Enteric bacteria such as *Escherichia coli* are able to use C₄-dicarboxylates, such as fumarate, malate, and aspartate, for anaerobic growth (29). Under anaerobic conditions, the C₄-dicarboxylates are converted to fumarate, which is then used for fumarate respiration. Conversion of malate and aspartate to fumarate is catalyzed by the dehydratases fumarase and aspartase (7, 13, 16). Due to repression of the citric acid cycle under these conditions, succinate cannot be oxidized further and is excreted (for reviews, see references 2, 12, 19, and 27). The transport of the C₄-dicarboxylates is effected by carriers which are specifically produced under anaerobic conditions (5, 12, 26, 32, 33, 34). DcuB functions as a C₄-dicarboxylate/succinate antiporter which catalyzes electroneutral antiporting of the external C₄-dicarboxylates (generally fumarate, malate, and aspartate) against succinate as the end product of fumarate respiration (6). DcuB is synthesized under conditions of fumarate respiration, i.e., under anaerobic conditions, in the presence of fumarate-synthesizing pathways (8, 9, 12, 14, 29, 33). DcuB can be replaced or supported by the carrier DcuA or DcuC. DcuA is homologous to DcuB but is expressed constitutively (8). DcuC normally functions as a succinate efflux carrier during glucose fermentation, but it can take over fumarate/succinate antiporting when required (32, 34).

Anaerobic tartrate degradation was recognized early as a significant microbiological process, but it has not been studied in much detail (1, 10, 21, 25, 30). Utilization of tartrate requires the presence of an oxidizable cosubstrate, such as glucose or glycerol. L-Tartrate is dehydrated by L-tartrate dehydratase (TtdAB) to oxaloacetate, which is converted via malate to fumarate (Fig. 1) (24). The reducing equivalents are required for the function of malate dehydrogenase and fumarate reductase. The carrier for the uptake of L-tartrate (and the export of succinate) in tartrate fermentation is not known. DcuB would be an obvious candidate for the transport of L-tartrate, since it has a broad substrate specificity and transports C₄-dicarboxylates, such as fumarate, malate, and aspartate, in antiport against succinate (5, 6). In addition, DcuB is expressed under anaerobic conditions in the presence of tartrate (14). From database analysis, it has been suggested, however, that the *ygjE* (the putative *ttdT* gene) gene, which is located downstream of the *ttdA* and *ttdB* genes (encoding L-tartrate dehydratase), encodes a specific secondary carrier for tartrate/succinate antiporting (22). It was tested, therefore, whether DtdT is required for tartrate/succinate antiporting or whether one of the Dcu carriers is used for this purpose. It turned out that L-tartrate, the major form of tartrate in nature, requires TtdT for transport. The properties of L-tartrate transport by the TtdT carrier and the differences from the general Dcu carriers were determined.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** The strains used in this study are shown in Table 1. Growth of the bacteria for mRNA isolation and transport experiments was performed in M9 medium (17) supplemented with acid-hydrolyzed casein (Gibco BRL) (0.1% [wt/vol]) and L-tryptophan (0.005% [wt/vol]) (15). For anaerobic growth, cultures were incubated at 37°C in degassed medium in rubber-stoppered infusion bottles under N₂. For growth tests, strains with a deleted *ttdT* gene or inactivated genes for other C₄-dicarboxylate carriers were grown in supplemented M9 medium under anaerobic conditions. A 20 mM concentration of L-tartaric acid (potassium-sodium salt), L-tartaric acid (sodium salt), *meso*-tartrate (sodium salt), or fumarate (fumaric acid disodium salt) was included as an electron acceptor, and 20 mM of glyceraldehyde was included as an electron donor.

**Genetic methods.** (i) Inactivation of *ttdT* (*ygjE*). The *ttdT* gene was deleted by the method of Datysenko and Wanner (4), using the PCR product of the chloramphenicol resistance (*cmr*) cassette from plasmid pKD3 flanked by FRT sequences. For inactivation of *ttdT*, primers ttdTH1p2 (5'-TAA CCC TCC CCG AGA GGC TCA CCC TCC TCC TTT TTC GCA GGC ATA ACA CGG TGT AGG CTT GAG CATG CTT C3') and ttdTH2P2 (5'-TGC GTA AAA CTA TTG GGT GCC CAA GAG CATC3' TCC TCA CTC ATA TGA ATA TCC TCC TTA G-3'), which contain parts of the regions adjacent to

