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

TIGHT BINDING OF GutR TO ITS BINDING SITE

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Glucitol induction in Bacillus subtilis requires a transcription activator, GutR, and a sequence located upstream of the gut promoter. To understand the initial steps involved in the GutR-mediated transcription activation process and the physiological roles of glucitol, GutR was overproduced and purified. In the absence of glucitol, GutR exists as a monomer and binds directly to its binding site in the gut regulatory region. This binding site was mapped to a 29-base pair imperfect inverted repeat located between −78 and −50, and there is only one GutR binding site within the regulatory region. The kinetic parameters of the interaction between GutR and its binding site were monitored in real time using surface plasmon resonance. The half-life of the GutR-DNA complex in the absence of glucitol was estimated to be 6.8 min. In contrast, in the presence of glucitol, the half-life of the complex was extended to longer than 19 h by affecting only the off-rate but not the on-rate. This effect is glucitol-specific. These data indicate that glucitol binds to GutR and induces GutR to have an extremely tight binding at its binding site. The physiological relevance of this process in transcription activation is discussed.

With the addition of glucitol to Bacillus subtilis, a set of glucitol-inducible genes is selectively turned on (1). These inducible genes include gutA- and gutB-encoding glucitol permease and glucitol dehydrogenase, respectively. They are the members of the gut (glucitol utilization) operon and are arranged in the order from gutB to gutA. This operon is subject to both negative and positive regulations at the transcription level with the positive regulation playing a major role in the induction process. The regulatory region of the gut operon can be divided into three parts (2). The central portion is an unusual promoter with its −10 and −35 elements similar to those recognized by the major RNA polymerase (EσA) separated from each other by a 15-bp spacer rather than a typical 17-bp spacer. An inverted repeat sequence located downstream of the promoter serves as a negative regulatory element (2). Although deletion of this sequence does not result in the constitutive expression of the gut operon in the absence of glucitol, the induced expression from this system increases by almost 4-fold. A region (from −126 to −48 with the transcription start site as +1) located upstream of the promoter is required for glucitol induction and is suggested to contain binding sites for a transcription activator, GutR. The structural gene encoding GutR is located upstream of the gut operon and is transcribed in an opposite direction relative to that of the gut operon (3, 4). Inactivation of this gene abolishes the glucitol induction of the gut operon.

Several different motifs can be found in GutR, including a typical helix-turn-helix motif for DNA binding at the N-terminal region, motifs A and B for a nucleotide binding site, and tetratricopeptide repeats at the C-terminal region (4). To understand the regulatory mechanism of glucitol induction in B. subtilis, we report in this study the overproduction and purification of GutR. This protein indeed binds specifically to the regulatory region upstream of the gut promoter, and there is only a single GutR binding site in that region. The precise boundary of this binding site was mapped. Interestingly, GutR can form a complex with its binding site even in the absence of glucitol. However, in the presence of glucitol, the real-time kinetic measurement with a BIAcoreX biosensor demonstrates that GutR binds extremely tightly to its binding site and that this tight binding is the result of a change in the off-rate (dissociation rate constant) of the binding reaction. Molecules other than glucitol are unable to induce a similar effect. Possible steps involved in the initial stage of the GutR-mediated transcription are discussed.

EXPERIMENTAL PROCEDURES

Construction of the GutR Expression Vector—The expression vector used in this study is the B. subtilis pUB18-P43 vector (5). This vector is a pUB110 derivative with a kanamycin resistance marker and a strong, constitutively expressed B. subtilis promoter P43 to drive the transcription of genes of interest inserted in the polylinker region located downstream of the promoter. gutR was obtained from pU1 (4), which carries the chromosomal gutR region by SacI and BamHI digestion. The resulting gutR gene was inserted into the polylinker region of Small/BamHI double-digested Escherichia coli Bluescript vector (pBS) to generate pBSGUTR. The digestion of pBSGUTR by KpnI and BamHI allowed the release of the gutR fragment that could be inserted into the KpnI- and BamHI-digested pUB18-P43 vector to generate pUB18P43GUTR.

