JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4576–4581
0021-9193/88/104576-06$02.00/0

Vol. 170, No. 10

# Purification of *Clostridium thermocellum* Xylanase Z Expressed in *Escherichia coli* and Identification of the Corresponding Product in the Culture Medium of *C. thermocellum*

OLIVIER GRÉPINET, MARIE-CHRISTINE CHEBROU, AND PIERRE BÉGUIN*

*Unité de Physiologie Cellulaire, Déparetment des Biotechnologies, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France*

Received 7 March 1988/Accepted 5 July 1988

**An endoxylanase encoded by the *xynZ* gene of *Clostridium thermocellum* was purified from *Escherichia coli* harbouring a fragment of the gene cloned in pUC8. The purified enzyme showed two active bands of $M_r$ 41,000 and 39,000, the latter one presumably derived from the former through proteolysis. The enzyme was highly active on xylan and *para*-nitrophenyl-β-D-xylobioside. The major end product of xylan hydrolysis was xylobiose. With an antiserum raised against the enzyme purified from *E. coli*, an immunoreactive polypeptide of $M_r$ 90,000, corresponding to the entire *xynZ* gene product, was detected in a culture supernatant from *C. thermocellum* grown on cellulose. By immunological detection, xylanase Z was shown to be associated with a cellulose-binding, high-molecular-weight fraction whose properties coincided with those described previously for the cellulose-degrading complex of *C. thermocellum* known as the cellulosome.**

Cellulose and hemicellulose, which are closely associated in most natural cellulosic substrates, are the two most abundant and biologically renewable resources for bioconversion into ethanol and feedstock chemicals (24, 25). A major fraction of hemicellulose is composed of D-xylans, consisting of a backbone of xylose residues with short side chains of arabinose or glucuronic acid (3). Furthermore, xylans of several wood species are acetylated. Complete degradation of xylan requires the action of several types of enzymes (3). Endoxylanase, which splits internal bonds in the polysaccharide backbone, and β-xylosidase, which hydrolyzes xylooligosaccharides to D-xylose, are the best-characterized enzymes. α-Glucuronidase and α-arabinofuranosidase cleave the branched sugars. Esterases which deacetylate acetyl xylan have been identified in some fungal cellulolytic systems.

The gram-positive anaerobic thermophile *Clostridium thermocellum* is considered one of the most efficient microorganisms for saccharification of microcrystalline cellulose (9). *C. thermocellum* secretes a highly active and thermostable multicellulase complex, termed the cellulosome (12, 13), which displays endoglucanase, cellobiohydrolase, and glucohydrolase activity (6).

Garcia-Martinez et al. (5) reported that *C. thermocellum* secretes xylanase activity when cells are grown on cellobiose, but they failed to detect growth on xylan. Wiegel et al. (23) found that *C. thermocellum* eventually utilizes xylan as a carbon source, but only after a lag phase of more than 100 h, during which xylose and xylooligomers of $n = 2$ to 5 accumulate in the culture medium. Subsequently, xylooligomers, but not xylose, are utilized.

Among the 10 clones described by Millet et al. (15) as containing *C. thermocellum* cellulose degradation (*cel*) genes, two clones, pCT1200 and pCT1300, were later identified as encoding xylanase activity (data not shown). Although both clones hydrolyze 4-methylumbelliferyl-β-D-cellobioside (MU-β-D-cellobioside), this activity most likely reflects unspecific cleavage of the agluconic bond.

This paper describes the purification and characterization of xylanase Z produced in *Escherichia coli* carrying the pCT1208 plasmid (7). The corresponding enzyme present in the culture supernatant of *C. thermocellum* was identified by Western blotting (immunoblotting) and was found to be associated with high-molecular-weight complexes which bind to cellulose.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The *C. thermocellum* strain was NCIB 10682. Plasmid pCT1208 contains a 2.15-kilobase (kb) *Acc*I-*Cla*I fragment derived from pCT1200 (15), which was inserted into pUC8 (7, 21). *E. coli* TG1 (22) carrying pCT1208 was grown in the presence of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG).

