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Auxin-regulated Invertase Activity in Strawberry Fruits

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Abstract. **Extracts of strawberry (*Fragaria ×ananassa* Duch.) receptacles contain a soluble invertase and a bound invertase which can be extracted with 1 M NaCl. Both soluble and salt-extracted invertases had a pH optimum of 4.6. The soluble invertase was more sensitive than salt-extracted invertase to inhibition by I_2 and $HgCl_2$. The soluble invertase activity was high at anthesis and decreased markedly during receptacle growth. In contrast, salt-extracted invertase activity increased 80% to 100% during receptacle growth induced either by pollination or by the application of NAA. The possible role of salt-extracted invertase in establishing sink activity during auxin-induced strawberry receptacle growth is discussed. Chemical names used. 1-naphthaleneacetic acid (NAA).**

Studies have been performed using etiolated grass coleoptiles and stems of dicotyledonous seedlings to understand the regulation of plant growth by auxin. Very little is known, however, about the mechanism by which auxin regulates fruit growth. Nitsch (12) demonstrated that achenes on the surface controlled strawberry receptacle development and that auxin could be substituted for the achenes. Subsequent analyses of the receptacles and achenes for auxin content by bioassay (13) and HPLC, using fluorimetric and isotope dilution methods (2, 4), support the idea that achenes serve as the source of auxin for the receptacles. Achenes can be removed with little injury to the receptacle, making the system useful for studying the effect of exogenous auxin with little or no influence of endogenous auxin (10). An auxin binding protein of strawberry receptacle membrane has been studied to understand its possible role as an auxin receptor (11). This paper reports the effects of pollination and auxin treatment on the regulation of the level of invertase, an important enzyme in sucrose metabolism of plants.

Auxin-induced cell enlargement during receptacle growth requires extensive cell wall synthesis. The increased requirement for sugars to support the growth processes, such as the synthesis of cell wall, makes the receptacle a strong sink for sugars. Invertase activity present in the free space has been shown to play an important role in the import of sucrose in sink tissues. In sugar cane stalk, the movement of sucrose from the phloem

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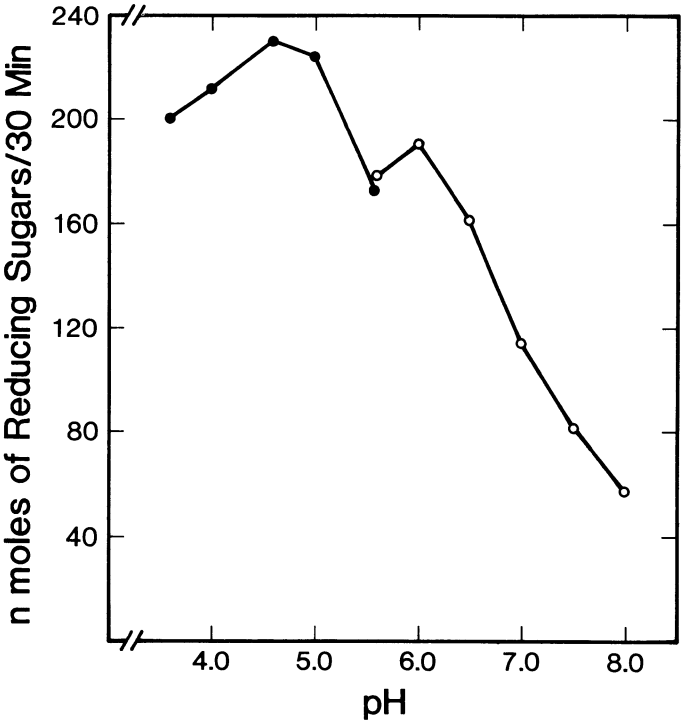

Fig. 1. Effect of pH on soluble invertase activity from strawberry receptacles. Acetate buffer was used for the pH range between 3.5 and 5.5 and Mes-NaOH buffer was used for the pH range between 5.5 and 8.0. Soluble protein fraction from the receptacles of 10-day-old fruit was used.

