K⁺ transport in *Vibrio alginolyticus*: isolation of a mutant defective in an inducible K⁺ transport system

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When grown in a synthetic medium containing more than 3 mM K⁺, the marine bacterium *Vibrio alginolyticus* exhibited a K⁺ transport system with apparent K⁺ concentrations and maximum velocity (V_{max}) of 3.0 mM and 1.5 μmol min⁻¹ (mg cell protein)⁻¹, respectively. The growth rate of this organism in synthetic medium containing less than 0.2 mM K⁺ was dependent on K⁺ concentration and was half-saturated at about 50 μM K⁺. The cells grown at low concentrations of K⁺ induced another K⁺ transport system with Kₐ and Vₘₐₓ values of 0.3 mM and 0.6 μmol min⁻¹ (mg cell protein)⁻¹, respectively. The high-affinity system appeared when cells were grown at concentrations less than 2.0 mM K⁺ and was fully induced at 0.1 mM K⁺ and below. A mutant strain (FS181) unable to grow at 0.1 mM K⁺ was isolated and found to be defective in the inducible K⁺ transport system.

Keywords: *Vibrio alginolyticus*, potassium transport, inducible ion transport

INTRODUCTION

Potassium ions have two important cellular functions; the activation of a number of enzymes and the maintenance of cell osmolality (Walderhaug et al., 1987). Since the only way to supply K⁺ is by accumulation, K⁺ transport is indispensable for all living organisms. K⁺ transport by *Escherichia coli* has been well characterized (Epstein & Davies, 1970; Epstein & Kim, 1971; Dosch et al., 1991; Bakker, 1992a). *E. coli* has constitutive low-affinity and inducible high-affinity K⁺ transport systems. The inducible high-affinity K⁺ transport system, designated Kdp (Kₐ = 2 μM), comprises three proteins encoded by the kdpABC operon, and a Kdp-like system has been detected in many Gram-negative bacteria (Hesse et al., 1984; Walderhaug et al., 1989). The constitutive K⁺ transport systems, TrkD (KupD), TrkG, TrkH and TrkF, have also been recognized (Bossemeyer et al., 1989; Schlösser et al., 1991; Bakker, 1992b). Except for TrkF, the other systems are well characterized and belong to low-affinity K⁺ transport systems with apparent Kₐ values of 0.3–3 mM (Dosch et al., 1991). The existence of constitutive low-affinity and inducible high-affinity K⁺ transport systems have been reported in *Paracoccus denitrificans* (Erecinska et al., 1981), *Bacillus acidocaldarius* (Bakker et al., 1987) and *Rhodobacter sphaeroides* (Abee et al., 1992).

In contrast, K⁺ transport by marine bacteria has not been investigated in detail. Hassan & MacLeod (1975) reported an Na⁺-dependent K⁺ uptake system in *Alteromonas haloplanktis* and the importance of K⁺ uptake for the regulation of cytoplasmic pH in a marine *Vibrio alginolyticus* was demonstrated (Tokuda et al., 1981). *V. alginolyticus* has a respiration-driven Na⁺ pump (Tokuda & Unemoto, 1982), a K⁺/H⁺ antiporter that functions as a regulator of cytoplasmic pH (Nakamura et al., 1984, 1992) and an electrogenic Na⁺/H⁺ antiporter (Nakamura et al., 1992). Since K⁺ uptake has been shown to play a central role in the regulation of cellular K⁺, Na⁺ and H⁺, its properties were studied in this paper. In addition to a constitutive low-affinity K⁺ transport system, this organism was found to have an inducible K⁺ transport system, which does not resemble those reported in *E. coli* and other bacteria.

METHODS

Bacterial strain and growth media. *V. alginolyticus* strain 138-2 was grown aerobically at 37 °C in a synthetic medium composed of 0.3 M NaCl, 2 mM Na₂HPO₄, 15 mM (NH₄)₂SO₄, 10 μM FeSO₄, 5 mM MgSO₄, 1% (w/v) glycerol, 50 mM Tris/HCl (pH 7.5) and the desired concentration of KCl. In the absence of added KCl, the above medium contained about 5 μM K⁺ as a contaminant. To determine the K⁺-dependent growth at low K⁺ concentrations, precultured cells...
were washed twice with synthetic medium without K⁺ by centrifugation before inoculating a K⁺-limited fresh medium. Culture growth was monitored by measuring OD₆₀₀ in a Perkin-Elmer model 35 spectrophotometer and the growth rate was expressed as the growth rate constant defined as 0.693/doubling time in h.

