*Biochimica et Biophysica Acta* 850 (1986) 458–465
Elsevier

BBA 42005

# The sodium cycle. II. $Na^+$-coupled oxidative phosphorylation in *Vibrio alginolyticus* cells

P.A. Dibrov, R.L. Lazarova, V.P. Skulachev * and M.L. Verkhovskaya

*A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899 (U.S.S.R.)*

(Received August 28th, 1985)
(Revised manuscript received January 28th, 1986)

Key words: $Na^+$ pump; Oxidative phosphorylation; Membrane potential; *(V. alginolyticus)*

**The role of $Na^+$ in *Vibrio alginolyticus* oxidative phosphorylation has been studied. It has been found that the addition of a respiratory substrate, lactate, to bacterial cells exhausted in endogenous pools of substrates and ATP has a strong stimulating effect on oxygen consumption and ATP synthesis. Phosphorylation is found to be sensitive to anaerobiosis as well as to HQNO, an agent inhibiting the $Na^+$-motive respiratory chain of *V. alginolyticus*. $Na^+$ loaded cells incubated in a $K^+$ or $Li^+$ medium fail to synthesize ATP in response to lactate addition. The addition of $Na^+$ at a concentration comparable to that inside the cell is shown to abolish the inhibiting effect of the high intracellular $Na^+$ level. Neither lactate oxidation nor $\Delta\psi$ generation coupled with this oxidation is increased by external $Na^+$ in the $Na^+$-loaded cells. It is concluded that oxidative ATP synthesis in *V. alginolyticus* cells is inhibited by the artificially imposed reverse $\Delta p$*Na*, i.e., $[Na^+]_{in} > [Na^+]_{out}$. Oxidative phosphorylation is resistant to a protonophorous uncoupler (0.1 mM CCCP) in the $K^+$-loaded cells incubated in a high $Na^+$ medium, i.e., when $\Delta p$*Na* of the proper direction ($[Na^+]_{in} < [Na^+]_{out}$) is present. The addition of monensin in the presence of CCCP completely arrests the ATP synthesis. Monensin without CCCP is ineffective. Oxidative phosphorylation in the same cells incubated in a high $K^+$ medium ($\Delta p$*Na* is low) is decreased by CCCP even without monensin. Artificial formation of $\Delta p$*Na* by adding 0.25 M NaCl to the $K^+$-loaded cells ($Na^+$ pulse) results in a temporary increase in the ATP level which spontaneously decreases again within a few minutes. $Na^+$ pulse-induced ATP synthesis is completely abolished by monensin and is resistant to CCCP, valinomycin and HQNO. 0.05 M NaCl increases the ATP level only slightly. Thus, *V. alginolyticus* cells at alkaline pH represent the first example of an oxidative phosphorylation system which uses $Na^+$ instead of $H^+$ as the coupling ion.**

## Introduction

In the preceding paper [1] it was shown that motility of the marine alkalotolerant *Vibrio alginolyticus* can be supported by an enzymatically formed or artificially imposed $\Delta\bar{\mu}_{Na^+}$. It was also

* To whom correspondence should be addressed.

Abbreviations: $\Delta\bar{\mu}_{H^+}$, $\Delta\bar{\mu}_{Na^+}$, electrochemical gradients of $H^+$ and $Na^+$, respectively; $\Delta\psi$, transmembrane electric-potential difference; $\Delta$pH and $\Delta p$*Na*, transmembrane differences in concentrations of $H^+$ and $Na^+$, respectively; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; $PCB^-$, phenyldicarbaundecaborane; $TPP^+$, tetraphenylphosphonium; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid; Taps, 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulphonic acid; Ches, 2-(cyclohexylamino)ethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Caps, 3-(cyclohexylamino)-1-propanesulphonic acid.

0005-2728/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

revealed in this study that besides the $Na^+$-motive respiration [2], there is an anaerobic arsenate-sensitive mechanism producing the energy utilized by the $Na^+$-motor. $Na^+$-ATPase hydrolyzing glycolytic ATP may in principle play the role of such a mechanism.

The $Na^+$-motive ATPase was described in *Propionigenum modestum* [3], *Streptococcus faecalis* [4,5], *Mycoplasma mycoides* [6,7] and in some animal tissues where it coexists with $Na^+$,$K^+$-ATPase [8]. In *Propionigenum modestum* subbacterial vesicles it was found that $Na^+$-ATPase can catalyze ATP synthesis utilizing $\Delta\bar{\mu}_{Na^+}$ which is supplied by the $Na^+$-motive methylmalonyl-decarboxylase. Unfortunately, the degree of coupling in the vesicles was rather poor so that the rate of ATP formation was $10^4$-times slower than the rate of decarboxylation [3].

In *V. alginolyticus*, $Na^+$-ATPase, if it is really inherent in this bacterium, may in principle be reversed by the respiratory chain-produced $\Delta\bar{\mu}_{Na^+}$ to form ATP at the expense of the respiration energy. To verify this suggestion [9–11], the ATP synthesis in *V. alginolyticus* cells was studied. The results of the study confirming the idea of the $Na^+$-coupled oxidative phosphorylation will be described below.

