Short Communication

PURIFICATION AND CRYSTALLIZATION OF INVERTASES FROM *FUSARIUM* *OXYSPORUM*

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Invertases from various sources have been purified and investigated in detail(1). In many cases they are glycoproteins in nature and difficult to obtain in a crystalline form. However, by using a detergent, NEGORO (2) was able to crystallize a saccharase secreted into the medium by a bacillus.

We have attempted to purify the invertase localized in the cell walls of *Fusarium oxysporum* in order to examine the chemical nature of the enzyme and to compare its properties with those obtained from various sources. We succeeded in crystallization of invertase and found that at least two forms of the isoenzyme, both of which were glycoproteins, existed in this fungus.

Fusarium oxysporum IAM 5009 was grown in a medium containing sucrose, KNO₃, and yeast extract as described previously (3). The cells were harvested at the end of the exponential growth phase. Both mycelia and microconidia (wet weight, 4 kg) suspended in cold distilled water were disrupted by a sonic oscillator at 10 kHz for 10 min in the cold. Cell extracts were obtained by centrifugation at 7,000×*g* for 20 min to remove cell debris and unbroken cells, 500 ml of 5% tannic acid solution adjusted to pH 6.6 was added to 6.75 l of the cell extract, and the mixture was kept for a few hours in an ice bath. The precipitate formed was collected by centrifugation at 7,000×*g* for 20 min and washed with cold acetone exclusively until color of the precipitate disappeared. The precipitate was dissolved in 700 ml of distilled water and 1% dodecyldimethylbenzylammonium chloride (DN-CI) was added to the maximum concentration which did not inactivate invertase activity (90 ml in this experimental condition). The precipitate thereby formed was discarded by centrifugation at 7,000×*g* for 20 min.

Cold acetone (−20°) was added to the supernatant to the final concentration of 70% and the solution was kept in a refrigerator. The precipitate was collected by centrifugation at 10,000×*g* for 20 min, dissolved in 100 ml of distilled water,

and the solution was dialyzed against 0.01 M phosphate buffer (pH 7.4). The dialyzate was loaded onto DEAE-cellulose column (5×30 cm) which was equilibrated with 0.02 M phosphate buffer (pH 7.4). Linear gradient elution was carried out with 0.02 M (500 ml) to 0.2 M (500 ml) phosphate buffer (pH 7.4), and fractions of 20 ml each were collected. Two peaks of invertase activity were observed; peak 1 (P-1) enzyme was eluted by 0.07–0.08 M phosphate buffer and peak 2 (P-2) enzyme by 0.14 M phosphate buffer. Both fractions were dialyzed against distilled water overnight at 4°, and P-1 and P-2 were concentrated to 25 ml and 40 ml, respectively, by a rotary evaporator at 30°. P-1 and P-2 were rechromatographed twice as described above (column, 2×30 cm; gradient buffer, 0.02 M, 300 ml to 0.2 M, 300 ml; fraction, 10 ml). The elution profiles of protein and activity were identical at this stage and both fractions were lyophilized and dissolved in 10 ml of distilled water. The enzyme solutions were centrifuged at 7,000×*g* for 20 min. Cold acetone was added dropwise into the supernatant solutions. When the solutions became cloudy, it was kept in a refrigerator overnight. Crystallization of the invertase was observed in both fractions. Typical purification procedures are shown in Table 1 and a photograph of crystalline P-2 enzyme is shown in Fig. 1.

Table 1. Purification of invertase of *Fusarium oxysporum*.

Fraction	Total protein (mg)	Total activity (μmol glucose/hr)	Specific activity (μmol glucose/hr/mg protein)	Yield (%)
Crude extract	2.26×10^5	4.33×10^6	19.2	100
Extract of tannic acid precipitate	1.51×10^4	1.69×10^6	112	39
Before DEAE-cellulose column chromatography	3,086	1.08×10^6	350	25
Third chromatography				
Peak 1	22.2	4.24×10^4	1,910	0.98
Peak 2	36.4	9.09×10^4	2,500	2.1
Crystalline product				
Peak 1			3,400	
Peak 2			4,020

Fig. 1. Crystals of P-2 invertase (×270).

A preliminary chemical analysis of the enzymes showed that both types of the enzymes were glycoproteins. They contained carbohydrates, the amount of carbohydrates in P-1 and P-2 enzymes differing and varying slightly from preparation to preparation. Therefore, we need further study to describe precisely the chemical components of *Fusarium* invertase isoenzymes.

Invertase of *Fusarium* was proved to be localized in the cell-wall fraction (3). Therefore, the enzymic carbohydrates are expected to be involved in the localization or secretion of the enzyme. It is interesting to note that P-1 enzyme was present in mycelia and P-2 in microconidia and the third invertase was secreted into the medium of an old culture which showed the same elution pattern from DEAE-cellulose column chromatograph as P-2.

This fact is intriguing and could be a useful system to study the relationship between differentiation and gene expression. The experiment on the relationship between growth phase and production of invertase isoenzymes and their properties will be published in a subsequent paper.

REFERENCES

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