Note

Isoforms of Invertase in Grape Berries

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Plant invertases (EC 3.2.1.26) are classified as acid and alkaline invertases on the basis of their pH optima. They are also classified as soluble and bound invertases on the basis of their intracellular localization. Recently, invertases were separated by DEAE-cellulose chromatography in some plant tissues, such as oat seedlings,¹⁾ carnation petals,²⁾ sugar beets³⁾ and tomato fruit.⁴⁾

It was reported that grape berries (*Vitis vinifera* cv. Ohanez) had soluble and bound invertases.⁵⁾ When we tried to extract both enzymes, we could get in the soluble form more than 99% of the whole activity. We found that it was separated into four isoforms by DEAE-cellulose

chromatography and ConA-Sepharose column chromatography.

Invertase activity was assayed by the methods of Nakagawa *et al.*⁴⁾ One unit of invertase activity was defined as the amount of enzyme that liberated one μmol of fructose from sucrose per min. Protein was measured by the methods of Lowry *et al.*⁶⁾ and absorbancy at 280 nm, using BSA as a standard protein.

Ripe grape berries (*Vitis vinifera* cv. Delaware) were harvested in the experimental farm of the faculty of horticulture, Chiba University, and stored at -20°C . Peeled frozen berries (880 g) were homogenized with 18 ml of 0.1 M EDTA (pH 7.0) and 440 ml of 0.1 M Na_2CO_3 by a Waring blender and were stirred for 30 min. The homogenate was centrifuged at $9,500 \times g$ for 20 min. The supernatant was adjusted to pH 5.5 and was stirred for 15 min. After centrifugation at $9,500 \times g$ for 20 min, the supernatant was brought to 0.1 saturation with solid $(\text{NH}_4)_2\text{SO}_4$ and the mixture was centrifuged. After removal of the precipitate by centrifugation, solid $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to 0.8 saturation. The resulting precipitate was collected by centrifugation, dissolved, and dialyzed against 20 mM sodium phosphate buffer (pH 6.5) containing 1 mM EDTA (Buffer A). The dialyzed solution (73.5 ml) was put on a column of DEAE-cellulose (2×26.5 cm) previously equilibrated with buffer A, and fractions of 4 ml were collected. The elution

Fig. 1. Elution Profile of Invertase from Grape Berries on DEAE-Cellulose Ion-exchange Chromatography.

Open circles, protein concentration (mg per ml); closed circles, activity (units per ml). The column (2×26.5 cm) was washed with 2 volumes of the buffer A and then eluted by to a 500 ml linear gradient of 0~0.25 M NaCl in buffer A. Four-ml fractions were collected.

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Fig. 2a, b. Elution Profile of Invertase I on ConA-Sepharose Affinity Chromatography.

Marks are the same as Fig. 1. A Con A-Sepharose column (1×12 cm) previously equilibrated with buffer B. The column was washed with 2 volumes of the buffer B and then the enzymes were eluted with 50 ml linear gradient of 0~10% (Fig. 2a) or 0~15% (Fig. 2b) α -methyl-D-glucoside in buffer B. Fractions of 1 ml were collected.

profile of invertase from DEAE-cellulose chromatography (Fig. 1) revealed the presence of two peaks, one eluting with 170 mM NaCl (invertase I) and the other eluting with 225 mM NaCl (invertase II). Each peaks containing invertase activity was dialyzed overnight against buffer B (20 mM sodium phosphate containing 0.5 M NaCl, pH 6.5) and put on a column of ConA-Sepharose (1×12 cm) previously equilibrated with buffer B. Fractions of 1 ml were collected. The elution pattern of invertase I shown in Fig. 2a reveal the presence of two forms of the enzyme, one eluting with 4% α -methylglucoside (invertase Ia) and the other eluting with 8% α -methylglucoside (invertase Ib). The elution profile of invertase II in Fig. 2b also revealed the presence of two forms of the enzyme, one eluting with 2.5% α -methylglucoside (invertase IIa) and the other eluting with 5% α -methylglucoside (invertase IIb).

The pH optima for invertase Ia, Ib, IIa, and IIb were 5.0, 4.5, 4.5, and 3.5, respectively. The pH optima of the four invertase isoforms were a little different. At acidic pH, the activity of invertase Ia was higher than activities of the other invertases.

The four invertase isoforms hydrolyzed sucrose, raffinose, and stachyose but not melibiose and melezitose. These findings suggest that these enzymes are β -fructofuranosidases. Several reagents known to inhibit plant invertase were tested against invertase isoforms. Mercuric

chloride (0.2 mM) completely abolished the four invertase activities. Potassium iodide (0.4 mM) did not inhibit invertase Ia, IIa, or IIb but slightly inhibited invertase Ib. Pyridoxine (0.95 mM) and pyridoxal (0.95 mM) inhibited the four enzymes.

Thiol reagents such as pCMB, NEM (N-ethylmaleimide), and MIA (monoiodo acetic acid) inhibited four enzymes, suggesting that the enzymes have sulfhydryl group(s) in their catalytic sites. When the enzymes inactivated by pCMB were treated with 30 mM 2-mercaptoethanol, their activity were almost completely restored.

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