RESEARCH LETTER

Functional characterization of the glxR deletion mutant of Corynebacterium glutamicum ATCC 13032: involvement of GlxR in acetate metabolism and carbon catabolite repression

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Corynebacterium glutamicum; glxR; cyclic AMP receptor protein; acetate metabolism; carbon catabolite repression.

Abstract
Recently, a cyclic AMP receptor protein homologue, GlxR, was reported to bind to the upstream regions of several genes involved in the regulation of diverse physiological processes in Corynebacterium glutamicum. However, the function of GlxR has not yet been explored in C. glutamicum in vivo using a glxR deletion mutant. Therefore, this study examines the role of GlxR as a repressor in glyoxylate bypass and carbon catabolite repression (CCR) using a deletion mutant. The disruption of glxR resulted in a severe growth defect, but growth was restored by complementation with the glxR and crp genes from C. glutamicum and Streptomyces coelicolor, respectively. The production of isocitrate lyase (ICL) and malate synthase (MS) was significantly increased in the glxR mutant. The specific activities of both enzymes were increased in the glxR mutant, regardless of the carbon source. In accordance, the promoter activities of ICL and MS using lacZ fusion were derepressed in the glxR mutant. In addition, the glxR mutant exhibited derepression of the gluA gene for glutamate uptake in the presence of glucose, thereby relieving CCR by glucose. These results indicate that GlxR plays an important role in CCR as well as in acetate metabolism.

Introduction
Corynebacterium glutamicum is widely used for the large-scale fermentation of amino acids such as lysine and glutamic acid. Thus, due to its industrial importance, extensive studies have already been conducted on its cellular physiology and metabolism (Ikeda, 2003). However, despite numerous studies of sugar metabolism and its regulation, the molecular mechanism of global carbon regulation is still not clearly understood in C. glutamicum, in contrast to that in Escherichia coli and Bacillus subtilis (Moon et al., 2007; Arndt & Eikmanns, 2008). The cyclic AMP receptor protein (CRP) is a global transcriptional regulator of carbon metabolism and contains a cyclic AMP (cAMP)-binding domain and helix–turn–helix DNA-binding motifs (Green et al., 2001). CRP regulates the expression of target genes in response to the concentration of intracellular cAMP in Gram-negative bacteria (Brückner & Titgemeyer, 2002). Yet, the function of CRP has not been clearly demonstrated in Gram-positive bacteria, due to the low level of cAMP and minimal differences in the cAMP level under various culture conditions (Chatterjee & Vining, 1981). In the case of high GC Gram-positive actinomycete species, including corynebacteria, mycobacteria and streptomycetes, knowledge of the functional role of the CRP–cAMP complex is very limited (Derouaux et al., 2004a; Titgemeyer et al., 2007). Recent studies have identified many genes involved in the putative CRP regulon in Mycobacterium tuberculosis, which encodes 16 putative class III adenylate cyclases (Shenoy et al., 2004). In addition, the cAMP–CRP signal transduction system involved in the control of virulence and starvation in M. tuberculosis has also been reported (Bai et al., 2005; Rickman et al., 2005). Plus, the cAMP–CRP system of Streptomyces coelicolor has been reported to modulate complex physiological processes, such as germination and morphological development (Derouaux et al., 2004a). Therefore,
these studies indicate that the CRP family of proteins may play an important role as a global regulator in high GC Gram-positive bacteria.

*Corynebacterium glutamicum* has a single adenylate cyclase and three predicted CRP homologue genes in the genome (Brune et al., 2005; Cha et al., 2010). One of the CRP homologues, glxR (cg0350), has been reported to regulate the gene expression of glyoxylate bypass enzymes involved in acetate metabolism, aceB, [encoding malate synthase (MS)] (Kohl et al., 2004). Letek et al. (2006) showed the possibility that GlxR acts as a transcriptional regulator of the catabolite repression of two genes, gntP (encoding gluconate permease) and gntK (encoding gluconate kinase), involved in gluconate catabolism. Recently, GlxR has been reported to bind to the upstream regions of several genes involved in central carbon metabolism, including glycolysis, gluconeogenesis and the tricarboxylic acid cycle (Han et al., 2007, 2008). In addition, Kohl et al. (2008) identified 51 binding sites *in vitro* using electrophoretic mobility shift assays, where the sites were selected from 201 potential GlxR-binding sites based on *in silico* analysis of the *C. glutamicum* genome. Thus, GlxR has been suggested to be an important transcriptional regulator involved in the regulation of several metabolic genes. However, a *C. glutamicum* mutant deficient in the glxR gene has not yet been characterized, due to the difficulties involved in constructing such a mutant. Accordingly, in this study, a glxR deletion mutant was constructed and characterized to analyse its role in *C. glutamicum*. The resulting data revealed that GlxR acts as a transcriptional repressor of the aceA [encoding isocitrate lyase (ICL)] and aceB genes involved in acetate metabolism. In addition, the derepression of the gluA gene of the glutamate uptake system in the glxR mutant on glucose medium suggests that GlxR plays a role as a global regulator controlling both carbon catabolite repression (CCR) and acetate metabolism.