**Preparation of K⁺-depleted cells.** Unless otherwise noted, K⁺-depleted cells were prepared by a Tris-EDTA treatment according to Stewart et al. (1985). The cells were harvested at the late exponential phase of growth, washed twice with, and suspended in 0.5 M Tris/HC1 (pH 8.0). The suspension was incubated for 1 min at 37 °C, and then sodium EDTA (pH 8.0) was added to a final concentration of 1 mM. After incubation for another 2 min at 37 °C, the cells were washed once with 0.5 M Tris/HC1 (pH 8.0) and twice with synthetic medium lacking K⁺ and glycerol. The cells were resuspended in the above synthetic medium at a hundredth volume of the growth medium. The cellular K⁺ concentration was reduced to less than 10 nmol (mg cell protein)⁻¹, which corresponded to 3 mM. Protein was determined by the method of Lowry with bovine serum albumin as a standard.

The replacement of cellular cations with Na⁺ (or Li⁺) was performed under iso-osmotic conditions as described previously (Nakamura et al., 1982). Briefly, harvested cells were treated twice with 0.4 M NaCl (or LiCl) containing 50 mM diethanolamine hydrochloride (pH 8.5) for 10 min at 25 °C. The K⁺-depleted and diethanolamine-loaded cells were treated twice with 0.4 M NaCl (or LiCl) containing 50 mM HEPES/NaOH (or HEPES/LiOH) at pH 7.0. By these treatments, the cellular K⁺ was reduced to less than 3 mM and the cells contained about 500 mM Na⁺ (or Li⁺).

The depletion of cellular K⁺ by hypotonic treatment (Mg²⁺-washed cells) was performed as described by Hassan & MacLeod (1975). Harvested cells were treated twice with 50 mM MgSO₄, and resuspended in the same medium. By these treatments, the cellular Na⁺ and K⁺ were reduced to 3 and 40 mM, respectively.

**Measurement of K⁺ uptake.** A filtration method (Nakamura et al., 1982) was used for the measurement of initial velocity and accumulation of K⁺ uptake. The reaction was carried out at 37 °C in synthetic medium containing different concentrations of K⁺ at a cell density of 0.8-1.0 mg cell protein ml⁻¹. K⁺ was determined by flame photometry using a Perkin-Elmer 403 atomic absorption spectrophotometer.

**Isolation of mutants unable to grow at low K⁺.** Cells of V. alginolyticus were treated with 10 μg NTG ml⁻¹ for 20 min at 37 °C in a medium containing 0.4 M NaCl, 44 mM maleic acid and 50 mM Tris (pH 6.0). NTG was removed by washing with the above buffer. About 70% of the cells were killed by this treatment. NTG-treated cells were cultured overnight at 30 °C with gentle shaking in a medium containing (all w/v) 0.1% yeast extract, 0.1% polypeptone, 1% glycerol and 50 mM Tris/HC1 (pH 6.0) supplemented with 0.1 M NaCl and 0.4 M KCl (medium A). The cells were washed with the above complex medium supplemented with 0.5 M NaCl (medium B), which contained about 0.7 mM K⁺. The cells were grown in medium B at 37 °C for 1 h, 100 μg aztreonam ml⁻¹, a penicillin analogue, was then added to the culture and incubated for 2 h. The cells were washed with, and suspended in medium A and were incubated at 37 °C for 6 h. The above aztreonam treatment was repeated. The cells were finally plated on medium A agar plates. A mutant, FS181, was selected by replica plating from colonies that were able to grow in synthetic medium containing 2.0 mM, but not 0.1 mM K⁺.

**RESULTS AND DISCUSSION**

**Effects of K⁺ on the growth and K⁺ uptake of V. alginolyticus**

When the marine V. alginolyticus was cultured in synthetic medium, more than 0.2 mM K⁺ was required for optimal growth (Fig. 1). At concentrations below 0.2 mM, the growth rate of this organism was dependent on external K⁺ concentration. The K⁺-dependent growth rate was not influenced much by the concentration of K⁺ in the preculture, and the growth rate was half-saturated at about 50 μM K⁺. Thus, compared with E. coli (Epstein & Davies, 1970), V. alginolyticus required relatively high concentrations of K⁺ for optimal growth. Furthermore, the K⁺-dependent growth observed here was very similar to that of a Kdp-deficient mutant (Frag 5) of E. coli (Epstein & Davies, 1970), suggesting the absence of an inducible Kdp-like high-affinity K⁺ transport system in this organism. The lag phase time required to grow at low K⁺ was also unaffected by the K⁺ concentration of the preculture (data not shown).

