Modulation of Monomer Conformation of the BglG Transcriptional Antiterminator from *Escherichia coli*

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The BglG protein positively regulates expression of the *bgl* operon in *Escherichia coli* by binding as a dimer to the *bgl* transcript and preventing premature termination of transcription in the presence of β-glucosides. BglG activity is negatively controlled by BglF, the β-glucoside phosphotransferase, which reversibly phosphorylates BglG according to β-glucoside availability, thus modulating its dimeric state. BglG consists of an RNA-binding domain and two homologous domains, PRD1 and PRD2. Based on structural studies of a BglG homologue, the two PRDs fold similarly, and the interactions within the dimer are PRD1-PRD1 and PRD2-PRD2. We have recently shown that the affinity between PRD1 and PRD2 of BglG is high, and a fraction of the BglG monomers folds in the cell into a compact conformation, in which PRD1 and PRD2 are in close proximity. We show here that both BglG forms, the compact and noncompact, bind to the active site-containing domain of BglF. BglG with IIB*bd* or BglF is mediated by PRD2. Both BglG forms are detected as phosphorylated proteins after in vitro phosphorylation with IIB bgl and are dephosphorylated by BglF in vitro in the presence of β-glucosides. Nevertheless, genetic evidence indicates that the interaction of IIB*bd* and BglG with the compact form is seemingly less favorable. Using in vivo cross-linking, we show that BglF enhances folding of BglG into a compact conformation, whereas the addition of β-glucosides reduces the amount of this form. Based on these results we suggest a model for the modulation of BglG conformation and activity by BglF.

The expression of the *bgl* operon in *Escherichia coli*, whose products are required for β-glucoside utilization, is regulated by two of its gene products, BglG, a transcriptional regulator, and BglF, a membrane-bound sugar sensor (4). Transcription from the *bgl* promoter initiates constitutively but, in the absence of β-glucosides, most transcripts terminate prematurely at one of the two *p*-independent terminators that flank the first gene of the operon. In the presence of β-glucosides, BglG prevents termination of transcription at these sites (20, 26) by binding to specific sites on the *bgl* transcript, which partially overlap with each of the terminators, and stabilizing an alternative secondary structure of the RNA that enables RNA polymerase to proceed (15). BglG activity is regulated by BglF, an enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), which reversibly phosphorylates BglG according to β-glucoside availability (1, 3, 25), thus modulating its dimeric state (2). Hence, in the absence of β-glucosides, BglG exists as an inactive, phosphorylated monomer, whereas in the presence of β-glucosides in the growth medium, BglG exists as an active nonphosphorylated dimer (2). A recent publication from our laboratory demonstrated that BglF recruits BglG to form a precomplex at the cell membrane and releases it to the cytoplasm upon the addition of β-glucosides (17).

BglG represent a family of transcriptional antiterminators that regulate the expression of bacterial genes and operons, whose products are required for utilization of PTS carbohydricates (reference 27 and references therein). The BglG-like antiterminators are composed of three domains, an RNA-binding domain followed by two homologous domains, PTS regulation domain 1 (PRD1) and PRD2 (31). Each PRD contains two conserved histidines, although BglG lacks the second histidine in PRD2. The conserved histidines are essential for the regulation of the antiterminators by the PTS via phosphorylation. BglF negatively regulates BglG activity by phosphorylating it on the conserved histidine in PRD2 (9). However, the conserved aspartate adjacent to the first histidine and the second conserved histidine, both in PRD1, are also required for this regulation (1, 19). Negative regulation by enzymes II of PTS has been demonstrated or suggested also for other BglG-like regulators (16, 21, 29, 24, 31). Positive regulation via phosphorylation of the conserved histidines by the general PTS protein HPr has been established or suggested for several antiterminators and was proposed to be part of a mechanism of carbon catabolite repression that operates mainly in gram-positive bacteria (31). The structure of the PRD region of a mutant of LicT, a BglG homologue from *Bacillus subtilis*, in which the two conserved histidines in PRD2 were replaced by aspartates, was solved by X-ray crystallography (32). The structure is a homodimer, each monomer containing two analogous α-helical domains. The interactions within the dimer are PRD1-PRD1 and PRD2-PRD2. The phosphorylation sites are totally buried at the dimer interface and hence inaccessible to the PTS (de)phosphorylating enzymes, suggesting a major conformational change upon reversible phosphorylation or a significant difference between the structure of the mutant LicT and that of the phosphorylated wild-type protein. The formation of BglG dimers was predicted to initiate with PRD2 dimerization, followed by zipper up of two BglG monomers to
create the active RNA-binding domain (5, 13). A similar mechanism was suggested for LicT (10).

