Roles of Glucitol in the GutR-mediated Transcription Activation Process in Bacillus subtilis

GLUCITOL INDUCES GutR TO CHANGE ITS CONFORMATION AND TO BIND ATP*

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GutR is a 95-kDa glucitol-dependent transcription activator that mediates the expression of the Bacillus subtilis glucitol operon. Glucitol allows GutR to bind tightly to its binding site located upstream of the gut promoter. In this study, a second functional role of glucitol is identified. Glucitol induces GutR to change its conformation and triggers GutR to bind ATP efficiently. After sequential binding of glucitol and ATP to GutR, GutR adopts a conformation by forming a compact structure that is resistant to trypsin digestion. Under this condition, the ATP-glucitol-GutR complex can dissociate slowly from the gutR-binding site (t\text{1/2} = 274 min). Interestingly, if ATP in the ATP-glucitol-GutR complex is replaced by ADP, GutR adopts another conformation and can dissociate from the gutR-binding site even faster (t\text{1/2} = 82 min). In all these GutR-DNA binding studies in the presence of different ligands (glucitol, ATP, or ADP), only the off-rate is affected. The vital role of ATP in the GutR-mediated transcription activation process is reflected by the poor transcription from the gut promoter with GutR(D285A) which has a mutation in the motif B of the putative ATP-binding site. A working model for this transcription activation process is presented.

Induction of the Bacillus subtilis glucitol utilization (gut) operon is regulated by a transcription activator, GutR, in the presence of glucitol (1–4). GutR is a 95-kDa protein (829 amino acids) with several putative motifs including an N-terminal helix-turn-helix motif for DNA binding, Walker motifs A and B for a nucleotide-binding site, and several C-terminal tetrapeptide repeats for possible intra- and intermolecular interactions (5). In the absence of any ligand, purified GutR in the concentration range of 0.5 to 5 μM exists in the monomeric form. It binds specifically to a single 29-base pair imperfect palindrome located upstream of the target site. After the subsequent binding of ATP to GutR, the GutR complex can dissociate slowly from the gutR-binding site with a half-life of longer than 19 h in the presence of glucitol (7). Considering B. subtilis as a soil microorganism living in an environment with limited nutrients available most of the time, a generation time of 380 min or longer is not uncommon for this organism under such growth conditions. Consequently, a half-life of 6.8 min of the complex is considered insignificant relative to the generation time of this organism. In sharp contrast, GutR binds to its target site with a half-life of longer than 19 h when in the presence of glucitol (7). The effect of glucitol specific and affects only the off-rate but not the on-rate of the binding reaction. It is interesting to know why such tight binding is necessary? Can GutR in the presence of glucitol be able to release from its binding site? In this study, glucitol is found to induce GutR to have a conformational change which allows the subsequent binding of ATP to GutR. The tight binding of glucitol-GutR to DNA allows this complex to have plenty of time to bind ATP. Binding of ATP triggers GutR to have another conformational change and GutR is now able to dissociate from its target site with a half-life of 274 min. If ATP is replaced by ADP, GutR can dissociate from its target site at an even faster rate and the half-life of the GutR-DNA complex under this condition is 82 min. AMP seems unable to bind to the glucitol-GutR complex. A working model describing the initial steps that lead to the GutR-mediated transcription activation is presented.

