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The role of protonic and sodium potentials in the motility of *E. coli* and *Bacillus FTU*

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The motility of *Escherichia coli* and of alkalo- and halotolerant *Bacillus FTU* has been studied. It is found that *Bac. FTU* motility (i) requires Na^+, (ii) is resistant to the protonophorous uncoupler pentachlorophenol (PCP) if cells grow at high pH, and is sensitive to the uncouplers at neutral pH, (iii) is sensitized to the uncouplers with the addition of monensin, (iv) sensitive to amiloride and (v) can be supported by an artificially imposed Na^+ gradient in the presence of uncoupler, cyanide and arsenate. On the other hand, *E. coli* motility (a) does not require Na^+, (b) is always uncoupler-sensitive, (c) is amiloride-resistant, and (d) can be supported by an artificially-imposed gradient of H^+, not Na^+. It is concluded that the motilities of *Bac. FTU* and *E. coli* are due to the operation of the Na^+ and the H^+ motors, respectively. In *Bac. FTU* growing at alkaline pH, the Na^+ motors are assumed to be energized by $\Delta\bar{\mu}_{Na^+}$ produced by the Na^+-motive respiratory chain, and therefore $\Delta\bar{\mu}_{H^+}$ is not involved in the motility process. As to *Bac. FTU* growing in a neutral medium, $\Delta\bar{\mu}_{Na^+}$ is produced secondarily, via the Na^+/H^+-antiporter, i.e., at the expense of $\Delta\bar{\mu}_{H^+}$ formed by the H^+-motive respiratory chain.

Introduction

The concept of the sodium cycle [1,2] assumes that, under certain conditions, $\Delta\bar{\mu}_{Na^+}$ (i) is produced by primary Na^+ pumps and (ii) is utilized to support three types of membrane-linked work, i.e., chemical (ATP synthesis), osmotic (accumulation of solutes) and mechanical (motility). Recently, this suggestion was experimentally proved in studies on various marine bacteria (for reviews, see Refs. 3–7).

An Na^+-dependent motility was observed for the first time in 1977 by Kodama and Taniguchi in experiments on *Pseudomonas stutzeri* [8]. Unfortunately, the problems of $\Delta\bar{\mu}_{Na^+}$ formation and utilization have never been studied in this bacterium.

There is at least one example of a bacterium performing mechanical work at the expense of $\Delta\bar{\mu}_{Na^+}$, generated by a primary Na^+ pump. Dibrov in our group showed in 1983–1987 that the motility of *Vibrio alginolyticus* (1) occurs only in the presence of Na^+, (2) can be supported by an artificially-imposed $\Delta p Na$ in a monensin-sensitive fashion (under the same conditions, ΔpH was ineffective) and (3) is observed at a lowered but measurable rate in the presence of a very high concentration of a protonophore, such as $1 \cdot 10^{-4}$ M CCCP. A 100-fold lower concentration of the protonophore completely arrests the motility if the medium is supplemented with monensin. Added without CCCP, monensin decreases the respiration-supported motility but slightly. It was concluded that the flagellar motor of *V. alginolyticus* is driven by $\Delta\bar{\mu}_{Na^+}$ rather than by $\Delta\bar{\mu}_{H^+}$ [5,9–12] (see also Refs. 13–15).

The $\Delta\bar{\mu}_{Na^+}$-supported motility was demonstrated in alkalophilic Bacilli by Imae and coworkers in 1981–1989. *Bacillus* sp. YN-1 and *Bacillus firmus* were studied for this purpose [16–22]. In the *Bacillus* sp. YH-1, studied in much detail, Na^+ requirement for motility and partial resistance of motility to the protonophore were shown. It was also found that $\Delta p Na$ and $\Delta\Psi$, generated enzymatically, have an equivalent effect in supporting motility. An artificially-imposed $\Delta\bar{\mu}_{Na^+}$ was not studied. Since the primary Na^+pumps have not been yet described in these microorganisms, it is assumed that the electrogenic Na^+/nH^+ antiport is the only mechanism of $\Delta\bar{\mu}_{Na^+}$ generation [23].

