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STUDIES ON INVERTASE FROM *MANGIFERA INDICA*

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Abstract—There are two types of invertase in mango tissues, one active at 0° and the other at 37°. These invertases have been partially purified and some of their properties studied.

INTERESTING observations have been reported about the enzyme invertase from a number of sources.[1–8] The presence of multiple forms of invertase was demonstrated in baker's yeast by Hoshino *et al.*,[1] in *Neurospora crassa* by Bates and Woodward[2] and in *Fusarium oxysporum* by Maruyama *et al.*[3] Pressey reported double pH optima of invertase from potato tissues.[4] Presence of acid and neutral or alkaline invertases was shown in sugarcane[5] and carrot root.[6] Recently Sasaki *et al.*[7] reported the presence of five types of invertases when potato tubers were stored at 4°. Earlier we[8] reported the presence of invertase activity in mango which showed a double temperature optima, one at 0° and the other at 37°. Investigations were therefore carried out to study further the characteristics of this enzyme preparation and the results obtained are now reported.

To study the mango enzymes in detail, it was necessary to achieve a partial purification. The crude extract was precipitated with ammonium sulphate (60% saturation) and the supernatant examined chromatographically. Using Sephadex G(75–40) column chromatography, three peaks of invertase were obtained. Fraction 6 had activity of invertase at 0° and also at 37°; fractions 11 and 12 showed enzymic activity only at 37°; whereas fraction 15 showed activity only at 0° (Fig. 1). Invertase active at 0° was purified 333-fold whereas 68-fold purification was achieved of the enzyme active at 37° (Table 1).

Using DEAEcellulose column chromatography 37°-invertase was eluted in the first 3–7 fractions. No 0°-invertase was detected in these fractions. However, fractions 45 and 53 (eluted with buffered 0·2 and 0·3 M NaCl respectively) showed some 0°-invertase activity (Table 2). It seems that invertase which is active at 0°- is not eluted easily from the anionic exchanger DEAE-cellulose. Hoshino *et al.*[1] have also reported that the third form of invertase they detected in baker's yeast was retained on DEAE-cellulose. It was also reported by Sasaki *et al.*[7] that some of the forms of invertase of potato stored at 4° could be eluted

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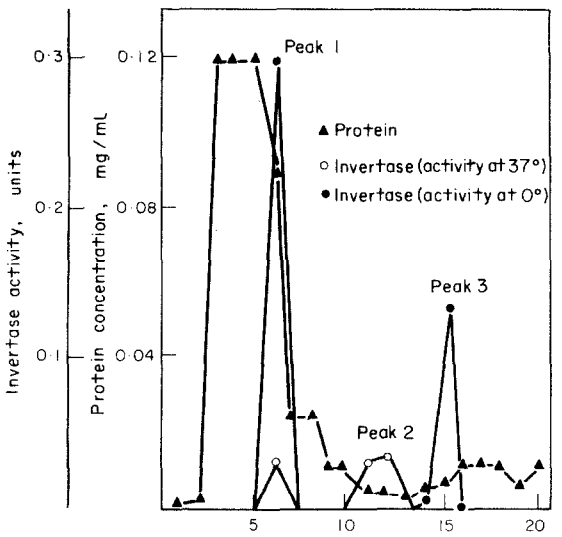

FIG. 1. ELUTION PROFILE ON SEPHADEX G(75–40) OF INVERTASE FROM *Mangifera indica* TISSUES.

TABLE 1. INVERTASE PURIFICATION BY SEPHADEX G(75–40)

Fraction	0		37	
	Invertase (sp. act.)*	Fold purification	Invertase (sp. act.)*	Fold purification
Crude†	0·10	1	0·13	1
$(NH_4)_2SO_4$ (0–60) Supernatant	0·60	6	0·48	3·7
Sephadex G(75–40)				
Fraction No. 6	3·3	33	0·30	2·3
11	—	—	8·9	68·4
12	—	—	8·4	64·6
15	33·3	333	—	—

– Not detected.
* Specific activity is mg hexose liberated/hr/mg protein.
† Protein 1·6 mg/ml, total protein 144·00 mg.

from DEAE-cellulose. To elute the enzyme active at 0°, the DEAE-cellulose gel was removed from the column and ground in 0·1 M phosphate buffer (containing 0·2 M NaCl), pH 7·0 and was applied to Sephadex G (75–40) column after centrifugation, concentration and dialysis. Invertase, active at 0° was eluted in the first 4 fractions (Table 2). None of these fractions had enzyme activity at 37°.

