Characterization of an extensin-modifying metalloprotease: N-terminal processing and substrate cleavage pattern of Pectobacterium carotovorum Prt1

Compared to other plant cell wall-degrading enzymes, proteases are less well understood. In this study, the extracellular metalloprotease Prt1 from Pectobacterium carotovorum (formerly Erwinia carotovora) was expressed in Escherichia coli and characterized with respect to N-terminal processing, thermal stability, substrate targets, and cleavage patterns. Prt1 is an autoprocessing protease with an N-terminal signal pre-peptide and a pro-peptide which has to be removed in order to activate the protease. The sequential cleavage of the N-terminus was confirmed by mass spectrometry (MS) fingerprinting and N-terminus analysis. The optimal reaction conditions for the activity of Prt1 on azocasein were at pH 6.0, 50 °C. At these reaction conditions, KM was 1.81 mg/mL and kcat was $1.82 \times 10^7$ U M$^{-1}$. The enzyme was relatively stable at 50 °C with a half-life of 20 min. Ethylenediaminetetraacetic acid (EDTA) treatment abolished activity; Zn$^{2+}$ addition caused regain of the activity, but Zn$^{2+}$ addition decreased the thermal stability of the Prt1 enzyme presumably as a result of increased proteolytic autolysis. In addition to casein, the enzyme catalyzed degradation of collagen, potato lectin, and plant extensin. Analysis of the cleavage pattern of different substrates after treatment with Prt1 indicated that the protease had a substrate cleavage preference for proline in substrate residue position P1 followed by a hydrophobic residue in residue position P1′ at the cleavage point. The activity of Prt1 against plant cell wall structural proteins suggests that this enzyme might become an important new addition to the toolbox of cell-wall-degrading enzymes for biomass processing. © 2014 Springer-Verlag Berlin Heidelberg.

General information
Publication status: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, University of Southern Denmark, University of Copenhagen
Pages: 10077–10089
Publication date: 2014
Peer-reviewed: Yes

Publication information
Journal: Applied Microbiology and Biotechnology
Volume: 98
ISSN (Print): 0175-7598
Ratings:
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.71 SJR 1.332 SNIP 1.444
Web of Science (2014): Impact factor 3.337
Web of Science (2014): Indexed yes
Original language: English
Keywords: Applied Microbiology and Biotechnology, Biotechnology, Autolytic processing, Glycan microarray, Plant extension, Potato lectin, Proline preference, Thermal stability
DOIs:
10.1007/s00253-014-5877-2
Source: FindIt
Source-ID: 2200693803
Research output: Contribution to journal › Journal article – Annual report year: 2014 › Research › peer-review