Expression and Purification of GutR—B. subtilis WB600 is a six-extracellular protease-deficient strain that is used here routinely for expression study (6). WB600(pUB18P43GUTR) and the negative control strain WB600(pUB18P43) were cultivated in superrich medium (7) with kanamycin at a level of 10 μg/ml at 37 °C. Cells were harvested at different time points. Both total and fractionated cellular proteins (sol-
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Glut10 induces a tight binding of GutR to its binding site

uble and insoluble) were analyzed by 12% SDS-polyacrylamide gel electrophoresis (PAGE). For GutR purification, WB600(pUB18P43GUTR) was cultivated under the above-mentioned conditions for 8 h with a cell density around 800 Klett units, and cells were collected by centrifugation for 10 min at 9,000 × g. Cell pellets from a 500-ml culture were resuspended in 40 ml of Buffer A (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) and lysed by French press (10,000 p.s.i. with three or four passes). Cell debris was removed by centrifugation at 27,000 × g for 20 min at 4 °C. The soluble fraction was applied through a DEAE-52 cellulose column (2.5 × 40 cm) equilibrated with Buffer A, followed by washing the column with 4 column volumes of buffer A until a value of A280 nm of 0.005 or less was achieved. The column was then eluted with a linear NaCl gradient (0–0.5 M) in Buffer A. Fractions were analyzed by SDS-PAGE, and the GutR-containing fractions were pooled and dialyzed extensively with several changes in Buffer A. The dialyzed fraction was applied to a heparin-Sepharose column (3 ml of bed volume). After washing with a 2-column volume of Buffer A to lower the value of A280 nm to 0.055, the column was eluted with a linear NaCl gradient (0–0.5 M) in Buffer A. Fractions containing pure GutR were pooled and stored at −20 °C.

Molecular Weight Determination—To determine the molecular weight of GutR in solution, a Superose 6 HR column (Amersham Pharmacia Biotech) was used in the Bio-Rad Biologic work station (Bio-Rad). The column was calibrated with a molecular weight marker kit (Amersham Pharmacia Biotech). The V0 is defined as the void volume of the column (24 ml). Vr is the total bed volume of the column (24 ml). Vr/V0 is the volume of the column, and using blue dextran, it was determined to be 6.4 ml. Vr is defined as the volume at which the protein elutes.

Gel Mobility Shift Assay and in Vivo Mapping of GutR Binding Site—Specific DNA fragments for gel mobility shift assays were generated by PCR and labeled at the 5′ ends with T4 polynucleotide kinase and [γ-32P]ATP as described previously (8). The boundary for these fragments was specified in Fig. 3. An EcoRI site was introduced at the 5′ end of these fragments, and a BamHI site was introduced at the 3′ end of these fragments. DNA binding reactions were performed in a total volume of 30 μl in a binding buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM dithiothreitol, 5 mM EDTA, 3% Ficoll, 1 μg of sonicated salmon sperm DNA, a 6 nm DNA probe, and 0–15 nm purified GutR. The reaction mixtures were incubated at room temperature for 15 min and then loaded onto a 6% polyacrylamide gel in 0.5 × TBE buffer (45 mM Tris-borate, pH 8.0, 1 mM EDTA). The gel was run at 175 V in 0.5 × TBE buffer for 3 h and then dried for autoradiography. Some of these fragments were also inserted in front of the promotorless lacZ in pDH32 (9), a B. subtilis integration vector, to generate transcriptional fusions. The information of pDH32 derivatives to chromosomal DNA and selection of integrants were performed as described by Ye and Wong (2). The β-galactosidase activity from integrants was assayed in the presence of 5 mM isopropyl-b-D-thiogalactoside (IPTG) as described by Sambrook et al. (10), a constitutive expression system in B. subtilis, in WB600(pUB18P43GUTR), was transformed to a six-extracellular protease-deficient B. subtilis strain WB600, and the transformants were used to produce bound streptavidin from the cell, several injections of 10 μl of 0.05% SDS were performed, and the residual SDS molecules were then removed by several washes with TEN buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.05% surfactant P20).