The alkalo- and halotolerant *Bacillus FTU* was discovered in our laboratory as a contaminant to the culture of marine *V. alginolyticus* [24,25]. It was found

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Abbreviations: $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$, differences in the electrochemical potentials of H^+ and Na^+, respectively; ΔpH and $\Delta p Na$, differences in the concentrations of H^+ and Na^+, respectively; PCP, pentachlorophenol.

[26,27] that this microorganism possessed an Na^+-motive respiratory chain when growing at low $\Delta\bar{\mu}_{H^+}$ (i.e., at high pH, in the presence of a protonophore or of a low concentration of cyanide which specifically inhibits the H^+-motive respiration [28]). Under high $\Delta\bar{\mu}_{H^+}$ conditions, only the H^+-motive respiratory chain was operative [26,27] and $\Delta\bar{\mu}_{Na^+}$ was formed secondarily, i.e., by means of the Na^+/H^+ antiporter exporting Na^+ from the cell at the cost of the respiration-produced $\Delta\bar{\mu}_{H^+}$ [26].

As it will be reported in the present paper, the motility of *Bac. FTU* is $\Delta\bar{\mu}_{Na^+}$-driven and independent of the $\Delta\bar{\mu}_{H^+}$ level when the cells grow at high pH. It is concluded that the Na^+, rather than the H^+ motors, are operative in *Bac. FTU* irrespective of the growth conditions. For comparison, the motility of *E. coli* growing at alkaline pH was studied. The H^+ motors were found to be responsible for the motility of this bacterium both at neutral and high pH values.

Materials and Methods

Bac. FTU was isolated in our laboratory [25]. *E. coli* GR70N was kindly provided by Professor R.B. Gennis. Before motility experiments, bacteria were grown for 12–14 h on 0.2% agar containing 1% tryptone, 1% NaCl, 0.5% yeast extract, streptomycin, 100 μg/ml (pH 7.3) (*E. coli*) or synthetic growth medium B (pH 8.6) (see below) supplemented with 0.5% yeast extract (*Bac. FTU*). Then bacteria were grown in a liquid synthetic growth media of the following composition: *E. coli*, 22 mM potassium phosphate, 20 mM sodium phosphate, 10 mM NaCl, 10 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 50 mM glycylglycine, 50 mM sodium succinate, 0.05% yeast extract, thiamin ($1 \cdot 10^{-6}$ g ml^{-1}) (pH 8.6) (medium A); *Bac. FTU*, 2 mM potassium phosphate, 10 mM KCl, 0.5 M NaCl, 5 mM $MgSO_4$, 0.01 mM $FeSO_4$, 0.1 mM EDTA, 15 mM $(NH_4)_2SO_4$, 50 mM Tris sulfate, 60 mM sodium succinate, 0.05% yeast extract (pH 8.6 or 7.5) (medium B). In the liquid medium, the cells were grown to $A_{600} = 0.5$–1.2.

To study the effect of artificially-imposed ion gradients, the *Bac. FTU* was exhausted in endogenous substrates by being kept for 10 min at 20°C in 0.5 M KCl, 10 mM Tris-sulfate, 50 mM diethylamine (pH 8.6) and washed by 0.5 M KCl and 10 mM Hepes (pH 7.5). This procedure was repeated twice. To exhaust *E. coli*, the cells were kept for 2 h at 20°C in 0.15 M NaCl, 5 mM $MgSO_4$ and 25 mM Tris-sulfate (pH 8.6) and washed by a medium of the same composition.

The motility rate was measured according to Shoesmith [29] at room temperature using a phase-contrast microscope. In these experiments, number of the cells per ml was $(1–2) \cdot 10^8$. Each measurement took 1 min or 10 s when enzymatically-produced or artificially imposed $\Delta\bar{\mu}_{Na^+}$ ($\Delta\bar{\mu}_{H^+}$) were used as the driving forces for the flagellar motor, respectively. In the former and in the latter cases, the values given in figures and in the table, represent the average of 10 and 25 measurements.

