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# Potassium Transport System of *Rhodopseudomonas capsulata*

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*Rhodopseudomonas capsulata* required potassium (or rubidium or cesium as analogs of potassium) for growth. These cations were actively accumulated by the cells by a process following Michaelis-Menten saturation kinetics. The monovalent cation transport system had $K_m$'s of 0.2 mM $K^+$, 0.5 mM $Rb^+$, and 2.6 mM $Cs^+$. The rates of uptake of substrates by the potassium transport system varied with the age of the culture, although the affinity constant for the substrates remained constant. The maximal velocity of uptake of $K^+$ was lower in aerobically grown cells than in photosynthetically grown cells, although the $K_m$'s for $K^+$ and for $Rb^+$ were about the same.

The energy-dependent accumulation of potassium has been reported for a number of bacteria (3, 6, 9, 10, 22, 25, 28) and was recently reviewed by Harold and Altendorf (8). In all bacteria studied, the transport of potassium was linked to energy metabolism. Cells tend to extrude $Na^+$ from the cytoplasm, concomitant with $K^+$ accumulation (8, 25). Bacterial potassium transport systems are under genetic as well as physiological controls. For instance, in *Escherichia coli*, nine genes affecting three independent transport systems are involved in the control of potassium transport (4, 21).

I present evidence that *Rhodopseudomonas capsulata*, a photosynthetic bacterium, possesses a potassium transport system similar to those previously described for other bacteria. Many species of *Rhodospirillaceae* (the purple nonsulfur bacteria), including *R. capsulata*, are able to grow either by respiration in the dark or by photosynthesis (anaerobic in the light). Photosynthetically grown *R. capsulata* forms intracellular chlorophyll-containing membranes (chromatophores) whose synthesis is influenced by $O_2$ tension and light intensity (1, 17). In the only previous report of potassium accumulation by whole cells of *Rhodospirillaceae*, it was found that potassium was maintained at high levels by *Rhodospirillum rubrum* anaerobically in the light, and potassium was lost over several days under dark-anaerobic conditions (24). That study measured total cellular potassium, chiefly in stationary-phase cells. There also was a brief report of $K^+$ fluxes in isolated chromatophores (12). The work reported here defines the potassium transport system of *R. capsulata* and, in addition, clearly shows for the first time that $Rb^+$, a frequently used analog of $K^+$, and probably $Cs^+$ are accumulated by the system primarily responsible for $K^+$ uptake.

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## MATERIALS AND METHODS

**Bacterium and media.** *R. capsulata* strain Z-1 is an arsenate-resistant spontaneous mutant (29) of *R. capsulata* strain St. Louis and was obtained from Howard Gest.

The synthetic growth medium RCV-B contained 4 g of DL-malic acid, 1 g of $(NH_4)_2SO_4$, and 10 μg of thiamine hydrochloride per liter of 0.01 M K-$PO_4$ buffer, pH 6.8, and additional inorganic salts as specified by Ormerod et al. (19). For transport experiments, cells were centrifuged and suspended in mediumidentical to RCV-B but lacking the additional inorganic salts and containing 0.01 M Na-$PO_4$ buffer instead of K-$PO_4$ ($K^+$-free suspension medium). Modified growth media were also prepared which contained less potassium phosphate than RCV-B to vary the concentration of potassium in the growth medium. Sodium phosphate was added to such media to keep the total phosphate concentration at 0.01 M. When cells grown in RCV-B were used as inocula for cultures to be grown on low-potassium media or with rubidium or cesium replacing potassium, they were first washed and suspended in $K^+$-free suspension medium.

Cultures referred to as light grown were grown in screw-cap test tubes completely filled to obtain anaerobic conditions and illuminated with one GTE Sylvania 60-W Lumiline lamp at about 8 mW/cm². Dark-grown cultures were grown in flasks covered with aluminum foil and/or in a dark room and were aerated by vigorous shaking. All cultures were incubated at 30°C. Growth was followed as increase in turbidity as measured in a Klett-Summerson colorimeter with a no. 54 filter for dark-grown cells and a no. 66 filter for light-grown cells.

**Transport assays.** Five percent inocula of 24-h cells were used to start cultures for transport assays. Cultures were incubated at 30°C until into midloga-

rithmic phase. Portions were then centrifuged 10 min at 5,000 × *g* in a Sorvall GLC-1 centrifuge at room temperature and suspended in $K^+$-free suspension medium. Radioactive cations and other chemicals were then added. Alternatively, the pelleted cells were suspended in a small volume of medium and subsequently diluted into reaction vials containing additional medium, radioactive cations, and other chemicals.

The cells were incubated at 30°C in the light or dark; and, at various times after the addition of the radioisotope, 0.3- to 0.5-ml samples were rapidly filtered through 25-mm membrane HA filters (Millipore Corp.; pore size, 0.45 μm) and washed twice with 5 ml of the suspension medium. For incubation in the light during transport assays, four 60-W incandescent light bulbs giving a light intensity of 18 $mW/cm^2$ were placed on the outside of a glass aquarium used as a water bath. Incubation in the light was carried out in glass scintillation vials whose caps were fitted with oven injection ports (no. 5336730, Packard Instrument Co., Downers Grove, Ill.; now available as no. HGC-138, half-hole septums from Analabs, Inc., New Haven, Conn.), through which were passed needles for gassing with a 95% argon–5% carbon dioxide mixture and syringes for withdrawing samples. Incubation in the dark was carried out in a shaking water bath in scintillation vials covered with aluminum foil and with the passage of oxygen into the vials.

