Distribution of chitinases and characterization of two chitinolytic enzymes from one-year-old Korean Ginseng (Panax ginseng C.A. Meyer) roots

Jong-Kook Moon¹, Beom-Ku Han², T. Doohun Kim¹ & Do-Hyun Jo¹,*

¹Department of Molecular Science and Technology, Graduate School, Ajou University, Suwon 442-749, ²Avicore Biotechnology Institute, Optifarm Solution Inc., Hanlim, Gyeonggi-do 435-050, Korea

We report the tissue-specific distribution of chitinolytic activity in Korean ginseng root and characterize two 31-kDa chitinolytic enzymes. These two enzymes (SBF1 and SBF2) were purified 70- and 81-fold with yields of 0.75 and 1.25%, respectively, and exhibited optimal pH and temperature ranges of 5.0-5.5 and 40-50°C. With [3H]-chitin as a substrate, K_m and V_max values of SBF1 were 4.6 mM and 220 mmol/mg-protein/h, respectively, while those of SBF2 were 7.14 mM and 287 mmol/mg-protein/h. The purified enzymes showed markedly less activity with p-nitrophenyl-N-acetylglucosaminide and fluorescent 4-methylumbelliferyl glycosides of D-N-acetylglucosamine oligomers than with [3H]-chitin. End-product inhibition of both enzymes demonstrated that both are endochitinases with different N-acetylglucosaminidase activity. Furthermore, the NH₂-terminal sequence of SBF1 showed a high degree of homology with other plant chitinases whereas the NH₂-terminal amino acid of SBF2 was blocked. [BMB reports 2010; 43(11): 726-731]

INTRODUCTION

Panax ginseng is renowned for its beneficial effects. Ginsenosides isolated from P. ginseng reportedly serve as functional ligands of glucocorticoid receptors (1), regulate the induction of tyrosine amino transferase gene transcription (2), and potentiating NO-mediated neurogenic vasodilatation (3). In addition, oligopeptides of Panax bind metals (4) and have a somnogenic effect (5). P. ginseng induces cell-proliferation (5) and anti-lipolytic (6) effects.

Although the potential roles of P. ginseng in human health have been well studied, less is known about the chitinases found in its roots (12, 13). Chitinases are important components of pathogenesis-related (PR) proteins that are induced by pathogenic attack. Investigation of these enzymes could result in the development of effective methods for preventing fungal invasion. In particular, root rot diseases caused by Fusaria, Botrytus, and Cylindrocarpon (14) result in devastating losses of cultivated ginseng. The potential for controlling these diseases rests in part on a better understanding of natural protection phenomena such as the role of chitinases. This paper reports the distribution of chitinolytic activity in rhizomes and main and lateral roots of 1-year-old ginseng seedlings, and describes the biochemical properties of two 31-kDa chitinolytic enzymes.

RESULTS AND DISCUSSION

Chitinolytic activity within ginseng roots

The ginseng root is composed of the rhizome, main roots, and lateral roots, and each of which was examined separately. As shown in Supplemental Table 1, the lateral roots had the highest chitinase activity (139 mmol/mg protein/h), followed by main roots (103 mmol/mg protein/h) and rhizomes (88 mmol/mg protein/h). Activity band staining after SDS-PAGE of crude extracts revealed three types with molecular weights of 43, 31, and 29 kDa and varying degrees of activity (Supplemental Fig. 1). In the main roots, the 31-kDa type was the primary chitinase with the other two being minor constituents. However, both the 31-kDa and 29-kDa types were major constituents of the lateral roots and rhizome.

Purification of two 31-kDa chitinolytic enzymes

As shown in Fig. 1A, a stepwise gradient of pH was used to isolate two major active fractions (QF1 and QF2) accounting for 52% and 25% of the total activity, respectively. As shown in Fig. 1B, one unbound fraction (SF1) containing 21% of the total activity and three bound fractions (SBF1, 2, and 3) comprising 30, 21, and 9% of the total activity, respectively, were obtained. SF1 and SBF3 exhibited single activity bands at 43
kDa and 29 kDa, respectively (data not shown). In contrast, SBF1 and SBF2 shared the same activity band at 31 kDa. Further purification of SBF1 and SBF2 on an HIC column resulted in a single major active fraction for each (Figs. 1C, D). The two purified enzymes (SBF1 and SBF2) showed a single band by SDS-PAGE corresponding to a molecular weight of 31 kDa (Fig. 1E), which correlated positively with the aforementioned activity band shown in Supplemental Fig. 1. The HIC fraction of SBF1 was purified 70-fold with a yield of 0.75% and that of SBF2 was purified 81-fold with a yield of

Fig. 1. Chromatographic purification of the two 31-kDa chitinolytic enzymes; the number in parentheses is the percentage of the total activity exhibited by each enzyme: (A) Anion exchange chromatogram (Source-Q, 2.6 x 15 cm), (B) Cation exchange chromatogram of QF1 (Source-S, 2.6 x 15 cm), (C) Hydrophobic interaction chromatogram of SBF1 (Phenyl Sepharose HP, 1 ml), (D) Hydrophobic interaction chromatogram of SBF2 (Phenyl Sepharose HP, 1 ml), (E) SDS-PAGE pattern of purified SBF-1 and SBF-2, (F) Chitinolytic activity staining of purified SBF-1 and SBF-2, (G) MW determination of each enzyme. (—) absorbance at 280 nm, (―) chitinase activity, (―) pH-salt gradient.