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Na^+-COUPLED ATP SYNTHESIS IN A MUTANT OF Vibrio parahaemolyticus LACKING H^+-TRANSLOCATING ATPase ACTIVITY

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SUMMARY: An H^+-translocating ATPase-defective mutant of Vibrio parahaemolyticus YS-1 grew well on lactate as a sole source of carbon at pH 8.5 under aerobic conditions, but not under anaerobic conditions. Both wild type cells and the mutant cells could grow on lactate at pH 8.5 even in the presence of an H^+ conductor, carbonylcyanide m-chlorophenylhydrazone (CCCP), but not at pH 7.5. Oxidative phosphorylation resistant to CCCP in the mutant occurred at pH 8.5. These findings suggest the existence of Na^+-coupled oxidative phosphorylation which is functional at alkaline pHs in V. parahaemolyticus. In fact, we observed ATP synthesis driven by an artificially imposed Na^+ gradient in YS-1 cells, which was resistant to CCCP.

Cells of Vibrio parahaemolyticus, as well as V. alginolyticus (1), possess a respiratory Na^+ pump which extrudes Na^+ from cells under alkaline conditions (2) in addition to the respiratory H^+ pump. An electrochemical potential of Na^+ established by this pump is utilized to drive flagella rotation (3), active transport of nutrients (2,4,5). Thus, the Na^+ circulation across the cytoplasmic membrane plays an important role in energy transduction in V. parahaemolyticus under alkaline conditions.

We reported previously that V. parahaemolyticus cells possess an H^+-translocating ATPase which synthesizes ATP by oxidative phosphorylation (6). On the other hand, we isolated an H^+-translocating ATPase-defective mutant YS-1 (6). The H^+-translocating (F_1F_0) ATPase activity and H^+ translocation elicited by ATP hydrolysis, which were observed in everted

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membrane vesicles prepared from wild type cells, were not detected in the membrane vesicles prepared from the mutant cells. Also, ATP synthesis driven by an artificially imposed H^+ gradient hardly occurred in the mutant cells, although large ATP synthesis was observed in the wild type cells under the same conditions. However, the mutant cells showed considerable oxidative phosphorylation which was resistant to CCCP (6).

These findings suggest that not only H^+-coupled ATP synthesis but also another mechanism of ATP synthesis (perhaps Na^+-coupled ATP synthesis) is involved in the oxidative phosphorylation in V. parahaemolyticus.

MATERIALS AND METHODS

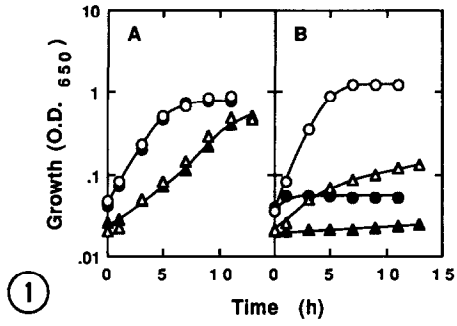
Bacterium and Growth V. parahaemolyticus AQ3334 (2) and an H^+-translocating ATPase-negative mutant YS-1 (6) were used. Cells were grown aerobically in medium S (7) (the buffer in this medium is either 100 mM Tris-H_2SO_4 (pH 8.5) or 100 mM Mops-KOH (pH 7.0)) supplemented with 40 mM lactate (K^+ salt) at 37 °C. To prevent flock-formation, 0.01 % Tween 80 was added to the medium. For Na^+ pulse experiments, cells were grown in a modified medium S in which 0.2 M NaCl was replaced with 0.4 M KCl plus 10 mM NaCl, and supplemented with 40 mM lactate (K^+ salt).

ATP Synthesis Cells of YS-1 were harvested at middle exponential phase of growth and washed with a buffer containing 50 mM Tricine-KOH (pH 8.5), 0.4 M KCl and 25 mM $MgSO_4$, and resuspended in the same buffer at about 3-6 mg protein/ml. The cells washed with this buffer had an intracellular ATP level lowered to about 0.1 mM, which was suitable for the measurement of ATP synthesis. The cell suspension was incubated under anaerobic conditions by bubbling with N_2 gas. The Na^+ gradient-driven ATP synthesis was carried out under anaerobic conditions. The cell suspension was diluted 2-fold with a similar buffer solution (bubbled with N_2 gas) containing 0.5 M NaCl instead of 0.4 M KCl to impose the Na^+ gradient. A similar buffer solution containing 0.5 M KCl was used as a control. Aliquots were taken at intervals, and ATP was extracted and determined as described previously (6).

Others Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Wild type AQ3334 cells and mutant YS-1 cells grew on lactate as a sole source of carbon under aerobic conditions (Fig. 1). Both types of cell grew on lactate even in the presence of an H^+ conductor, CCCP, at pH 8.5 but not at pH 7.0. Thus it seems that only H^+-coupled oxidative phosphorylation is operative at pH 7.0 and another mechanism of ATP synthesis which is operative at pH 8.5 is present in V. parahaemolyticus. Tokuda et al. have reported that cells of V. alginolyticus grew even in the



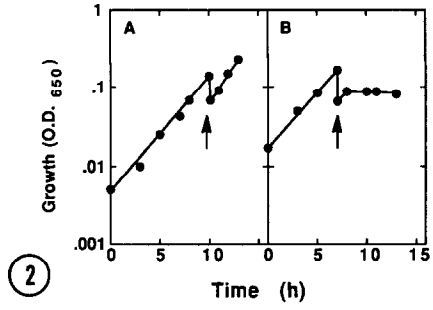


Fig. 1. Effects of CCCP and pH on growth of wild type cells and mutant (YS--1) cells on lactate. Wild type cells (circles) and the mutant cells (triangles) were shaken at 37 °C in the medium S supplemented with 40 mM lactate at pH 8.5 (A) or 7.0 (B) in the absence (open symbols) or presence (closed symbols) of 5 μM CCCP. Cell growth was monitored turbidimetrically at 650 nm.

