A Kdp-like, high-affinity, K⁺-translocating ATPase is expressed during growth of *Rhodobacter sphaeroides* in low potassium media

**Distribution of this K⁺-ATPase among purple non-sulphur phototrophic bacteria**

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Received April 8, 1992/Accepted May 25, 1992

**Abstract.** Cells of the purple non-sulphur bacterium *Rhodobacter sphaeroides* express a high-affinity K⁺ uptake system when grown in media with low K⁺ concentrations. Antibodies against the catalytic KdpB protein or the whole KdpABC complex of *Escherichia coli* cross-react with a 70.0 kDa *R. sphaeroides* protein that was expressed only in cells grown in media with low K⁺ concentrations. In membranes derived from *R. sphaeroides* cells grown with low K⁺ concentrations (induced cells), a high ATPase activity could be detected when assayed in Tris-HCl pH 8.0 containing 1 mM MgSO₄. This ATPase activity increased upon addition of 1 mM KCl from 166 to 289 μmol ATP hydrolysed × min⁻¹ × g protein⁻¹ (1.7-fold stimulation). The K⁺-stimulated ATPase activity was inhibited approximately 93% by 0.5 mM vanadate but hardly by N,N'-dicyclohexylcarbodiimide (DCCD). These results indicate that the inducible K⁺-ATPase in *R. sphaeroides* resembles the Kdp K⁺-translocating ATPase of *Escherichia coli*. This Kdp-like transport system is also expressed in *R. capsulatus* and *Rhodospirillum rubrum* during growth in media with low K⁺ concentrations suggesting a wide distribution of this transport system among phototrophic bacteria.

**Key words:** Potassium transport – High-affinity transport system – Kdp-like potassium ATPase – Expression – Immunological cross-reactivity – Internal pH regulation – Photosynthetic bacteria (*Rhodobacter sphaeroides, Rhodobacter capsulatus* and *Rhodospirillum rubrum*)

The purple nonsulfur bacterium *Rhodobacter sphaeroides* can obtain metabolic energy for growth by aerobic or anaerobic respiration and by anoxygenic photosynthesis (Abee et al. 1988b, Chory et al. 1984, Ferguson et al. 1987). At high external K⁺ the constitutive low affinity K⁺ transport system, described previously by Hellingwerf et al. (1982), plays a central role in the regulation of the intracellular pH (Abee et al. 1988a). Subsequently, several transport systems were found to be regulated by the internal pH. The initial rates of uptake of the non-metabolizable analogues of l-alanine (AIB) and of lactose (TMG), increased with the internal pH in *R. sphaeroides* strain 4P1 with pKₐ values of 7.8 for AIB and 7.2 for TMG (Abee et al. 1989b).

In addition to its role in pH homeostasis (Booth 1985), K⁺ plays an important role in the maintenance of cell turgor in prokaryotes (Epstein 1986; Walderhaug et al. 1987). In *Escherichia coli* the cytoplasmic K⁺ concentration can vary between 0.2 and 1.0 M and is carefully balanced by the action of several uptake and efflux systems (Bakker 1988). Above external K⁺ concentrations of approximately 0.2 mM, *E. coli* can accumulate this cation via three constitutive transport systems, Kup, TrkG and TrkH (Bakker 1990; Dosch et al. 1991). During growth of *E. coli* at low external K⁺, the cell turgor diminishes due to the K⁺ limitation in the medium. Under these conditions the organism synthesizes a high-affinity K⁺-ATPase (Kdp). This enzyme transports K⁺ with high affinity, which allows the cell to scavenge the medium for K⁺ to restore cell turgor (Laimins et al. 1981; Walderhaug et al. 1987). The kdpABC operon which has been cloned and sequenced encodes the three cytoplasmic membrane proteins KdpA, B and C with molecular weights of 59.189, 72.112 and 20.267, respectively, as derived from the DNA sequences (Hesse et al. 1984). The *E. coli* Kdp-ATPase has been purified in a functional form (Siebers and Altendorf 1988). The KdpB
subunit was shown to be the phosphorylated intermediate which indicates that the Kdp ATPase belongs to the class of P-type ATPases (Siebers and Altendorf 1989; Epstein et al. 1990).

