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A NOVEL MECHANISM FOR UTILIZATION OF EXTRACELLULAR AMP IN Vibrio parahaemolyticus

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SUMMARY: Vibrio parahaemolyticus could grow with AMP, ADP or ATP as the sole source of carbon. In the presence of Cl^-, a membrane-bound Cl^--dependent 5'-nucleotidase seemed to hydrolyze the nucleotides extracellularly, and then the cells took up the resulting adenosine. In the absence of Cl^-, although no significant dephosphorylation of the nucleotides occurred, the cells could still grow with AMP, but not with ADP or ATP. Moreover, in the presence of Cl^-, Zn^{2+} inhibited the 5'-nucleotidase, and inhibited growth of the cells with ADP or ATP, but not with AMP, as the carbon source. V. parahaemolyticus was unable to grow with adenine or ribose 5-phosphate. These results suggested that the cells might have an AMP transport system. In fact, Na^+ uptake was observed on addition of AMP to a cell suspension in the absence of Cl^-, indicating Na^+-AMP cotransport.

Vibrio parahaemolyticus, a slightly halophilic marine bacterium, can utilize ATP, ADP, AMP or adenosine as the sole carbon source. In bacteria, extracellular nucleotides are generally believed to be cleaved to nucleosides or bases outside the cytoplasmic membrane before uptake into the cells (1). Hayashi et al. (2) and Bengis-Garber and Kushner (3) reported the presence of membrane-bound 5'-nucleotidases that require Mg^{2+} and Cl^- for activity in V. alginolyticus and V. costicola, respectively. V. parahaemolyticus possesses a membrane-bound 5'-nucleotidase, and hydrolyzes externally added 5'-nucleotides in the presence of Mg^{2+} and Cl^- (manuscript in preparation). This organism also possesses a transport system for adenosine that is stimulated by Na^+ (manuscript in preparation). Thus it seemed reasonable to suppose that 5'-nucleotides are dephosphorylated by the 5'-nucleotidase outside the cells, and then the resulting adenosine is taken

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up via the adenosine transport system in this organism too. However, the results described in this paper suggest that AMP, but not ADP or ATP, could be utilized by the cells without its dephosphorylation. In addition, our data indicate the existence of Na^+-AMP cotransport in V. parahaemolyticus.

MATERIALS AND METHODS

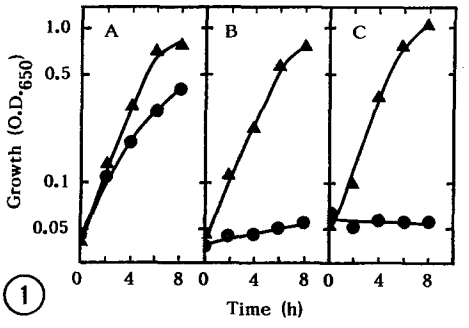
Bacterium and Growth V. parahaemolyticus AQ3334 was used. Cells were grown aerobically in medium S2 consisting of 50 mM Mops-Tris (pH 7.5), 0.2 M NaCl, 25 mM $MgSO_4$, 10 mM KCl, 1 mM $CaCl_2$, 0.01 mM $FeSO_4$, 0.33 mM K_2HPO_4 and 10 mM $(NH_4)_2SO_4$, supplemented with an appropriate carbon source at 37°C. When necessary, Cl^- salts were replaced by SO_4^{2-} salts. Growth was monitored turbidimetrically at 650 nm.

Assay of 5'-Nucleotidase Activity The standard assay mixture (0.6 ml) consisted of medium S2 (SO_4^{2-} salts), 4 mM AMP and about 4 μg protein of cells. Assay mixtures were incubated at 37°C for 15 min, and then the inorganic phosphate released was determined (4).

Assay of Na^+ Uptake Na^+ uptake was measured using a Na^+-electrode as described previously (5) with the minor modifications that the concentration of the buffer was 0.2 M and that of NaCl was 50 μM.