We have recently shown that the affinity between the separate PRD1 and PRD2 domains of BglG is high and that they heterodimerize efficiently in vitro and in vivo (13). We also showed that a fraction of the BglG protein is present in the cell in a compact conformation, identified as a faster migrating band on gels, in which PRD1 and PRD2 are in very close proximity, probably due to bending of the linker that connects the two domains. Formation of the compact form was demonstrated by chemical cross-linking between cysteines in PRD1 and PRD2 in vitro and in vivo and by the formation of a disulfide bridge (zero length cross-linking) upon oxidation. It should be emphasized that the cross-linked cysteines, although close to each other, do not bond in vivo. These cysteines, which were instrumental in identifying the compact form, are not conserved in BglG homologues. The compact form is present mainly in BglG monomers (12). Thus far, no prediction is available for a BglG-like monomer structure.

In this study, we investigated the interaction of the two forms of the BglG protein, the compact and noncompact, with the BglF sensor and attempted to identify factors that affect the switch between the two forms.

MATERIALS AND METHODS

Strains. The following E. coli K-12 strains were used: BL21(DE3) [harDC mlb
(xc857 ind1 Sim7 mini)] lacUV5/T7 gen1], obtained from Novagen, and SI3009, obtained from QIAGEN, were used for the expression of His-tagged proteins. MC1061 [harDC, mcrB, arsD, 139Q(araABC-leu) 7879 ΔlacO174 galK polk rpsL thi], was used during the purification of proteins tagged with maltose-binding protein (MBP); SU202, which harbors a chromosomal copy of the lacZ gene under the control of a hybrid LexA operator (op408/op1+1), was used to study heterodimerization; K38 [HarC repK thi-1] was used as a host for the preparation of membrane fractions.

Media. Luria-Bertani (LB) medium, M63 salts minimal medium, and M9 minimal medium were prepared essentially as described by Miller (22). Ampicillin (200 μg/ml), kanamycin (30 μg/ml), and chloramphenicol (25 μg/ml) were included in the medium for growing strains containing plasmids that confer resistance to either one of these antibiotics.

Plasmids. All plasmids used in this study and the proteins they encode are listed in Table 1. Plasmid pLZZ-F encodes BglF expressed from the P7T7 promoter. A 1.938-bp fragment, encoding BglF and containing the Ace651 site at one end and the MluI site at the other, was generated by PCR by using pT7AC-F (1) as a template. This fragment was ligated to the 4.211-bp Ace651-MluI fragment of pZS tet/etp/etf, pZS tet/etp/etf, a derivative of pZS tet/etp/etf, was obtained from New England Biolabs. Peroxidase-conjugated AffiniPure goat anti-mouse was obtained from Jackson ImmunoResearch Laboratories Inc. Anhydrotretracycline hydrochloride was obtained from Acros.

Protein purification. MBP-tagged BglG, PRD1, or PRD2 was expressed in MC1061 and purified as described previously for MBP-BglG (12). His-tagged enzyme I (E1), HPr, and IPP (2) were expressed in BL21(DE3). His-tagged IIA (2) was expressed in SI3009. SI3009's tagged proteins were purified as described previously (7), except that the extracts were incubated for only 1 h with the Ni-nitrilotriacetic acid resin. 4-(2-Aminoethoxy)benzenesulfonil fluoride hydrochloride was used during the purification procedure instead of phenylmethylsulfonyl fluoride.

Far-Western analysis. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were subjected to far-Western analysis as described previously (23) or stained with Coomassie blue. No denaturing agents were used throughout the entire procedure performed to study the interaction of the BglG complex and noncompact forms with IPP (2) by far-Western analysis.

Two-hybrid analysis. For a quantitative assay of heterodimerization, the SU202 strain, which harbors a chromosomal copy of the lacZ gene under the control of a hybrid LexA operator (op408/op1+1), was cotransformed with the derivatives of pDP804 and pMS604 (11). Cells were grown in a minimal medium containing 0.4% succinate as the carbon source and 0.5 mM isopropyl-β-D-thiogalactopyranoside. Assays for β-galactosidase activity were carried out as described by Miller (22).

