Kinetic Studies on Cesium Transport in *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. Strain CS402

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Uptake of cesium, potassium, and rubidium by *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402 followed Michaelis–Menten saturation kinetics. The $K_s$'s for uptake of these monovalent cations by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were 136 and 436 µM for Cs$^+$, 65 and 101 µM for K$^+$, and 102 and 113 µM for Rb$^+$, respectively. These values were significantly lower than those of *Rhodobacter capsulatus* and the Kup system in *Escherichia coli*. Potassium was a competitive inhibitor of cesium uptake by these strains, suggesting that cesium was accumulated by the potassium transport system. Although an uncoupler, FCCP, inhibited the cesium transport system, this system was not repressed by high concentrations of potassium in both *Rhodococcus* strains. However, the specificity in both *Rhodococcus* strains was different from the Trk system. These results suggest that the potassium transport system which can transport cesium in both *Rhodococcus* strains may be novel.

A large amount of radioactive cesium was released during the nuclear reactor accident at Chernobyl.1,2 Radioactive cesium was accumulated by freshwater plants,3 green algae,4,5 mushrooms,6,7 and marine fishes.8 However, the cesium accumulation mechanism is poorly understood.

Previously, in our laboratory, two strains of cesium-accumulating bacteria were isolated from soil and identified as *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402, respectively.9 We also analyzed the growth and cesium accumulation characteristics of these *Rhodococcus* strains and found that the cesium accumulation was inhibited by potassium and rubidium.9 Several researchers also reported similar inhibition phenomena. Avery et al. observed that cesium accumulation by *Synechocystis* PCC 680310) was inhibited by external potassium and rubidium. Plato and Denovil3) reported that the concentration factors for $^{137}$Cs by *Chlorella pyrenoidosa* decreased as the external potassium ion concentration increased. From these results, it was suggested that cesium might be accumulated via potassium transport systems. Concerning the potassium transport systems, there have been several reports.11–14 Bossemeyer et al.13) reported that *Escherichia coli* containing a potassium transport system encoded by the trkD gene could accumulate much cesium.

In this report, we studied the uptake kinetics of cesium and other monovalent cations by cesium-accumulating bacteria and discussed cesium transport mechanisms.

Materials and Methods

Microorganisms and growth conditions. All experiments were done with *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. CS402, both previously isolated as cesium-accumulating bacteria.9 These strains were cultured in BS medium containing various potassium concentrations. The composition of BS medium was as follows (all units in mg/liter): CH$_3$COONa, 2,000; MgSO$_4$, 7H$_2$O, 200; Na$_2$HPO$_4$, 100; FeSO$_4$, 7H$_2$O, 10; CaCl$_2$, 2H$_2$O, 10; MnSO$_4$, 4·5H$_2$O, 0.6; Co(NO$_3$)$_2$, 6H$_2$O, 0.6; ZnSO$_4$, 7H$_2$O, 0.1; CuSO$_4$, 5H$_2$O, 0.06; NiSO$_4$, 7H$_2$O, 0.06; H$_2$BO$_3$, 0.05; H$_2$SeO$_4$, 0.04; NaMoO$_4$, 2H$_2$O, 0.01; and thiamin 0.2. Cells equivalent to 2–3 mg dry weight were inoculated into 100 ml of BS medium and incubated at 30°C with shaking. Cell growth was measured by optical density at 550 nm.

Transport assays. To measure the kinetic parameters for cation uptake and observe the effects of the uncoupler carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), both *Rhodococcus* strains were cultured in 100 ml of BS medium containing 10 µM K$^+$ and harvested. The cells were washed 2–4 times and resuspended at 0.2 mg dry cells/ml in buffer solution and incubated for 10 min at 25°C. The buffer solution contained 50 mM Na$^+$, pH 8.5, 5 mM CH$_3$COONa, and 0.1 mM MgSO$_4$. After the first incubation, cells were added and the mixture incubated at 25°C for 10 min. During this period, the uptake of cesium, potassium, and rubidium proportionally increased in both *Rhodococcus* strains. The supernatant was obtained by centrifugation (16,000 × g, 2 min) and the cation concentration in the supernatant was measured with a Shimadzu AA640-12 atomic absorption spectrophotometer (Shimadzu Co., Kyoto, Japan). To observe the effects of the FCCP, cation uptake was measured after incubation for 30 min at 25°C with an inhibitor. To observe the effects of culture conditions on cesium uptake, cells were cultured in 100 ml of medium with various K$^+$ concentrations.

Chemicals. FCCP was purchased from Sigma Chemical Co., St. Louis, Mo. Other reagents were of analytical grade.

