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YOUNG AUTHOR'S AWARD

FOR EXCELLENT PRESENTATION

Presented to

Shoko Shinya

for the presentation entitled

Binding Mode of Chitosan Oligosaccharides to Novel Chitosan-specific Carbohydrate-binding Modules (CBM32) of a Chitosanase from Paenibacillus Sp. IK-5

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Binding Mode of Chitosan Oligosaccharides to Novel Chitosan-Specific Carbohydrate-Binding Modules (CBM32) of a Chitosanase from *Paenibacillus* sp. IK-5

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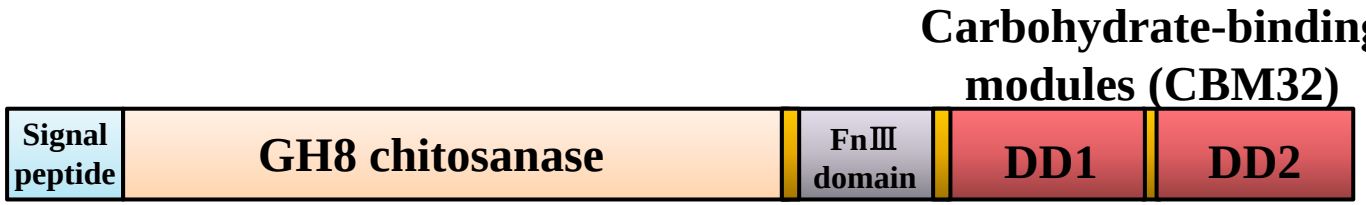
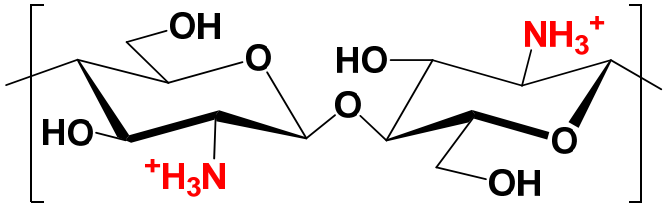
A chitosanase isolated from *Paenibacillus* sp. IK-5 has two carbohydrate-binding modules (DD1 and DD2) in its C-terminus [1]. Chitosan oligosaccharides, $(GlcN)_n$, were found to most strongly bind to DD1 and DD2 among the oligosaccharides derived from chitin, chitosan, cellulose, and laminarin. This was the first report of carbohydrate-binding modules specific to chitosan. To more closely define the binding mode of $(GlcN)_n$ to DD1 and DD2, we tried to estimate the $(GlcN)_n$ binding site of the modeled module structures and to quantitatively determine the affinities between the binding modules and $(GlcN)_n$, by molecular modeling, NMR spectroscopy, and isothermal titration calorimetry (ITC).

The modeled structures of DD1 and DD2 were constructed based on the sequence homology with CBM32 domain of α-*N*-acetylglucosaminidase from *Clostridium perfringens* as a template using the MODELLER program. To define the binding site of $(GlcN)_n$, the NMR titration experiments were performed using ^{15}N-labelled DD1 and DD2. ^{1}H-^{15}N HSQC spectra were recorded at 300 K with a Bruker AV-500 spectrometer. Based on the chemical shift perturbations of the HSQC signals, the amino acid residues affected by $(GlcN)_n$ binding were mapped on the modeled structures of the individual modules. $(GlcN)_n$ were found to bind to the loop region extruded from the core β-barrel in both DD1 and DD2. ITC experiments were also conducted, and the K_a values for $(GlcN)_3$ were 3.86×10^5 M^{-1} for DD1 and 5.21×10^3 M^{-1} for DD2 [2]. Although the binding site of $(GlcN)_n$ for DD1 was similar to that for DD2, the affinity of $(GlcN)_n$ to DD1 was much higher than that to DD2. Site-directed mutagenesis of DD1 and DD2 suggested that amino acid substitution of the glutamic acid (DD1) with tyrosine (DD2) at the 36th position may be responsible for the reduced affinity of DD2.

[1] Kimoto, H. et al., J. Biol. Chem. 277, 14695-702, 2002
[2] Shinya, S. et al., J. Biol. Chem. 288, 30042-30053, 2013

Keywords: chitosan, carbohydrate-binding module, CBN32, NMR, ITC

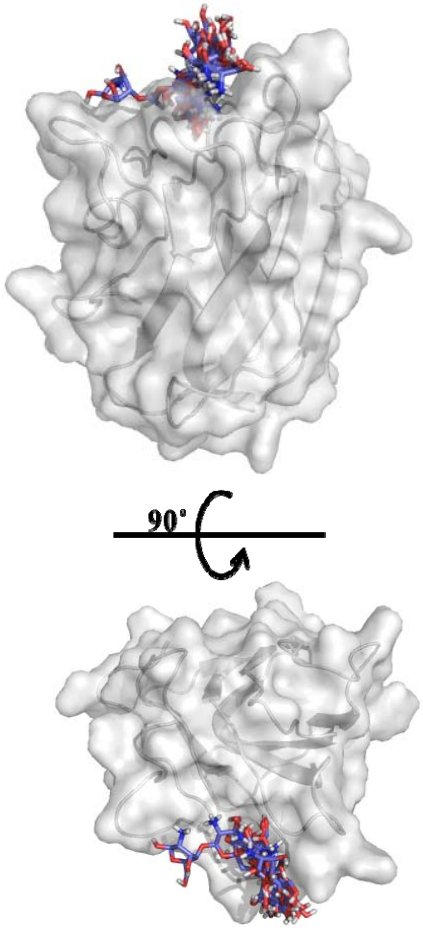




Chitosan

DD1

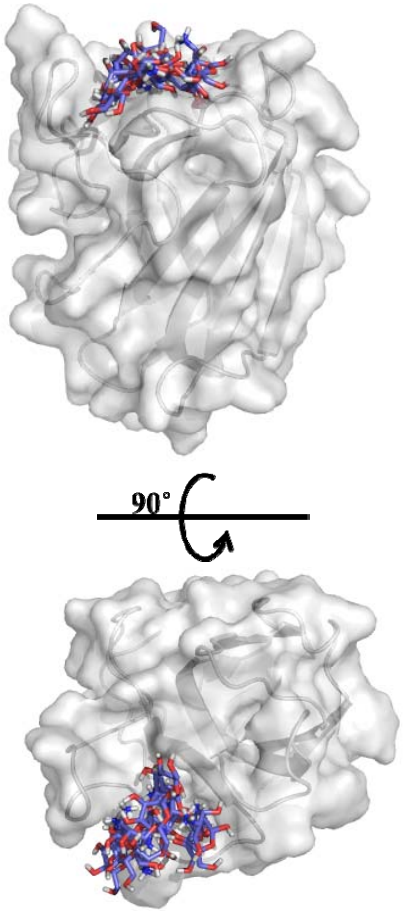
Side view



Top view

DD2

Side view



Top view