Specific Cesium Transport via the Escherichia coli Kup (TrkD) K⁺ Uptake System

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Escherichia coli cells which contain a functional Kup (formerly TrkD) system took up Cs⁺ with a moderate rate and affinity. Kup is a separate K⁺ uptake system with relatively little discrimination in the transport of the cations K⁺, Rb⁺, and Cs⁺. Regardless of the presence or absence of Kup, K⁺-replete cells took up Cs⁺ primarily by a very low affinity mode, proportional to the ratio of the Cs⁺ and K⁺ concentrations in the medium.

One of the striking consequences of the Chernobyl reactor catastrophe is the prolonged occurrence of radioactive cesium (Cs⁺) in animals, plants, and fungi. Higher animal cells show relatively little discrimination between the transport across the cytoplasmic membrane of K⁺ (ionic radius, 0.133 nm) and the larger monovalent cations Rb⁺ (radius, 0.148 nm) or Tl⁺ (radius 0.143 nm) (5). The Na⁺/K⁺-ATPase catalyzes uptake of Cs⁺ (radius, 0.169 nm) (24). By contrast, microorganisms and especially bacteria possess alkali cation transport systems that are much more selective for the different cations (13, 22). The constitutive K⁺ uptake system Trk from Escherichia coli shows approximately a 10-fold discrimination between K⁺ and Rb⁺ or Tl⁺ (1, 8, 20), and the inducible, high-affinity K⁺ uptake ATPase Kdp is even more selective (8, 20). Since very little is known about Cs⁺ accumulation by microorganisms besides what is in a few reports (7, 15) and since knowledge about such accumulation is essential for the estimation of the extent to which microorganisms may contribute to the prolonged retention of radioactive Cs⁺ by soil, we determined which E. coli K⁺ uptake systems accept Cs⁺. This is the only microorganism for which a number of well-defined K⁺ transport mutants are available (10, 19, 23). We report here that Cs⁺ is only taken up via the TrkD system. This observation supports the original but later abandoned view (cf. reference 19 with references 11 and 23) that the TrkD system is a separate K⁺ uptake system. It possesses a low specificity for the alkali cations transported and is thereby clearly distinct from the two main K⁺ uptake systems Trk and Kdp. To avoid future confusion with the Trk system we will refer to the TrkD system, which is the product of the trkD gene(s), as Kup (mnemonic for K⁺ uptake).

Bacterial strains and growth conditions. All of the strains used are derivatives of E. coli K-12 kindly provided by W. Epstein, The University of Chicago, Chicago, Ill. The genotypes of these strains and the K⁺ uptake systems expressed under the different growth conditions are listed in Table 1. The growth media were those described by Epstein and Kim (10).

Plasmid pJG1. The trkD-containing plasmid pJG1, described by Lopilato et al. (16), was obtained from W. Epstein. It consists of a 6.3-kilobase EcoRI-HindIII fragment cloned into the larger EcoRI-HinII fragment of pBR322 and contains the trkD gene (D. C. Dosch, Ph.D., thesis, The University of Chicago, Chicago, Ill. 1985), as well as the rbsA gene (14).

K⁺ depletion and transport assays. Growing cells were harvested by centrifugation after they had reached an optical density at 578 nm between 0.7 and 1.0. These cells were depleted of K⁺ by Tris-EDTA treatment as described previously (4). Transport assays were carried out at 23 to 25°C with EDTA-treated cells suspended at 1 mg (dry wt) ml of medium⁻¹ consisting of 200 mM sodium HEPES (N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid) (pH 7.5) and 10 mM glucose (4). After a preincubation of 15 min, uptake assays were started by the addition of KCl, ⁸⁶RbCl, or CsCl at the concentration indicated at the particular experiment. Cells from 1.0-ml samples were centrifuged through silicone oil. The K⁺ and Cs⁺ contents of the cell pellets were determined in a flame-photometer 700 (Eppendorf Geratebau, Hamburg, Federal Republic of Germany) and at 852.1 nm in the emission mode of an atomic absorption spectrophotometer 357 (Instrumentation Laboratory Inc., Lexington, Mass., respectively). The ⁸⁶Rb⁺ content of the cell pellets was determined in a 460C-liquid scintillation counter (Canberra-Packard, Frankfurt, Federal Republic of Germany).