Determination of the Association and Dissociation Rate Constants of GutR with Its Target DNA Using BIAcoreX Biosensor—Biotinylated polynucleotide sequence was immobilized to the first flow cell at a final quantity of 84 resonance units to serve as a negative control for GutR binding. The biotinylated GutR binding site-containing fragment was immobilized in the second flow cell to 71 resonance units. With this level of the GutR binding site immobilized, no mass transport effect was observed. The kinetic measurement was observed (10). GutR in a binding buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% surfactant P20, and 20 mM MgCl2) was injected into the sensor chip and washed from the first flow cell to the second flow cell. The binding of GutR was monitored by the change of resonance units with time. The on-rate (association rate constant, k1) was determined via a two-step analysis. In the first step, the binding rate, expressed in terms of dR/dt (where R represents the resonance unit), was plotted against the response (R).

Plasmid isolation from and DNA transformation to E. coli and B. subtilis, DNA subcloning, PCR amplification, and SDS-PAGE were performed as described previously (2). The amount of protein was determined by measuring the absorbance at 280 nm under denaturing conditions as described by Gill and von Hippel (11). The molar extinction coefficient at A280 nm of 149,550 M−1 cm−1 was used to estimate the amounts of GutR. DNA concentration was determined as described by Sambrook et al. (12). N-terminal sequencing of purified GutR was performed as described by Ng et al. (13).

RESULTS

Overproduction of GutR—gutR was inserted into a high copy number B. subtilis plasmid pUB18-P43 with the strong and constitutively expressed B. subtilis P43 promoter instilled in the vector to drive the transcription of gutR. The resulting vector, pUB18P43GUTR, was transformed to a six-extracellular protease-deficient B. subtilis strain WB600, and the transformants were found to overproduce and stably accumulate a 95-kDa protein with the molecular mass agreeable with the calculated molecular mass (95,076) of GutR. This protein was stable in the intracellular environment at least up to 12 h after inoculation (Fig. 1A). Fractionation studies indicated that more than 95% of this protein was in the soluble intracellular fraction (data not shown). Overproduction of GutR in E. coli using T7 RNA polymerase systems (14, 15) was not successful. The lack of GutR overproduction in E. coli is not attributable to the presence of rare arginine codons in gutR because the use of strains that overproduce rare arginine tRNAs does not improve the situation (16).

Development of a Two-step Scheme for GutR Purification—The isoelectric point of GutR was estimated to be 6.1. By adjusting the pH to 8.0, GutR should be negatively charged and would be able to bind to the positively charged DEAE-dextran column (DE52). Therefore, the first step in this purification scheme was loading the cell lysate from WB600(pUB18P43GUTR) to a DE52 column (Fig. 1B). The bound GutR protein was eluted with a linear salt gradient from 0 to 0.5 M NaCl. This step served three purposes. First, it could concentrate GutR from the crude cell lysate into a few fractions that were rich in GutR.
Second, chromosomal DNA could be separated from GutR because GutR was eluted from the column at the salt concentration of 0.15 M whereas chromosomal DNA was eluted from the column at a salt concentration of 0.45 M. Third, many cellular proteins could also be separated from GutR. Fractions containing GutR were pooled and dialyzed to remove salt. The sample was then applied to a heparin-agarose column based on the assumption that GutR is a DNA-binding protein and should have an affinity to heparin. Indeed, this step was very effective in removing other contaminating proteins (Fig. 1B). The eluted GutR protein was estimated to be at least 95% pure. The identity of this purified protein as GutR was confirmed by N-terminal sequencing of the first five amino acid residues of this protein. Approximately 4–6 mg of purified GutR can be obtained from one liter of cell culture. Because there were not that many endogenous 95-kDa proteins in the crude extract, purification of GutR was monitored by analyzing the presence of the 95-kDa protein in the eluted fractions. With the purification of GutR and the generation of GutR-specific antibodies, subsequent rounds of GutR purification were monitored by Western blot.