**Experimental Procedures**

**UV Cross-linking of [γ-32P]ATP to GutR**—This procedure is modified from Zhong and Tai (8). Purified GutR (1 μg), [γ-32P]ATP (1 μCi, 3,000Ci/mmol) with or without glucitol (or other sugars/sugar alcohols) were added to a binding buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, and 5 mM dithiothreitol) in a final volume of 20 μl in a 0.5-ml microcentrifuge tube. A series of binding reactions was set up and the final concentrations of glucitol in these tubes ranged from 0 to 2%. ATP molecules bound to GutR were UV cross-linked to GutR by short-wave UV using a handheld UV lamp (model UVGL-25, UVP, Inc., Upland, CA) positioned 2 cm above the microcentrifuge tubes with the lights on. Trypsin digestion was done at room temperature in a total volume of 200 μl. Trypsin (type II-S from Sigma, Canada) was prepared in 0.1 mM HCl at 1 mg/ml and divided into 10 μl/microcentrifuge tube. The enzyme was aliquoted and stored at −20 °C until use. Four μg of GutR and 0.1 μg of trypsin were used in the reaction. The conditions for each digestion reaction were identical with respect to time, buffer components, and GutR concentration. The variable manipulated was the ligand added. These ligands included glucitol, xylitol, mannitol, glucose, ATP, ADP, and AMP. The final concentrations of sugars/sugar alcohols and nucleotides were 2% and 1 mM, respectively.

1 The abbreviation used is: PAGE, polyacrylamide gel electrophoresis.
Plasmid pBSGUTRN was used as the template and inverse polymerase chain reaction (10) was performed using a pair of primers, MDB (5′- CAGTATCAATGCTGGCAACGATCAG-3′) and GUTRBF (5′- GTCTTGCACTGTTAAGTACTTTAAA-3′) to change Asp-288 to Asn (2). One of the resulting mutated vectors was selected for further characterization and was designated pGutRM. Since SsuI and PstI sites are the unique restriction enzyme sites that are flanking the mutagenic oligonucleotide, the degree of cross-linking depended on the concentration of glucitol and reached the peak level in the presence of 0.5–1% glucitol. This peak level (lanes 7–9) was about 10 times higher than the basal cross-linking level (lanes 4 and 5). Three independent binding studies were performed and a consistent 6–10-fold increase in cross-linking could be observed in the presence of glucitol at a concentration of 0.5% or higher. To further determine the specificity of the glucitol-mediated ATP binding to GutR, the effects of xylitol, mannotol, and glucose were examined. The effect mediated by 2% xylitol, a 5-carbon sugar alcohol, was minimal (lane 2). A more obvious effect was observed in the presence of mannotol, a 6-carbon epimer of glucitol at the C-2 position. However, even at a final concentration of 2%, the stimulated ATP binding to GutR (lane 3) was weaker than that mediated by 0.05% glucitol. In the presence of 2% glucose (lane 4), the degree of cross-linking was comparable to the basal level. All these data indicate that GutR can bind ATP in a glucitol-dependent manner and this effect is relatively glucitol specific.

To gain information concerning the specificity of the nucleo-
The presence of 1 mM ATP is presented in Fig. 3.

**Glucitol Induces a Conformational Change in GutR**—Since GutR can bind ATP to a significant level only in the presence of glucitol, it is logical to predict that binding of glucitol to GutR can induce GutR to have a conformational change. To examine this possibility, limited trypsin digestion of GutR in the presence or absence of various ligands was studied. The first step in this study was to establish an optimal trypsin-to-GutR weight ratio to generate trypsin fragments that could be monitored in a reasonable time frame. This ratio was established to be 1:40. In the absence of ligands, GutR was cleaved by trypsin to a 75-kDa fragment in 18–24 min (Fig. 2A). This species was resistant to further digestion. In contrast, GutR became highly susceptible to protease digestion in the presence of glucitol. Several clusters of bands were generated with time. After a 60-min digestion, two clusters of bands remained to be dominant (Fig. 2B). A doublet designated “a” and a triplet designated “b” with the apparent molecular masses around 55 and 45 kDa, respectively, were observed. These two clusters could be observed more obviously by the Western blot analysis as shown in Fig. 2C. The difference in the digestion profiles of GutR in the presence or absence of glucitol indicates that GutR can bind glucitol and there is a conformational change in GutR after glucitol binding. This conformational change was relatively glucitol specific. Addition of glucose, galactose, mannitol, or xylitol to GutR at a final concentration of 2% failed to show any significant changes in both the pattern and the kinetics of the GutR digestion profiles relative to those observed for GutR in the absence of any ligand. A typical digestion profile for GutR in the presence of glucose is shown in Fig. 2D.