## Methods and Materials

*V. alginolyticus* 138-2 were grown at 37°C and pH 8.6 under anaerobic conditions in the presence of 0.5% glucose (for other conditions, see the preceding paper [1]). To measure ATP, the luciferase system and Pico-ATP luminometer (Jobin-Yvon) were used (for details, see Ref. 12). Respiration and external $K^+$ concentration were measured polarographically and with a radiometer $K^+$-sensitive electrode, respectively. To estimate the $Na^+$ content in the cells, they were centrifuged, suspended in a small volume of $H_2O$, sonicated and treated with 2.5% trichloroacetic acid. In supernatant, $Na^+$ was measured by means of a flame spectrophotometer.

To obtain $K^+$- or $Li^+$-loaded cells, a slightly modified procedure of Tokuda and Unemoto [2] was employed. Instead of methylamine, 50 mM diethylamine was used in some experiments. The final cell precipitate was washed twice with 0.4 M KCl (or LiCl)/25 mM Tris-HCl/(pH 8.6) and then suspended and stored in a solution of the same composition. In the $Na^+$ pulse experiments, preparation of the $K^+$-loaded cells was carried out in the presence of 10 mM $MgCl_2$.

Measurements of the intracellular [$Na^+$] showed that in the $K^+$-loaded cells it was about 2 mM.

$Na^+$-loaded cells were obtained by storing the cells for 12 h at −5°C in a solution containing 0.5 M NaCl/5 mM $MgSO_4$/25 mM Tris-HCl (pH 8.6).

Concentration of the bacterial protein in the incubation mixture was 0.05–0.35 mg · $ml^{-1}$. In Fig. 3B it was increased up to 1.1 mg · $ml^{-1}$.

## Results

The procedure of the replacement of intracellular cations by outer $K^+$ or $Na^+$ (see Methods) was found to be accompanied by exhaustion of the pools of endogenous substrates and ATP. The addition of the respiratory substrate, lactate, to the exhausted cells entailed strong activation of respiration (see below Fig. 3A) and a manifold increase in the intracellular ATP (Fig. 1). The rate of ATP synthesis coupled to lactate oxidation in the $Li^+$-loaded cells proved to be much higher than in the $Na^+$-loaded cells (cf. Fig. 1 and 2, upper curve). Note that $Na^+$ concentrations in the medium in these two experiments were the same.

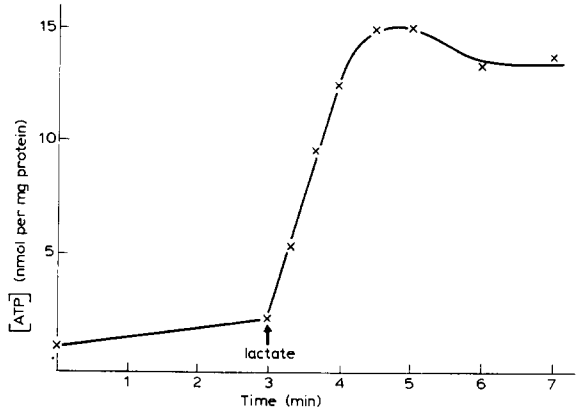


Fig. 1. Kinetics of ATP synthesis coupled with lactate oxidation by $Li^+$-loaded *V. alginolyticus* cells. Incubation mixture contained 0.5 M NaCl, 10 mM KCl, 25 mM Tris-HCl (pH 8.6), 5 mM $MgSO_4$, $Li^+$-loaded cells and 3 or 50 mM NaCl. 75 mM D,L-lactate was added.

TABLE I

EFFECT OF INHIBITORS ON OXIDATIVE PHOSPHORYLATION IN $K^+$-LOADED CELLS OF *V. ALGINOLYTICUS*

| Conditions | ΔATP (nmol per mg protein) |
|---|---|
| aerobic | 6.0 |
| anaerobic | 0.0 |
| aerobic, 0.1 mM HQNO | 0.0 |
| aerobic, 0.1 mM CCCP | 6.0 |
| aerobic, 0.05 mM monensin | 5.0 |
| aerobic, CCCP + monensin | 0.0 |

ATP synthesis proved to be completely abolished by anaerobiosis as well as by HQNO, inhibitor of the $Na^+$-motive NADH-mena-(ubi)quinone reductase activity (Table I).

In Table II we compared ATP synthesis in the $K^+$- and $Na^+$-loaded cells incubated in media differing in ionic composition. One can see that ATP synthesis was obtained after lactate addition to the $K^+$-loaded cell incubated in a high $Na^+$ medium as well as in low $Na^+$ media where $Na^+$ was substituted by $K^+$ or $Li^+$. An ATP synthesis was also found in the $Na^+$-loaded cells incubated in the high $Na^+$-medium. At the same time, $Na^+$-loaded cells in the low $Na^+$ medium ($Na^+$ was substituted by $K^+$ or $Li^+$) did not respond to lactate addition by any ATP formation.