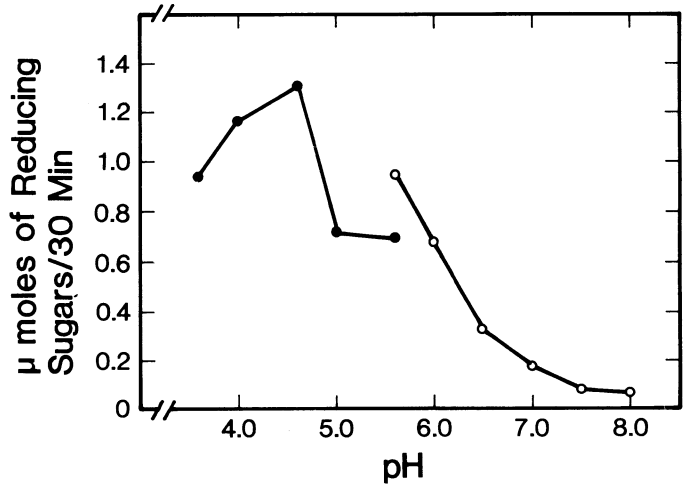

Fig. 2. Effect of pH on salt-extracted invertase activity from strawberry receptacles. Acetate buffer was used for the pH range between 3.5 and 5.5 and Mes-NaOH buffer was used for the pH range between 5.5 and 8.0. Salt-extractable protein fraction from the receptacles of 9-day-old fruit was used.

to the storage cells of the stalk is accompanied by hydrolysis of sucrose by an invertase in the free space, and the hexoses subsequently are used for the synthesis of sucrose in the symplast (1, 5, 6). Therefore, the presence of wall-bound invertase activity in a tissue offers an advantage in the utilization of sucrose from the phloem (3). Accordingly, tissues growing in response to hormones such as GA_3 (8), auxin (15), and cytokinin (7) exhibited higher levels of invertase activity.

Table 1. Effects of inhibitors on the soluble and the salt-extracted invertases from strawberry receptacles. Soluble proteins and salt-extractable proteins were obtained from the receptacles of 10-day fruits.

Inhibitor	Concentration (μM)	Inhibition (%) Soluble invertase	Inhibition (%) Salt-Extracted invertase
Pyridoxine	2500	29	32
$HgCl_2$	100	76	70
$HgCl_2$	2	74	0
$AgNO_3$	100	0	0
$AgNO_3$	20	0	0
I_2	100	49	0
I_2	4	0	0

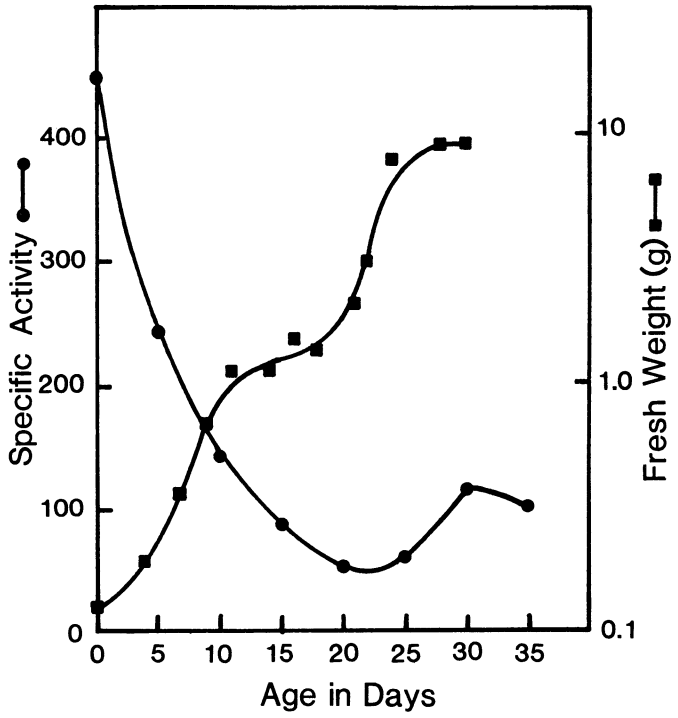

Fig. 3. Changes in soluble invertase activity at different stages of strawberry fruit development. Specific activity of invertase is expressed as μmol reducing sugar released per mg protein at 30°C for 30 min. Fresh weights of receptacles are means of 15–20 replicates and are plotted on a log scale.