RESULTS AND DISCUSSION

Growth with AMP V. parahaemolyticus grew with AMP, ADP or ATP as the sole source of carbon in the presence of Cl^- (Fig. 1). In the absence of Cl^-, however, the cells were unable to grow with ADP or ATP. Since V. parahaemolyticus possesses membrane-bound Cl^--dependent 5'-nucleotidase that is oriented outward (manuscript in preparation) like the 5'-nucleotidase of V.



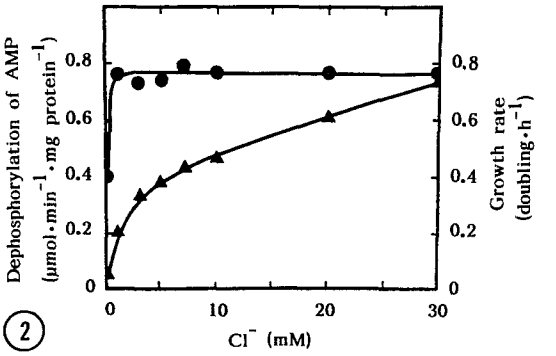
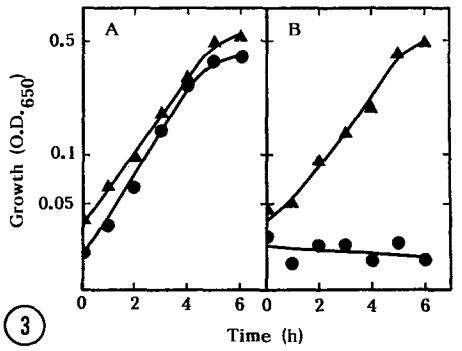


Fig. 1. Effect of Cl^- on the growth of V. parahaemolyticus with adenine nucleotides as the sole carbon source. Cells were shaken at 37°C in medium S2 containing either Cl^- salts (▲) or SO_4^{2-} salts (●). The carbon source was 20 mM AMP (A), ADP (B) or ATP (C).

Fig. 2. Effects of Cl^- concentration on dephosphorylation of AMP and on cell growth with AMP. For dephosphorylation assay, cells grown in medium S2 (SO_4^{2-} salts) supplemented with 20 mM AMP were used. Dephosphorylation of AMP (▲) and the growth rate of cells (●) with AMP (20 mM) were measured in medium S2 (SO_4^{2-} salts) containing various concentrations of Cl^-. The total concentration of NaCl plus Na_2SO_4 was 0.2 M.

costicola (1), these results are consistent with the view that the 5'-nucleotides in the culture medium were dephosphorylated by the Cl^--dependent 5'-nucleotidase outside the cells and the adenosine produced was taken up by the cells. Unexpectedly, however, we found that the cells could grow with AMP in the absence of Cl^-, although at slightly lower rate than in its presence (Fig. 1A). This finding suggested that the Cl^--dependent 5'-nucleotidase was not always essential for the utilization of external AMP. As shown in Fig. 2, dephosphorylation of AMP by the cells strongly depended on Cl^- present in the assay medium. In the absence of added Cl^-, little dephosphorylation of AMP was observed. On the other hand, added Cl^- was not essential for growth of cells with AMP as the sole carbon source, although it had some effect. Even with low concentrations of Cl^- (a few mM) at which little dephosphorylation of AMP occurred, the cells grew fairly well. These results indicate that there is no close correlation between dephosphorylation of AMP and cell growth with AMP. Thus it seemed very likely that in the absence of Cl^-, AMP was taken up without being dephosphorylated, whereas in the presence of Cl^-, AMP was dephosphorylated, and the adenosine produced (and perhaps residual AMP too) was utilized. Since addition of Cl^- increased the growth rate, utilization of adenosine was apparently more efficient than that of AMP. An additional possibility is that Cl^- is involved in AMP transport or metabolism in the cytoplasm.