GutR Exists as a Monomer in Solution—To determine whether GutR exists as a monomer or an oligomer in solution in the absence of glucitol, the molecular mass of purified GutR was determined via gel filtration. GutR has an apparent molecular mass of 115 kDa (data not shown). Because this value is much smaller than that expected for a dimeric GutR, the result suggests that GutR exists as a monomer in solution and is likely to be a slightly elongated molecule rather than a globular molecule. The same apparent molecular mass was obtained when tested with a wide range of GutR concentrations (0.5–5 μM). Because the highest intracellular GutR concentration under the induced condition was estimated to be less than 1 μM based on the Western blot analysis, GutR in the cellular environment in the absence of glucitol should exist as monomers.

GutR Binds Specifically to a Sequence Located Upstream of the Promoter of the gut Operon in a Glucitol-independent Manner—Previous studies indicated that the sequence upstream of the gut promoter is required for the expression of the gut operon (2) and that GutR is the transcription activator that is hypothesized to bind to the upstream regulatory sequence (4). To examine this possibility, a gel mobility shift experiment was performed with two PCR-amplified, 5' end-labeled fragments. One fragment had the sequence corresponding to the −132 to −14 region from the regulatory region of the gut operon with the transcription start site at +1. The other fragment was a 168-bp fragment amplified from the polylinker region of the E. coli Bluescript plasmid (pBS) to serve as a negative control. In the presence of excess nonlabeled sonicated salmon sperm DNA, each of these labeled fragments was incubated with an increasing concentration of GutR from 5 to 50 nM. As shown in Fig. 2, GutR bound specifically to the regulatory region of the gut operon but not to the polylinker region from the Bluescript vector. With increasing concentrations of GutR, there was a corresponding increase for the GutR-DNA complex. This binding did not require the presence of glucitol.

In Vitro Deletion Mapping of the GutR Binding Site—Examining the upstream region of the gut promoter, a 29-bp imperfect inverted repeat sequence located from positions −78 to −50 is likely to be the GutR binding site (Fig. 3A). Because this sequence is highly AT-rich and the entire regulatory region of the gut operon is also AT-rich, it would be important to determine both the number and the location of the GutR binding site within this region. Gel mobility shift assays were performed using two series of DNA fragments carrying the regulatory region of the gut operon for the study. These DNA fragments were systematically shortened either at the left or the right arm (Fig. 3B). For fragments with deletion at the left arm, three deletions were made. Fragments L1 to L3 contained the intact inverted repeat sequence. In fragment L3, all of the nucleotide sequence upstream of this inverted repeat was deleted. In fragment L4, half of the inverted repeat sequence in the left arm was also deleted. For the right arm deletion series, fragments R1 to R3 contained the intact inverted repeat whereas fragment R4 had the right arm of the inverted repeat sequence deleted. Mobility shift assays with GutR concentration ranging from 1 to 15 nM demonstrated that DNA fragments (fragments L1 to L3 and R1 to R3) carrying the intact inverted repeat sequence. In fragment L3, all of the nucleotide sequence upstream of this inverted repeat was deleted. In fragment L4, half of the inverted repeat sequence in the left arm was also deleted. For the right arm deletion series, fragments R1 to R3 contained the intact inverted repeat whereas fragment R4 had the right arm of the inverted repeat sequence deleted. Mobility shift assays with GutR concentration ranging from 1 to 15 nM demonstrated that DNA fragments containing either the left (fragment L4) or the right (fragment R4) half of the inverted repeat sequence were either not retarded at all or only weakly retarded by GutR at the concentration range 1–15 nM. These data have three implications. First, the 29-bp inverted repeat serves as the GutR binding site. Second, there is only one GutR binding site in the region tested (from −143 to +1) because all sequences upstream and downstream of this inverted repeat could be deleted without affecting the binding affinity of GutR to these fragments. Furthermore, only one retarded complex could be observed in all these mobility shift studies. These observations
strengthened the idea that there was a single GutR binding site in the gut operon regulatory region. The last implication is that GutR should bind to its binding site in the form of a dimer or a higher oligomer because GutR could form an extremely weak complex with the sequence that covered only half of the intact GutR binding site.

