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Comparison of domains function between cellobiohydrolase I and endoglucanase I from *Trichoderma pseudokoningii* S-38 by limited proteolysis

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Abstract

Two homogeneous catalytic domains (CD) and cellulose-binding domains (CBD) of cellobiohydrolase I (CBHI) and endoglucanase I (EGI) from *Trichoderma pseudokoningii* S-38 are obtained from the products of limited proteolysis by papain and purified followed a series of gel filtration. A comparison of the catalytic activities and binding capacities between these two intact cellulases and their CD and CBD were made. Binding and de-sorption experiments indicate intact CBHI has stronger binding capacity on cotton fibers than others, and cannot be released from cotton fibers upon simple dilution by 20 mM NaAc, pH 4.8 buffer, while both CBDs can be completely eluted by the same buffer. Non-hydrolytic disruption of cotton fibers after adsorption of CBHI-CBD was clearly observed by scanning tunneling microscope. FT-IR spectroscopy studies demonstrate that those disruptions were driven by weakening and splitting of hydrogen bonds in cellulose. The binding capacities, as a measure of the maximum amounts of binding enzymes, and hydrolysis rates of both intact enzymes are all higher than those of the combination of their two corresponding domains in equal mole. Similarly, the effect of synergism between CBHI and EGI in hydrolysis rate of cotton fibers are also higher than those of the combination of their four domains. These results clearly suggest that the binding and catalytic functions of a cellulase molecule is mainly dependent on the coupling work of its two domains and any one domain alone is not sufficient for its full function as in an intact enzyme.

Keywords: Cellobiohydrolase I; Endoglucanase I; Cellulose-binding domain; Catalytic domain

1. Introduction

Cellulose is the most abundant renewable polysaccharide in nature. Its biodegradation by microorganisms is one of the major steps of carbon cycle on earth. Therefore, the efficient utilization of this process would provide a significant contribution to solve the environmental and ecological problems. As a skeletal component in plant cell wall, cellulose has high tensile strength and rigidity, which makes it much more difficult to be hydrolyzed by chemicals or enzymes. As it was known that the enzymatic hydrolysis of cellulose is a complicated heterogeneous reaction, in which the binding/adsorption of cellulases to cellulose surface is a crucial prerequisite step [1,2,26]. According to the widely accepted view, efficient hydrolysis of crystalline cellulose requires the synergistic action

Abbreviations: CBHI, cellobiohydrolase I; CBD, cellulose-binding domain; FT-IR spectrum, Fourier transform-infrared spectrum; STM, scanning tunneling microscopy; NaAc, sodium acetate

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of cellobiohydrolase I (CBHI) and endoglucanase I (EGI) in fungal cellulase systems [1,2]. Both CBHI and EGI consist of two distinct domains connected by a linker region: a relatively small non-catalytic cellulose-binding domain (CBD) and a catalytic domain (CD). Several investigations clearly showed that CBDs of cellulases appeared to assist in the hydrolysis of insoluble crystalline cellulose, because lower activities were generally observed following their removal by proteolysis or being deleted through in vitro DNA manipulation [3,4]. But, how they perform this function is not yet full understood. Even though there are many successful results in this field, several different even contrary reports on the function of CBD were also presented. Din et al. [5,6] first reported that the binding domain of endoglucanase A (CeA) from a bacterium *Cellulomonus fimi*, belonging to family II, could cause non-hydrolytic disruption of cellulose fibers and release small particles. Similar studies from *Trichoderma pseudokoningii*, *Trichoderma reesei* and *Penicillum janthinellum* were obtained in our laboratory [7–10]. And there are also some controversies on the interaction mode of CBD with cellulose [11]. In Linder and Carrard [11] and Teeri's report [12], binding is a totally-reversible process for CBHI-CBD from *T. reesei*, while it is irreversible for CBDs from *C. fimi* according to Jervis et al. [13]. This contradiction of interaction of CBDs with cellulose may be due to the difference of bacterial and fungal CBD with different adsorption properties as suggested by Reinikainen [4].

In the present investigation, the binding behavior and hydrolysis rates of intact CBHI and its CD and CBD, intact EGI and its corresponding domains on cotton fibers were compared.

2. Materials and methods

2.1. Cellulosic substrates

De-waxed cotton fiber was used as substrate. The fibers were simply cut, and the fraction with 60–80 meshes was collected, and then dried for 3 days in vacuum oven at 45 °C before each experiment. Crystalline cellulose (Avicel, PH101, Sigma), CF11 cellulose powder (Whatman), CMC-Na (medium viscosity, Sigma), and phosphoric acid swollen cellulose prepared according to Wood [14], were used as substrates for estimation of enzymatic activity. All other chemicals were reagent grade.

2.2. Enzyme purification and determination of activities

CBHI and EGI were purified from the culture filtrate of the fungus *T. pseudokoninigii* S-38 [15], by a series of chromatography procedures as described in the previous reports [16,17]. The specific activities of intact enzymes and their domains were determined in 50 mM NaAc, pH 4.8 at 40 °C using various cellulose substrates with different incubation period, 240 min for cotton fibers, 120 min for CF11 cellulose and swollen cellulose, and 30 min for CMC-Na and *p*-nitrophenyl-β-D-cellobioside (pNPC), respectively. Concentration of each substrate was 1.0 mg/ml, and the final concentration of enzymes and their domains was about 0.75 μM. The total reaction volume was 2 ml. The released reducing sugar was measured by the dinitrosalisylic acid (DNS) method using glucose as standard. The specific activities were defined as the amount of reducing sugar (in microgram) produced by 1 μmol enzyme/min.

For the study on synergistic action in hydrolysis, the experiments were performed at 40 °C for 120 h in 50 mM NaAc buffer, pH 4.8 containing 1 mg/ml cellulosic substrates, 30 μM enzymes and 0.01% (w/v) NaN_3.

