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

Tatsunosuke Nakamura, Fumihiro Suzuki, Mizuhiro Abe, Yasuhiro Matsuba and Tsutomu Unemoto

When grown in a synthetic medium containing more than 3 mM K⁺, the marine bacterium *Vibrio alginolyticus* exhibited a K⁺ transport system with apparent $K_m$ 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 $K_m$ and $V_{max}$ 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 osmolarity (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 ($K_m = 2 \mu$M), 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 $K_m$ 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* (Abec 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 0.5 M Tris/HCl (pH 8.0), 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/HCl (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/HCl (pH 8.0) supplemented with 0.1 M NaCl and 0.04 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.](image-url)

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.
I+ transport in Vibrio alginolyticus

Fig. 2. Time courses of K+ uptake by cells precultured in synthetic medium containing 35 μM (○), 0.69 mM (△ or ■) or 140 mM (□) K+. Cellular K+ was depleted by Tris-EDTA treatment. K+ uptake was measured in synthetic medium containing 10 mM (closed symbols) or 10 mM (open symbols) K+

Fig. 3. Accumulation of K+ by cells precultured in synthetic medium containing 25 μM (△) or 50 mM (●) K+. Cellular K+ was depleted by Tris-EDTA treatment. The amounts of K+ accumulated in 3 min were determined at different K+ concentrations.

Fig. 4. Effects of K+ in the pre-culture on the induction of the high-affinity K+ transport system. The cells were precultured at different K+ concentrations, and then cellular K+ was depleted by Tris-EDTA treatment. The amounts of K+ accumulated in 3 min were measured in the presence of 1.0 mM and 10 mM K+, and the ratio (1 mM K+/10 mM K+) was plotted against the K+ concentration in the pre-culture.

Cellular K+ was depleted by Tris-EDTA treatment. K+ uptake was measured in synthetic medium containing 1.0 mM (closed symbols) or 10 mM (open symbols) K+

Fig. 5. Effects of K+ in the pre-culture on the induction of the high-affinity K+ transport system. The cells were precultured at different K+ concentrations, and then cellular K+ was depleted by Tris-EDTA treatment. The amounts of K+ accumulated in 3 min were 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_max) were calculated to be 3.0 mM and 1.5 μmol min⁻¹ (mg cell protein)⁻¹, respectively. When Rb+ was used instead of K+, the apparent K_m value for Rb+ and V_max were estimated to be 10 mM and 0.43 μmol min⁻¹ (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_max were calculated to be 0.3 mM and 0.6 μmol min⁻¹ (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_max of 0.35 μmol min⁻¹ (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 μM reported in E. coli and other

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.

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bacteria (Erecinska et al., 1981; Hesse et al., 1984; Bakker et al., 1987; Abee et al., 1992). Moreover, the induced system actively took up Rb\(^+\) in contrast to the Kdp system. 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 MgSO\(_4\), and thus the cells were plasmolysed. To 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 (Mg\(^{2+}\)-washed cells), external Na\(^+\) was required for K\(^+\) uptake. The light scattering at 500 nm of the suspension of Mg\(^{2+}\)-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 [(\mu)mol min(^{-1}) (mg cell protein)](^{-1})</th>
<th>NaCl medium</th>
<th>LiCl medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)-loaded cells</td>
<td>0.82</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Li(^+)-loaded cells</td>
<td>0.81</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Mg(^{2+})-washed cells</td>
<td>0.23</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

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 precultured 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 system. The apparent \(K_m\) value and \(V_{\text{max}}\) of FS181 grown at 0·25 mM K\(^+\) were estimated to be 4 mM and 0·24 \(\mu\)mol min\(^{-1}\) (mg cell protein\(^{-1}\)), respectively. Strain FS181 apparently lost the high-affinity system, but the \(V_{\text{max}}\) of the constitutive K\(^+\) translocating ATPase was still higher than that of the wild type strain.

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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