A feature specific for conditions at which no ATP was formed consists in that here there was a $\Delta p Na$ of the direction opposite to the natural one, i.e., $[Na^+]$ inside was much higher than $[Na^+]$ outside the cell. As measurements of $[Na^+]_{in}$ showed, this value was 2 mM and about 0.5 M in the $K^+$- and $Na^+$-loaded cells, respectively.

It is these relationships that were predicted in a concept assuming an $Na^+$-coupled oxidative phosphorylation in *V. alginolyticus* cells [10,11]. According to this concept, the $Na^+$-motive respiration forms $\Delta\psi$ (negative inside) and $\Delta p Na$ (lower $[Na^+]$ inside) which are consumed by the $Na^+$-driven ATP-synthase. If $\Delta\psi$ is counterbalanced by the oppositely directed $\Delta p Na$ (higher $[Na^+]$ inside), ATP synthesis must be inhibited.

Below some details of the inhibiting effect of the reverse $\Delta p Na$ are given. In Fig. 2 one can see that the inhibition disappears at rather high $[Na^+]_{out}$, the critical $Na^+$ concentration being about 100 mM. At 60 mM $[Na^+]_{out}$ some phosphorylation became measurable after a rather long incubation period (Fig. 2A). This may be explained by dissipation of the reverse $\Delta p Na$. The rate of lactate oxidation in 0.5 M NaCl where ATP formation occurred was the same as in 60 mM NaCl where oxidative phosphorylation was strongly inhibited (Fig. 3A). Moreover, it was found that $\Delta\psi$ formation coupled with oxidation of added lactate does not require high $[Na^+]_{out}$. $\Delta\psi$ was measured with valinomycin + $K^+$ (Fig. 3B) or with $TPP^+$ (not shown).

Fig. 4 shows the effect of a protonophore (CCCP) upon oxidative phosphorylation in *V. alginolyticus*. The $K^+$- or $Na^+$-loaded cells were incubated in a high $Na^+$ medium containing different amounts of CCCP with or without monensin. It was found that ATP synthesis is resistant to very high (0.1 mM) concentration of CCCP if

TABLE II

ATP SYNTHESIS SUPPORTED BY LACTATE OXIDATION IN THE CELL OF *V. ALGINOLYTICUS* AT VARIOUS $[Na]_{in}/[Na]_{out}$ RATIOS

| Cells | Incubation mixture | ATP (nmol per mg protein) | | |
|---|---|---|---|---|
| | | without lactate | with lactate | ΔATP |
| Experiment 1 | | | | |
| $K^+$-loaded | 500 mM NaCl | 1.0 | 9.0 | 8.0 |
| | 3 mM NaCl, 500 mM KCl | 1.0 | 5.5 | 4.5 |
| $Na^+$-loaded | 500 mM NaCl | 1.5 | 6.5 | 5.0 |
| | 3 mM NaCl, 500 mM KCl | 1.5 | 1.5 | 0.0 |
| Experiment 2 | | | | |
| $K^+$-loaded | 3 mM NaCl, 500 mM LiCl | 1.0 | 13.5 | 12.5 |
| $Na^+$-loaded | 3 mM NaCl, 500 mM LiCl | 3.0 | 3.0 | 0.0 |

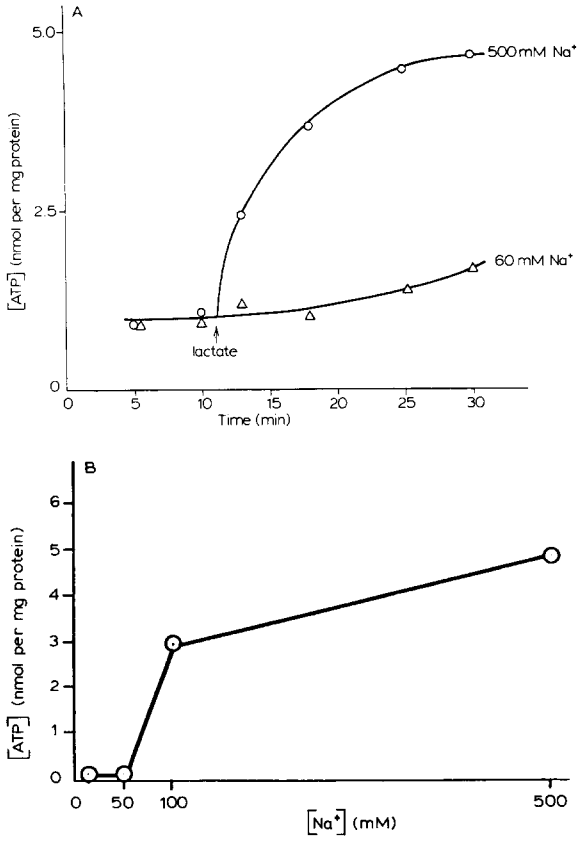


Fig. 2. Effect of $Na^+$ on oxidative ATP synthesis in $Na^+$-loaded *V. alginolyticus* cells. In the samples with 0.5 M NaCl, KCl was omitted. Other conditions as in Fig. 1.