2.3. Limited proteolysis of CBHI and EGI, and isolation of their catalytic domains (CD) and cellulose-binding domains (CBD)

The procedure was similar as reported previously [7,18] with some modification. Briefly, a concentrated CBHI or EGI solution (20 mg/ml in 50 mM NaAc buffer, pH 5.0) was treated with papain (Sigma p3120, 10 mg/ml in 20 mM phosphate buffer, pH 7.0 contained 5 mM L-cysteine and 2 mM EDTA). The ratio of CBHI to papain (w:w) was 5:1, and EGI:papain was 10:1. The mixture was incubated at 37 °C for 120 min. Then, the digests were separated by gel filtration on Sephadex G-75 column (1.5 cm × 100 cm). The fraction containing catalytic domain of CBHI or EGI was further purified on ion exchange DEAE-A50 column. The fraction of CBHI-CBD was mixed with microcrystalline cellulose (PH101) at a concentration

of 10 mg/ml and incubated at 4 °C overnight for CBD absorbing [8]. For adsorbing EGI-CBD, CF11 cellulose used as substrate. The cellulose pellet adsorbing CBD was collected by centrifugation at 10,000 × g for 30 min, then re-suspended in 50 mM phosphate buffer (pH 6.0) containing 2 M urea, and incubated at 4 °C for 30 min with agitation for CBD de-sorption. Cellulose powder was removed by centrifugation as described above. The supernatant containing CBD was dialyzed against acetate buffer (pH 5.0, 50 mM) and concentrated by ultrafiltration with a cut-off of 5000 Da (PM 10 membrane, Amicon, USA), then loaded on Sephadex LH-20 column for further purification [8,10].

2.4. Binding and de-sorption experiments

The binding of CBHI, EGI and their domains to cellulose were performed by mixing the protein solution with cotton fiber suspension (1 mg/ml in 20 mM NaAc, pH 4.8) at 4 °C for 90 min as full equilibrium reached. The substrate was collected by centrifugation at 10,000 × g for 10 min. In de-sorption experiments, the cellulose pellet was re-suspended in the same buffer with the same volume and incubated at 4 °C for another 90 min, then centrifuged as above. The de-sorption% as calculated following Palonen et al. [27]. In all experiments, the adsorbed and de-sorbed percentages of enzymes were determined by UV absorbency at 280 nm. The binding isotherm data of the intact enzymes and their domains were fitted with one-site saturation binding model [19]. Analyzed by the method of non-linear least squares to determine the apparent relative equilibrium dissociation constant (K_d) and the maximum amounts of binding enzyme (B_{max}).

2.5. Determination of changes in structure parameters of cotton fibers after binding of CBHI-CBD and EGI-CBD

A reaction mixture containing 2 mg/ml of de-waxed cotton fiber powder, 0.05 mg CBHI/EGI-CBD in 50 mM sodium acetate buffer (pH 6.0) with a total volume of 1.0 ml was incubated at 45 °C for appropriate period with gentle agitation. The cellulose fibers were collected on a glass filter and washed with distilled water, then dried in an oven at 45 °C. The treated cotton fiber was suspended in anhydrous ethanol, dropped on a freshly cleaved highly oriented pyrolytic graphite (HOPG), dried in air, and carried out with a domestic STM setup CSPM-930A (manufactured by Institute of Chemistry, Academia Sinica, Beijing) as described before [20]. The IR spectra were determined on a FT-IR 710 infrared spectrophotometer (Nicolet Instrument Corp., USA). Spectra were recorded using a deuterated triethylene glycol sulfide (D-TGS) detector in a transmission mode with a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}.

3. Results

3.1. Proteolytic digest of CBHI and EGI and isolation of their catalytic domains and cellulose-binding domains

Under the given conditions with CBHI:papain ratio (w:w) as 5:1 and EGI:papain as 10:1, the mixture was incubated at 37 °C for 90 min (for CBHI) or 120 min (for EGI), the intact enzymes were completely cleaved into catalytic domains and cellulose-binding domains [7,18]. The digests were separated by gel filtration on Sephadex G-75 column for primary purification of these two domains. The catalytic domains were further purified on DEAE-A50 column as described for purification of CBHI and EGI [16,17]. Homogeneous fractions of two CBDs were obtained after adsorption and de-sorption on Avicel/CF11 cellulose, concentrated by ultrafiltration, and re-chromatography on Sephadex LH-20 column. According as the properties of CBDs bound to cellulose and which could be released upon simple dilution, the purified CBDs were easily obtained [8].

According to the gel filtration data on Biogel P-100 column, the molecular weight of CBHI-CD was estimated as about 58 kDa and EGI-CD about 60 kDa; thus a peptide with the molecular weight of about 7–8 kDa for CBHI would be CBHI-CBD, and 9–10 kDa for EGI would be EGI-CBD. Because, the proteolysis site by papain in cellulase is adjacent to the N-terminus of linker [7], thus, the CBDs purified in present should be combined with the linker region. As reported by Srisodsuk et al. [40] that the linker is necessary for full binding capacity and hydrolysis rate of CBHI.

3.2. Binding properties of intact CBHI and EGI, and comparison with those of their CDs and CBDs

The adsorption isotherm in the Scatchard plot shown that the curves of both intact cellulase and its CD were non-linear, and both CBDs were nearly linear (Fig. 1). Which were similar to many reports of cellulase binding studies [4,26,27]. Thus, the B_{max} and K_d of all components as determined by linear regression of Scatchard data would be far from their true values, and estimation of the adsorption parameters with non-linear regression will be more accurate.