Although K⁺ plays an important role in the overall metabolism in R. sphaeroides, as is also evident from the strong chemotactic response to K⁺ (Poole et al. 1990), nothing is known about the ability of R. sphaeroides and other phototrophic bacteria to grow at low external K⁺ concentrations. In this report it is shown that R. sphaeroides can grow in a minimal succinate medium at very low (µM) K⁺ concentrations. K⁺ transport studies revealed the presence of a second, high affinity, K⁺ uptake system. Subsequent immunological studies with antibodies against the catalytic KdpB protein or the whole KdpABC complex of E. coli showed cross-reactivity with a 70.0 kDa Rs sphaeroides protein that was expressed only in cells grown in media with low K⁺ concentrations. This Kdp-like transport system is also expressed in R. capsulatus and Rhodospirillum rubrum during growth in media with low K⁺ concentrations suggesting a wide distribution of this transport system among phototrophic bacteria.

**Methods**

*Rhodobacter sphaeroides* was grown aerobically in the dark in batch cultures (strains ML and NCIB8253) and in a K⁺-limited continuous culture (strain ML) at 30 °C in a mineral medium (pH 7.0) containing MgSO₄·7H₂O (0.5 g/l), NaCl (0.4 g/l), CaCl₂·2H₂O (0.02 g/l), KC1 (0.037 g/l) (K⁺ limitation), Vi Shniac trace elements (1 ml/l), sodium phosphate (Na-phosphate) pH 7.0 (200000 x g, 4 °C), resuspended in 2 ml 30 mM Na-phosphate pH 7 containing 5 mM EDTA, 0.5 mM PMSF and chloramphenicol (50 μg/ml) and stored in liquid nitrogen. *R. sphaeroides* membranes used for ATPase measurements were prepared by ribi-press treatment as described previously (Siebers et al. 1988). The membrane vesicle preparation obtained was suspended in 50 mM HEPES-Tris pH 7.5 and stored in liquid nitrogen.

The protein composition of the isolated membranes was analysed by SDS-PAGE as described (Laemmli 1970). Gels were stained with Coomassie-brilliant blue.

Proteins were transferred by electrophoresis from (SDS) polyacrylamide gels to nitrocellulose sheets as described (Towbin et al. 1979). The sheets were subsequently incubated with antibodies raised in rabbits against either the *Escherichia coli* KdpB subunit or against the intact KdpABC complex (Siebers and Altendorf 1988). Antigen-antibody reactions were visualized on the sheets by the activity of alkaline-phosphatase (Blake et al. 1984) coupled to mouse anti-rabbit IgG.

ATPase activity was measured as described (Arnold et al. 1976) at 37 °C in 50 mM Tris-HCl pH 8.0 containing 1 mM MgCl₂ and 1 mM KCl unless indicated otherwise. For studies with inhibitors the enzyme preparations were preincubated for 2 min with 0.5 mM vanadate or 15 min with 0.02 mM DCCD at 57 °C in a total volume of 99.9 ml before the reaction was started by the addition of 0.1 ml of a 100 mM Tris-ATP solution.

Protein was determined as described (Lowry et al. 1951) with BSA as a standard or by the bicinchoninic acid method (Smith et al. 1985).

**Results**

**Growth of Rhodobacter sphaeroides at low K⁺ concentrations**

*R. sphaeroides* can grow aerobically in the dark in a minimal medium containing salts, vitamins, trace elements and succinate as a carbon and/or energy source (Abee et al. 1989a). We investigated whether the organism could grow in this minimal medium with very low concentrations of K⁺. *R. sphaeroides* was grown in this medium containing 100 mM KCl or without added KCl (Fig. 1). The growth rate under both conditions was comparable (t₉₀ approximately 4.8h). However, growth with low K⁺ concentrations ceased rapidly at suboptimal cell density (OD₆₆₀nm 0.05) which was conceivably due to K⁺ limitation. Growth resumed after the addition of 50 μM KCl indicating that K⁺ was indeed the limiting substrate. A second addition of KCl (50 μM) was sufficient to reach approximately the same final optical density as the culture grown with high K⁺ concentrations (OD₆₆₀nm 0.8). Apparently, *R. sphaeroides* is very well able to grow at K⁺ concentrations as low as 0.1 mM.

**Kinetics of K⁺ transport in high and low K⁺ grown cells**

*R. sphaeroides* was grown in a continuous culture with high K⁺ concentrations and under K⁺-limiting conditions. K⁺ uptake experiments in these high and low K⁺ grown cells revealed that *R. sphaeroides* contains at least two different K⁺ transport systems (Fig. 2). A high capacity system (Vₘₐₓ approximately 1000 nmol/min - mg protein) with a low affinity for K⁺ (Kₘ, approximately