**Purification of xylanase Z.** Unless otherwise stated, all operations were performed at 4°C. One and a half liters of an overnight culture of *E. coli* TG1(pCT1208) in LB medium containing 0.5 mM IPTG and carbenicillin (100 μg/ml) was centrifuged at 5,000 × *g* (maximum) for 20 min. Cells were resuspended in 100 ml of 40 mM Tris hydrochloride buffer (pH 7.5) and disrupted by sonication in a Branson B-12 sonifier. The extract was centrifuged at 8,000 × *g* for 15 min to remove cell debris.

A 10-ml amount of a 100-mg/ml streptomycin sulfate solution in 40 mM Tris hydrochloride (pH 7.5) per g of protein contained in the crude extract was slowly added. After being stirred for 45 min, the precipitate was removed by centrifugation at 8,000 × *g* for 15 min.

The supernatant was heated with gentle stirring in a 60°C water bath. After 10 min, the suspension was chilled in ice, and precipitated proteins were centrifuged at 8,000 × *g* for 15 min. Traces of residual material were removed by filtration through a Whatman GF/D filter.

Powdered ammonium sulfate was added to the heat-treated extract (0.164 g/ml, 30% saturation). After being stirred for 45 min, the precipitate was removed by centrifugation at 8,000 × *g* for 15 min. The enzyme was precipitated by adding 0.181 g of ammonium sulfate per ml of supernatant (60% saturation). After being stirred for 45 min, the suspen-

* Corresponding author.

sion was centrifuged at 8,000 × g for 15 min and the precipitate was dissolved in 5 ml of 40 mM Tris hydrochloride (pH 7.5). The solution was dialyzed three times against 900 ml of the same buffer.

The dialyzed sample was loaded on a column (2.5 by 12 cm) of DEAE-Trisacryl (LKB) previously equilibrated with 40 mM Tris hydrochloride (pH 7.5). The resin was washed with the same buffer until the $A_{280}$ returned to the baseline and then eluted with a 120-ml linear NaCl (0 to 0.5 M) gradient in the same buffer at a flow rate of 20 ml/h. Active fractions were pooled and concentrated by ultrafiltration on an Amicon PM10 membrane.

The enzyme was precipitated by adding 0.6 g of ammonium sulfate per ml of concentrate (90% saturation) and redissolved in 0.3 ml of 100 mM Tris-acetate (pH 7.0). The sample was loaded on a Perkin-Elmer 3B liquid chromatograph equipped with a TSK-G3000SW column (0.75 by 60 cm; Ultropac) and a TSK-SWP precolumn (0.75 by 7.5 cm; Ultropac). The column was eluted with 100 mM Tris-acetate (pH 7.0) with a flow rate of 0.1 ml/min, and 0.7-ml fractions were collected. Active fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10) and pooled.

**Purification of the cellulosome.** *C. thermocellum* was grown anaerobically at 60°C in complete medium (17) containing 10 g of cellulose MN300 (Macherey, Nagel and Co., Düren, F.R.G.) per liter. The cellulosome purification method was adapted from that of Lamed, Bayer, and coworkers (1, 12). Unless otherwise stated, subsequent steps were performed at 4°C under normal aerobic conditions. One liter of culture was cleared by centrifugation at 5,000 × g for 20 min. Traces of contaminating pellet material were removed from the supernatant by filtration through a Whatman GF/D filter. Proteins were precipitated with 2.5 liters of technical ethyl alcohol. After standing overnight, most of the supernatant was siphoned off. The remaining suspension was centrifuged at 8,000 × g for 20 min. The precipitate was resuspended in 34 ml of 20 mM Tris hydrochloride buffer (pH 7.7). After being gently stirred overnight, the solution was centrifuged at 12,000 × g for 20 min, and the supernatant was dialyzed three times against 900 ml of 50 mM Tris hydrochloride (pH 7.5).

The concentrated culture supernatant was diluted with 100 ml of 50 mM Tris hydrochloride (pH 7.5) and 20 g of Avicel (Macherey, Nagel and Co., Duren, F.R.G.) was added. After being stirred for 2 h at room temperature, the suspension was packed into a column (2.5 by 10 cm). The column was washed with 50 mM Tris hydrochloride (pH 7.5) until the $A_{280}$ returned to the baseline and then eluted with 1% triethylamine. The nonadsorbed fractions and the fractions eluted by triethylamine were pooled separately, concentrated by the addition of 2 volumes of acetone, and centrifuged at 12,000 × g for 30 min. The precipitates were resuspended in 15 and 5 ml, respectively, of 50 mM Tris hydrochloride (pH 7.5) and centrifuged at 6,000 × g for 15 min to remove residual insoluble material. The concentrated triethylamine-eluted pool was loaded on a column (1.5 by 80 cm) of Sepharose CL-4B (Pharmacia) and eluted with 50 mM Tris hydrochloride (pH 7.5) at 10 ml/h; 1.5-ml fractions were collected.

