INVERTASE & INVERTASE INHIBITOR IN POTATO [1]

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The pattern of changes in sugar composition which occur when potatoes are stored at low temperatures or are irradiated (18, 19) suggests that the reducing sugars may arise as the consequence of the hydrolytic action of invertase on sucrose. During cold temperature storage there is a rapid increase in sucrose content followed by an increase in reducing sugars relative to the sucrose content. Furthermore a survey of the literature indicates that, for many varieties of potatoes, the ratio of glucose to fructose is close to unity during at least part of the time of storage (17). If invertase is indeed a factor in the accumulation of reducing sugars, information concerning the characteristics of this enzyme and the inhibitor here reported could conceivably lead to means for controlling the reducing sugar content of potatoes (20).

The literature on potato invertase is rather sparse. In 1903, Kastle and Clark (9) detected invertase in sprouted but not in unsprouted potatoes. In 1928, McGuire and Falk (13) showed that the invertase action of potato juice was higher at pH 4 than at pH 5 and 6. Denny et al (5) found that the invertase activity of potato juice from tubers treated with ethylene chlorohydrin was significantly greater than that from untreated controls. A few reports have suggested that the level of invertase activity depends on the elemental nutrition of the plant [potassium (24), boron (4), chlorine & sulfur (10)]. Bois and Savary (3) and McCready (12) in more recent years have detected invertase in potatoes, although the latter author could find activity only in sprouted and not in unsprouted tubers.

The present paper describes our experience in attempting to detect invertase in crude potato macerates. In general, the results confirm the scattered findings of the older literature but also point to the presence of an endogenous inhibitor of the enzyme. A preliminary report of some of the present results has been published elsewhere (21).

MATERIALS & METHODS

SOURCE OF TUBERS. Four different lots of tubers were used in this investigation. Lot A consisted of freshly harvested (2–3 weeks) tubers of the White Rose variety. Lot B, the same variety, had been stored at 4° C for about one year and had not sprouted. Lot C consisted of tubers from Lot B which were removed from cold storage after 1 year and allowed to remain at room temperature in a humid atmosphere (about 90 % R.H.) for 18 days prior to assay for enzyme activity. Sprouts 1 to 2 inches long had developed. Lot D consisted of Russet Burbank tubers which were assayed about 4 to 6 weeks after harvest.

PREPARATION OF EXTRACT. Five to ten tubers were hand-peeled and diced. Three hundred grams of the dice were mechanically blended with 20 to 30 g of ice and 50 mg of ascorbic acid in a Waring Blendor for 5 minutes. The slurry was passed through cheese cloth and the resultant extract filtered through Celite. The clear filtrate was used for the determination of invertase activity.

INVERTASE DETERMINATION. The invertase reaction mixture contained varying amounts of the above filtrate, buffer, (0.05–0.2 M) water and sucrose (0.065 M final concentration) in a total volume of 7 ml. This concentration of sucrose is quite adequate to saturate the enzyme. Sucrose, when present at a final concentration of 0.040, 0.050, 0.065, 0.080 or 0.140 M, saturated the potato invertase. The same maximum rate of inversion was obtained with each concentration of sucrose. The K_M of crude, dialyzed potato invertase prepared from cold stored potatoes was found to be 0.003 M for sucrose at pH 6.2. The estimated K_M for leaf invertase has been reported as 0.004 M (25). Zero-time controls and controls containing no added sucrose were run, along with the experimental digests at 30° C in the presence of toluene. After the designated time intervals, including a time close to 24 hours, the action of the enzyme was terminated by addition to the reaction mixture, with mixing, of 150 mg of air-dried cation exchange resin [Amberlite IR–120, (H)] plus 2 ml ice water. After 10 minutes at 0° C, 500 mg of anion exchange resin were added [Amberlite IR–4 (OH)]. After 2 hours at 0° C the sample was diluted to 100 ml and filtered through Whatman no. 2 filter paper. This method halts the reaction by creation of a controlled acidic environment (pH 2) at low temperature and precipitates protein in a form which renders the latter easily removable. A satisfactory replication of assays was obtained. The results with the controls demonstrated that enzyme activity was minimal after addition of the