To examine possibilities of an induction of K⁺ transport system in the cells grown at low K⁺, uptake of K⁺ by this organism was compared with the cells precultured at 35 μM, 0.69 mM and 140 mM K⁺. When the uptake of K⁺ was assayed at 1.0 mM K⁺, the cells precultured at lower K⁺ exhibited a faster initial rate of K⁺ uptake and a higher extent of K⁺ accumulation (Fig. 2). However, when assayed at 10 mM K⁺, these cells accumulated K⁺ to nearly the same extent. Since K⁺ accumulation reached a steady state in 3 min, the effects of external K⁺ on the extent of K⁺ accumulation were examined with the cells precultured at 25 μM and 50 mM K⁺ (Fig. 3). The cells precultured at 25 μM K⁺ showed a high ability to accumulate K⁺ at low external K⁺. These results implied

**Fig. 1. Effects of K⁺ on the growth rate of V. alginolyticus.** Cells were precultured in synthetic medium containing 50 mM (■), 15 mM (○) or 20 μM (△) K⁺, and then grown in synthetic medium containing different concentrations of K⁺. The growth rate constant was calculated as described in Methods.
an induction of a high-affinity K\(^+\) transport system in the cells precultured at low K\(^+\).

Since the cells precultured at 0.69 mM K\(^+\) showed an intermediate rate of K\(^+\) uptake in the presence of 1.0 mM K\(^+\) (see Fig. 2), a partial induction of the high-affinity K\(^+\) transport system was assumed. From the results of Figs 2 and 3, the induction of the high-affinity system was reflected by the difference in the extent of K\(^+\) accumulation measured in the presence of 1.0 mM and 10 mM K\(^+\). Thus, the ratio of K\(^+\) accumulation at 10 mM K\(^+\) to that at 1.0 mM K\(^+\) was determined with the cells precultured at different K\(^+\) concentrations (Fig. 4). The high-affinity system appeared when grown at concentrations less than 2.0 mM K\(^+\), and it was fully induced at 0.1 mM K\(^+\) and below.

The initial rate of K\(^+\) uptake increased with the increase in external K\(^+\) and the activation curve conformed to Michaelis–Menten kinetics (Fig. 5). With cells precultured at 15 mM K\(^+\), the apparent K\(_m\) value for K\(^+\) and the maximum velocity (V\(_{\text{max}}\)) were calculated to be 3.0 mM and 1.5 \(\mu\text{mol min}^{-1} (\text{mg cell protein})^{-1}\), respectively. When Rb\(^+\) was used instead of K\(^+\), the apparent K\(_m\) value for Rb\(^+\) and V\(_{\text{max}}\) were estimated to be 10 mM and 0.43 \(\mu\text{mol min}^{-1} (\text{mg cell protein})^{-1}\), respectively. In contrast, with the cells precultured at 0.2 mM K\(^+\), the apparent K\(_m\) value for K\(^+\) and V\(_{\text{max}}\) were calculated to be 0.3 mM and 0.6 \(\mu\text{mol min}^{-1} (\text{mg cell protein})^{-1}\), respectively (Fig. 5). These cells could also take up Rb\(^+\) with an apparent K\(_m\) value of 2 mM and V\(_{\text{max}}\) of 0.35 \(\mu\text{mol min}^{-1} (\text{mg cell protein})^{-1}\). Cs\(^+\) uptake, however, was not observed with the cells precultured at any K\(^+\) concentration.

