BglF, the Escherichia coli β-Glucoside Permease and Sensor of the bgl System: Domain Requirements of the Different Catalytic Activities

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The Escherichia coli BglF protein, an enzyme II (EI) of the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS), catalyzes concomitant transport and phosphorylation of β-glucosides (11). In addition, BglF regulates bgl operon expression by controlling the activity of the transcriptional regulator BglG. In the absence of β-glucosides, BglF phosphorylates BglG, thus inactivating it; in the presence of β-glucosides, BglF dephosphorylates BglG, which can then function as a transcriptional antiterminator and enable bgl operon expression (1, 2, 3, 24). Thus, BglF is the β-glucoside phosphotransferase, the BglG kinase, and the phosphorylated BglG (BglG-P) phosphatase. A dimeric form of BglF can catalyze all these activities (7).

Like other EIIs of the PTS, BglF is composed of three domains. IIAbgl possesses the first phosphorylation site, His547 (site 1), which is phosphorylated by HPr; IIBbgl possesses the second phosphorylation site, Cys24 (site 2), which accepts the phosphoryl group from IIAbgl and transfers it to β-glucosides; and IICbgl, the membrane-spanning domain, presumably forms the sugar translocation channel and at least part of the sugar-binding site (9, 25). The order of these domains in BglF is IICBAbgl (reviewed in references 17 and 20). BglF uses site 2, Cys24 on the IIBbgl domain, to phosphorylate the two substrates, β-glucosides and BglG (9), and to dephosphorylate BglG-P (10). A rearranged BglF protein which contains the three domains in the order IICBAbgl (scrambled-BglF) catalyzes BglG phosphorylation but fails to carry out the sugar-induced reactions, i.e., sugar phosphotransfer and BglG-P dephosphorylation (8). These findings suggest that the structural requirements for the sugar-induced reactions differ from those for the noninduced function. The key to BglF stimulation, i.e., the sugar-induced change which switches it from a BglG kinase mode to a BglG-P phosphatase and sugar phosphotransferase mode, is not understood.

To define the domain(s) required for catalysis of the different functions of BglF, we subcloned and expressed the three individual domains, IIAbgl, IIBbgl, and IICbgl, as well as truncated BglF proteins which lack one domain (IIICbgl and IICAbgl). We show that phosphorylated IIBbgl alone can phosphorlylate BglG in vitro and negatively regulate its activity as a transcriptional antiterminator in vivo. In contrast, phosphorylated IIBbgl alone can catalyze BglG inactivation by phosphorylation. Thus, the sugar-induced and noninduced functions have different structural requirements. Our results suggest that catalysis of the sugar-induced functions depends on specific interactions between IIBbgl and IICbgl which occur upon the interaction of BglF with the sugar.

MATERIALS AND METHODS

Strains. The following E. coli K-12 strains were used: K38 (HfrC trpR thi λ') was obtained from C. Richardson. LM1 contains mutations in the nagE and cor genes, which code for EIInag and IIAglc, respectively (13). ZSC112G contains a deletion of the ptsG gene, which codes for IICBgl and IICAglc (9). We show that phosphorylated IIBbgl alone can phosphorylate BglG in vitro and negatively regulate its activity as a transcriptional antiterminator in vivo. In contrast, IICbgl is required for β-glucoside phosphorylation in vitro and for β-glucoside utilization in vivo. The same holds true for BglG-P activation by dephosphorylation. These results suggest that the immediate environment of the BglF active site changes upon sugar stimulation. This change induces specific interactions between the active site-containing domain, IIBbgl, and the membrane-bound domain, IICbgl.

