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 (EIi) of the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS), catalyzes concomitant transport and phosphorylation of β-glucosides (11). In addition, BglF regulates bgl operon expression, thus inactivating BglG. In the absence of β-glucosides, it phosphorylates BglG, a positive regulator of bgl operon transcription, thus inactivating BglG. In the presence of β-glucosides, it activates BglG by dephosphorylating it and, at the same time, transports β-glucosides into the cell and phosphorylates them. BglF is composed of two hydrophilic domains, IIAβbgl and IIBβbgl, and a membrane-bound domain, IICβbgl, which are covalently linked in the order IIBCAβbgl. Cys-24 in the IIBβbgl domain is essential for all the phosphorylation and dephosphorylation activities of BglF. We have investigated the domain requirement of the different functions carried out by BglF. To this end, we cloned the individual BglF domains, as well as the domain pairs IIBCβbgl and IICαβbgl, and tested which domains and which combinations are required for the catalysis of the different functions, both in vitro and in vivo. We show here that the IIB and IIC domains, linked to each other (IIBCβbgl), are required for the sugar-driven reactions, i.e., sugar phosphotransfer and BglG activation by dephosphorylation. In contrast, phosphorylated IIBβbgl 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 IIBβbgl and IICβbgl, which occur upon the interaction of BglF with the sugar.

The *Escherichia coli* BglF protein (EIβbgl), an enzyme II (EIi) of the phosphoenolpyruvate (PEP)-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. IIAβbgl possesses the first phosphorylation site, His-547 (site 1), which is phosphorylated by HPr; IIBβbgl possesses the second phosphorylation site, Cys-24 (site 2), which accepts the phosphoryl group from IIAβbgl and transfers it to β-glucosides; and IICβbgl, 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 IIBCβbgl (reviewed in references 17 and 20). BglF uses site 2, Cys-24 on the IIBβbgl 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 IICβbgl (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 functions 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 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, IIAβbgl, IIBβbgl, and IICβbgl, as well as truncated BglF proteins which lack one domain (IIICβbgl and IICαβbgl). We show that phosphorylated IIBβbgl alone can phosphorylate BglG in vitro and negatively regulate its activity as a transcriptional antiterminator in vivo. In contrast, IICβbgl 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, IIBβbgl, and the membrane-bound domain, IICβbgl.

**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 crr genes, which code for EIiβbgl and IIAβbgl, respectively (13). ZSC112AG contains a deletion of the *psgG* gene, which codes for IICβbgl (6). PPA501 contains a mutation in the *bgl* gene and carries a *bgl-lacZ* fusion on its chromosome (9). SG13009 was obtained from Qiagen.

**Plasmids.** The plasmids that encode the BglF derivatives used in this study are listed in Table 1. Plasmid pACYC184 was obtained from New England Biolabs. Plasmid pQE-30, which contains a translation start site followed by a sequence coding for six histidines and a multicloning site, and plasmid pREP4, which carries the *lacI* gene encoding the *lac* repressor, were obtained from Qiagen. Plasmid pT712, which contains the phage T7 late promoter, and plasmid pGP1-2, which carries the T7 RNA polymerase gene under the control of the *λ* rep repressor, were obtained from Bethesda Research Laboratories. Plasmid pT7OAC-F carries the entire BglG gene cloned downstream of the T7 promoter in pT712 (1). Plasmid pT7CQ-F1, a derivative of pT7OAC-F, encodes BglF with

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His-S47 mutated to Arg (H547R) (9). Plasmid pMN5 carries the entire bglF gene cloned in pBR322 (14). Plasmids pT7OC-F, pT7Q-F4, pT7Q-F3, pT7Q-F6, and pT7Q-F8 encode derivatives of T712 which code for IIB<sup>B</sup>, H<sup>C</sup>β<sup>D</sup>, H<sup>C</sup>β<sup>H</sup>, and H<sup>C</sup>β<sup>II</sup>, respectively, from the T7 promoter (7). Additional plasmids were constructed as described below.

pACQ-F3, which encodes IIB<sup>B</sup>, was constructed by ligating a 593-bp EcoRI-PvuII fragment from pT7Q-F3 to a 3,388-bp EcoRI-SalI fragment from pACQ184.

pACQ-F6, which encodes ICA<sup>B</sup>β<sup>D</sup>, was constructed by ligating a 2,065-bp EcoRI-PvuII fragment from pT7Q-F6 to a 3,388-bp EcoRI-SalI fragment from pACQ184.

pACQ-F8, which encodes IBC<sup>B</sup>β<sup>D</sup>, was constructed by ligating a 986-bp PstI-EcoRI fragment from pT7Q-F8 to a 3,091-bp PstI-SalI fragment from pACQ184.