**Endoglucanase and xylanase activity.** Endoglucanase and xylanase activities were assayed by incubating the enzyme at 60°C in PC buffer (50 mM $K_2HPO_4$, 12.5 mM citric acid, pH 6.3) containing either 1.5% (wt/vol) carboxymethylcellulose (CMC) (Sigma, medium viscosity) or 0.5% (wt/vol) xylan (larchwood xylan; Sigma). The appearance of reducing sugars was assayed by the Somogyi-Nelson method (16). One unit of endoglucanase or xylanase activity is defined as the amount of enzyme that released 1 μmol of glucose or xylose equivalent, respectively, per min. Protein concentration was determined by the Coomassie blue G-250 binding assay (18) with bovine serum albumin as a standard.

**Other enzyme assays.** Activity towards various aryl-β-glycosides was measured by incubating the enzyme at 60°C in 1.5 ml of PC buffer containing 1.1 mM *para*-nitrophenyl (pNP)-β-D-cellobioside (Sigma), 20 mM pNP-β-D-glucoside (Sigma), 1.2 mM pNP-β-D-xylobioside (gift from M. Claeyssens), or 13 mM pNP-β-D-xyloside (Sigma). The reaction was stopped by adding 0.5 ml of 1 M $Na_2CO_3$, and the release of pNP was measured at 420 nm. One unit of activity is defined as the amount of enzyme liberating 1 μmol of pNP per min.

**Preparation of antiserum.** A 500-μg amount of enzyme purified up to the high-pressure liquid chromatography (HPLC) step was submitted to SDS-PAGE (10) on the whole width of the gel. The enzyme was localized by staining two strips cut at each side of the gel with Coomassie blue R-250. The zone containing xylanase was cut out from the unstained gel and crushed in a mortar with 10 ml of 0.154 M NaCl. Portions containing 40 μg of protein were mixed with an equal volume of Freund complete adjuvant and injected into New Zealand White rabbits. Second and third injections were done with Freund incomplete adjuvant at 3-week intervals. Serum was collected 5 weeks after the third injection.

**Immunological detection of xylanase Z in culture supernatant.** Western blotting was performed as described by Towbin et al. (20) after SDS-PAGE (10). The anti-xylanase Z antiserum was saturated with extracts of TG1(pUC8) or TG1(pCT1208) purified up to the heat precipitation step, as described above. Antiserum (600 μl) was incubated in the presence of 400 μl of TG1(pUC8) extract (1.55 mg of protein per ml) or TG1(pCT1208) extract (1.94 mg of protein per ml) in PBS buffer (137 mM NaCl, 2.68 mM KCl, 8.09 mM $Na_2HPO_4$, 1.47 mM $KH_2PO_4$, 3.08 mM $NaN_3$) for 1 h at 37°C. The precipitate was removed by centrifugation. The saturation was repeated with 100 μl of supernatant and 900 μl of TG1(pUC8) or TG1(pCT1208) extract in PBS buffer for 3 h at 37°C, and the whole mixture was used for Western blotting.

**Analysis of reaction products.** Xylanase Z (0.1 U) was incubated for 16 h at 60°C in 1 ml of PC buffer containing 0.5% xylan or cellobiose, cellotriose, cellotetraose, or cellopentaose (gifts from R. Longin). Xylodextrin-containing samples were deionized by shaking with 100 mg of Bio-Rad RG501-X8 resin for 10 min at 37°C and filtered through a 0.45-μm pore size HV Millipore membrane. Cellodextrin-containing samples were only filtered. Fractions (100 μl) were analyzed in a Perkin-Elmer 3B liquid chromatograph equipped with an HPX-42A column (Bio-Rad Laboratories, Richmond, Calif.) and a microguard column (Cation H; model 125-0129; Bio-Rad) at 60°C. Products were eluted with water at 0.6 ml/min and detected by differential refractometry.