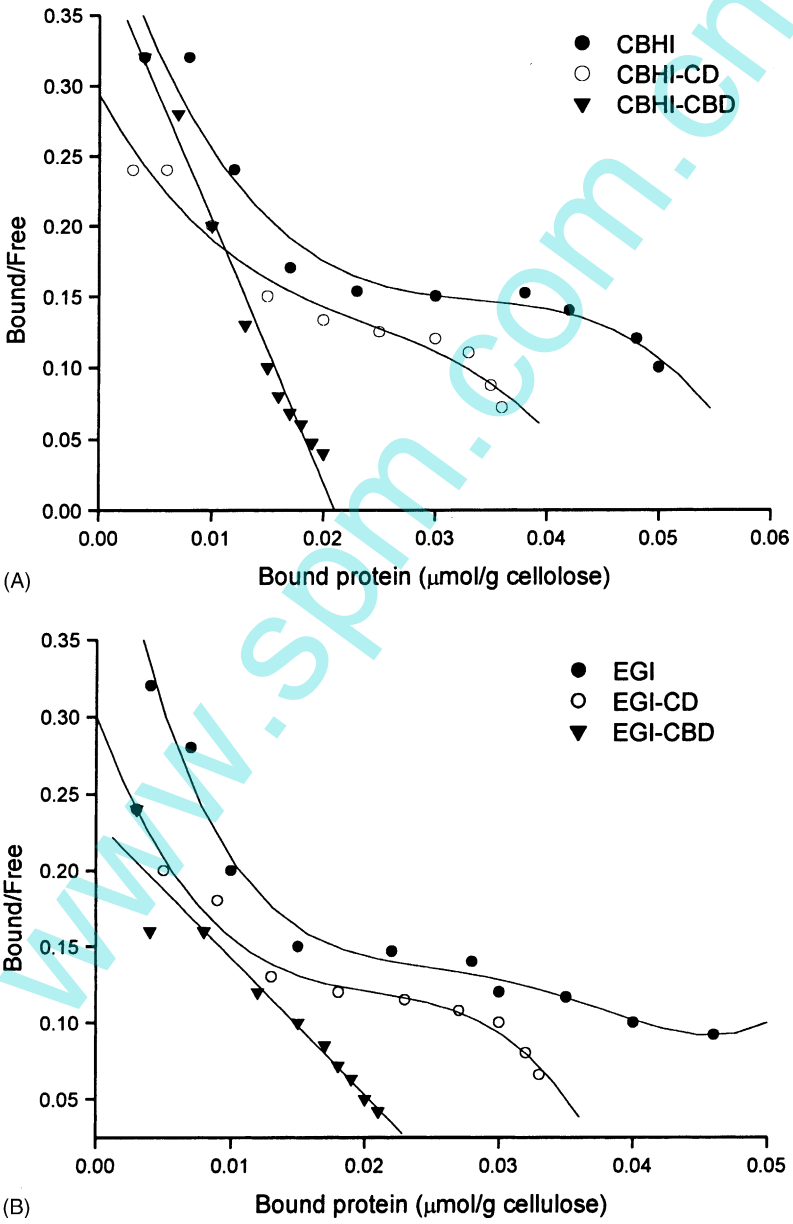

Fig. 1. Scatchard plots analysis for adsorption data of CBHI (A), EGI (B) and their domains binding to cotton fibers.

Table 1
Adsorption parameters for the binding of CBHI, CBHI-CD, CBHI-CBD, EGI, EGI-CD, and EGI-CBD to cotton fibers

Enzyme	B_{max} (μmol/g cotton)	K_d (μmol/l)	Goodness-of-fit (R^2)
Intact CBHI	0.0923	0.389	0.98
CBHI-CD	0.0555	0.238	0.99
CBHI-CBD	0.0216	0.0602	0.99
Intact EGI	0.0810	0.397	0.99
EGI-CD	0.0521	0.255	0.99
EGI-CBD	0.0260	0.112	0.99

B_{max}: maximum amounts of adsorbed ligand; K_d: equilibrium dissociation constant (l/mol).

Table 2
De-sorption of adsorbed enzymes on cotton fibers by 20 mM NaAc buffer, pH 4.8

	De-sorption%[a]
Intact CBHI	<5
CBHI-CD	≈75
CBHI-CBD	≈100
Intact EGI	≈63
EGI-CD	≈85
EGI-CBD	≈100

[a] De-sorption% = (binding enzymes − de-sorbed enzyme)/bound enzyme [27]; all tests were set-up in triplicates.

In the present work, non-linear curve fitting to the experimental isotherms were performed using the one-site binding model to determine B_{max} and K_d. Results are shown in Fig. 2 and Table 1. The B_{max} of both intact CBHI and EGI are higher than those of its domains and approximated the sum of its two domains, respectively. But, the K_d of both CBDs is smaller than those of its CD and intact enzyme. Similar contradiction is also presented in another report [40]. How can we measure the affinity or binding capacity of a binding system? In general, K_d or K_a has been selected as the index for estimating the affinity, i.e. "the lower the K_d, the higher the affinity" [41], while the distribution coefficient and the B_{max} have also been selected as the index of binding capacity [42]. The relations between them are so complex, as reviewed by Fuchs and Gessner "the result of equilibrium constant strongly depends on the evaluation method used and on the type of experimental errors" [39].

3.3. De-sorption of enzymes by buffer dilution and then for competitory binding experiments

The de-sorption data (Table 2) shown that CBDs of CBHI and EGI can be completely eluted from cotton cellulose by simple dilution with 20 mM NaAc buffer, and both CDs and intact EGI can be partially eluted. These results indicate that the adsorption at 4 °C in NaAc buffer pH 4.8 is a binding-reversible process for CBDs and CDs as well as EGI, which is in agreement with previous reports [12,21–23]. And suggested that CBD alone is not responsible for the irreversible binding of intact enzyme. CBHI has strong affinity to cellulose substrates and it is difficult to release from these substrates.

Present results shown a fully irreversible binding of intact CBHI on cotton cellulose. It is different from those results obtained using Avicel, BMCC and filter paper as substrates [24,26]. However, it is similar with the reports [21,22,28] using wood cellulose, filter paper and newspaper as substrates, respectively. Much further research is required to clarify this contradiction. Since CBHI cannot be eluted from cotton cellulose with 20 mM NaAC buffer, this property can be used for investigation of competitive binding between CBHI and other cellulase components.

A similar procedure of adsorption as described above is followed, then the cotton fiber adsorbed with CBHI is collected and used as substrate in re-adsorption test by other components, such as EGI or its CBD with a saturate amount. The activity of CBHI in bulk solution is also estimated by pNPC.