there is high $\Delta p Na$ of proper direction ($K^+$-loaded cells without monensin). The monensin-induced $\Delta p Na$ collapsing (of small effect without CCCP) completely inhibited oxidative phosphorylation in the presence of CCCP. The $Na^+$-loaded cells incubated in the $Na^+$-medium ($\Delta p Na$ should be small, with $\Delta\bar{\mu}_{Na^+}$ being mainly in the form of $\Delta\psi$) respond to CCCP addition by a strong decrease in ATP synthesis apparently due to partial $\Delta\psi$ dissipation by the CCCP-mediated $H^+$ influx. Again, a combination of CCCP and monensin brought ATP synthesis down to the zero level. In Fig. 5, pH dependence of oxidative phosphorylation in the *V. alginolyticus* cells is given. It is seen that the process decreases when pH lowers, the effect being more pronounced in the $Na^+$-loaded cells than in $K^+$-loaded ones.

In the last series of experiments, the ATP

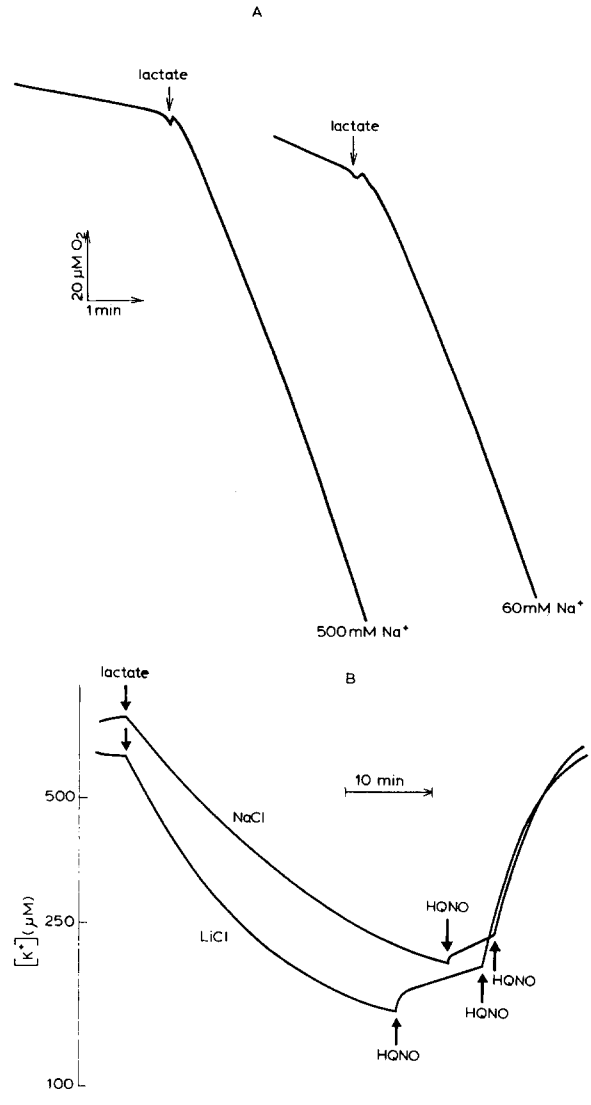


Fig. 3. Oxygen consumption (A) and $K^+$ uptake (B) induced by lactate addition to $Na^+$-loaded *V. alginolyticus* cells. (A) The same sample of the cells as in Fig. 2A was used. All the conditions as in Fig. 2A, except that a closed polarographic chamber was employed instead of open vessel used when ATP synthesis was measured. (B) Incubation mixture contained 0.5 M NaCl or LiCl, 25 mM Tricine-KOH, 5 mM $MgSO_4$ and $Na^+$-loaded cells. Additions: 50 mM D,L-lactate (lithium salt), 20 $\mu$M valinomycin and 0.1 mM HQNO.

synthesis supported by an artificially imposed $\Delta p Na$ was demonstrated. To this end, the $K^+$-loaded cells were incubated in the KCl or LiCl medium for 5 min. Then the mixture was diluted by the equal volume of 0.5 M NaCl medium so that the final $Na^+$ concentration proved to be 0.25

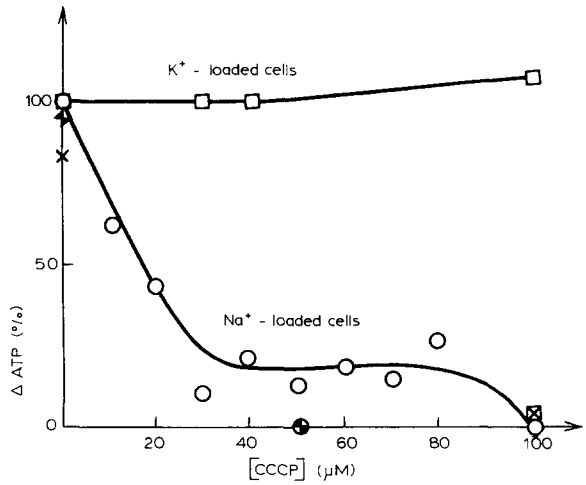


Fig. 4. Effects of CCCP and monensin on oxidative ATP synthesis in the *V. alginolyticus* cells. For conditions, see Fig. 1. ×, □, $K^+$-loaded cells incubated with or without $5 \cdot 10^{-5}$ M monensin, respectively. ●, ○, $Na^+$-loaded cells incubated with or without monensin, respectively.