Preparation of membrane fractions. Membrane fractions enriched for BglF were prepared as described previously (1). BglF was expressed from its gene cloned under the T7 promoter in pT7OAC-F. The expression of T7 RNA polymerase from plasmid pGPI-1, which is compatible with the pT7 plasmid, was induced thermally. The E. coli K38 strain was used as a host.

In vitro phosphorylation and dephosphorylation. In vitro (de)phosphorylation was carried out essentially as described before (8). Briefly, to study phosphorylation of BglG, [γ-32P]ATP was prepared and separated from [32P]ATP. BglG (2 μg) was labeled by incubation at 30°C in a mixture containing E1 (0.2 μg), HPr (1.5 μg), IIA (2 μg), [32P]ATP (10 μM), and PLB buffer (50 mM Na2HPO4, pH 7.4), 0.5 mM MgCl2, 1 mM NaF) lacking diithiothreitol, in a final volume of 10 μl. After incubation for 10 min, MBP-BglG (5 μg) was added, and the reaction mixtures were further incubated in PLB buffer for 10 min at 30°C. To study dephosphorylation of BglG by β-glucosidase, the reaction was dialyzed against PLB buffer lacking diithiothreitol to separate 32P-BglG from residual [32P]ATP. Subsequently, 0.1% salicyl and BglF-containing membranes (400 μg) were added to the dialyzed 32P-BglG (80 μg), and incubation was continued at 30°C. Aliquots were removed at various times. Reactions were terminated by the addition of electrophoresis sample buffer containing or lacking β-mercaptoethanol. Equal amounts of samples were subjected to SDS-PAGE.

In vivo cross-linking of MBP-BglG in the presence of BglF and arbutin. MC1061 cells, harboring plasmids pANSMG and pLZZ-F, were grown with aeration at 37°C in M9 minimal medium containing 0.4% glycerol as a carbon source. When indicated, BglF expression from pLZZ-F was induced by the addition of anhydrotretracycline hydrochloride to a final concentration of 100 ng/ml. When specified, 0.5% arbutin (hydroxyphenyl-β-D-glucopyranoside) was added to the medium. When cells reached an optical density at 600 nm of 0.6, in vivo cross-linking with p-PDM was performed as described before (12). Cells were subsequently handled and analyzed as previously described (21), except that when cells were grown in the presence of arbutin, 0.5% arbutin was maintained in all solutions throughout the procedure.

Table 1. Plasmids used in this study and the proteins they encode

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Protein encoded</th>
<th>Reference</th>
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<tbody>
<tr>
<td>pANSMG</td>
<td>MBP·BglG</td>
<td>17</td>
</tr>
<tr>
<td>pANSM-PRD1</td>
<td>MBP·BglG PRD1(aa 65–172)</td>
<td>13</td>
</tr>
<tr>
<td>pANSM-PRD2</td>
<td>MBP·BglG PRD2(aa 175–278)</td>
<td>13</td>
</tr>
<tr>
<td>pANSHIB</td>
<td>His6·IB</td>
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</tr>
<tr>
<td>pOE-F5</td>
<td>His6·IAB</td>
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</tr>
<tr>
<td>pLFHis-HPr</td>
<td>His6·HPr</td>
<td>12</td>
</tr>
<tr>
<td>pLFHis-EI</td>
<td>His6·EI</td>
<td>12</td>
</tr>
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<td>pTOAC-F</td>
<td>BglF</td>
<td>5</td>
</tr>
<tr>
<td>pGPI-2</td>
<td>T7 RNA polymerase</td>
<td>28</td>
</tr>
<tr>
<td>pMS604</td>
<td>LexA·BglG WT</td>
<td>11</td>
</tr>
<tr>
<td>pDP804</td>
<td>LexA·BglG(mutant)-Jun leu zipper</td>
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</tr>
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<td>pL1</td>
<td>LexA·BglG(mutant)</td>
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<td>pLLI-G</td>
<td>LexA·BglG(mutant)-BglG</td>
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<tr>
<td>pLF3-PRD2</td>
<td>LexA·BglG WT-BglG PRD2(aa 175–278)</td>
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<tr>
<td>pLL-F</td>
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<td>17</td>
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<tr>
<td>pLLZ-F</td>
<td>BglF</td>
<td>5</td>
</tr>
</tbody>
</table>

a LexA·BglG WT is a fragment of wild-type LexA repressor (amino acids [aa] 1 to 187), which contains a domain that binds to wild-type LexA operator.
b LexA·BglG(mutant) is a fragment of LexA 408 repressor (amino acids 1 to 87), which contains a domain that binds to an altered LexA operator (op 45).
**Coomassie stain**

