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Short Communication

Development of Soluble and Insoluble Invertase Activity in Washed Storage Tissue Slices

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Recently Palmer (7) published some observations on the development of invertase activity in washed beetroot disks, but made no reference to previous work with this tissue. The appearance of invertase in washed disks of storage tissue was first observed with beet (1). Further, the desirability of using aseptic conditions has been stressed by Bacon et al. (2). The absence of any effect of added penicillin and streptomycin, which was taken by Palmer as evidence that contaminating micro-organisms were not contributing to the metabolic changes he observed must be viewed in the light of the observations of Leaver and Edelman (5) that these antibiotics were of little use, permitting the growth of 5×10^6 organisms/g fresh weight in carrot disks washed for 24 hours.

Palmer's measurements of invertase activity rest on the assumption (shown to be incorrect by the observations reported below) that the enzyme is located only in the cell wall. As far as can be made out his assays were made at 25° and pH 6.5, although the pH optimum of this invertase lies in the range 4.5 to 5.0 (see below). Even when allowance is made for these differences, the activities he reports (0.1 mg sucrose hydrolysed/g fr wt hr) are of a different order of magnitude from those previously reported [e.g. Bacon et al. (2) give 3.0 mg/g fr wt hr].

It is the purpose of this communication to show that any study of invertase development in washed storage tissue must take account of the existence of a soluble as well as an insoluble (cell wall) invertase.

Storage tissue disks of carrot (*Daucus carota* L.), potato (*Solanum tuberosum* L.) and red beet (*Beta vulgaris* L.) were cut and aged (washed) aseptically. Invertase activity was measured by incubating 10 disks in 10 ml of 0.1 M citrate-phosphate buffer, pH 5.0 containing 5% sucrose, at 35° for 1 hour and estimating the reducing sugars formed with a Technicon autoanalyser. The pH optimum for invertase activity in each of the 3 tissues studied was found to be between 4.0 and 5.0 (fig 1) and there was no evidence of the existence of an alkaline invertase. The disks were then subjected to ethyl acetate treatment and their invertase activity again determined. With each tissue, aging in water resulted in an increase in invertase activity during the first 3 days (table I). Ethyl acetate treatment prior to assay further increased the invertase activity, but the effect was more marked for beet and potato than for carrot. Ethyl acetate does not act simply by preventing the uptake of reducing sugars from the assay medium by the disks, since only 0.25 mg glucose

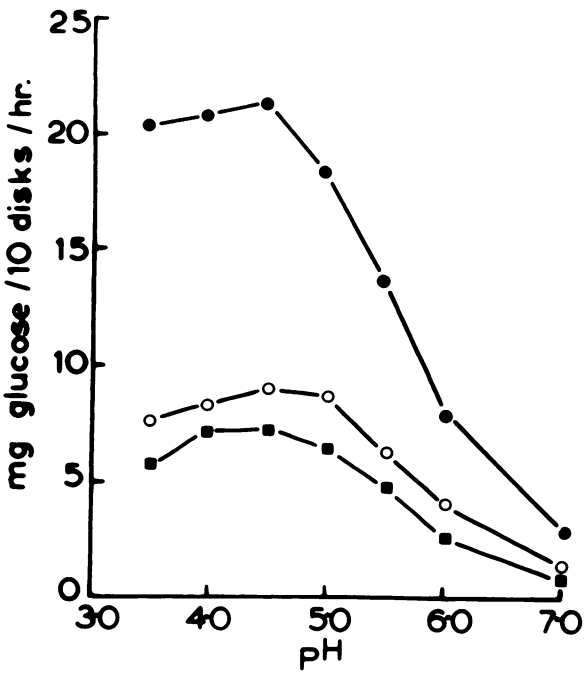

FIG. 1. Effect of pH on the invertase activity of disks of carrot (— ● —), potato (— ■ —) and beet (— ○ —) aged for 3 days in water. Reaction mixture contained 10 disks in 10 ml of 0.1 M citrate-phosphate buffer. Glucose formed was measured after incubation for 1 hour at 35°.