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*Escherichia coli* ferments L-tartrate under anaerobic conditions in the presence of an additional electron donor to succinate. The carrier for L-tartrate uptake and succinate export and its relation to the general C₄-dicarboxylate carriers DcuA, DcuB, and DcuC were studied. The secondary carrier TtdT, encoded by the *ttdT* (previously called *ygjE*) gene, is required for the uptake of L-tartrate. The *ttdT* gene is located downstream of the *ttdA* and *ttdB* genes, encoding the L-tartrate dehydratase TtdAB. Analysis of mRNA by reverse transcription-PCR showed that *ttdA*, *ttdB*, and *ttdT* are cotranscribed. Deletion of *ttdT* abolished growth by L-tartrate and degradation of L-tartrate completely. Bacteria containing TtdT catalyze L-tartrate or succinate uptake and specific heterologous L-tartrate/succinate antiporting. −/TtdT is not a substrate for TtdT. TtdT operates preferentially in the direction of tartrate uptake and succinate excretion. The Dcu carriers do not support anaerobic growth on L-tartrate or L-tartrate transport. TtdT is related in sequence and function to CitT, which catalyzes heterologous citrate/succinate antiporting in citrate fermentation.
FIG. 1. Comparison of the pathways for anaerobic conversion of L-tartrate and other C₄-dicarboxylates (fumarate, malate, and aspartate) to L-succinate in Escherichia coli. The pathways require a supply of external electron donors for fumarate and oxaloacetate reduction. The enzymes and carriers for the pathways are (i) a C₄-dicarboxylate/succinate antiporter (DcuB), (ii) fumarase (FumB), (iii) aspartase (AspA), (iv) fumarate reductase (Frd), (v) (putative) tartrate carrier (TtdT, YgjE), (vi) L-tartrate dehydratase (TtdAB), and (vii) malate dehydrogenase (MdH), menaquinone (MK), and menaquinol (MKH₂).

FRT and of the target gene ttdT, were used. The PCR product was purified, concentrated, and used for transformation. Cm' colonies were tested for loss of the helper plasmid (pKD46) by ampicillin sensitivity. For deletion of the Cm' cassette, the ttdT::Cm' mutant was transformed with the FLP helper plasmid pCP20 and selected at 30°C (3). The ttdT::Cm' and ΔttdT mutant genotypes were verified by PCRs with test primers ttdT_test_frd (5'-CGA CAA CCA GTA TGC GTA AG-3') and ttdT_test_rev (5'-GTC ATC GCC ATT ATT GCT CT-3'). The cDNA was amplified by PCRs using three forward (ttdA_frd_RT [5'-CGA TCA AAG CTG AAG ATC TG-3']) and reverse (ttdT_rev_RT, and the product cDNA was used for PCRs using primers located in the ttdA, ttdB, and ttdT genes in various combinations (see lower part of the figure). The primer combinations are given at the top of each track, and the calculated lengths of the products (bp) are given below the bands. M, 1-kb DNA ladder.

Script III reverse transcriptase (Invitrogen), using primer ttdT_rev_RT (5'-CGT ATG ATG TAA CAG TTA CG-3'), and ttdT_frd_RT (5'-GAG ATG TCT ACC GTC TCA GCC AAT CC-3'), cat_rev (5'-CTC TGG AGT GAA TAC CAC GAC-3'), and cat_mitte (5'-CTG AGT ACG AAT TTC ACC AC-3'). For construction of mutants IMW258 and IMW259, dcuB::Spc, dcuB::mini-Tn10 (Cm'), and citT::Kan' were transduced successively by bacteriophage P1 in strains LJ1 and IMW252 (Table 1).

(ii) RT-PCR. Total mRNA was isolated from E. coli LJ1 grown anaerobically on L-tartrate (50 mM) and glycerol (50 mM) to an optical density at 578 nm (OD₅₇₈) of 0.7, using Protect Bacteria reagent (QIAGEN) and an RNeasy Mini kit (QIAGEN). The mRNA was transcribed into cDNA with SuperScript III reverse transcriptase (Invitrogen), using primer ttdT_rev_RT (5'-CGT ATG ATG TAA CAG TTA CG-3'), and ttdT_frd_RT (5'-GAG ATG TCT ACC GTC TCA GCC AAT CC-3'), cat_rev (5'-CTC TGG AGT GAA TAC CAC GAC-3'), and cat_mitte (5'-CTG AGT ACG AAT TTC ACC AC-3'). For construction of mutants IMW258 and IMW259, dcuB::Spc, dcuB::mini-Tn10 (Cm'), and citT::Kan' were transduced successively by bacteriophage P1 in strains LJ1 and IMW252 (Table 1).