![Coomassie stain](image)

**Far-Western Probe: IIB\textsuperscript{bgld}**

1 - βME + βME
Non-compact

2 - βME + βME
Compact

3 - βME + βME

4 - βME + βME

FIG. 1. The two forms of BglG, the compact and the noncompact, interact with the active site-containing domain of BglF in vitro. (A) Purified MBP-BglG was analyzed by SDS-PAGE in the absence or presence of β-mercaptoethanol (βME) and stained with Coomassie blue. (B) As for panel A, but the proteins were blotted onto a nitrocellulose membrane and probed with His-tagged IIB\textsuperscript{bgld} and then with anti-His antibodies. The use of denaturing agents was avoided throughout the entire procedure. Arrowheads indicate the positions of the compact and noncompact forms of MBP-BglG.

SDS-PAGE, Western blot analysis, and autoradiography. Proteins were incubated with electrophoresis sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 0.01% bromophenol blue, and, only when indicated, 5% β-mercaptoethanol. Electrophoresis of proteins was carried out by SDS-PAGE (8 or 10% polyacrylamide). Samples were fractionated next to SeeBlue Plus2 prestained marker (Invitrogen). After electrophoresis, gels were either stained with Coomassie blue, blotted onto a nitrocellulose filter for Western blot analysis as described before (8), or dried and exposed to Kodak X-OMAT X-ray film at −70°C.

RESULTS

The active site-containing domain of BglF binds to the BglG compact form in vitro. In a recent publication from our laboratory it was shown that the transcriptional antiterminator BglG can fold into a compact conformation in which the PRD1 and PRD2 domains are in close proximity (12). In this conformation, two cysteines, one in each PRD, are brought to a distance that enables them to form a disulfide bond as a result of air oxidation upon cell rupture. Consequently, a fraction of the BglG protein can be detected as a faster migrating form when analyzed in the absence of reducing agents. Another recent publication from our laboratory demonstrated that BglG interacts with the BglF membrane sensor and with its active site-containing domain, IIB\textsuperscript{bgld} (17). One tool used for studying this interaction was the far-Western technique. We showed that BglG, which was analyzed by the standard SDS-PAGE procedure, i.e., in the presence of β-mercaptoethanol, and blotted onto a membrane, reacted with IIB\textsuperscript{bgld}. Hence, we established that the active site-containing domain of BglF binds to the noncompact form of BglG, the only BglG form that was detected under these conditions. To examine whether BglF can bind also to the compact form of BglG, we used the following modification of the far-Western technique: we separated the two forms of BglG on a nonreducing gel, blotted both forms onto a filter, and tested the ability of IIB\textsuperscript{bgld} to bind to them. Hence, purified MBP-BglG (BglG fused to MPB) was analyzed on an SDS-polyacrylamide gel with no reducing agents and blotted onto a nitrocellulose filter. The membrane was incubated with His-tagged IIB\textsuperscript{bgld} (the IIB\textsuperscript{bgld} domain fused to six histidines) and then with antibodies against the His tag (Fig. 1A, lane 3). Both BglG forms, the slower migrating noncompact form and the faster migrating compact form, reacted with IIB\textsuperscript{bgld}. The lower intensity observed with the compact form can be attributed, at least in part, to its relative low amount (Fig. 1, lane 1, Coomassie blue stain). BglG analyzed in the presence of β-mercaptoethanol served as a control (Fig. 1, lanes 2 and 4). When MBP alone was probed with His-IIB\textsuperscript{bgld}, no binding was observed (reference 17 and data not shown), ruling out the possibility that the MBP moiety mediates the interaction. These results demonstrate that the active site-containing domain of BglF interacts with both forms of BglG. These interactions are direct and do not require additional proteins, at least in vitro. Because the far-Western technique is not sensitive enough to give a quantitative estimate for the interaction, we cannot draw any conclusion concerning the relative affinity of IIB\textsuperscript{bgld} for the two BglG forms. Hence, we cannot rule out the possibility that IIB\textsuperscript{bgld} interacts preferentially with one of the BglG forms.

