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EFFECTS OF SUGARS, GIBBERELLIC ACID AND KINETIN ON ACID INVERTASE OF DEVELOPING CARROT ROOTS

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Abstract—The activities of acid invertase carrot roots 32, 50 and 60 days old were, respectively, 5.7, 1.4 and 0.5 nkat/g fr. wt. When portions of such roots were excised and incubated in water for 20 hr the activities of the enzyme rose, respectively, to 9.7, 14.4 and 18.4. Fructose (50 mM), GA (30 μM) and kinetin (50 μM) affected the rise in invertase activity, GA stimulating it and fructose and kinetin decreasing it. The magnitude of these effects varied, however, with the age of the roots. Fructose had the highest effect in young non-tuberized roots while the effects of kinetin and GA were highest in mature tuberous roots. A 48 hr incubation of discs from mature roots in fructose plus kinetin reduced the rise in invertase activity by 75%; nevertheless, fructose plus kinetin could not abolish, even after 66 hr of incubation, the *ca* 10% increase in invertase activity produced by a 1 hr GA pulse treatment applied at 0 hr.

INTRODUCTION

It has been proposed that acid invertase (E.C. 3.2.1.26) plays a role in the regulation of sucrose metabolism in plant tissues, the activity of the enzyme being very low in mature organs that store sucrose [1–6]. A marked rise in the activity of this enzyme is observed in slices cut from storage organs and incubated in aerated water [1, 3, 7, 8]. There are indications that the enzyme is synthesized *de novo* on tissue slicing [9, 10]. In slices cut from mature carrots high activities of acid invertase can develop in the presence of appreciable concentrations (50 mM) of exogenous glucose [11]. Similar results were obtained with tissue cultures of carrot, *Acer* and *Convolvulus* [11–14]. In contrast to these is the observation that 30 mM glucose has a high suppressive effect on the activity of the enzyme in slices of immature sugar cane stems [8].

It could be that sugar cane metabolism differed substantially from that of the other tissues; more likely, the differences reported could result from the pattern of interactions between the exogenously applied sugars and the endogenous metabolites and hormones, whose concentrations may vary with the type and age of the tissue. That hormones affect the development of acid invertase activity has been demonstrated for several tissues [10, 15, 16]. The present work reports experimental results bearing on the effect exerted by sugars and hormones on acid invertase activity of carrot roots at different stages of development.

RESULTS AND DISCUSSION

Experiments with mature roots

Incubation of carrot slices either in glucose, fructose or sucrose (50 mM solutions) persistently showed a negligible effect (0–20% inhibition) upon the development of acid invertase activity. Conversely, kinetin showed a marked inhibitory effect on such development when at a concentration of 50 μM or higher (Table 1). The effect exerted by the prolonged slice incubation (up to 66 hr) in kinetin (50 μM), fructose (50 mM) and kinetin plus fructose is shown in Fig. 1. This last treatment markedly inhibited the development of invertase activity.

In excised plant tissues GA was shown to stimulate the activity of acid invertase [15, 16] and also of other hydrolases [15, 17]. It has been shown that this hormone is synthesized upon tissue wounding [18], its concentration progressively increasing with time at the surface layers of peeled tuberous organs [16]. The production of GA is considered to be a prerequisite for the synthesis of acid invertase in several tissues [15–17]. Thus, I have studied the interaction of kinetin and sugars with GA in relation to the development of acid invertase activity. When applied to the carrot slices as a one hr pulse immediately after slicing GA (30 μM) increased the rise in enzyme activity. Such stimulation was not abolished by subsequent slice incubation in kinetin, sugars or kinetin plus sugars and it could still be detected at least 66 hr from GA treatment (Fig. 1). It should be noted that not even the incubation in kinetin plus fructose, which greatly prevented the development of invertase activity, could abolish the GA stimulation of that activity. These results suggest that since GA has triggered invertase synthesis, kinetin and sugars can no longer prevent such synthesis from occurring.

It was postulated that kinetin suppresses invertase synthesis by acting at the translational level while GA stimulates such synthesis by stabilizing the enzyme *m*RNA (see [15]). Consequently, it would seem reasonable to expect that the prolonged incubation (up to 66 hr) of carrot slices in kinetin should eventually abolish the

Table 1. Effect of kinetin concentration on the development of invertase activity (nkat/g fr. wt) of discs* of mature carrot roots

Freshly cut discs	Discs incubated for 22 hr in kinetin (μM)				
	0	2	10	50	100
1.1 ± 0.4†	10.4 ± 0.3	10.6 ± 0.1	10.6 ± 0.3	5.5 ± 0.4	0.8 ± 0.2

* The discs were 10 mm in diameter, 1 mm thick. † Means ± s. e. of activities of two different extracts.