Results

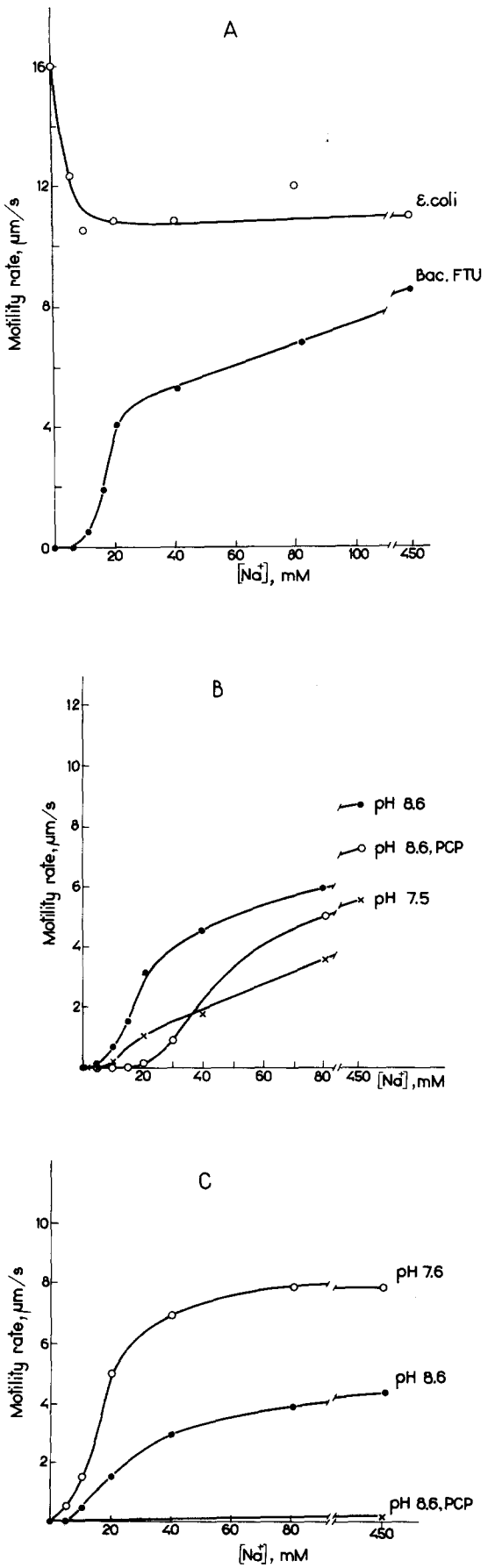
Shown in Fig. 1 are the motility rates of *Bac. FTU* and *E. coli* as functions of $[Na^+]$. It is seen that *Bac. FTU* is motionless in a Na^+-free medium in spite of, as was previously shown by our group, the membrane potential level without Na^+ being as high as in the presence of Na^+ [30]. On the other hand, the maximal motility rate of *E. coli* was observed without Na^+ (Fig. 1A). In *Bac. FTU*, grown in an alkaline medium, a decrease in pH of the incubation mixture from 8.6 to 7.5 slightly lowered the swimming rate and increased the half-maximal Na^+ concentration. The latter parameter increased especially strongly when the protonophorous uncoupler PCP was added at pH 8.6 (Fig. 1B). Quite different relationships were obtained for *Bac. FTU* grown at neutral pH (Fig. 1C). Here PCP completely arrested its motility, whereas pH decrease from 8.6 to 7.6 resulted in an increase in the motility rate, with the half-maximal $[Na^+]$ not being affected.

In *E. coli*, PCP strongly inhibited the motility rate even in cells growing at high pH. At any PCP concentration, Na^+ failed to prevent the inhibition (Fig. 2) in spite of the fact that Na^+ was shown to prevent inhibiting effect of low concentrations of uncouplers on the growth and the membrane potential level of the *E. coli* cells [31,32]. In agreement with this observation, we found that much lower concentrations of PCP inhibit motility than the growth rate of *E. coli* (Fig. 3).

As one can see from Fig. 4, the addition of the Na^+/H^+ antiporter monensin rendered uncoupler-sensitive the motility of the *Bac. FTU* growing at alkaline pH. Monensin was almost ineffective without PCP. At the same time, motility of *Bac. FTU* growing at neutral pH was uncoupler-sensitive even without monensin. Such relationships could be revealed at either neutral or alkaline pH of the incubation mixture.

