The Periplasmic Cyclodextrin Binding Protein CymE from *Klebsiella oxytoca* and Its Role in Maltodextrin and Cyclodextrin Transport

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*C. oxytoca* M5a1 has the capacity to transport and to metabolize α-, β- and γ-cyclodextrins. Cyclodextrin transport is mediated by the products of the *cymE*, *cymF*, *cymG*, *cymD*, and *cymA* genes, which are functionally homologous to the *malE*, *malF*, *malG*, *malK*, and *lamB* gene products of *Escherichia coli*. CymE, which is the periplasmic binding protein, has been overproduced and purified. By substrate-induced fluorescence quenching, the binding of ligands was analyzed. CymE bound α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin, with dissociation constants (*Kd*) of 0.02, 0.14 and 0.30 μM, respectively, and linear maltohexaose, with a *Kd* of 70 μM. In transport experiments, α-cyclodextrin was taken up by the *cym* system of *K. oxytoca* three to five times less efficiently than maltohexaose by the *E. coli* maltose system. Besides α-cyclodextrin, maltohexaose was also taken up by the *K. oxytoca cym* system, but because of the inability of maltodextrins to induce the *cym* system, growth of *E. coli mal* mutants on linear maltodextrin was not observed when the cells harbored only the *cym* uptake system. Strains which gained this capacity by mutation could easily be selected, however.

Cyclodextrins (CDs) are α-1,4-glycosidically linked, cyclic maltotriosaccharides. The main forms are α-, β- and γ-CDs, which have 6, 7, and 8 glucose residues, respectively (5, 24). Their three-dimensional structure is torus shaped, with a hydrophilic outside ring and an interior hydrophobic cavity. Dependent on the size of the hydrophobic cavity of the respective CD, inclusion complexes with a variety of guest molecules can be formed, which is the basis of broad applications in industry (5, 24). CDs are formed enzymically from starch by CD-glycosidase (CGTases) a subgroup of the α-amylase class of enzymes (27, 35). One of the producers of CGTases is *Klebsiella oxytoca* M5a1 (1).

*K. oxytoca* can utilize starch as the sole carbon and energy source via two metabolic routes. The first one involves the extracellular degradation into linear maltodextrins by hydrolysis of the α-1,6-glycosidic bonds via the cell surface-associated pullulanase (1, 32) and the subsequent cleavage of the α-1,4-glycosidic linkages by the disproportionation activity of the extracellular α-CGTase (1). *Escherichia coli* (11, 15, 16, 36) and *Klebsiella pneumoniae* (7, 44) can then take up these linear maltodextrins (maltose up to maltoheptaose) via a binding protein-dependent ABC transporter (12) consisting of maltoporin (LamB), the maltodextrin-binding protein (MalE), the cytoplasmatic membrane proteins (MalF and MalG), and the ATP-binding protein (MalK). Intracellularly, the linear maltodextrins are degraded into glucose and glucose-1-phosphate by the enzymes amyłomaltesa (MalQ) (31), the maltodextrin phosphorylase (MalP) (43), and maltodextrin glucosidase (MalZ) (30).

In the second pathway, present only in *K. oxytoca*, starch is converted extracellularly into CDs by the cyclization activity of the α-CGTase, forming first and predominately α-CD, which later on is transformed into the thermodynamically favored β-CD (3, 35). The growth of *K. oxytoca* with CD as its sole carbon and energy source (2) is based on the uptake of the α- and β-CD via the *cym* system (17). Intracellularly, CDs are linearized by a cyclodextrinase (CymH) into linear maltotriosaccharides (14) which enter the maltose degradation pathway (17).

The genes responsible for CD metabolism were localized at the 5′ side of the gene coding for the α-CGTase (6, 17). Sequence analysis demonstrated the homology of the gene products of *cymE*, *cymF*, *cymG*, and *cymD* to MalE, MalF, MalG, and MalK, respectively (17). In addition, a functional homology could be shown between the gene product of *cymA* and the maltoporin LamB (29). A mutational analysis showed that *cymE*, *cymF*, *cymD*, and *cymA* were essential for growth at the expense of CDs (17, 29).