TABLE 1. Bacterial strains and plasmids used for this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Reference or cross</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LJ1</td>
<td>MG1655, but fur^+</td>
<td>K. Jahreis (Osnaprück)</td>
</tr>
<tr>
<td>JRG2814</td>
<td>AN387, but dcuA::Spc' dcuB::Kan'</td>
<td>26</td>
</tr>
<tr>
<td>IMW157</td>
<td>AN387, but dcuA::mini-Tn10 (Cm')</td>
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</tr>
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<td>IMW158</td>
<td>AN387, but dcuA::Spc dcuA::mini-Tn10 (Cm')</td>
<td>11</td>
</tr>
<tr>
<td>IMW277</td>
<td>AN387, but dcuA::Spc dcuB::Kan' dcuC::mini-Tn10 (Cm') citT::Kan'</td>
<td>This work</td>
</tr>
<tr>
<td>IMW516</td>
<td>MG1655, but dcuA::Spc' dcuA::Spc' dcuB::Kan' ttdT::Cm'</td>
<td>P1 (JRG2814) × LJ1</td>
</tr>
<tr>
<td>IMW518</td>
<td>LJ1, but dcuA::Spc' dcuB::Kan'</td>
<td>P1 (IMW156) × LJ1</td>
</tr>
<tr>
<td>IMW519</td>
<td>LJ1, but ttdT::Cm'</td>
<td>P1 (IMW157) × IMW518</td>
</tr>
<tr>
<td>IMW520</td>
<td>LJ1, but dcuA::Spc' dcuB::Kan' dcuC::Tn10 (Cm')</td>
<td>P1 (JRG2814) × IMW519</td>
</tr>
<tr>
<td>IMW521</td>
<td>LJ1, but dcuA::Spc' dcuB::Kan' ttdT::Cm'</td>
<td>P1 (IMW157) × IMW522</td>
</tr>
<tr>
<td>IMW522</td>
<td>IMW521, but ΔttdT</td>
<td>This work</td>
</tr>
<tr>
<td>IMW524</td>
<td>LJ1, but dcuA::Spc' dcuB::Kan' dcuC::Tn10 (Cm') ΔttdT</td>
<td>P1 (IMW157) × IMW522</td>
</tr>
<tr>
<td>IMW528</td>
<td>LJ1, but dcuA::Spc dcuB::Kan' dcuC::Tn10 (Cm') citT::Kan'</td>
<td>P1 (IMW277) × IMW518</td>
</tr>
<tr>
<td>IMW529</td>
<td>LJ1, but dcuA::Spc dcuB::Kan' dcuC::Tn10 (Cm') citT::Kan' ΔttdT</td>
<td>P1 (IMW277) × IMW522</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pKD3</td>
<td>oriR6 cat bla ∆(phoB-phoR)S80 galU95 ∆uidA3::pir ∆endA::FRT</td>
<td>4</td>
</tr>
<tr>
<td>pKD46</td>
<td>oriR101 repA101(ts) arrA-16-gum-Bet-exo bla</td>
<td>4</td>
</tr>
<tr>
<td>pCP20</td>
<td>FLP' xcatI585 x pΔRep' cat bla</td>
<td>4</td>
</tr>
</tbody>
</table>
buffer, which was subsequently degassed. Glucose was added, and the suspension was incubated at 37°C for 5 min (energization). If required, the protonophore CCCP (carboxyl cyanide m-chlorophenylhydrazone; Sigma) (20 μM) was added after 3 min of energization and incubated for 2 min before use in transport measurement. For the other experiments, anaerobic solutions and suspensions were used, but the transport experiments were performed under air. The identity and purity of D,L-[14C]tartrate (D,L-[1,4-14C]tartaric acid at 3,700 MBq/mmol; American Radiolabeled Chemical, Inc.) were tested and verified by high-performance liquid chromatography (HPLC) on an Aminex HPX87H column (300 × 7.8 mm; Bio-Rad), with authentic t- and l-tartrate, and by thin-layer chromatography on Merck F254 silica gel 60 with the solvent n-propanol–saturated ammonium hydroxide–distilled water (6/3/1 [vol/vol]) (http://www.nat-working.uni-jena.de/NatWorkingMaterialien.htm).