In Vivo Deletion Mapping of the GutR Binding Site—To strengthen the two conclusions concerning the GutR binding site in the gut operon regulatory region, fragments L1 to L4 were individually inserted in front of a promoterless lacZ reporter gene in the B. subtilis integration vector pDH32. The resulting gut-lacZ transcriptional fusions were integrated at the amyE locus in the chromosome. As shown in Fig. 4, integrants with fragments L1–L3 carrying the intact inverted repeat sequence showed comparable β-galactosidase activities (1428–1609 Miller units) under the induction condition. In the absence of glucitol, only a background level (2–3 Miller units) of β-galactosidase activity was observed. These observations indicate that the sequence located between 2143 and 279 is not required for the glucitol-inducible expression. Consistent with the in vitro observations, the integrant with fragment L4, which had the left half of the inverted repeat deleted, showed the expression at the basal level. This indicates that the inverted repeat sequence is indeed required for glucitol induction.

Glucitol Induces a Tight Binding of GutR to Its Binding Site—In vivo studies indicate that glucitol serves as an inducer to turn on the gut operon in B. subtilis. However, the roles of glucitol in the GutR-mediated transcription activation are unknown. To explore the possible functional roles of glucitol in this induction process, the rate constants for both the binding and dissociation reactions of GutR to its binding site in the absence and presence of glucitol were determined using the BIAcoreX biosensor. In this study, a GutR binding site containing a fragment (−295 to −215) with the nucleotide at position −215 biotinylated was generated by PCR amplification and uni-directionally immobilized to biosensor chips that had been coupled with streptavidin. The GutR binding site located at the distal end of the immobilized fragment would be available for GutR binding. The sequence between −49 and −15 served as a spacer between the site of immobilization and the GutR binding site. Fig. 5A showed the plots of $k_a$ versus GutR concentration, and the slopes of these plots represent the association rate constants. With the plots of $k_d (R/R_0)$ versus time, the slopes of these lines represent the dissociation rate constants (Fig. 5B).

### Fig. 3. In vitro deletion mapping of the GutR binding site. A, sequence of the regulatory region of the gut operon. +1, the transcription start site. B, two series of fragments generated for the in vitro GutR binding study. L1–L4, fragments with deletions in the left arm. R1–R4, fragments with deletions in the right arm. C, gel mobility shift studies by GutR using fragments shown in B. In these binding reactions, increasing GutR concentrations were used (0, 1, 3, 8, 12, and 15 nM). D, estimation of the concentration of GutR required to shift 50% of the labeled DNA fragments.

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| GutR  | α-glucosidase activity | GutR | α-glucosidase activity |
|-------|------------------------|-------|------------------------|
| w/o Glucitol | 2% Glucitol | w/o Glucitol | 2% Glucitol |
| I     | +1 lacZ                | I     | +1 lacZ                |
|       | 1.7 ± 0.5              | I     | 1.558 ± 110            |
| II    | +1 lacZ                | II    | 2.0 ± 1.0              |
|       | 1.428 ± 212            | II    | 1.609 ± 43             |
| III   | +1 lacZ                | III   | 2.1 ± 0.7              |
|       | 2.5 ± 1.0              | III   | 2.3 ± 0.9              |
| IV    | +1 lacZ                | IV    | 3.2 ± 1.0              |

**Fig. 4.** In vivo mapping of the GutR binding site. DNA fragments carrying different lengths of the gut regulatory region were inserted upstream of a promoterless lacZ reporter gene in an integration vector, pH32, to create gutB-lacZ fusions. Each of these integration vectors was integrated at the amyE locus in the B. subtilis 168 genome. Integrants I-III carry the intact GutR binding site, whereas integrant IV has only half of the intact GutR binding site. *β*-Galactosidase activity was expressed in Miller units (2).