**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>pT7QAC-F</td>
<td>Wild-type BglF (IIBCA)&lt;sup&gt;β&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>pT7Q-F1</td>
<td>H547R</td>
<td>9</td>
</tr>
<tr>
<td>pT7Q-F3</td>
<td>IIB&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>pT7Q-F4</td>
<td>H&lt;sup&gt;C&lt;/sup&gt;β&lt;sup&gt;H&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>pT7Q-F5</td>
<td>IIB&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>pT7Q-F6</td>
<td>ICA&lt;sup&gt;B&lt;/sup&gt;β&lt;sup&gt;D&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>pT7Q-F8</td>
<td>IBC&lt;sup&gt;B&lt;/sup&gt;β&lt;sup&gt;D&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>pMN5</td>
<td>Wild-type BglF (IIBCA)&lt;sup&gt;β&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>pACQ-F3</td>
<td>IIB&lt;sup&gt;B&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>pACQ-F6</td>
<td>ICA&lt;sup&gt;B&lt;/sup&gt;β&lt;sup&gt;D&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>pACQ-F8</td>
<td>IBC&lt;sup&gt;B&lt;/sup&gt;β&lt;sup&gt;D&lt;/sup&gt;</td>
<td>This work</td>
</tr>
<tr>
<td>pOE-F5</td>
<td>IIA&lt;sup&gt;B&lt;/sup&gt;β&lt;sup&gt;D&lt;/sup&gt; tagged with six histidine</td>
<td>This work</td>
</tr>
</tbody>
</table>

Molecular cloning and β-galactosidase assay. All manipulations with recombinant DNA were carried out by standard procedures (21). Assays for β-galactosidase activity were carried out as described by Miller (16). Cells were grown in minimal medium supplied with 0.4% succinate as a carbon source.

**RESULTS**

To define the domains required for the different functions of BglF, we subcloned the individual domains, IIA<sup>B</sup>β<sup>D</sup>, IIB<sup>B</sup> and IIC<sup>B</sup>β<sup>D</sup> as well as the domain pairs, IICA<sup>B</sup>β<sup>D</sup> and IIBC<sup>B</sup>β<sup>D</sup>. 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 EI (E1) 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 <sup>35S</sup>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<sup>B</sup> and IIC<sup>B</sup>β<sup>D</sup> 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<sup>B</sup>β<sup>D</sup> produced under the control of the T7 promoter, we engineered histidine-tagged IIA<sup>B</sup>β<sup>D</sup> expressed from an IPTG-inducible promoter (see Materials and Methods). The His-tagged IIA<sup>B</sup>β<sup>D</sup> polypeptide was purified almost to homogeneity by affinity chromatography (Fig. 2A). The ability of His-tagged IIA<sup>B</sup>β<sup>D</sup> 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<sup>B</sup>β<sup>D</sup> was incubated with <sup>32P</sup>PEP, purified EI and HPr, and membranes of strain LM1 (with deletions of the crr and nagE genes) enriched for a BglF mutant protein which lacks phosphorylation site 1 (H547R). The results presented in Fig. 2B demonstrated that IIB<sup>B</sup> and IIC<sup>B</sup>β<sup>D</sup> expressed from the heat-inducible T7 promoter are very stable.

**IIB<sup>B</sup>β<sup>D</sup> is sufficient for BglG phosphorylation, whereas IIBC<sup>B</sup>β<sup>D</sup> is required for β-glucoside phosphorylation in vitro.** It was previously shown that His-S47 in IIA<sup>B</sup>β<sup>D</sup> accepts the phosphoryl group from HPr and transfers it to Cys-24 in IIB<sup>B</sup>β<sup>D</sup>, 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 IIBCbgl phosphorylation and dephosphorylation, we produced the protein in strain ZSC112ΔG, which has a deletion of the ptsG gene. This strain does not contain IICbgl, which is present in membrane preparations of E. coli strains, such as LM1, in significant amounts (9) and which migrates very close to IIBCbgl on SDS-polyacrylamide gels. Mem-

FIG. 1. Individual BglF domains and domain pairs are stable. Expression of wild-type BglF, IIBCbgl (B), IICbgl (C), IICAbgl (CA), and IIBCbgl (BC), was induced from pT7OC-F, pT7CQ-F3, pT7CQ-F4, pT7CQ-F6, and pT7CQ-F8, respectively, in E. coli K38 cells harboring pGPl-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.

