

Characterization of *Nocardiopsis* β -1,3-glucanase with additional carbohydrate-binding domains

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ABSTRACT

β -1,3-Glucanase F (BgIF) from alkaliphilic *Nocardiopsis* sp. F96 is a single domain enzyme composed of only a catalytic domain. Chimeric BgIFs with some carbohydrate-binding domains were constructed and characterized. By connecting the C-terminal additional domain of β -1,3-glucanase H from *Bacillus circulans* IAM1165 and the chitin-binding domain of chitinase J from alkaliphilic *Bacillus* sp. J813, binding ability and hydrolyzing activity toward insoluble β -1,3-glucans were both improved.

INTRODUCTION

β -1,3-Glucan, a polymer of β -1,3-linked glucose, is the main constitute of botanical and fungal cell walls. β -1,3-Glucanase hydrolyzes β -1,3-bonds of β -1,3-glucan. Alkaliphilic *Nocardiopsis* sp. F96, a producer of chitinolytic enzymes, was isolated from a soil sample [1]. We have found that strain F96 also secretes β -1,3-glucanase F (BgIF) [2]. The gene encoding BgIF has been cloned and sequenced, indicating that BgIF is a single domain enzyme composed of a glycoside hydrolase family 16 catalytic domain.

Certain polysaccharide-degrading enzymes have one or several carbohydrate-binding domains. The carbohydrate-binding domains have been known to bind to insoluble polysaccharides and enhance their degradation. By adding carbohydrate-binding domains to a single domain enzyme, improvement of insoluble polysaccharide-degrading activity should be expected. In this study, chimeric BgIFs with additional carbohydrate-binding domains were constructed and characterized.

RESULTS AND DISCUSSION

As carbohydrate-binding domains, C-terminal additional

domain (CAD) of β -1,3-glucanase H (BglH) from *Bacillus circulans* IAM1165 [3] and chitin-binding domain (ChBD) of chitinase J (ChiJ) from alkaliphilic *Bacillus* sp. J813 [4] were selected. These binding domains exhibited binding ability toward insoluble substrates. The amino acid sequence of CAD, comprising of 193 residues, has not been found in any other β -1,3-glucanases and related enzymes. The deduced amino acid sequence of ChBD exhibited the highest homology to those of carbohydrate-binding module family 5.

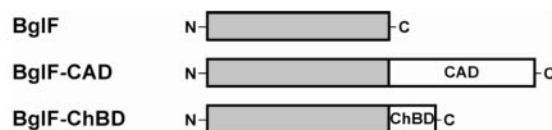


Fig. 1 Schematic structure of BgIF and chimeric enzymes.

The DNA fragments encoding CAD and ChBD were PCR-amplified from cloned *bglH* [3] and *chiJ* genes [4], respectively, and fused to *bglF* structural gene to construct chimeric genes. The chimeric genes based on *bglF* were successfully expressed in *Escherichia coli* BL21(DE3) under the control of T7 promoter.

Binding ability of BgIF and chimeric enzymes constructed in this study (BgIF-CAD and BgIF-ChBD; Fig. 1) toward several insoluble polysaccharides was assessed. *E. coli* cell extracts containing BgIF and chimeric enzymes were gently mixed with polysaccharides at 4°C in the presence of BSA. After 1 h, the mixture was centrifuged. The enzyme activity left in the supernatant was measured by the standard assay using laminarin (soluble β -1,3-glucan) as a substrate. The amount of enzyme bound to the polysaccharide was estimated by the subtracting of the activity from the original activity. The binding ability of BgIF-CAD was distinctly increased toward curdlan and pachyman compared to that of BgIF (Table 1). The ability

Table 1 Binding ability of BglF and chimeric enzymes toward insoluble polysaccharides.

Polysaccharide	Binding rate (%)		
	BglF	BglF-CAD	BglF-ChBD
Curdlan (β -1,3-Glucan)	N.D.*	38	N.D.
Pachyman (β -1,3-Glucan)	N.D.	46	55
Lichenan (β -1,3-1,4-Glucan)	N.D.	16	51
Avicel (β -1,4-Glucan)	N.D.	N.D.	43
Chitin (β -1,4-Glycan)	26	22	96

*N.D.: Not detected.

of BglF-ChBD increased remarkably toward chitin, and moderately toward avicel, pachyman and lichenan. It is noteworthy that ChBD, a chitin-binding domain, adhered toward some species of β -1,3- and β -1,3,1,4-glucans.

Hydrolyzing activity toward insoluble polysaccharides (curdlan, pachyman and lichenan) was examined for BglF and chimeric enzymes. Same amount of activity against soluble β -1,3-glucan (0.08 U each enzyme) was used for hydrolysis of each insoluble polysaccharide. BglF-CAD showed higher activity toward curdlan than BglF (Fig. 2). This result might be explained that adhesion to curdlan by CAD tended to increase enzyme concentration around the substrate. The activity of BglF-ChBD was almost the same as BglF. The activity of both BglF-CAD and BglF-ChBD toward pachyman was increased compared with that of BglF. On the other hand, both chimeric enzymes exhibited almost the same activity toward lichenan as BglF, although CAD and ChBD had distinct binding ability to lichenan.

Fungal cell wall has a complex structure composed typically of β -1,3-glucan, β -1,6-glucan, chitin, mannan and proteins. Although wall composition frequently varies markedly between species of fungi, since hydrolysis of β -1,3-glucan could weaken the cell wall of fungi, chimeric enzymes constructed in this study might be useful for anti-fungal reagents.

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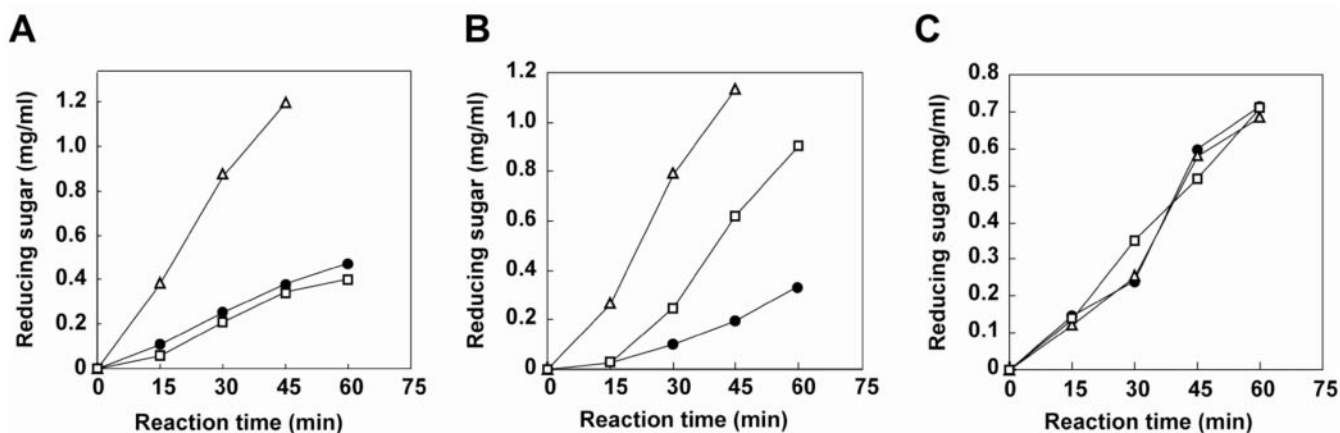


Fig. 2 Time-course of hydrolysis of curdlan (A), pachyman (B) and lichenan (C) by BglF and chimeric enzymes. Symbols: closed circles, BglF; open triangles, BglF-CAD; open squares, BglF-ChBD.