Fig. 5 shows the effect of amiloride on the motility of two bacterial species. The data indicate that the motility proved to be amiloride-resistant in *E. coli* and amiloride-sensitive in *Bac. FTU* growing at pH 8.6 or 7.5. It is also seen, that amiloride was more effective in neutral than in alkaline incubation mixture which is consistent with an assumption that the inhibitor is active in its protonated form [33].

In a final series of experiments, the motility supported by an artificially-imposed ΔpH or Δp*Na* was studied. Cells, grown at high pH and partially exhausted in endogenous substrates, were used for this purpose. It was found that in *E. coli* an H^+ pulse stimulated the motility (Fig. 6A), whereas a Na^+ pulse



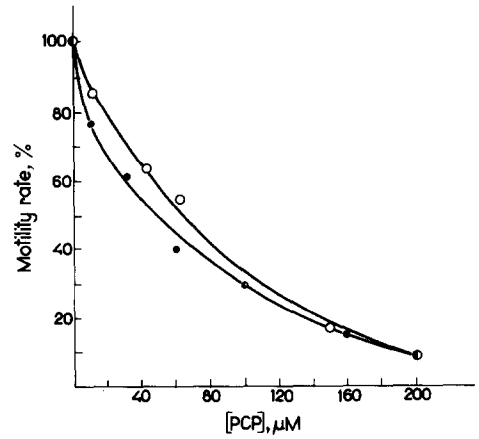


Fig. 2. Effect of PCP on the motility of *E. coli* grown at pH 8.6. Incubation mixtures: (○), 0.2 M sucrose, 10 mM Tris-sulfate (pH 8.6); (●) 0.2 M NaCl, 10 mM Tris-sulfate (pH 8.6).

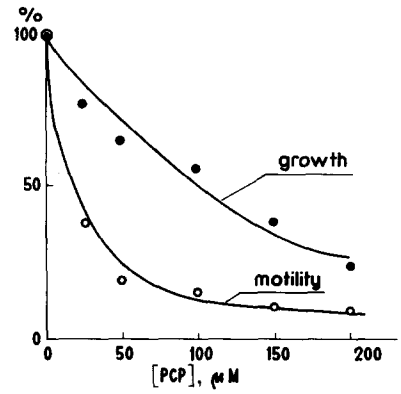


Fig. 3. Effect of PCP on the growth rate and motility of *E. coli* at pH 7.5. 100% was 0.55 h^{-1} (1/generation time) and 21 $\mu m\ s^{-1}$ for the growth and motility rates, respectively.

was ineffective (not shown). On the other hand, the Na^+ pulse was found to exert a stimulating effect on the *Bac. FTU* motility (Fig. 5B). To exclude any possible explanation of the Na^+ pulse effect by a conversion of Δp*Na* to ΔpH of proper direction, the experiment, shown in Fig. 5B, was carried out in the presence of protophore ($7 \cdot 10^{-5}$ M PCP). Moreover, pH of the medium was increased from 7.6 to 9.0 when Na^+ was added, to form an opposite ΔpH (interior acidic) and generate $\Delta\Psi$ of proper direction (inside negative) due to the PCP-mediated H^+ efflux.

Yet another explanation of the Na^+ pulse effect could be that the *Bac. FTU* motor specifically requires

Fig. 1. Na^+-dependence of *Bac. FTU* and *E. coli* motility. Cells were grown at pH 8.6 (A,B) or 7.5 (C). Incubation mixtures: A, 0.25 M (*E. coli*) or 0.5 M (*Bac. FTU*) KCl, 25 mM Tris-sulfate (pH 8.6); B and C, *Bac. FTU*, 0.5 M KCl, 0.1 M Tris-sulfate (pH 8.6 or 7.5), and, where indicated, 0.1 mM PCP, and where indicated, 0.1 mM PCP. When the Na^+ concentration was increased, KCl was decreased for constant osmolarity of the medium.

