AN ENDOGLUCANASE GSCELA FROM *GEOBACILLUS* SP. UNDERGOES AN INTRIGUING SELF-TRUNCATION PROCESS FOR ENHANCING ACTIVITY AND THERMOSTABILITY

Tuan-hua David Ho, Institute of Plant and Microbial Biology
tho@sinica.edu.tw
Mei-Huey Wu, Institute of Plant and Microbial Biology
Su-May Yu, Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan

An endoglucanase, GsCelA, was isolated and cloned from a thermophilic *Geobacillus* sp. 70PC53 grown in a rice straw compost in southern Taiwan. It was observed that highly purified GsCelA was able to self-truncate, removing a segment of 53 amino acid residues from its C-terminus. The purified GsCelA does not possess any protease activity and this self-truncation process is insensitive to standard protease inhibitors except EDTA and EGTA. This unique self-truncation process takes place at a temperature higher than 10C with an optimal pH between 6-7, and can be further enhanced with certain divalent ions such as Ca^{+2} and Mg^{+2}. Crystal structure of GsCelA has a typical TIM-barrel configuration with 8 alpha-helices and 8 beta-strands, but with the presence of a divalent ion. Mutations of amino acids residues surrounding this metal ion do not affect the self-truncation process, but some of these mutants have enhanced enzymatic activities. Mutation of the cleavage site between K315 and G316 does not affect the self-truncation process. However, a deletion of ten amino acids near the cleavage site, i.e. from amino acid 310 to 320, slows down the truncation process but does not block it, and a truncated form around 315 amino acids in length eventually appears. This intriguing observation indicates that the self-truncation process is not site specific, but capable of measuring 315 amino acids from the N-terminus as the cleavage site. This self-truncation process also occurs in the native host of this enzyme, *Geobacillus* sp. 70PC53, with almost all secreted form of this enzyme being self-truncated. The 53 amino-acid-long C-terminal segment removed by this self-truncation process has binding affinity toward both crystal and amorphous cellulose as well as the *s* cell walls, yet its sequence bears no apparent homology to any known carbohydrate binding motifs. Various other mutation analyses and the structure-based recombination process, SCHEMA, have been carried out, and both the activity and thermostabilty of this enzyme are further improved. The truncated and improved GsCelA has almost twice the activity as the un-truncated form, and its thermostability is also further enhanced with T_{50} reaching 86C and TA_{50} higher than 100C, making this enzyme extremely useful in industrial processes carried out at high temperatures, such as the pre-treatment of cellulosic animal feeds during the final drying step.

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