**Materials and methods**

**Bacterial strains, plasmids, oligonucleotides and growth conditions**

The bacterial strains, plasmids and oligonucleotides used in this study are listed in Table 1. The *E. coli* strain was grown in Luria–Bertani medium (10 g L\(^{-1}\) tryptone, 5 g L\(^{-1}\) yeast extract, 10 g L\(^{-1}\) NaCl) at 37 °C, and the *C. glutamicum* ATCC 13032 and glxR mutant strains were grown at 30 °C in MB medium (15 g L\(^{-1}\) tryptone, 5 g L\(^{-1}\) yeast extract, 5 g L\(^{-1}\) soytone, 5 g L\(^{-1}\) NaCl) (Follettie et al., 1993) or brain–heart infusion (BHI) medium (Eggeling & Reyes, 2004). As the carbon source, glucose, fructose, acetate, pyruvate or glutamate was added to the media at 1% (w/v). When appropriate, ampicillin, kanamycin and chloramphenicol were added at concentrations of 50, 20 and 10 μg mL\(^{-1}\), respectively. The oligonucleotides used in this study were purchased from Genotech (Korea).

**DNA manipulations and transformation**

Standard molecular cloning procedures were followed in this study (Sambrook et al., 1989). The chromosomal DNA from the *C. glutamicum* cells was isolated using a genomic DNA purification kit (Solgent, Korea), and the DNA fragments from the agarose gel were eluted using the Qiagen Gel Extraction Kit (Qiagen, Germany). The plasmids were introduced into *C. glutamicum* by electroporation (Tauch et al., 2002).

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and N-terminal amino acid sequence analysis**

After SDS-PAGE of the cell extract of *C. glutamicum*, the protein bands were electrophoretically transferred to a polyvinylidene difluoride membrane (BioRad). Without allowing the membrane to dry, it was washed in ddH\(_2\)O for 1 min. The blot was placed in 0.025% Coomassie blue in 40% MeOH and 5% acetic acid for only 1 min. It was then quickly destained for 1 min with a few changes of 40% MeOH and 5% acetic acid until the bands were visible and the background was clear, followed by washing for 5 min with ddH\(_2\)O. The protein bands of the derepressed enzymes in the glxR mutant were then cut out and the N-terminal amino acid sequence was analysed by the Edman degradation method using an Applied Biosystems model 476A Protein/Peptide sequencer (Applied Biosystems Inc.).

**Construction of the deletion mutant of the glxR gene and complementation experiment**

To construct the glxR mutant, a marker-free deletion based on a double cross-over was performed using plasmid pK18mobsacB (Schäfer et al., 1994). The two fragments, covering 456 bp upstream of glxR and 144 bp of the 5′ end of the glxR gene, and 302 bp at the 3′ end of glxR and 296 bp downstream of the stop codon, were amplified with the primer pair delF1/delR1 and delF2/delR2, respectively, using the *C. glutamicum* genomic DNA as the template (Table 1). The two PCR products were annealed in the overlapping regions and amplified by PCR using the primers delF1 and delR2. The fused product was then digested with XbaI and cloned into pK18mobsacB. The recombinant plasmid pCRD was introduced into *C. glutamicum* by electroporation, and the integration of pCRD into the chromosome was tested by the selection of colonies on a BHI plate containing kanamycin (20 μg mL\(^{-1}\)). The glxR gene from the genome of *C. glutamicum* was deleted by homologous recombination according to the protocol described by Schäfer et al. (1994),
and the kanamycin-resistant colonies were screened by growing overnight in liquid BHI and spreading on BHI plates containing 10% (w/v) sucrose. A sucrose-resistant and kanamycin-sensitive cell (glxR deletion mutant) was selected. For complementation of the glxR mutant, the glxR gene including a 275-bp upstream region was amplified by PCR using the primers pFR1 and pRR1. Meanwhile, the crp gene of S. coelicolor was amplified by PCR using the primers pFS1 and pRS1 from the genomic DNA. The glxR gene (1.3 kb) of C. glutamicum and the crp gene (1.7 kb) of S. coelicolor were then cloned into the E. coli lacZ transcription fusion vector pRS415 digested with BglII and XbaI. The DNA fragment containing 588 bp from the translational start site (487 bp from the transcriptional start site) was used to construct pTac-lacZ. The fragment containing 216 bp from the translational start site (78 bp from the transcriptional start site) was cloned into the lacZ fusion plasmid pRS415 digested with EcoRI and BamHI. The 4.8-kb DraI fragment of the

