

Functional Properties of Mouse Chitotriosidase Expressed in the Periplasmic Space of *Escherichia coli*

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Keywords : Chitotriosidase, chitinolytic activity , *Escherichia coli*, physiological conditions

Outline

Chitotriosidase (Chit1) is an enzyme associated with various diseases, including Gaucher disease, chronic obstructive pulmonary disease, Alzheimer disease and cystic fibrosis. In this study, Chit1 tagged with Protein A at the N-terminus and V5-His at the C-terminus, was expressed in the periplasmic space of *E. coli* as a soluble protein and successfully purified (Fig. 1). We found that its activity was comparable to CHO cells-expressed Chit1-V5-His (Fig. 2). Optimal conditions for the *E. coli*-produced Chit1 were pH 5.0 at 50° C (Fig. 3A, B). Chit1 was stable after 1 h incubation at pH 5.0~11.0 on ice and its chitinolytic activity was lost at pH 2.0, although the affinity to chitin remained unchanged (Fig. 3C, D). The degradation of 4NP-(GlcNAc)₂ and (GlcNAc)₃ was less evident at pH 7.0~8.0, while (GlcNAc)₂ production from colloidal chitin and (GlcNAc)₆ at these pH conditions remained strong at the neutral conditions (Fig. 4). Our results indicate that Chit1 degrades chitin substrates under physiological conditions and suggest its important pathophysiological roles *in vivo*.

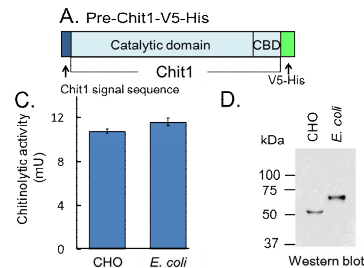
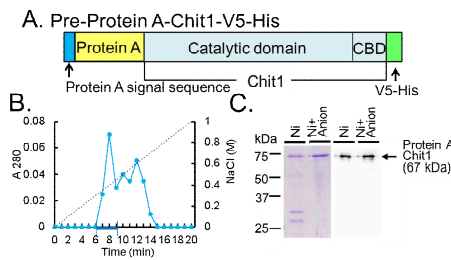


Fig 1. (A) Schematic representation of the *E. coli*-expressed pre-Protein A-Chit1-V5-His. (B) Chromatogram of Hitrap Q HP columns. (C) CBB and Western blot.

Fig 2. (A) The schematic representations of the CHO-expressed pre-Chit1-V5-His. (B) Chitinolytic activity of the enzyme preparations from CHO cells and *E. coli*. (C) Western blot of the enzyme preparations

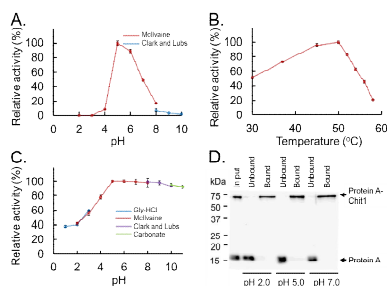


Fig 3. (A) pH profile, (B) temperature profile, (C) pH stability profile, (D) affinity of Protein A-Chit1-V5-His to chitin beads.

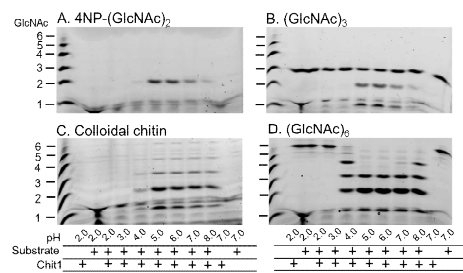


Fig 4. Chit1 chitinase activity was investigated by incubating the enzyme with 4NP-(GlcNAc)₂ (A), (GlcNAc)₃ (B), colloidal chitin (C) or (GlcNAc)₆ (D) as substrates at pH 2.0~8.0.

Novelty

Our results indicate that Chit1 degrades chitin substrates under physiological conditions and suggest its important pathophysiological roles *in vivo*.

Application

Further investigation of biomedical roles of Chit1.

Related information

● Original paper: Kimura, M., Wakita, S., Ishikawa, K., Sekine, K., Yoshikawa, S., Sato, A., Okawa, K., Kashimura, A., Sakaguchi, M., Sugahara Y., Yamanaka, D., Ohno, N., Bauer, P.O. and Oyama, F. (2016) Functional properties of mouse chitotriosidase expressed in the periplasmic space of *Escherichia coli*. *PLoS ONE* 11, e0164367.

● URL <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0164367>