**Effects of ATP on the Conformation of GutR**—In the presence of ATP, the tryptic digestion pattern of GutR was similar to that observed for GutR in the absence of any ligand (data not shown). This suggests that, in the absence of glucitol, ATP cannot bind to GutR and thus does not affect the GutR conformation. This observation was consistent with the observation of the UV cross-linking study which indicated that ATP could bind to GutR only when glucitol was present.

To determine whether ATP can induce the glucitol-GutR complex to have a conformational change, different concentrations (1 nM, 1 μM, 10 μM, 100 μM, and 1 mM) of ATP were added to glucitol-GutR. At low concentration (1 nM) of ATP, both the pattern and kinetics of digestion profile of GutR were the same as those for GutR in the presence of glucitol. However, different digestion patterns were observed with the ATP concentration ranging from 1 μM to 1 mM. Since the microbial physiological cellular concentration of ATP ranges from 1 to 5 mM (12), the tryptic digestion profile of the glucitol-GutR complex in the presence of 1 mM ATP is presented in Fig. 3A. Under this condition, two sets of bands were observed. The first set was a doublet with the intact 95-kDa GutR protein as the major band. The second set was a 75-kDa GutR fragment. Even with a 60-min trypsin digestion, the intact form (95 kDa) of GutR still represented the dominant band. These data indicate that ATP indeed can bind to the glucitol-GutR complex to trigger GutR to have another conformational change. In this case, GutR adopts a more compact conformation so that it is more resistant to trypsin digestion. Consistent with the UV-cross-linking experiments, GTP at a final concentration of 1 mM could also induce glucitol-GutR to adopt the trypsin resistant conformation although most as well as ATP (Fig. 3B) while CTP could offer only some degree of protection (Fig. 3C).

**Effects of ADP and AMP on the Conformation of GutR**—In the presence of glucitol, addition of AMP (1 mM) to GutR did not protect GutR from trypsin digestion (data not shown). Both the kinetics and digestion profiles were similar to those observed for GutR in the presence of glucitol (i.e., with 55- and 45-kDa fragments as the major bands) suggesting that AMP either cannot bind to GutR even in the presence of glucitol or that it binds but fails to trigger a conformational change in GutR. Fig.
FIG. 2. Binding of nucleotides to the glucitol-GutR complex as probed by limited trypsin digestion. Panels A–D show SDS-PAGE analyses of the digestion kinetics and the digestion profiles of GutR in the presence of ATP, GTP, CTP, and ADP, respectively. The final concentration of each of these nucleotides is 1 mM. To determine the position of the 75-kDa GutR fragment is marked by an arrow.

3D shows the digestion profile of GutR in the presence of both glucitol and ADP, wherein a major band of a 75-kDa fragment and a minor band of a 95-kDa GutR were seen. This indicated ADP could bind to glucitol-GutR. Since the major band is a 75-kDa fragment which is similar to that observed for GutR in the absence of glucitol, it is tempting to speculate that binding of ADP to GutR may cause the dissociation of glucitol from the ADP-glucitol-GutR complex.