The existence of a specific transport system for CDs, which are rigid molecules of considerable size (ranging from 1.37 nm for α-CD to 1.69 nm for γ-CD [outer diameter]), was unexpected. Since their transfer through the outer membrane and the cytoplasmic membrane may involve novel mechanisms, we have initiate the biochemical analysis. Here we report on the biochemical characterization of CymE as a periplasmic CD binding protein as well as the kinetic analysis of the *cym* transport system.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids, media, and growth conditions.** The bacterial strains and the plasmids used in this study are listed in Table 1, together with their source or derivation. The rich medium used was Luria-Bertani (LB) medium (26). *mal* and *cym* mutants were discriminated on MacConkey agar plates enriched with 0.5% (wt/vol) maltose or α-CD. The minimal medium employed was salt solution P supplemented with 12 mM ammonium sulfate (18). If not indicated otherwise, particular carbon sources were added at concentrations of 0.2 or 0.5%. The concentrations of antibiotics added and growth conditions were described previously (17).
TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Klebsiella oxytoca derivatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M5a1</td>
<td>Wild type</td>
<td>This work</td>
</tr>
<tr>
<td>CYME</td>
<td>Wild type, Δ(cymE)</td>
<td></td>
</tr>
<tr>
<td><strong>Escherichia coli K-12 derivatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JM109</td>
<td>F′ (traD36 proAB′ lacP ZM155) recA1 endA1 gyrA96 thi-1 lamD17 relA1 supE44 (lac-proAB) λ supE44 (lac-proAB) λ</td>
<td>46</td>
</tr>
<tr>
<td>MC4100</td>
<td>F′, Δ(argF-lac) U169 amm1A385 fosF25 deoC1 relA1 thiB5301 rpsL150 λ</td>
<td>13</td>
</tr>
<tr>
<td>GM1</td>
<td>Like MC4100, malE::Tn10 (Kan′)</td>
<td>17</td>
</tr>
<tr>
<td>JV2000</td>
<td>Like MC4100, malE::Tn10 (Tet′) phto48 rbsR</td>
<td>W. Boos</td>
</tr>
<tr>
<td>GM15</td>
<td>Like GM1, malE::Tn10 (Tet′)</td>
<td>This work</td>
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<tr>
<td><strong>Escherichia coli B derivatives; BL21 (DE-3)</strong></td>
<td>F′ hasD gal λ (DE-3)</td>
<td>41</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT7-Hs-TRXFUS</td>
<td>Ap′ Hs-trxA</td>
<td>22</td>
</tr>
<tr>
<td>pCYME2 (pT7-Hs-TRXFUS derivative)</td>
<td>Ap′ Hs-trxA ’cymE’</td>
<td>This work</td>
</tr>
<tr>
<td>pSU2719</td>
<td>Cm′ lacZa</td>
<td>25</td>
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<tr>
<td>pGM200 (pSU2719 derivative)</td>
<td>Cm′ lacA-J</td>
<td>17</td>
</tr>
<tr>
<td>pCYMÆ (pSU2719 derivative)</td>
<td>Cm′ lacA-D (Δ(cymE)) cymF-J</td>
<td>17</td>
</tr>
</tbody>
</table>

**Standard genetic procedures.** Standard genetic procedures were adopted from Miller (26) and Sambrook et al. (33). Enzymes for recombinant DNA techniques were used according to the recommendations of the manufacturers.

**Immunoblotting analysis.** Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23) and subjected to immunoblotting analysis (34) with rat anti-CymH monoclonal antibodies at a dilution of 1:10,000 as described previously (14).

**Construction of a K. oxytoca cymE-deletion mutant.** The wild-type K. oxytoca strain was transformed with the plasmid pCYME carrying an in-frame deletion in cymE (17). The transformants were grown overnight in LB medium without antibiotics, and the resulting cultures were diluted to cell densities of 1 × 10⁹ to 2 × 10⁹ cells/ml and plated on MacConkey plates supplemented with 0.5% NaCl and the entire antibiotic marker of the plasmid used. The presence of the deletion was verified by PCR employing oligonucleotides priming up- and downstream of the deletion (MC5. 5′-CCCATTTCCGGAATAGAT-3′; MC15. 5′-ACGCAAGTTAGATTAC-5′). Finally, the strains were tested for complementation of the cym phenotype by transformation with a wild-type copy of the gene.