**Flame photometry.** To determine net cellular potassium, cells were harvested, washed once with water, and recentrifuged. The pellets were dried overnight at 100°C and hydrolyzed with a 2:1 (vol/vol) mixture of concentrated nitric and sulfuric acids with heating until the solutions cleared. After dilution into a LiCl internal standard solution, potassium content was measured in an IL model 143 flame photometer (Instrumentation Laboratory, Inc., Boston, Mass.). In some experiments, intact cells were diluted into the LiCl solutions, and potassium was measured on undisrupted cells. Identical values for cell potassium were obtained with and without prior acid hydrolysis.

**Radioisotopes.** $^{42}K$ was obtained from International Chemical and Nuclear Corp., Irvine, Calif., at high specific activity (2 to 5 Ci/g of K). $^{86}Rb$ (3 Ci/g) and carrier-free $^{137}Cs$ were purchased from New England Nuclear Corp., Boston, Mass. $^{42}K$ and $^{86}Rb$ were counted either on a low-background gas-flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.) or by Cerenkov radiation in water in a Packard model 3325 liquid scintillation spectrometer using the "$^3H$ preset" channel or a gain setting of 50%. $^{137}Cs$ was counted in a toluene-Triton X100-based scintillation fluid in the liquid scintillation spectrometer with a gain setting of 5% and a 50 to 1,000 window setting.

**Chemicals.** Dinitrophenol and *N,N'*-dicyclohexylcarbodiimide (DCCD) were purchased from Sigma Chemical Co., St. Louis, Mo. Sodium cyanide was purchased from Fisher Scientific Co., Fair Lawn, N.J., and *m*-chlorophenyl carbonylcyanide hydrazone was purchased from both Sigma Chemical Co. and Calbiochem, Los Angeles, Calif. Reagent grade compounds were used throughout.

**Kinetic analyses.** Kinetic constants were calculated and plotted using a Wang 720B computer equipped with a plotting output writer (Wang Laboratories, Inc., Tewksbury, Mass.).

## RESULTS

**Potassium requirement and content during growth.** Potassium is required for the growth of *R. capsulata*. The normal medium contained 15 mM $K^+$. Cells grew anaerobically in the light as rapidly in 100 μM $K^+$ as in high $K^+$ and reached the same ultimate cell yield (Fig. 1). With less $K^+$ (5, 20, or 50 μM), there was a lower cell yield. Dark-grown cells also showed a $K^+$-dependent lag phase before doubling at about the same rate with 5 to 100 μM $K^+$ as with 15 mM $K^+$. There was no significant difference in cell potassium (micromoles per milligram of cell mass) between the light- and dark-grown cells in high-$K^+$ medium (Fig. 2). The content of potassium in cells varied only slightly during the growth cycle; the cellular potassium content was highest at the end of logarithmic growth and decreased during stationary phase. Much more rapid loss of cellular potassium in stationary phase was reported for *Bacillus subtilis* (3) and *E. coli* (5), but *R. rubrum* maintained high intracellular $K^+$ for several days in stationary phase (24).

Rubidium could replace potassium during photosynthetic growth of cells, as could cesium (although less efficiently; Fig. 3). The optimum $Rb^+$ concentration for growth yield was 1.0 mM $Rb^+$; 10 mM $Rb^+$ was slightly less effective as a $K^+$ substitute. The length of the lag phase increased as the amount of $Rb^+$ present in the

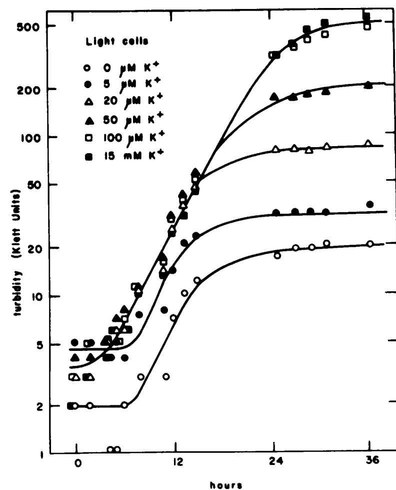


FIG. 1. *Growth of R. capsulata in media containing varying concentrations of potassium. Light-grown cells from cultures grown 14 h in RCV-B were washed and suspended in $K^+$-free medium. One percent inocula of these washed cells were added to media containing varying concentrations of potassium, and the cultures were incubated at 30°C under light anaerobic conditions. Growth was followed as an increase in turbidity.*

culture increased. Thus, the lag was shortest for the culture that contained 0.1 mM $Rb^+$, despite the fact that less growth was achieved by this culture than by the cultures that contained 1.0 or 10 mM $Rb^+$. $Cs^+$ at 1.0 mM supported significant growth, but 10 mM $Cs^+$ was growth inhibitory under $K^+$-starvation conditions. The cultures grown in $Rb^+$-containing medium (Fig. 3A) had the same growth rate as did cells grown in high-$K^+$ medium. However, cells grown in $Cs^+$-containing medium had lower growth rates at all tested concentrations. *R. capsulata* was grown with rubidium as a substitute for potassium for over 50 generations, indicating that these cells can be grown indefinitely on $Rb^+$. In medium containing 1.0 mM $Cs^+$ instead of $K^+$, *R. capsulata* was able to grow for at least 13 generations. After adaptation to $Rb^+$ or $Cs^+$ (5 generations of growth), *R. capsulata* had a lower growth rate than was observed during the first few generations (Fig. 3). Cells grown in medium containing 1.0 mM $Rb^+$ increased their doubling time to approximately 4.8 h, while those grown with 1.0 mM $Cs^+$ had a doubling time of 15.5 h. The doubling time of *R. capsulata* in 15 mM $K^+$ RCV-B medium under these phototrophic growth conditions was between 2.7 and 3 h.