Breakdown of sucrose is catalyzed by invertase and sucrose synthetase in higher plants. Invertases are known to exist in multiple forms (1). A bound invertase present in the free space and active at acidic pH range usually is associated with hydrolysis of sucrose in the free space, whereas a soluble invertase active at neutral or alkaline pH range is associated with the hydrolysis of sucrose in cytoplasm. Invertase is an important enzyme in the regulation of plant growth, and its activity is influenced by stimuli such as hormones (7, 8, 15). A naturally occurring proteinaceous inhibitor of invertase has been purified from potato tubers and has been implicated in the regulation of invertase activity (14).

In this investigation, the soluble and bound forms of invertase in the strawberry receptacle were studied in order to understand the mechanism by which auxin could regulate the sink activity in a growing fruit.

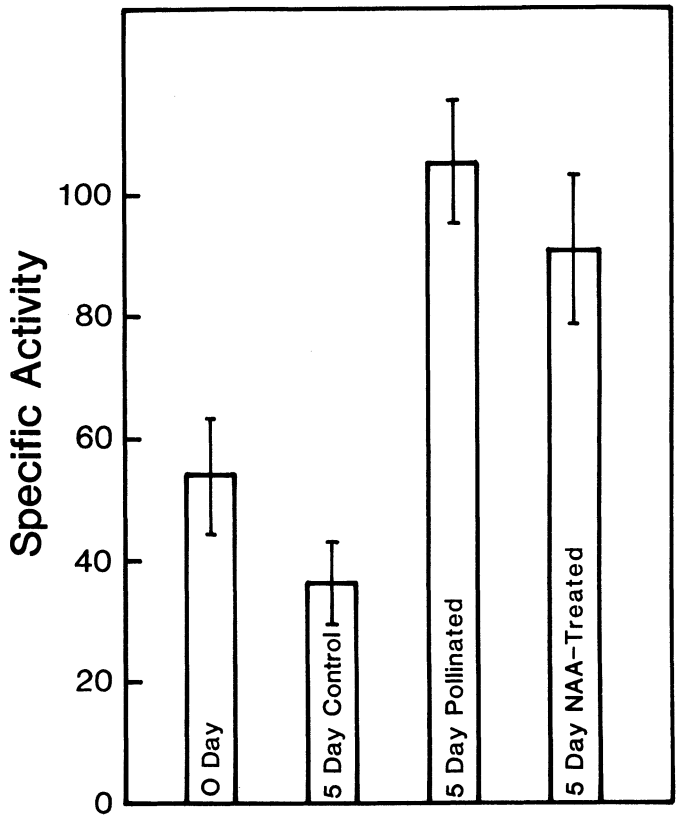

Fig. 4. Changes in salt-extracted invertase activity in receptacles during fruit set in strawberry. Flower receptacles were harvested before fertilization (0 day, 120 mg/receptacle); 5 days after flower opening but not pollinated (5-day control, 122 mg/receptacle); 5 days after pollination (5-day pollinated, 260 mg/receptacle) and NAA-induced fruit set (5-day NAA-treated, 440 mg/receptacle). Four replicate batches of receptacles were used for each treatment. Bars indicate SD.

Materials and Methods

Everbearing strawberries 'Ozark Beauty' were grown in a greenhouse under 16 h/d photoperiod in 15 cm pots with a mixture of 1 peat : 1 perlite : 1 soil (by volume). Fully opened flowers were hand pollinated, and the age of the fruit was determined starting from the day of pollination. Achenes on chilled receptacles were removed with a flat spatula, and receptacles were used for extracting enzymes. Receptacle development in the absence of pollination was induced by emasculating the flowers one day before flower opening and by applying a few drops of a solution of 1 mM NAA in 1% dimethylsulfoxide (v/v) and 0.1% Tween-20 (v/v). The receptacles were covered with aluminum foil to prevent cross pollination.