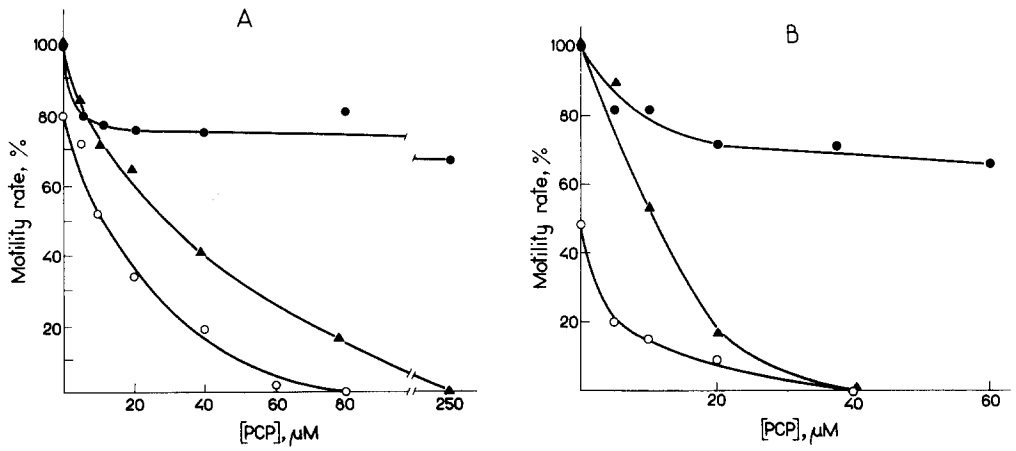


Fig. 4. Effect of PCP and monensin on the motility of *Bac. FTU* grown at pH 8.6 (●, ○) or at pH 7.5 (▲). Incubation mixtures: A, 0.5 M NaCl, 50 mM Tris-sulfate (pH 8.6); curve (○), the incubation mixture was supplemented with $2 \cdot 10^{-5}$ M monensin; B, 0.5 M NaCl, 50 mM Hepes-KOH (pH 7.5).

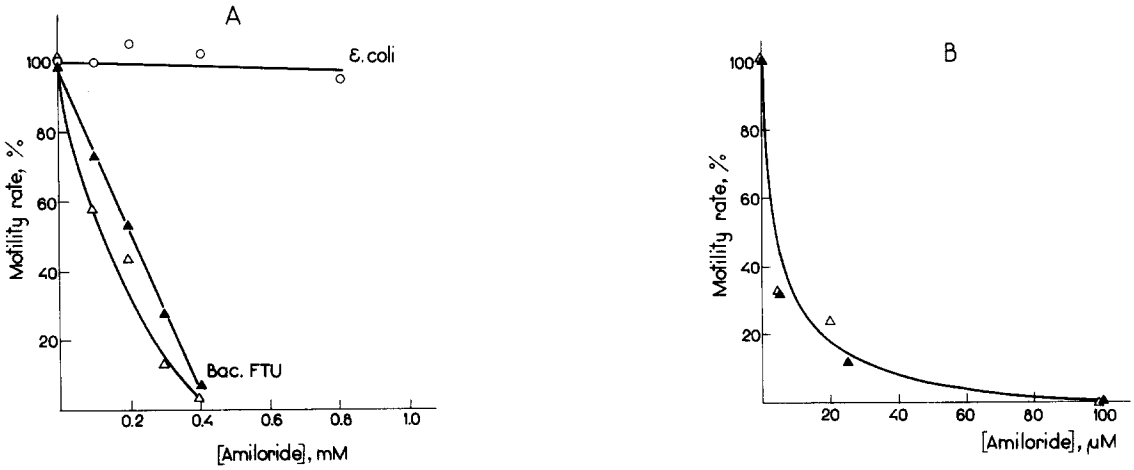


Fig. 5. Effect of amiloride on the motility of *E. coli* (○) and *Bac. FTU* grown at pH 8.6 (▲) or *Bac. FTU* grown at pH 7.5 (△). Incubation mixtures: A, 0.2 M sucrose, 20 mM NaCl, 10 mM Tris-sulfate (pH 8.6); B, 0.2 M sucrose, 20 mM NaCl, 10 mM Hepes-KOH (pH 7.6).