M. As seen in Fig. 6, such a $Na^+$ pulse resulting in the formation of $\Delta p$*Na* of proper direction gives rise to a fast increase in the ATP level. In 1–2 min, the ATP concentration returns to that observed before the $Na^+$ pulse. Subsequent addition of lactate caused a steady increase in the ATP level.

An inhibitor analysis of the effects of the $Na^+$ pulse and lactate revealed the following.

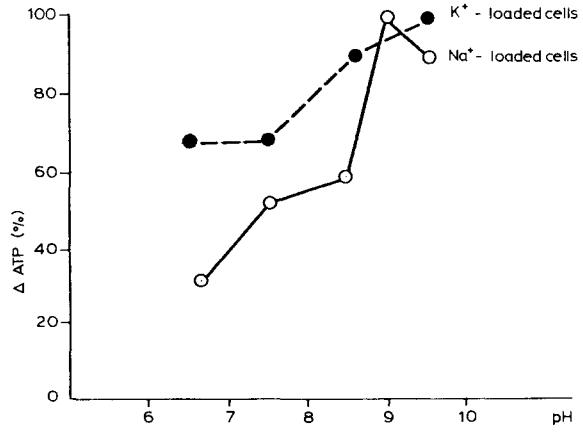


Fig. 5. pH dependence of oxidative ATP synthesis in *V. alginolyticus* cells. To obtain given pH, Mes, Tricine, Caps and Hepes buffers were used. The total buffer concentration was 25 mM. For other conditions, see Fig. 1.

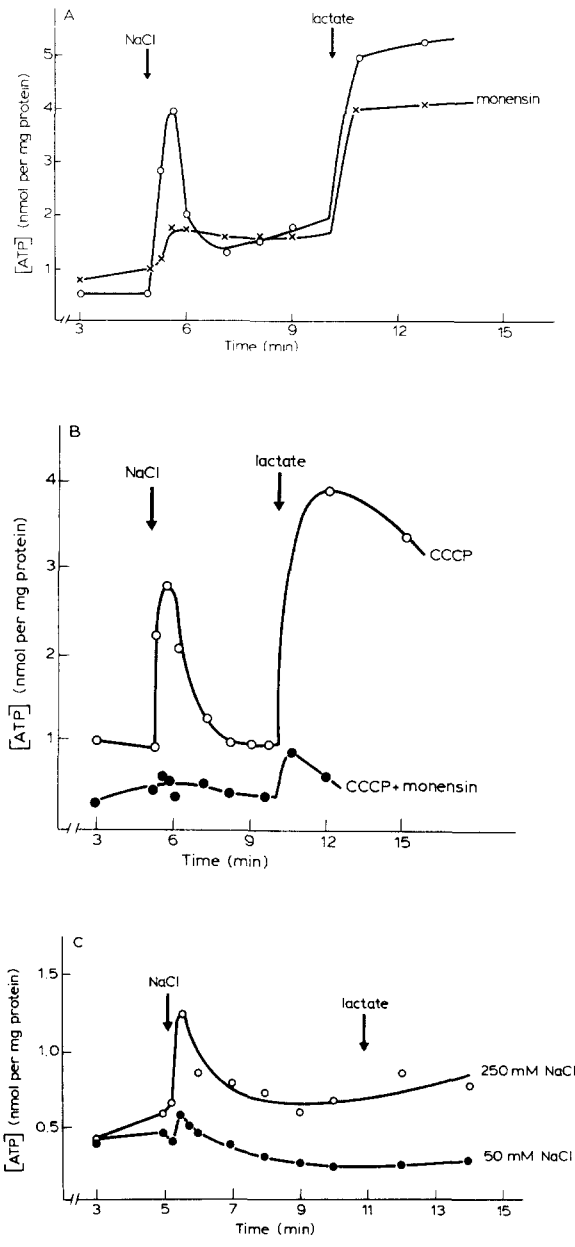


Fig. 6. The $Na^+$ pulse-driven ATP synthesis in the $K^+$-loaded *V. alginolyticus* cells. (A,B) Incubation mixture containing 0.4 M KCl/10 mM $MgCl_2$/25 mM Tris-HCl (pH 8.6) was 2-fold diluted at 5 min by solution of 0.5 M NaCl/5 mM $MgCl_2$/25 mM Tricine-KOH (pH 8.6). (C) Incubation mixture: 0.5 M LiCl/5 mM $MgSO_4$/25 mM Tricine-KOH (pH 8.6)/$3 \cdot 10^{-4}$ M HQNO. At 5 min, the mixture was 2-fold diluted by 5 mM $MgSO_4$, 25 mM Tricine-KOH (pH 8.6), $5 \cdot 10^{-6}$ M valinomycin and 0.5 M or 0.1 M NaCl (upper or lower curves, respectively). Where indicated, $5 \cdot 10^{-5}$ M monensin, $5 \cdot 10^{-5}$ M CCCP were added at zero time. D,L-lactate concentration was 75 mM.