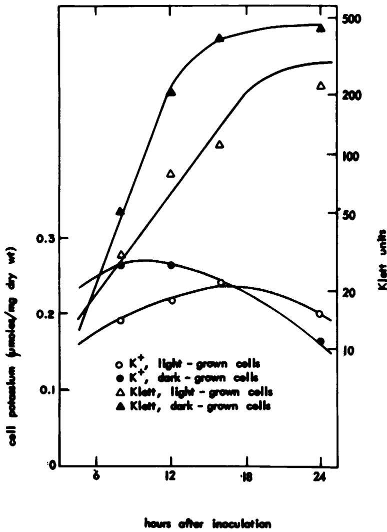


FIG. 2. *Cellular potassium content of R. capsulata during different stages of growth. Cultures were incubated either anaerobically in the light or aerobically in darkness at 30°C. Portions were removed for turbidity measurements, and 400 ml of cells were centrifuged, washed, and suspended in deionized water. The potassium content was determined by flame photometry.*

To demonstrate that rubidium was able to support growth rather than growth being due to contamination of the medium by potassium, the $K^+$ and $Rb^+$ contents of cells grown with 0.1, 1.0, or 10 mM $Rb^+$ were directly determined (Table 1). The intracellular concentration of $Rb^+$ ranged from 70 mM in cells grown with 0.1 mM $Rb^+$ to 116 mM in cells grown with 10 mM $Rb^+$. This represents a 12- to 700-fold concentration of $Rb^+$ from the medium. The intracellular $K^+$ concentration in the cells grown with 10 mM $Rb^+$ was only 0.4 mM, or less than 0.4% of that in cells grown in 15 mM $K^+$ medium. The $K^+$ content of cells grown with 1.0 or 0.1 mM $Rb^+$ was higher, but still 10% or less than the $K^+$ level in cells grown in RCV-B.

**Energy dependence of $K^+$ transport.** The

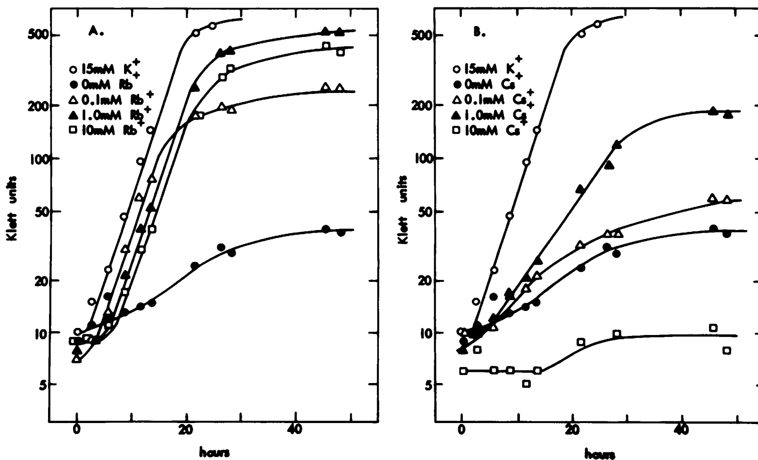


FIG. 3. *Growth of R. capsulata in media containing rubidium or cesium. Light-grown stationary-phase cells were washed and suspended in $K^+$-free medium. One percent inocula were added to media with varying concentrations of RbCl or CsCl and 0.01 M Na-$PO_4$. The cultures were incubated at 30°C under anaerobic conditions in the light.*

TABLE 1. *Ion content of cells grown with* $Rb^{+}$ [a]

| $Rb^{+}$ in medium (mM) | Intracellular $Rb^{+}$ [b] (mM) | Intracellular $K^{+}$ [c] (mM) |
|---|---|---|
| 10 | 116 | 0.4 |
| 1 | 88 | 6.4 |
| 0.1 | 70 | 11.0 |
| 0 (RCV-B) | | 110 |

[a] Cells grown on RCV-B (containing 15 mM $K^{+}$) were washed and suspended in $K^{+}$-free medium. A 1% inoculum of these washed cells was added to each of six tubes of rubidium-containing growth medium. Two tubes contained 10 mM RbCl, two contained 1.0 mM RbCl, and two contained 0.1 mM RbCl. One of the tubes at each concentration of $Rb^{+}$ contained $^{86}Rb^{+}$. Cells were grown photosynthetically to early stationary phase.

[b] Samples were taken from each $^{86}Rb^{+}$-containing culture, filtered through membrane filters (Millipore Corp.), washed, and counted. From the amount of $^{86}Rb^{+}$ taken up by the cells, the intracellular concentration of rudibium was calculated, using the value of 1.8 ml of cellular water per g of dry weight of cells.

[c] Portions of cells from nonradioactive cultures were washed once with $K^{+}$-free medium. The potassium content of the washed cells was measured by flame photometry. For comparison, the intracellular concentration of $K^{+}$ of cells grown with RCV-B medium (15 mM $K^{+}$) is shown.

accumulation of $K^{+}$ (and $Rb^{+}$) by *R. capsulata* was energy dependent and followed Michaelis-Menten saturation kinetics. Compounds such as *m*-chlorophenyl carbonylcyanide hydrazone and 2,4-dinitrophenol, which uncouple phosphorylation from both respiratory and photosynthetic electron transport (7, 11), completely inhibited transport of $K^{+}$ and $Rb^{+}$ by both anaerobically and aerobically growing cells (data not shown). DCCD, which inhibits bacterial $Mg^{2+}$-adenosine triphosphatases, inhibited uptake of $K^{+}$ and $Rb^{+}$ by cells grown either aerobically or anaerobically by more than 95% when added at a concentration of 100 $\mu$M (data not shown). This contrasts with the relative lack of effect of 100 $\mu$M DCCD on $Mn^{2+}$ uptake shown in the next paper (13). DCCD at 100 $\mu$M also causes the net loss of previously accumulated $K^{+}$ or $Rb^{+}$ by anaerobically grown cells (data not shown; not measured with aerobic cells).

