

# Acidic mammalian chitinase is a proteases-resistant glycosidase in mouse digestive system

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**Keywords:** acidic mammalian chitinase, chitin, digestive enzyme, gastrointestinal environments, protease resistant glycosidase

**Outline** Chitinases are enzymes that hydrolyze chitin, a polymer of  $\beta$ -1, 4-linked *N*-acetyl-D-glucosamine (GlcNAc). Chitin is the second most abundant naturally occurring polysaccharide. Since it has been thought to be not degraded in the digestive system, it has been considered dietary fiber. In this study, we provide evidence that acidic mammalian chitinase (AMCase) can function as a major digestive enzyme that constitutively degrades chitin substrates and produces (GlcNAc)<sub>2</sub> fragments in the mouse gastrointestinal environment. AMCase was resistant to endogenous pepsin C digestion and remained active in the mouse stomach extract at pH 2.0 (Fig. 1). The AMCase mRNA levels were much higher than those of four major gastric proteins and comparable to the level of pepsinogen C in the mouse stomach tissues (Fig.2a). Furthermore, AMCase was expressed in the gastric pepsinogen-synthesizing chief cells (Fig.2b). The enzyme was also stable and active in the presence of trypsin and chymotrypsin at pH 7.6, where pepsin C was completely degraded (Fig.3). Mouse AMCase degraded polymeric chitin substrates in the gastrointestinal environments in presence of the proteolytic enzymes (Fig.4).

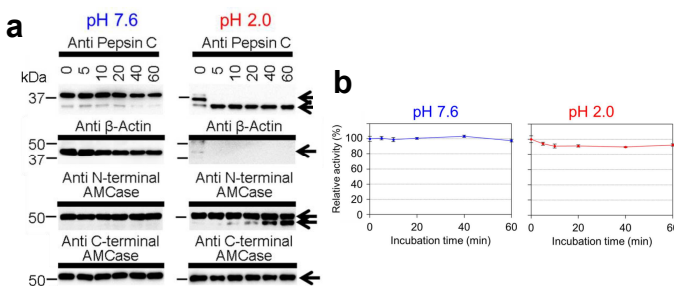


Fig. 1. Soluble proteins obtained from mouse stomach were incubated at 37° C for 0, 5, 10, 20, 40 and 60 min at pH 7.6 or 2.0. (a) Western blot, (b) chitinolytic activity.

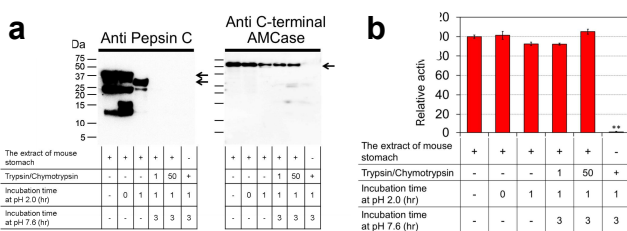


Fig. 3. Soluble proteins fraction from mouse stomach was incubated at pH 2.0, followed by at pH 7.6 containing trypsin and chymotrypsin. (a) Western blot and (b) chitinolytic activity.

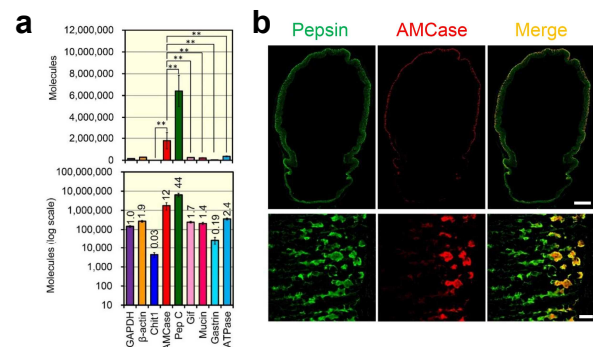


Fig. 2. (a) The mRNA levels of nine genes were quantified by qPCR. (b) Expression and co-localization of pepsin C (green) and AMCase (red) proteins in mouse stomach sections shown by immunohistochemistry.

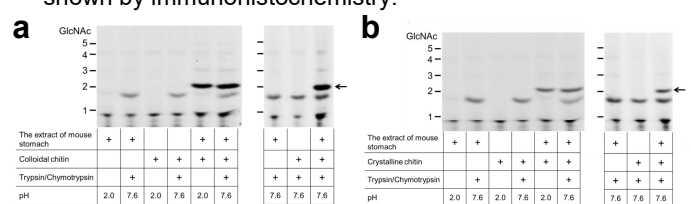


Fig. 4. Degradation products were generated during incubation of colloidal (a) and crystalline chitin (b) with the extract of mouse stomach at pH 2.0 for 1 hour and subsequently at pH 7.6 for 3 hours after addition of equal amount of trypsin and chymotrypsin.

**Novelty** Our results clearly show that AMCase is able to digest chitin polymers even in the presence of pepsin C, trypsin and chymotrypsin. The main degradation product, (GlcNAc)<sub>2</sub>, can be then uptaken in mouse GIT, providing the primary source of carbon, nitrogen and energy.

**Application** Chitin can be used in feeds for murine breeding.

**Related information** ● Original paper Ohno, M., Kimura, M., Miyazaki, H., Okawa, K., Onuki, R., Nemoto, C., Tabata, E., Wakita, S., Kashimura, A., Sakaguchi, M., Sugahara, Y., Nukina, N., Bauer, P.O. and Oyama, F. (2016) Acidic mammalian chitinase is a proteases-resistant glycosidase in mouse digestive system. *Sci. Rep.* 6, 37756.

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