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RESEARCH PAPER

# Effect of Straw Apoplast Protein on Cellulase Activity

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**Abstract:** The alteration of the maize straw apoplast proteins involved in the process of preservation was studied and the effects of the apoplast proteins on *Penicillum expansum* cellulase activities were analyzed. The results obtained by the study and the analysis showed a gradual decrease in the extractable apoplast proteins that occurred during the preservation of the maize straw. Meanwhile, their synergistic effects on *P. expensum* cellulase were also attenuated. The apoplast proteins that were extracted from the fresh maize straw possess endogenous EG activities but these were unstable and completely disappeared after 6 months of preservation. The apoplast proteins that were extracted from the preserved straw exhibited a large amount of synergistic effects on FPA, cotton lyase, and β-glucosidase. The maximum synergistic values were 95.32 %, 102.06 %, and 96.6 %, respectively. They inhibit the CMCase activity at a maximum value of 49.52 %. The apoplast proteins show distinctive synergistic effects with βG and EG, but have no effects on the CBH activity. When the synergistic effects on endogenous EG were eliminated, the apoplast proteins extracted from fresh maize straw showed more enhanced synergistic or inhibiting effects on FPA, cotton lyase, βG, and CMCase than in the case where the apoplast proteins extracted from the preserved straw. On the basis of the observation, the extracted apoplast proteins play important roles in the regulation of cellulase activities. The detailed analysis of the related mechanisms will greatly benefit the studies of natural biomaterial hydrolysis.

**Key Words:** apoplast proteins of straw; cellulose; cellulase activity; cell wall bound proteins

It has been more than 50 years since the different components of homologous cellulase that synergistically hydrolyze cellulose have been discovered[1]. The common view was that the cellulase hydrolytic process was the result of the cooperation among endoglucanases (EG, EC 3.2.1.4), cellobiohydrolases or exocellulases (CBH, EC 3.2.1.91), and β-glucosidase (βG, E.C.3.2.1.21), but there are still many disputations regarding the rational synergistic mechanism. Synergistic effects became complex as results published in the literature are inconclusive, even contradictory sometimes[2]. Therefore, the method of synergistic action has not been entirely acknowledged. The action of cellulases on natural substrates has been more difficult to evaluate and the results have often been controversial. In most cases, the competitive and the non-competitive inhibitions were both observed[3]. This was partly owing to the unsatisfactory purity of the individual cellulases and the complexity of the nature of the bio-substance.

The investigation of the plant apoplast proteins is relatively complex in plant physiology. Subsequent to the development in the experimental approaches and tools in the recent decade, genome-scale assessments predicted the existence of hundreds of extra-cellular proteins[4]. The extra-cellular proteins take up approximately 10 % of the cell wall mass[5] and are involved in the regulation of cell growth and development, support of cell wall architecture, and respond to biotic and abiotic stresses. The size and complexity of the wall proteome underlines its multifunctional nature and the tightly regulated expression of cell wall proteins was observed in a broad range of events in plants, such as the growth, metabolic processes,

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and the response to the external stimuli[6–9]. The multi-functional nature of apoplast proteins inside the cell wall probably had effects on the activity of cellulase.

The research presently focuses on the supramolecular structure, that is, the polymeric structure for the effect of raw substrates on the hydrolysis activity of cellulase. However, the roles of the active components of the plant cell wall in regulating cellulase activity have not been defined. In this study, maize (*Zea mays*) straw was selected in order to study the regulatory roles of the apoplast protein in cellulase activity from a new perspective.

## 1 Materials and methods

### 1.1 Chemicals, reagents, substrates and enzymes

Phenylmethyl Sulfonyl Fluoride (PMSF) was purchased from the Amresco Co. (USA) and other biochemical reagents were from the Sigma Chemical Co. (USA). The cellulase obtained from *Penicilllum expansum* was provided by the Sunson Co. (China).