In all the re-adsorption tests, the adsorbed CBHI is not replaceable by any of EGI components. During re-binding, there are about 0.015 μmol CBHI-CBD or EGI-CBD and 0.05 μmol EGI can be binding to one gram of cotton, which is about the half of its original (Table 1). The results clearly demonstrate that there exist, at the list, some partial distinct binding sites for CBHI and EGI, and there are also extra sites for CBHI-CBD and EGI-CBD binding after intact CBHI bound. These results show good agreement with the previous reports of Ryu et al. [43] and Kyriacou et al. [44].

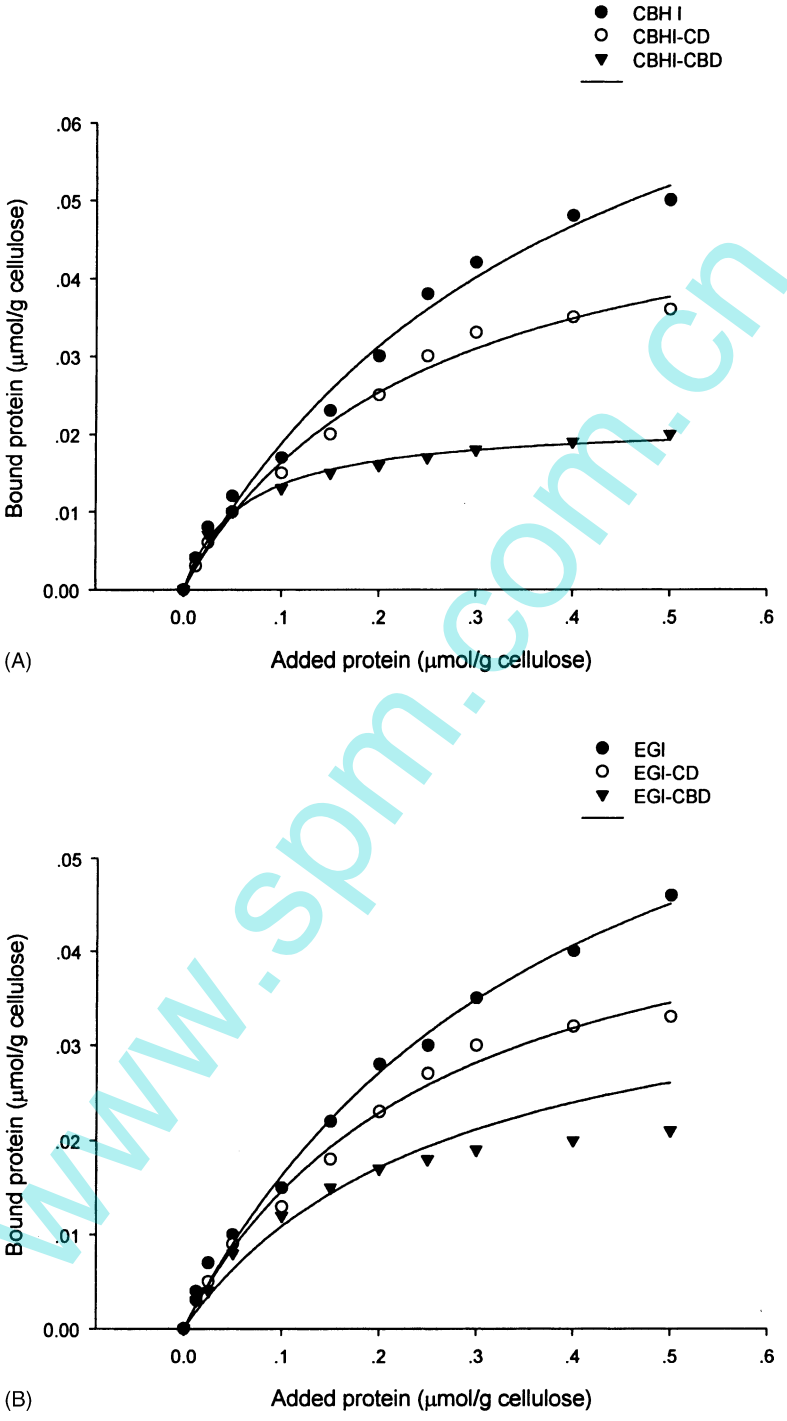

Fig. 2. Non-linear regression analysis for adsorption data of CBHI (A), EGI (B) and their domains binding to cotton fibers.

3.4. Structural changes of cotton cellulose after binding of CBHI-CBD and comparison with other cellulose components

3.4.1. STM observation

These results were clearly observed at the microfibril(s) level at high magnification (Fig. 3).

Fig. 3A shows a bundle of microfibrils with the diameter of 11.2 nm, which consists of 4–5 elementary fibrils with the diameter of about 2 nm. The structural changes after adsorption of CBHI clearly observed that the microfibril bundle becomes more dispersed and disorderly (Fig. 3B). Same structural changes of cotton fiber after adsorption by EGI were also observed (figure omitted). After hydrolysis in synergism by CBHI and EGI, the structure of cotton cellulose became completely disrupted (Fig. 3C).

3.4.2. FT-IR spectra analysis

Fig. 4 shown an IR spectrum of native cotton fibers, in "finger-print region" 1500–900 cm^{-1}, it exhibits the characteristics of high crystalline cellulose [30]. There are about 11 absorbing bands which have been assigned. After binding by CBDs, its position and peak shape have little or no changes. However, only the relative intensities of some bands near 1030, 1060, 1116, 1160 and.1370 cm^{-1} appear decrease tendency, which assigns as C–O stretching. And bands near 1430 cm^{-1}, which were assigned as CH_2 scissoring, also decreased. These changes may be reflects the occurrence of transition of cellulose I to cellulose II during hydrolysis process [30,31]. But, its true value can not be estimated, because the limitation of the KBr disc method.