Regardless of whether cells were grown aerobically or anaerobically, cyanide inhibited $Rb^{+}$ transport under aerobic assay conditions but not under anaerobic conditions, as expected (Fig. 4). When switched to aerobic, dark conditions, $Rb^{+}$ previously accumulated in the light in the presence of NaCN was released from the cells (Fig. 4B). Cells accumulating $^{86}Rb^{+}$ in the light in the absence of cyanide exhibited a small transient release of previously accumulated $Rb^{+}$ shortly after the change to aerobic, dark assay conditions; they then resumed accumulation of $Rb^{+}$ (Fig. 4B). Dark-grown cells in the presence of NaCN began to accumulate $Rb^{+}$ immediately upon transfer to the light (Fig. 4D).

**Kinetics of potassium transport.** Uptake of potassium by both photosynthetically and aerobically grown cells followed Michaelis-Menten saturation kinetics. The $K_m$ for potassium transport in log-phase light-grown cells was 200 $\mu$M, and the $V_{max}$ was 8 $\mu$mol/min per g of dry weight of cells (Fig. 5). The $K_m$ for potassium transport by log-phase dark-grown cells was similar (180 $\mu$M $K^{+}$), but the $V_{max}$ was considerably lower than that for light-grown cells and varied from experiment to experiment, depending upon the growth history of the cells. This change in rate during growth will be discussed below after consideration of the use of $^{86}Rb^{+}$ as a potassium analog.

Since $Rb^{+}$ can substitute for $K^{+}$ during growth of *R. capsulata* and has been frequently used as a convenient $K^{+}$ analog in transport studies in other bacteria (half-life for $^{86}Rb$ of 18.7 days versus 12.3 h for $^{42}K$), the kinetic parameters of $^{86}Rb^{+}$ transport were compared with those for $^{42}K^{+}$. Figure 5 shows a Lineweaver-Burk plot of the initial rates of $Rb^{+}$ uptake by light-grown cells. The $V_{max}$ was 5.9 $\mu$mol/min per g, just slightly lower than that for $K^{+}$ uptake by photosynthetically grown cells. The $K_m$ for $Rb^{+}$ was, however, more than twice that for $K^{+}$: 520 $\mu$M $Rb^{+}$.

Rubidium uptake was inhibited by $K^{+}$, but not by a number of divalent cations, including $Mn^{2+}$, $Mg^{2+}$, and $Ca^{2+}$ (data not shown). Similarly, $Rb^{+}$ and $Cs^{+}$ inhibited the uptake of $K^{+}$. From Lineweaver-Burk plots of the initial rates of $^{42}K^{+}$ uptake in the presence or absence of 2 mM $Rb^{+}$ or $Cs^{+}$, it was determined that both $Rb^{+}$ and $Cs^{+}$ were competitive inhibitors of $K^{+}$ uptake with inhibitor constants ($K_i$'s) estimated to be 0.56 mM $Rb^{+}$ and 2.7 mM $Cs^{+}$ (data not shown). An alternate method for determining $K_i$'s and demonstrating competitive inhibition is that of Dixon (2) shown in Fig. 6 for $Rb^{+}$ as an inhibitor of $K^{+}$ accumulation. $Rb^{+}$ is a competitive inhibitor with a $K_i$ of 0.46 mM $Rb^{+}$. Similar experiments gave a $K_i$ of 2.75 mM $Cs^{+}$. The $K_i$ for $Rb^{+}$ is quite similar to the half-maximal uptake constant ($K_m$) in the Lineweaver-Burk plot (Fig. 5). $Rb^{+}$ thus appears to be an alternate substrate for the system which accumulates $K^{+}$.

Initial rates of $^{86}Rb^{+}$ accumulation in the presence of $K^{+}$ or $Cs^{+}$ were also measured. Both $K^{+}$ and $Cs^{+}$ were competitive inhibitors of $Rb^{+}$ accumulation, with $K_i$'s of 0.2 mM $K^{+}$ and 2.6 mM $Cs^{+}$ (Fig. 7). The $K_i$ for $K^{+}$ as an inhibitor of $Rb^{+}$ accumulation is very similar to the $K_m$ for the accumulation of $K^{+}$, supporting the conclu-