## RESULTS

**Purification of xylanase Z.** The various steps of purification are summarized in Table 1. The TG1(pCT1208) clone chosen for the purification of xylanase Z encodes the same truncated fragment of xylanase Z as pCT1211, with translation initiated at an internal site within the coding sequence (7). Analysis

TABLE 1. Purification of xylanase Z produced by *E. coli* TG1(pCT1208)

| Step | Volume (ml) | Total protein (mg) | Total activity (U) | Sp act on xylan (U/mg) | Yield (%) |
|---|---|---|---|---|---|
| Crude extract | 90 | 675 | 1,008 | 1.5 | 100 |
| Streptomycin sulfate supernatant | 96 | 494 | 969 | 1.9 | 96 |
| Heat-treated extract | 82 | 122 | 653 | 5.3 | 65 |
| Ammonium sulfate precipitate after dialysis | 9.1 | 53.1 | 645 | 12.1 | 64 |
| Pool from DEAE column (concentrated) | 2.6 | 3.70 | 510 | 136 | 50 |
| Pool from HPLC column | 4.2 | 1.47 | 244 | 166 | 24 |

by SDS-PAGE showed two major protein bands of $M_r$ 39,000 and 41,000 (Fig. 1, lane 4). The two bands coincided with two bands of xylanase activity which were detected on a xylan-containing agar replica stained with Congo red (2) (data not shown). Since the 2.15-kb fragment carried by pCT1208 encodes a single polypeptide having a theorical molecular weight of 41,767 (7), the $M_r$-39,000 protein most likely results from proteolysis of the $M_r$-41,000 protein.

The ratios between specific activities on xylan, pNP-β-D-cellobioside, and pNP-β-D-xyloside were constant through all steps of purification (data not shown). Specific activities for the enzyme purified up to the HPLC step were 166 U/mg for xylan, 340 U/mg for pNP-β-D-xylobioside, 7.83 U/mg for pNP-β-D-xyloside, 13.4 U/mg for pNP-β-D-cellobioside, and 0.86 U/mg for pNP-β-D-glucoside. Release of fluorescent 4-methylumbelliferone (MU) was detected with several glycosides, such as MU-β-D-cellobioside, MU-α-L-arabinoside, MU-β-D-xyloside, and MU-β-D-fucoside. The high activity of the enzyme towards pNP-β-D-xylobioside compared with that towards pNP-β-D-xyloside was correlated with the fact that the major final products of xylan degradation were xylobiose (31%) and xylotriose (15%), with only small amounts of xylose (2.5%). Unidentified products eluting faster than xylodextrins (32%) were also present. At shorter incubation times (30 min), xylan degradation products included xylodextrins of degree of polymerization 3 to 7 (data not shown), suggesting that the enzyme is an endo-β-1,4-xylanase.

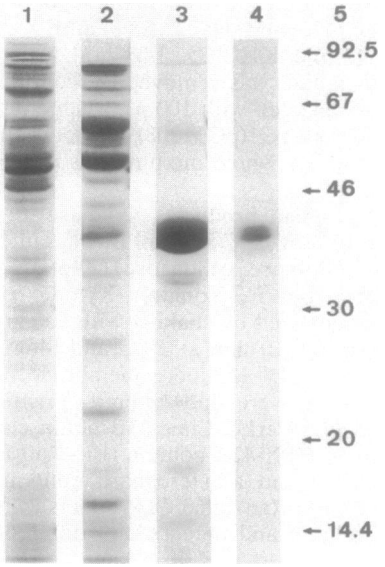


FIG. 1. Electrophoretic monitoring of the major steps of the purification of xylanase Z. Electrophoresis was done in a 10% polyacrylamide gel in the presence of 0.1% SDS (10). Lane 1, Crude extract (75 μg of protein). Lane 2, Ammonium sulfate precipitate (70 μg of protein). Lane 3, DEAE column effluent (36 μg of protein). Lane 4, HPLC column effluent (5 μg of protein). Lane 5, Standard proteins: phosphorylase B, 92.5 kDa; bovine serum albumin, 67 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; lysozyme, 14.4 kDa.