The receptacles were homogenized in a mortar with pestle in a homogenizing buffer (6 ml/gfw) containing 50 mM 2-[N-morpholino]ethanesulfonic acid (Mes)-NaOH, pH 7.0, 1 mM EDTA, 0.1 mM $MgCl_2$ and 0.5% 2-mercaptoethanol (v/v). All operations were performed at 4°C. During homogenization of each 1 g receptacle, 1 g sand, and 0.4 g prewetted insoluble polyvinylpolypyrrolidone were added. The homogenate was centrifuged at 8000 × g for 10 min. The supernatant was centrifuged at 130,000 × g for 10 min and the resulting supernatant designated as the soluble protein fraction. The pellet after 8000 × g centrifugation was washed twice by suspending in the homogenizing buffer and centrifuging at 8000 × g. The final pellet was suspended in the homogenizing buffer containing 1 M NaCl and agitated in a wrist action shaker at 4°C for 12 hr. The suspension was centrifuged at 8000 × g, and the supernatant is referred to as the salt-extracted protein fraction. The soluble and salt-extracted protein fractions were dialyzed against 10 mM acetate buffer, pH 4.6.

Invertase activity was measured using 20 mM sucrose as the substrate, and the assay was performed at 30°C in 10 mM acetate buffer, pH 4.6 for 30 min. Reaction was stopped by leaving the tubes in a boiling water bath for 5 min. Reducing sugars formed were estimated by the method of Somogyi (16). Under these assay conditions, a linear increase in enzyme activity was obtained with increasing amounts of the extract. Proteins were estimated by the method of Lowry et al. (9). Specific activity of invertase is expressed as μmoles of reducing sugars released per mg protein at 30° for 30 min.

Results and Discussion

Plant invertases have been classified as acid or alkaline invertases on the basis of their pH optima. The activity of the soluble and salt-extracted invertases at various pH values was studied. Acetate buffer was used for 3.5 to 5.5 pH range and Mes-NaOH buffer was used for the 5.5 to 8.0 pH range. Both invertases showed maximum activity at pH 4.6 (Fig. 1 and 2). Since both invertases exhibited the same pH optimum, their sensitivity to different inhibitors was investigated to resolve any possible difference between them. Inhibitors were added to the reaction mixture at desired concentrations before the addition of enzyme. Pyridoxine inhibited the 2 invertases similarly (Table 1), and $AgNO_3$ inhibited neither. Iodine at 100 μM concentration inhibited soluble invertase 49% without inhibiting the salt-extracted invertase. $HgCl_2$ at 100 μM concentration inhibited both invertases similarly but, at 2 μM concentration, inhibited the soluble form less than the salt-extracted form. Varying effects of these inhibitors on the 2 invertases indicate that the soluble invertase is different from the salt-extracted invertase, although both exhibit the same pH optimum. Soluble invertases investigated in other plant systems have been shown to be active at neutral or alkaline pH range (1). The significance of the acidic pH optimum for the strawberry soluble invertase is not clear.

Soluble invertase activity was high at anthesis and decreased rapidly during the growth of the fruit (Fig. 3). At the time of ripening, there was a small increase in its activity. Strawberry receptacles exhibited linear increase in fresh weight for up to 10 days after pollination (Fig. 3). During this period of rapid growth, there was a decline in the soluble invertase activity suggesting a lack of correlation between receptacle growth and increase in soluble invertase.

The role of auxin in the regulation of salt-extracted invertase levels was studied during fruit set. Fully opened flowers were harvested, and receptacles were used for measuring initial invertase activity. Receptacles also were obtained from the 5-day-old fruit induced by pollination or NAA treatment. A set of control flowers was left for this period without pollination or NAA application. After pollination, receptacles of 5-day-old fruit exhibited a 2-fold increase in fresh weight and about 80% higher salt-extracted invertase activity over the 0-day receptacles (Fig. 4). Similarly, the NAA-treated receptacles showed a 3.5-fold increase in fresh weight, and the salt-extracted invertase activity was 65% higher. If NAA was not applied, receptacles of unpollinated flowers failed to grow, and salt-extracted invertase activity remained low. Therefore, an increase in salt-

extracted invertase activity was associated with auxin-induced receptacle growth.

Arnold (3) suggested that the presence of invertase activity in the cell wall of a growing tissue could favor the utilization of sucrose which is the major translocating sugar in plants. Bound invertase activity has often been associated with the cell wall (1). The salt-extracted invertase of strawberry also is probably bound to the cell wall. The increase in the activity of this bound invertase during auxin-induced receptacle growth suggests that auxin-regulated increase in invertase activity could play an important role in establishing a strong sink activity in the growing strawberry receptacles. The concomitant decrease in the soluble invertase activity following fruit set also suggests that these two enzyme activities may be regulated differently by auxin.

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