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His-S477 mutated to Arg (H547R) (9). Plasmid pMN5 carries the entire bglF gene cloned in pBR322 (14). Plasmids pT7OAC-F, pT7CQ-F3, pT7CQ-F5, pT7CQ-F6, and pT7CQ-F8 are derivatives of pT712 which code for IIB bgl, IIC bgl, IIA bgl, and IIBC bgl, respectively, from the T7 promoter (7). Additional plasmids were constructed as described below. pACO-F5, which encodes IIB bgl, was constructed by ligating a 933-bp EcoRI-PvuII fragment from pT7CQ-F3 to a 3388-bp EcoRI-SalI fragment from pACYC184. pACO-F6, which encodes IICA bgl, was constructed by ligating a 2065-bp EcoRI-PvuII fragment from pT7CQ-F6 to a 3388-bp EcoRI-SalI fragment from pACYC184. pACO-F8, which encodes IIBC bgl, was constructed by ligating a 1730-bp EcoRI-PvuII fragment from pT7CQ-F8 to a 3388-bp EcoRI-SalI fragment from pACYC184. pQF-E5, which encodes IIA bgl tagged with six histidines, was constructed by ligating a 968-bp PstI-PvuII fragment from pT7CQ-F5 to a 3091-bp PstI-PvuII fragment from pQF-E30. Chemicals. [32P]P-ATP (7000 Ci/mmol) was obtained from ICN. [35S]Methionine (1200 Ci/mmol) was obtained from Du Pont. PEP, pyruvic acid, and pyruvate kinase were obtained from Sigma. [32P]PEP was prepared and separated from [32P]ATP as described before (1). Purified enzyme I (EI) and HPr were obtained from Qiagen.

**TABLE 1. Plasmids encoding BglF derivatives**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Encoded BglF derivative</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT7OAC-F</td>
<td>Wild-type BglF (IICA bgl)</td>
<td>1</td>
</tr>
<tr>
<td>pT7CQ-F3</td>
<td>IIBbgl</td>
<td>7</td>
</tr>
<tr>
<td>pT7CQ-F4</td>
<td>IICbgl</td>
<td>7</td>
</tr>
<tr>
<td>pT7CQ-F5</td>
<td>IIA bgl</td>
<td>7</td>
</tr>
<tr>
<td>pT7CQ-F6</td>
<td>IICA bgl</td>
<td>7</td>
</tr>
<tr>
<td>pT7CQ-F8</td>
<td>IIBC bgl</td>
<td>7</td>
</tr>
<tr>
<td>pMN5</td>
<td>Wild-type BglF (IICA bgl)</td>
<td>14</td>
</tr>
<tr>
<td>pACO-F3</td>
<td>IIB bgl</td>
<td>This work</td>
</tr>
<tr>
<td>pACO-F6</td>
<td>IICA bgl</td>
<td>This work</td>
</tr>
<tr>
<td>pACO-F8</td>
<td>IIBC bgl</td>
<td>This work</td>
</tr>
<tr>
<td>pOE-F5</td>
<td>IIA bgl tagged with six histidine</td>
<td>This work</td>
</tr>
</tbody>
</table>

**RESULTS**

To define the domains required for the different functions of BglF, we subcloned the individual domains, IIB bgl, IIC bgl, and IIBC bgl, as well as the domain pairs, IICA bgl and IIBC bgl. We then examined the ability of the different polypeptides (i) to be phosphorylated in vitro in the presence of PEP and the general PTS enzymes protein I (EI) and HPr; (ii) once phosphorylated, to transfer the phosphoryl group to β-glucosides and to BglG in vitro; and (iii) to mediate β-glucoside utilization and modulate BglG activity in vivo.

The stability of the different plasmid-encoded polypeptides was assayed with pulse-chase experiments. The proteins were labeled with [35S]methionine and chased with unlabeled methionine. Samples removed at different times were analyzed by SDS-polyacrylamide gel electrophoresis. The results (Fig. 1) demonstrate that IIB bgl, IIC bgl, IICA bgl, and IIBC bgl expressed from the heat-inducible T7 promoter are very stable, like wild-type BglF, although they are produced at variable levels.

Due to the low level of IIA bgl produced under the control of the T7 promoter, we engineered histidine-tagged IIA bgl expressed from an IPTG-inducible promoter (see Materials and Methods). The His-tagged IIA bgl polypeptide was purified almost to homogeneity by affinity chromatography (Fig. 2A). The ability of His-tagged IIA bgl to accept a phosphoryl group from HPr and to deliver it to site 2 of BglF was tested in vitro as follows. Purified His-tagged IIA bgl was incubated with [32P]PEP, purified EI and HPr, and membranes of strain LM1 (with deletions of the crp and nagE genes) enriched for a BglF mutant protein which lacks phosphorylation site 1 (HS747R). The results presented in Fig. 2B demonstrated that His-tagged IIA bgl enabled efficient phosphorylation of HS747R (lane 5). HS747R was not phosphorylated in this in vitro phosphorylation system when His-tagged IIA bgl was omitted (Fig. 2B, lane 2). His-tagged IIB bgl was engineered and purified in a similar manner.

