Isolation of Enzyme Subunit (X40) of Multienzyme Complex from Paenibacillus curdlanolyticus B-6


Abstract

Paenibacillus curdlanolyticus B-6, a facultative anaerobic bacterium isolated from anaerobic digester, produces an extracellular multienzyme complex when grown on xylan under aerobic conditions. A multienzyme complex is a large protein complex with high molecular weight approximately 1,450 kDa containing several xylanolytic and cellulolytic enzymes subunits bound to core protein called cellulosome. The 1,450 kDa multienzyme complex of P. curdlanolyticus B-6 was isolated by four steps of chromatographies. The multienzyme complex was separated and the 40 kDa protein was found on SDS-PAGE in all steps of purification. The results indicated that the 40 kDa protein was a major subunit of the multienzyme complex because it was hold together with core protein along all of purification steps. The 40 kDa subunit showed the majority of xylanase activity.

Keywords: enzyme subunit, multienzyme complex, Paenibacillus curdlanolyticus B-6, xylanase

Introduction

The utilization of plant biomass in human culture produces wastes, the major source of environmental pollution in Thailand. The plant cell wall is a complex polysaccharides, cellulose and hemicellulose tightly interaction with lignin and other components, called lignocellulosic substance. Bioconversion is one of alternative to change the plant biomass to valuable products by enzyme. The plant cell wall degradation requires cellulolytic enzymes co-operated with xylanolytic enzymes and side chain-cleaving enzymes. Paenibacillus curdlanolyticus B-6, a facultative anaerobic bacterium isolated from an aerobic digester fed with pineapple wastes and identified by 16S rRNA gene analysis, produces an extracellular multienzyme complex. When P. curdlanolyticus B-6 was grown on xylan under aerobic conditions, the bacterium produced two extracellular multienzyme complexes with high molecular weights estimated at 1,450 and 400 kDa (Pason et al., 2006). Most studies on the subunit of multienzyme complex have been confined to anaerobic cellulolytic microorganisms. Reports on the multienzyme complex of Paenibacillus curdlanolyticus B-6 have not been reported.
complex produced by a facultatively anaerobic bacterium such as *P. curdlanolyticus* B-6 are limited and scant. In the present study, we describe the isolation of one of the subunits of multienzyme complex with high molecular weight produced by *P. curdlanolyticus* B-6.

**Materials and Methods**

*Paenibacillus curdlanolyticus* B-6 was grown in Berg’s mineral salts medium at pH 7.0 containing 0.5% commercial oat spelt xylan as a carbon source in a rotary incubator at 200 rpm for 3 days at 37°C. The culture was harvested by centrifugation (10,000 rpm, 10 min, 4°C). An enzyme subunit (X40) was purified by four steps. The first step was separation of cellulose-binding proteins by using Avicel PH101. The next step was isolation of the enzyme complex with high molecular weight from the other cellulose-binding proteins by gel-filtration chromatography using HiPrep 26/60 Sephacryl S-300 high resolution. In the third step, the fraction with high molecular weight was applied to anion-exchange column chromatography (HiLoad 16/10 Q-Sepharose High Performance) equilibrated with 0.05 M sodium phosphate buffer (pH 6.0). The multienzyme complex was eluted with a linear gradient of 0 to 1.0 M NaCl. Five fractions corresponding to protein peaks (280 nm) were designated I1 through I5. The last step was concentration of the sample and it was applied to a hydrophobic-interaction chromatography column (HiPrep 16/10 Phenol). The fractions corresponding to absorbance peaks (280 nm) were designated H1 through H3. Then they were concentrated and desalted by ultrafiltration. The carboxymethyl cellulase (CMCase) and xylanase activity were measured by the method of Ratanakhanokchai *et al.* (1999). The reaction mixture consisted of 0.5 ml of 1% CMC or oat spelt xylan in 100 mM sodium phosphate buffer (pH 7.0) and 0.1 ml enzyme. After incubation for 10 min at 50°C, reducing sugar was determined by using the Somogyi–Nelson method with xylose as a standard (Somogyi, 1952). One unit of the xylanase activity was defined as the amount of enzyme that liberated 1 μmol of reducing sugar in 1 min under the above conditions. Protein concentration was measured as described by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The protein content of the eluate was measured at 280 nm. The pattern of proteins and enzymes were analyzed by Native-PAGE, SDS-PAGE (Laemmli, 1970) and zymogram.