Cs⁺ uptake by K⁺ uptake mutants. Figure 1 gives rates with which K⁺-depleted cells of the different K⁺ transport mutants showed net Cs⁺ uptake. Strains that possess a functional Kup system (i.e., FRAG-1, FRAG-5, or TK1001 [Fig. 1, closed symbols]) took up Cs⁺ fairly rapidly (Vmax, 15 to 25 mmol min⁻¹ g [dry wt] of cells⁻¹; Km, 5 to 7 mM). Strains that only possess a Kdp system (strains TK2240 or TK2242) or Trk system (TK1001) took up Cs⁺ as slowly as did strain TK2205, which is deleted for the Kdp system and carries point mutations in both the trkA and trkD genes (Fig. 1, open symbols).

Enhanced Cs⁺ uptake by cells carrying the trkD gene on a multicopy plasmid. Cells that were either kdp trkA trkD, kdp trkD trkE trkG, or kdp trkD trkG trkH and contained the trkD gene on the multicopy plasmid pJG1 (16) took up K⁺ several times faster than did cells that carried a functional trkD gene in the chromosome (D. Bossemeyer, I. R. Booth, and E. P. Bakker, Short Rep. Fifth Eur. Bioenergetics Conf. Aberystwyth, Wales, 1988, p. 199). The same was true for Cs⁺ uptake (Fig. 1). Since Cs⁺ is only taken up via the Kup system (see above), these results suggest that the E. coli chromosomal DNA of plasmid pJG1 alone is sufficient to encode a functional Kup system.

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Table 1. K⁺ uptake mutants of E. coli K-12 and their properties

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>[K⁺] during growth (mM)</th>
<th>K⁺ transport systems expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAG-1 gal</td>
<td></td>
<td>30</td>
<td>Trk, Kup</td>
</tr>
<tr>
<td>FRAG-5 kdpABC5 gal</td>
<td></td>
<td>5</td>
<td>Trk, Kup</td>
</tr>
<tr>
<td>TK1001 kdpABC5 trkD1 gal</td>
<td></td>
<td>5</td>
<td>Trk</td>
</tr>
<tr>
<td>TK1110 kdpABC5 trkA405 gal</td>
<td></td>
<td>5</td>
<td>Kup</td>
</tr>
<tr>
<td>TK2205 kdpABC5 trkA405 gal nagA</td>
<td></td>
<td>115</td>
<td>None</td>
</tr>
<tr>
<td>TK2240 trkA405 trkD1 nagA</td>
<td></td>
<td>100 μM⁺</td>
<td>Kdp</td>
</tr>
<tr>
<td>TK2242 kdp-42 trkA405 trkD1 nagA</td>
<td></td>
<td>5</td>
<td>Kdp⁺</td>
</tr>
</tbody>
</table>

- Genetic data are from references 10, 12, and 19.
- All of the strains are also F⁻ lacZ rha thi.
- The slow K⁺ uptake observed with this strain may be mediated by a fourth uptake system, TrkF (19).
- Cells of strain TK2240 were grown overnight at 0.1 mM K⁺. On the next day, they were diluted 1:20 into fresh medium with added K⁺, which was contaminated with about 25 μM K⁺. After growth had ceased, 25 μM KCl was added to the suspension. After growth had ceased again, K⁺ was added at 100 μM. The cells were harvested after growth had resumed.
- The Kdp⁺ system possesses a greatly diminished affinity for K⁺ (12).