**Construction of plasmid pCYME2.** Plasmid pCYME2 is a derivative of the plasmid pT7-Hs-TRXFUS (22). It carries a translational fusion of the gene coding for a His-tagged thioredoxin, followed by an enterokinase cleavage site, and the cymE gene lacking its signal sequence coding region (bp 1 to 60). For construction, the ‘cymE’ part was amplified via PCR with the oligonucleotide 5′-CCGTCGACAATAACATAGTTACTCCT-3′; MC7, 5′-CCCATTTCCGGAATAGAT-3′; MC15, 5′-ACGCAAGTTAGATTAC-5′. The resulting fragment was phosphorylated and cleaved with SalI. The pT7-Hs-TRXFUS vector part was restricted with KpnI, treated with Klenow fragment, subsequently cleaved by SalI, and dephosphorylated. Finally, both fragments were ligated. All fusion joints and the entire ‘cymE’ gene region were verified by sequencing.

**Overproduction of the His-tag-thioredoxin–CymE fusion protein.** For the cleavage of the fusion protein, the dialysate was incubated with enterokinase (Boehringer Mannheim GmbH) for 6 h at 37°C at a ratio between fusion protein and enterokinase of 1:20 (wt/wt). To separate the mature form of CymE from the His-tag-thioredoxin part of the fusion protein and from the noncleaved fusion protein and minor impurities, ion-exchange chromatography through a Mono-Q HR 5/5 column (1-ml bed volume) was performed. The column was equilibrated with buffer B; after the sample was loaded, the column was washed with 5 ml of buffer B, developed with a 20-ml linear gradient reaching from 0 to 1 M KCl, and finally washed with 5 ml (each) of buffer B and buffer C (50 mM Tris-HCl, pH 7.6). The flow rate was 0.5 ml/min. Fractions containing the mature form of CymE were pooled and dialyzed against buffer B. The solution was frozen in liquid nitrogen and stored at −80°C.

**Fluorescence spectroscopy.** Fluorescence spectroscopy was performed to measure the binding of substrates by the purified CymE (42). Fluorescence measurements were carried out in an LS 80B Luminescence spectrometer (Perkin-Elmer, Norwalk, Conn.) with excitation and emission slits between 5 and 10 nm and at an excitation wavelength of 280 nm. The emission spectrum was monitored between 300 and 400 nm. Changes in the fluorescence intensity as a function of ligand concentrations were recorded at an emission wavelength of 355 nm. All measurements were performed at room temperature in 1 ml of 50 mM Tris-HCl (pH 7.6), which also served as a reference. Protein concentrations ranged from 0.8 to 8 μg/ml, and the substrates were added in small volumes. Changes in the intensity of fluorescence emission due to dilutions were corrected with a control containing the same concentration of protein and receiving buffer instead of substrate. For determination of the respective Kᵢ values, a series of substrate concentrations were examined for the degree of quenching of fluorescence. The results reported are the mean values of three consecutive measurements for 5 min each. Maximal quenching was set at 1. The substrate concentration resulting in half-maximal quenching was defined as the Kᵢ and was derived from Line-Weaver-Burk plots.

**Chromatographic techniques.** Linear and cyclic maltodextrin and cyclodextrin standards were analyzed qualitatively by thin-layer chromatography (TLC) as described previously (14).

**Transport assays.** In order to measure transport, the bacteria were first adapted in a preculture to the carbon source employed. For the characterization of CD uptake, 0.5% α-CD or a mixture of 1% sucrose plus 0.1% α-CD was used as the carbon source, whereas for studying the maltose system, 0.2% maltose was used as the carbon source. After inoculation of the main culture, cells were grown to the late exponential growth phase, harvested by centrifugation, washed three times with minimal medium without a carbon source, resuspended in the same medium, and adjusted in terms of their cell density. For transport measurements, samples of 3 or 6 ml of this stock solution were adjusted to 25°C. To start the uptake reaction, a small volume of the labeled substrate was added.
Their radioactivity was determined in a TRI-CARB 2100 TR liquid scintillation analyzer (Packard, Dreieich, Germany) with the Ultima Gold scintillation cocktail (Packard). The rate of uptake was taken from the linear portion of the resulting curve (initial rate of uptake).

