Reactivities of cellulases from fungi towards ribbon-type bacterial cellulose and band-shaped bacterial cellulose

Yoshihiko Amano1,*, Kouichi Nozaki1, Takashi Araki1, Hideki Shibasaki2, Shigenori Kuga2 and Takahisa Kanda1
1Department of Chemistry and Material Engineering, Faculty of Engineering, Shinshu University, 380-8553 Nagano, Japan; 2Department of Biomaterial Science, School of Agricultural and Life Sciences, The University of Tokyo, 113-8657 Tokyo, Japan; *Author for correspondence (e-mail: yoamano@gipwc.shinshu-u.ac.jp; phone: +81-26-269-5394; fax: +81-26-269-5395)
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Abstract

We have investigated the reactivities of various cellulases on ribbon-type bacterial cellulose (BC I) and band-shaped bacterial cellulose (BC II) so as to clarify the properties of different cellulases. BC I was effectively hydrolyzed by exo-type cellulases from different fungi from twice to four times as much as BC II, but endo-type cellulases showed little difference in reactivity on those substrates. One of the endo-type cellulases, EG II from Trichoderma reesei, degraded BC II more rapidly than exo-type cellulases even in the production of reducing sugars. The degree of polymerization (DP) of BC II was rapidly decreased by endo-type cellulases at an early stage, while exo-type cellulases did not cause the decrease of DP at the initial stage, though the decrease of DP was observed after an incubation of 24 h. All exo-type cellulases adsorbed on BC I and BC II, while endo-type cellulases except for EG II adsorbed slightly on both substrates. It was interesting to observe EG II adsorbed on BC I but not on BC II. It is suggested that the adsorption of enzyme on cellulose is important for the degradation of BC I, but not for BC II. It is proposed that the ratio of a specific activity of each enzyme between BC I and BC II represents the difference in the mode of action of cellulase. Furthermore, the value, which we can calculate from the decrease of DP/reducing sugar produced, is effective for discriminating the mode of action of cellulase, especially the evaluation of randomness in the hydrolysis of cellulose by endo- and exo-type cellulases.

Introduction

It is well known that certain bacteria such as Aceto- bacter sp. (Brown 1886), Agrobacterium tumefaciens (Matthysse 1983), and Pseudomonas sp. (Kawahara et al. 1996) synthesize extracellular cellulose. Acetobacter xylinum is an exceptionally good producer of bacterial cellulose in comparison with other bacteria, and this cellulose has been used in industrial materials (Masaoka et al. 1993) because of its characteristic properties.

Bacterial cellulose (cellulose I) has been found to have a characteristic structure that is different from higher plant cellulose. It is composed of sub-microfibrils, which is a so-called ribbon-like structure of crystalline cellulose I microfibrils (Muhlethaler 1949) and has an ultra fine network structure (Yoshinaga et al. 1997). The ribbons have been extruded parallel to the longitudinal axis of the cell and have a size of approximately 3.0–3.5 nm thickness and 40–60 nm wide. Each ribbon is composed of a number of microfibrils that have been observed with transmission electron microscopy (Brown et al. 1976; Zaar 1977; Hirai et al. 1998). It has been reported that bacterial cellulose with a different crystalline structure (cellulose II) is produced by a mutant strain of Acetobacter
xylinum (Kuga et al. 1993; Hirai et al. 1997). This cellulose is known as ‘band shaped fibrils’. This band cellulose is composed of a strand-like structure with a lateral dimension of 10 nm, as examined by transmission electron microscopy (Kuga et al. 1993; Shibasaki et al. 1995).

There are some reports on the decomposition of bacterial cellulose I by various cellulase components. Scheu et al. (1993) reported that bacterial cellulose I could be degraded easily by cellulases produced from microorganisms in soil and litter. Recently it was reported that native bacterial cellulose was more susceptible than acid-treated bacterial cellulose by the synergistic action of CBH I and EG II from Trichoderma reesei, Exo-A from Aspergillus niger, and three endo-type cellulases (En-1 from I. lacteus, Endo-2 from T. reesei, and EG-1 from A. niger) from the mode of action on soluble and insoluble cellulotic substrates (Amano et al. 1996; Shiroishi et al. 1997; Kanda et al. 1994). From our study the partial amino acid sequence of Endo-2 coincided with that of EG II from T. reesei as reported previously (Saloheimo et al. 1988) and the properties of Endo-2 are also similar to EG II (Amano et al. 1996; Shiroishi et al. 1997; Kanda et al. 1994). So Endo-2 is referred to as EG II in this paper.

Materials and methods

Enzymes

Various cellulase components from different origins were purified according to the methods reported previously (Amano et al. 1996; Shiroishi et al. 1997). Those highly purified cellulases were classified as two groups in which there were four exo-type cellulases (Ex-1 from Irpex lacteus, CBH I and CBH II from Trichoderma reesei, Exo-A from Aspergillus niger), and three endo-type cellulases (En-1 from I. lacteus, Endo-2 from T. reesei, and EG-1 from A. niger) from the mode of action on soluble and insoluble cellulotic substrates (Amano et al. 1996; Shiroishi et al. 1997; Kanda et al. 1994). From our study the partial amino acid sequence of Endo-2 coincided with that of EG II from T. reesei as reported previously (Saloheimo et al. 1988) and the properties of Endo-2 are also similar to EG II (Amano et al. 1996; Shiroishi et al. 1997; Kanda et al. 1994). So Endo-2 is referred to as EG II in this paper.

Substrates

The bacterial cellulose (cellulose I, BC I, Lot No. 0023-1) produced from A. xylinum subsp. saccharofermentans BPR201 as reported previously (Toyosaki et al. 1995), was donated by Bio-Polymer Research Co., Ltd. The other type of bacterial cellulose (cellulose II, BCII) was produced by the mutant strain of A. xylinum ATCC 23769 using Schramm-Hestrin (SH) medium (Kuga et al. 1993). This cellulose was treated as follows: after production of this bacterial cellulose, cellulose II, a milky layer was removed and then boiled for 30 min in 2% NaOH to remove the cell followed by washing with water. The structure of BC II was determined by electron diffraction analysis as described previously (Kuga et al. 1993).

Enzyme activity

The reaction mixture consisted of 2.5 mg/ml of BC II and 3.4 μM of cellulase dissolved in 40 mM buffer at optimal pH for individual enzymes (Exo-A and EG-1: pH 3.0, CBH I and En-1: pH 4.0, CBH II and EG II: pH 4.5, Ex-1: pH 5.0). After incubation at 30 °C for an appropriate period, reducing sugars produced were measured per ml of reaction mixture by the methods of Somogyi (1952) and Nelson (1944). Specific activity of each enzyme on BC I and BC II was calculated from reducing power produced with incubation for 1 h. Reaction products liberated from BC I and BC II were analyzed by thin layer chromatography (TLC) and by high performance liquid chromatography (HPLC) quantitatively as reported previously (Shiroishi et al. 1997).