Thus, cells grown at high K\(^+\) exhibited a constitutive low-affinity K\(^+\) transport system, which was similar to the TrkH system of E. coli with respect to the K\(_m\) value (Dosch et al., 1991). When grown at low K\(^+\), a high-affinity system could be detected. Although the induced system showed a higher affinity for K\(^+\) than that of the constitutive one, its K\(_m\) value was still very large compared to an inducible Kdp-type high-affinity system with a K\(_m\) value of 2 \(\mu\text{M}\) reported in E. coli and other
bacteria (Erecinska et al., 1981; Hesse et al., 1984; Bakker et al., 1987; Abee et al., 1992). Moreover, the induced system actually took up Rb⁺ in contrast to the Kdp system of E. coli (Bossemeyer et al., 1989). These results implied that the induced system did not resemble to Kdp-type K⁺-translocating ATPase.

Properties of the K⁺ transport system

The marine bacterium, A. haloplanktis was reported to require Na⁺ for active uptake of K⁺ and the cotransport of K⁺ with Na⁺ was proposed (Hassan & MacLeod, 1975). In the Hassan & MacLeod (1975) experiments, K⁺-depleted cells were prepared by suspending the cells in 50 mM MgSO₄, and thus the cells were plasmolysed. Examine the effect of plasmolysis on K⁺ uptake, K⁺-depleted cells prepared by different methods were compared (Table 1). When K⁺-depleted cells were prepared under hypotonic conditions (Mg²⁺-washed cells), external Na⁺ was required for K⁺ uptake. The light scattering at 500 nm of the suspension of Mg²⁺-washed cells decreased by more than 20% from the original value during K⁺ uptake, indicating the occurrence of deplasmolysis by K⁺ accumulation. In contrast, K⁺-depleted cells prepared under iso-osmotic conditions actively took up K⁺ without significant changes in light scattering of the cell suspension and even in the absence of Na⁺ (the Li⁺-loaded cells in LiCl medium). Thus, Na⁺ was not obligatory for the active uptake of K⁺. Similar results were described in E. coli (Bakker et al., 1984). Extrusion of some cations is necessary for the accumulation of K⁺. Since the Li⁺-loaded cells in LiCl medium showed lower activity, Na⁺ was apparently better than Li⁺ as a counter cation for K⁺ uptake.

**Table 1. Comparison of the initial rate of K⁺ uptake by K⁺-depleted cells of V. alginolyticus prepared by different methods**

<table>
<thead>
<tr>
<th>K⁺-depleted cells</th>
<th>K⁺ uptake [μmol min⁻¹ (mg cell protein)⁻¹] in:</th>
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<tr>
<td></td>
<td>NaCl medium</td>
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<tr>
<td>Na⁺-loaded cells</td>
<td>0.82</td>
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<tr>
<td>Li⁺-loaded cells</td>
<td>0.81</td>
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<tr>
<td>Mg²⁺-washed cells</td>
<td>0.23</td>
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In most of our experiments, cellular K⁺ was depleted by the Tris-EDTA treatment described in Methods. Since this method was relatively simple and the K⁺-depleted cells always showed a high K⁺ transport activity, it was used throughout the experiments.

**Isolation of a mutant defective in the inducible K⁺ transport system**

NTG-induced mutants were enriched by using aztreonam as described in Methods. A mutant strain, FS181, was isolated which was unable to grow in the synthetic medium containing 0.1 mM K⁺ on an agar plate. The rate of reverse mutation of FS181 was estimated to be about 10⁻⁴-10⁻⁷, suggesting that FS181 was obtained by a single mutation.

The wild type and mutant strain FS181 were preincubated in synthetic medium containing 0.2 mM K⁺ and their abilities to accumulate K⁺ were compared (Fig. 6). Under those conditions, the wild type cells accumulated K⁺ at 0.75 mM, indicating the induction of the high-affinity K⁺ transport system (compare with Fig. 3). In contrast, the mutant strain FS181 was unable to accumulate K⁺ at 0.75 mM, implying that it was defective in the induction of the high-affinity K⁺ transport system. The apparent Km value and Vₘₐₓ of FS181 grown at 0.25 mM K⁺ were estimated to be 4 mM and 0.24 μmol min⁻¹ (mg cell protein)⁻¹, respectively. Strain FS181 apparently lost the high-affinity system, but the Vₘₐₓ of the constitutive K⁺ transport system was also reduced. Thus, growth at low K⁺ seems to either inhibit or repress the low-affinity system. Alternatively, since K⁺ transport systems are generally composed of multi-subunits in E. coli (Bakker, 1992b), a deficiency in one component may possibly affect another K⁺ transport system. The mutant isolated in this paper will help to further characterize these K⁺ transport systems.
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REFERENCES


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