For the $V_{\text{max}}$ and $K_m$ determination of $\alpha$-CD and maltodextrin uptake, $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ (1,890 mCi/mmol) and $[\beta\text{-}{}^{14}\text{C}]\text{CD}$ (2 mCi/mmol) were synthesized as described by Pajatsch et al. (28) $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ of low specific radioactivity (2 mCi/mmol) obtained from Wacker Chemicals (Munich, Germany) was also used. $K_m$ and $V_{\text{max}}$ values were deduced from Lineweaver-Burk plots. Competition of $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ uptake by unlabeled cyclodextrins or linear maltodextrins was measured with a saturating concentration (20 μM) of the $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ of the specific radioactivity (2 mCi/mmol) mixed with the indicated molar excess of unlabeled compounds.

To observe the metabolism of the $\alpha$-CD taken up by the cells, chromato-
graphic analysis of the intracellular low-molecular-weight compounds was performed. To this end, cells were incubated with a sufficient amount of the $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ (2 mCi/mmol) to ensure maximal uptake rates over the entire incubation time. At the times indicated, samples were harvested by filtration, washed, frozen immediately in liquid nitrogen, and stored at −80°C. The cells were broken by resuspension in 1 ml of a solution containing 2.5% SDS and 1% chloroform. The solution was clarified by centrifugation, and the supernatant was desalted by the mixed bed ion-exchange material Serdolit MB-1 (Serva, Heidelberg, Germany). Finally, the solution was lyophilized, dissolved in a small volume of water, and separated by TLC. The TLC plate was then autoradiographed and developed.

Special chemicals. The unlabeled $\alpha$, $\beta$, and $\gamma$-CDs, as well as the $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ of low specific radioactivity (2 mCi/mmol), were gifts from Wacker Chemicals. $[\alpha\text{-}{}^{14}\text{C}]\text{CD}$ of high specific radioactivity (1,890 mCi/mmol) and $[\beta\text{-}{}^{14}\text{C}]\text{malto}-
hexaose$ (2 mCi/mmol) were synthesized as described previously (28). Nonradio-
actively labeled linear maltodextrins were purchased from Sigma Chemicals (Deisenhofen, Germany). $[^{35}\text{S}]\text{dATP}$ used for sequencing was delivered by NEN/ DuPont (Dreieich, Germany). Oligonucleotide primers were synthesized by MWG (Ebersberg, Germany). Molecular biological reagents were from Boehr-
ger Ing Mannheim GmbH, Pharmacia (Freiburg, Germany), or New England Biolabs (Schwalbach, Germany).

RESULTS AND DISCUSSION

Overproduction and purification of CymE. A fragment containing the part of the cymE gene corresponding to its mature (leaderless) form (17) plus 22 bp downstream of the coding region was amplified via PCR and cloned into the vector pET-H6-TRXFUS to generate a translational fusion with the gene coding for a His-tagged thioredoxin, which at its C-terminus contains the cleavage site for enterokinase. Considerable at-
tempts to overproduce native CymE from other vector systems and under many growth conditions only led to insoluble pro-
tein. For overproduction of the CymE fusion protein, the re-
sulting plasmid, pCYME2, was transformed into E. coli BL21 (DE-3), and the expression was induced in LB medium at 37°C by the addition of IPTG. SDS-PAGE of a crude cellular extract showed that a protein of the expected molecular mass was overproduced (Fig. 1, lane 1), the major part in soluble form (data not shown). The purification involved the preparation of a 30,000 × g supernatant, affinity chromatography on a Zn$^{2+}$-chelating Sepharose FF column, cleavage of the His-tag–thio-
redoxin–CymE fusion protein by enterokinase, and ion-ex-
change chromatography through a Mono-Q-HR5/5 column. A symmetrical peak consisting solely of the mature form of CymE was eluted from the final column (data not shown). Figure 1 demonstrates the course of purification as analyzed by SDS-PAGE of the pooled fractions from each step. From 200 ml of culture, 1 mg of purified CymE was obtained.

Specificity and affinity of substrate binding by CymE. The CymE protein contains six tryptophan residues, which, when excited at a wavelength of 280 nm in the absence of ligand, induce a fluorescence emission spectrum with a maximum of 338 nm (Fig. 2). The addition of 0.1 mM substrate such as $\alpha$-CD (Fig. 2A) decreased the fluorescence intensity and caused a shift of the emission spectrum to shorter wavelengths, in the case of $\alpha$-CD by about 4 nm. Addition of 0.1 mM $\beta$- and $\gamma$-CD and of 0.1 mM maltodextrins also quenched fluorescence, with a shift of the emission peak to shorter wavelengths, although this shift becomes less prominent with increasing size of the CDs or decreasing chain length of the maltodextrins, respectively (data not shown). Addition of maltose (Fig. 2B) was without effect. Since the substrate-induced quenching was maximal at 355 nm (Fig. 2A), this wavelength was used in further experiments.