FIG. 2. Histidine-tagged IIAbgl is functional. (A) Expression of His-tagged IIAbgl was induced by IPTG from plasmid pQE-F5 in strain SG13009 harboring plasmid pREP4 (lane 1). His-tagged IIAbgl 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 IIAbgl. 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\(^{\text{bgl}}\) 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.

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

<table>
<thead>
<tr>
<th>Plasmid(s)</th>
<th>Complementation of bglF mutant strain PPA501 (expressing IIA&lt;sup&gt;lacZ&lt;/sup&gt; and EI&lt;sup&gt;lacZ&lt;/sup&gt;)</th>
<th>β-Galactosidase activity (U)&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Without salicin</th>
<th>With 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>IIB&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>+</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>pT7CQ-F4</td>
<td>IC&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>175</td>
<td>178</td>
</tr>
<tr>
<td>pT7CQ-F5</td>
<td>IIA&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>225</td>
<td>255</td>
</tr>
<tr>
<td>pACO-F6</td>
<td>IICA&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>724</td>
<td>637</td>
</tr>
<tr>
<td>pACO-F8</td>
<td>IIBC&lt;sup&gt;bgd&lt;/sup&gt; + IIB&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>+</td>
<td>10</td>
<td>415</td>
</tr>
<tr>
<td>pACO-F6-2</td>
<td>IICA&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>pT7CQ-F3</td>
<td>IICC&lt;sup&gt;bgd&lt;/sup&gt; + IIB&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>pBR322</td>
<td>IIB&lt;sup&gt;bgd&lt;/sup&gt;</td>
<td>-</td>
<td>447</td>
<td>456</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<sup>b</sup> Determined with strain PPA501, which carries a <i>bgl</i>-<i>lacZ</i> transcriptional fusion and a defective <i>bglF</i> gene and which constitutively expresses IIA<sup>bgd</sup> and EI<sup>bgd</sup>, encoded by genes <i>ccr</i> and <i>nagE</i>, respectively. The values represent the averages of four independent measurements. Salicin at 7 mM was added to the growth medium when indicated.

membranes that lacked BglF and that were prelabelled 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<sup>bgd</sup> alone can accept the phosphoryl group from phosphorylated IIA<sup>bgd</sup> and transfer it to BglG. IIC<sup>bgd</sup> is not involved in these phosphotransfer reactions. However, phosphorylated IIB<sup>bgd</sup> separated from IIC<sup>bgd</sup> is incapable of delivering its phospho group to β-glucosides. In contrast, phosphorylated IIBC<sup>bgd</sup> can catalyze β-glucoside phosphorylation.

IIIB<sup>bgd</sup> can negatively regulate BglG antitermination activity, whereas IIBC<sup>bgd</sup> is required for β-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 different combinations of BglF domains to transfer β-glucosides into the cell while phosphorylating them and to negatively regulate BglG activity as a transcriptional antiterminator.

We first tested whether plasmids which encode the individual BglF domains or domain pairs can complement a <i>bglF</i> mutant strain. To this end, the different truncated BglF proteins or certain combinations encoded by compatible plasmids were produced in the <i>bglF</i> strain PPA501. This strain produces IIA<sup>bgd</sup> and EI<sup>bgd</sup>, which can substitute for IIA<sup>bgd</sup> and EI<sup>bgd</sup> on the cotranscribed <i>bglI</i> gene, whose transcriptional terminator is expressed when 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.

The ability of BglF to inactivate the transcriptional antiterminator BglG in the absence of β-glucoside starts from its ability to phosphorylate BglG. The relief of BglG inhibition upon β-glucoside addition is due to the dephosphorylation of BglG by sugar-stimulated BglF (1). Therefore, to examine the ability of individual BglF domains, domain pairs, and combinations of domains to phosphorylate and dephosphorylate BglG in vivo, we tested their ability to regulate the activity of BglG as a transcriptional antiterminator. To this end, we made use of strain PPA501, which carries a chromosomal fusion of the <i>bgl</i> promoter and transcriptional terminator to <i>lacZ</i> and which lacks a functional <i>bglF</i> gene. BglG is not negatively regulated by phosphorylation in this strain, and high β-galactosidase activity is measured whether or not β-glucosides are added to the growth medium (Table 2, line 9). The expression of plasmid-encoded wild-type BglF in strain MA200-1 renders <i>lacZ</i> expression inducible; in the absence of β-glucosides, the <i>lacZ</i> gene is not transcribed because BglG is inactivated by phosphorylation; upon the addition of β-glucosides, BglF dephosphorylates BglG, allowing it to block transcription termination, and the <i>lacZ</i> gene is expressed (Table 2, line 1).

