A Fraction of the BglG Transcriptional Antiterminator from *Escherichia coli* Exists as a Compact Monomer*

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Expression of the *bgl* operon in *Escherichia coli*, induced by β-glucosides, is positively regulated by BglG, a transcriptional antiterminator. In the presence of inducer, BglG dimerizes and binds to the *bgl* transcript to prevent premature termination of transcription. The dimeric state of BglG is determined by BglF, a membrane-bound enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), which reversibly phosphorylates BglG according to β-glucoside availability. BglG is composed of an RNA-binding domain followed by two homologous PTS regulation domains (PRD1 and PRD2). The predicted structure of dimeric LicT, a BglG homologue from *Bacillus subtilis*, suggests that the two PRDs adopt a similar structure and that the interactions within the dimer are PRD1-PRD1 and PRD2-PRD2. We have shown recently that the PRD1 and PRD2 domains of BglG can form a stable dimer. We report here, based on *in vitro* and *in vivo* cross-linking experiments, that a fraction of BglG is present in the cell in a compact form in which PRD1 and PRD2 are in close proximity. The compact form is present mainly in the BglG monomers. Our results imply that the monomer-dimer transition involves a conformational change. The possible role of the compact form in preventing untimely induction of the *bgl* operon is discussed.

BglG represents a family of bacterial RNA-binding proteins that bind to specific sequences partially overlapping rho-independent terminators within transcripts of PTS-related genes, and prevent premature termination by stabilizing an alternative secondary structure of the RNA (1). Negative regulation via reversible phosphorylation by enzymes II, which catalyze the phosphotransfer of PTS carbohydrates, has been established for several BglG-like antiterminators (for examples, see Refs. 2–6) and suggested for many others (7). Positive regulation via phosphorylation by HPr, a general PTS protein, has been demonstrated for several antiterminators and was proposed to be part of a mechanism of carbon catabolite repression that operates mainly in Gram-positive bacteria (7). The BglG-like proteins are composed of three domains, namely an RNA-binding domain followed by two homologous domains known as PTS regulation domains (PRD1 and PRD2) (7). Four conserved histidines, two in each PRD, are essential for regulation of BglG-like antiterminators by the PTS proteins (7). BglG-like proteins bind to their RNA targets and activate transcription as dimers. The structure of the RNA-binding domain of LicT and SacY, two BglG homologues from *Bacillus subtilis*, has been determined with and without the RNA target site (8, 9, 10). The structure of the PRDs region of a mutant of LicT, in which two of the conserved histidines were replaced by aspartates, was solved by x-ray crystallography (11). The structure is a homodimer in which the two PRDs adopt a similar structure, and the interactions are of PRD1 and PRD2 from one monomer with PRD1 and PRD2 of the other monomer, respectively (i.e. PRD1-PRD1 and PRD2-PRD2). The phosphorylation sites are totally buried at the dimer interface and, hence, are 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 monomer structure of BglG-like antiterminators has not, to date, been determined.

BglG enables expression of the *Escherichia coli bgl* operon, which encodes proteins that are required for β-glucoside utilization, by transcription antitermination only in the presence of β-glucosides in the growth medium (12). BglF, an enzyme II of PTS, regulates BglG activity by reversible phosphorylation (2, 3). In the absence of the inducing sugar, BglG is present in the cell as a phosphorylated monomer; following inducer addition, BglG is dephosphorylated, undergoes dimerization, binds to its RNA target sites, and allows readthrough of transcription of the *bgl* operon (13). BglF phosphorylates BglG on His-208, a conserved histidine located in PRD2 (14), but the two conserved histidines in PRD1 are required for this phosphorylation reaction (2). We have shown recently that the affinity between PRD1 and PRD2 is high and that they hetero-dimerize efficiently *in vitro* and *in vivo* (15). The interaction between PRD1 and PRD2 offers an explanation for the requirement of conserved residues in PRD1 for the phosphorylation of PRD2 by BglF. We show here that PRD1 and PRD2 can be cross-linked *in vitro* and *in vivo*, indicating that a fraction of BglG is present in the cell in a compact form in which PRD1 and PRD2 are in close proximity. The compact form is present mainly in BglG monomers. The residues in PRD1 and PRD2, which can be cross-linked, are predicted to be far apart in the dimer, based on the three-dimensional structure of dimeric LicT. Hence, our results imply that the monomer-dimer transition involves a conformational change, which most probably occurs by the bending of the flexible linker that connects the two PRDs in the...
monomer molecule to bring PRD1 and PRD2 to a close proximity. We suggest a role for the compact form of BglG in preventing induction of the bgl operon in the absence of the inducing sugar.

