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SHORT COMMUNICATION

MULTIPLE FORMS OF INVERTASE OF POTATO TUBER STORED AT LOW TEMPERATURE

T. SASAKI, K. TADOKORO and S. SUZUKI

Food Research Institute, Ministry of Agriculture and Forestry, Tokyo, Japan

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Abstract—Five types of invertase in cold treated potato tuber are separable by DEAE-cellulose column chromatography. Invertase I and II are different from each other on several grounds (pH, foaming, heat stability and K_m).

INTRODUCTION

THE PRESENCE of multiple forms of invertase (β-fructofuranosidase) has been demonstrated in Baker's yeast,[1] *Fusarium oxysporum*[2] and *Neurospora crassa*[3]. In higher plants, however, the multiplicity of this enzyme has not yet been reported.

The experiment reported here shows the presence of five types of invertase in potato tuber stored at 4°. Separation of these different types of the enzyme was carried on DEAE-cellulose column, and some properties of the main two types were examined.

RESULTS AND DISCUSSION

The invertase activity in tubers stored at 4° for three weeks was increased approximately 4-fold to that of tubers at 20°. Sugars were formed in cold treated tubers about ten times more than in the control, and the amounts of glucose and fructose are higher than that of sucrose and their ratio is about 1:1 (Table 1). This indicates that sucrose formed at low temperature was hydrolysed by invertase. These phenomena in cold treated tuber have long been known,[4,5] but it is not certain whether increasing invertase acivity depends on induction or activation of a latent enzyme.

Invertase activity in tubers given cold treatment was separated using DEAE-cellulose column. As shown in Fig. 1, five types of invertase were obtained, peaks I and II showing remarkably high enzyme activity. The whole activity of enzyme could not be recovered completely from the column, suggesting the possible retention of some other invertase on the column. After elution of the major peaks (I–IV) the DEAE-cellulose was chopped up and the invertase activity in each fragment was assayed. Invertase activity was found only on the top of the column (invertase V). It is interesting that three types of invertase are present in yeast and one of them remains absorbed on cellulose.[1] The elution pattern of enzyme obtained from tuber stored at 20° showed similar patterns with regard to peaks I, II and V, though their activities were lower than those cold treated tuber. Peaks III and IV were not detected in significant amounts. It is known that invertase activity in potato tuber is increased by 'foaming' treatment of invertase solution.[5] In our experiments the maximum

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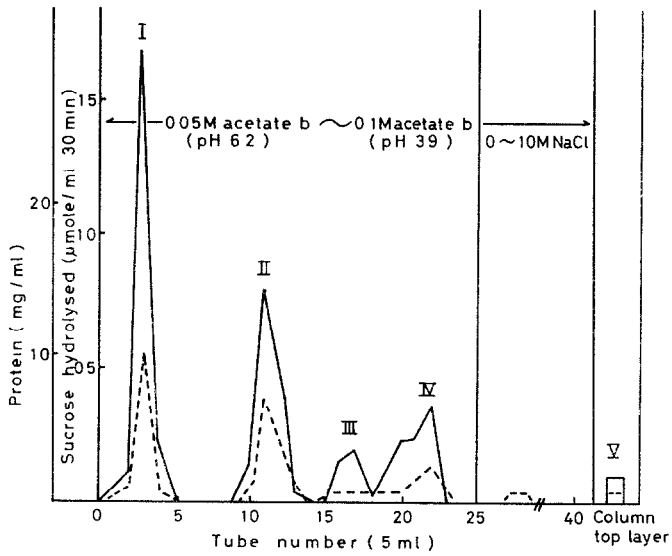

FIG. 1. DEAE-CELLULOSE COLUMN CHROMATOGRAPHIC PATTERN OF INVERTASE

activity was obtained by 10 min foaming treatment of the extract (Fig. 2). The foamed extract gave the same five types of invertase as the control by the fractionation. Activity of invertases I, III and IV were unchanged, but the activity of invertase II and V were increased about 1·5 and 20 times respectively by the treatment (Fig. 3).

Invertase I and II was not separated by CMC- and Sephadex chromatography. The activities of purified invertase I and II as a function of pH are shown in Fig. 4. The optimum pH of invertase I was 4·5, while that of invertase II was at a broad pH range 3·0–4·5. Heat stability of invertase I and II was determined after heating at 50°. Invertase II was found to be more stable (Fig. 5). Michaelis constants of invertase I and II were determined from the Lineweaver–Burk plot using sucrose as a substrate. The value obtained for K_m were 23 mM for invertase I and 12 mM for invertase II. The activity of foaming treatment invertase I was not changed, while that of invertase II increased approximately 35% by control. Although a release of inhibitor by foaming treatment has been reported by Pressey *et al.*,[5] the result mentioned above suggests that the increase of invertase II activity by foaming treatment is based on an unfolding of the enzyme molecule.

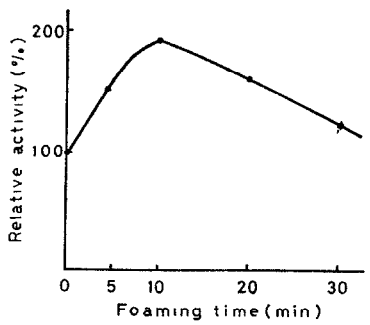

FIG. 2. EFFECT OF FOAMING ON THE ACTIVITY OF INVERTASE.