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Construction of lacZ transcription fusion vectors

The promoter probe transcription fusion vector pXMJ2 was constructed as follows: the C. glutamicum–E. coli shuttle vector pXMJ19 was digested with NarI and EcoRI to remove the ptac promoter and the lacI gene, and the ends were filled in with the Klenow enzyme. The filled-in pXMJ19 was then ligated with the DraI fragment containing the lacZ gene from the lacZ fusion plasmid pRS415 (Simons et al., 1987), yielding the promoter probe vector pXMJ2. The promoter fragments of the aceA and aceB genes were generated by PCR using primers tagged with EcoRI and BamHI and cloned in front of the lacZ of pXMJ2. The DNA fragment containing 598 bp from the translational start site (487 bp from the transcriptional start site) was used to construct paceA-lacZ. The fragment containing 216 bp from the translational start site (78 bp from the transcriptional start site) was cloned into the lacZ fusion plasmid pRS415 digested with EcoRI and BamHI. The 4.8-kb DraI fragment of the

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Table 1. List of the plasmids, oligonucleotide primers and strains used in this study

<table>
<thead>
<tr>
<th>Plasmid/strain</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK18mobbacB</td>
<td>Integration vector, oriV, oriT, mob, sacB, Kmr</td>
<td>Schäfer et al. (1994)</td>
</tr>
<tr>
<td>pXMJ19</td>
<td>Escherichia coli–Corynebacterium glutamicum shuttle vector, Cmr</td>
<td>Jakoby et al. (1999)</td>
</tr>
<tr>
<td>pCR1</td>
<td>pXMJ19 with a 1.3-kb glxR from C. glutamicum ATCC 13032</td>
<td>This study</td>
</tr>
<tr>
<td>pCR2</td>
<td>pXMJ19 with a 1.7-kb crp from Streptomyces coelicolor</td>
<td>This study</td>
</tr>
<tr>
<td>pXMJ1</td>
<td>pXMJ19 digested with NarI/HindIII</td>
<td>This study</td>
</tr>
<tr>
<td>pXMJ2</td>
<td>pXMJ19 derivative carrying lacZ without the tac promoter</td>
<td>This study</td>
</tr>
<tr>
<td>pAL</td>
<td>pXMJ2 with a 0.6-kb aceA promoter</td>
<td>This study</td>
</tr>
<tr>
<td>pBL</td>
<td>pXMJ2 with a 0.26-kb aceB promoter</td>
<td>This study</td>
</tr>
<tr>
<td>pGL</td>
<td>pXMJ2 with a 0.25-kb gluA promoter</td>
<td>This study</td>
</tr>
<tr>
<td>pCRD</td>
<td>pK18mobbacB with a 0.2-kb deletion fragment of glxR</td>
<td>This study</td>
</tr>
</tbody>
</table>

Oligonucleotides

delR1 5’-TATCTAGAATTCGTCCTCGGAAGGTGC-3’ Construction of the glxR mutant

delR2 5’-CCCATCCTAATAAATCAATCAAGATTTCCGTG-3’ Construction of the glxR mutant

delF2 5’-TGTATAGTTGATGGATGGAAGAATGTCGCTGCTTA-3’ Construction of the glxR mutant

delR2 5’-TATCTAGAAGATTGATGTGATACAC-3’ Cloning of glxR

pR1 5’-CATCTAGAAGATTGATGTGATACAC-3’ Cloning of glxR

Strains

*E. coli* XL1 Blue  *supE44 hsdR17 recA1 endA1 gyrA96 thi relA1* Stratagene

*E. coli* S17-1  Mobilizing donor strain, *hsd pro recA* Schäfer et al. (1994)

*C. glutamicum* ATCC 13032  Type strain ATCC

ΔglnR  *glbR* deletion mutant of *C. glutamicum* ATCC 13032 This study

Underlined sequences indicate the recognition sites for restriction enzymes.
pgluA-lacZ was then ligated into the EcoRI- and BamHI-digested pXMJ1, which originated from the pXMJ19 digested with Nari/HindIII, yielding pGL.

