Functional Characterization of CRP Homologue (GlxR) and Adenylate Cyclase Genes in *C. glutamicum*; Involvement in the Regulation of Carbon Metabolism

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Corynebacterium glutamicum is of major importance in the fermentative production of amino acids and nucleotides. Therefore, extensive genome-wide analysis and strain improvements have been conducted on this industrially important organism. However, despite extensive investigations of cellular metabolism and its regulation in *C. glutamicum*, relatively few studies have been conducted on carbon catabolite repression (CCR) and cAMP signaling pathway [1,2,3]. CCR has extensively been studied in model bacteria such as *E. coli* and *B. subtilis* [4]. However, the molecular mechanism of CCR in *C. glutamicum* seems to be quite different from that of those model bacteria [5,6]. GlxR, the glyoxylate bypass regulator in *C. glutamicum* is supposed to be the CRP homologue of *E. coli.* GlxR is involved in acetate and gluconate metabolism in *C. glutamicum*, and it has been reported that the purified GlxR could bind to the promoter of the genes for malate synthase (*aceB*), isocitrate lyase (*aceA*), gluconate permease (*gntP*) and gluconate kinase (*gntK*) *in vitro* in a cAMP-dependent manner, thereby repressing transcription of these genes during growth on glucose [7,8]. Although several target genes of GlxR have been reported, only the experimental evidence of cAMP binding to the GlxR *in vitro* has been known [9].

To elucidate functional roles of adenylate cyclase and GlxR in *C. glutamicum*, first we constructed the deletion mutants of *glxR* and *cyaB*. Interestingly, the *cyaB* mutant displayed growth defect on acetate medium. Similarly, it showed growth defect on glucose-acetate mixture minimal medium, and the utilization of glucose was inhibited in the presence of acetate. On the other hand, the disruption of *glxR* resulted in severe growth defect, regardless of carbon sources, which was restored by complementation with the *glxR* and *crp* gene from *C. glutamicum* and *Streptomyces coelicolor,* respectively. The *glxR* and *cyaB* mutants showed higher activities of glyoxylate bypass enzymes encoded by *aceA* and *aceB* in acetate medium, compared to that of wild-type. In addition, both the mutants showed derepression of *gluA* of *gluABCD* operon, which encodes enzymes for glutamate uptake system, when grown in the presence of glucose.

We present experimental evidence for the involvement of GlxR and cAMP in the expression of glyoxylate pathway and glutamate transport genes. The data obtained from our study suggests that GlxR and adenylate cyclase plays an essential role in the acetate metabolism of *C. glutamicum*. The observation that glyoxylate

bypass enzymes are derepressed in both *cyaB* and *glxR* mutants indicates the involvement of GlxR in the repression of *aceB* and *aceA* in a cAMP dependent manner. Though the presence of an adenylate cyclase gene in *C. glutamicum* indicates the possibility of sharing the similar cAMP-CRP system for catabolite repression with *E. coli*, *C. glutamicum* seems to have somewhat different cAMP signaling mechanisms.

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