**Results and Discussion**

*P. curdlanolyticus* B-6 was grown on xylan for 3 days and harvested by centrifugation. In the first step, multienzyme complex was separated from cellulose-unbound proteins by affinity purification on Avicel. Cellulose-unbound proteins were removed by washing with phosphate buffered saline and cellulose-binding proteins were eluted by 1% triethylamine and analyzed by polyacrylamide gel electrophoresis and zymograms (Data not shown). The presence of multiple cellolyses and xylanases in the cellulose-binding protein fraction was revealed by native-PAGE, SDS-PAGE and zymograms. The cellulose-binding proteins indicated that at least 12 proteins with molecular masses in the range of 48 to 224 kDa showed xylanase activities and 9 proteins showed CMCase activities on zymograms (Data not shown). After preparation of the cellulose–binding fraction, the fraction was applied to gel-filtration column chromatography. Chromatogram of gel-filtration showed that cellulose-binding protein fraction was exhibited to 4 protein peaks as shown in Figure 1. The peak I, peak II, peak III and peak IV were estimated to be 1,450, 400, 160 and 40 kDa, respectively. The peak I and peak II containing both xylanase and CMCase activities were isolated. The peak I and peak II were estimated to be molecular mass 1,450 and 400 kDa, respectively. Native-PAGE and SDS-PAGE of each peak showed only 3 protein bands in native-PAGE but in SDS-PAGE analysis peaks I, II, III and IV consisted of 8, 7, 3 and 2 protein subunits, respectively (data not shown). This result indicated that peaks I and II were multienzyme complexes I and II due to high molecular mass and they composed of various subunits including xylanases and CMCases. Peak I showed that it has not only a high
molecular weight with high xylanase activity but also it was composed of several proteins with xylanase and CMCase activities. Thus, peak I was collected and concentrated by freeze-drying after desalting.

Figure 1 Sephadryl S-300 chromatography of the isolated multienzyme complex from the culture supernatant of *P. curdlanolyticus* B-6, grown on xylan.

The Peak I was separated into five protein peaks (OD₁₉₀) by anion-exchange chromatography and designated as F1 to F5 (data not shown). F1 had the highest xylanase activity, while F2 and F5 showed moderate activity. On the other hand, F3 and F4 showed a little xylanase activity. Each purified protein fraction F1 to F5 showed difference in protein composition (Figure 2). From anion-exchange chromatography, F1 was apparently the most suitable fraction to study the subunit of multienzyme complex of *P. curdlanolyticus* B-6 because F1 had the highest xylanase activity and its subunit composition was clearly shown on SDS-PAGE (data not shown).

Figure 2 SDS-PAGE (5-20% gradient gels) analysis of the ion-exchange protein fraction.

The F1 fraction from anion-exchange chromatography (Figure 1) was subjected to hydrophobic-interaction chromatography and separated 3 protein peaks (data not shown). The major fraction 1 exhibited only one protein band on native PAGE and three major bands (280, 260, and 40 kDa) plus additional three minor bands (65, 60, and 48 kDa) on SDS-PAGE (Figure 3). These results indicated that one protein on Native-PAGE is a protein complex comprising six subunits.
Figure 3 Protein pattern of purified protein fraction from HiPrep 16/10 Phenol. Peak 1 to 3 (lane 1-3) were analyzed by Native-PAGE (A) and 10-20% SDS-PAGE (B) analysis. The 40 kDa protein were purified and analyzed by SDS-PAGE (XA) and zymogram for xylanase activity (XB)(C).

Summary

The results showed that the 40 kDa protein has a tightly bound with protein complex through many steps of purification and majority of xylanase activity resided in the 40 kDa subunit indicating that the 40 kDa may be the most important catalytic subunit of the multienzyme complex. In the next study, the 40 kDa will be cloned and studied physico-chemical properties of recombinant proteins.

Literature cited