Although xylanase Z purified from TG1(pCT1208) was active on MU-β-D-cellobioside, pNP-β-D-cellobioside, and pNP-β-D-glucoside, the enzyme had no detectable activity on cellobiose, cellotriose, cellotetraose, cellopentaose, or CMC. Activity towards MU-β-D-cellobioside, pNP-β-D-cellobioside, and pNP-β-D-glucoside reflects the nonspecific cleavage of the agluconic bond.

**Identification of xylanase Z in *C. thermocellum*.** In order to determine whether the *xynZ* gene was expressed in *C. thermocellum*, a Western blot was performed with a culture supernatant of *C. thermocellum* grown on cellulose, with an antiserum raised against xylanase Z purified from *E. coli*. A radioactive band of $M_r$ 90,000 (Fig. 2B, lane 2) was detected with immune serum saturated with a crude extract of TG1(pUC8). No band was observed with preimmune serum (data not shown) or with anti-xylanase Z antiserum saturated with a crude extract of TG1(pCT1208) (Fig. 2A, lane 2).

**Localization of xylanase Z in various subfractions of *C. thermocellum* culture medium.** To investigate whether xylanase Z was associated with the cellulosome, the presence of the enzyme was assayed after performing the two steps which were described by Lamed et al. (12) for purification of the cellulosome. The first step of purification is affinity chromatography on cellulose. Table 2 shows the percentages of nonadsorbed and triethylamine-eluted CMCase and xylanase activities. Total recovery of protein and enzymatic activities was about 55%; the rest of the sample remained bound to the column. The percentages of triethylamine-eluted CMCase and xylanase activities were similar. Comparing autoradiograms exposed for 6 and 18 h, Western blotting with anti-xylanase Z antiserum indicated that less than 30% of the antigen went unadsorbed through the column. The triethylamine-eluted fraction was enriched at least 2.5-fold in xylanase Z compared with the nonadsorbed fraction (data not shown).

To verify whether xylanase Z was associated with a high-molecular-weight fraction having a size compatible with that of the cellulosome (2.1 million), the triethylamine-eluted fraction was submitted to gel filtration. The elution profile is shown in Fig. 3A. The major part of the protein was eluted between the void volume and the elution peak of thyroglobulin ($M_r$ 669,000). CMCase activity followed a similar profile (data not shown). SDS-PAGE analysis revealed that the same polypeptides were present in fractions 31 to 48 (Fig. 3C). The positions and relative intensities of the bands

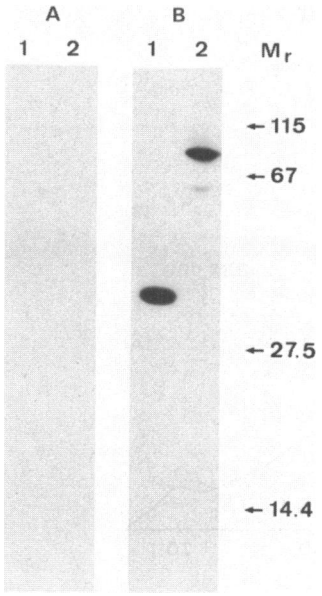


FIG. 2. Detection of the *xynZ* gene product of *C. thermocellum*. Samples were electrophoresed on an SDS gel containing 10% polyacrylamide and analyzed by Western blotting (20). Blots were incubated with anti-xylanase Z antiserum saturated with heat-treated extract of (A) TG1(pCT1208) or (B) TG1(pUC8). Lanes 1; 0.3 μg of xylanase Z purified from *E. coli* up to the DEAE column step. Lanes 2, 20 μl of a concentrated culture supernatant (23 μg of protein) of *C. thermocellum* grown on cellulose. Standard proteins: β-galactosidase, 115 kDa; bovine serum albumin, 67 kDa; adenylate kinase, 27.5 kDa; lysozyme, 14.4 kDa.

resembled the pattern obtained by Lamed et al. (12) for the purified cellulosome. Material eluting after fraction 50 had a molecular weight smaller than 1 million and was depleted in intact S1 subunit. This material may result from partial degradation of the cellulosome. The broad distribution profile of the material eluting from the column reflects the true molecular weight heterogeneity of the sample rather than poor resolution of the column, since molecular weight markers were eluted as peaks with a normal profile (data not shown).