### 1.2 Plant material

The stem tissue obtained from the fourth to the sixth internodes (lignifying tissue) from branches that had seven internodes in all were collected from the maize plants (*Z. mays*) grown in the croplands in Beijing, in the autumn season and were used as the source of cell wall proteins. This freshly collected material was separated into two parts. One part was stored at −20 °C immediately after harvesting until used; the other part was air dried at room temperature in the dark. The stored maize stem was ground with pulp refiner before use while the air-dried maize stem was milled using an ultrafine grinder.

### 1.3 Preparation of cell walls

The ground stem tissue was homogenized in the grinding buffer that contained 50 mmol/L sodium acetate, pH 4.8, 50 mmol/L NaCl, 30 mmol/L ascorbic acid, and 100 mg PVPP with liquid nitrogen in a mortar. The stem tissue was then filtered through nylon mesh (47 $\mu m^2$) membranes. The cell wall was then separated by sequential washes with the ice-cold grinding buffer, 0.1 mol/L NaCl, $dH_2O$, −20 °C acetone, $dH_2O$, and 50 mmol/L sodium acetate[10]. The residue so obtained which was the cell wall was used for extracting apoplast proteins.

### 1.4 Extraction of straw apoplast proteins

The detailed methods for the extraction of apoplast proteins from maize straw can be found in the reference [11]. Three extracts were combined and mixed with equal volumes of −20 °C EtOH that was incubated at 4 °C overnight and then centrifuged at 4 000 g/min to harvest the precipitation. This was then dried in the air and the precipitation was resuspended in a 4-time volume 50 mmol/L sodium acetate buffer (pH 4.8) for 2 h at 4 °C. The insoluble material was removed by a second centrifugation at 16 000 g/min for 20 min. The supernatant was the extract of apoplast. The extraction ratio of the apoplast proteins was defined as the percentage of the apoplast proteins in the dry weight of the total cell wall.

### 1.5 Protein determination

The protein concentrations were estimated by the Coomassie Brilliant Blue method (Bradford, 1976) using the bovine serum albumin as the standard.

### 1.6 Enzyme activity assay

The methods of measuring activities of filter paper cellulase, β-glucosidase, cotton lyase, and CMCase are described in the reference [12]. The activity of CMCase was determined according to the traditional measurement of EG enzyme activity. All these activities were measured at 50 °C in 50 mmol/L NaOAc buffer (pH 4.8). The reducing sugar content was measured by the DNS Reagent[13]. One unit of enzyme activity was defined as the amount of enzyme that can liberate 1 mmol of reducing sugar per minute under the assay conditions.

### 1.7 Cellulase activity assay effected by apoplast protein

Varying amounts of column fractions, apoplast protein supernatant were mixed sufficiently with a defined amount of *P. expansum* cellulase before the addition of different substrates deciding in which enzyme activity were mensurated in 50 mmol/L NaOAc buffer (pH 4.8). The reaction was incubated for 10 min at room temperature. The aliquots were analyzed by a colorimetric assay in 520 nm after a defined time point (see 1.6) in order to determine the amount of reducing sugar generated by the reaction. The substrates were reduced with 50 mmol/L NaOAc buffer (pH 4.8) in advance to minimize the background in the reducing sugar assay. The endogenetic cellulase activity assay of apoplast proteins were analyzed in the same way but with the exclusion of the addition of *P. expansum* cellulase into the response system.

### 1.8 Heat inactivation of apoplast proteins assay

Following a 10-min boiling bath, the extract of apoplast protein was placed at room temperature until the original temperature was obtained. The *P. expansum* cellulases that were mixed with apoplast proteins were heated and unheated, respectively, were process cellulase activity assay (see 1.6). The results compare with the blank control of the *P. expansum* cellulase.

## 2 Results and analysis

### 2.1 Effect of preserved straw apoplast proteins on cellulase activity

The apoplast proteins extracted from the six-month preserved maize straw were used to perform the cellulase activity assays with four different kinds of substrates, namely, 1 % CMC, 1 % salicin, filter paper, absorbent cotton as shown in Fig. 1a and 1b.

When the concentration of apoplast protein from the preserved straw tissue is 1.043 7 mg/mL in the reaction, the