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The influence of supramolecular structure of cellulose allomorphs on the interactions with cellulose-binding domain, CBD3b from *Paenibacillus barcinonensis*

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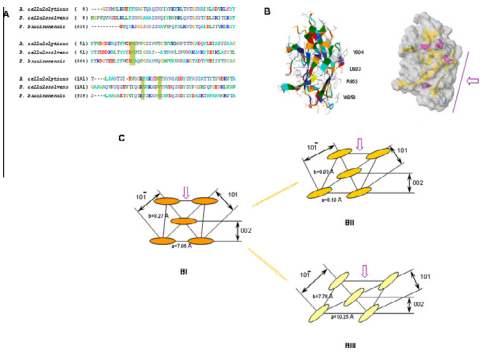
HIGHLIGHTS

- The recombinant CBD of endoglucanase Cel9B from *Paenibacillus barcinonensis* is used.
- The highest CBD adsorption capacity is recorded for the most crystalline cellulose I.
- The CBD adsorption kinetic is rendered by surface area and porosity of cellulose.
- The CBD desorption is related to hydrophobic interactions with cellulose allomorphs.
- Temperature and pH medium have important influence on CBD desorption.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of recombinant cellulose-binding domains (CBDs) of endoglucanase Cel9B from *Paenibacillus barcinonensis* with different cotton cellulose allomorphs (I, II and III) has been investigated, in order to bring new insights regarding the CBD adsorption and desorption processes. The highest CBD adsorption capacity was recorded for cellulose I, confirming the affinity of proteins to the most crystalline substrate. The weakening and splitting of the hydrogen bonds within cellulose structure after CBD adsorption, as well as a decrease of the crystallinity degree were identified by ATR-FTIR spectroscopy and XRD. The CBD's adsorption kinetic was shown to be rendered by properties as, specific surface area and porosity, being confirmed by dynamic vapor sorption measurements. An important influence of temperature (25, 37 and 50 °C) and/or pH medium (4, 5.5, 7 and 10) on the CBD desorption capacity was confirmed, being related to the hydrophobic interactions formed between the CBD and the cellulose allomorphs.

1. Introduction

Cellulose, the most abundant polymer in nature, plays a major role in daily life by representing an important economic investment. The physical properties of cellulose, as well as their chemical behavior and reactivity, are strongly influenced by the arrangement of the cellulose molecules with respect to each other and to the fiber axis. Several crystal structures of cellulose are recognized which differ in unit cell dimensions and chain polarity. The natural form of cellulose, so-called cellulose I, was found to be a composite of cellulose Iα and Iβ crystalline forms. This can easily

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be converted into the crystalline form of cellulose II by regeneration or mercerization processes. Another crystalline form known as cellulose III can be prepared in anhydrous liquid ammonia or in organic amines. Amorphous cellulose has often been used to understand the behavior of the non-crystalline domains within cellulose, under various conditions. Generally, it is prepared by ball-milling of cellulose, deacetylation of cellulose acetate under non-aqueous alkaline conditions and regeneration of cellulose solution within aqueous media or into non-aqueous media (Ciolacu et al., 2011). The structural differences in cellulose in terms of crystalline and amorphous contents and the sizes and shapes of the crystallites, allow a variable target for the binding modules and the enzymes action on the cell wall.

Most cellulases display a modular structure that, besides a catalytic domain, comprises accessory domains as carbohydrate-binding modules (CBMs), linker or repeat regions, and Fn3 modules (Chiriac et al., 2010). A carbohydrate-binding module (CBM) is defined as a contiguous, independently-folding sequence of amino acids found within the primary structure of a carbohydrate-active enzyme which interacts with a carbohydrate but does not chemically modify its structure (Abbott and Boraston, 2012). CBMs, previously known as cellulose binding domains (CBDs), can significantly contribute to the activity of the enzymes against cellulosic substrates by increasing enzyme–substrate proximity, enhancing accessibility, and modifying the surfaces of the cellulose crystals. It was also found that this module has a determinant role in the adsorption of proteins onto different lignocellulosic substances, as lignin preparations (Pareek et al., 2013). Three basic properties have contributed to CBDs being perfect candidates for many applications: CBDs are usually independent folding units and therefore can function autonomously in chimeric proteins since the attachment matrices are abundant and inexpensive and have excellent chemical and physical properties, and the binding specificities can be controlled, and therefore the right solution can be adapted to an existing problem (Shoseyov et al., 2006).

Due to industrial reasons and to the considerable potential in biotechnology, applied microbiology and immunology, there is much interest in the nature of the binding of CBDs on cellulose. Nowadays there are still controversies about the interaction mode of CBD with cellulose. CBDs from the same organism can differ in their binding specificity and occasionally, two CBDs located on the same enzyme can also exhibit this distinction (Ciolacu et al., 2010). It was established that the binding sites of families I, II and III CBDs are adapted to bind to a surface, while family IV CBD binds to a single molecule (Shoseyov and Warren, 1997). Moreover, the binding process takes place on both crystalline and amorphous regions from cellulose for families I, II and III CBDs, while family IV CBDs do not bind to crystalline cellulose.