For measurements of uptake activity, 50 μl of energized cell suspension was added to 50 μl of D,L-[14C]tartrate (3,700 MBq/mmol) or [2,3,14C]succinate (Moravek Biochemicals, Inc.) (1,628 MBq/mmol) at various concentrations at 37°C in 1.5-ml reaction tubes. After various times, the reaction was stopped by the addition of 0.5 ml of ice-cold 0.1 M LiCl followed by rapid vacuum filtration through membrane filters (mixed cellulose ester, type ME 24; diameter, 25 mm; 0.2-μm pore size) (Schleicher & Schuell MicroScience). The filters were washed three times with ice-cold 0.1 M LiCl, transferred to scintillation vials with 4 ml of scintillation liquid, and counted for D,L-[14C]tartrate or [14C]succinate. Generally, all transport assays and experiments were performed at least in triplicate.

In the antiport assay, energized bacteria from the anaerobic cell suspension (460 μl) were preloaded by being mixed with 460 μl of [14C]succinate or D,L-[14C]tartrate (200 μM). After 10 min, 405 μl of the loaded cell suspension was removed and added to 8.2 μl of antiport substrate (l-tartrate or d-tartrate potassium sodium salt, succinate disodium salt, fumarate disodium salt, citrate trisodium salt, and butyrate sodium salt at 100 mM [each]) to give a final concentration of 2 mM antiport substrate. The reaction was stopped after 20 s and 1, 2, and 5 min by mixing 100 μl of the cell suspension with LiCl as described above, followed by vacuum filtration to determine the amount of intracellular [14C]succinate or [14C]tartrate. The transport activities were calculated from the changes in the intracellular concentration of the D,L-[14C]tartrate or [14C]succinate (25.7 and 33.4 Bq/nmol, respectively) by measuring the radioactivity of the cells, assuming that an OD₅₇₈ of 1 corresponds to 281 mg dry weight/liter of E. coli cells (32).

Identification of fermentation products by HPLC. The fermentation products in the supernatants of media from growth experiments were determined after removal of bacteria by centrifugation. The substrates and products in the supernatants were analyzed by HPLC on an Aminex HPX87H column (300 × 7.8 mm; Bio-Rad) at 65°C with buffer (6.5 mM H₂SO₄) at a flow rate of 550 μl/min (28) and were quantified by UV (215 nm) and refractive index detection.

RESULTS AND DISCUSSION

The ttdT (or yggE) gene is part of the ttd operon. The ttdA and ttdB genes encode the O₂-liable l-tartrate dehydrogenase (l-Ttd or TdDAB) (24). The yggE (presumptive ttdT) gene downstream of ttdAB shows similarity to genes for secondary carriers of the CitT family and was suggested to encode a tartrate carrier for anaerobic growth (22). Total mRNA was isolated from a wild-type strain of E. coli grown anaerobically on l-tartrate plus glycerol. The ttd mRNA was reversely transcribed by RT-PCR, using a primer specific for the 3′ end of the ttdT gene. The cDNA was then amplified with three forward and three reverse primers in various combinations, one of which was located in each of the three genes (Fig. 2). This should allow reverse transcription of each of the genes (ttdA, ttdB, and ttdT) separately, or in pairs, or as the complete set of genes, depending on the mRNA present. Each of the primer pairs provided products, including transcripts corresponding to ttdA-ttdB, ttdB-ttdT, and the ttdA-ttdB-ttdT total transcript. The products were of the predicted sizes if one assumes cotranscription of the corresponding single genes. The amounts of the cDNAs containing ttdA and the complete fragment ttdABT were comparable, indicating that the ttdA-ttdB-ttdT mRNA is not a minor form.

Prediction of promoter regions by PRODORIC (18; http://www.prodoric.de), which is based on the G+C content and the calculated stacking energy of DNA segments to predict T+A-rich promoter regions, suggested a promoter region in front of ttdA only, not in front of the other ttd genes. In addition, the intergenic region between ttdB and ttdT is small (47 bp), and the ttdA and ttdB genes even overlap by 4 bp, supporting the view that no additional promoter site is present in the ttdABT region.