Hydroxyl is a main functional group in forming hydrogen bonds with various hydrogen bonding acceptors. In IR spectra around the broad bands of 3600–3200 cm^{-1}, corresponding to the strong –OH stretching and flexural vibration frequencies of intra- and inter-molecular hydrogen bonds of cellulose, and can be used for estimating the relative amounts of free and bonds (ordered) hydroxyl groups [30]. However, its type is poorly resolved by general KBr disc method, because the hydroxyl absorption

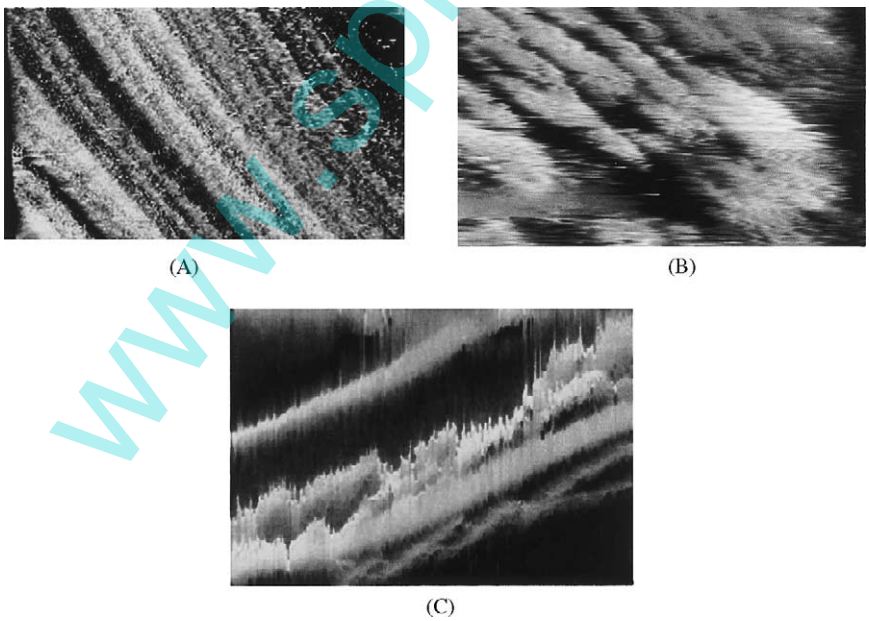

Fig. 3. STM images of native cotton fiber. (A) Native cotton fiber, I_{ref} = 0.28 nA, V_{bias} = 420 mV, scan area = 32 nm × 18 nm; (B) native cotton fiber by binding of CBHI and incubation for 24 h, I_{ref} = 0.10 nA, V_{bias} = 266 mV, scan area = 24 nm × 14 nm; (C) native control fibers by hydrolysis of CBHI plus EGI incubation for 24 h, I_{ref} = 0.28 nA, V_{bias} = 420 mV, scan area = 80 nm × 46 nm.

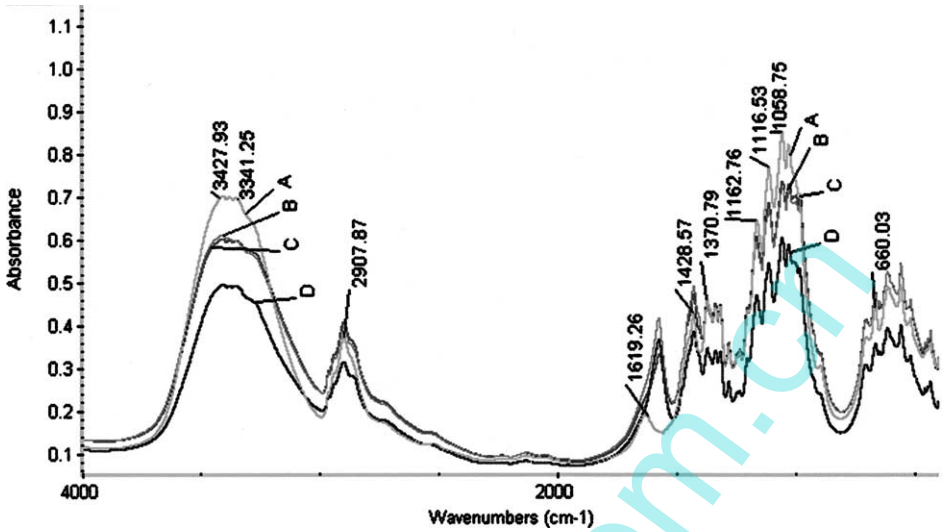

Fig. 4. Show a characteristic IR spectrum of native highly crystalline cellulose (A); after binding with intact CBHI and incubation for 20 h (B); with intact EGI (C); and after hydrolysis by CBHI plus EGI for 25 h (D).

peak is broad and overlaps with that of water effect. Here, we use OMNIC software package for correct and conversion the data of FT-IR spectroscopy, such as used second derivative and de-convoluted by using a Gaussian–Lorenzian curve fitting analysis to obtain relative quantitative information in 3600–3200 cm^{-1} bands. And then, the intensity ratio of 2900 cm^{-1}/1372 cm^{-1} that has been used as the crystallinity index [25] and the intensity of 2900 cm^{-1} was selected as an internal standard for estimated the changes of relative intensity of these hydrogen bonds. As shown in Fig. 5 and Table 3, after binding of CBHI, the crystallinity index estimated by the ratio of 2900 cm^{-1}/1372 cm^{-1} has a little change, while the relative intensity in 3600–3200 cm^{-1} estimated by same way appears obviously decrease tendency.

3.5. Comparison of specific activities of intact CBHI, EGI and their domains

Results are summarized in Tables 4 and 5, which are similar to those of other reports [27,32].

There is no significant difference between the specific activities of both intact enzymes and their catalytic domains on soluble substrates, but removal of CBD dramatically reduces their activities towards crystalline cellulose. That clearly indicated the important function of CBD in hydrolysis of crystalline cellulose.