Ion specificity of the Kup system. That the Kup system accepts Cs⁺ suggests that it does not discriminate strongly between K⁺ and Cs⁺. This differs from previous findings that the TrkD system distinguishes even more strongly between K⁺ and Rb⁺ than does the Trk system (20). We cannot confirm this earlier result. First, the kinetic parameters of K⁺ and Rb⁺ via Kup were almost identical (Table 2). Second, the uptake of tracer amounts of ⁸⁶Rb⁺ by the cells in the presence of large amounts of either KCl or RbCl indicate that ⁸⁶Rb uptake mimics bulk K⁺ uptake by the Kup system very well (results not shown). Table 2 also shows a large difference between the transport of K⁺ and Rb⁺ via the Kup system and that of Cs⁺ in the approximately 10-fold-higher Kₘ value of the latter. Thus, in contrast to Trk or Kdp, the Kup system does not strongly distinguish between the alkali cations K⁺, Rb⁺, or even Cs⁺ (cf. Fig. 1 and Table 2 with references 1 and 20).

Inhibition of K⁺ uptake by Cs⁺. Even a compound not translocated by a transport system may still inhibit the transport of the natural substrate. This effect was very small for Cs⁺ acting on K⁺ uptake by the Kdp system. A 30 mM concentration of Cs⁺ inhibited the net uptake of K⁺ (added at 2 mM) via the altered Kdp system (Table 1) of strain TK2242 by less than 10%. The effect of Cs⁺ on K⁺ uptake via the Trk system was larger with competitive inhibition and a Kᵢ of 30 mM Cs⁺. As expected, the strongest effect of Cs⁺ was observed on K⁺ uptake via the Kup system. The inhibition was competitive with a Kᵢ of 7 mM Cs⁺ (results not shown).

Table 2. Cation specificity of E. coli K⁺ uptake systems

<table>
<thead>
<tr>
<th>System</th>
<th>K⁺ uptake</th>
<th>Rb⁺ uptake</th>
<th>Cs⁺ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>βₚ</td>
<td>Vₘₚ</td>
<td>Kₘ</td>
</tr>
<tr>
<td>Kdp</td>
<td>0.002</td>
<td>150</td>
<td>Low activity</td>
</tr>
<tr>
<td>Trk</td>
<td>0.9–1.5</td>
<td>190–500</td>
<td>0.35</td>
</tr>
<tr>
<td>Kup</td>
<td>0.37 ± 0.13a</td>
<td>27 ± 5a</td>
<td>0.38 ± 0.07a</td>
</tr>
</tbody>
</table>

- Data from strains TK2240, TK1001, and TK1110 for the Kdp, Trk, and Kup systems, respectively.
- Kₘ in millimolar; Vₘ in micromoles minute⁻¹ gram⁻¹ (dry wt) of cells.
- ND, No activity detected.
- Average from four experiments with standard deviations.
- Average from two experiments with standard deviations.
FIG. 2. K⁺-Cs⁺ exchange by E. coli. To glucose-metabolizing, K⁺-depleted cells of strain FRAG-5 (A) or TK1001 (B) were added, at zero time, either 5 mM KCl (●), 30 mM CsCl (●) or 5 mM KCl plus 30 mM CsCl (●, △); circles, K⁺ content; triangles, Cs⁺ content.

Mulder (M. M. Mulder, Ph.D. thesis, University of Amsterdam, Amsterdam, The Netherlands, 1988) drew a conclusion similar to ours.

Very slow Cs⁺ uptake. Cells that do not possess a functional Kup system took up Cs⁺ very slowly (Fig. 1). This Cs⁺ uptake nevertheless saturated at high Cs⁺ concentrations (V_max. 10 to 20 μmol min⁻¹ g⁻¹; K_m, 100 to 150 mM; data not shown) and may even reflect uptake via a fourth, very low affinity K⁺ uptake system, TrkF (19), or reflect a leak current through the cytoplasmic membrane driven by the internally negative membrane potential (19, 23). The second point illustrated by Fig. 2 is that in the presence of both K⁺ and Cs⁺, the uptake of Cs⁺ by K⁺-loaded cells occurred via this fourth system, since regardless of the presence or absence of a functional Kup system the two strains showed the same rate of Cs⁺ uptake (Fig. 2, open triangles). Since this system also shows almost no discrimination between K⁺ and Cs⁺ (data not shown), the extent of accumulation of radioactive Cs⁺ by wild-type, K⁺-replete E. coli should be proportional to the ratio of the Cs⁺ and K⁺ concentrations present in the medium.

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LITERATURE CITED