**Enzyme assays**

To determine the enzyme activities, the strains were cultivated in MB medium containing glucose or acetate. The cells were harvested in the exponential phase, washed in 50 mM Tris–HCl (pH 7.0) and suspended in the same buffer containing 10 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol and 30% (v/v) glycerol. The cell suspension was mixed with glass beads (Sigma-Aldrich) and subjected to mechanical disruption using a RiboLyser (Hybaid, Heidelberg, Germany) at 4°C. After the disruption, the glass beads and cellular debris were removed by centrifugation (13 000 g, 4°C, 15 min), and the supernatant was used for the assays. The protein concentration was measured using the Bradford method (BioRad). The ICL activity was assayed by monitoring the formation of glyoxaldehyde from glyoxylate at 324 nm (Dixon & Kornberg, 1959). The assay mixture consisted of 1 mL of 0.1 M KH₂PO₄ (pH 7.5) containing 5 mM MgCl₂, 3 mM phenylhydrazine, 2 mM cysteine and 2 mM isocitrate. One unit of ICL activity corresponds to the formation of 1 mol of glyoxylate per min at 30°C. Meanwhile, the MS activity was assayed following the increase of TNB (1,3,5-trinitrobenzene) at 412 nm in 1 mL of 50 mM Tris (pH 7.4) containing 5 mM MgCl₂, 2 mM glyoxylate, 0.1 mM acetyl CoA and 20 μg of DTNB (5,5′-dithio-bis-2-nitrobenzoic acid), as described previously (Dixon & Kornberg, 1959). One unit of MS activity corresponds to the production of 1 μmol of malate per min at 30°C. The β-galactosidase activity in the strains harbouring the paceA/paceB-lacZ fusion plasmids was determined in permeabilized cells using the Miller method (Miller, 1972) with the modifications described by Shimotsu & Henner (1986) and expressed as Miller units.

**Results**

**Site-specific disruption of glxR**

Whereas *C. glutamicum* GlxR shares only 27% amino acid sequence identity with the CRP of *E. coli*, it shows a high similarity to the CRP from the high GC Gram-positive bacteria *M. tuberculosis* and *S. coelicolor*, sharing 78% and 53% identity, respectively (Kim et al., 2004). cAMP has been reported to be essential for the interaction of GlxR with target genes such as *aceB* and *aceA* in vitro (Kim et al., 2004; Kohl et al., 2008). Thus, to explore the physiological function of GlxR in vivo, the internal region of the glxR gene encoding the cAMP-binding motif was site-specifically deleted by double cross-over recombination. The glxR mutants grew very slowly, forming tiny colonies on the sucrose selection plate after 3 days of incubation. The deletion of the glxR gene in the mutant strain was verified by PCR (Fig. 1) and Southern blot (data not shown).

**Growth defect phenotype of glxR mutant**

To explore the growth phenotype of the glxR mutant, it was grown in MB medium containing various carbon sources, including glucose, sucrose, acetate, pyruvate and glutamate. The glxR mutant displayed a significantly reduced growth rate compared with that of the wild type, regardless of the carbon source (μ, 0.74–0.8 vs. 0.19–0.21 h⁻¹) (Fig. 2a), which was consistent with the growth on the agar plate. The growth yields of the glxR mutant were about 75% of those of the wild type at the stationary phase when acetate or glutamate was used as the carbon source. Whereas the wild-type strain exhibited a similar growth yield and growth rate in the MB medium, independent of the carbon source, the glxR mutant entered the stationary phase earlier when the medium contained glucose or pyruvate rather than acetate or glutamate. To further verify that the phenotype observed was solely due to the deletion of the glxR gene, the mutant strain was complemented with the recombinant plasmids pCR1 and pCR2, containing the glxR and *S. coelicolor* crp genes, respectively. The complemented strains displayed a growth phenotype similar to that of the wild-type strain when cultivated in the MB medium (Fig. 2b). Thus, these findings indicate that the mutation in the glxR gene is responsible for the impaired growth phenotype, suggesting that GlxR is important for the growth of *C. glutamicum*.