These results indicate that there are two separable forms of invertase, one showing activity at 0° and the other at 37°. This could be due to either the occurrence of isozymes of invertase or the dissociation of the enzyme into subunits, some of which were active at 0° and others at 37°. Gel electrophoresis of invertase at pH 8·3 and 5·0 showed only one band. However, when the crude enzyme was incubated with sodium dodecylsulphate and 2-mercapto-ethanol,4 bands of invertase were obtained. It seems therefore that there

are at least 4 subunits of invertase. Metzenberg[9] has also shown in *Neurospora* two forms of invertase, heavy and light forms.

Incubation at 0° perhaps brings about changes in the enzyme so that it is adapted and shows activity at 0°. It was shown by Grant and Alburn that freezing can accelerate a number of catalyzed reactions including hydrolysis, aminolysis, dehydration, oxidation and peroxide decomposition.[10]

TABLE 2. PURIFICATION OF INVERTASE FROM *Mangifera indica* TISSUES

Fractions	0° Invertase (sp. act.)*	0° Fold purification	37° Invertase (sp. act.)*	37° Fold purification
I Crude (Sup.)†	0·3		0·5	
II $(0–60)(NH_4)_2SO_4$ (Sup)	1·48	4·9	0·8	1·6
III DEAE-cellulose Fraction No. 3	—	—	12·6	25·2
5	—	—	5	10·0
6	—	—	2	4·0
7	—	—	1·1	2·2
45	1·72	5·7	—	—
53	1·40	3·6	—	—
IV Sephadex G(75–40) Fraction No. 1	18	60	—	—
2	3·9	13	—	—
3	0·73	2·4	—	—
4	0·28	1	—	—

—Not detected.
* Specific activity is mg hexose liberated/hr/mg protein.
† Protein 1·84 mg/ml, total protein 165·6 mg.

Both invertases differ from each other in heat stability and K_m value. 0°-invertase shows a K_m of 5×10^{-3} M whereas 37°-invertase has K_m $0{\cdot}9 \times 10^{-3}$ M. Invertase active at 37° is comparatively more stable at 50° than 0°-invertase. Studies on the subunits of invertase are in progress.

EXPERIMENTAL

Alfanso mangoes (*Mangifera indica*) used for this study were obtained from the local market. The method employed for the preparation of cell-free extracts and assay of invertase were essentially same as described earlier,[8] except for the determination of invertase activity was used: the reaction mixture in a total volume of 1 ml, contained, in μmol: acetate buffer (pH 5·0) 10 and sucrose 10. The enzyme reaction was terminated by the method of Pressey and the hexose liberated was estimated quantitatively by the arsenomolybdate method.[8]

Purification of invertase. The purification of invertase by DEAE-cellulose was according to the method of Sasaki *et al.*[7] The column was previously equilibrated with 0·05 M sodium acetate buffer(pH 6·2). The fractions of 10 ml each were eluted with increasing concentrations of NaCl 0·01–0·3 M in buffer. For the purification of invertase with Sephadex G (75–40), column was equilibrated with 0·01 M phosphate buffer of pH 7·0. The fractions of 10 ml each were eluted with 0·01 M phosphate buffer(pH 7).

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Polyacrylamide gel electrophoresis of invertase. The method used for polyacrylamide gel electrophoresis and invertase staining was essentially the same as described by Dahlqvist and Brun.[11] For SDS electrophoresis, the enzyme was preincubated with SDS (0·1%) and 2-mercaptoethanol for 15 min at 0–5° and the enzyme preparation then applied to the gels.

Protein was estimated by the method of Lowry *et al.*[12]

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