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SEASONAL CHANGES IN THE INVERTASE AND HYDROLASE ACTIVITIES OF JERUSALEM ARTICHOKE TUBERS

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Abstract—Soluble protein was extracted from Jerusalem artichoke tubers during a complete season, from tuber formation through dormancy to sprouting, and its invertase and hydrolase activities measured. Invertase activity increased markedly during tuber formation, fell to a very low value during dormancy and increased during sprouting. The increase in hydrolase activity which occurred during tuberization continued for some weeks during dormancy and thereafter decreased continuously even when the tubers had sprouted. Whereas the changes in hydrolase activity may be accounted for in terms of fructosan metabolism, the precise significance of the invertase change is uncertain.

INTRODUCTION

ALTHOUGH Jerusalem artichoke tubers have been extensively used in studies on the mode of action of plant growth regulators, work on seasonal changes in the chemistry and biochemistry of the tubers has been largely restricted to examination of changes in the stored carbohydrates. Sometimes this has involved a detailed examination of changes in the individual sugars during part of the season such as dormancy[1] or growth[2] and sometimes it has been less detailed but has covered a complete season from tuber formation through dormancy to sprouting.[3]

The researches of Edelman and his school have shown the presence in Jerusalem artichoke tubers of the enzymes transfructosylase,[4] invertase[5] and hydrolase,[5] all involved in sugar transformations. However, no detailed information is available on any seasonal changes in the amount and activity of these enzymes which could account for the carbohydrate changes observed.

The work described here is a study of the changes which occur during a complete cycle—from tuber formation to dormancy through to sprouting—in the two enzymes invertase and hydrolase, both of which would be expected to be associated with the carbohydrate changes which have been found to occur in Jerusalem artichoke tubers.

RESULTS

The percentage dry matter in the Jerusalem artichoke tubers remained at approximately 16 per cent throughout each season and all results are based on the unit of 1 mg dry wt. of tissue.

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[2] T. G. JEFFORD and J. EDELMAN, *J. Exptl. Botany* **12**, 177 (1961).
[3] J. S. D. BACON and R. LOXLEY, *Biochem. J.* **51**, 208 (1952).
[4] J. EDELMAN and A. G. DICKERSON, *Biochem. J.* **98**, 787 (1966).
[5] J. EDELMAN and T. G. JEFFORD, *Biochem. J.* **93**, 148 (1964).

Chromatography on DEAE cellulose[6] resolved the total soluble extracted protein into a number of fractions, some of which possessed hydrolase activity and only one showed invertase activity. Since the activities of both the single invertase fraction and the sum of the activities of the hydrolase fractions were very similar to the values obtained with unfractionated protein, averages of the activities for unfractionated and fractionated protein are presented in Figs. 1 and 2. Very similar changes were found in both the invertase and hydrolase activities of the soluble protein extracted from the Jerusalem artichoke tubers during the two successive seasons (Figs. 1 and 2).

Figure 1 shows that there was a considerable increase in invertase activity during the initial stages of tuber formation in September and October. In the later stages of tuberization, invertase activity fell until it reached a very low value during dormancy. There was very

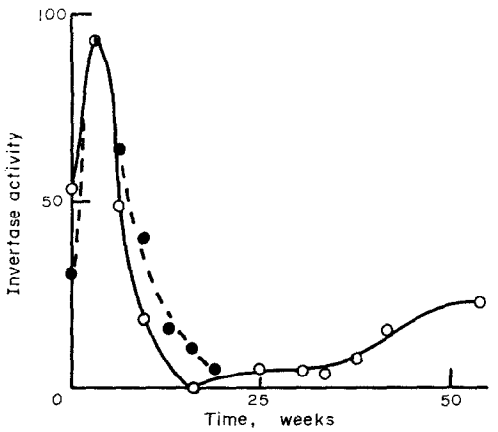

FIG. 1. SEASONAL VARIATION IN THE INVERTASE ACTIVITY OF THE SOLUBLE PROTEIN EXTRACTED FROM JERUSALEM ARTICHOKE TUBERS.
(○)1967–8; (●) 1968–9. 1 unit of invertase activity represents the liberation at 25° of 2 μmoles hexose/min/mg initial dry wt. × 10^6.

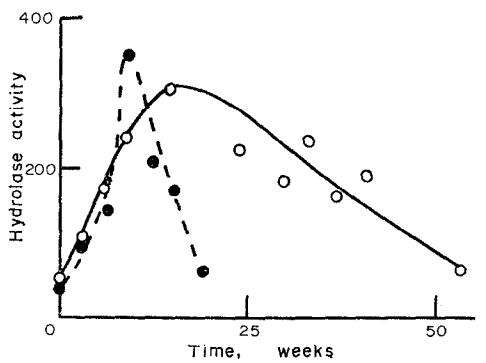

FIG. 2. SEASONAL VARIATION IN THE HYDROLASE ACTIVITY OF THE SOLUBLE PROTEIN EXTRACTED FROM JERUSALEM ARTICHOKE TUBERS.
(○) 1967–8; (●)1968–9. 1 unit of hydrolase activity represents the liberation at 25° of 1 μmole hexose/min/mg of initial dry wt. × 10^6.

[6] A. E. FLOOD, P. P. RUTHERFORD and E. W. WESTON, *Phytochem*, **9**, 2431 (1970).

little change until after the tubers sprouted in the following spring when invertase activity again increased. Hydrolase activity (Fig. 2) also increased rapidly during tuber formation, but this increase continued during the initial stages of cold storage and then it gradually decreased.