Growth defects in ttdT mutants. Strains with a deleted ttdT gene were tested for anaerobic growth on l-tartrate, with glycerol as an electron donor (Fig. 3). For the experiments, a strain deleted in genes encoding DcuA, DcuB, DcuC, and CitT, representing the carriers for C₄-dicarboxylates in anaerobic growth (12, 26, 29, 32) and for citrate/succinate antiporting (22), was used as the genetic background to avoid interference from other C₄-dicarboxylate carriers. The dcuA dcbB dcuC citC ttdT strain was only slightly impaired for growth on l-tartrate with glycerol (Fig. 3A). A mutant with a deleted ttdT gene in the dcuA dcbB dcuC citC background completely lost the capability for growth by l-tartrate fermentation (Fig. 3A). The same result was observed for a ttdT deletion in the wild-type background. Anaerobic growth on fumarate plus glycerol, on the other hand, was not affected by inactivation of ttdT (Fig. 3B) but required the presence of the dcuA, dcbB, and dcuC genes, as described earlier (26, 32). Therefore, growth by l-tartrate...
fermentation depends specifically on the presence of TtdT, which cannot be replaced by the Dcu carriers, and vice versa.

**Kinetics of tartrate and succinate transport by the TtdT carrier.** The kinetics of anaerobic tartrate transport was analyzed with cell suspensions of bacteria grown under inducing conditions in the presence of L-tartrate. To exclude interference from other known C4-dicarboxylate carriers, a strain lacking carriers DcuA, DcuB, DcuC, and CitT was used. After the addition of D,L-[14C]tartrate, representing a mixture of D-[14C]- and L-[14C]tartrate, uptake started rapidly, and cellular contents reached constant levels after about 2 min (Fig. 4A). In the *ttdT* mutant which lacked TtdT in addition to the Dcu and CitT carriers, uptake was not significantly impaired. This is presumably due to alternative uptake carriers (YeaV, YfaV, and others) for tartrate and other C4-dicarboxylates which are formed in addition to the Dcu and DctA carriers when C4-dicarboxylates are present (unpublished data). Succinate, the proposed antiporter substrate of L-tartrate, was taken up by the *TtdT/H11001* strain as well (Fig. 4B), but at a significantly lower rate. However, in the *ttdT* mutant, the succinate uptake rate was decreased to very low levels, indicating that succinate uptake in the *dcuA-, dcuB-, dcuC-, and citT*-negative strain depends on the presence of TtdT.

Succinate uptake was strongly induced in L-tartrate-grown bacteria and repressed in glucose-grown bacteria (Fig. 4). In contrast, succinate did not induce (not shown). Induction by tartrate and repression by glucose are compatible with a role of TtdT in L-tartrate metabolism. Tartrate uptake was also induced by tartrate and repressed by glucose, though to a lesser degree. The background levels without induction and under glucose repression corresponded to about one-half the maximal activity. This background activity and the high residual activity after deletion of *ttdT* suggest that the bacteria contain a further tartrate uptake carrier which supports tartrate uptake but not antiporting and growth by tartrate fermentation. Expression analysis and transport assays support the presence of a further uptake carrier for tartrate and other C4-dicarboxylates (11; unpublished data). The alternative carriers which do not support growth by L-tartrate fermentation are presumably uptake carriers and lack tartrate/succinate antiporting capacity (unpublished data).

[14C]succinate and [14C]tartrate accumulated to final concentrations of 640 μM and 4 mM, respectively, within the bacteria, corresponding to 6.4- and 40-fold accumulations of the substrates (100 μM) in the external medium. The transport rates were highest around neutral pH (pH 7 to 8), exceeding the activities at pHs 5 to 6 by factors of 1.8 to 3.7. The initial rates for tartrate and succinate uptake increased with increases...
ing concentrations with Michaelis-Menten-type kinetics and showed a linear relation of 1/V to 1/S in a Lineweaver-Burk plot. The $K_m$ and $V_{max}$ were about 700 µM and 110 µmol/min/g dry weight for tartrate (L- and D-tartrate mixture) and 400 µM and 16 µmol/min/g dry weight for succinate in L-tartrate-grown bacteria.