N-terminal Sequencing of the Trypsin-digested GutR Fragments—Three major GutR fragments were isolated for N-terminal sequence determination. They were the 75-kDa fragment generated by digestion of GutR in the absence of any ligand and one of the doublet (55 kDa) and one of the triplet (45 kDa) fragments generated by the trypsin digestion of GutR in the presence of glucitol. The first 5 amino acids from each of these fragments were determined (Fig. 4A). The 75-kDa fragment was found to miss the first 145 amino acids from the N-terminal region which contained the putative helix-turn-helix motif for DNA binding. If this is the only exposed trypsin cleavage site in GutR under this condition, the C-terminal GutR fragment (amino acid residues 146–829) that shows resistance to trypsin should have a theoretical molecular mass of 79 kDa. It is not sure whether this 75-kDa fragment has an apparent molecular mass less than the actual one or there is at least one extra cleavage at the C-terminal region. The 55- and 45-kDa fragments were missing both the putative DNA- and ADP-binding sites (Fig. 4A). If there are no extra trypsin cleavage sites at the C-terminal end of GutR, the theoretical molecular masses of these two fragments should be 59 and 50 kDa, respectively. Both the 75- and 45-kDa fragments were generated by trypsin digestion. However, the 55-kDa fragment was likely to be generated by chymotrypsin contaminated in the trypsin preparation since the cutting site was between Tyr-322 and Arg-323.

Effects of Nucleotide Binding on the On-rate and Off-rate of the Glucitol-GutR Complex to Its Target Binding Site—We have previously demonstrated that GutR can bind to its target site in the absence of glucitol (7). However, in the presence of glucitol, the glucitol-GutR complex binds to its target sequence so tight that the off-rate is not measurable using the BIAcoreX biosensor. The on-rate for the binding reaction is not changed significantly whether glucitol is present or not. It would be interesting to examine whether nucleotides would have any effect on the GutR-DNA interaction. As shown in Table I, both the on-rate and off-rate of GutR to its target sequence in the presence of 1 mM ATP without any glucitol were comparable to those observed for GutR in the absence of glucitol. Once again, these data strengthen the idea that ATP cannot bind to GutR in the absence of glucitol. In contrast, in the presence of 2% glucitol and 1 mM ATP, GutR was able not only to bind but also to dissociate from its target DNA. Although the on-rate was not changed significantly in comparison with other conditions, the off-rate of the ATP-glucitol-GutR ternary complex from its target site became measurable. In comparison with the off-rate of the GutR-DNA complex in the absence of glucitol, it was about 40 times lower. Replacement of ATP with GTP got comparable results (Table I). All these data indicate that GutR can bind ATP and GTP in the presence of glucitol. If ATP was replaced by ADP, dissociation of the ADP-glucitol-GutR ternary complex from the GutR-binding site was even faster than that of the ATP-glucitol-GutR-DNA complex. This observation strengthens the idea that ADP binds to the glucitol-GutR complex and affects the dissociation of GutR from its target site.

GutR(D288A) Activates Transcription from the Gut Operon Poorly—To determine whether ATP plays a role in the GutR-mediated transcription activation, the conserved aspartic residue in the putative motif B (5, 13) of the ATP-binding site in GutR (Fig. 4) was changed to alanine by site-directed mutagenesis. The expression vector pUB18P43GUTR(D288A) for the production of GutR(D288A) was transformed into B. subtilis WB1104. This strain is a derivative of WB1101 which has the chromosomal gutR inactivated (5). WB1104 carries a gut-lacZ cassette (6) integrated in the amyE locus in the genome. This gut-lacZ cassette has the regulatory region of the gut operon including the gut promoter fused to the promoterless lacZ reporter gene. WB1104 carrying a plasmid vector (pUB18-P43)
showed an 83% reduction in 12 Miller units). In contrast, WB1101[pUB18P43GUTR(D288A)] expression vector showed a high WB1104[pUB18P43GUTR] which overproduced GutR from the without any transcription activation. This result suggests that ATP is required for the GutR mediated transcription activation. This was not because of the low-level production of GutR(D288A). This indicates that ATP can have the same conformation as the ATP-binding site in the GutR complex if the ATP-binding site in GutR is preformed. Closed triangle and closed circle represent the N-terminal domain of GutR and ATP, respectively. The glucitol-binding site is tentatively assigned to the C-terminal domain of GutR. 