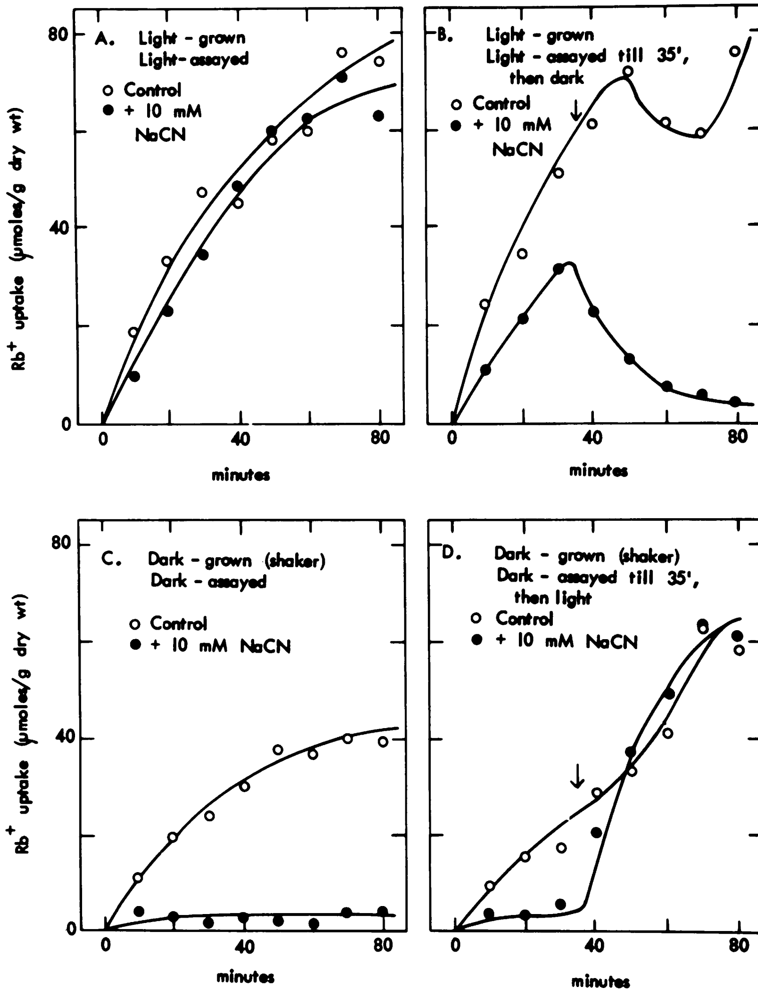


FIG. 4. *Dependence of NaCN-induced inhibition of rubidium transport on dark, aerobic assay conditions. Cells were grown anaerobically with light or aerobically in the dark, harvested by centrifugation, and suspended in $K^+$-free medium. Cells were added to medium containing 300 µM $^{86}Rb^+$; half the vials also contained 10 mM NaCN. Final density of cells was 160 to 180 µg/ml (light-grown cells, A and B) or 85 µg/ml (dark-grown cells, C and D). The cells were assayed under the same conditions as for growth until 35 min after addition of the cells; then two vials of dark-grown cells were changed to anaerobic, light conditions (D), and two vials of light-grown cells were changed to aerobic, dark conditions (B). All assays were performed at 30°C.*

sion that $Rb^+$ is accumulated by the transport system normally responsible for the accumulation of $K^+$. I also measured low-level uptake of $^{137}Cs^+$ (data not shown) and concluded that the *R. capsulata* potassium transport system has the capability of accumulating $Cs^+$. The system has the highest affinity (lowest $K_m$) for potassium and the lowest affinity (highest $K_m$) for cesium. The kinetic parameters of the *R. capsulata* potassium transport system are summarized in Table 2. Because of the low affinity of the $K^+$ transport system for $Cs^+$, the $Cs^+$ values in Table 2 are only estimates obtained by fitting the $^{137}Cs^+$ kinetic data to the $K_i$ (= $K_m$) determined with $Cs^+$ as a competitive inhibitor of $Rb^+$ and of $K^+$ uptake. Kinetic parameters of potassium transport for two other bacteria are shown for comparison.

The rate of accumulation of $Rb^+$ (hence, the rate of functioning of the potassium transport system in general) was not constant but varied with the age of the cells (Fig. 8). The highest rate of $^{86}Rb^+$ accumulation occurred in light-grown cells toward the end of logarithmic growth, and the highest rate for dark-grown cells occurred also during the latter part of logarith-

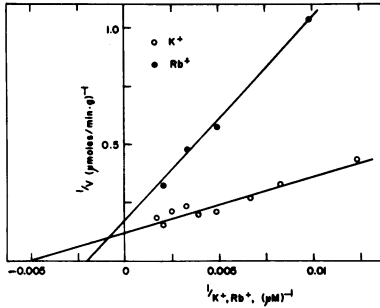


FIG. 5. *Kinetic parameters of potassium and rubidium transport anaerobically in the light. Cells grown photosynthetically were harvested, washed, and added to medium containing (○) 80 to 600 μM $K^+$ ($^{42}K^+$) or (●) 100 to 500 μM $Rb^+$ ($^{86}Rb^+$) at a final cell density of 170 to 200 μg/ml. Samples were filtered and washed, and the initial rates of accumulation at 30°C were calculated.*

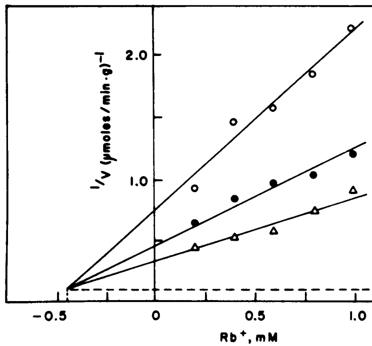


FIG. 6. *Competitive inhibition of potassium transport by rubidium. Cells grown photosynthetically were harvested, washed, and added to medium containing 40 (○), 75 (●), or 105 (△) μM $^{42}K^+$ and RbCl (0.2 to 1.0 mM $Rb^+$) at a final cell density of 170 to 200 μg/ml. Samples were filtered and washed at intervals at 30°C under anaerobic conditions in the light. The initial rates of uptake were calculated and plotted according to the method of Dixon (2).*

mic growth (Fig. 8). The "peak" rate of $^{86}Rb^+$ accumulation was almost three times higher for light-grown cells than for dark-grown cells. The kinetic parameters in Table 2 were all for cells in late logarithmic phase. The results in Fig. 8B indicate that the $K_m$ for $Rb^+$ transport remained the same during growth, but that the $V_{max}$ increased from 6.0 (6-h cells) to 17.2 μmol/min per g (16-h cells) as the cells approached stationary phase.

