Functional Exchangeability of the ABC Proteins of the Periplasmic Binding Protein-Dependent Transport Systems Ugp and Mal of Escherichia coli

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The periplasmic binding protein-dependent transport systems Ugp and Mal of Escherichia coli transport sn-glycerol-3-phosphate and maltose, respectively. The UgpC and MalK proteins of these transport systems, which couple energy to the transport process by ATP-hydrolysis, are highly homologous, suggesting that they might be functionally exchangeable. Complementation experiments showed that UgpC expression could restore growth of a malK mutant on maltose as a carbon source, provided that it was expressed at a sufficiently high level in the absence of the integral inner membrane components UgpA and/or UgpE of the Ugp system. Conversely, MalK expression could complement ugpC mutants and restore the utilization of sn-glycerol-3-phosphate as a phosphate source. The hybrid transporters appeared to be less efficient than the wild-type systems. The complementation of ugpC mutations by MalK was strongly inhibited by the presence of glucose or α-methylglucoside, which are substrates of the phosphotransferase system. This inhibition is probably due to hypersensitivity of the hybrid UgpBAE-MalK transporter to inducer exclusion. UgpC expression did not complement the regulatory function of MalK in mal gene expression. The exchangeability of UgpC and MalK indicates that these proteins do not contribute to a substrate-binding site conferring substrate specificity to the transporter. These are the first examples of functional, hybrid periplasmic permeases in which the energy-coupling components could be functionally exchanged.

Escherichia coli K-12 possesses two major uptake systems for sn-glycerol-3-phosphate (G3P): the GlpT system and the Ugp system. The synthesis of the GlpT system is induced by the presence of G3P in the growth medium (10). GlpT is a low-affinity antipporter that mediates the uptake of G3P by exchange against cellular P3 (1, 16). Therefore, the GlpT system is geared for the use of G3P as a carbon source. The Ugp system is a high-affinity, periplasmic binding protein (BP)-dependent transport system encoded by the ugp operon (Fig. 1) at min 76 of the chromosomal map (35). Like many other BP-dependent transport systems (for reviews, see references 3 and 19), the Ugp system consists of a periplasmic substrate-BP, encoded by ugpB, and two hydrophobic integral inner membrane proteins, encoded by ugpA and ugpE, that form a substrate-translocating complex with probably two copies of the ugpC gene product. The latter protein is a more hydrophilic ATP-binding protein which is a member of the ATP-binding cassette (ABC) protein family (22). By analogy to the corresponding proteins of related transport systems (2, 11), it is supposed to be peripherally bound to the inner membrane and to energize the transport system by ATP-hydrolysis. The fifth gene of the ugp operon, ugpQ (23, 45), encodes an enzyme that hydrolyzes sn-glycerophosphoryl diesters which are also transported by the Ugp system (8).

As a member of the pho regulon, the Ugp system has synthesis induced by P3, starvation (4). Physiologically, the Ugp system is geared for the use of G3P as a phosphate source and not as a carbon source (6–8). Nevertheless, induction of the operon by carbon starvation has also been demonstrated (24, 44).

The UgpB, A, E, and C proteins have the same general characteristics as the corresponding proteins of other BP-dependent transport systems. However, a substantially higher sequence homology was observed with the MalE, F, G, and K proteins, respectively, of the maltose-maltodextrin transport system (35). Especially, the energy-coupling components of the systems, UgpC and MalK, are much more closely related to each other than to the corresponding components of other transport systems. In addition to the higher sequence homology in the N-terminal part, which contains the ATP-binding site (approximately 60% sequence identity), both proteins have a C-terminal extension, which also shows some homology (35). Apart from its role in energizing maltose transport, MalK serves as the target for enzyme III8 of the phosphotransferase system (PTS) in the process of inducer exclusion (13, 32, 39), resulting in inhibition of transport. Furthermore, MalK negatively regulates the expression of mal genes (14, 21, 37). The C-terminal extension has been suggested to function as the regulatory domain of MalK in mal gene expression (27, 40) and (partly) in inducer exclusion (13, 27). Since UgpC and MalK appear so significantly related, we considered in the present study the possibility that they could complement each other’s functions.