![Diagram](https://example.com/diagram.png)

**FIG. 4. Organization of domains within GutR.** Panel A shows the domain organization of GutR. HTH marks the putative helix-turn-helix motif to serve as a DNA-binding domain. Walker motifs A and B for the ATP-binding domain, and the C-terminal domain of GutR, respectively. A, B, and C represent glucitol and ATP, respectively. The glucitol-binding site is marked by an asterisk. Arrows A, B, and C represent the cleavage sites within GutR that are susceptible to protease digestion under the specified conditions. Horizontal lines represent both the intact and the protease-resistant GutR fragments (cut at sites A, B, and C), respectively. The theoretical molecular masses (expressed in kDa) of these fragments observed were shown on the right. Numbers bracketed represent the apparent molecular masses of these fragments observed by SDS-PAGE analysis. Panel B shows a working model to illustrate the conformational changes of GutR under different conditions. d, a, and c represent glucitol and ATP, respectively. The glucitol-binding site is tentatively assigned to the C-terminal domain of GutR.

**TABLE I**

| Conditions          | $k_a$ ($s^{-1}$) | $k_d$ ($s^{-1}$) | $t_{1/2}$ (min) | $K_d$ ($M$) | Ref. |
|---------------------|-----------------|-----------------|----------------|------------|-----|
| GutR                | $1.8 \times 10^6$ | $1.7 \times 10^{-3}$ | 6.8            | $9.4 \times 10^{-10}$ | 7   |
| GutR + glucitol     | $2.1 \times 10^6$ | ND              | 7.8            | $7.1 \times 10^{-10}$ | This study |
| GutR + ATP          | $1.6 \times 10^6$ | $1.5 \times 10^{-3}$ | 274            | $1.3 \times 10^{-10}$ | This study |
| GutR + glucitol + ATP | $8.0 \times 10^6$ | $4.2 \times 10^{-5}$ | 82             | $5.6 \times 10^{-10}$ | This study |
| GutR + glucitol + ADP | $1.1 \times 10^6$ | $1.4 \times 10^{-4}$ | 205            | $5.6 \times 10^{-10}$ | This study |
| GutR + glucitol + GTP | $1.0 \times 10^6$ | $5.6 \times 10^{-5}$ | ND             | 7          | This study |

*ND, not determined because of the tight binding of GutR to its binding site.*

without any gutR insert served as a negative control. When cultivated in a defined medium in the presence of 2% glucitol (5), its β-galactosidase activity was $0.963 \pm 0.086$ Miller units. WB1104[pUB18P43GUTR] which overproduced GutR from the expression vector showed a high β-galactosidase activity ($452 \pm 12$ Miller units). In contrast, WB1101[pUB18P43GUTR(D288A)] showed an 83% reduction in β-galactosidase activity ($79 \pm 8$ Miller units) in reference to that of WB1104[pUB18P43GUTR]. Since SDS-PAGE analysis demonstrated that the cellular levels of GutR and GutR(D288A) from WB1104[pUB18P43GUTR] and WB1104[pUB18P43GUTR(D288A)], respectively, were comparable (data not shown), the observed low β-galactosidase activity was not because of the low-level production of GutR(D288A). This result suggests that ATP is required for the GutR mediated transcription activation.

**DISCUSSION**

Induction of the glucitol operon in B. subtilis requires both GutR, the transcription activator, and glucitol. Our previous studies demonstrate that one of the functional roles of glucitol is to induce GutR to bind tightly to its target site upstream of the gut operon with a half-life of 19 h or longer. In this study, another important functional role of glucitol is identified. GutR is shown to be a nucleotide-binding protein. It binds ATP and GTP more efficiently than CTP and has low or poor binding capability to UTP. This nucleotide binding capability is dependent on the presence of glucitol. GutR has to bind glucitol first. Subsequently, the glucitol/GutR complex has the capability to bind ATP as illustrated by the UV cross-linking experiments. The idea for sequential binding is further supported by both the limited trypsin digestion experiments and the biosensor studies. The ATP induced changes in both the GutR conformation and the off-rate of GutR-DNA complex can be observed only in the presence of glucitol.