IIB bgl is sufficient for BglG phosphorylation, whereas IIBC bgl is required for β-glucoside phosphorylation in vitro. It was previously shown that His-S477 in IIA bgl accepts the phosphoryl group from HPr and transfers it to Cys-24 in IIBC bgl, which can then deliver it to β-glucosides or to BglG (9). It has also been
demonstrated that the dephosphorylation of BglF in the presence of β-glucosides in vitro is a good indication of the ability of BglF to transfer the phosphoryl group to the sugar (1). We therefore tested the ability of different combinations of BglF domains to be phosphorylated in vitro and then to be dephosphorylated by the β-glucoside salicin.

To test IIBC<sup>bgl</sup> phosphorylation and dephosphorylation, we produced the protein in strain ZSC112ΔG, which has a deletion of the <i>ptsG</i> gene. This strain does not contain IIC<sub>bgl</sub>, which is present in membrane preparations of <i>E. coli</i> strains, such as LM1, in significant amounts (9) and which migrates very close to IIBC<sub>bgl</sub> on SDS-polyacrylamide gels. Mem-

![Image of figure 1](https://example.com/figure1.png)

**FIG. 1.** Individual BglF domains and domain pairs are stable. Expression of wild-type BglF, IIB<sup>bgl</sup> (B), IIC<sup>bgl</sup> (C), IICA<sup>bgl</sup> (CA), and IIBC<sup>bgl</sup> (BC), was induced from pT7OAC-F, pT7CQ-F3, pT7CQ-F4, pT7CQ-F6, and pT7CQ-F8, respectively, in <i>E. coli</i> K38 cells harboring pGPI-2. The plasmid-encoded proteins were pulse-labeled with [35S]methionine for 2 min and chased by the addition of unlabeled methionine to the growth medium. Aliquots, removed at the times indicated, were analyzed on SDS–10% polyacrylamide gels (A) or on tricine–SDS–16.5% polyacrylamide gels (B). Autoradiograms of the gels are shown. An unstable protein (a truncated BglG protein) was used as a control for chase success (data not shown). Molecular masses of protein standards are given in kilodaltons.

![Image of figure 2](https://example.com/figure2.png)

**FIG. 2.** Histidine-tagged IIA<sup>bgl</sup> is functional. (A) Expression of His-tagged IIA<sup>bgl</sup> was induced by IPTG from plasmid pQE-F5 in strain SG13009 harboring plasmid pREP4 (lane 1). His-tagged IIA<sup>bgl</sup> was purified on an Ni-NTA column (lane 2) (see Materials and Methods). Samples were analyzed on tricine–SDS–16.5% polyacrylamide gels, followed by Coomassie blue staining. (B). Membranes of LM1 cells that overproduced wild-type (WT) BglF or H547R proteins were incubated with [32P]PEP and purified EI and HPr for 10 min without (lanes 1 and 2) or with (lanes 4 and 5) His-tagged IIA<sup>bgl</sup>. No BglF, membranes from cells which did not overproduce BglF but which were otherwise identical to the other membrane preparations used in this experiment. Samples were analyzed on SDS–10% polyacrylamide gels, followed by autoradiography. Molecular masses of protein standards are given in kilodaltons.
branes of ZSC112ΔG enriched for IIBC\textsuperscript{bgl} were incubated with \[^{32}\text{P}]\text{PEP} and purified EI and HPr for 10 min. Incubation was continued with (+) or without (−) 0.2% salicin for 5 min. Samples were analyzed on SDS–10% polyacrylamide gels (A) or on tricine–SDS–8 to 20% gradient polyacrylamide gels (B). Autoradiograms are presented. Molecular masses of protein standards are given in kilodaltons. Control, membranes from cells which did not overproduce BglF or any of its domains but which were otherwise identical to the other membrane preparations used in this experiment. EI and BglF comigrated in the gel system used in panel A.