Fig. 2. Effects of anaerobiosis on growth of YS-1. Cells of YS-1 were cultured under aerobic conditions at 37 °C in the medium S (pH 8.5) supplemented with 20 mM glucose (A) or 40 mM lactate (B). At the time point indicated by the arrows, an equal volume of each fresh medium was added, and stirred slowly at 37 °C under anaerobic conditions. Cell growth was monitored turbidimetrically at 650 nm.

presence of CCCP at alkaline pHs, although glucose was required for the growth (9). They suggested that ATP was synthesized by a substrate level phosphorylation under such conditions.

We tested the effect of anaerobiosis on the growth to clarify whether the growth on lactate was supported by ATP synthesized by oxidative phosphorylation or not (Fig. 2). The mutant YS-1 cells grew well on glucose and the growth was not affected by anaerobiosis. Thus ATP synthesis in the glycolytic pathway seems to be efficient enough to support normal growth of *V. parahaemolyticus*. On the other hand, growth of YS-1 on lactate stopped under anaerobic conditions. Similar results were obtained with wild type cells (data not shown). These results suggest that ATP is synthesized by oxidative phosphorylation when lactate is the sole source of carbon in the mutant YS-1, which lacks the H^+-translocating ATPase activity, and the ATP synthesis occurs more efficiently at an alkaline pH than at a neutral pH.

At pH 8.5, ATP synthesis resistant to CCCP occurred by oxidative phosphorylation in YS-1 cells (6). This CCCP-resistant ATP synthesis is

consistent with the CCCP-resistant growth of this organism on lactate at alkaline pHs.

We suggested previously that ATP synthesis is coupled to Na^+ under alkaline conditions in V. parahaemolyticus, and addition of Na^+ to the cell suspension slightly promoted ATP synthesis (6). We tried to improve the system. We cultured cells of YS-1 on lactate at an alkaline pH in a medium containing a high concentration of K^+ (400 mM) and a low concentration of Na^+ (10 mM). The concentration of intracellular Na^+ in such cells is expected to be low. Then by the addition of a high concentration of Na^+ to such cells, a large Na^+ gradient (inwardly directed) should be produced. ATP synthesis occurred rapidly and in large amounts when 0.25 M NaCl was added to such a cell suspension (Fig. 3). ATP synthesis was more rapid in the presence of CCCP than in its absence. This would be due to dissipation by CCCP of the back pressure established by the Na^+ influx (interior positive). In wild type cells, similar ATP synthesis was driven by an artificial Na^+ gradient, which was resistant to CCCP (data not shown).

Our findings support the idea of the existence of an Na^+-coupled ATP synthetase in cells of V. parahaemolyticus. We observed some growth of YS-1 cells on lactate at pH 7.0 and this growth was sensitive to CCCP (Fig.

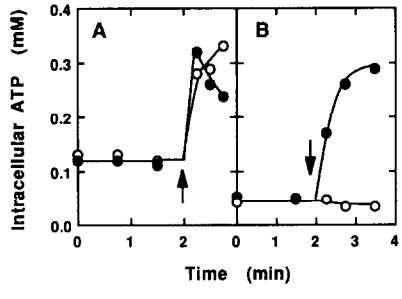


Fig. 3. ATP synthesis driven by an artificial Na^+ gradient in YS-1. Cells were incubated as described in the text. In A, an equal volume of a buffer containing high Na^+ (25 mM Tricine-KOH (pH 8.5), 10 mM $MgSO_4$, and 0.5 M NaCl) was added in the absence (○) or presence (●) of 5 μM CCCP at the time point indicated by the arrow. Final concentration of Na^+ was 0.25 M. In B, at the time point indicated by the arrow, an equal volume of a buffer containing high Na^+ (25 mM Tricine-KOH (pH 8.5), 10 mM $MgSO_4$, and 0.5 M NaCl) (●) or high K^+ (25 mM Tricine-KOH (pH 8.5), 10 mM $MgSO_4$, and 0.5 M KCl) (○) was added. Samples were taken at intervals, and ATP was measured.

1). Thus, it seems that an electrochemical potential of H^+ established by the respiratory chain is converted to that of Na^+ by the Na^+/H^+ antiporter, and the electrochemical potential of Na^+ would be the driving force for ATP synthesis under such conditions.

Recently, Dimroth et al. reported that an Na^+-translocating ATPase in Propionigenium modestum couples to H^+ instead of Na^+ when the Na^+ concentration is low (10). At present, we are not sure whether there are two different ATP synthetases (H^+-coupled one and Na^+-coupled one) in V. parahaemolyticus or there is only one ATP synthetase which utilizes two ions (H^+ and Na^+) similar to the ATPase of P. modestum. We can not exclude the possibility that YS-1 might be a mutant which possesses altered F_1F_0 and lost the ability to utilize H^+ as a coupling ion.

We could not observe ATP synthesis driven by an Na^+ gradient in V. parahaemolyticus AQ3334 cells grown on glucose under anaerobic conditions although large ATP synthesis driven by an Na^+ gradient has been reported for V. alginolyticus 138-2 cells grown on glucose under anaerobic conditions (11).

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