MATERIALS AND METHODS

Strains and growth media. Bacterial strains used are listed in Table 1. The malK mutant strains CE1350 and CE1356 were constructed by P1 transduction (33) with ME347 as the donor strain and K10 and C86, respectively, as acceptor strains. The ugpB::cat, ugpA::cat, and ugpE::cat mutations were similarly introduced in Lin221 by using SH7498, SH7500, and SH7505, respectively, as donor strains, resulting in strains CE1365, CE1366, and CE1367. The ugpA::cat and ugpE::cat mutations were also introduced in CE1356, resulting in strains CE1357

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and CE1358. The ugpC::kan, ugpQ::kan, and ugpCQ-orfQ::kan mutations were first constructed on plasmids (see description below). These mutations were introduced in the chromosome of recBC sbcB strain AM1095 by homologous recombination after transformation with linear plasmid DNA fragments, selection for kanamycin-resistant transformants, and screening for ampicillin sensitivity.

When grown on G3P as the sole phosphate source was tested, a synthetic medium in which the phosphate concentration can be varied (46) was used. This medium was supplemented with a carbon source and with 1 mM Na-G3P (Sigma) as a phosphate source and solidified with 0.8% agarose (Sigma) when required. For induction or repression of the phospho regulon, the medium without G3P supplemented with 45 μM (low-Pi) or 5 mM (high-Pi) K₂HPO₄ as a phosphate source was used. As carbon sources, 0.2% glucose, galactose, or maltose, 0.26% potassium gluconate, and 0.4% (wt/vol) glycerol were used. When required, α-methylglucoside (αMG; Sigma) was added to a concentration of 20 mM. Media were supplemented when necessary with the appropriate antibiotics, i.e., ampicillin, 75 μg/ml; chloramphenicol, 25 μg/ml; spectinomycin, 100 μg/ml; and kanamycin, 20 μg/ml. When required, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added.

Plasmids and DNA manipulations. DNA manipulations and transformation of strains were performed essentially as described previously (31). In the transformation procedure for recombination of linear plasmid DNA fragments into the chromosome, bacteria were incubated for 30 min at 0°C after heat shock. Restriction endonucleases and other DNA-modifying enzymes were purchased from Pharmacia (Uppsala, Sweden) or New England Biolabs. Plasmids used are listed in Table 2. Plasmids pH19, pH10, and pH20 were constructed by cloning the PstI-BglII fragment from pH12 containing ugpC (Fig. 1) into the PstI-BamHI-digested multiple cloning sites of the high-, medium-, and low-copy-number vectors pUC19, pHG329, and pCL1920, respectively. In all three resulting plasmids, ugpC is under lac promoter control. Plasmid pH30 was constructed by cloning the BamHI fragment of pH11, containing malK under tre promoter control, into the BamHI site of pCL1920. In pH30, the lac promoter is oriented opposite to malK. The ugp::kan mutations were constructed on plasmids with the Kan’ cassette of pUC4K. This cassette was cloned as a HindII fragment into the unique EcoRV site in ugpC on plasmid pH10, resulting in plasmid pH41, and as a BamHI fragment into the unique BglII site in ugpQ on plasmid pH37, resulting in plasmid pH42. Furthermore, this cassette, as a HindII fragment, was substituted for the EcoRV fragment of pH12 encoding (part of) ugpC, ugpQ, and orfQ (Fig. 1), resulting in plasmid pH40. Plasmids

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<td>A. Garen⁴</td>
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⁴ Via E. coli Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, Conn.
TABLE 2. Plasmids used in this study

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<td>pCL1920, Plac ugpC'</td>
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<td>pCL1920, Prc-malK'</td>
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<td>pSH12, Δ(ugpCQ-orfQ-kan)</td>
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<td>pDH41</td>
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<td>pDH42</td>
<td>pJP371, ugpQ::kan</td>
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* Amp, ampicillin; Spec, spectinomycin; Cam, chloramphenicol; Kan, kanamycin.

with the Kan' gene in the same orientation as the ugp genes were used to construct chromosomal ugp::kan mutations by gene replacement as described above.

