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Masahiro Matsumiya, J Exp Food Chem 2017, 3:2 (Suppl)
DOI: 10.4172/2472-0542-C1-009

2nd International Conference on

FOOD CHEMISTRY & NUTRITION

July 24-26, 2017 Vancouver, Canada

Chitinolytic enzymes from the marine organisms: Enzymatic production of *N*-acetyl-D-glucosamine using crude enzyme from the liver of squid

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Chitin is an amino polysaccharide containing *N*-acetyl-D-glucosamine (GlcNAc) units connected with *β*-1,4 linkages. It is a renewable biological resource that is abundantly present over the natural world and is found in the exoskeletons of arthropods, cell walls of fungi, and the epidermis of nematodes. The majority of naturally occurring chitin exists in the rigid, α-crystalline structure that is insoluble in common solvents, thus rendering it difficult for use. However, degradation products of chitin exhibit various bioactivities, which have been attributed to the length and solubility of a polymer; these include the promotion of bifidobacteria proliferation and immunostimulatory effect in chitooligosaccharides ((GlcNAc)n) and improvement of skin quality and alleviate osteoarthritis in GlcNAc. End- and exo-type chitinolytic enzymes, chitinase (EC 3.2.1.14) and *ß-N-acetylhexosaminidase* (Hex, EC 3.2.1.52), are necessary for enzymatic production of GlcNAc. We investigated enzyme properties of chitinase and Hex from the liver of Japanese common squid *Todarodes pacificus* and enzymatic production of GlcNAc by using the crude enzyme prepared from the liver. Two chitinase isozymes were purified from the liver by ammonium sulfate fractionation and column chromatographies with Chitopearl Basic BL-03, CM-Toyopearl 650S, and Bio-Gel HTP. A Hex was purified from the liver by ammonium sulfate fractionation and column chromatographies with Butyl-Toyopearl 650S and Toyopearl HW-55SS. Crude chitinolytic enzyme was prepared from the liver by ammonium sulfate fractionation (0-65%). GlcNAc was prepared by incubation at 37^0C with colloidal chitin suspension and the crude enzyme solution. The purified chitinase isozymes were basic chitinases with molecular masses of 38 and 42 kDa. The N-terminal amino acid sequences of both chitinases were different each other. Both chitinases hydrolyzed $GlcNAc_n$ (n=4, 5, and 6) and released $GlcNAc_n$ (n=2, 3, and 4). The molecular mass of the purified Hex was estimated to be 120 kDa by gel filtration and 54 kDa by SDS-PAGE in non-reducing condition. The Hex released GlcNAc from the non-reducing end side of $GlcNAc_n$. The ratio for the activities of chitinase and Hex of the crude chitinolytic enzyme was 1:19. The crude enzyme, corresponding to 2 g of liver weight, produced 26.8 mg of reducing sugar from 50 mg of colloidal chitin during 5 days of incubation at 37^0C. The main product of the produced reducing sugar, analyzed by HPLC, was GlcNAc. These results suggest that the squid liver could be a source of chitinolytic enzyme for the enzymatic production of GlcNAc.

Biography

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