Table 3
Comparison of the ratio of some peaks intensity of cotton fibers after adsorption by CBHI

Cellulose sample	1372 cm^{-1}	2900 cm^{-1}	3600–3200 cm^{-1}	Relative crystallinity index 1372 cm^{-1}/2900 cm^{-1}		3600–3200 cm^{-1}/2900 cm^{-1}	
					Changes ± %		Changes ± %
Native cotton fibers	7.25	6.28	140.5	1.15		22.3	
Native cotton fibers after adsorption by CBD	7.50	6.40	115.6	1.17	+1.7%	18.0	−19.6%

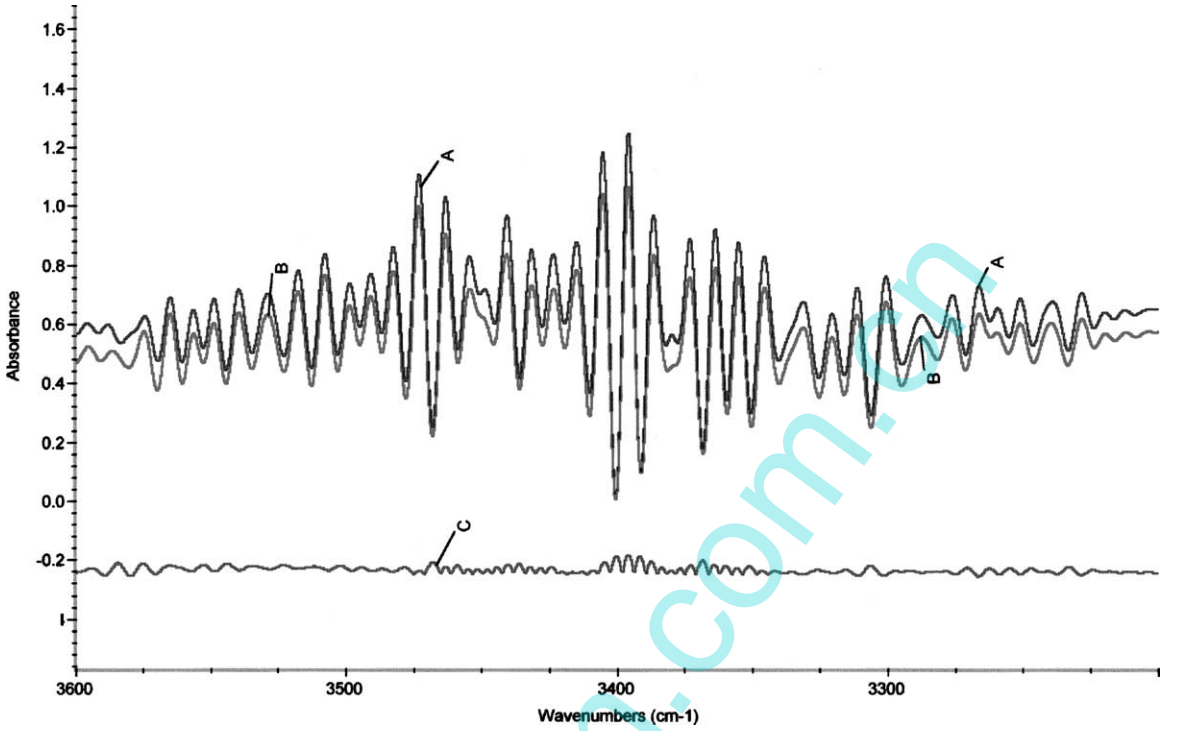

Fig. 5. Comparison of second-derivative FT-IR spectra in the region 3600–3200 cm^{-1} of native cotton fibers (A) and after adsorption by intact CBHI (B); (C) is their difference spectra.

3.6. Synergistic action between CD and CBD of CBHI and EGI, comparison with intact enzymes

The synergistic behavior of the combination of limited proteolytic domains as well as the comparison with their intact enzymes is investigated during prolonged hydrolysis of cellulolytic substrates (Fig. 6A–C). These results reveal that the hydrolysis rates of both intact enzymes and their combinations are much higher than those of the combination of CBDs and CDs in equivalent mole. These studies have confirmed the current view that an organized three-dimensional structure of an intact enzyme is necessary for its full catalytic function.

Table 4
Comparison of specific activities of intact CBHI with its CD and CBD on various celluloses[a]

Substrate	CBHI	CBHI-CD	CBHI-CBD
Cotton fiber	0.18	Trace	ND[b]
CF11 cellulose	0.53	0.31	ND
Avicel	0.71	0.13	ND
Swollen cellulose	1.38	0.96	ND
CMC-Na	ND	ND	ND
pNPC	8.75	8.43	ND

[a] Assays are performed as described in Section 2. Specific activity is defined as the amount of reducing sugars (μg, D-glucose equivalent) produced by 1 mg enzyme/min.
[b] None have detected.

Table 5
Comparison of specific activities of intact EGI with its CD and CBD on various celluloses[a]

Substrate	EGI	EGI-CD	EGI-CBD
Cotton fibers	0.06	Trace	ND[b]
CF11 cellulose	2.43	2.13	ND
Avicel	0.18	0.09	ND
Swollen cellulose	2.89	2.31	ND
CMC-Na	11.7	9.36	ND
pNPC	ND	ND	ND

[a] Assays are performed as described in Section 2. Specific activity is defined as the amount of reducing sugars (μg, D-glucose equivalent) produced by 1 mg enzyme/min.
[b] None have been detected.