**DISCUSSION**

Transcriptional regulation of glucitol induction in *B. subtilis* is mainly regulated via transcription activation. GutR serves as a transcription activator in this process (4), and a region upstream of the gut promoter is also required for induction (2). It is logical to predict that GutR binds to this upstream regulatory region and activates transcription. In this study, with overproduction and purification of GutR, GutR was demonstrated to bind directly to the upstream region of the gut promoter. This binding did not require the presence of glucitol. The GutR binding site was mapped to the 29-bp imperfect inverted repeat located at positions −78 to −50 of the gut operon. This site is AT-rich. Both the in vitro and in vivo deletion studies indicate that there is only a single GutR binding site in this system. Because the entire *B. subtilis* genome has been sequenced (17), a pattern search of the SubtiList database (18) for this 29-nucleotide GutR binding sequence was performed. With an allowance even up to a seven-nucleotide mismatch in this 29-nucleotide region during the search, the GutR binding site located upstream of the gut operon is the only sequence that can be found.

With GutR as the transcription activator for the gut operon, another logical prediction is that GutR should serve as the sensor for the presence of the inducer (glucitol) of this system. Isolation of a gutR1 mutant that results in the constitutive expression of the gut operon even in the absence of glucitol supports this idea (1). In this mutant, a C to A change at the nucleotide level leads to the substitution of Ser-289 by an arginine in GutR (4). In the present study using a biosensor with purified GutR, the idea for glucitol to bind directly to GutR is further strengthened. In the presence of glucitol, GutR binds extremely tightly to its target site. The on-rate of the binding reaction is not significantly changed, whereas the off-rate is significantly reduced to a degree that cannot be measured by the biosensor system. This effect is glucitol-specific. If glucitol was replaced by either 2% xylitol or 2% glucose in the binding reactions, the dissociation rate constants under these conditions were comparable with those observed for GutR in the absence of any ligand. These data suggest that glucitol can bind to GutR and trigger GutR to have an extremely tight binding under these conditions.

**Table I**

| Sample          | $k_a$ | $k_d$ | $t_{1/2}$ | $K_d$ |
|-----------------|-------|-------|-----------|-------|
| GutR            | 1.8 ± 10^6 | 1.7 ± 10^-3 | 6.6 | 9.4 ± 10^-10 |
| GutR + glucitol | 2.1 ± 10^6 | ND^a | 5.2 | 2.0 ± 10^-9 |
| GutR + xylitol  | 1.1 ± 10^6 | 2.2 ± 10^-3 | 5.2 | 2.0 ± 10^-9 |
| GutR + glucose  | 9.3 ± 10^6 | 2.3 ± 10^-3 | 5.2 | 2.5 ± 10^-9 |

^a Half-life of the GutR-DNA complex estimated based on the equation ($t_{1/2} = 0.693/k_d$) described by Neri et al. (31).

^b ND, not determined because of the extremely tight binding of GutR to its target sequence.

