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Note

Separation of cellobiase (E.C. 3.2.1.21) from the crude cellulase system (E.C. 3.2.1.4) of *Trichoderma viride* using affinity chromatography on concanavalin A bound to agarose

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Different methods for carrying out chromatography of cellulases have been described over the last few years (see, *e.g.*, refs. 1–4). The most convenient method for separating all the different types of cellulases produced by the fungus *Trichoderma viride* is that described by Pettersson and co-workers[5,6]. For the separation of one of the enzymes of the cellulase system and for some analytical and kinetic studies, the relatively simple and fast method of partial purification may be advantageous. One such type of purification is affinity chromatography of cellulase on cross-linked cellulose, as described by Weber *et al.*[7]. Endoglucanases were separated from the other types of cellulolytic enzymes by this method. As all the cellulases of *T. viride* except for the cellobiase are glycoproteins, affinity chromatography of the mixture using concanavalin A (Con A)–Sepharose (Con A covalently bound to agarose gel) is able to separate cellobiase from the rest of the system by a simple one-step procedure.

In this paper, we describe a method for partial purification of cellobiase of *T. viride* using one-step affinity chromatography.

EXPERIMENTAL

The enzyme was obtained from *T. viride* B-7 (a mutant of the wild type isolated from soil) cultivated under the usual conditions[8]. The mycelia were filtered off and the cultivation broth was precipitated at 5°C using 2.5 times the volume of isopropanol. For the chromatography the precipitate was dissolved in the starting buffer.

The adsorbent was Con A–Sepharose (Pharmacia, Uppsala, Sweden)]. The cellulolytic activity was determined by the methods of filter paper activity (FPA)[9] and carboxymethylcellulase[10] (CMC-ase) determination. The cellobiase activity was determined from the amount of glucose formed from the cellobiose solution (2%). Glucose was determined using the "Bio-La-Test–glucose" reagent set (Lachema, Brno, Czechoslovakia), a product based on the glucose oxidase–peroxidase system. Chromatography was performed using commercial chromatographic columns (Pharmacia) and the proteins in the effluent were monitored using a UV analyser (Developing Workshops of Czechoslovak Academy of Sciences, Prague, Czechoslovakia).

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RESULTS AND DISCUSSION

A 204-mg sample of a crude cellulase preparation (189.5 mg of protein), having a CMC-ase activity of 7956 U, an FPA activity of 448.8 U and a cellobiase activity of 47.2 U, was dissolved in 5 ml of 0.01 *M* Tris–HCl buffer (pH 7.4). The solution was then applied to a column (30 × 0.9 cm, bed volume 19 ml) equilibrated with the same buffer. The column was washed with the same buffer containing 0.5 *M* aqueous sodium chloride whereupon all the unadsorbed proteins were eluted. After these, the glycoproteins bound to the gel were eluted using a 0.1 *M* borate buffer (pH 6.5). Attempts at eluting these glycoproteins using a solution of α-methylglucoside (0.1 *M* in the starting buffer) were not successful. The results of the cellobiase separation are summarized in Table I. Although a good separation of cellobiase from the mixture was obtained, the cellobiase was contaminated with both the CMC-ase and FPA activity. No cellobiase appeared in the second peak. The first peak containing cellobiase gave, after rechromatography under the same conditions, two peaks, the first of which contained cellobiase activity and the second CMC-ase activity only. The second peak obtained in the first chromatography gave a mixture of FPA and CMC-ase activity after rechromatography.

TABLE I

YIELDS OF CELLOBIASE FROM AFFINITY CHROMATOGRAPHY OF A CRUDE CELLULASE MIXTURE FROM *TRICHODERMA VIRIDE*

Chromatography on concanavalin A bound to agarose gel.

Chromatographic step	*Yield of enzymic activity (%)*			
	*Resolution**	*Peak I (cellobiase)*	*Peak II*	
			CMC-ase	*FPA*
First chromatography	1.41	65.4	67.1	28.2
Rechromatography of peak I	1.28	51.4	29.4	–
Rechromatography of peak II	1.54	–	57.8	–
Total yield		51.4	87.2	–

* The resolution (R_s) was calculated from the relation of the difference between the elution volumes (ΔV_E) of the individual peaks to the sum of the widths of the individual peaks (w_1, w_2): $R_s = \Delta V_E / (w_1 + w_2)$.

The isoelectric point of cellobiase obtained by this method was determined using electrofocusing. The value found was 5.22, which is rather different from the value obtained by Pettersson[5] (5.74) by the same method. This difference could be explained by differences between the two strains of *T. viride* employed.

The method described may be used for the rapid separation of cellobiase from other types of cellulase produced by *T. viride*. To obtain a higher purity of the enzyme, rechromatography is necessary.

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