The substrate specificity of CymE was analyzed by measur-
ing the effect of addition of 0.1 mM cyclic and linear maltodextrins and of several mono- and disaccharides (Fig. 3). The mono- and disaccharides, including maltose, only marginally quenched fluorescence, which may be due to maltodextrin impurities. Linear maltodextrins of a chain length of 3 glucose units and longer, however, had a significant effect. The CDs displayed the highest degree of quenching.

The $K_d$ values of CymE for the three CDs and for malto-
heptaose were then determined by titrating the protein with a series of concentrations of the ligands and measuring the effect on fluorescence. As measured by fluorescence quenching, the affinity of CymE for the different sugars decreased with in-
creasing size of the CDs as follows. The $K_d$ values for $\alpha$-CD, $\beta$-CD, and $\gamma$-CD were 0.02, 0.14, and 0.30 μM, respectively. The affinity for maltoheptaose ($K_{d'}$ 70 μM) was much lower than those for the CDs.

CymE dependence of transport of CDs and maltodextrins into K. oxytoca and recombinant E. coli cells. To gain informa-
tion on whether the binding affinity of CymE determines the in

FIG. 1. Course of purification of the mature form of CymE followed by SDS-PAGE of the respective fractions. Lanes: 1, crude extract; 2, S30 fraction; 3, eluate of the Zn$^{2+}$-chelating Sepharose FF column; 4, eluate of the Mono-Q column; 5, molecular mass standard (97, 85, 55, 39, 27, 19, and 14 kDa). Proteins were stained with Coomassie brilliant blue.

FIG. 2. Fluorescence quenching of CymE induced by $\alpha$-CD (A) and maltose (B). Excitation was at 280 nm. The emission spectrum of CymE (A, 0.8 μg/ml; B, 4 μg/ml) before (solid lines) and after (dotted lines) the addition of 0.1 mM substrate is shown.
vivo affinity for cellular uptake, transport experiments with radioactively labeled α-CD and maltohexaose were conducted at different substrate concentrations. Several strains were used: *K. oxytoca* (wild type and a cymE mutant), *E. coli* GM15/pGM200 harboring a plasmid that encodes the cym genes except that for the cyclodextrin glucanotransferase (cgt), and *E. coli* GM15/pCYMΔE with a deletion in cymE of pGM200 (both strains lack the *E. coli* maltose transport system because of a chromosomal mutation in malE). *E. coli* MC4100 served as control strain for the determination of the uptake of maltohexaose by the mal system. All strains, with the exception of MC4100, were grown under conditions used to induce the cym system. Table 2 gives the apparent $K_m$ and $V_{max}$ values for α-CD and maltohexaose. The results allow the following conclusions. (i) Under $V_{max}$ conditions, the recombinant *E. coli* strain transports the two substrates at nearly identical rates. (ii) The apparent $K_m$ for α-CD transport in *K. oxytoca* as well as in the recombinant *E. coli* strain is higher than the $K_d$ of the binding protein (1 versus 0.02 mM). (iii) In the recombinant *E. coli* strain, the $K_m$ for α-CD is nearly 10-fold higher than that for maltohexaose. Also, transport of maltohexaose via the *E. coli* mal system is more efficient than that via the *K. oxytoca* cym system.

Since only the initial rate of uptake was used, the kinetic constants of Table 2 reflect the properties of the uptake system and are not influenced by subsequent metabolism or by a possible feedback inhibition due to the accumulating substrate. We also monitored the intracellular fate of radioactive α-CD during the course of an uptake experiment with the *K. oxytoca* wild type. Figure 4 shows that α-CD is accumulated more rapidly than it is hydrolyzed, which indicates that it is taken up in an unaltered form. Finally, we have carried out α-CD uptake competition experiments in the absence and presence of competing substrates (Fig. 5). Competition decreased with increasing size of CDs and with decreasing chain length of linear maltooligosaccharides; maltose, again, was without any effect. This pattern roughly reflects the binding affinities of CymE towards these substrates.