**TdtT-dependent L-tartrate/succinate antiporting.** TdtT is suggested to function as an L-tartrate/succinate antiporter, taking up tartrate and excreting succinate. To test the antiporter capacity, anaerobically grown and L-tartrate-induced bacteria were loaded with [14C]succinate or [14C]tartrate by incubation with the substrate. Up to 1.3 or 8.4 µmol succinate or tartrate, respectively, per g dry weight was taken up by bacteria which contained the TdtT carrier but were deficient in the Dcu and CitT carriers (Fig. 5). After the substrates reached maximal levels, unlabeled substrates were added in excess, and the effects on the intracellular levels of the loaded substrates was determined. The [14C]succinate-loaded cells released most of the internal succinate rapidly after the addition of external L-tartrate, which was the most efficient antiporter agent, followed by succinate and fumarate (Fig. 5A and Table 2). Citrate showed a linear relation of $1/V$ to $1/S$ reaction (L-tartrate in/succinate ex) is not efficient.

**TABLE 2. Efflux of [14C]succinate or D,L-[14C]tartrate from loaded cells of E. coli TdtT" CitT" (IMW528 [dcaA dcaB dcaC citT] mutant) after the addition of 2 mM external antiporter substrate.**

<table>
<thead>
<tr>
<th>Antiporter substrate (2 mM)</th>
<th>[14C]succinate</th>
<th>D,L-[14C]tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Tartrate</td>
<td>2.6</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Succinate</td>
<td>2.0</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1.5</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.2</td>
<td>ND*</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.1</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*Activities were determined as described in the experiments shown in Fig. 5 as the release of internal [14C]succinate or D,L-[14C]tartrate 20 s after the addition of the external exchange substrate.

*ND, not detectable.

When TtdT as well as CitT was missing in addition to the Dcu carriers, the release of intracellular [14C]succinate was very low in similar experiments (not shown).

**Competitive inhibition of [14C]tartrate transport.** For more information on the substrate specificity of [14C]succinate and [14C]tartrate uptake by L-tartrate-grown *E. coli*, the uptake was measured in the presence of alternative unlabeled substrates (Fig. 7). The unlabeled substrates were added in a 10-fold excess of the amount of [14C]succinate. In strain IMW528 (dcaA dcaB dcaC citT), which contains only TdtT among the known anaerobic C4-dicarboxylate carriers, the activity for the uptake of [14C]succinate was higher, by a factor of 1.9, than DcuC but containing CitT, the succinate uptake was decreased about 50% (Fig. 6). The addition of citrate caused the most efficient release of succinate (1.32 µmol/min/g dry weight), followed by L-tartrate (0.84 µmol/min/g dry weight) and succinate (0.81 µmol/min/g dry weight), suggesting that in this strain the most efficient antiporter is the citrate/succinate antiporter and that the tartrate/succinate antiporter in strains lacking TtdT indeed relies on the presence of this carrier. When TtdT as well as CitT was missing in addition to the Dcu carriers, the release of intracellular [14C]succinate was very low in similar experiments (not shown).

**FIG. 6. Efflux of [14C]succinate from *E. coli* TdtT" CitT" strain by the addition of external antiporter substrate (arrow). Degassed cell suspensions of *E. coli* IMW524 (dcaA dcaB dcaC ttdT mutant) (OD$_{600}$ = 4) were incubated with 100 µM [14C]succinate (open circles) for 10 min. A 2 mM concentration of L-tartrate (■), succinate (●), fumarate (▲), citrate (▼), or butyrate (▼) was added to the suspension. At the time points indicated, samples were drawn, and the amount of internal [14C]succinate was determined by a filtration assay.