The compact BglG form is detected in the phosphorylated fraction of BglG and is dephosphorylated by BglF. It has previously been shown that the phosphorylated forms of intact BglF and its active site-containing domain, IIB\textsuperscript{bgld}, can phosphorylate BglG in vitro (1, 7). In contrast, either the entire BglF protein or its truncated IIBC\textsuperscript{bgld} derivative (IIB\textsuperscript{bgld} fused to the membrane domain IIC\textsuperscript{bgld}) but not IIB\textsuperscript{bgld} alone is required for BglG-P dephosphorylation in the presence of β-glucosides (7). At this time, we asked whether the compact form of BglG is detected also in vitro phosphorylation. Because MBP-BglG and BglF are similar in size and comigrate in SDS-PAGE, the detection of \(^{32}\)P-BglG after incubation with intact \(^{32}\)P-BglF is not possible. Therefore, we used IIB\textsuperscript{bgld}, the active site-containing domain of BglG, to follow phosphorylation of the compact form of BglG. To this end, MBP-BglG was added to \(^{32}\)P-IIB\textsuperscript{bgld}, which was prelabeled in a reaction mixture containing \(^{32}\)PEP and purified His-tagged EI, HPr, IIA\textsuperscript{bgld}, and IIB\textsuperscript{bgld}. After further incubation, the products were subjected to SDS-PAGE analysis in the absence or presence of β-mercaptoethanol (Fig. 2A, lanes 1 and 2, respectively). Both forms of BglG, the compact and the noncompact, were detected as phosphorylated proteins by this analysis (Fig. 2A, lane 1). However, we cannot rule out the possibility that the compact form was obtained due to oxidation of the noncompact form subsequent to the phosphorylation. Although we cannot proclaim that IIB\textsuperscript{bgld} directly phosphorylates the compact form of BglG, this result demonstrates that the compact BglG form can exist as a dephosphorylated protein.

Next, we tested the ability of the BglG compact form to be dephosphorylated by BglF in the presence of β-glucosides. The results shown in Fig. 2B demonstrate that the two \(^{32}\)P-BglG forms, the compact and the noncompact, are dephosphorylated in a time-dependent manner when incubated with BglF-enriched membranes and the β-glucoside salicin (Fig. 2B, lanes 2 to 4). Therefore, the two forms of BglG are both recognized and dephosphorylated by BglF in the presence of β-glucosides. Binding of BglG to BglF is mediated by its PRD2 domain and is hampered by PRD1. To characterize the interaction of BglG with BglF, we aimed at determining the domain in BglG which mediates the interaction. To this end, we studied the interaction of the separated PRDs of BglG with BglF or with its active site-containing domain, IIB\textsuperscript{bgld}, both in vivo.

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and in vitro, and compared the results to those obtained with the entire BglG protein.

First, we tested the interaction of purified BglG or the separated PRDs with purified IIB\(^{\text{bgl}}\) by the far-Western technique. To this end, similar amounts of BglG, PRD1, and PRD2 fused to MBP (MBP-BglG, MBP-PRD1, and MBP-PRD2, respectively) were subjected to SDS-PAGE (Fig. 3, Coomassie stain). The proteins were then blotted onto a nitrocellulose filter and incubated with His-tagged IIB\(^{\text{bgl}}\) and antibodies against the His tag (Fig. 3, far-Western). A strong signal was detected for the interaction of either intact BglG or its PRD2 domain with IIB\(^{\text{bgl}}\) (Fig. 3, lanes 4 and 6, respectively). A relatively weak signal was detected for the interaction of the PRD1 domain with IIB\(^{\text{bgl}}\) (Fig. 3, lane 5). When MBP alone was probed with His-IIB\(^{\text{bgl}}\), no binding was observed (data not shown), ruling out the possibility that the interaction with IIB\(^{\text{bgl}}\) is mediated by the MBP moiety. Hence, PRD2 recognizes and interacts with IIB\(^{\text{bgl}}\) much better than PRD1, suggesting that the PRD2 domain is responsible for the interaction of BglG with IIB\(^{\text{bgl}}\).