There are two possible models to explain the observed glucitol-dependent ATP binding in GutR. In the first model, the ATP-binding site in GutR is not preformed. The binding of glucitol to
GutR is required to induce GutR to have a conformational change so that critical residues involved in ATP binding can be positioned in proper locations. A similar mechanism has been observed for a transcription regulator, BirA, in E. coli. BirA, a bifunctional protein, serves as a repressor for the biotin biosynthetic operon as well as an enzyme for protein biotinylation with both biotin and ATP as the substrates (14, 15). X-ray crystallography studies show that the ATP-binding site in BirA is not preformed in the absence of biotin (16). After the binding of biotin to BirA, key residues involved in ATP binding are repositioned and BirA gains the ATP binding capability. In the second model, the ATP-binding site in GutR is preformed. However, this site is not accessible for nucleotide binding because of the physical blockage by other domains in GutR. Binding of glucitol to GutR induces GutR to have a conformational change so that the ATP-binding site is now exposed and becomes accessible for ATP binding. This situation is similar to that observed in XylR, a transcription activator that mediates the expression of genes involved in toluene biodegradation in Pseudomonas putida (17). In this case, binding of the inducer, m-xylene, to the N-terminal reception domain in XylR exposes the ATP-binding site at the central domain and allows XylR to bind and hydrolyze ATP. Deletion of this N-terminal domain results in the constitutive expression of the toluene biodegradation operon (18, 19). A combination of these two models (i.e., physical blockage of a non-preformed ATP-binding site by other domains from GutR in the absence of glucitol) can also be possible (Fig. 4B). To differentiate these models, x-ray crystallographic studies of GutR in the presence and absence of glucitol may provide insightful information.

Besides BirA and XylR, GutR is another transcription regulator that binds the inducer/substrate and ATP in a sequential manner. Although GutR shares similarities to transcription activators such as MalT (20, 21) and AcoK (22) in the 100-kDa transcription activator family in terms of their size, ATP binding capability, and the ability to activate transcription from the major RNA polymerase rather than the $\sigma^{34}$ containing RNA polymerase, none of the members in this family has been shown to bind the inducer and ATP in a sequential order. In fact, MalT binds ATP even in the absence of maltotriose (23).

GutR contains 100 sites that can potentially be cleaved by trypsin if all these sites are exposed. In the absence of glucitol, cleavage mainly occurs in site A (Fig. 4A). The expected cleavage products are the 16-kDa fragment corresponding to the first 145 amino acids from the N-terminal region of GutR and the 79-kDa C-terminal fragment. The observation of the 79-kDa GutR fragment indicates most, if not all, of the sequence between residue 146 and residue 829 forms a tightly folded structure. This includes the putative ATP-binding site with the Walker motif A (residues 200–207) and Walker motif B (residues 283–291). In contrast, in the presence of glucitol, tryptic cleavages occur in many sites with sites B and C as two of the dominant cleavage sites (Fig. 4A). It is particularly interesting to note that cleavage site B (between residues 322 and 323) is only 31 residues downstream from the putative motif B of the ATP-binding site. This provides appealing evidence to suggest that glucitol induces GutR to have a conformational change and the ATP-binding site in GutR is now more accessible. In combination with the UV cross-linking experiments, the tryptic digestion experiments provide a mechanistic basis to explain the observed glucitol dependence for GutR to bind ATP. These data also provide insights to explain the properties of the gutR1 mutant (2). gutR1 contains a single point mutation (Cys to Ala) that results in a substitution of serine 289 to arginine (5). This mutation is located within the putative motif B region of the ATP-binding site. This gutR1 mutant can constitutively turn on the gut operon even in the absence of glucitol (2). It is possible that this mutation induces a conformational change in GutR1 and allows this protein to bind ATP in the absence of glucitol.