In all fractions, Western blot analysis with anti-xylanase Z antiserum showed the presence of the $M_r$-90,000 immunoreactive polypeptide, which was more abundant in fractions 31 to 42 (Fig. 3B). Thus, xylanase Z coelutes with fractions having the same apparent molecular weight as the cellulosome.

## DISCUSSION

The larger form of xylanase Z purified from *E. coli* TG1(pCT1208) had an $M_r$ of 41,000, in close agreement with the 41,767 predicted from the nucleotide sequence of pCT1208 (7). The sequence of *xynZ* contains a putative internal translation initiation site which is apparently recognized by *E. coli* (7). Translation was probably initiated from this site in *E. coli* TG1(pCT1208), which had lost the original initiation codon of *xynZ*. Xylanase activity was 5-fold higher in TG1(pCT1208) than in TG1(pCT1202) (7) and 30-fold higher than in *E. coli* MC1061(pCT1200) (data not shown), two strains which carry the intact gene cloned in pUC8 and pACYC184, respectively. However, after purification of xylanase Z had been completed, further constructions fusing the 3′ end of *xynZ* in frame with the 5′ end of *lacZ* carried by pUC19 were obtained. Such clones expressed up to 30-fold-higher xylanase activity than TG1(pCT1208) (7) and should certainly facilitate purification of the enzyme.

TABLE 2. Adsorption of a concentrated culture supernatant from *C. thermocellum* to cellulose and triethylamine elution of endoglucanase and xylanase activities

| Portion | Protein, mg (%) | Activity, U (%) | |
|---|---|---|---|
| | | Endoglucanase | Xylanase |
| Loaded | 56.5 (100) | 405 (100) | 195 (100) |
| Nonadsorbed | 22.8 (40.3) | 49.5 (12.2) | 28.5 (14.6) |
| Triethylamine eluted | 8.7 (15.4) | 164.5 (40.6) | 64.0 (32.8) |

In spite of the lack of more than half of the sequence encoded by the *xynZ* gene, the truncated protein purified from TG1(pCT1208) was highly active on xylan, with a specific activity of 166 U/mg. The enzyme also exhibited activity towards a broad range of chromogenic and fluorogenic substrates, such as pNP-β-D-xylobioside, pNP-β-D-xyloside, pNP-β-D-glucoside, pNP-β-D-cellobioside, MU-β-D-cellobioside, and MU-α-L-arabinoside. Thus, cleavage of the heterosidic bond appears to be less specific than cleavage of the holosidic bond, since xylanase Z is inactive towards CMC and cellodextrins.

Immunological detection of xylanase Z in the culture supernatant of *C. thermocellum* revealed a polypeptide of $M_r$ 90,000, which is in close agreement with the 92,159 calculated for the polypeptide encoded by the *xynZ* gene (7). The function of the sequence preceding the C-terminal catalytic domain responsible for xylan hydrolysis is unknown; this region would certainly be large enough to accommodate a second catalytic site.

Our data suggest that xylanase Z is indeed associated with high-molecular-weight fractions which bind to Avicel and have a protein composition resembling that reported for the cellulosome (12). The size heterogeneity of the complexes eluted from cellulose has been reported by previous authors (8, 11–13). Thus, the 2.1-megadalton complex which was termed cellulosome by Lamed and co-workers was obtained after sampling fractions from a polydisperse peak (11, 13). However, as shown in Fig. 3, the polypeptide composition of the fractions eluting from the gel filtration column was remarkably similar over a wide range of apparent molecular weights. Therefore, the broad elution profile from gel filtration columns may be explained by the presence of multimeric forms of a basic motif (4, 14) and by conformational variants showing heterogeneous hydrodynamic properties (11). Electron microscope studies confirm the presence in cellulosome-containing fractions of complexes with a variety of different shapes (12, 14). However, in the absence of well-defined criteria for the purity of the cellulosome, it cannot be ruled out that the high-molecular-weight fractions eluted from cellulose affinity columns may consist of different populations of complexes with distinct protein composition but similar size distribution. Therefore, it is not impossible that xylanase Z is associated with structures which bind to cellulose and have the same size as the cellulosome while being distinct from the complex actually responsible for the degradation of cellulose. Until such hypothetical structures are identified and separated from the cellulosome, a more simple interpretation of our data remains that xylanase Z is part of the cellulosome.