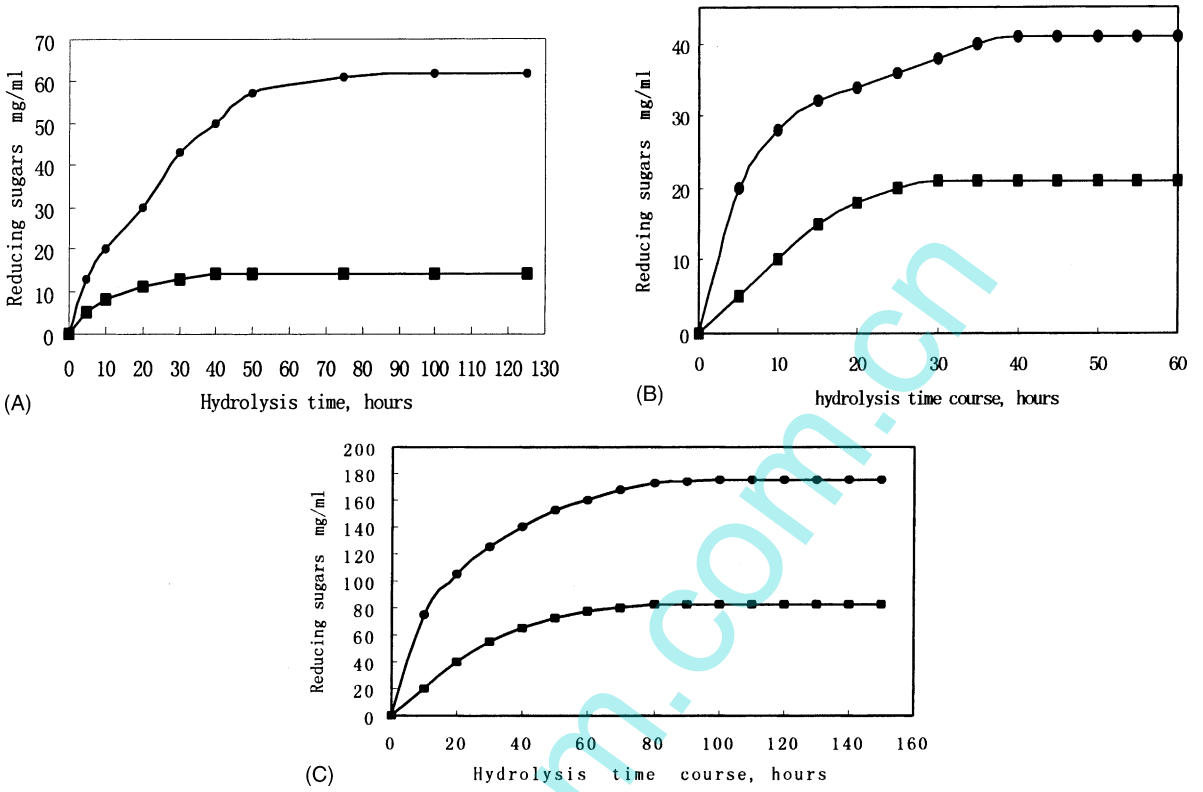

Fig. 6. Synergistic effect of CBHI-CBD and CBHI-CD with different molar ratios. (A) Hydrolysis of cotton fibers by intact CBHI (●) and the mixture of CBH-CD and CBHI-CBD in equivalent mole (■); (B) hydrolysis of CF11 cellulose by intact EGI (●) and the mixture of EGI-CD and EGI-CBD in equivalent mole (■); (C) hydrolysis of cotton fibers by intact CBHI combined with EGI (●) and the mixture of CBHI-CD, CBHI-CBD, EGI-CD and EGI-CBD in equivalent mole (■).

4. Discussion

4.1. The binding and catalytic functions of a cellulase are mainly dependent on the intact structure of enzyme molecule

The "induced-fit" hypothesis [32] postulates that conformational changes induced by substrate binding could orient functional group of an enzyme so as to enhance the efficiency of the subsequent chemical process. For insoluble substrates such as cellulose, hemi-cellulose, starch, and chitin, the relevant enzymes are typically modular enzymes with two distinct domains—a catalytic domain and a substrate-binding domain, connected by a linker region. Studies of the present work indicated that CDs alone can appear certain binding and hydrolysis function, CBDs alone appear binding function, but only an intact CBHI or EGI can exhibits effective function, any one domain alone and even the combination of both separated domains in equivalent mole have not sufficient for this function of intact enzyme. As is well known that the CD of CBHI and EGI is joined to CBD by an extended *O*-glycosylated interdomain-linker peptide. What is the role of linker peptide? Srisodsuk et al. [40] reported that deletion of linker would reduce the binding capacity and hydrolysis rate of CBHI. Present results are good agreement with those report. These phenomena suggested that the structural entirety in an enzyme is necessary for effective coupled working of its domains and conformational transition. Thus, it appears to us, in analogical studies of enzyme, only focus on its catalytic center is not sufficient.

4.2. Adsorption of CBD on insoluble substrate could be weakening and splitting of hydrogen bonding

As mentioned above, conformational changes induced by substrate binding could orient functional group of an enzyme for recognizing of catalytic site in substrate. But, this theory is mainly based on the study of soluble substrates in which binding requires loss conformational entropy [33]. In contrast, the conformation of each cellulose chain on the surface of cellulose fiber is essentially fixed, and the conformational change cannot spontaneously occurred by itself. In consequence of which, the relevant enzymes, for insoluble substrate hydrolysis, are typical modular enzymes with two distinct domains. The substrate-binding domain appears to assist in hydrolysis of insoluble substrates. But, how the two domains work together and how they interact with the insoluble substrates is not understood yet [32–34]. Reese et al. [35] first proposed that the existence of a hydrogen bond breaking enzyme was needed for the highly crystalline cellulose decomposition. Unfortunately, so far, our knowledge is still limited for this hypothesis. Recently, a novel protein termed “expansin” isolated from germinating seed of cucumber, which can induce plant cell wall enlargement, has been assumed to possess the capacity of disruption of hydrogen bonding [29]. And then, a bacterial CBD found appears to have same effect as “expansin” [36]. But, these disrupt effect of “expansin” and CBD to hydrogen bonding have not check to evidence by direct experiment. So far, only CBDs of family II (Cex and Cen CBD) have been proven to enhance the physical disruption of cellulose fibers and release small particles [5,6]. In present work, STM provide visible morphological changes of cotton fibers after treatment with CBHI-CBD. These observations are also consistent with the investigations carried out with IR observation that the intensity of hydrogen bonds appears decreased following the binding by CBD. All of these confirm that CBHI-CBD adsorbing on insoluble substrate was not limited by locating the cellulase close to cellulose and could be weakening and splitting of hydrogen bonding of cellulose.