The total protein content of each extract and the protein content of fractions possessing either invertase or hydrolase activity are shown in the Table. 'Inactive protein' refers to protein having neither invertase nor hydrolase activity. Although there was some variation in the amounts of the various proteins, there appears to be no major seasonal change.

DISCUSSION

In spite of some scatter, the variation in the amounts of total soluble protein extracted from the Jerusalem artichoke tubers could not account for the changes in either invertase or hydrolase activities observed throughout the whole season. Even though fractionation did not effect complete purification of the two enzymes, variations in the amounts of protein possessing either invertase or hydrolase activity could hardly account for the changes which were measured in the activities of these enzymes.

Although some workers have been unable to detect any invertase activity during the late summer and early autumn,[7] our measurements show that there is a very large increase in invertase activity during September and October at a time when inulin synthesis is most active.

As the rate of inulin synthesis decreased so too did the invertase activity and by December, shortly after the tubers became dormant, very little invertase activity was detectable. The invertase activity remained at a negligible value throughout the dormant period during which time little change in the sugars has been shown to take place.[1] Once sprouting was observed during the following spring and early summer, invertase activity began to increase.

It is possible to relate these changes in invertase activity to fructosan metabolism in the tuber but the work of Edelman and his colleagues[8] would suggest that invertase is not significant at any stage of fructosan metabolism. The seasonal changes in activity recorded here may therefore be confined to more general aspects of carbohydrate metabolism, e.g. the availability of sucrose for polymer synthesis.

Hydrolase activity also increased considerably during tuber formation in September and October, and continued to increase into the initial stages of dormancy. Then it began to decrease, continuing even after the tubers had started to sprout in May.

Unlike the invertases, the function of hydrolases in fructosan metabolism is well recognized[8] and the seasonal changes recorded here accord with the conversion of inulin to oligosaccharides particularly during cold storage.

EXPERIMENTAL

Biological Material

Jerusalem artichoke tubers were obtained from plants grown from two original tubers. Between September and November 1967, up to 6 tubers from each clone were lifted at suitable intervals and the protein extracted. Then, towards the end of November, all the remaining tubers were lifted and stored in moist peat at $3\pm1°$. This procedure was repeated in 1968. In the 1967/68 season, a single tuber from each clone was removed from the cold store at intervals of about 3 weeks and analyzed. The sampling was continued for 9 months, thus including a period of dormancy (November to April) and of sprouting (May to September). In the 1968/69 season, sampling was continued for 2 months only after lifting.

Dry weights were determined by oven drying at 100°.

[7] J. Edelman, *Bull. Soc. Chim. Biol.* **42**, 1737 (1960).
[8] J. Edelman and T. G. Jefford, *New Phytologist* **67**, 517 (1968).

TABLE 1. SOLUBLE PROTEIN EXTRACTED FROM JERUSALEM ARTICHOKE TUBERS THROUGHOUT THE YEAR

Season	Date sampled	Time in weeks from September 19	Protein*			
			Total	Invertase	Hydrolase	Inactive†
I	19 September 1967‡	0	—	—	—	—
	10 October 1967	3·0	10·3	5·2	5·0	0·1
	31 October 1967	6·0	16·4	6·3	5·9	4·2
	21 November 1967§	9·0	14·8	4·3	5·1	5·4
	2 January 1968	15·0	16·7	3·6	6·8	6·3
	5 March 1968	24·0	17·3	4·8	7·5	5·0
	16 April 1968¶	30·0	12·8	3·7	6·6	2·5
	7 May 1968	33·0	16·1	4·4	7·7	4·0
	4 June 1968	37·0	12·4	3·4	5·6	3·4
	2 July 1968	41·0	13·4	3·5	5·9	4·0
	28 September 1968	53·6	10·6	4·0	3·1	3·5
II	23 September 1968‡	0·6	11·1	4·0	4·7	2·4
	14 October 1968	3·6	13·3	5·3	4·7	3·3
	4 November 1968	6·6	12·9	5·2	4·4	3·3
	25 November 1968§	9·6	11·9	3·4	6·4	2·1
	16 December 1968	12·6	10·1	2·9	4·5	2·7
	6 January 1969	15·6	15·6	4·0	7·8	3·8
	27 January 1969	18·6	9·0	2·9	5·2	0·9

* mg protein $\times 10^6$/mg dry wt. of tuber tissue.
† Values obtained by difference.
‡ Tubers just beginning to form.
§ Tubers lifted and put into cold store.
¶ First visible signs of sprouting.

Protein Extraction, Fractionation, Determination and Assay for Invertase and Hydrolase Activity

Freshly peeled tuber (40 g) from each clone was cut into small pieces and the soluble protein extracted and fractionated by methods previously described.[6, 5] The invertase and hydrolase activities of the unfractionated and fractionated protein were measured[9] and the amount of protein was determined by the method of Lowry *et al.*[10]

[9] P. P. RUTHERFORD, E. W. WESTON and A. E. FLOOD, *Phytochem.* **8**, 1859 (1969).
[10] O. H. LOWRY, N. J. ROSEBROUGH, A. LEWIS FARR and R. J. RANDLE, *J. Biol. Chem.* **193**, 265 (1951).