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学位論文題目	Structure-function relations of family 1 carbohydrate-binding module from white-rot fungi (白色腐朽菌に由来する family 1 糖質結合モジュールの構造と機能の相関)
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論　文　内　容　の　要　旨

Carbohydrate-binding module family1 (CBM1) is known as a cellulose-binding domain (CBD) which contained in fungal cellulolytic and associated proteins. It can facilitate the adsorption of proteins on cellulose and enhance the degradation of microcrystalline cellulose. It is well known that a white-rot fungi, *Irpex lacteus* and *Trametes hirsuta* produce several cellulases and hemicellulases which have several types of CBM1s, and has significant potential for plant cell wall-degrading system as they are works well synergistically. Therefore, it is used for the production of commercial enzyme preparation, Driselase from *I. lacteus* by KYOWA-HAKKO BIO Co., Ltd. It seems that the adsorption of enzyme on cellulose is the first step of cellulosic biomass degradation. Therefore, CBM1 is pivotal domain for cellulose degradation, especially under the diluted substrate concentration. In the present study, the role of CBM was investigated using *T. hirsuta* endoglucanase which was the typical CBM with three important aromatic amino acids (tryptophan-tyrosine-tyrosine, WYY) as reported in the chapter 2. In the chapter 3 and chapter 4, the cellulose-binding proteins in Driselase were fractionated in the differences of their adsorption abilities using a cellulose affinity column. In this thesis a new type of CBM1 from xylanase with a unique binding motif (W-YYY) was identified, and the binding characteristic of this type of CBM1 was determined using fusion proteins of various CBM1s and Green Fluorescence Protein (CBM-GFP).

To investigate adsorption behavior, CBM1 region of *T. hirsuta* endoglucanase (*Th*EG1) was expressed as a fusion protein with GFP (CBM$_{ThEG1}$-GFP). The adsorption rate of this protein on cellulose was increased in proportion to salt concentration of buffer. *Th*EG1 activity on insoluble microcrystalline cellulose was decreased when CBM of *Th*EG1 was removed. It is suggested that microcrystalline cellulose degradation activity is depending on adsorption amount of enzymes by CBM1. Therefore, regulating of adsorption of CBM1 on cellulose would influence strongly to hydrolytic activity of cellulase.

Proteins from Driselase were separated into four fractions using a cellulose affinity column, and then 42 kDa protein (Xyn10B) with strong adsorption to cellulose was isolated. To clarify the strong adsorption of Xyn10B on crystalline cellulose, only the CBM1 region of Xyn10B was expressed as a fusion protein with GFP (CBM_{Xyn10B}-GFP). CBM_{Xyn10B} region was amplified from *I. lacteus* cDNA by PCR and inserted to pRSET/EmGFP. CBM_{Xyn10B}-GFP expression system was constructed and expressed in *Escherichia coli* strain B. CBM_{ThEG1}-GFP was used as the control of normal type of CBM1. F42 and/or Y52 of CBM_{Xyn10B} were mutated to serine protein (CBM_{F42S}-GFP, CBM_{Y52S}-GFP) and also constructed by point mutation.

Xyn10B (a 42 kDa protein) exhibited irreversible adsorption on cellulose and desorption was difficult under standard conditions. Xyn10B is consist of N-terminus CBM1 region (CBM_{Xyn10B}) and C-terminus catalytic domain. The essential residues of CBM_{Xyn10B} for cellulose binding were considered to be W24, Y50, and Y51, and the additional aromatic residue of Y52 was located in juxtaposition to the continuous aromatic residues that formed part of the binding motif. In addition, F42 of CBM_{Xyn10B} is also characteristic aromatic amino acid. CBM-GFPs expression system and adsorption behavior to cellulose was evaluated. As a result, A_{max} (maximal concentration of bound protein) and K_{ad} (association constant for the binding site) values of CBM_{Xyn10B}-GFP (7.8 μmol/g and 2.0 l/μmol, respectively) were approximately 2-fold higher than those of CBM_{ThEG1}-GFP (3.4 μmol/g and 1.2 l/μmol, respectively), even though both proteins had the same binding motif (W-YY). This result suggested that the strong adsorption of Xyn10B could be attributed to CBM1, not the catalytic domain. The mutant protein, CBM_{Y52S}-GFP, was constructed to investigate the role of the additional Y52 residue. The adsorption isotherms of CBM_{Y52S}-GFP were significantly lower than those of the native protein, and were similar to those observed for CBM_{ThEG1}-GFP. These results suggested that the aromatic residue Y52 plays an important role for binding affinity. Thus, CBM_{Xyn10B} contains a unique motif (W-YYY) that facilitates strong cellulose adsorption. CBM-GFPs adsorbed on cotton were examined under a fluorescence microscope and the distribution of the fusion proteins was also observed. The adsorption of CBM_{ThEG1}-GFP and the mutant protein, CBM_{Y52S}-GFP, was spread uniformly on cotton surfaces. On the other hand, the fluorescence of CBM_{Xyn10B}-GFP was detected as a spot on the cotton due to bunched adsorption. These results suggested that the adsorption behaviors of the native and mutant CBMs were completely different to each other. Interestingly, many W-YYY motifs in CBM1 were found from other hemicellulases, not only Xyn10B. These hemicellulases can have the strong adsorption to cellulose as well as Xyn10B from *I. lacteus*. It is noteworthy that the strong adsorption of Xyn10B might contribute for effective degradation of hemicellulose composed with cellulose such as cellulosic biomass in nature.

In conclusion, the adsorption ability of Xyn10B with a unique W-YYY CBM1 motif on crystalline cellulose was stronger than that of cellulase with normal type CBM1. The CBM1 of Xyn10B had an additional aromatic amino acid, Y52 which may form another binding face in addition to flat face of normal CBM1. The strong cellulose adsorption properties of the CBM1 of xylanase from *I. lacteus* may provide a new insight into biomass degradation system by fungi.