To study the interaction of the PRDs with BglF or with IIB\(^{\text{bgl}}\) in vivo, we used the bacterial LexA-based two-hybrid system (11). In this system, the proteins of interest are fused either to the DNA-binding domain of the wild-type LexA repressor (LexADBD) or to an altered-specificity LexADBD and introduced into a strain that harbors a chromosomal copy of lac\(Z\) under the control of a LexA hybrid operator (SU202). Transcriptional repression is achieved upon coexpression of both hybrid proteins, provided that they bind to each other. We fused BglF, IIB\(^{\text{bgl}}\), BglG and the two PRDs to LexADBD, wild type or mutant, and calculated the transcriptional repression obtained when different combinations of these chimeras were coexpressed in SU202. The results are presented in Table 2. The leucine zipper domains of Fos and Jun, fused to the wild-type and mutant LexADBD, respectively, served as positive controls, and the two LexADBDs served as a negative

control. As shown before (17), coexpression of either IIB\(^{\text{bgl}}\) or BglF with the entire BglG protein fused to the LexADBDs resulted in formation of stable heterodimers that functioned as repressors. The lower repression obtained with BglF is attributed to the constraint caused by anchoring the BglF-LexADBD chimera to the membrane. Replacing BglG with the PRD1 domain either reduced or almost eliminated formation of heterodimers with IIB\(^{\text{bgl}}\) or BglF, respectively. In contrast, not only did PRD2 bind to IIB\(^{\text{bgl}}\) and to BglF, as indicated by the high transcriptional repression obtained when the respective combinations of chimeras were coexpressed, but these interactions were stronger than those obtained when the entire BglG was coexpressed with IIB\(^{\text{bgl}}\) or BglF. Hence, in

![Coomassie stain](http://jb.asm.org/)

**FIG. 2.** Reversible phosphorylation of the compact and noncompact forms of BglG in vitro. (A) Phosphorylation of BglG was carried out by incubating purified MBP-BglG with \(^{32}\)P-IIB\(^{\text{bgl}}\) for 10 min. IIB\(^{\text{bgl}}\) was prelabeled by incubating purified EI, HPr IIA\(^{\text{bgl}}\), and IIB\(^{\text{bgl}}\), all His-tagged, with \(^{32}\)P-PEP for 10 min. (B) Dephosphorylation of BglG was obtained by incubating \(^{32}\)P-MBP-BglG, phosphorylated as described in panel A and then separated from the residual \(^{32}\)P-PEP by dialysis, with membranes containing BglF and 0.1% salicin. Aliquots were removed at the indicated time points. Samples were analyzed by SDS-PAGE in the absence or presence of \(\beta\)-mercaptoethanol (\(\beta\)ME) followed by autoradiography. Arrowheads indicate the position of the compact form of MBP-BglG on the gels.

![Far-Western analysis](http://jb.asm.org/)

**FIG. 3.** Far-Western analysis of the interaction between the PRDs of BglG and the active site-containing domain of BglF. (A) Purified BglG, PRD1, and PRD2, each fused to MBP, were analyzed by SDS-PAGE and stained with Coomassie blue. (B) As for panel A, but the proteins were blotted onto a nitrocellulose membrane, probed with His-tagged IIB\(^{\text{bgl}}\), and then with anti-His antibodies.
TABLE 2. Analysis of the interaction between BglG derivatives and BglF or its active site-containing domain, IIb\(^{ab}\), by the two-hybrid LexA-based system*

<table>
<thead>
<tr>
<th>Protein fused to WT or mutant LexA-DBD</th>
<th>Interactions(^c) (% repression of Plac::UV5-lacZ(^d)) with:</th>
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<tr>
<td></td>
<td>IIb(^{ab})</td>
</tr>
<tr>
<td>Negative control</td>
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<td>Positive control</td>
<td>98</td>
</tr>
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<td>BglG</td>
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<td>PRD1</td>
<td>51</td>
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<td>PRD2</td>
<td>93</td>
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*The experiment was performed with E. coli strain SU202 which carries a hybrid LexA operator op408/op+::lacZ fusion on its chromosome (11). The standard deviation ranged from 1% to 5% repression.

\(^{ab}\)The two LexADBD (expressed from pLL1 and pPLL3) served as a negative control. The leucine zipper domains of Fox and Jun fused to wild-type (bT) and mutant LexADBD (expressed from pMS604 and pDP804), respectively, served as a positive control. pLL1 and pPLL3 are derivatives of pDP804 and pMS604, respectively.