Sinnott [37] proposed that the glucan chain must be left from the surface of a cellulose crystallite before the glycosidic bond was cleaved, because the long tunnel of CBHI had evolved to accommodate a single glucan chain [38]. Our results may provide an evidence for this hypothesis.

These studies can further provided an opinion that the function of an enzyme in decreases active energy for catalytic reaction is derived from the conformational regulation by intact enzyme molecule itself.

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References

[1] M. Claeyssens, H.V. Tilbeurgh, P.T. Tomme, T.M. Wood, Biochem. J. 261 (1989) 819.
[2] P. Béguin, J.P. Aubert, FEMS Microbiol. Rev. 13 (1994) 25.
[3] H.V. Tilberugh, P. Tomme, M. Claeyssens, R. Bhikhabhai, G. Perrersson, FEBS Lett. 204 (1986) 223.
[4] T. Reinikainen, Technical Research Center of FinlVTT, vol. 206, University of Turku Espoo, 1994.
[5] N. Din, N.R. Gilkes, B. Tekant, R.C. Miller, R.A.J. Warren, G. Kilburn, Biotechnology 9 (1991) 1096.
[6] N. Din, H.G. Damude, N.R. Gilkes, R.C. Miller, R.A.J. Warren, G. Kilburn, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 11383.
[7] B.X. Yan, Y.Q. Sun, J. Protein Chem. 16 (1997) 59.
[8] T.H. Wang, C.H. Wang, P.J. Gao, Y.X. Ling, Acta Microbiol. Sinica 38 (1998) 269.
[9] P.J. Gao, G.J. Chen, T.H. Wang, Y.S. Zhang, J. Liu, Acta Biochim.: Biophys. Sinica 33 (2001) 13.
[10] Z.H. Xiao, P.J. Gao, Y.N. Qu, T.H. Wang, Biotechnol. Lett. 23 (2001) 711.
[11] G. Carrard, M. Linder, Eur. J. Biochem. 262 (1999) 637.
[12] M.T.T. Teeri, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 12251.
[13] E.T. Jervis, C.A. Haynes, D.G. Kilbum, J. Biol. Chem. 272 (1997) 24016.
[14] T.M. Wood, Preparation of crystalline, amorphous, dyed cellulose substrates, in: W.A. Word, S.T. Kellogg (Eds.), Methods in Enzymology, Academic Press, San diego, p. 19.
[15] D.B. Ma, P.J. Gao, Z.N. Wang, Enzyme Microbiol. Technol. 12 (1990) 631.
[16] B.X. Yan, Y.Q. Sun, P.J. Gao, J. Protein Chem. 18 (1997) 681.
[17] B.X.P.J. Gao, Chin. Biochem. J. 13 (1997) 362.
[18] B.X. Yan, P.J. Gao, Chin. Biochem. J. 13 (1997) 580.
[19] J.G. Norby, P. Ottolenghi, J. Jensen, Anal. Biochem. 102 (1980) 318.
[20] Y.Z. Zhang, J. Liu, P.J. Gao, L.P. Ma, D.X. Shi, S.J. Pang, Appl. Phys. A 67 (1998) 483.
[21] M.L. Mattinen, M. Linder, T. Drakenberg, A. Annila, Eur. J. Biochem. 256 (1998) 279.

[22] B. Nidetzky, W. Steiner, M. Claecyssens, Biochem. J. 303 (1994) 817.
[23] A. Kyriacon, R.J. Neufeld, C.R. MacKenzi, Enzyme Microbiol. Technol. 10 (1988) 675.
[24] J. Stahlberg, G. Johansson, G. Pettersson, Biochim. Biophys. Acta 1157 (1993) 107.
[25] V.W. Tripp, Measurement of crystallinity, in: N.M. Bikales, L. Segel. (Eds.), Cellulose and Cellulose Derivatives, Wiley, New York, pp. 319–320.
[26] G. Beldman, A.G.J. Vorangen, F. Rombouts, M. Searl, M.F. Leeuwen, Biotechnol. Bioeng. 30 (1987) 251.
[27] H. Palonen, M. Tenkanen, M. Linder, Appl. Environ. Microbiol. 65 (1999) 5229.
[28] M.C.R. Wilke, Biotechnol. Bioeng. 22 (1980) 1037.
[29] S. McQueen-Mason, J. Cosgrove, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 6574.
[30] T. Sugiyama, J. Person, H. Chanzy, Macromolecules 24 (1991) 2461.
[31] Y. Marechal, H. Chanzy, J. Mol. Struct. 523 (2000) 183.
[32] C.B. Post, Biochemistry 34 (1995) 15881.
[33] A. Greagh, E. Ong, E. Jervis, D. Kilburm, C.A. Haynes, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 12229.
[34] G. Carrard, A. Koivula, H. Derlund, P. Béguin, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 10342.
[35] E.T. Reese, R.G.H. Siu, H.S. Leyinson, J. Bacteriol. 59 (1950) 485.
[36] E. Shpigel, L. Roiz, R. Goren, O. Shoseyov, Plant Physiol. 117 (1998) 1185.
[37] M.L. Sinnott, Biochem. Soc. Trans. 26 (1998) 160.
[38] T.T. Teeri, A. Koivula, M. Linder, G. Wahlfahrt, C. Divne, T.T. Jonest, Biochem. Soc. Trans. 26 (1998) 173.
[39] H. Fuchs, R. Gessner, Biochem. J. 359 (2001) 411.
[40] M. Srisodsuk, T. Reinikainen, M. Penttila, T.T. Teeri, J. Biol. Chem. 268 (1993) 20756.
[41] M.K. Agarwal, Principles of Receptorolgoy, Salter De Gruyter, Berlin, 1983.
[42] J. Medve, J. Stahlberg, T. Jerneld, Appl. Biochem. Biotechnol. 66 (1955) 39.
[43] D.Y. Ryu, C. Kim, M. Mandels, Biotechnol. Bioeng. 26 (1984) 488.
[44] A. Kyriacou, R.J. Neufeld, C.R. Mackenzie, Biotechnol. Bioeng. 33 (1989) 631.