(i) The effect of the $Na^+$ pulse, but not of lactate was abolished by monensin (Fig. 6A).

(ii) The effect of lactate but not of the $Na^+$ pulse was abolished by HQNO (Fig. 6C).

(iii) CCCP did not abolish either the $Na^+$ pulse- or lactate-induced [ATP] increases (Fig. 6B).

(iv) CCCP + monensin almost completely inhibited both the $Na^+$ pulse and lactate effects (Fig. 6B).

(v) Valinomycin does not prevent the action of the $Na^+$ pulse (Fig. 6C).

(vi) A decrease in the concentration of added $Na^+$ from 0.25 M down to 0.05 M almost completely prevented the $Na^+$ pulse-supported [ATP] rise (Fig. 6C).

## Discussion

The above-mentioned data indicate that *V. alginolyticus* possesses a system of the $Na^+$-coupled oxidative phosphorylation. The following observations are consistent with such a conclusion.

(i) The ATP synthesis coupled with lactate oxidation is abolished by the artificially imposed reverse $\Delta p Na$ ($[Na^+]_{in} > [Na^+]_{out}$) without any decrease in respiration rate and coupled $\Delta\psi$ generation.

(ii) It is resistant to 0.1 mM CCCP under conditions when high $\Delta p Na$ of the proper direction is present ($[Na^+]_{in} < [Na^+]_{out}$).

(iii) It is decreased by CCCP when $\Delta\bar{\mu}^+_{Na}$ is in the form of $\Delta\psi$ rather than $\Delta p Na$, i.e., when monensin is added, or when $[Na^+]_{in}$ is of the same order of magnitude as $[Na^+]_{out}$.

Alternative explanations of these facts could consist in the assumption that the transport of lactate into the *V. alginolyticus* cell or lactate oxidation rather than ATP synthesis is $\Delta\bar{\mu}_{Na^+}$-dependent. These explanations are at variance with the finding that the rates of oxidation of added lactate by the $Na^+$-loaded cells are similar in 60 mM and 500 mM NaCl although ATP synthesis occurred in the latter case only (Figs. 2A and 3). It was also found that $\Delta\psi$ level supported by lactate oxidation in the $Na^+$-loaded cells was the same or even higher in the low $Na^+$ medium as compared with the high $Na^+$ medium ($\Delta\psi$ was monitored with $K^+$ + valinomycin or with $TPP^+$).

The above-mentioned data cannot be explained by a suggestion that only a small portion of the cells is energized by lactate oxidation. As shown by separate experiments, the large part of bacteria became motile after lactate addition under condition of estimation of oxidative phosphorylation.

The inhibition of oxidative phosphorylation by reverse $\Delta p Na$ (low $[Na^+]_{out}$ versus high $[Na^+]_{in}$) cannot be explained by a direct unfavourable action of high $Na^+$ concentration in the cytoplasm, since the inhibition can be abolished by high $[Na^+]_{out}$. It is obvious that high $[Na^+]_{out}$ must prevent high $[Na^+]_{in}$ from being dissipated. It is likely that low $[Na^+]_{out}$, rather than high $[K^+]_{out}$ is required to inhibit ATP synthesis in the $Na^+$-loaded cells. As seen in Table II, the use of $Li^+$ instead of $K^+$ in the low $Na^+$ medium does not activate oxidative phosphorylation.

The data presented above cannot be accounted for by suggestion that the ATP synthesis is supported by $H^+$-coupled oxidative phosphorylation and the inhibitory action of the reverse $\Delta p Na$ is due to formation of the reverse $\Delta pH$ mediated by an endogenous $Na^+/H^+$-antiporter. At least two facts are at variance with such a speculation. (i) In the $Na^+$ medium, the ATP synthesis by the $K^+$-loaded cells in response to the addition of lactate cannot be decreased even by such a high concentration of CCCP as 0.1 mM. This seems to be impossible if an ordinary protonic energy coupling were operative. (ii) The same cells do not synthesis ATP at all when CCCP is added together with monensin which is almost without effect if CCCP is absent. The latter observation exclude the possibilities that the *V. alginolyticus* membrane per se is resistant to CCCP. It is also obvious that there is no active endogenous $Na^+/H^+$-antiporters in this bacterium, since, if it were the case, monensin would not be necessary. At the same time, the uncoupling effect of CCCP + monensin on $Na^+$-energetics may be easily explained in terms similar to that of valinomycin + nigericin on $H^+$ energetics.