Results

Kinetics of cesium, potassium, and rubidium uptake

The relationship between uptake rate of cesium, potassium, and rubidium, and their concentrations in *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 fitted the Michaelis–Menten saturation kinetics model, and their Lineweaver-Burk plots are shown in Figs. 1 and 2. The $K_s$'s for cesium, potassium, and rubidium transport by *R. erythropolis* CS98 were 136, 65, and 102 µM, and the $V_{max}$ were 16, 26, and 17 µmoles/min/g dry cells, respectively (Fig. 1). The $K_s$'s for cesium, potassium, and rubidium

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Abbreviations: FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; TAPS, N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid; $K_s$, inhibition constant.
transport by *Rhodococcus* sp. strain CS402 were 434, 101, and 113 μM, and the \( V_{\text{max}} \) were 25, 30, and 34 μmoles/min/g dry cells, respectively (Fig. 2). These kinetic parameters and those by *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) and *E. coli* are summarized in Table I. The \( K_m \) for cesium uptake by *Rhodococcus* sp. strains CS402 was 3 times higher than that by *R. erythropolis* CS98, suggesting that the cesium uptake system of *R. erythropolis* CS98 has a higher affinity than that of *Rhodococcus* sp. strains CS402. For both strains, the \( K_m \) for cesium uptake was highest, that for rubidium was intermediate, and that for potassium was lowest. The \( K_m \)'s for cesium were 2 and 4 times larger than those for potassium in *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402, respectively. The strains have almost same the \( V_{\text{max}} \) for those three cations.

**Competitive inhibition of cesium uptake**

A Dixon plot\(^{13}\) showed that potassium was a competitive inhibitor of cesium uptake by both strains (Figs. 3 and 4). The \( K_i \) for potassium as an inhibitor of cesium uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were 43 and 50 μM, respectively. Those \( K_i \)'s were smaller than the \( K_m \)'s for the accumulation of potassium (65 and 101 μM) by the two strains.

**Effects of FCCP on cation uptake activity**

The effects of an uncoupler (FCCP) on cation uptake were studied (Table II). DMSO (dimethyl sulfoxide), used as a solvent for FCCP, did not affect cation uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402. FCCP inhibited the uptake of three cations by both strains. Especially, *Rhodococcus* sp. strain CS402 was more sensitive than *R. erythropolis* CS98.

**Effects of potassium on cation uptake activity**

We investigated the effects of potassium concentrations on cation uptake rates by the *Rhodococcus* strains (Table III). No differences in cesium and rubidium uptake rates for either strain grown at low (0.01 mM) or high (10 mM) potassium concentrations were observed. The potassium uptake rate of *R. erythropolis* CS98 cells grown in 10 mM potassium was 55% of that of cells grown in 0.01 mM potassium. These data suggest that *R. erythropolis* CS98 might have another potassium transport system repressed by high concentrations of potassium.

**Discussion**

There are only a few reports on the kinetics of cesium uptake. Bossemeyer et al.\(^{13}\) showed that the Kup system, the low affinity potassium transport system encoded by the trkD gene in *E. coli*, allowed high cesium accumulation.
The $K_m$ of the Kup system for cesium uptake was 5 mM. *Rhodococcus capsulatus* could also accumulate cesium with a $K_m$ for cesium uptake of 3 mM. In this study, the $K_m$'s for cesium uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were obtained as 136 and 436 $\mu$M, respectively. These values were less than one-twentieth and one-sixth of the $K_m$'s of the Kup system in *E. coli* and *R. capsulatus*, respectively. Therefore, both *Rhodococcus* strains have a high affinity cesium uptake system.

Jasper reported that rubidium and cesium were competitive inhibitors of potassium uptake by *R. capsulatus* and the $K_i$ for rubidium and cesium as the inhibitors of potassium accumulation were very similar to the $K_m$ for the accumulation of rubidium and cesium, respectively. Then, it was concluded that the rubidium and cesium were accumulated by the transport system normally responsible for the accumulation of potassium. Moreover, Avery et al. reported that the potassium transport systems in *Synechocystis PCC6803* and *Chlorella emersonii* acted in cesium accumulation. In this study, potassium was a competitive inhibitor of cesium uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402, suggesting that cesium was transported by the uptake system normally responsible for the accumulation of potassium in both *Rhodococcus* strains. However the $K_i$'s for potassium as an inhibitor of cesium accumulation were not similar to the $K_m$'s for the potassium accumulation. These results suggested that an additional novel potassium uptake system that could transport cesium might exist in both *Rhodococcus* strains. Therefore it seems that the $K_m$'s for potassium accumulation were apparent value.

Potassium uptake by *R. erythropolis* CS98 was partially repressed by high concentrations of potassium, but cesium uptake was not repressed. These results suggested that *R. erythropolis* CS98 had at least two potassium uptake systems; one was constitutive, and concerned with cesium transport, and another was inducible and was not concerned with cesium transport. On the other hand, potassium, rubidium and cesium uptake on *Rhodococcus* sp. strain CS402 were not repressed by high potassium concentrations. Therefore, it seemed that *Rhodococcus* sp. strain CS402 did not have an inducible potassium transport system that was induced by low potassium concentrations.