Binding of ATP to glucitol-GutR induces the complex to have another conformational change. The entire ATP-glucitol-GutR ternary complex forms a compact structure which is resistant to trypsin digestion as illustrated in Fig. 3A. Unlike the glucitol-GutR-DNA complex, the ATP-glucitol-GutR-DNA complex at least can dissociate from the gutR-binding site ($t_{1/2} \approx 274$ min). For the case of the ADP-glucitol-GutR complex, it can dissociate from the gutR-binding site even faster ($t_{1/2} \approx 82$ min). This suggests that different forms of nucleotide can play a role in modulating the affinity of the GutR-DNA complex.

A weak ATPase activity was observed from our purified GutR preparation. However, the possibility for this activity to be derived from other contaminated ATPases cannot be eliminated. In all these binding reactions, the on-rates were not significantly changed and only the off-rates were affected (Table 1). Since GutR adopts a different conformation under each condition, the degree of interaction between GutR and its target site via hydrogen bonding and other noncovalent interactions can be different. This can provide a possible explanation for the observation changes in off-rate under each situation.

To illustrate that ATP really plays a role in the GutR mediated transcription activation, the conserved aspartic acid residue (Asp-288) in the putative motif B of GutR (5) was mutated. A typical motif B sequence in a nucleotide-binding protein contains four hydrophobic residues followed by a conserved aspartate residue (13). Structural studies of several nucleotide-binding proteins (24–26) suggest that this conserved aspartate and a serine or threonine residue in the Walker motif A are the two key residues to stabilize $\mathrm{Mg}^{2+}$ in the $\mathrm{Mg}^{2+}$-ATP or $\mathrm{Mg}^{2+}$-GTP complex. This $\mathrm{Mg}^{2+}$ ion is essential for hydrolysis of ATP or GTP (27, 28). Therefore, mutation of this conserved aspartate in the nucleotide binding pocket of E. coli DnaA (29) and Salmonella typhimurium NtrC (30) has been shown to abolish the nucleotide binding capability of DnaA and the ATPase activity of NtrC, respectively. In the cases of the high affinity ATP-binding sites in both B. subtilis and E. coli SecA (28), equivalent mutations reduce both the ATP binding affinity and the ATPase activity. Because of the important role of this residue in nucleotide binding and hydrolysis, aspartate 288 in GutR was changed to alanine. GutR(D288A) was produced at a high level from a high-copy number plasmid and its production level was comparable to that of the wild type GutR. However, the low transcription activation capability of GutR(D288A) in vivo suggests that ATP plays an important role in the GutR mediated transcription activation. In the case of E. coli MalT (23), only ATP binding but not hydrolysis is required for the formation of open complex. Transcription activation mediated by Rhodobacter capsulatus NtrC also requires only ATP binding but not hydrolysis (31). In contrast, NtrC from enteric bacteria requires both ATP binding and hydrolysis for transcription activation (32). Since the gut promoter has an unusually short spacer (15 base pairs rather than 17 base pairs) between the −35 and −10 hexameric sequences (6), it is tempting to speculate that GutR may use the energy from ATP to realign the −35 and −10 elements in the proper relative orientation so that this promoter can be recognized by RNA polymerase. Further characterization of different ATP-binding site mutants would provide insights to understand this transcription activation process.

A working model that shows the early steps in the GutR mediated transcription activation is summarized in Fig. 4B. In this process, glucitol, the inducer, plays a vital role. It binds to
GutR and induces GutR to change its conformation and adopt a more open structure. This leads to two significant consequences. First, GutR binds tightly its target site. This provides plenty of time for GutR to bind the second ligand, ATP. Second, under this condition, GutR is in the appropriate conformation to bind ATP. ATP binding, hydrolysis, or a combination of both can turn GutR to the active state to function as the transcription activator.

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