ROLE OF NCgl1221 MECHANOSENSITIVE CHANNEL IN GLUTAMATE PRODUCTION BY *Corynebacterium glutamicum*

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Introduction. *Corynebacterium glutamicum* was isolated in 1956 as an L-glutamate-producing bacterium by Japanese researchers. The mechanism of L-glutamate secretion by *C. glutamicum* is unique. The presence of biotin, which is required by *C. glutamicum* for growth, inhibits L-glutamate production in the culture medium, while production is induced under biotin-limited conditions. Although it has been suggested that the secretion of L-glutamate by *C. glutamicum* is mediated by a carrier system in the cytoplasmic membrane, the nature of L-glutamate exporter has long been unclear. We have recently shown that the NCgl1221 gene encoding a mechanosensitive channel homolog is involved in the mechanism of L-glutamate secretion (1). The NCgl1221 mechanosensitive channel mediates L-glutamate secretion by sensing changes in membrane tension caused by inducing treatments such as biotin limitation and penicillin addition.

The NCgl1221 protein has an N-terminal domain (1 – 286 a.a.) homologous to the *E. coli* MscS and a long C-terminal domain (287 – 533 a.a.) of unknown function. In order to investigate a role of the C-terminal domain in L-glutamate secretion, we constructed a series of C-terminally truncated mutants of NCgl1221 (2).

Methods. The PHD.htm program predicts that the C-terminal domain of NCgl1221 consists of three parts, a cytoplasmic domain (287 – 401 a.a.), a transmembrame segment (402 – 419 a.a.) and an extracytoplasmic domain (420 – 533 a.a.) (Fig. 1). Based on this prediction, we constructed following three truncated mutants; NCgl1221-419 lacking the extracytoplasmic domain, NCgl1221-401 lacking the C-terminal transmembrane segment and extracytoplasmic domain, and NCgl1221-286 lacking the whole C-terminal domain. We also constructed additional two mutants; NCgl1221-224 and NCg1221-110, in which the N-terminal domain was truncated further (Fig. 1). Plasmids expressing these truncated mutants were introduced into the ΔNCgl1221 strain, and glutamate production was induced by biotin limitation.

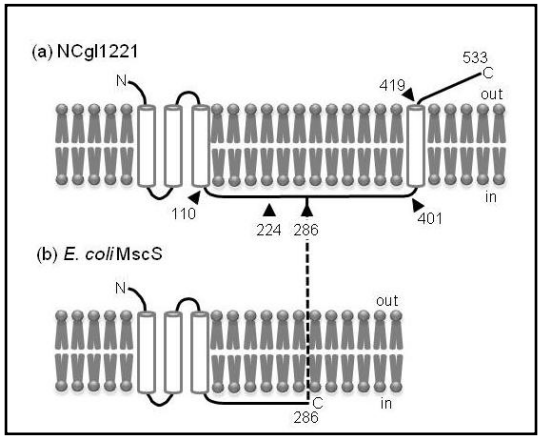

Fig.1 Membrane topology of the NCgl1221 and *E. coli* MscS. Arrow heads indicate the positions of truncation constructed in this study.

Results. The N-terminal half NCgl1221-286 homologous to the *E. coli* MscS could secrete L-glutamate (23.8 g/l) at almost comparable levels to the full length NCgl1221 (26.7 g/l). The NCgl1221-401 also secreted L-glutamate (15.8 g/l), although lower than NCgl1221-286. On the other hand, the strains expressing the shorter N-terminal domains NCgl1221-110 and NCgl1221-224 could not secrete L-glutamate (0.82 and 0.58 g/l, respectively). Interestingly, the NCgl1221-419 lacking the C-terminal extracytoplasmic domain (420 – 533 a.a.) produced L-glutamate without any inducing treatments.

Conclusions. These results clearly indicate that the N-terminal domain (1 – 286 a.a.) homologous to the *E. coli* MscS is necessary and sufficient to secrete L-glutamate in response to inducing treatments.

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