The simplest explanation of all the above data consists in that $\Delta\bar{\mu}_{Na}$ formed by the $Na^+$-motive respiratory chain is utilized by $Na^+$-ATP synthase.

Suggestion that such an activity is really inherent in *V. alginolyticus* was directly proved by the $Na^+$-pulse experiments. As it is shown in Fig. 6, addition of NaCl to the $K^+$-loaded cells incubated

in a $Na^+$-free medium results in fast transient increase in the ATP level. Monensin, abolishing $\Delta pNa$, completely prevents this effect. In the same sample, monensin fails to abolish the lactate oxidation-supported ATP synthesis which apparently is $\Delta\psi$ -driven in the presence of this antibiotic. The situation proved to be opposite when the respiratory chain inhibitor HQNO was used. Now lactate-induced ATP synthesis, but not the $Na^+$ pulse-induced one, was specifically abolished. CCCP and valinomycin could not arrest either the $Na^+$ pulse or lactate-supported phosphorylations.

Apparently, the $\Delta pNa$ value produced by 0.25 M NaCl pulse was close to the threshold critical for operation of $Na^+$-ATP-synthase, since a 5-fold decrease in the concentration of added NaCl results in the almost complete disappearance of the pulse-induced ATP formation. Oxidative phosphorylation in the same $K^+$-loaded cell was still quite good in a medium containing 0.05 M NaCl.

As it was already mentioned in the Introduction, there are some indications that $Na^+$-ATPase exists in some bacteria. The fact that action of ion-translocating ATPases can be reversed by ion gradients was first established by Garrahan and Glynn in 1966 who studied reversal of $Na^+$, $K^+$-ATPase in erythrocytes [13]. This observation was later extended by Makinose and Hasselbach to $Ca^{2+}$-ATPase of sarcoplasmic reticulum [14,15]. Perhaps the same mechanism is responsible for ATP synthesis in cells of the halotolerant methanogenic bacterium *Methanococcus voltae* studied by Crider et al. [22]. The authors reported that ATP synthesis can be induced by an acid-to-base transition in the presence but not in the absence of a potent protophorous uncoupler, SF 6847. Valinomycin plus external $K^+$ eliminated phosphorylation. The ATP synthesis could also be shown when valinomycin was added to the $K^+$-loaded cells incubated in a $K^+$-free medium. In this case a protonophore was not necessary. It was concluded that the observed ATP synthesis was mediated by reversal of an ion-translocating ATPase not involving either $H^+$ or $K^+$, which was supported by $\Delta\psi$ (interior negative). Crider et al. suggested that the enzyme in question is $Na^+$-ATPase. Maybe it is responsible for the ATP synthesis coupled with formation of $CH_4$ from $H_2$ and $CO_2$, since this electron transfer-linked phosphorylation proved to resistant to the protonophore. In *M. voltae*, $Na^+$-ATPase probably co-exists with an $H^+$-ATPase so long as a base to acid transition is also competent in ATP formation, the process being uncoupler-sensitive.

$Na^+$ seems to be a good candidate as an ion involved in the methanogenesis-driven phosphorylation. In methanobacteria studied by Thauer and co-workers, $Na^+$ was shown to be necessary for methanogenesis [16] and ATP synthesis [23].

An indication of the reversal of $Na^+$-ATPase by the enzymatically produced $\Delta\bar{\mu}_{Na}$ was recently published by Dimroth and his colleagues [3] who investigated another anaerobic bacterium, *Propionigenum modestum* decarboxylating methylmalonyl CoA in a $Na^+$-motive fashion. The $Na^+$-ATPase proved to be sensitive to DCCD like $H^+$-ATPase and $Ca^{2+}$-ATPase of sarcoplasmic reticulum.

Summarizing our data and those obtained by Tokuda and Unemoto [2] we may conclude that (i) *V. alginolyticus* possesses a $Na^+$-motive respiratory chain [1,2] and (ii) $\Delta\bar{\mu}_{Na^+}$ produced by this chain can be utilized to perform all the three types of the membrane-linked work, i.e., chemical work (ATP synthesis – see this paper), mechanical work (rotation of flagellum by the $Na^+$ motor [1]) and osmotic work (uphill import of metabolites [2]).

So, we may now answer the question how *V. alginolyticus* survives at alkaline pH when $\Delta\bar{\mu}_H$ cannot be effectively used. The problem is solved by substituting $Na^+$ for $H^+$ as the coupling ion so that the $Na^+$ cycle instead of the $H^+$ cycle is employed. It is the $Na^+$ cycle that couples respiration to the work of various types.

In this context, it should be mentioned that the first indication that respiration and the three above mentioned types of work are somehow related to $Na^+$ was published in 1977 by Kodama and Taniguchi [17]. The authors showed that intact cells of *Pseudomonas stutzeri* require $Na^+$ ions for (i) high rate of respiration, (ii) incorporation of $H_3^{32}PO_4$ into nucleoside triphosphates, (iii) accumulation of various solutes and (iv) motility. Unfortunately, $Na^+$ transport was not studied so that it was not clear whether the above effects, in fact quite similar to those in *V. alginolyticus*, are associated with the sodium cycle or, alternately, with

some other functions of $Na^+$. To solve this problem, further studies on this bacterium are necessary.