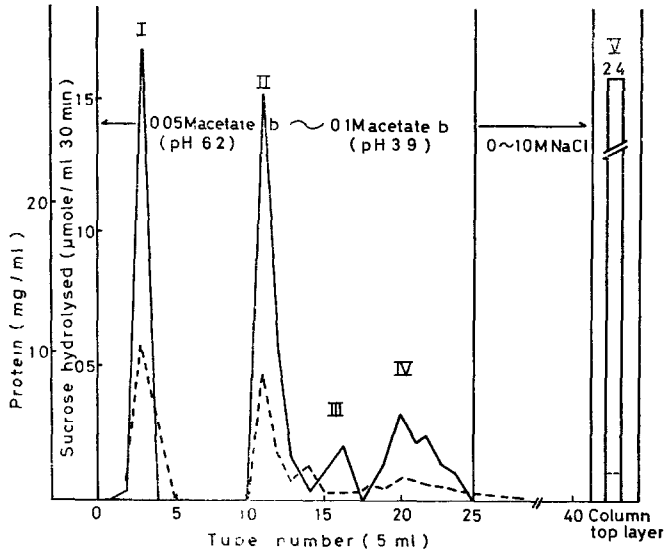

FIG. 3. DEAE-CELLULOSE COLUMN CHROMATOGRAPHIC PATTERN OF FOAMED INVERTASE.

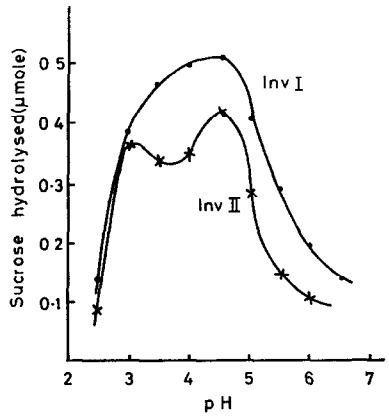

FIG. 4. EFFECT OF pH ON THE ACTIVITY OF INVERTASE I AND II.

TABLE 1. CHANGE OF INVERTASE ACTIVITY AND SUGAR FORMATION IN POTATO TUBER STORED AT 4° AND 20° FOR 3 WEEKS

	4°	20°
	Relative activity (%)	
Invertase	100	26
	Fresh weight (%)	
Total sugar*	2·36	0·22
Sucrose	0·32	0·08
Glucose	1·10	0·14
Fructose	0·97	0·01

* Sugar in tuber was extracted with ethanol method and separated by paper chromatography (Acetate: Ethyl acetate: H_2O=3:3:1). Total sugar and the sugars were analysed by Anthrone method.

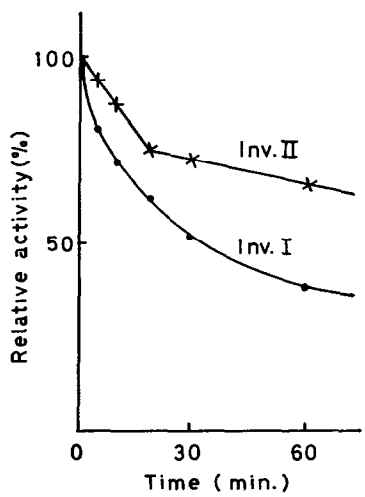

FIG. 5. HEAT STABILITY OF INVERTASE AND H AT 50°.

EXPERIMENTAL

Preparation of Enzyme

The potato tubers (var. Danshaku) were obtained at local market and placed at 20°. After sprouting, the tubers were stored at 4° and 20° for three weeks. Enzyme extracts were prepared according to the procedure described by Pressey,[5] the tubers were washed, peeled and squeezed by a juicer. After adding 5 ml of 1·0 M Na_2SO_4 to 100 ml of the juice, the homogenate was centrifuged for 30 min at 28,000 *g* and the supernatant was fractionated at 0·8 saturation of ammonium sulfate. The resulting precipitate was collected and dialysed overnight against 0·05 M sodium acetate buffer (pH 6·2) containing 0·01 M Na_2SO_4.

DEAE-cellulose Column

The fractionation of invertase was carried out according to the procedure described by Hashimoto[1] with slight modification. A column (1·5 × 30 cm) of DEAE-cellulose was used for the chromatographic separation of invertase. The enzyme extract (5 ml) was charged on the column previously equilibrated with 0·05 M sodium acetate buffer (pH 6·2). The invertase was eluted by a linear gradient of changing molarity and pH with 0·05 M acetate buffer, pH 6·2 and 0 1 M acetate buffer, pH 3 9, then a linear gradient of 0–1·0 M NaCl.

Enzyme Activity

Invertase activity was analysed according to the procedure described by Pressey[5] with slight modification. An aliquot of the enzyme solution was incubated, in a final volume of 1·0 ml, with 0·5 ml of 0·2 M sodium acetate buffer (pH 4·7) and 100 μmoles of sucrose at 37° for 30 min The reactions were terminated by addition of 1 ml of 0·5 M Na_2HPO_4 and heating at 100° for 2 min. The solution (0·5 ml) was then analysed for reducing sugars by heating with 0·5 ml of copper reagent in a boiling water bath for 10 min. The solution was cooled and 0·5 ml of arsenomolybdate reagent was added. Invertase activity was expressed as μmoles of surcrose hydrolysed per mg protein 30 min under the conditions of the assay Protein content was estimated according to the biuret method, using bovine serum albumin as reference standards. Foaming treatment of enzyme solution was carried on according to the procedure described by Pressey[5] with slight modification.