EXPERIMENTAL PROCEDURES

Strains—The following E. coli K12 strains were used: (i) MA152 and MA200, which both carry a bgl-lacZ fusion on their chromosome (λ bglR7 bglG [lacz’ galY’]) but, whereas the first is Δbgl, the second is bgl’ (16); (ii) BL21(DE3) (hsdS gal [λAeta1T7 ind1 Sam7 nin5] lacUV5-T7 gene1), obtained from Novagen, and SG13009, obtained from Qiagen, which were used for expression of His-tagged proteins; and (iii) MC1061 (lacIq recA1 leuB6 lacProC1 ara-C13 (lac2) mcrB araD134 leuB6 trpC1 femB25 (from stock solutions of 50 mM dissolved in N,N-dimethyl-formamide) for 20 min at 30°C. The reaction was quenched by the addition of a reducing agent, β-mercaptoethanol, together with the electrophoresis sample buffer. Equal amounts of protein (2 μg) were subjected to SDS-PAGE analysis followed by Coomassie Blue staining.

In Vivo Cross-linking—20 μg of purified MBP-BglG or its derivatives were diluted in 100 μl PBS, pH 7 (20 mM sodium phosphate buffer and 150 mM NaCl, pH 7), and incubated with 1 mM BMH, p-PDM, or o-PDM (from stock solutions of 50 mM dissolved in N,N-dimethyl-formamide) for 30 min at 30°C. The ability of the MBP-BglG mutants to antiterminate transcription was essentially as described by Chen et al. (23). Briefly, [32P]phosphoenolpyruvate was prepared and separated from [32P]ATP. HBII (2 μg) was labeled by incubation at 30°C in a mixture containing EI (0.8 μg), HPr (1.5 μg), IIA^et (2 μg), [32P]phosphoenolpyruvate (10 μM), and PLB buffer (50 mM NaH2PO4, pH 7.4), 0.5 mM MgCl2, 1 mM NaF, and 2 mM dithiothreitol) in a final volume of 10 μl. After incubation for 10 min, MBP-BglG (2.5 μg) or its derivatives were added, and the reaction mixtures were further incubated in PLB buffer for 10 min at 30°C. Reactions were terminated by the addition of electrophoresis sample buffer.

In Vitro Phosphorylation—In vitro phosphorylation was carried out essentially as described by Chen et al. (23). Briefly, [32P]phosphoenolpyruvate was prepared and separated from [32P]ATP. HBII (2 μg) was labeled by incubation at 30°C in a mixture containing EI (0.8 μg), HPr (1.5 μg), IIA^et (2 μg), [32P]phosphoenolpyruvate (10 μM), and PLB buffer (50 mM NaH2PO4, pH 7.4), 0.5 mM MgCl2, 1 mM NaF, and 2 mM dithiothreitol) in a final volume of 10 μl. After incubation for 10 min, MBP-BglG (2.5 μg) or its derivatives were added, and the reaction mixtures were further incubated in PLB buffer for 10 min at 30°C. Reactions were terminated by the addition of electrophoresis sample buffer.

