanolamine and egg phosphatidylcholine 3:1 (w/w) in 10 mM postassium phosphate, 100 mM KCl, 5 mM $MgCl_2$ (pH 7.0). Proton permeability (●) was measured by diluting liposomes loaded with 100 μM pyranine 100-fold in the same buffer with Na^+ instead of K^+. H^+ influx in response to a membrane potential was induced by the addition of the potassium ionophore valinomycin (200 nM) and measured as a change in fluorescence of internal pyranine. Na^+ permeability (○) was estimated from the uptake of $^{22}Na^+$ as determined by the filtration method. The external Na^+ concentration was 1 mM.

and *C. fervidus* follow different strategies to deal with these high proton leaks. In *B. stearothermophilus* high oxidation rates catalyzed by electron transport chains provide enough proton pumping capacity to maintain a high protonmotive force across the membrane [18]. *C. fervidus* deals with the high proton leak through the membrane by eliminating a proton cycle altogether and using an Na^+ cycle instead. Comparison of Na^+ and H^+ influx into liposomes, prepared from a mixture of *E. coli* phosphatidylethanolamine and egg phosphatidylcholine, shows that between 18°C and 38°C the rate constant for Na^+ influx is 10 to 11 orders of magnitude lower than the rate constant for H^+ (Fig. 2). At physiological concentrations of the substrates (pH 7 and 15 mM Na^+) the difference in net influx will be 5 to 6 orders of magnitude.

5. Discussion

The available data on the secondary transporters suggest no selective advantage for the use of H^+ or Na^+ as the coupling ion at the level of enzyme catalysis. This is most likely true for all enzymes involved in the bioenergetic cation cycles. Apparently, nature can built enzymes for a specific task just as easy with H^+ or Na^+ as the coupling ion. In fact, the structural differences between these enzymes are likely to be very small and the data indicate that the catalytic efficiency would not be different. Hence, the evolution of H^+ and Na^+-dependent enzymes is a consequence of the adaptation of the organisms to a changing environment as opposed to an optimization of catalytic efficiency. Therefore, the reason for using H^+ or Na^+ as the coupling ion must be in the conditions the organism is facing or has been facing in earlier stages of evolution in its natural habitat. The requirement to keep the internal pH constant and the internal concentration of toxic Na^+ low should be taken into consideration as well as the availability of metabolic energy.

The amount of energy *Clostridium fervidus* would have to spend in order to compensate for passive leaks of H^+ through the membrane so that a high proton gradient for driving metabolic processes can be maintained may have played a crucial role in the choice of Na^+ over H^+. The yield of metabolic energy per molecule of substrate in this anaerobic thermophile is much less than in an aerobic thermophile like *B. stearothermophilus*. The much lower permeability of phospholipid membranes for Na^+ than for H^+ implies that with Na^+ as coupling ion much less of the sparsely available energy has to be invested to maintain a high driving force for energy-requiring processes. This advantage will be valid only if no H^+ cycling is present at the same time. This turns out to be the case in *C. fervidus*. The price the organism has to pay is that it cannot control the internal pH. As a result, changes in the external pH will affect the activity of pH-sensitive metabolic processes and ultimately growth. Clearly, *C. fervidus* has a very sharp optimum for growth between pH 6.3 and 7.7. When conditions get less favorable *C. fervidus* will form spores that will outgrow again when the situation improves.

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