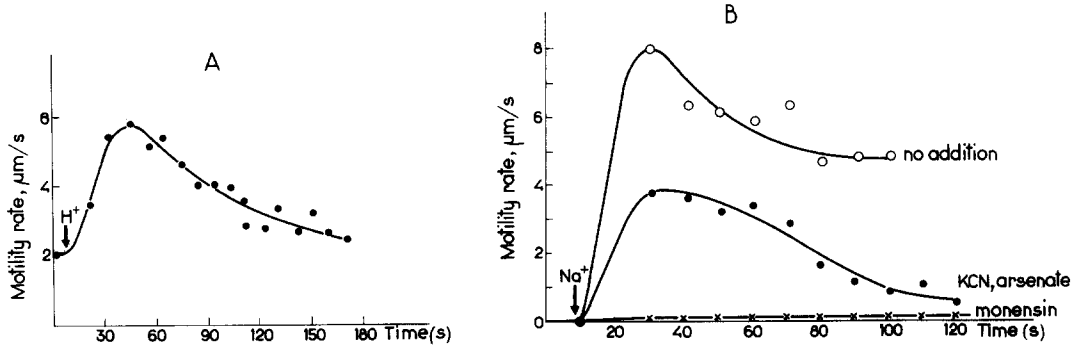


Fig. 6. Motility of *E. coli* (A) or *Bac. FTU* (B) supported by artificially-imposed H^+ or Na^+ gradients, respectively. Cells were grown at pH 8.6 and, after partial exhaustion in the endogenous substrates, were incubated in media containing 0.1 M K_2SO_4, 5 mM $MgSO_4$ and 10 mM Tris-sulfate (pH 8.6) (A), 0.2 M sucrose, 10 mM Hepes (pH 7.6), and $7 \cdot 10^{-5}$ M PCP (B). At the moment indicated by the arrow the cells were diluted 10-fold by 0.1 M K_2SO_4, 5 mM $MgSO_4$, 0.1 M Mops (pH 6.5), $1 \cdot 10^{-5}$ M valinomycin (A), or by 0.6 M NaCl, 0.1 M Tris-sulfate (pH 9.0), $7 \cdot 10^{-5}$ M PCP (B). Additions, 4 mM KCN, 20 mM potassium arsenate and $2 \cdot 10^{-5}$ M monensin. Samples with arsenate were pre-incubated with this inhibitor for 10 min.

TABLE I

Effect of metabolic inhibitors on the enzymatically supported motility of Bac. FTU grown at pH 8.6

Incubation mixture, 0.5 M NaCl, 0.05 M Tris-sulfate, pH 8.6. Additions, 0.1 mM PCP, 20 mM K_2HAsO_4, 4 mM KCN. In the case of K_2HAsO_4, the cells were preincubated with the inhibitor for 10 min.

Additions	Motility rate	
	$\mu m\ s^{-1}$	%
–	9.6	100
PCP	7.2	75
PCP, K_2HAsO_4	5.0	52.5
PCP, KCN	2.1	22
PCP, K_2HAsO_4, KCN	0	0

Na^+ rather than $\Delta\bar{\mu}_{Na^+}$ To exclude such a possibility, the effects of KCN and arsenate on the Na^+ pulse-supported motility were studied. In a sample without inhibitors (Fig. 6B, the upper curve), the cells were motionless without Na^+ but became motile with the addition of Na^+. The motility rate, attaining its maximum immediately after the Na^+ pulse, decreased to some steady-state level within a minute. Apparently, this final motility level was supported by the oxidation of endogenous substrates and/or by ATP hydrolysis. In the presence of cyanide and arsenate, however, the steady state was absent (Fig. 6B, the middle curve), as if all the motility were supported by an artificially-imposed $\Delta\bar{\mu}_{Na^+}$. This conclusion is confirmed by Table I, showing that combined action of PCP, cyanide and arsenate resulted in cessation of the enzymatically supported motility. As for the Na^+-pulse supported motility, this was completely inhibited by monensin, discharging of the Na^+ and H^+ gradients (Fig. 6B, the lower curve).