Preparation of UgpC antisera. UgpC was isolated from ompT strain UTS600 harboring plasmid pDH19, since the protein was degraded when isolated from an ompT+ strain. After cell disruption by ultrasonication and centrifugation for 1 h at 12,000 × g, the heavily overproduced UgpC pelleted, probably as inclusion bodies, with the crude membrane fraction. The pellet was dissolved in 8 M urea and centrifuged for 1 h at 250,000 × g to remove insoluble debris. Thus, dissolved UgpC was purified from preparative sodium dodecyl sulfate (SDS)-polyacrylamide gels and used to immunize a rabbit by the procedures described previously (26). UgpC antiserum was preadsorbed for 6 h with an equal volume of a crude extract of ugpC mutant strain CE1368. The extract was prepared by ultrasonic disruption and boiling for 15 min of a 20-fold concentrated overnight culture in phosphate-buffered saline (PBS) containing 0.1% SDS–0.1 mM EDTA–8% Triton X-100. After preadsorption and centrifugation for 45 min at 16,000 × g, the supernatant obtained was used for immunoblotting.

Immunoblotting. Cells were boiled in sample buffer, and total cellular proteins (equivalents of 100 μl of the culture at an optical density at 660 nm of 1) were separated by SDS-polyacrylamide gel electrophoresis (29) and subsequently transferred onto nitrocellulose filters (Schleicher and Schuell BA85; 0.45-μm pore size) by using a semidyry blotting system (LKB2117 Multiphor II). The blots were blocked by incubation for 1 h in 0.5% nonfat dried milk (Protifar; Nutricia, Zoetermeer, The Netherlands) in PBS and subsequently incubated overnight at 4°C with antiseras, washed three times with water and 0.1% nonfat dried milk in PBS for 5 min, and incubated for 1 h with peroxidase-conjugated secondary antibodies (TAGO, Burlingame, Calif.). After washing, the peroxidase activity was visualized with a solution of 0.5 mg of 4-chloronaphthol per ml in 15% methanol–85% PBS–0.01% H2O2.

β-Galactosidase assay. After a 1:20 dilution of an overnight culture into fresh LB medium, cells were grown for 2 h. β-Galactosidase activity was determined essentially as described previously (33).

RESULTS

UgpC expression can complement a malK mutation for growth on maltose. Since UgpC is expressed mainly under P starvation, possible complementation of malK mutations by UgpC would previously have remained undetected in the commonly used high-phosphate media. To test for possible complementation, a malK mutation was introduced in phoS strain C86, which expresses the pho regulon and consequently the Ugp system constitutively. However, the resulting strain, CE1356, did not grow on maltose (Fig. 2, segment 4). The lack of possible complementation could still be explained by too low an expression level of UgpC or, alternatively, by UgpC having a different affinity for the integral cytoplasmic membrane proteins of the Ugp system than for those of the Mal system. In the latter case, complementation of the malK mutation might have been observed by expression of UgpC independent of UgpA and/or UgpE. Indeed, ugpA and ugpE derivatives of CE1356 (i.e., strains CE1357 and CE1358, respectively) did grow on maltose as a carbon source (Fig. 2, segments 5 and 6), although somewhat more slowly than the malK+ parental strain C86 (Fig. 2, segment 3). If this complementation of the malK mutation is indeed the result of expression of the ugpC gene, the mutations in ugpA and ugpE, which are insertions of the cat gene, apparently do not negatively affect the expression of the downstream-located ugpC gene. Western blot (immunoblot) analysis revealed that the UgpC protein was even somewhat

FIG. 2. Complementation of a malK mutation by UgpC expression. Bacteria were grown on minimal maltose media for 2 days (left- and right-hand plates) or for 3 days (middle plate). The right-hand plate contained IPTG. Segments: 1, malK mutant CE1350; 2, wild-type K10; 3, phoS mutant C86; 4, malK phoS mutant CE1356; 5, malK phoS ugpA mutant CE1357; 6, malK phoS ugpE mutant CE1358; 7, wild-type K10 containing vector pHG329; 8 to 12, malK mutant CE1350 containing plasmids (8, pDH10 [ugpC]; 9, pHG329 [vector]; 10, pDH30 [malK]; 11, pDH20 [ugpC]; 12, pCL1920 [vector]).
FIG. 3. Western immunoblot on total cellular proteins probed simultaneously with UgpC- and MalK-specific antisera. All cells were grown on LB medium supplemented as indicated below. Lanes: 1, wild-type K10 grown with maltose; 2, malK mutant CE1350; 3 and 12, phoS mutant C86 grown with maltose; 4, malK phoS mutant CE1356; 5, malK phoS ugpA mutant CE1357; 6, malK phoS ugpE mutant CE1358; 7 to 11, malK mutant CE1350 containing plasmids (7, pDH20; 8, pDH20 induced with IPTG; 9, pDH10; 10, pDH30; 11, pCL1920). All lanes contained equal quantities of cells.