IIBC<sup>bgd</sup> (which could be phosphorylated by IIA<sup>bgd</sup> and EI<sup>bgd</sup> in PPA501) behaved like wild-type BglF, allowing <i>lacZ</i> expression only upon the addition of β-glucosides (Table 2, line 6). Thus, as expected from the in vitro results, IIBC<sup>bgd</sup> could inhibit BglG activity by phosphorylation and could relieve the inhibition by dephosphorylation in vivo. IIIB<sup>bgd</sup> (which could also be phosphorylated by IIA<sup>bgd</sup> and EI<sup>bgd</sup> in PPA501) inhibited BglG activity by phosphorylation, as indicated by the low β-galactosidase levels obtained in the absence of β-glucosides. However, low β-galactosidase levels were also recorded in the presence of β-glucosides, indicating that IIIB<sup>bgd</sup> could not relieve the inhibition of BglG by dephosphorylating it (Table 2, lines 2, 7, and 8). IIC<sup>bgd</sup> was not required to enable IIIB<sup>bgd</sup> to act as a BglG negative regulator in vivo (Table 2, compare line 2 with lines 7 and 8), nor could it act in trans with IIB<sup>bgd</sup> to implement BglG dephosphorylation (Table 2, lines 7 and 8). As expected, the production of IIBC<sup>bgd</sup>, IIA<sup>bgd</sup>, or IICA<sup>bgd</sup> in PPA501 did not affect the constitutive nature of <i>lacZ</i> expression (Table 2, lines 3 to 5); i.e., these polypeptides cannot regulate BglG activity by phosphorylation.

DISCUSSION

The ability of the same active site on BglF to phosphorylate β-glucosides and to reversibly phosphorylate BglG prompted us to investigate the structural basis for sugar-controlled differential phosphorylation. Dimerization of BglF could not explain the β-glucoside-induced switch in BglF activity, since β-glucoside does not affect the BglF dimeric state (7). In addition, both the sugar-induced and noninduced functions could be catalyzed by BglF dimers, which seem to form spontaneously (7).

The requirements for β-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
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<sub>bgl</sub> is critical for the ability to implement β-glucoside phosphorylation and BglG dephosphorylation, whereas BglG phosphorylation can be catalyzed by IIB<sub>bgl</sub> alone. IIC<sub>bgl</sub> could not rescue IIB<sub>bgl</sub> and enable it to catalyze the sugar-stimulated functions, unless it was linked to it, i.e., IIBC<sub>bgl</sub>. Nonetheless, the domain requirements for the nonstimulated function are quite loose; i.e., the domain which contains the active site, IIB<sub>bgl</sub>, is sufficient. Phosphotransfer from IIA<sub>bgl</sub> to IIB<sub>bgl</sub> is the same in the presence and absence of IIC<sub>bgl</sub>, suggesting that the activity of IIB<sub>bgl</sub> as a phosphoryl acceptor is not affected by IIC<sub>bgl</sub>.

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<sub>bgl</sub> and IIC<sub>bgl</sub> 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 BglG-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 dephosphorylated IIB domain.

TABLE 3. Summary of the demonstrated activities of BglF domain polypeptides

<table>
<thead>
<tr>
<th>BglF domain(s)</th>
<th>β-Glucoside phosphorylation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BglG phosphorylation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>BglG dephosphorylation&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCA (wild-type BglF)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
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<sup>a</sup> Ability to phosphorylate β-glucosides in vitro (Fig. 3) and/or to complement *bgl* mutant strains (Table 2).

<sup>b</sup> Ability to phosphorylate BglG in vitro (Fig. 4) and/or to negatively regulate BglG activity in vivo (Table 2; β-galactosidase activity without salicin).

<sup>c</sup> Ability to restore BglG activity in vivo in the presence of β-glucosides (Table 2, β-galactosidase activity with salicin).

A number of successful gene dissection and complementation experiments have been reported for other PTS sugar permutases. For example, IIA<sub>mut</sub> could restore the activity of a IICB<sub>mut</sub> protein which lacks the first phosphorylation site (27), IIA<sub>mut</sub> from *Bacillus subtilis* complemented *E. coli* IICB<sub>ec</sub> (18), and IIA<sub>ec</sub> 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<sub>mut</sub>, IIB<sub>mut</sub>, and IIC<sub>mut</sub> showed 4% the activity of the mannitol permease, which contains all three domains (19). Similarly, a mixture of IIA<sub>ec</sub>, IIB<sub>ec</sub>, and IIC<sub>ec</sub> 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<sub>ec</sub>-IIB<sub>ec</sub>-IIA<sub>ec</sub>-HPc-ElI 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.

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