**GlxR is a repressor of glyoxylate bypass genes**

Previously, it has been speculated that GlxR represses the genes of the glyoxylate bypass enzymes in the presence of
concentration of 10 strains were cultivated in MB medium containing chloramphenicol at a concentration of 100 μg mL^{-1}. GlxR plasmids pCR1 and pCR2 contained the GlxR and the crp gene from Corynebacterium glutamicum and the crp gene from S. coelicolor, respectively. The strains were cultivated in MB medium containing chloramphenicol at a concentration of 100 μg mL^{-1}. ■, wild-type strain harbouring pXMJ19; △, glxR mutant harbouring pCR1; ●, glxR mutant harbouring pCR2; and A, glxR mutant harbouring pXMJ19.

GlxR represses glutamate transport in the presence of glucose

CRP is a representative global regulator for CCR, which establishes the priorities in carbon metabolism, in E. coli. However, not much experimental evidence for CCR in C. glutamicum is available, even though the CCR phenomenon has been reported in glutamate uptake (Krämer & Lambert, 1990; Kronemeyer et al., 1995), ethanol utilization (Arndt & Eikmanns, 2007) and gluconate utilization (Letek et al., 2006; Frunzke et al., 2008). To explore whether GlxR is involved in CCR related to the glutamate uptake system encoded by the gluA operon, the β-galactosidase activity was examined in the glxR mutant and the wild type harboursing the gluA promoter–lacZ fusion plasmid pGL. In agreement with previous results (Parche et al., 2001), the expression of gluA was repressed fivefold when the wild-type strain was grown in a medium containing glucose, or glucose and glutamate when compared with the expression with the glutamate-grown wild type (Table 2). In contrast, the glxR mutant derepressed the expression of gluA in the presence of glucose, as the intracellular concentration of cAMP, a modulator of GlxR activity, is higher in glucose than in acetate-grown C. glutamicum (Kim et al., 2004; Cha et al., 2010). However, the glxR mutant showed a similar derepression in the case of ICL and MS, irrespective of the carbon source (Fig. 3). Thus, the transcriptional regulation of the aceB and aceA genes was further investigated in an acetate and glucose medium using the glxR mutant. The mutant showed a 15- and 4-fold increase in β-galactosidase activity when the transcription of the promoterless lacZ gene was driven, respectively, by the promoters of aceB (pBL) and aceA (pAL) in the glucose medium, whereas it relieved less than twofold β-galactosidase activity in the acetate medium (Fig. 4). Therefore, these results indicate that GlxR represses aceB and aceA not only in the presence of glucose but also in the presence of acetate.
presence of glucose, showing 74% activity of glutamate-grown cells (Table 2). These results confirm that glutamate uptake is regulated by CCR, and that GlxR represses the utilization of glutamate in the presence of glucose.

**Discussion**

Recently, a potential GlxR regulon that covers diverse cellular processes including central carbohydrate metabolism was reported (Kohl et al., 2008). However, little is known about the functional role of the CRP homologue, GlxR, in vivo, as the construction of a glxR mutant is difficult due to the growth defect phenotype. Inactivation of the glxR gene leads to an increased production of ICL and MS in the mutant, regardless of the carbon source, suggesting that the expression of ICL and MS is repressed by GlxR, even though ICL and MS are essential for growth on acetate. In addition to GlxR, two additional transcriptional regulators, RamB and RamA, are also involved in regulating the expression of aceB and aceA (Gerstmeir et al., 2004; Cramer et al., 2006). However, in contrast to RamB, which only represses aceB and aceA genes in the presence of glucose, GlxR repressed both genes, regardless of the carbon source. RamA is an activator of aceB and aceA in the presence of acetate (Arndt & Eikmanns, 2008). The involvement of the three regulators GlxR, RamA and RamB or even more regulator(s) in the
C. glutamicum suggests that the catabolite repression of glutamate uptake. The derepression in vivo (Lambert, 1990; Kronemeyer C. glutamicum was related to glutamate uptake (Kramer &

group under the large CRP–FNR superfamily. Interestingly, C. glutamicum similarities between the two CRP homologues from

plementation, there is a strong possibility of functional CRPs under the same CRP subfamily and successful com-
ation and morphological development, via a Cya–

S. coelicolor. As in the case of does not have any developmental processes, such as mor-

same aceB–aceA intergenic region would appear to make the regulation of both genes more complex (Cramer et al., 2006).