## DISCUSSION

Potassium is required for the growth of *R. capsulata*. Cells grown photosynthetically in low $K^+$ appeared to grow at a normal rate but stopped growing earlier than cells in $K^+$-rich medium (100 μM or greater $K^+$). Similar results have been reported for *E. coli* (26), which grew in medium containing limiting $K^+$ at the maximum rate until all the extracellular $K^+$ had been consumed. The rates of growth and of glucose and oxygen consumption then decreased abruptly in *E. coli*, and the cellular concentrations of potassium and phosphorus gradually declined (26).

$Rb^+$ and, less efficiently, $Cs^+$ can replace $K^+$ during growth of *R. capsulata*. It was previously reported that $Rb^+$, but not $Cs^+$ or $Li^+$, could satisfy the $K^+$ growth requirement of another nonsulfur photosynthetic bacterium, *Rhodopseudomonas spheroides* (23). The growth rate of *R. spheroides* with $Rb^+$ was the same as that with $K^+$ for three or four generations (23); this was also true of *R. capsulata* grown for a few generations with $Rb^+$. After longer periods of growth on $Rb^+$-containing medium, the doubling time was 50% longer than with $K^+$. For *E. coli*, in contrast, $Rb^+$ was a poor replacement for $K^+$. The molar growth yield on $Rb^+$ was only 5% of that on $K^+$ (20).

Measurements of $Rb^+$-grown cells showed that cells grown without added $K^+$ nevertheless contained significant amounts of intracellular $K^+$ (Table 1). Since the cells used for the measurements in Table 1 were only three to four generations removed from growth on 15 mM $K^+$, the $K^+$ found in the cells may have been conserved during growth of the cells. If all the $K^+$ contained by 15 mM $K^+$-grown cells were conserved during growth on $Rb^+$, one would find at the time of measurement (i.e., after 3.3 generations or a 10× increase in mass) that the cells would still contain 12 mM $K^+$. Thus cells grown in 0.1 mM $Rb^+$ probably retained most of the $K^+$ from the inoculum cells, whereas cells grown in 10 mM $Rb^+$ exchanged the $K^+$ for $Rb^+$. A concentration of 10 mM $Rb^+$ is well above the half-saturation constant for $Rb^+$ uptake ($K_m$ = 0.5 mM $Rb^+$), whereas 0.1 mM $Rb^+$ is well below the $K_m$ for $Rb^+$ transport. Cells in 0.1 mM $Rb^+$ would be able to "reaccumulate" exchanged $K^+$, whereas cells in 10 mM $Rb^+$ would not be able to do so.

The results of the kinetic studies indicate that the potassium transport system is a single system with three substrates: $K^+$, $Rb^+$, and $Cs^+$. The affinity of the system is greatest for $K^+$ and least for $Cs^+$. Studies were not performed with $Li^+$ or $Na^+$ to see if they were also substrates of the potassium transport system, but with other organisms, this is unlikely (8, 15, 16). The relatively low affinity and high $V_{max}$ of potassium transport by *R. capsulata* as compared with the transport of divalent cations by different systems (13) is expected, since $K^+$ is the most abundant

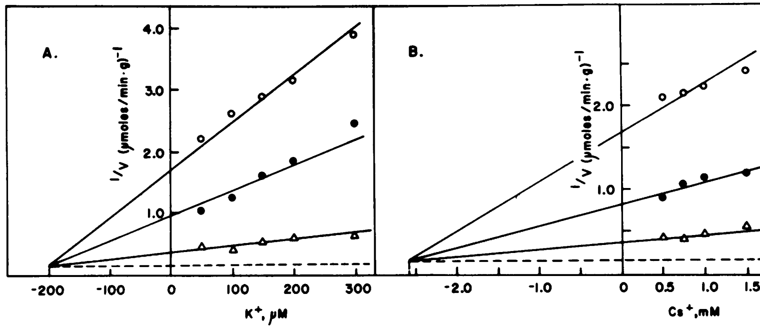


FIG. 7. *Competitive inhibition of rubidium transport by potassium and by cesium. Cells were grown anaerobically in the light, washed, and added to medium (A) containing 40 (○), 60 (●), or 160 (△) μM $^{86}Rb^+$ and KCl (0.05 to 0.3 mM $K^+$) or (B) containing 40 (○), 60 (●), or 150 (△) μM $^{86}Rb^+$ and CsCl (0.5 to 1.5 mM $Cs^+$) at a final cell density of 180 to 220 μg/ml. Samples were filtered and washed at intervals at 30°C under anaerobic conditions in the light.*

TABLE 2. *Kinetic parameters of bacterial potassium transport systems*[a]

| Bacterium | Cation transported | $K_m$ (mM) | $V_{max}$ (μmol/min per g) | Competitive inhibitors |
|---|---|---|---|---|
| *R. capsulata* | $K^+$ | 0.20 | 8.0 | $K_i$ = 0.56 mM $Rb^+$<br>$K_i$ = 2.8 mM $Cs^+$ |
| | $Rb^+$ | 0.52 | 5.9 | $K_i$ = 0.2 mM $K^+$<br>$K_i$ = 2.6 mM $Cs^+$ |
| | $Cs^+$ | 3 | 2 | |
| *B. subtilis* | $K^+$ | 0.20 | 26.0 | $Rb^+$, $Cs^+$ |
| *E. coli* *trkA* | $K^+$ | 1.5 | 550 | |
| *trkD* | | 0.50 | 40 | $Rb^+$ |
| *kdp* | | 0.002 | 150 | |

[a] Kinetic parameters for *R. capsulata* are for photosynthetically grown cells. The kinetic parameters of the transport of potassium by *E. coli* (4, 21) and *B. subtilis* (E. Eisenstadt, Ph.D. thesis, Washington University, St. Louis, Mo., 1971) are previously reported values.