Discussion

The above data confirm some previous observations indicating that the motility of *E. coli* growing aerobically at neutral pH is due to the operation of the H^+-motors (for review, see Ref. 5). This is in agreement with the fact that, under these conditions, *E. coli* employs the H^+-motive respiratory chain as a membrane energization mechanism [26]. On the other hand, *E. coli*, growing at alkaline pH or in the presence of uncoupler, was found to possess the Na^+-motive respiratory chain in addition to the H^+-motive one [26,31,32]. The question was, what kind of the flagellar motor, H^+-driven or Na^+-driven, was used in this case. The above data clearly indicate that at high pH or with PCP, too, the *E. coli* flagellar motors are H^+-driven. In fact, the motility of *E. coli* proved to be Na^+-independent, uncoupler-sensitive and amiloride-resistant at alkaline pH of the growth medium. Moreover, the artificially imposed ΔpH, not Δp*Na*, proved to be competent in supporting the motility of the *E. coli* cells growing in this medium.

But it was quite the reverse with *Bac. FTU*. Here flagellar motors proved to be Na^+-driven independently of the pH value in the growth medium. The motility of this microorganism (i) always required Na^+, (ii) was uncoupler-resistant when cells grew at high pH, (iii) was uncoupler-sensitive in the presence of monensin and (iv) could be actuated by an artificially imposed Δp*Na*. Moreover, higher concentrations of Na^+ proved to be necessary for motility when an uncoupler was present (Fig. 1B), something that could have been predicted, assuming that the uncoupler-mediated influx of H^+ ions discharges the Na^+-motive respiration-generated $\Delta\Psi$, converting $\Delta\Psi$ to Δp*Na*. The inhibiting effect of monensin on the motility in the presence (but not in the absence) of uncouplers (Fig. 4) could be accounted for by the Δp*Na* discharge via Na^+/H^+ antiport [28]. As to the uncoupler sensitivity of motility of *Bac. FTU* grown at neutral pH, it was most probably due to the fact that in this case, the H^+-motive respiration is the only aerobic mechanism of membrane energization. To convert the respiration-produced $\Delta\bar{\mu}_{H^+}$ to $\Delta\bar{\mu}_{Na^+}$, an endogenous Na^+/H^+ antiporter seems to be employed; as a result, the following chain of events may take place: respiration $\rightarrow \Delta\bar{\mu}_{H^+} \rightarrow \Delta\bar{\mu}_{Na^+} \rightarrow$ motility. Unfortunately, the Na^+/H^+ antiporter inhibitor amiloride cannot be used in *Bac. FTU* to verify the antiporter involvement since the *Bac. FTU* Na^+ motor per se proved to be amiloride-sensitive. Such a conclusion is based on the fact that the motility was amiloride-inhibited even in cells grown at high pH (Fig. 5) where the endogenous Na^+/H^+ antiporter seemed to be not operative [26,28]. In this respect *Bac. FTU* resembles an alkalophilic *Bacillus* previously studied by Imae's group [34].

Thus, both in *E. coli* and *Bac. FTU* the respiration switchover from H^+ to Na^+ with a pH increase is not accompanied by a similar switchover in the motility mechanism. The motility of *E. coli* is H^+-driven, and that of *Bac. FTU* Na^+ -driven at all the investigated pH values. This is different from the situation described quite recently by Imae and coworkers in the alkalotolerant *V. alginolyticus* [35]. This bacterium was found to engage the Na^+ motor to rotate its single flagellum, when living, e.g., in sea water. On the other hand, the H^+-motors were induced when *V. alginolyticus* grew in a viscous medium. (Under these conditions, bacteria became peritrichial.) Apparently, *E. coli* and *Bac. FTU*, in contrast to *V. alginolyticus*, are incapable of changing the motility mechanism. At the same time, both *E. coli* and *Bac. FTU* can, depending on growth conditions, switch from the H^+-motive to the Na^+ -motive respiration [26,27,31] and, from the H^+-driven to the Na^+-driven ATP-synthesis (unpublished data).

This means that the possibility of an $H^+ \rightarrow Na^+$ switchover in *E. coli* and *Bac. FTU* is restricted to such a vital function as oxidative phosphorylation, whereas the less important function, motility, remains unchanged in spite of a change in the $\Delta\bar{\mu}_{H^+}$ level. It is also noteworthy that the threshold $\Delta\bar{\mu}_{H^+}$ value for oxidative phosphorylation is much higher than one for motility [5]. Therefore a decrease in the $\Delta\bar{\mu}_{H^+}$ level must result, first of all, in the inhibition of ATP synthesis and, much later, in the motility rate decrease.

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