The crp gene from S. coelicolor successfully complemented the glxR mutant of C. glutamicum; thus, the growth defect phenotype was restored to that of the wild type. Derouaux et al. (2004b) suggested that the CRP homologues of the actinomycetes species, including S. coelicolor, C. glutamicum and mycobacterial strains, belong to the same CRP sub-
group under the large CRP–FNR superfamily. Interestingly, Derouaux et al. (2004a) also reported that the CRP of S. coelicolor does not play any role in CCR, and yet modulates complex physiological processes such as germination and morphological development, via a Cya–
cAMP–CRP system. Based on the classification of both CRPs under the same CRP subfamily and successful comple-
mentation, there is a strong possibility of functional similarities between the two CRP homologues from C. glutamicum and S. coelicolor, even though C. glutamicum does not have any developmental processes, such as morpho-

gical differentiation. As in the case of S. coelicolor, the growth defect phenotype of the glxR mutant indicates that GlxR plays an important role in cell viability.

Based on physiological and molecular genetic studies, and bioinformatic analyses of the whole genome sequence of C. glutamicum, it would appear that the molecular mechanism of global carbon regulation such as CCR is quite different from that in Gram-negative or low GC Gram-

positive bacteria (Moon et al., 2007; Arndt & Eikmanns, 2008; Cha et al., 2010). The first report of CCR in C. glutamicum was related to glutamate uptake (Krämer & Lambert, 1990; Kronemeyer et al., 1995). However, there is no in vivo experimental evidence that GlxR is involved in the catabolite repression of glutamate uptake. The derepression of pgluA-lacZ in the glxR mutant in the glucose medium suggests that the gluABCBD operon is repressed by GlxR. In C. glutamicum, the enzymes involved in glucose catabo-

lism (gntP and gntK), phosphoenolpyruvate carboxykinase (pck) and alcohol dehydrogenase (adhA), are also subjected to CCR by glucose (Letek et al., 2006; Han et al., 2007; Kohl et al., 2008). The presence of potential GlxR-binding sites (TGTGA-N6-TCAAC) in the promoter regions of the genes encoding these enzymes indicates that GlxR is a repressor of these genes. In addition, consensus sequences for GlxR binding have been reported upstream of the phosphotrans-

ferase system genes (ptsG, ptsI, fruR), gapA (glycerol-3-phosphate dehydrogenase), pyc (pyruvate carboxylase), ldh (lactate dehydrogenase) and adhCAB (saccharate dehydro-

genase operon) genes involved in central carbon metabolism (Han et al., 2007; Kohl et al., 2008; Bussmann et al., 2009). Accordingly, in addition to acetate metabolism, GlxR would also appear to be involved in the regulation of a large number of carbon metabolic pathways (Kohl et al., 2008).

In this study, a glxR knockout mutant was constructed and characterized to examine the functional role of GlxR in C. glutamicum. The resulting data using the glxR mutant confirmed earlier reports that glxR plays a key role as a global regulator of carbohydrate metabolism in C. glutamicum. However, further studies are still needed to address many questions regarding the physiological function of GlxR, the cellular or environmental signal involved in the activation of GlxR and the GlxR-dependent regulon.

Acknowledgement

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References


Table 2. glxA expression in wild-type and glxR mutant strains grown in different carbon sources

<table>
<thead>
<tr>
<th>Strain</th>
<th>Glutamate</th>
<th>Glucose</th>
<th>Glutamate + glucose</th>
<th>Repression fold*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>1126 ± 172</td>
<td>245 ± 48</td>
<td>243 ± 48</td>
<td>0.22</td>
</tr>
<tr>
<td>AglxR</td>
<td>1285 ± 110</td>
<td>923 ± 14</td>
<td>919 ± 67</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*The cells were grown to the exponential growth phase in MB medium containing 1% glutamate, glucose, or glucose and glutamate. The enzyme activity is expressed in Miller units. All the values are the average of three independent measurements with SDs.

† The fold repression was calculated as the value in glutamate divided by the value in glucose.


Eng Biot et al. (2004b) Crp of *Corynebacterium glutamicum* crp is the third transcription factor of the large CRP-FNR superfamily able to bind cAMP. *Biochem Bioph Res Co* **325**: 983–990.


