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SHORT COMMUNICATION

Expression and localization of the *Corynebacterium glutamicum* NCgl1221 protein encoding an L-glutamic acid exporter

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Summary

Corynebacterium glutamicum strains are used for the fermentative production of L-glutamate. Biotin limitation and addition of fatty acid ester surfactants can induce L-glutamic acid secretion. However, the mechanism of L-glutamate secretion remains unclear. It was recently reported that the NCgl1221 protein, a mechanosensitive channel homolog, was considered to be functional as an important L-glutamate exporter. However, the structure of the NCgl1221 protein has not been studied recently. In this study, we predicted the topology structure of the NCgl1221 protein by TopPred. We further analyzed the expression and localization of the NCgl1221 protein in the cytoplasmic membrane of both *Escherichia coli* and *C. glutamicum* cells by fusing green fluorescence protein (GFP) in the C terminus of the NCgl1221 protein. We found that the expressed fusion protein NCgl1221/GFP was visualized in the periphery of both *E. coli* and *C. glutamicum* cells under confocal microscope, which is consistent with a cytoplasmic membrane location. In contrast, GFP was ubiquitous in the cytoplasm of bacterial cells expressing GFP only. We herein provide the straightforward and obvious evidence to prove that NCgl1221 is confined to the cytoplasmic membrane. And NCgl1221 is a membrane protein having four transmembrane segments with its C terminus in the cytoplasm.

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Introduction

Corynebacterium glutamicum is an aerobic, nonpathogenic, biotin-auxotrophic Gram-positive soil bacterium that was isolated during a screening program for L-glutamate-producing bacteria (Kinoshita et al. 1957). Different strains of this species are used for the fermentative production of L-glutamate and several other amino acids (Hermann 2003; Leuchtenberger et al. 2005). On the metabolism and molecular mechanisms underlying glutamate overproduction of *C. glutamicum* many works have been carried out in the past years, particularly on terms of changes in metabolic flux. Many studies suggest that changes in glutamate biosynthesis play a decisive role in glutamate formation. A recent study showed that a strain deleted the *odhA* gene and completely lacking 2-oxoglutarate dehydrogenase complex (ODHC) activity produced L-glutamate as efficiently as the induced wild type, suggesting that L-glutamate overproduction can be caused by the change in metabolic flux alone and the ODHC branch point is the bottleneck of glutamate biosynthesis pathway (Asakura et al. 2007). Recently, a novel regulatory mechanism involving oxoglutarate dehydrogenase inhibitor protein OdhI and serine/threonine protein kinase G (PknG) was reported to control the ODHC in *C. glutamicum* (Niebisch et al. 2006; Schultz et al. 2007). Disruption of the *dtsR1* gene, which encodes a protein homology to the β subunit of the biotin enzyme acetyl-coenzyme A (CoA) carboxylase complex, indirectly contributes to glutamate biosynthesis by reducing the activity of ODHC (Kimura et al. 1997). Previous studies also found that the amount of DtsR protein declined in response to biotin limitation or Tween-40 addition (Kimura et al. 1999).

Glutamate secretion can be induced by incubating the biotin-auxotrophic wild type in a biotin-limited medium (Shiio et al. 1962). Lack of biotin decreases the activity of the fatty acid synthase, which results in a decreased amount of available fatty acids and thus phospholipids in the plasma membrane (Takinami et al. 1968). This imbalance would then lead to altered physical properties of the membrane which becomes permeable to glutamate, which is designated the ''leak model'' (Takinami et al. 1965). The addition of selected detergents, such as Tween-60 (Duperray et al. 1992) or penicillin (Nunheimer et al. 1970) and the cell wall arabinogalactan synthesis inhibitor Ethambutol (Radmacher et al. 2005) causes glutamate efflux. It has been suggested that secretion of glutamate in *C. glutamicum* is mediated by a special efflux carrier system (Hoischen and Krämer 1989; Hoischen and Krämer 1990). However, no L-glutamate exporter has been identified in the past decades only recently additional mutations in the NCgl1221 gene have been found in *odhA* disruptants, and thus suggested that the NCgl1221 gene encodes a putative L-glutamate exporter and hence it is proposed that treatments inducing glutamate secretion trigger a structural transformation of the NCgl1221 protein (Nakamura et al. 2007). The function of the NCgl1221 protein was also analyzed by disrupting and amplifying the NCgl1221 gene in *C. glutamicum* (Nakamura et al. 2007). Some other amino acid carriers exporting L-lysine, L-threonine and L-isoleucine, which are encoded by the *lys*E, *thr*E and *brn*FE genes, respectively, have also been identified based on the understanding of the molecular basis of amino acid secretion (Vrljic et al. 1996; Simic et al. 2001; Kennerknecht et al. 2002).

Although it has been suggested that the NCgl1221 protein functioned as a putative L-glutamate exporter (Nakamura et al. 2007), the NCgl1221 structure has not been studied. In this study, we report successful expression of the NCgl1221 protein fused with green fluorescence protein (GFP) in both *Escherichia coli* cells and *C. glutamicum* cells, and visualization of the fusion protein located in the periphery of the cells under confocal microscope, which is consistent with a cytoplasmic membrane location and a cytoplasmic C terminus. We herein provide a visual evidence to prove that the NCgl1221 protein, the major exporter of L-glutamate in *C. glutamicum*, is located in the cytoplasmic membrane.

Materials and methods

Strains and plasmids

The bacterial strains, plasmids and primers used in this study are listed in Table 1.

Chemicals, media and growth conditions

Enzymes for molecular biology were from TAKARA biotechnology Co. Ltd. (Dalian, China). *E. coli* strains were grown in luria-bertani (LB) medium at 37 °C. *C. glutamicum* strains were cultivated aerobically at 30 °C with LB medium (Eggeling and Bott 2005). When necessary, kanamycin was added at a final concentration of 50 (*E. coli*) or 25 mg l^{-1} (*C. glutamicum*). Chloramphenicol was added at a final concentration of 34 mg l^{-1}.