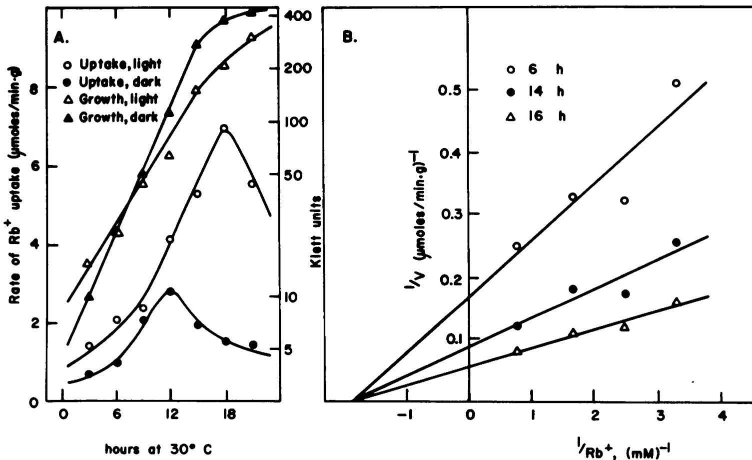


FIG. 8. *Rate of $Rb^+$ uptake during different stages of growth. Two percent inocula of light- or dark-grown cells were used to start cultures to grow in the light or dark, respectively. Portions of cells were removed periodically for turbidity measurements; additional portions were removed, and the cells were concentrated or diluted to 180 μg/ml in $K^+$-free medium. $^{86}Rb^+$ was added at 0.3 mM $Rb^+$ (A) or 0.3 to 1.3 mM $Rb^+$ (B), and samples were filtered and washed at intervals during light, anaerobic or dark, aerobic incubation (A) or in the light (B).*

cellular cation and is present at reasonable levels under most growth conditions.

Given the essentially constant level of cellular $K^+$ (Fig. 2) under high-$K^+$ growth conditions where the potassium transport system would be functioning at its maximum rate, the differences in rates shown in Fig. 8 will bring about changes in the turnover or exchange rate of intracellular for extracellular $K^+$. With the lowest measured $V_{max}$ of about 1 μmol/min per g for early log-phase dark-grown cells and an internal $K^+$ content of 250 μmol/g of cells (Fig. 2), then 250 min would be required for the accumulation of a cellular equivalent of $K^+$. This being about the doubling time for logarithmically growing cells, one might suggest that $K^+$ accumulation is at least close to being growth limiting with early cultures of aerobically growing cells. One can also expect that, once accumulated, cellular $K^+$ is retained and not turned over at this stage of growth. At the peak $K^+$ ($Rb^+$) transport rate for light-grown cells of 17.2 μmol/min per g of cells (Fig. 8) and with the comparable $K^+$ content of 200 μmol/g from Fig. 2, one can calculate that only 12 min is required to accumulate a cellular equivalent of potassium. Under those conditions of slow postlogarithmic increase in cell mass, intracellular potassium would be expected to exchange for extracellular potassium perhaps 50 times per generation. These are only estimates comparing rates of uptake of $Rb^+$ over a few minutes with $K^+$ concentrations during growth; however, these calculations demonstrate the changes in cellular metabolism of $K^+$ under usual laboratory growth conditions.

Active transport of monovalent cations can be coupled to energy either from photosynthetic electron transport or respiratory electron transport. With cells incubated aerobically in the dark (the respiratory electron transport pathway), cyanide blocks energy production and the transport of cations. Photosynthetic cells contain cyanide-sensitive terminal oxidases as well, but these oxidases are not utilized during photosynthetic electron transport (14, 27). Upon transfer from photosynthetic to aerobic conditions, cells immediately utilize the respiratory electron transport chain; electron transport is blocked immediately in the presence of cyanide, and energy for ion translocation is unavailable (Fig. 4D). Since cells grown in the dark with shaking in air produce small quantities of bacteriochlorophyll and components of the photosynthetic electron transport pathway, these cells are able to carry out photophosphorylation immediately upon transfer to anaerobic light conditions. They can therefore immediately begin anaerobic uptake of $K^+$ even in the presence of cyanide (Fig. 4B).

The results reported here are the first detailing the properties of a cation transport system in *Rhodospirillaceae*. Previous studies of cations in the *Rhodospirillaceae* dealt with total potassium content of cells over long periods of time (days) (24) and with the movement of $K^+$ across chromatophore membranes in response to antibiotics (12, 18). It is shown here that *R. capsulata* has an energy-dependent system for the accumulation of potassium and its analogs, and this system has been defined kinetically. This report is the first demonstration that $Rb^+$ and $Cs^+$ (commonly used $K^+$ analogs) are actually transported by a bacterial $K^+$ transport system. With regard to $Rb^+$ uptake, there appears to be a major difference between the $K^+$ transport systems of *R. capsulata* and *E. coli* (22).