Generation and Characterization of BglG Mutants with Pairs of Cysteines—We have shown recently that the separated PRD1 and PRD2 domains hetero-dimerize efficiently in vitro and in vivo (15). To test whether PRD1 and PRD2 can come into a close proximity within the entire BglG protein, we carried out cross-linking experiments. To this end, we made use of the cysteine residues present on BglG and dimaleimide reagents, which cross-link cysteine residues. The BglG protein contains only three cysteines, two in the PRD1 domain in positions 76 and 102 and one in the PRD2 domain in position 243. We mutated these cysteines to serines, one at a time, to construct three BglG mutants, each containing two of the three original cysteines. The BglG variants were tagged with MBP to enable their purification by affinity chromatography. To check whether the di-cysteine BglG mutants fold correctly and function as wild type BglG and, therefore, can be compared with the latter, we tested their ability to antiterminate transcription and to be negatively regulated by BglF.

The ability of the MBP-BglG mutants to antiterminate transcription of the bgl operon in vivo was tested in the E. coli strain MA152, which is deleted for the bgl operon and carries a
chromosomal fusion of the bgl promoter and terminator to the lacZ gene (16). Expression of lacZ in MA152 depends on introduction of a plasmid encoding an antiterminator. The ability of MBP-BglG and its di-cysteine mutants to enable lacZ expression in MA152 was tested by observing colonies color on MacConkey lactose plates and measuring β-galactosidase levels. As shown in Table I, MBP-BglG and all three di-cysteine mutants antiterminated transcription at the bgl-lacZ fusion. MBP-BglG(C76S) and MBP-BglG(C243S) antiterminated transcription to the exact same extent as MBP-BglG, whereas MBP-BglG(C102S) activity was somewhat reduced. These results also imply that the di-cysteine mutants fold properly and bind to the RNA target site.

Next, we tested the ability of the di-cysteine BglG mutants to be negatively regulated by BglF in vivo. To this end, we made use of strain MA200, which carries the same bgl-lacZ fusion as MA152 but also the bgl operon on its chromosome (16). Expression of lacZ in this strain is inducible and occurs only upon the relief of BglF inhibition by the addition of β-glucosides to the growth medium. Expression of a plasmid-encoded BglG mutant that cannot be regulated by BglF renders growth medium. Expression of a plasmid-encoded BglG mutant was shown before to be the reason for the antiterminated transcription at the bgl-lacZ fusion (16). To this end, we made use of strain MA200, which carries the same bgl-lacZ fusion as MA152 but also the bgl operon on its chromosome (16). Expression of lacZ in this strain is inducible and occurs only upon the relief of BglF inhibition by the addition of β-glucosides to the growth medium. Expression of lacZ in this strain is inducible and occurs only upon the relief of BglF inhibition by the addition of β-glucosides to the growth medium.

### Table I

| Plasmid       | Plasmid encoded BglG derivative | Phenotype on MacConkey lactose plates | β-gal activity units<sup>a</sup> | Plasmid | Plasmid encoded BglG derivative | Phenotype on MacConkey lactose plates | β-gal activity units<sup>a</sup> |
|---------------|--------------------------------|--------------------------------------|-------------------------------|----------|--------------------------------|--------------------------------------|-------------------------------|
| pANSMG       | MBP-BglG                       | Red                                  | 215                           |         |                                 | White                                | 5                             |
| pLFMG(C76S)  | MBP-BglG(C76S)                 | Red                                  | 213                           |         |                                 | White                                | 21                            |
| pLFMG(C102S) | MBP-BglG(C102S)                | Red                                  | 149                           |         |                                 | White                                | 32                            |
| pLFMG(C243S) | MBP-BglG(C243S)                | Red                                  | 214                           |         |                                 | White                                | 24                            |
| pST6 1        | MBP-BglG                       | White                                | 5                             |         |                                 | White                                | 7                             |

<sup>a</sup> Expression of the plasmid-encoded BglG derivatives was not induced by isopropyl-β-D-thiogalactopyranoside.

<sup>b</sup> MA152 and MA200 both carry a bgl-lacZ transcriptional fusion on their chromosome, but whereas the first is Δbgl, the second is bgl<sup>−</sup> (16).