Table I. *Development and Location of Invertase Activity in Washed Storage Tissue Slices*
Results are the mean values of 4 separate determinations.

Tissue	Location of invertase	Day of aging 0	1	2	3	4
		mg Glucose formed per g fr wt tissue per hr				
Carrot	Whole disks	0.6	18.4	23.8	24.3	20.2
	Ethyl acetate treated disks	0.6	21.3	26.3	26.8	22.8
	Cell walls (F1)	0.4	18.6	22.7	23.9	19.5
	Supernatant (F2)	0.2	2.0	2.6	3.3	2.1
	Combined fractions (F1+F2)	0.6	20.6	25.3	27.2	21.6
Potato	Whole disks	0.3	4.1	4.8	5.7	4.4
	Ethyl acetate treated disks	0.5	6.4	9.1	9.9	8.7
	Cell walls (F1)	0.3	4.5	5.2	5.9	4.5
	Supernatant (F2)	0	1.4	3.3	4.5	4.4
	Combined fractions (F1+F2)	0.3	5.9	8.5	10.4	8.9
Beet	Whole disks	0.4	4.2	9.4	11.9	11.6
	Ethyl acetate treated disks	0.4	6.2	18.6	26.2	25.3
	Cell walls (F1)	0.3	4.4	9.1	12.3	12.1
	Supernatant (F2)	0	1.3	8.7	12.9	12.5
	Combined fractions (F1+F2)	0.3	5.7	17.8	25.2	24.6

was taken up by 10 disks in 1 hour from 10 ml of 0.05 % glucose.

To investigate further the effect of ethyl acetate 10 disks were homogenized in 15 ml of 0.1 M citrate-phosphate buffer, pH 5.0 and separated into a cell wall and supernatant fraction by centrifugation at 1000 × *g*. The cell wall invertase was assayed by incubating the cell wall preparation with 10 ml of 5 % sucrose in citrate-phosphate buffer, pH 5.0 at 35°. Samples (1.0 ml) were removed at 0, 30, 60 and 120 minutes, centrifuged at 1000 × *g* for 2 minutes, and the reducing sugar content of the solution was estimated using a Technicon autoanalyser. The soluble invertase was assayed by adding 0.75 g of sucrose to the supernatant fraction and incubating at 35°. Samples were taken as described for the cell wall fraction, but the reaction was stopped by the addition of 1 drop of 0.1 % $HgCl_2$ solution and the samples were not centrifuged prior to measuring their reducing sugar contents.

It was found that invertase activity developed in both the cell wall and supernatant fractions and both invertases, irrespective of the tissue, had a similar pH optimum between 4.0 and 5.0. The results show that the invertase activity of intact aged disks is approximately equal to the activity of the cell wall fraction, while the sum of the cell wall and supernatant invertase activities is almost identical with the activity of aged disks pre-treated with ethyl acetate (table I). The effect of ethyl acetate, therefore, is probably to destroy the cell membranes [a purpose for which it has been used in Baker's yeast (4, 6)] and so render the supernatant invertase within the cell accessible to the sucrose of the incubation medium. That ethyl acetate only slightly affects the assay of invertase activity in carrot disks can thus be explained in terms of a lesser development of the supernatant invertase in this tissue. However in potato and beet the supernatant invertase eventually develops to about the same extent as in the cell wall, although in both tissues there is only a minimal development of the supernatant invertase by the end of the first day.

In addition to invertase activity Palmer also measured O_2 uptake and Pi uptake as indicators of the effect of the growth regulating substances on the metabolism of the tissue. Since there was no evidence that the disks were free from micro-organisms the use of such a dilute phosphate solution (3 μM) leaves his conclusions open to question (3). Further, the rates of O_2 uptake in the presence of high concentrations of growth substances were substantially lower than those for fresh disks, suggesting that these concentrations were toxic.

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