As shown in Table II, FCCP inhibited the uptake of three cations by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402. This uncoupler is known to inhibit the expression of a transmembrane proton motive force. This suggests that cation uptake by *R. erythropolis* CS98 and

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**Table II.** Inhibition by FCCP of Cation Uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. Strain CS402

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cation uptake ratea (µmoles min⁻¹ g dry cells⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R. erythropolis</em> CS98</td>
</tr>
<tr>
<td>None</td>
<td>Cs⁺</td>
</tr>
<tr>
<td>1.3</td>
<td>5.2</td>
</tr>
<tr>
<td>1.3 (25µM)</td>
<td>5.2</td>
</tr>
<tr>
<td>+ DMSO (1%)b</td>
<td>2.7</td>
</tr>
</tbody>
</table>

a The respective assay solutions were contained 20 µM of each cation.
b These sample were treated for 30 min at 25°C with DMSO or with the inhibitor.

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**Table III.** Cation Uptake Rates by *Rhodococcus* Species Grown at Various Potassium Concentrations

<table>
<thead>
<tr>
<th>Strain</th>
<th>K⁺ concn in medium (mM)</th>
<th>Cation uptake rateb (µmoles min⁻¹ g dry cells⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodococcus erythropolis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS98</td>
<td>0.01</td>
<td>Cs⁺</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Rb⁺</td>
</tr>
<tr>
<td><em>Rhodococcus</em> sp. strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS402</td>
<td>0.01</td>
<td>Cs⁺</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Rb⁺</td>
</tr>
</tbody>
</table>

b Cells used for transport assay were grown at various potassium concentrations.

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*Fig. 3.* Competitive Inhibition of Cesium Uptake in *R. erythropolis* CS98 by Potassium. ○, 25 $\mu$M; △, 50 $\mu$M; □, 75 $\mu$M; ●, 100 $\mu$M CsCl.

*Fig. 4.* Competitive Inhibition of Cesium Uptake in *Rhodococcus* sp. Strain CS402 by Potassium. ○, 50 $\mu$M; △, 100 $\mu$M; □, 200 $\mu$M; ●, 400 $\mu$M CsCl.
Rhodococcus sp. strain CS402 might require the proton motive force. Additionally, cesium uptake was more inhibited than potassium and rubidium uptakes by FCCP in R. erythropolis CS98, suggesting that the potassium uptake system that could also transport cesium was more sensitive to FCCP than those that transport only potassium in R. erythropolis CS98.

Three potassium uptake systems (Kdp, Trk, and Kup) were reported in E. coli. Among them, cesium was transported only via the Kup potassium uptake system in E. coli. The Kup system was not thought to be the main potassium transport system and little is known about its characteristics. Bakker reported that potassium uptake by the Kup system in E. coli was almost independent of the culture pH. In contrast, both Rhodococcus strains had the maximal cesium accumulation under alkaline conditions (pH 8.5). Moreover, the differences between the $K_m$'s of cesium uptake by both Rhodococcus strains and the Kup systems were large. From these results, both Rhodococcus strains might take up cesium via a mechanism different from that of the E. coli Kup system.

The Kdp system in E. coli transported potassium with high affinity, but didn't transport rubidium or cesium. This system was repressed by high potassium concentrations. Although the cesium uptake systems of both Rhodococcus strains showed relatively high affinity for potassium, both Rhodococcus strains transported not only potassium but also rubidium and cesium. Moreover, in both Rhodococcus strains, potassium uptake systems concerned with cesium transport were not repressed by high potassium concentrations (Table III). Therefore, the characteristics of the potassium uptake system in both Rhodococcus strains were different from those of the Kdp system.

There are several reports on the Trk system in E. coli and Trk-like systems in other bacteria as potassium transport systems. The Trk system is known to be constitutive with low affinity for potassium. Potassium and rubidium uptake via the Trk system in E. coli was accompanied by alkalinization of the medium, and this system was inhibited by the protonophore FCCP. In our previous paper, both Rhodococcus strains had maximal cesium accumulation under alkaline conditions (pH 8.5). And FCCP inhibited the uptake of the three cations by R. erythropolis CS98 and Rhodococcus sp. strain CS402 (Table II). Furthermore, both Rhodococcus strains expressed cesium uptake activity in the presence of a high concentration of potassium (10 mM K$^+$). These observations indicate that the potassium uptake system of both Rhodococcus strains have characteristics like the Trk system. However, the Trk system in E. coli could transport rubidium, but could not transport cesium. In Halobacterium halobium and Bacillus acidocaldarius, rubidium was taken up via the Trk-like potassium transport systems, but cesium was not, but both Rhodococcus strains transported these cations as described above. So the Trk-like systems in the Rhodococcus strains were different from other Trk systems in substrate specificity.

We concluded that the Rhodococcus strains studied here have potassium transport systems different from the potassium transport systems reported previously. Further work is needed to clarify the characteristics of the cesium transport system of Rhodococcus strains.

References