<sup>c</sup> The values of β-galactosidase (β-gal) units represent the average of at least four independent measurements.

<sup>d</sup> 0.4% salicin was added to the growth medium when indicated.

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**Phosphorylation of the different MBP-BglG derivatives, i.e. wild type (WT), MBP-BglG(C76S), MBP-BglG(C102S), and MBP-BglG(C243S), was obtained by incubating the purified proteins with [32P]phosphoenolpyruvate for 10 min. MBP-BglG(C76S) was cross-linked after incubation by BMH, the maleimide groups are connected by an aromatic ring, and, therefore, the span widths do not vary too much in solution. The most probable distance is 9.6 Å, but it can range from 9.2 to 12.3 Å, whereas with p-PDM, the most probable distance is 9.6 Å, and the range in solution is 7.7–10.5 Å (25).

**FIG. 1. In vitro phosphorylation of the di-cysteine BglG mutants.** Phosphorylation of the different MBP-BglG derivatives, i.e. wild type (WT), MBP-BglG(C76S), MBP-BglG(C102S), and MBP-BglG(C243S), was obtained by incubating the purified proteins with [32P]phosphoenolpyruvate for 10 min. Samples were analyzed on 10% SDS-polyacrylamide gel followed by autoradiography. Arrowheads indicate the positions of the EI and MBP-BglG derivatives.

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**The Two PRD Domains of BglG Can Be Cross-linked in Vitro**—To examine the possibility that the PRD1 and PRD2 domains of BglG can come near each other, we asked whether the cysteine residues in wild-type MBP-BglG and in the three di-cysteine mutants can be cross-linked. In case the cysteines are available for interaction and can come in close proximity, we expected to get cross-linked product(s) that are more compact than the wild-type protein and might migrate faster on an SDS-polyacrylamide gel. As cross-linkers we used three homobifunctional reagents, the dimaleimides BMH, p-PDM, and o-PDM, which cross-link cysteine residues by adding the sulfhydryl group of the cysteines to the double bond in the maleimide groups (Fig. 2A). In BMH, the maleimide groups are connected by a flexible hexyl chain, and, hence, although the most probable distance between the thiols is 11.5 Å, the span width can vary between 3.5 and 15.6 Å in solution (25). The maleimides in p-PDM and o-PDM are connected by an aromatic ring, and, therefore, the span widths do not vary too much in solution. The most probable S-S distance with p-PDM is 11.4 Å, but it can range from 9.2 to 12.3 Å, whereas with o-PDM, the most probable distance is 9.6 Å, and the range in solution is 7.7–10.5 Å (25).

We first performed cross-linking experiments with wild-type BglG fused to MBP. Two cross-linked products were observed with all three reagents (Fig. 2B, lanes 2–4). The first, designated S, migrated slightly faster than the non-cross-linked protein, and the other, designated F, migrated significantly faster. With BMH and p-PDM, significant amounts of the two cross-linked products were observed (Fig. 2B, lanes 2 and 3, respectively); yet, the amount of F was significantly higher than that of S. With o-PDM the overall efficiency of the cross-linking was reduced, but, whereas the amount of F was still significant, the amount of S was negligible (Fig. 2B, lane 4). Varying the concentration of the cross-linking reagents between 0.5 and 1 mM did not affect the results.
To determine which cysteines in BglG cross-link to give the two products, we repeated the experiments with the three BglG mutants, each containing two of the three original cysteines. As can be seen in Fig 2B, only the faster migrating product, F, was detected with MBP-BglG(C76S) (lanes 6–8), indicating that this product results from cross-linking between Cys-102 in PRD1 and Cys-243 in PRD2. The efficiency of the cross-linking between Cys-102 and Cys-243 in this mutant was high with the three reagents, although with o-PDM it was somewhat lower, the same as was observed for wild-type BglG. With MBP-BglG(C102S), only the slower migrating cross-linked product S was observed, indicating that it results from bridging between Cys-76 in PRD1 and Cys-243 in PRD2. The efficiency of the cross-linking between Cys-76 and Cys-243 was low and occurred only with BMH and p-PDM (Fig. 2B, lanes 10–12), indicating that these cysteines, although they can come to close proximity, seem to be more than 10 Å apart. No cross-links were observed with MBP-BglG(C243S) (Fig. 2B, lanes 14–16).

