Characterization of the *Escherichia coli*-expressed Protein A-AMCase-V5-His activities

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Outline

We establish an *E. coli* expression system that allows for the periplasmic production of an active AMCase fused to Protein A, V5 epitope and $(His)_6$ tag (Protein A-AMCase-V5-His) (Figure 1). To obtain insight into the characteristics of *E. coli*-expressed AMCase, we examined the chitinolytic activity of the Protein A-AMCase-V5-His using 4-nitrophenyl *N,N'*-diacetyl-β-D-chitobioside as a substrate (Figure 2). The recombinant protein showed a robust peak of activity with a maximum observed activity at pH 2.0, where an optimal temperature was 54° C (Figure 3A and 3B). When this protein was preincubated between pH 1.0 and pH 11.0 on ice for 1 h, full chitinolytic activity was retained (Figure 3C). This protein was also heat-stable till 54° C, both at pH 2.0 and 7.0 (Figure 3D).

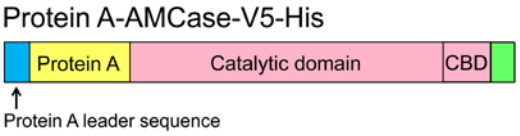

Figure 1. The schematic representations of the *E. coli*-expressed mouse AMCase fusion protein.

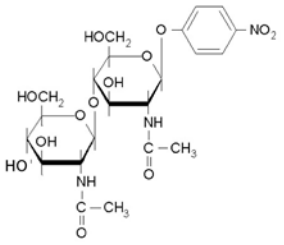

Figure 2. The schematic representations of 4-nitrophenyl *N,N'*-diacetyl-β-D-chitobioside

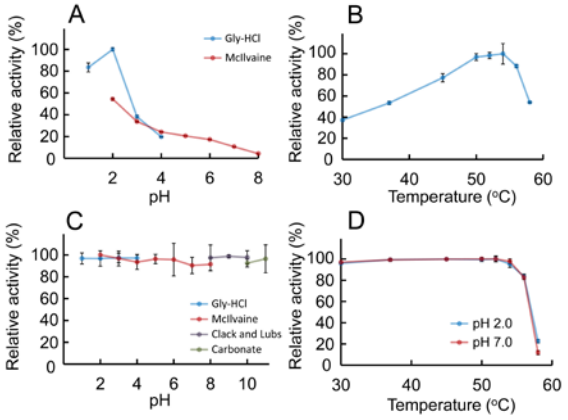

Figure 3. Characterization of the *E. coli*-expressed AMCase activities. (A) pH profile, (B) temperature profile, (C) pH stability profile and (D) thermostability profile.

Novelty

The *E. coli*-expressed AMCase had the highest activity at pH 2.0 and exhibited robust stability under basic as well as acidic conditions. In addition, recombinant AMCase is heat stable both in acidic and neutral conditions.

Application

Because inhibition of AMCase has been suggested as a therapeutic strategy against asthma, the substrate specificity and analysis of the product using the recombinant AMCase reported in this study are of medical interest.

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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