As in animal cells (6), Zn^{2+} strongly inhibited the 5'-nucleotidase of V. parahaemolyticus (manuscript in preparation). Thus if the action of the 5'-nucleotidase is not necessary for the utilization of AMP, Zn^{2+} should not inhibit the growth of cells with AMP even in the presence of Cl^-. Consistent with this idea, Zn^{2+} (1 mM) did not significantly inhibit cell growth with AMP, although it strongly inhibited growth with ADP as the carbon source (Fig. 3). These results indicate that the action of the 5'-nucleotidase is necessary for the utilization of ADP, but not of AMP. Furthermore, Zn^{2+} (1 mM) did not seem to have any significant effect on other metabolic pathways of V. parahaemolyticus. It should be noted that Escherichia coli possesses a



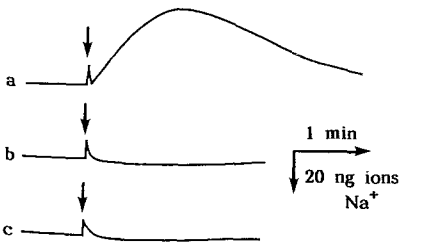


Fig. 3. Effect of Zn^{2+} on cell growth. Cells were grown in medium S2 (Cl^- salts) supplemented with 10 mM AMP (A) or ADP (B), in the absence (▲) or presence (●) of 1 mM $ZnCl_2$.

Fig. 4. Na^+ uptake caused by AMP. Cells were grown in medium S2 (SO_4^{2-} salts) supplemented with 20 mM AMP. Changes in extracellular Na^+ concentration were monitored with a Na^+-electrode under anaerobic conditions. At the times indicated by arrows, 40 μM AMP (a), ADP (b) or ATP (c) was added. An upward deflection represents uptake of Na^+.

system for the utilization of nucleosides by which nucleosides are cleaved to bases and ribose 1-phosphate, and the bases produced are taken up (7). So, it seemed possible that AMP was degraded to adenine and ribose 5-phosphate, and then taken up by the cells. However, V. parahaemolyticus could not grow with adenine or ribose 5-phosphate (data not shown). Therefore it seems very likely that AMP itself was taken up.

Na^+-AMP Cotransport V. parahaemolyticus possesses a Na^+-coupled cotransport system for adenosine (manuscript in preparation) and for serine (8). In halophilic bacteria, Na^+-substrate cotransport seems to be a major mechanism of transport of nutrients (9). Therefore, we tested whether there is a Na^+-AMP cotransport system in V. parahaemolyticus. One of the most convincing and convenient methods to test for the existence of a Na^+-substrate cotransport is, in our opinion, to measure Na^+ uptake caused by substrate influx using a Na^+-electrode (5). Fig. 4 shows that addition of AMP (0.112 μmole in 2.8 ml: 40 μM) to a cell suspension under anaerobic conditions caused uptake of Na^+, indicating the existence of a Na^+-AMP cotransport system. Addition of ADP or ATP did not cause Na^+ uptake. It should be noted that the AMP preparation used contained less than 0.1 % of contaminating adenosine, and we could not detect any Na^+ uptake on addition

of such a low concentration of adenosine (data not shown). Thus the possibility that the observed Na^+ uptake was due to contaminating adenosine could be excluded. Furthermore, hydrolysis of AMP in the assay mixture was less than 0.01 μmole/min under the present conditions, and the small amount of resulting adenosine did not cause detectable Na^+ uptake. No AMP-induced Na^+ uptake was observed when excess adenosine was present in the assay mixture (data not shown). Very recently, we isolated mutants with a defective Na^+-adenosine cotransport system. Since these mutants did not show Na^+ uptake when AMP was added, we conclude that AMP was taken up with Na^+ *via* the Na^+-adenosine cotransport system. We also observed Na^+ uptake caused by IMP and GMP (data not shown).

In microorganisms, membrane transport of nucleotides has been observed in intracellular parasites, which utilize nucleotides of the host cells (10-13). In bacteria, an ADP/ATP exchange system has been suggested to be present in the membrane of heavy chromatophores of *Rhodopseudomonas capsulata* (14) and in membrane vesicles of *Methanobacterium thermoautotrophicum* (15).

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