In many activator-dependent operons, activators gain the ability to bind to the target sites only in the presence of a specific ligand such as catabolite activator protein with cAMP (19). Alternatively, activators can bind to the target site with equal affinity no matter whether a specific ligand is present or not as observed for SoxR, an activator that activates genes in response to oxidative stress. However, only the Fe-SoxR but not the apo-SoxR activator can stimulate transcription (20). In the case of GutR, it has a relatively tight binding to its target site ($K_d = 9 × 10^{-10}$ M) even in the absence of glucitol. If this is the case, what is the physiological role of glucitol? With an off-rate of $1.7 × 10^{-3}$ s^-1 determined at 25 °C, the half-life ($t_{1/2}$) of the GutR-DNA complex in the absence of glucitol is estimated to be 6.8 min (Table I). Consider that *B. subtilis* as a soil bacterium and that the nutrients present in the soil are not always at high levels, the generation time for cell division can be relatively long. By cultivating *B. subtilis* in a defined medium (SP1) (21) at 25 °C, the generation time is determined to be 380 min (6.3 h). Therefore, a half-life of 6.8 min of the GutR-DNA complex would not be considered to be significant. In contrast,
the BIACoreX biosensor can measure the off-rate as low as $1 \times 10^{-5}$ s$^{-1}$, and this translates to a half-life ($t_{1/2}$) of the complex to 19 h. In the presence of glucitol, the dissociation rate constant for the GutR-DNA complex should be less than $1 \times 10^{-5}$ s$^{-1}$ because it was beyond the limit that could be measured by the BIACoreX biosensor. Therefore, the GutR-DNA complex in the presence of glucitol had the off-rate decreased by 100-fold or more and had the ability to bind to its binding site without dissociation before the cell divided. This tight binding raises an important question. Why would GutR remain bound to its target site? This tight binding is hypothesized here to allow GutR to be positioned very close to the promoter as one of the necessary steps in a multistep process to mediate the transcription activation process. Our study indicates that the next step in the activation process is the binding of ATP to GutR. Under this condition, GutR has positioned itself at the proper location to wait for the next step (ATP binding) to occur. Therefore, one of the functions of glucitol as the inducer is to induce GutR to have a tight binding to its target site so that subsequent reactions can occur and lead to the activation of the gut operon.

A tight binding can be achieved by an increase in the on-rate, a decrease in the off-rate, or a combination of both. The on-rate is a second order reaction governed mainly by diffusion and long range electrostatic interactions. In contrast, the off-rate is a first order reaction determined by many short range interactions between the two interacting molecules, such as hydrogen bonding and hydrophobic interactions. In the case of GutR, only the off-rate of the GutR binding reaction in the presence of glucitol is affected. This can be achieved by at least three possible mechanisms. 1) The binding of glucitol to GutR can induce GutR to have conformational changes so that more residues in the DNA binding region of GutR are available to make extensive contacts with the GutR binding site. 2) Besides the helix-turn-helix region, extra contacts possibly be made between GutR and the sequences flanking the GutR binding site via DNA wrapping. DNA wrapping around the hexameric arginine repressor has been suggested to account for the tight binding of the arginine repressor to its operator (22). 3) Glucitol may induce GutR to oligomerize. The presence of multiple DNA binding domains in these oligomers will make sure that the GutR binding site is always in contact with some of the GutR DNA binding domains in the GutR oligomer.

Although the sequence search of GutR did not find any known regulatory proteins that show significant homology to GutR, this protein shares some similarities to the properties of the members of the 100-kDa transcription activator family, such as MalT (23, 24) and AcoK (25). They all are transcription activators with a molecular mass in the range of 100 kDa and there is a nucleotide binding site in each of these transcription activators. They activate promoters recognized by the major RNA polymerase (i.e. the E. coli family rather than the E. coli family), and their binding sites are located slightly upstream of the −35 region of the promoters. In the absence of inducer, both MalT (26) and GutR exist as monomers in solution. However, the GutR system has its unique aspects that are different from the MalT system. MalT can bind to its binding sites only in the presence of both the inducer (maltotriose) and ATP (27, 28). This is not the case for the GutR system. Multiple MalT binding sites are located upstream of the mal promoters (29, 30), whereas only a single GutR binding site is in the gut operon. Furthermore, the putative DNA binding site in GutR is located at the N-terminal region rather than at the C-terminal region as observed for MalT (29). All these differences indicate that GutR may mediate its transcription activation by a mechanism that is different from other known systems, and the elucidation of this mechanism would provide insights to understand this transcription activation system.

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