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LITERATURE CITED

1. **Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier.** 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**:25–68.
2. **Dixon, M.** 1953. The determination of enzyme inhibitor constants. Biochem. J. **55**:170–171.
3. **Eisenstadt, E.** 1972. Potassium content during growth and sporulation in *Bacillus subtilis*. J. Bacteriol. **112**:264–267.
4. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
5. **Epstein, W., and S. G. Schultz.** 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. J. Gen. Physiol. **49**:221–234.
6. **Epstein, W., and S. G. Schultz.** 1966. Cation transport in *Escherichia coli*. VI. K exchange. J. Gen. Physiol. **49**:469–481.
7. **Geller, D. M.** 1962. Oxidative phosphorylation in extracts of *Rhodospirillum rubrum*. J. Biol. Chem. **237**:2947–2954.
8. **Harold, F. M., and K. Altendorf.** 1974. Cation transport in bacteria: $K^+$, $Na^+$, and $H^+$, p. 1–50. *In* F. Bronner and A. Kleinzeller (ed.), Current topics in membranes and transport, vol. 5. Academic Press Inc., New York.
9. **Harold, F. M., and J. R. Baarda.** 1967. Inhibition of potassium transport by sodium in a mutant of *Streptococcus faecalis*. Biochemistry **6**:3107–3110.
10. **Hassan, H. M., and R. A. MacLeod.** 1975. Kinetics of $Na^+$-dependent $K^+$ ion transport in a marine pseudomonad. J. Bacteriol. **121**:160–164.
11. **Heytler, P. G.** 1963. Uncoupling of oxidative phosphorylation by carbonyl cyanide phenylhydrazones. I. Some characteristics of *m*-Cl-CCP action on mitochondria and chloroplasts. Biochemistry **2**:357–361.
12. **Jackson, J. B., A. R. Crofts, and L.-V. von Stedingk.** 1968. Ion transport induced by light and antibiotics in chromatophores from *Rhodospirillum rubrum*. Eur. J. Biochem. **6**:41–54.
13. **Jasper, P., and S. Silver.** 1978. Divalent cation transport systems of *Rhodopseudomonas capsulata*. J. Bacteriol.

133:1323–1328.

14. **LaMonica, R. F., and B. L. Marrs.** 1976. The branched respiratory system of photosynthetically grown *Rhodopseudomonas capsulata*. Biochim. Biophys. Acta **423**:431–439.
15. **MacLeod, R. A., and E. Onofrey.** 1957. Nutrition and metabolism of marine bacteria. III. The relation of sodium and potassium to growth. J. Cell. Comp. Physiol. **50**:389–401.
16. **MacLeod, R. A., and E. E. Snell.** 1948. The effect of related ions on the potassium requirement of lactic acid bacteria. J. Biol. Chem. **176**:39–52.
17. **Marrs, B., and H. Gest.** 1973. Regulation of bacteriochlorophyll synthesis by oxygen in respiratory mutants of *Rhodopseudomonas capsulata*. J. Bacteriol. **114**: 1052–1057.
18. **Nishimura, M., and B. C. Pressman.** 1969. Effects of ionophorous antibiotics on the light-induced internal and external hydrogen ion changes and phosphorylation in bacterial chromatophores. Biochemistry **8**: 1360–1370.
19. **Ormerod, J. G., K. S. Ormerod, and H. Gest.** 1961. Light-dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria; relationships with nitrogen metabolism. Arch. Biochem. Biophys. **94**:449–463.
20. **Rhoads, D. B., and W. Epstein.** 1977. Energy coupling to net $K^+$ transport in *Escherichia coli* K-12. J. Biol. Chem. **252**:1394–1401.
21. **Rhoads, D. B., F. B. Waters, and W. Epstein.** 1976. Cation transport in *Escherichia coli*. VIII. Potassium transport mutants. J. Gen. Physiol. **67**:325–341.
22. **Rhoads, D. B., A. Woo, and W. Epstein.** 1977. Discrimination between $Rb^+$ and $K^+$ by *Escherichia coli*. Biochim. Biophys. Acta **469**:45–51.
23. **Sistrom, W. R.** 1960. A requirement for sodium in the growth of *Rhodopseudomonas spheroides*. J. Gen. Microbiol. **22**:778–785.
24. **Stenn, K. S.** 1968. Cation transport in a photosynthetic bacterium. J. Bacteriol. **96**:862–864.
25. **Thompson, J., and R. A. MacLeod.** 1973. $Na^+$ and $K^+$ gradients and $\alpha$-aminoisobutyric acid transport in a marine pseudomonad. J. Biol. Chem. **248**:7106–7111.
26. **Weiden, P. L., W. Epstein, and S. G. Schultz.** 1967. Cation transport in *Escherichia coli*. VII. Potassium requirement for phosphate uptake. J. Gen. Physiol. **50**:1641–1661.
27. **Zannoni, D., A. Baccarini-Melandri, B. A. Melandri, E. H. Evans, R. C. Prince, and A. R. Crofts.** 1974. Energy transduction in photosynthetic bacteria. The nature of cytochrome *c* oxidase in the respiratory chain of *Rhodopseudomonas capsulata*. FEBS Lett. **48**:152–155.
28. **Zarlengo, M. H., and S. G. Schultz.** 1966. Cation transport and metabolism in *Streptococcus fecalis*. Biochim. Biophys. Acta **126**:308-320.
29. **Zilinsky, J. W., G. A. Sojka, and H. Gest.** 1971. Energy charge regulation in photosynthetic bacteria. Biochem. Biophys. Res. Commun. **42**:955–961.