**FIG. 7. Effects of competitor substrates on the uptake of [14C]succinate by *E. coli* IMW528 (dcaA dcaB dcaC citT" mutant) (black bars) and IMW529 (dcaA dcaB dcaC citT" ttdT mutant) (hatched bars). Uptake of 50 µM [14C]succinate was determined after incubation of anaerobic cell suspensions for 1 min with the substrate, without and in the presence of unlabeled competitors (500 µM). A 100% uptake activity corresponds to 0.53 µmol/min/g dry weight for *E. coli* IMW528 and to 0.28 µmol/min/g dry weight for IMW529.
that in the TtdT-deficient strain IMW529 (deuA deuB ducC citT ttdT). In the TtdT+ strain, the presence of 500 μM l-tartrate inhibited 85% of the uptake activity for [14C]succinate, whereas D-tartrate and fumarate had much smaller inhibitory effects. Unlabeled succinate (not shown) decreased the uptake of [14C]succinate in the same strain to 45% of the original activity. The presence of additional (uptake) carriers for succinate which function in the presence of high concentrations of succinate (11) can explain the relatively low degree of competitive inhibition. Overall, the competition experiments are complicated by (so far unknown) carriers for C₄-dicarboxylates which are present in E. coli in addition to the DctA, DcuA, DcuB, DcuC, CitT, and TtdT carriers, but the experiments support the specificity of TtdT for l-tartrate. In the ttdT-negative strain, the activity was not significantly inhibited by l-tartrate, and the effects of the other C₄-dicarboxylates were comparable to those in the TtdT+ strain (Fig. 7). Therefore, in this background, another C₄-dicarboxylate carrier which is not l-tartrate specific probably operates. However, interference from other carriers was only observed for tartrate or C₄-dicarboxylate uptake by TtdT, i.e., for partial reactions. No interference or nonspecificity was found for the complete reaction, as demonstrated by the lack of antiporting of l-tartrate/succinate in and of growth on l-tartrate in the ttdT deletion strain (Fig. 3A).

TtdT is the l-tartrate/succinate antiporter of l-tartrate fermentation in E. coli. The TtdT (previously called YgiE) carrier has been shown here to be the l-tartrate/succinate antiporter which operates in anaerobic l-tartrate fermentation. The carrier is essential for growth by l-tartrate fermentation and for anaerobic l-tartrate degradation, and it preferentially catalyzes heterologous l-tartrate/succinate antiporting, which proves its function as the l-tartrate/succinate antiporter of l-tartrate fermentation. The antiporter activity in the physiological direction (l-tartrate/succinate) is higher, by a factor of >13, than the reverse activity (succinate/l-tartrate). The transport experiments demonstrated that E. coli contains an additional (unknown) tartrate carrier which catalyzes uptake only, with no antiporting. This additional transport does not play a role in anaerobic growth on tartrate.

Transport of l-tartrate appears to be the only transport of a C₄-dicarboxylate in the anaerobic metabolism of E. coli which does not use the general Dcu carrier. DcuB has broad substrate specificity and accepts all physiologically relevant C₄-dicarboxylates (succinate, fumarate, malate, and aspartate) apart from l-tartrate. Surprisingly, the two vicinal -OH groups at l-tartrate obviously are not accepted by the Dcu carriers. TtdT is much more specific and accepts only l-tartrate, not D-tartrate, as the external substrate. In addition, TtdT is selective with respect to the transport mode and preferentially catalyzes heterologous antiporting (l-tartrate/succinate), similar to CitT catalyzing heterologous citrate/succinate antiporting. The Dcu carriers, in contrast, are able to shift between the uptake, antiporter, and efflux transport modes and catalyze homo- or heterologous antiporting (6, 12, 32). By the use of a tartrate-specific carrier (TtdT) and tartrate-specific transcriptional regulation (20), l-tartrate metabolism is physiologically separated from general C₄-dicarboxylate metabolism of fumarate, malate, and aspartate, although l-tartrate ends up in fumarate respiration as well.

TtdT is a member of the carbohydrate/C₄-dicarboxylate carrier family, which is a subgroup of the DASS family (divalent anion: Na⁺ symporter) (12, 23; http://www.tcdb.org/index.php). The best-characterized bacterial member of this family is the citrate/succinate antiporter CitT of E. coli (22), which shares 45% sequence identity with TtdT of E. coli. CitT can alternatively use citrate, fumarate, or tartrate as an antiporter substrate with low affinity and activity. Homologs of TtdT/YgiE have been identified in enteric and other bacteria (22, 31), and all preferentially catalyze heterologous antiporting of carboxylic acids. For CitT and TtdT/YgiE, 12 or 13 transmembrane helices are predicted, with similar arrangements. Both proteins show similar distributions of polar amino acid residues, which are concentrated in the loops between the transmembrane helices. The polar residues include a considerable number of positively or negatively charged amino acid residues, many of which are conserved in CitT and TtdT/YgiE. This conservation suggests a role in binding of the carboxylates and/or a structural role.

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