Even though it is obvious from the results with the first two mutants that the two cross-link products observed with wild-type MBP-BglG were not due to bridging between the two cysteines in PRD1, Cys-76 and Cys-102, we cannot rule out the possibility that cross-linking of these cysteines results in a product that migrates indistinguishably from the non-cross-linked protein. Taken together, these results indicate that the cysteines in PRD1, especially Cys-102, are in close proximity to Cys-243 in PRD2. Cys-76 is either less accessible to the cross-linkers than Cys-102 or more distant from Cys-243 than Cys-102.

The formation of cross-links with all three of the reagents used by us can be explained by the overlap in the range of S-S distances that can be bridged by these reagents. Alternatively, flexibility in the distance between the PRD1 and PRD2 domains could account for this result. To learn more about the distance between the PRD domains, we tested whether the cysteines in PRD1 and PRD2 can come close enough to form a disulfide bond that can be defined as a zero-length cross-linker. To this end, we analyzed MBP-BglG, MBP-BglG(C76S), and MBP-BglG(C102S) by SDS-PAGE in the absence of reducing agents. As shown in Fig. 3, wild-type MBP-BglG, which contains the three cysteines, and MBP-BglG(C102S), which contains Cys-102 and Cys-243, were able to form a disulfide that disappeared in the presence of β-mercaptoethanol. These results indicate that Cys-102 and Cys-243 can come in very close proximity, because the length of a disulfide bond is \( \sqrt{2} \) Å. The fact that Cys-102 and Cys-243 can be found in a distance that is either as short as 2 Å or as long as 12 Å or more suggests a high degree of flexibility in distance between these residues and the PRD domains.

**Inter-domain Cross-links Are Formed In Vivo in BglG**—The results of the cross-linking experiments in vitro indicate that BglG has the capacity to form a compact form in which the two PRD domains come to a very close proximity. Does the compact form exist in vivo? To answer that question, we incubated cells expressing MBP-BglG (without induction) with the same cross-linking reagents used in vitro, BMH, p-PDM, and o-PDM, which can penetrate cells. Following the incubation, the cells were washed, collected by centrifugation, resuspended in sample buffer, and analyzed by SDS-PAGE and Western blot analysis. The two forms of BglG were detected with anti-MBP
The Compact Form of BglG Is Found Mainly in the Monomer—The compact form of BglG observed in vitro and in vivo migrated on SDS-polyacrylamide gels as a monomer. However, during SDS-PAGE, dimers are denatured to monomers unless they are covalently bound. Because dimers of BglG-like proteins are not held together by covalent bonds (7), the question of whether the compact form is found in the monomeric or dimeric BglG fractions was left open. To address this question, we separated BglG monomers and dimers on a blue native gel (Fig. 5A), cut out gel slices that contained the two forms, immersed them in electrophoresis sample buffer in the presence and absence of β-mercaptoethanol, and loaded them on an SDS-polyacrylamide gel. An attempt was made to load similar amounts of the monomer (M, in Fig. 5) and dimer (D, in Fig. 5) on the denaturing gels. The compact form was clearly observed in the monomeric fraction (Fig. 5B, lane 1) only in the absence of β-mercaptoethanol. A negligible amount of the compact form was detected in the dimeric fraction (Fig. 5B, lane 2). Oxidation upon dimer denaturation could account for this residual amount. Taken together, these results indicate that the compact form of BglG is found mainly in the monomeric fraction, if not exclusively. Because only BglG dimers bind to RNA and catalyze transcriptional antitermination, our results imply that the compact form of BglG is not involved in this activity.

