Expression of mouse acidic mammalian chitinase in periplasmic space of *Escherichia coli*

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Outline

Acidic mammalian chitinase (AMCase) has been shown to be associated with asthma in mouse models, allergic inflammation and food processing. Here, we describe an *E. coli*-expression system that allows for the periplasmic production of active AMCase fused to Protein A at the N-terminus and V5 epitope and $(His)_6$ tag (V5-His) at the C-terminus (Protein A-AMCase-V5-His) in *E. coli* (Figure 1). The mouse AMCase cDNA was cloned into the vector pEZZ18, which is an expression vector containing the *Staphylococcus* Protein A promoter, with the signal sequence and truncated form of Protein A for extracellular expression in *E. coli*. Most of the Protein A-AMCase-V5-His was present in the periplasmic space with chitinolytic activity, which was measured using a chromogenic substrate, 4-nitrophenyl *N,N'*-diacetyl-β-D-chitobioside (Table 1). The Protein A-AMCase-V5-His was purified from periplasmic fractions using an IgG Sepharose column followed by a Ni Sepharose chromatography (Figure 2).

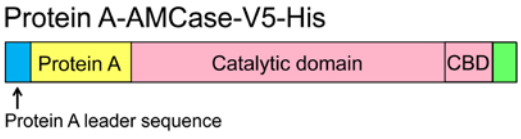

Figure 1. The schematic representations of the *E. coli*-expressed mouse AMCase fusion protein.

Table 1. Distribution of chitinolytic activity in *E. coli.*

Fraction	Total activity (U)	Distribution (%)	Total Protein (mg)	Specific activity (U/mg)
Medium	0.021 ± 0.005	4 ± 1.0	0.208 ± 0.07	0.107 ± 0.038
Periplasm 1 (Peri 1)	0.201 ± 0.063	33 ± 6.8	0.497 ± 0.09	0.410 ± 0.131
Periplasm 2 (Peri 2)	0.343 ± 0.149	54 ± 8.5	1.03 ± 0.11	0.349 ± 0.164
Cytoplasm	0.044 ± 0.009	7 ± 1.8	0.311 ± 0.10	0.151 ± 0.050
Insoluble	0.014 ± 0.009	2 ± 1.1	0.436 ± 0.11	0.030 ± 0.016

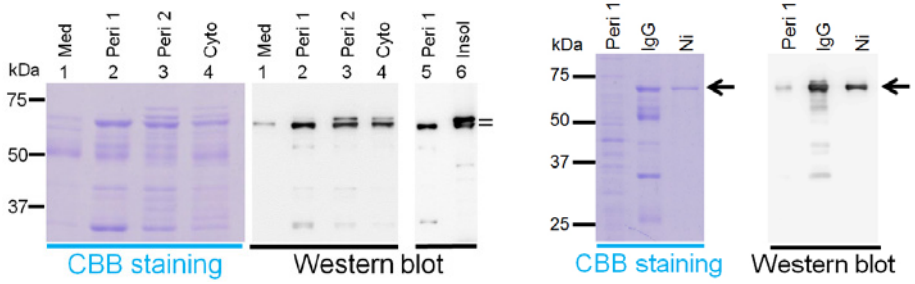

Figure 2. Analysis of localization of *E. coli*-produced fusion proteins.

Novelty

We established an *E. coli*-expression system that allows for the periplasmic production of mouse AMCase.

Application

This recombinant protein can be used to elucidate detailed biomedical functions of the mouse AMCase.

Related information

- Original paper: Kashimura, A., Okawa K., Ishikawa, K., Kida, Y., Iwabuchi, K., Matsushima, Y., Sakaguchi, M., Sugahara Y. and Oyama F. (2013) Protein A-mouse acidic mammalian chitinase-V5-His expressed in periplasmic space of *Escherichia coli* possesses chitinase functions comparable to CHO-expressed protein. ***PLoS ONE*** 8: e78669.
- URL: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0078669

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