The idea that $Na^+$ may, in principle, be employed as a coupling ion in oxidative phosphorylation was at first discussed in 1981 by Guffanti et al., who considered possible mechanisms of energy transduction in alkalophilic bacilli [18]. However, the authors concluded that this process is absent at least from these microorganisms [19], since (i) in subbacterial vesicles $Na^+$ addition is not required for oxidative phosphorylation which proved to be sensitive to $5 \cdot 10^{-7}$ M CCCP [20], and (ii) in intact bacteria 0.1 mM CCCP decreases the ATP level. The former effect, however, may be due to the loss of the $Na^+$-coupled process, but not of the $H^+$-coupled one, after decomposition of the bacterial cell provided that alkalophilic bacilli possess both $Na^+$ and $H^+$ energetics, the former being specifically employed at higher pH. Co-existence of $Na^+$ and $H^+$ cycles is also not excluded for *V. alginolyticus*. Such a possibility is now under study.

As to the ATP level decrease in intact cells of alkalophilic bacilli by very high CCCP concentration, it may be explained by a $\Delta\psi$ discharge and/or inhibition of respiration by an excess of uncoupler [21]. It was found by the Krulwich's group that an artificially imposed $\Delta\bar{\mu}_{H^+}$ does not support ATP synthesis in alkalophilic bacilli, whereas in the presence of $\Delta\bar{\mu}_{H^+}$ of the same size, but when the respiratory chain is operative, the ATP synthesis takes place [19]. Apparently there is an additional driving force formed by the respiratory enzymes. It may be $\Delta p Na$ if one assumes that the studied bacilli like *V. alginolyticus* possess $Na^+$-motive respiration and the $Na^+$-coupled oxidative phosphorylation system.

In conclusion, the data reported in this paper are sufficient to assume that for the first time, the $Na^+$-coupled oxidative phosphorylation has been demonstrated.

## References


1 Dibrov, P.A., Kostyrko, V.A., Lazarova, R.L., Skulachev, V.P. and Smirnova, I.A. (1986) Biochim. Biophys. Acta 850, 449–457
2 Tokuda, H. and Unemoto, T. (1982) J. Biol. Chem. 257, 10007–10014
3 Hilpert, W., Schink, B. and Dimroth, P. (1984) EMBO J. 3, 1665–1680
4 Heefner, D.L. and Harold, F.M. (1982) Proc. Natl. Acad. Sci USA 79, 2798–2802
5 Kinoshita, N., Unemoto, T. and Kobayashi, H. (1984) J. Bacteriol. 158, 844–848.
6 Benyoucef, M., Rigaud, J.-L. and Leblanc, G. (1982) Biochem. J. 208, 529–538
7 Benyoucef, M., Rigaud, J.-L. and Leblanc, G. (1982) Biochem. J. 208, 539–547
8 Del Castillo, J.R. and Robinson, J.W.L. (1985) Biochim. Biophys. Acta 812, 413–422
9 Chernyak, B.V., Dibrov, P.A., Glagolev, A.N., Sherman, M.Yu. and Skulachev, V.P. (1983) FEBS Lett. 164, 38–42
10 Skulachev, V.P. (1984) Trends Biochem. Sci. 9, 483–485
11 Skulachev, V.P. (1985) Biokhimiya 50, 179–183
12 Arshavsky, V.Yu., Baryshev, V.A., Brown, I.I., Glagolev, A.N. and Skulachev, V.P. (1981) FEBS Lett. 133, 22–26
13 Garrahan, P.J. and Glynn, I.M. (1966) Nature 211, 1414–1415
14 Makinose, M. (1971) FEBS Lett. 12, 269–270
15 Makinose, M. and Hasselbach, W. (1971) FEBS Lett. 12, 271–273
16 Perski, H.J., Schönheit, P. and Thauer, R.K. (1982) FEBS Lett. 143, 323–326
17 Kodama, T. and Taniguchi, S. (1977) J. Gen. Microbiol. 98, 503–510.
18 Guffanti, A.A., Bornstein, R.F. and Krulwich, T.A. (1981) Biochim. Biophys. Acta 635, 619–630
19 Krulwich, T.A. and Guffanti, A.A. (1984) Abstracts of the EBEC Meeting 3A, 5
20 Guffanti, A.A., Susman, P., Blanko, R. and Krulwich, T.A. (1978) J. Biol. Chem. 253, 708–715
21 Skulachev, V.P. (1969) Energy Accumulation in the Cell, Nauka, Moscow.
22 Crider, B.P., Carper, S.W. and Lancaster, J.R., Jr. (1985) Proc. Natl. Acad. Sci. USA 82, 6793–6796
23 Schönheit, P. and Perski, H.J. (1983) FEMS Lett. 20, 263–267