**DISCUSSION**

We have shown recently that the separated PRD1 and PRD2 domains of the BglG transcriptional antiterminator from *E. coli* hetero-dimerize efficiently in vitro and in vivo. In the current study we used homo-bifunctional reagents, three di-maleimides that cross-link cysteine residues (BMH, p-PDM, and o-PDM), to study the proximity of PRD1 and PRD2 in the entire BglG protein. We demonstrated that, in a fraction of the wild-type BglG protein, two cysteines in PRD1 can cross-link both in vitro and in vivo with a cysteine in PRD2, implying that these residues are in close proximity. The cross-linked and non-cross-linked forms can be separated by gel electrophoresis, because the cross-linked products are more compact and therefore migrate faster. Using di-cysteine BglG mutants, we assigned each
product to the specific cysteines that are cross-linked. The more compact cross-link product, which migrates the fastest (designated F) is the result of cross-linking between Cys-102 and Cys-243. The other product (designated S), which migrates slightly faster than the non-cross-linked protein, resulted from bridging between Cys-76 and Cys-243. The F product, which contains a bridge between Cys-102 and Cys-243, is formed much more efficiently in vitro than the S product and is observed with all three reagents. S, which contains a bridge between Cys-76 and Cys-243, is formed in vitro, albeit inefficiently, and is observed only with BMH and P-PDM. F is also the only cross-link product observed in vitro, although we could not rule out that the S form is formed in vitro but is masked by the heavy band of the non-cross-linked protein. The weak cross-linking of the Cys-76-Cys-243 pair might be because Cys-76 is less accessible to the cross-linker, not in close proximity of the domain interface, or further from Cys-243 than Cys-102.

The cross-linking that we observed did not result from changes in the BglG structure due to the mutagenesis, because the two cross-link products were observed with the wild-type BglG protein. The cross-linking cannot be attributed to the use of the dimaleimide reagents because, intriguingly, Cys-102 and Cys-243 are close enough to form a disulfide bond. Formation of the disulfide bridge was observed when analyzing a purified BglG protein or cell extract on non-reducing gels. Importantly, formation of the disulfide bond was spontaneous, that is, in the absence of a catalyst to initiate oxidation. The S-S bond is not observed after incubation of the cells with iodoacetamide, indicating that it is not formed in the cells but, rather, after the cells rupture. Nevertheless, its formation both in cell extract and after BglG purification is a good indication for the proximity for Cys-102 and Cys-243 and, hence, of PRD1 and PRD2.

The formation of cross-links with all three of the dimaleimide reagents used by us can be explained by the overlap in the range of distances that can be bridged by these reagents. However, a disulfide bond necessitates a closer proximity between the cysteines (2.03 Å; Ref. 26) than the cross-linking reagents. The fact that Cys-102 and Cys-243 can be found at a distance that is either as short as 2 Å or as long as 12 Å or more, suggests a high degree of flexibility in the distance between these residues and, hence, flexibility in the distance between the PRD1 and PRD2 domains. This flexibility in distance between the PRD domains can be explained by breathing of the protein, protein dynamics, or reagent dynamics. The results suggest that the BglG monomer can be found in two or more conformations and that the different conformers are in equilibrium. None of the conformers is expected to be stabilized by disulfide bridge formation, because the intracellular environment is too reducing to allow cysteine oxidation to the disulfide state.

Besides the existence of different conformers of BglG, the extent of cross-linking by dimaleimides and by a disulfide bridge might not represent the genuine proportion of the compact BglG form. There are several reasons that reduce the efficiencies of cross-linking and disulfide bond formation (see Ref. 27). First, cross-linking of two cysteines with one dimaleimide molecule is competitive with the binding of each of the two cysteines to a different dimaleimide molecule, which precludes cross-linking. Second, inactivation of one of the maleimide moieties of a dimaleimide by hydrolysis also precludes cross-linking. Third, the formation of disulfide bonds is competitive with terminal oxidation of thiols to sulfonates (28). Fourth, oxidative cleavage of the thiol groups might occur rapidly because of low stability of the disulfide bond, which is most readily explained by the unfavorable positioning (distance-wise or geometry-wise) of cysteines that do not interact naturally (29). Such bonds are difficult to form and unstable once formed (30).