FIG. 3. Phosphorylated IIBC\textsuperscript{bgl} is required for the phosphorylation of β-glucosides. Wild-type BglF and IIBC\textsuperscript{bgl} (BC) were overproduced in ZSC112ΔG, a \textit{ptsG} strain. IIB\textsuperscript{bgl} (B), IIC\textsuperscript{bgl} (C), and IICA\textsuperscript{bgl} (CA) were overproduced in LM1, a \textit{crr} and \textit{nagE} strain. His-tagged IIA\textsuperscript{bgl} (A) was purified on an Ni-NTA column. Mixtures of the indicated proteins were incubated with \[^{32}\text{P}]\text{PEP} and purified EI and HPr for 10 min. Incubation was continued with (+) or without (−) 0.2% salicin for 5 min. Samples were analyzed on SDS–10% polyacrylamide gels (A) or on tricine–SDS–8 to 20% gradient polyacrylamide gels (B). Autoradiograms are presented. Molecular masses of protein standards are given in kilodaltons. Control, membranes from cells which did not overproduce BglF or any of its domains but which were otherwise identical to the other membrane preparations used in this experiment. EI and BglF comigrated in the gel system used in panel A.

To check whether IIB\textsuperscript{bgl} not linked to IIC\textsuperscript{bgl} can be dephosphorylated by salicin, an LM1 cell extract enriched for IIB\textsuperscript{bgl} was incubated with \[^{32}\text{P}]\text{PEP}, EI, HPr, and (i) His-tagged IIA\textsuperscript{bgl}, (ii) His-tagged IIA\textsuperscript{bgl} and membranes of LM1 enriched for IIC\textsuperscript{bgl}, or (iii) membranes of LM1 enriched for IICA\textsuperscript{bgl}. The results are shown in Fig. 3B. IIB\textsuperscript{bgl} was phosphorylated in all cases (Fig. 3B, lanes 1, 3, and 5); i.e., it could accept the phosphoryl group from His-tagged IIA\textsuperscript{bgl} or from IICA\textsuperscript{bgl}. However, phosphorylated IIB\textsuperscript{bgl} was not dephosphorylated by salicin in any of these cases (Fig. 3A, lanes 2, 4, and 6). Therefore, IIB\textsuperscript{bgl} separated from IIC\textsuperscript{bgl} cannot phosphorylate β-glucosides. The same results were obtained when His-tagged IIB\textsuperscript{bgl} was used instead of a cell extract enriched for IIB\textsuperscript{bgl} (data not shown). No phosphorylation of MBP-BglG could be detected when MBP-BglG was added to

We next tested the ability of different combinations of BglF domains to phosphorylate BglG in vitro. Purified MBP-BglG (BglG fused to maltose-binding protein), which was previously shown to be phosphorylated by BglF on its BglG moiety in vitro (9), was added to mixtures of BglF domains which were prelabeled with \[^{32}\text{P}]\text{PEP}, EI, and HPr. The results (Fig. 4, lanes 3 to 5) showed that IIB\textsuperscript{bgl} phosphorylated by IICA\textsuperscript{bgl} or by His-tagged IIA\textsuperscript{bgl} could phosphorylate MBP-BglG. The same held true for IIB\textsuperscript{bgl} phosphorylated by His-tagged IIA\textsuperscript{bgl} (Fig. 4, lane 6). IIC\textsuperscript{bgl} was dispensable for phosphoryl transfer from phosphorylated IIA\textsuperscript{bgl} to IIB\textsuperscript{bgl} and from phosphorylated IIB\textsuperscript{bgl} to MBP-BglG (Fig. 4, lane 4). IIA\textsuperscript{bgl} was required for these phosphorylation reactions (Fig. 4, lane 8) but could not phosphorylate MBP-BglG in the absence of IIB\textsuperscript{bgl} (Fig. 4, lanes 7 and 9). The same results were obtained when His-tagged IIB\textsuperscript{bgl} was used instead of a cell extract enriched for IIB\textsuperscript{bgl} (data not shown). No phosphorylation of MBP-BglG could be detected when MBP-BglG was added to
membranes that lacked BglF and that were prelabeled in the in vitro phosphorylation reaction (Fig. 4, lane 1). This result is in agreement with our previous observation that phosphorylated EI and HPr cannot phosphorylate BglG (9).

Based on the results presented here, it can be concluded that IIB\textsubscript{bgl} alone can accept the phosphoryl group from phosphor-
lylated IIA\textsubscript{bgl} and transfer it to BglG. IIC\textsubscript{bgl} is not involved in these phosphotransfer reactions. However, phosphorylated IIB\textsubscript{bgl} separated from IIC\textsubscript{bgl} is incapable of delivering its phosph-
yl group to \(\beta\)-glucosides. In contrast, phosphorylated IIC\textsubscript{bgl} can catalyze \(\beta\)-glucoside phosphorylation.