Comparison with the protein bands stained with Coomassie blue indicated that xylanase Z comigrated with the band

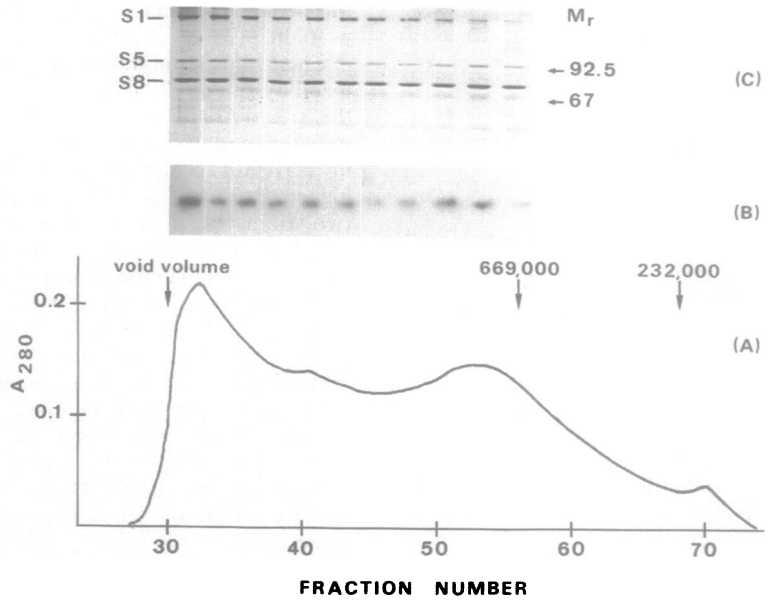


FIG. 3. Chromatography on a Sepharose CL-4B column of the concentrated triethylamine-eluted pool. (A) Elution profile. The elution positions of blue dextran (void volume), thyroglobulin (669 kDa), and catalase (232 kDa) are indicated by arrows. (B) Immunological detection of xylanase Z in fractions 31 to 57. Samples (10 μl) were analyzed by Western blotting (20) after SDS-PAGE (10). (C) SDS-PAGE analysis of fractions 31 to 57. Samples (25 μl) were electrophoresed in a 10% polyacrylamide gel in the presence of 0.1% SDS. Lanes in panels B and C are aligned with the respective fractions in panel A. Three major bands, designated S1, S5, and S8 (12), are indicated on the left. The molecular weight markers phosphorylase B (92.5 kDa) and bovine serum albumin (67 kDa) are indicated on the right.

designated S5 by Lamed et al. (12). The discrepancy between the reported molecular weights of the two proteins (90,000 versus 98,000 [12]) can be fully accounted for by the discrepancy of molecular weights ascribed to the phosphorylase B marker by various suppliers. A molecular weight of 90,000 would be closer to the value expected from the nucleotide sequence of *xynZ* (7). Synthesis of xylanase Z does not require induction by xylan, since mRNA hybridization (not shown) and Western blot analysis of a culture supernatant showed that *xynZ* was expressed when *C. thermocellum* was grown on cellobiose or cellulose without any xylan. Furthermore, xylan utilization by *C. thermocellum* requires a long adaptation period (23). Therefore, the major role of xylanase activity may be to make the cellulose contained in natural compounds accessible to cellulolytic enzymes. In this respect, it would make sense for the cellulosome to include some components responsible for hydrolysis of the hemicellulose fraction which is usually associated with cellulose. This would imply that the cellulosome may be conceived not only as a purely cellulolytic multienzyme complex, but as an integrated, multipurpose machine performing all steps of natural holocellulose degradation. It may be noted that in *Trichoderma reesei* culture supernatant, xylanase has been found to be tightly associated with a complex containing β-glucosidase and cellulase (19).

## ACKNOWLEDGMENTS

We thank G. Séverac for skillful technical assistance in preparing the antiserum, T. Msadek for his help with the manuscript, and J.-P. Aubert for helpful discussions.

This work was supported by grants ATP 955355 from CNRS and EN3B 0082-F from the Commission of European Communities and by research funds from the University of Paris 7.

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