FIG. 4. Complementation of a ugpC mutation by MalK expression. Bacteria were grown for 3 days on minimal media containing G3P as the sole phosphate source. The carbon sources and additives are as indicated. Segments: 1, 4, and 7, ugpCQ mutant CE1370 containing vector pCL1920; 2, 5, and 8, CE1370 containing ugpC on pDH20; 3, 6, and 9, CE1370 containing malK on pDH30.
promoter (Fig. 4, segments 2, 5, and 8). However, when the carbon source was glucose, UgpC expression in the strains harboring pH20 had to be induced by adding IPTG to the medium to enable growth on G3P as phosphate source. This was probably due to catabolite repression of the lac promoter.

When the possibility that MalK expression could complement the ugpC mutations was tested, an additional function of MalK had to be taken into consideration. In addition to its role in energizing maltose transport, MalK serves as the target for enzyme IIIGlc of the PTS in the process of inducer exclusion (13, 32, 39). Transport of the PTS substrate glucose or the nonmetabolizable glucose analog αMG leads to inhibition of maltose transport. This inhibition is mediated by the allosteric interaction of dephosphorylated enzyme IIIGlc with MalK. Strain CE1370 harboring pH30 did not grow on G3P as the sole phosphate source when glucose was present as the carbon source (Fig. 4, segment 3). However, when the non-PTS substrates, glycerol (Fig. 4, segment 6), galactose, and gluconate (results not shown), were used as carbon sources, significant growth was observed. Apparently, MalK expression restored G3P uptake by the formation of a functional hybrid UgpBAE-MalK transporter. This hybrid transporter, however, appeared to be less efficient than the wild-type Ugp transporter, since the colonies obtained were clearly smaller. Furthermore, the MalK-dependent growth was inhibited by addition of αMG (Fig. 4, segment 9), which also indicates that the hybrid transporter is sensitive to inducer exclusion. Glycerol transport and galactose transport are also regulated by inducer exclusion (36, 39). Nevertheless, growth inhibition of CE1370(pH30) by αMG was not due to reduced carbon transport, since addition of Pi (5 mM K2HPO4) restored growth of the cells on these carbon sources in the presence of αMG (results not shown). Therefore, the observed growth inhibition of strain CE1370(pH30) by αMG or glucose (Fig. 4) is due to reduced G3P transport via the hybrid transporter. In conclusion, MalK expression can complement a ugpC mutation to some extent, and the hybrid G3P transporter consisting of UgpBAE-MalK is very sensitive to inducer exclusion.

Strain CE1370 containing an intact mal regulon on the chromosome did not grow on minimal medium plates with maltose as the carbon source and G3P as the phosphate source (results not shown). The growth defect was not due to limiting carbon uptake, since addition of Pi (5 mM K2HPO4) restored growth. These results indicate that MalK is titrated under these circumstances, probably because of its higher affinity for MalF and MalG than for UgpA and UgpE.

UgpC does not complement the regulatory function of MalK. In addition to its role in energizing maltose transport and in inducer exclusion, MalK has a third function, i.e., it negatively regulates mal gene expression. In malK mutants, other mal genes are constitutively expressed (14, 21), whereas overproduction of MalK results in repression of the remaining mal genes (37). Mutations abolishing this regulatory function have been localized in the C-terminal part of MalK (27, 40).

Since strain BRE1162 contains a malK-lacZ operon fusion (5), malK promoter activity could be monitored by measuring β-galactosidase activity. The constitutive β-galactosidase activity of this strain (Fig. 5, bar 2) was repressed by expression of MalK from plasmid pH30 (Fig. 5, bar 3) but not by IPTG-induced expression of UgpC from plasmid pH20 (Fig. 5, bar 5) or by plasmid pH10 (results not shown). Apparently, UgpC does not complement the repressor function of MalK in mal gene expression.