IIB\textsubscript{bgl} can negatively regulate BglG antitermination activity, whereas IIC\textsubscript{bgl} is required for \(\beta\)-glucoside utilization and for the relief of BglG inhibition in vivo. To substantiate our in vitro results by in vivo studies, we analyzed the ability of dif-
ferent combinations of BglF domains to transfer \(\beta\)-glucosides into the cell while phosphorylating them and to negatively reg-
ulate BglG activity as a transcriptional antiterminator.

We first tested whether plasmids which encode the individ-
ual BglF domains or domain pairs can complement a \(bglF\) mutant strain. To this end, the different truncated BglF pro-
tins or certain combinations encoded by compatible plasmids 
were produced in the \(bglF\) strain PPA501. This strain produces
\(\alpha\)-glucosides. In contrast, phosphorylated IIC\textsubscript{bgl} cannot be catalyzed by BglF dimers, which seem to form spontane-
ously (7).

The requirements for \(\beta\)-glucoside phosphorylation and for BglG dephosphorylation, both stimulated by the sugar, are 
expected to be similar and to differ from the requirements for 
BglG phosphorylation, which occurs in the absence of the sugar. In light of our finding that only the sugar-stimulated

### TABLE 2. Biological functions of BglF individual domains or combinations of domains in vivo

<table>
<thead>
<tr>
<th>Plasmid(s)</th>
<th>Plasmid-encoded BglF derivative</th>
<th>Complementation(^a) of (bgl) mutant strain PPA501 (expressing IIA\textsubscript{bgl} and EI\textsubscript{(II})()</th>
<th>(\beta)-Galactosidase activity (U)(^f) with salicin</th>
<th>Without salicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMN5</td>
<td>Wild-type BglF</td>
<td>+</td>
<td>5</td>
<td>153</td>
</tr>
<tr>
<td>pACO-F3</td>
<td>IIC\textsubscript{bgl}</td>
<td>+</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>pT7CQ-F4</td>
<td>IIC\textsubscript{bgl}</td>
<td>+</td>
<td>175</td>
<td>178</td>
</tr>
<tr>
<td>pT7CQ-F5</td>
<td>IIA\textsubscript{bgl}</td>
<td>+</td>
<td>225</td>
<td>255</td>
</tr>
<tr>
<td>pACO-F6</td>
<td>IICA\textsubscript{bgl}</td>
<td>+</td>
<td>724</td>
<td>637</td>
</tr>
<tr>
<td>pACO-F8</td>
<td>IIC\textsubscript{bgl} + IIB\textsubscript{bgl}</td>
<td>+</td>
<td>10</td>
<td>415</td>
</tr>
<tr>
<td>pACO-F9</td>
<td>IIC\textsubscript{bgl} + IIB\textsubscript{bgl}</td>
<td>+</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>pT7CQ-F3</td>
<td>IIC\textsubscript{bgl} + IIB\textsubscript{bgl}</td>
<td>+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>pBR322</td>
<td></td>
<td></td>
<td>447</td>
<td>456</td>
</tr>
</tbody>
</table>

\(^a\) Complementation was indicated by two alternative methods: +, growth on minimal arbutin plates and red colonies on MacConkey arbutin plates; −, no growth on minimal arbutin plates and white colonies on MacConkey arbutin plates.

\(^b\) Determined with strain PPA501, which carries a \(bgl\)-lacZ transcriptional fusion and a defective \(bglF\) gene and which constitutively expresses IIA\textsubscript{bgl} and EI\textsubscript{II}, encoded by genes \(crr\) and \(nag\), respectively. The values represent the averages of four independent measurements. Salicin at 7 mM was added to the growth medium when indicated.
TABLE 3. Summary of the demonstrated activities of BglF domain polypeptides

<table>
<thead>
<tr>
<th>BglF domain(s)</th>
<th>β-Glucoside phosphorylation</th>
<th>BglG phosphorylation</th>
<th>BglG dephosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCA (wild-type BglF)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C + B</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Ability to phosphorylate β-glucosides in vitro (Fig. 3) and/or to complement bgf mutant strains (Table 2).
* Ability to phosphorylate BglG in vitro (Fig. 4) and/or to negatively regulate BglG activity in vivo (Table 2, β-galactosidase activity without salicin).
* Ability to restore BglG activity in vivo in the presence of β-glucosides (Table 2, β-galactosidase activity with salicin).