The crystal structure of the region that contains PRD1 and PRD2 from a constitutively active mutant form of LicT was determined (11). The mutations that enabled crystallization were formed by the replacement of the two conserved phosphorylatable histidines in PRD2 by aspartates. The truncated protein crystallized as a homodimer in which the PRD1 of one monomer dimerizes with the PRD1 of the other monomer and PRD2 dimerizes with PRD2. The two domains are connected by a flexible loop. No structural information is yet available on the inactive monomer of any of the BglG-like transcriptional anti-terminators. Assuming that the dimer structure of BglG is similar to that of dimeric LicT, interaction of Cys-243 in PRD2 with either Cys-76 or Cys-102, both of which are in PRD1, is not possible in the dimer form. Hence, the results presented here, demonstrating the proximity of Cys-243 to Cys-102 and, to a certain extent, also to Cys-76 in the monomer form, suggests that the structure of the BglG monomer differs from that of the dimer, implying that a conformational change is involved in the transition from the monomer to the dimer form. Bearing in mind that PRD1 and PRD2 are similar to each other, this conformational change might be a variation of the domain swapping described by Liu and Eisenberg (31). Importantly, the switch between activated (phosphorylated) and native (unphosphorylated) forms of LicT also seem to involve a conformational change and motions of the PRD domains, as indicated by the different fluorescence spectrum, proteolysis resistance, and small angle x-ray scattering exhibited by the two proteins (32). Also, mutations within PRD2 of LicT modified the activity of the RNA-binding domain and the local environment of residues within PRD1 as well as in the linker region between PRD1 and the RNA-binding domain (32). In the case of LicT, a switch between open and closed dimers was suggested (32), as opposed to a monomer-dimer switch demonstrated for BglG (13). What triggers the changes in BglG and LicT is a subject for future studies. PTS proteins, such as HPr and enzymes II might play a role in the switch. Experiments aimed at studying the effect of BglF, the BglG phosphorylase and dephosphorylase, on the formation of the compact form of BglG are currently underway. Differences in the structures of BglG and LicT cannot be ruled out. Crystallization of the dimer and monomer of wild-type BglG-like proteins will shed light on the nature of the monomer-dimer transition. The equilibrium between the different monomer conformers might make the obtaining of crystals that diffract at a good resolution a difficult goal to achieve.

What might be the possible role(s) of the compact BglG form or the reasons for its formation? One possible role might be preventing the untimely induction of the bgl operon, that is, when β-glucosides are not present in the growth medium and, hence, the products of this operon are not needed. We have recently shown that BglG is recruited to the cell membrane by BglF, in the absence of β-glucosides, and is released to the cytoplasm following the addition of the stimulating sugar (17). The formation of a pre-complex between BglF and BglG can guarantee rapid response to β-glucoside addition. It might also prevent BglG activity in the absence of β-glucosides, either by sequestering BglG at the membrane or by keeping it as a phosphorylated monomer, which is inactive. Yet, a minute quantity of BglG dimers is enough to induce expression of the bgl operon, leading to the production of more BglG, which is encoded by this operon. One question is what prevents the transition of monomers to dimers in the absence of the stimulating sugar. A slightly different question is what prevents the dimerization of phosphorylated BglG. 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, then formation of the compact monomers can prevent dimerization of BglG and, hence, transcriptional antitermination. According to this model, β-glucosides might play a role in shifting the equilibrium toward the formation of non-compact monomers. Alternatively, the non-compact form of the BglG monomer might be unstable, less structured, or too exposed to irrelevant factors. The compact form might serve as reservoir for intact and stable BglG monomers when β-glucosides are absent.

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