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TABLE I

SUMMARY OF INVERTASE ACTIVITY OF POTATO TUBERS

POTATO LOT*	BUFFER	pH	CONC OF POTATO EXTRACT**	INVERTASE ACTIVITY***
A	None	5.8	1.0	0
B	"	6.2	1.0	3
"	Acetate	4.9	1.0	4
C	Phosphate	4.4	0.13	26
"	"	5.3	0.13	22
"	"	6.3	0.13	17
"	Acetate	3.9	0.13	21
"	"	4.8	0.13	17
"	"	5.8	0.13	16
D	Phosphate	5.7	0.84	3.5
"	"	5.7	0.21	8.7
"	"	5.7	0.09	16.0
"	"	5.0	(0.0)	(26)†

* See text for description of potato lots.
** Expressed as grams of original potato tuber in the reaction mixture.
*** Expressed as mg of sucrose hydrolyzed per 24 hours per g of tuber tissue.
† Calculated from figure 1 and equation I (see text).

ion exchange resins. This ion exchange method eliminated a component of the potato extracts which reacted with copper reagent (used in the sugar analysis) to give rise to a garlic-like odor.

For the determination of sugar, 1 ml of the deionized and diluted enzyme digest was added to 1 ml of a copper-sugar reagent (23). After heating for 20 minutes in a boiling water bath the mixture was cooled and 1 ml of arsenomolybdate color reagent added (14). The optical density of the resulting solution was measured with an Evelyn colorimeter (520 mμ filter). Reducing sugar was estimated from a glucose standardization curve. Invertase activities are expressed as mg of sucrose hydrolyzed per 24 hours per gram of potato tissue.

RESULTS & DISCUSSION

Some typical results are summarized in table I. No invertase under the conditions of the present procedure was detected in freshly harvested White Rose tubers (lot A). An apparently significant invertase activity was detected in this same variety after long term cold storage (lot B). When allowed to remain at room temperature until sprouting started (lot C) considerable activity was present. This activity was about five times that found in the unsprouted tubers.

The results obtained with lot D, consisting of room-stored Russet Burbank potatoes, suggest that the difference between sprouted and unsprouted tubers may not be due entirely to a difference in actual invertase content of the tubers but may reflect the influence of the presence of a naturally occurring inhibitor of invertase activity in non-sprouted tubers. Data obtained with lot D using varying concentrations of potato extract shows that the apparent activity per unit weight of tuber increases with decreasing amount of extract added to the enzyme reaction mixture. Thus, the low values obtained with lot B might have been considerably higher if less than the equivalent of 1 g of tuber per 7 ml of reaction mixture had been used.

Deviation from linearity between enzyme concentration and rate of enzyme action may be interpreted in two ways: A, at higher concentrations of the enzyme the reaction is terminated at the later stage of the reaction where the rate progressively decreases due in part to inhibition by product and in part due to depletion of substrate, so that the overall measured rate per unit of enzyme added appears to be small; B, there is present in the enzyme preparation an inhibitor whose inhibitory effect decreases faster with dilution of the enzyme than does the enzyme activity (8). Situation A is unlikely because the maximum per cent sucrose hydrolyzed in the experiments described here did not amount to more than 7 %. To test the validity of situation B the following equation may be applied:[3]

$$E/V_I = 1/k_1 + cE \qquad I$$

where V_I is the observed enzyme activity in the presence of enzyme concentration E, and k_1 and c are constants. By plotting E/V_I against E, one obtains a straight line if situation B is valid. This straight line will have the intercept $1/k_1$ at E = O, where k is the specific activity in the absence of the inhibitor. A test of the validity of these equations for the invertase activity in crude potato extract is shown in figure 1. It will be noted that the calculated specific activity in the absence of the hypothetical endogenous inhibitor is eight times that in the presence of 0.84 g

of potato tissue in the reaction mixture.

Table II compares data on the magnitude of invertase activity of potato tubers as found in the present studies and in the literature with the invertase activity of other plant sources. On the average, potato tuber invertase activity runs about one order of magnitude lower than that of green leaf tissue. It is vanishingly small as compared with a rich invertase source such as yeast. The metabolic role of invertase in plant tissue, as manifested by its hydrolytic properties, is now a matter of interesting conjecture as the result of investigations of the labeling patterns of sugars obtained from plant organs infiltrated with radioactive sugars (11, 16, 22). Increase in activity upon storage, upon the initiation of sprouting and upon application of sprout inhibitors, as well as the presence of an endogenous inhibitor, suggest the potato tuber to be an excellent system for future studies on the elucidation of the metabolic role of invertase in plant tissue.