activities are sensitive to changing the domain order within BglF (8), we speculated that the domains required for the sugar-stimulated functions might differ from those required for the nonstimulated functions. Of course, the IIB domain, which contains the active site, is expected to be required for all functions. We therefore attempted to define the domains which are required for the different functions by assaying the catalytic activities of individual domains or combination of domains, either covalently linked or produced in trans. Our results (summarized in Table 3) showed that intact IIBC bgl is critical for the ability to implement β-glucoside phosphorylation and BglG dephosphorylation, whereas BglG phosphorylation can be catalyzed by IIB bgl alone. IIC bgl could not rescue IIB bgl and enable it to catalyze the sugar-stimulated functions, unless it was linked to it, i.e., IIC bgl. Nonetheless, the domain requirements for the nonstimulated function are quite loose; i.e., the domain which contains the active site, IIB bgl, is sufficient. Phosphotransfer from IIA bgl to IIB bgl is the same in the presence and absence of IIC bgl, suggesting that the activity of IIB bgl as a phosphoryl acceptor is not affected by IIC bgl.

These results agree with our previous observation that the order of the domains in BglF (BCA) is critical for β-glucoside phosphorylation and BglG dephosphorylation, whereas BglG phosphorylation can be catalyzed by a scrambled-BglF derivative with the domain order CBA (8). One possible explanation for our previously and currently reported results is that β-glucosides induce an interaction between the IIB bgl and IIC bgl domains. Such an interaction could induce a conformational change in BglF and might be the key to the sugar-induced signal transduction pathway that leads to the expression of the bgl operon. In fact, the catalysis of alternative functions due to different interactions between protein domains might be a general theme in the stimulation and/or regulation of diverse functions which are dictated by environmental or cellular conditions. Another possible explanation for our results is suggested by the reversible nature of the BglG phosphorylation reaction (10). The sugar substrate, by dephosphorylating BglF-P, shifts the equilibrium and leads to dephosphorylation of BglG-P. The role of the IIC domain in this process might be to present the sugar substrate to the phosphorylated IIB domain. The apparent requirement for a conformational change between the two domains might reflect a requirement that they be in proximity and properly oriented for sugar phosphorylation to occur efficiently. The two explanations are not mutually exclusive. It is possible that a sugar-induced conformational change of BglF orients IIC and IIB properly for sugar phosphorylation and BglG dephosphorylation.

A number of successful gene dissection and complementation experiments have been reported for other PTS sugar permeases. For example, IIA bgl could restore the activity of a IICBA bgl protein which lacks the first phosphorylation site (27), IIA bgl from *Bacillus subtilis* complemented E. coli IICB bgl (18), and IIA bgl complemented a BglF mutant lacking phosphorylation site 1 (9, 25). Separation of the B domain of the mannitol permease or of the glucose permease from the respective C domain had a more drastic effect, yet catalytic activity was retained to some extent upon their coexpression (6, 19, 27). Moreover, a circularly permuted derivative of the *E. coli* glucose permease in which the order of the domains is BC rather than CB, as in the wild-type protein, has activity comparable to that of the wild-type protein (15). Nevertheless, a mixture of IIA bgl, IIB bgl, and IIC bgl showed 4% of the activity of the mannitol permease, which contains all three domains (19). Similarly, a mixture of IIA bgl, IIB bgl, and IIC bgl showed 2% sugar phosphorylation activity (6). A fusion protein which incorporates all proteins and protein domains of the glucose-specific PTS into a single polypeptide chain with the domain order IIC bgl−IIA bgl−IIB bgl−HPr−EI increased phosphotransfer activity over that of an equimolar mixture of the isolated subunits. Therefore, the linking of functional domains confers certain advantages to a specific PTS permease by rendering it more efficient (15). We do not have enough evidence yet to decide whether sensitivity to domain splitting and splicing is a characteristic of permeases which recognize similar sugars, of permeases which have more than one function, or of permeases which are related, due to a different, yet unknown, reason.

ACKNOWLEDGMENTS

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