To the best of our knowledge there are no reports regarding CBD adsorption on different allomorphic forms of cellulose, even considering the studies regarding the binding preference of CBD for cellulose substrates. Therefore, there is a need to examine the adsorption of CBD on celluloses I, II and III. As the enzyme, a recombinant CBD from *Paenibacillus barcinonensis* was used which is a newly-identified species that shows a multiple-enzyme β-glycanase system correlated with its high polysaccharide degrading potential (Pastor et al., 2001; Sánchez et al., 2004, 2005). This recombinant CBD belongs to modular cellulase Cel9B from *P. barcinonensis*, and has been previously characterized and classified in family III (CBM3b) (Chiriac et al., 2010). The interactions of CBD with cellulose allomorphs were investigated regarding their binding capacity and the structural changes induced, which were characterized by ATR-FTIR spectroscopy and the X-ray diffraction method (XRD). Dynamic vapor sorption (DVS) was applied to provide more information about the adsorption process at the surface of cellulose. In addition, the desorption process under different conditions of temperature (25, 37 and 50 °C) and pH (4, 5.5, 7 and 10), has been investigated.

2. Methods

2.1. Materials

Three cellulose allomorphs were prepared: as *cellulose I* (BI), cotton cellulose (Arshad Enterprises, Pakistan) was used, having been extracted for 8 h in a Soxhlet extractor using an ethanol–benzene mixture; *cellulose II* (BII) was prepared by treating BI in 17.5% NaOH for 24 h at 15 °C; and *cellulose III* (BIII) was prepared by soaking BI in 100% ethylenediamine for 24 h at 15 °C. *Amorphous cellulose* (Ba) was prepared based on the SO_2-diethylamine-dimethylsulfoxide (SO_2-DEA-DMSO) solvent system for the dissolution of cellulose by using ethanol as the regeneration medium (Ciolacu et al., 2011). The CBD from *P. barcinonensis* (*Paenibacillus* sp. strain BP-23) was purified from the recombinant strain *Escherichia coli* BLR (DE3) pET28aCBD2, which expressed the isolated CBD3b (Chiriac et al., 2010). The apparent molecular weight of the recombinant CBD3b, as determined by SDS–PAGE, was 17 kDa. All the other chemicals used were of the highest purity available commercially.

2.2. CBD adsorption and desorption studies

The adsorption studies were performed mixing 3 mg of the different cellulose allomorphs (BI, BII, and BIII) and amorphous cellulose (Ba) with 1.8 mg of CBD in 1 ml of Tris buffer (50 mM, pH7) and incubated for 2 h at 25 °C, using gentle rotation (600 rpm). In parallel, the blank samples were prepared similarly but without CBD. Afterwards the fibers were centrifuged at 5000 rpm for 5 min and the CBD concentration in supernatant (P_F, μM) was measured using a TECAN Infinite M200 spectrophotometer. The protein concentration was determined by the Lowry method (Bio-Rad *DC* Protein assay) and the bound CBD (P_B, μM) calculated by Eq. (1):

$$P_B = [(P_T - P_F)/m] \times V \ (\mu mol/g) \quad (1)$$

where P_T – the initial concentration of CBD (μM); P_F – the free CBD concentration (μM); P_B – the bound CBD concentration (μM); m – the mass of cellulose fibers (g); V – the volume of the buffer (L).

The protein adsorption on cellulose was fitted to a Langmuir isotherm which assumes that the adsorption can be described by single adsorption equilibrium constant and a maximal adsorption capacity. The data were analyzed by non-linear regression analysis of $1/P_B$ versus $1/P_F$ and the distribution coefficient, R was defined as:

$$R = K_d \cdot P_{Max} \quad (2)$$

where P_{Max} – the maximum protein adsorption (μmol CBD/g); K_d – the dissociation constant (L/μmol).

The fibers were washed with the same volume of buffer, centrifuged at 5000 rpm for 5 min and lyophilized using a Mini Lyotrap freeze-dryer.

For desorption experiments 3 mg of different cellulose allomorphs treated with CBD (BI-CBD, BII-CBD, BIII-CBD) and CBD-treated amorphous cellulose (Ba-CBD) per mL of different buffer solutions (pHs of 4, 5.5, 7 and 10) were washed for 1 h at different temperatures (25, 35 and 50 °C), with gentle rotation (600 rpm). The blank samples were prepared in parallel Afterwards the fibers were centrifuged at 5000 rpm for 5 min and the CBD concentration in supernatant was measured in a TECAN Infinite M200 spectrophotometer. The desorbed protein was measured by the Lowry method.