\(^{c}\)To test the interaction with IIb\(^{ab}\), IIb\(^{ab}\) was cloned on pLL3 and the BglG derivatives on pLL1. To test the interaction with BglF, BglF was cloned on pLL1 and the BglG derivatives on pPLL3.

\(^{d}\)Percent repression was calculated as follows: \(1 - (\beta\text{-galactosidase activity with repressor/\beta\text{-galactosidase activity without repressor}) \times 100}\.

FIG. 4. Modulation of compact BglG monomer formation by BglF and \(\beta\)-glucosides in vivo. Cells expressing MBP-BglG (lane 1) or both MBP-BglG and BglF (lanes 2 to 3) were grown in minimal medium, lacking (lanes 1 to 2) or containing (lane 3) 0.5% arbutin, to an optical density at 600 nm of 0.6. Pelleted cells were washed and resuspended in phosphate-buffered saline, pH 7.0, with arbutin added only to cells grown with arbutin. 5-PDM, a cross-linker that penetrates cells and cross-links cysteines in the BglG compact monomer, was added to a final concentration of 1 mM. Following incubation at 30°C for 30 min, samples were collected by centrifugation, washed, resuspended in sample buffer, and analyzed by SDS-PAGE and Western blot analysis. The two forms of BglG were detected with anti-MBP antibodies. The relative intensities of the lower bands are 1.00:3.03:1.00. The arrowhead indicates the position of the compact form of MBP-BglG.
of LicT, the proximity of the crossed-linked cysteines in the monomer form and their availability for interaction suggest that the structure of the compact BglG monomer differs from the structure of the monomers that compose the dimer; this in turn implies that a major conformational change is involved in this transition, in addition to the motions of the PRDs (12). What triggers the change? We suggested that PTS proteins might play a role in the switch. In the present study we investigated the involvement of BglF, a membrane-bound enzyme II of PTS which controls BglG dimer-monomer transition via reversible phosphorylation, in modulation of the noncompact-compact transition. We demonstrated that both forms of the BglG monomer are recognized by the BglF active site-containing domain, IIB, in vitro, that the compact BglG form can be detected in the phosphorylated fraction, and that the phosphorylated compact form is dephosphorylated by BglF with the addition of β-glucosides. The limitations of the techniques that we used did not allow us to draw conclusions concerning the relative affinity of IIB for the two BglG forms.

To further characterize BglG-BglF interaction, we compared the interaction of the separated PRDs of BglG with BglF and/or with IIB in vitro and in vivo. Our results demonstrated that the PRD2 domain mediates BglG interaction with BglF and IIB. Not only does PRD1 interact poorly or not at all with IIB and BglF, respectively, but its presence on BglG also has a negative effect on the interaction with IIB and BglF. One possible explanation for the interference exerted by PRD1 is that it competes with BglF and IIB for the same binding site on PRD2. This is not very likely in light of the results demonstrating that the compact form of BglG, in which PRD1 and PRD2 are in close proximity, can bind to IIB and be de-phosphorylated by BglF. Alternatively, PRD1 might reduce the PRD2-BglF interaction by physically blocking the access to the binding site due to formation of the compact conformation, in which PRD2 is partially sequestered. PRD1 interference can also be attributed to the lower affinity of the compact form of BglG for BglF and IIB, compared to the affinity of the noncompact form, due to exposure of slightly altered binding sites. Hence, although the compact form of BglG is recognized by BglF, our results suggest that its interaction with IIB and BglF is less favorable.

The evidence for the involvement of BglF in modulation of BglG monomer conformation was obtained by in vivo cross-linking experiments, which demonstrated that BglF enhances the formation of the compact BglG monomer. The genuine amounts of the compact form in the cell are in fact higher than exhibited by the cross-linking experiments with dimaleimides, mainly because the efficiency of in vivo cross-linking experiments is low, as it depends on the diffusion of external reagents into intact cells, and also because cross-linking is precluded by binding of each cysteine to a different dimaleimide molecule, by inactivation of one of the maleimide moieties of a dimaleimide due to hydrolysis, and by terminal oxidation of thiols to sulfonates (30, 6). Nevertheless, the differences in the levels of the compact form in the various cultures (Fig. 4) are valid, as they were obtained under identical experimental conditions and demonstrated in several independent experiments. As discussed above, proximity of the cysteines in PRD1 and PRD2 and their availability for interaction imply that the monomer-dimer transition involves a conformational change. We suggested before that the flexible linker, which connects PRD1 and PRD2, is involved in this change (12). This idea is reinforced by our recent observation that BglG mutants with amino acid substitutions in the linker demonstrate reduced cross-linking ability in vitro in the absence of BglF (unpublished data). The fact that these mutants retained the ability to be regulated by BglF to a certain degree in vivo suggests that BglF contributes to the formation of the compact form. How BglF promotes folding of the linker remains to be studied.