Can the cym system support growth at the expense of maltooligosaccharides? The results described above have shown that CymE binds maltooligosaccharides, that maltooligosaccharides are transported through CymE, and that CymE may possibly support growth at the expense of these substrates. We were, however, unable to demonstrate growth on maltooligosaccharides through CymE. Since CymE is a highly specific enzyme, it may be possible that the cym system is not able to support growth on maltooligosaccharides.

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**TABLE 2.** Kinetics of cym system-dependent uptake of α-CD and maltohexaose

<table>
<thead>
<tr>
<th>K. oxytoca or E. coli strain</th>
<th>$V_{max}$ (nmol · min$^{-1}$)</th>
<th>$K_m$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-CD</td>
<td>Maltohexaose</td>
</tr>
<tr>
<td>Wild type</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>CYME</td>
<td>—</td>
<td>ND</td>
</tr>
<tr>
<td>GM15/pGM200</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>GM15/pCYMΔE</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MC4100</td>
<td>ND</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* K. oxytoca wild-type and CYME and *E. coli* GM15 strains were grown with a mixture of 1% succinate plus 0.1% α-CD as a carbon source, whereas for *E. coli* MC4100, the carbon source used was 0.2% maltose.

a Values are for $10^9$ cells.

b ND, not determined.

c –, from no uptake at all to about 3% of the $V_{max}$ of the respective cym strain.
Charides can be transported in a CymE-dependent fashion, and that they compete with the uptake of α-CD. Therefore, one would assume that the cym system would allow the cells to grow on these linear maltooligosaccharides. This assumption was tested with strain GM15/pGM200 (Fig. 6A). It grows well in α-CD-containing minimal medium, with a mean doubling time of about 2 h. When subcultured into medium with linear maltodextrins from maltotetraose to maltoheptaose, growth resumed and a resubculture grew without a lag. For maltohexaose and maltoheptaose, this lag phase lasted at least 36 h, and it was even longer on maltopentaose and maltotetrose. No growth took place at all on maltose and maltotriose (not shown). Growth on maltodextrins from maltotetrose to maltoheptaose was completely blocked when a mutation was present in cymE (not shown).

To test whether the resumption of growth is the consequence of a selection of mutants, we have taken samples at the two time points indicated in Fig. 6. Only 20% of the colonies retrieved from the culture at time 1 were able to grow on α-CD and on maltoheptaose without a lag phase. This proportion increased to 80% at time 2.

Figure 6B shows that the ability to grow on α-CD correlates precisely with the expression pattern of cymH, an enzyme essential for CD metabolism (14, 17). The results of the experiment therefore show that the cym system can be used for the uptake of linear maltodextrins, but linear maltodextrins are unable to induce cym gene expression. Mutations, however, can be selected which lead to expression. The underlying mutations will be characterized in the future.

In conclusion, the results from previous work—that cym genes code for products of a transport system specific for CDs—have been established now by the in vitro analysis of CymE, the periplasmic binding protein, as well as by transport studies. Meanwhile, the gene product of cymA also has been identified as the functional homolog of the maltoporin LamB (29). CymE binds CDs, especially α-CD, with high affinity. It also binds linear maltooligosaccharides with a chain length of 3 and higher. However, binding appears to be productive and competent for transport only for α-CD and β-CD and for the longer linear maltooligosaccharides. The metabolism of γ-CD requires the activity of CGTase (17), which allows the conclusion that γ-CD has to be converted into α- or β-CD or linearized (3, 4, 29) by this enzyme to be transported. The fact that γ-CD does not support growth (17) could reside in the fact that its complex with CymE does not acquire the conformation required for docking to the membrane CymFG partner (37, 39, 40).

A similar situation has been described for MalE. MalE binds α-CD and β-CD with high affinity (Kₐ values of 4 and 1.8 μM, respectively) (38), but in the form of an unproductive complex (17, 19–21, 37, 38). The Kₐ of CymE for α-CD binding is about 30-fold lower than the Kₐ in the uptake reaction. Since it appears that the outer membrane does not limit the diffusion (10), the uptake process must be limited at some other step, most probably at the interaction of the substrate-loaded CymE with the membrane complex. Possibly, the amount of CymE is not large enough to ensure equality of Kₐ with K₉ (8, 9).

ACKNOWLEDGMENTS

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