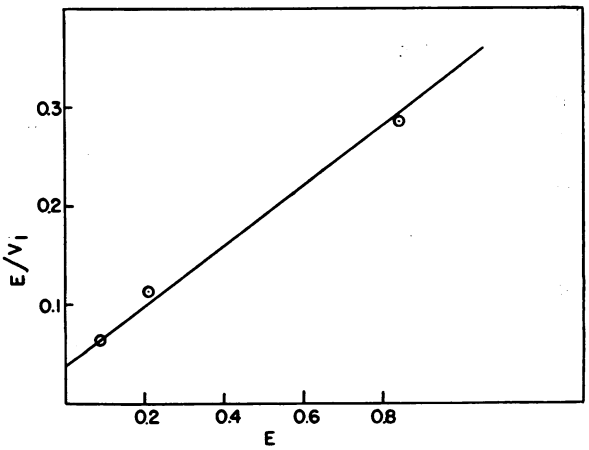

FIG. 1. Demonstration of the presence of an inhibitor of invertase activity in extracts of Russet Burbank potato tubers. E is expressed as g of tuber tissue in the enzyme reaction mixture, and E/V_I as reciprocal of enzyme activity (mg of sucrose hydrolyzed per 24 hr. per g of potato) At E = O, the ordinate is $E/V_0 = 1/k_1$ (see footnote [3]).

[3] The following is a simple derivation for the endogenous inhibition equation: For non-competitive inhibition (7):

$$\frac{V_0}{V_I} = 1 + \frac{I}{K_I} \qquad \text{II}$$

where V_0 = the velocity of enzyme action in the absence of inhibitor, V_I = the velocity in the presence of inhibitor at concentration I, and K_I the equilibrium constant of the inhibitor enzyme complex. Both V_0 and I are proportional to the total amount of enzyme present (E); $V_0 = k_1E$ and $I = k_2E$; k_1 and k_2 are proportionality constants. Substituting these values into equation II:

$$\frac{k_1E}{V_I} = 1 + \frac{k_2E}{K_I} \qquad \text{III}$$

$$\frac{E}{V_I} = \frac{1}{k_1} + cE$$

where $c = \frac{k_2}{k_1K_I}$

When E/V_I is plotted against E, a straight line is obtained and the intercept at E = O is $1/k_1$. The equation for competitive inhibition leads to an equation of the same form.

SUMMARY

The apparent invertase activity of crude extracts of potato tubers has been investigated as a function of post-harvest history, pH, and enzyme concentra-

TABLE II

INVERTASE ACTIVITY OF POTATO TUBERS AS COMPARED TO OTHER PLANT TISSUES

SOURCE	TEMP. 0° C	pH	INVERTASE ACTIVITY*	REFERENCES**
Potato tubers	30	4–6	0–38	Present results
" "	37	4–8	1–8	McGuire (13)
" "	25	6	0–60	McCready (12)
" "	20	...	60	Bois (3)
Potato leaves	38	...	500	Doby (6)
Barley leaves	30	...	320	Archbold (1)
Wheat leaves (seedling)	...	...	750	Ward (25)
Tobacco leaves	25	...	600	Nijholt (15)
Oak leaves	...	6	750	Blagoveschenski (2)
Apple leaves	25	6	300	"
Yeast	25	4.8	34,000	Weidenhagen (26)

* Estimated on the basis of mg of sucrose hydrolyzed 24 hour per gram of fresh tissue.
** Only name of first author given, followed by reference number in Literature Cited.

tion. The invertase activity was found to be about one order of magnitude lower than that recorded in the literature for leaves of potato and other green leaf tissue. The results corroborated previous reports that the apparent activity increases upon sprouting. Preliminary evidence for the presence of an endogenous inhibitor of invertase activity based upon kinetic considerations is presented as well as a simple derivation of the endogenous inhibition equation.

Note. Mention of commercial products throughout this report does not constitute recommendation by the Department of Agriculture.

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