It was recently shown by our group that BglG is recruited to the cell membrane by BglF in the absence of β-glucosides and is released to the cytoplasm upon addition of the stimulating sugar (17). Formation of the precomplex at the membrane can guarantee rapid response to the presence of β-glucosides in the growth medium, on one hand, and prevent the induction of bgl operon expression in the absence of β-glucosides, on the other hand. What prevents the small fraction of soluble unbound BglG from inducing bgl expression in the absence of the stimulating sugar? By computational analysis, phosphorylation of PRD2 of BglG was not predicted to lead to electrostatic repulsion between the PRD2 domains, suggesting that PRD2 phosphorylation is not the sole regulator of the monomer-dimer equilibrium (E. Ben-Zeev, L. Fux, O. Amster-Choder, and M. Eisenstein, unpublished results). Formation of the compact form can be an additional means that controls this equilibrium. Assuming that the conformational coupling between the PRD1 and PRD2 domains is mutually exclusive with the formation of active dimers, in which PRD1 couples with PRD1 and PRD2 with PRD2, we suggested that folding into the compact conformation can prevent dimerization of BglG, and hence transcriptional antitermination in the absence of β-glucosides (12). The fact that BglF, which regulates the BglG monomer-dimer transition according to β-glucoside availability, also modulates compact monomer formation suggests that the compact form of BglG is important, e.g., in preventing untimely activity of BglG. The compact form probably serves as a reservoir for BglG monomers that can be activated upon the addition of the stimulating sugar. Following the rationale of this hypothesis, we suggested that β-glucosides might play a role in shifting the equilibrium towards the formation of noncompact monomers. In the present study we provide evidence for this mode of regulation. Using in vivo cross-linking experiments, we showed that the level of the compact form drops when β-glucosides are added to the growth medium, depending on BglF expression in the cell. One possibility is that the β-glucosides trigger BglF to diminish the amount of BglG compact form by dephosphorylating it. Alternatively, the presence of β-glucosides interferes with the ability of BglF to enhance formation of the compact form. Whether β-glucosides lead to opening up of the compact conformation or prevention of its formation, their presence leads to increases in the level of the noncompact form and, hence, can promote BglG dimerization and activation. Based on our results, we suggest a model for the modulation of BglG conformation and activity by BglF. In the absence of β-glucosides, BglG is recruited to the cell membrane by BglF. The interaction between BglG and BglF is mediated by the PRD2 domain of BglG and the IIB domain of BglF. Disruption of the BglG dimers (PRD1-PRD1 and PRD2-PRD2 interactions) is probably accomplished by BglF, which recognizes and binds to both BglG monomers and
dimers (G. Monderer-Rothkoff and O. Amster-Choder, unpublished data); this disruption is necessary to expose the residues which are required for phosphorylation and are buried in the dimer interface. However, in order for BglG to be phosphorylated on PRD2 by BglF, residues on the PRD1 domain are also required. We suggest that BglF helps in aligning PRD1 and PRD2 by promoting bending of the linker. It seems reasonable that BglF should have a higher affinity for the noncompact BglG monomers, which should still be taken care of, than for the compact monomers that can be safely released to the cytoplasm, as their conformation is mutually exclusive with dimerization and, hence, with antitermination. Upon addition of β-glucosides, the immediate supply of active BglG comes from the fraction that stayed bound to BglF, which can be rapidly dephosphorylated. Because formation of the compact monomer by BglG is reversible, the compact monomers that were released to the cytoplasm can reach the membrane, be dephosphorylated (we showed that the compact monomer is dephosphorylated by BglF), open up, and serve as a secondary source for BglG. This does not need to be very efficient, because at that stage the bgl operon is induced and new BglG molecules are being produced in the cell.

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