**Discussion**

All BP-dependent transport systems have their own specific energy-coupling component(s) despite striking functional and structural similarities between these proteins (22, 34). The energy-coupling components have parts in common that are functionally interchangeable, as was shown by the construction of a functional chimeric HisP-MalK protein (40). Here, we have shown that even complete proteins are functionally exchangeable between two transporters with widely differing substrate specificities. UgpC expression could complement a malK mutation in maltose utilization, even though UgpC was less efficient than MalK, since cells grew more slowly when depending on UgpC for maltose transport. The energy-coupling components of the transporters are thought to be anchored to the membrane in a complex with the integral inner membrane proteins of the systems (12, 18, 25). Coexpression of UgpA and UgpE with UgpC could inhibit UgpC-mediated maltose utilization. Probably, UgpC has a higher affinity for UgpA and UgpE than for MalF and MalG. Therefore, UgpC had to be overproduced independently of UgpA and/or UgpE to complement the malK mutation. Similarly, MalK expressed from a plasmid in the absence of MalF and MalG could complement ugpC mutants for growth on G3P as the sole phosphate source, although less efficiently than UgpC. Also in this case, a lower affinity of MalK for the UgpA and UgpE proteins in the inner membrane may account for this lower efficiency. Probably, the slower growth phenotype of cells depending on a hybrid transporter for growth on G3P or maltose will enable the selection of more efficient mutants with improved interactions in the transport complex. This approach, which we are presently pursuing, is expected to lead to the identification of amino acid residues that are involved in these interactions.

The presence of either of the PTS substrates, glucose or αMG, strongly inhibited the G3P transport function of MalK. This is probably due to inducer exclusion (39), i.e., inhibition of the MalK transport function by enzyme IIIGlc of the PTS (13). The hybrid G3P transporter, consisting of UgpBAE-MalK, appeared to be hypersensitive to inducer exclusion, since in an otherwise wild-type PTS background, the utilization of G3P.
was inhibited by αMG, whereas glycerol utilization was unaffected. This hypersensitivity may be due to less efficient protein-protein interactions in the hybrid transport complex. Interestingly, UgpC and MalK share their C-terminal extensions a “hot spot” of homology (35) that is very similar to a conserved region that is found in several proteins regulated by inducer exclusion (GlpK, LacY, and MelB) and that possibly defines an enzyme IIIβSc-binding domain (13). Furthermore, even though the Ugp system is geared for the uptake of G3P as a phosphate source and not as a carbon source (8), ugp transcription is partially under crp control (24, 44) like other permeases regulated by inducer exclusion. This raises the question of whether UgpC is also regulated by inducer exclusion and actually binds IIIβSc. In a wild-type PTS background, however, glucose or αMG did not appear to interfere significantly with the transport function of UgpC, whether functioning in its own system (Fig. 4) or in maltose transport (result not shown). However, this result does not completely rule out the possibility that the Ugp system is sensitive to inducer exclusion. This possibility will be further evaluated in a PTS mutant background that renders non-PTS substrate transport hypersensitive to inducer exclusion (39).

MalK also has a negative regulatory role in mal gene expression (14, 21, 37). Mutations abolishing this regulatory function have been localized in the C-terminal extension of MalK (27, 40). UgpC and MalK share some homology (approximately 28%) in their C-terminal extensions. Despite these similarities, UgpC could not complement the repressor function of MalK in mal gene expression.

It has been suggested that HisP, the analog of UgpC and MalK in histidine permease, contributes to the formation of a substrate-binding site in the membrane-bound complex (42). Hence, HisP would be involved in the substrate specificity of the histidine transporter. Furthermore, it has recently been shown that the ATPase activity of purified MglA, the energy-coupling component of galactose permease, is stimulated by galactose (38), suggesting that MglA possesses a galactose-binding site. Since the substrate specificities of the Ugp and the Mal systems differ widely and the UgpC and MalK proteins could be functionally exchanged, the presence of a substrate-binding site on these proteins, which is essential for the functioning and the substrate specificities of the cognate permeases, seems to be highly unlikely. However, the presence of a secondary substrate-binding site exerting its effect on the efficiency of the permeases cannot be ruled out completely. The existence of such a secondary substrate-binding site on the energy-coupling components of the BP-dependent permeases would provide an alternative explanation for the observation that the hybrid transporters were less efficient than the wild-type systems.

In conclusion, we have shown that the conserved energy-coupling components of two BP-dependent transport systems can be functionally exchanged. By extrapolation, this suggests a highly conserved, strictly energy-coupling role for the ATP-binding component in the transport mechanism for all these transporters. Substrate specificity is apparently conferred not by the energy-coupling protein(s) but by the other components of the transport systems.

ACKNOWLEDGMENTS

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REFERENCES