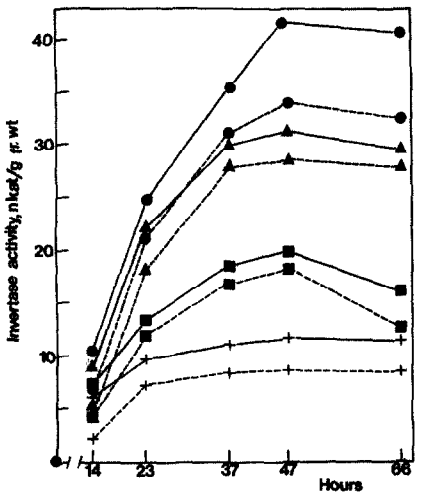

Fig. 1. Effect of a pulse treatment with GA (30 μM) and subsequent incubation in water (●), 50 mM fructose (▲), 50 μM kinetin (■), or kinetin plus fructose (+) on the development of invertase activity of discs of mature carrot roots. Immediately after cutting the discs (10 mm in diameter, 1 mm thick) received a one hr pulse treatment with GA were next washed with water and then transferred to the respective incubation media. The broken lines correspond to the discs which did not receive the GA pulse (incubated for 1 hr in water). Each point is the mean of activities from three different extracts; the standard errors were, at the maximum, ± 0.8 nkat/g fr. wt.

stimulatory effect produced by the one hr GA pulse treatment on invertase activity. Since that was not so, the results shown in Fig. 1 could be interpreted as suggesting that kinetin inhibited the synthesis of GA's, needed for invertase synthesis, but did not interfere with these hormones in the synthesis of the enzyme. In support of this interpretation is the suggestion that endogenous cytokinins control GA metabolism in higher plants [19]. It should also be noted that kinetin is known to suppress the synthesis not only of invertase but, yet, of other hydrolases, e.g.: protease, RNase, acid phosphatase, esterase [20], whose syntheses are likewise stimulated by GA [17].

Experiments with young roots

The effects produced by GA, kinetin and sugars on the development of acid invertase of very young roots (prior to tuberization) were quite distinct from those described above for mature roots (Tables 2 and 3). Fructose (or glucose) had a markedly inhibitory effect on the rise of enzyme activity; such inhibition could reach 100% (Table 3). Kinetin was also highly inhibitory. On the contrary, GA did not affect the development of invertase, neither could it reverse the inhibitory action of fructose (Table 2).

Influence of tissue age

Table 3 shows how root age determines both the magnitude of the development of invertase activity and the effect exerted on such development by fructose and kinetin. As previously found [3, 6] invertase activity of carrot roots decreased during growth. The subsequent rise in acid invertase activity, originated upon tissue slicing, was inversely related to the actual activity of the enzyme in the intact root. Fructose (or glucose) had a marked inhibitory effect on that rise of invertase activity only in tissues excised from non-tuberized roots. After tuber initiation sugars had a negligible effect on invertase activity of carrot roots. Therefore, only non-tuberized carrot roots appear to respond to hexoses in a manner similar to that described for immature sugar cane slices [8]. From Table 3 it can still be seen that, as opposed to sugars, kinetin inhibits the development of invertase activity increasingly as the root gets older.

The variation with root age of the effects exerted by exogenously applied sugars and hormones, which are reported in this article, is probably related to metabolic changes occurring during tuberization. Tuber induction and growth appear to involve important changes in the cellular contents of GA's, cytokinins, sugars, etc.

GA's inhibit tuber induction [21] and their content in sugar-beet root was highest at the early stages of development decreasing from then onwards [22]. In mature tubers of Jerusalem artichoke GA content was very low and its synthesis by the tuber slices was the limiting factor for the development of invertase activity in the slices [16]. Young roots are important centres of cytokinin synthesis [23] and these hormones are required for

Table 2. Effect of fructose (50 mM), GA (30 μM) and kinetin (50 μM) on the development of invertase activity (nkat/g fr. wt) of non-tuberized carrot roots (cv. Nantes)

Freshly harvested roots	Incubated roots					
	Water	GA	Kinetin	Fructose	Fructose plus kinetin	Fructose plus GA
*9.3 ± 0.3	14.1 ± 0.5	14.3 ± 0.1	11.7 ± 0.2	11.7 ± 0.1	11.2 ± 0.2	12.0 ± 0.2

Roots were 1 mm thick, from plants 22 days after germination; the roots were incubated as 5 cm long pieces. * Means ± s. e. of activities of two different extracts.

Table 3. Effect of fructose (50 mM) and kinetin (50 μM) on the development of invertase activity of carrot roots (cv. Nantes) of different age

Root age (days from germination)	Root diameter (mm)	Invertase activity of freshly cut tissue (nkat/g fr. wt)	Ratio of invertase activities: incubated tissue/freshly cut tissue‡			
			Water	Fructose	Kinetin	Kinetin plus fructose
32	1†	5.7 ± 0.2*	1.7	1.0 (44.4)	1.4 (21.4)	1.1 (36.4)
50	5–10	1.4 ± 0.1	10.3	9.4 (9.0)	6.8 (33.4)	5.8 (44.2)
60	7–12	0.5 ± 0.1	36.8	33.3 (9.5)	16.5 (55.1)	13.8 (62.6)

* Means ± s. e. of activities of two different extracts; for the incubated tissues the maximum s. e. was ±0.3. † Non-tuberized roots, incubated as 5 cm long pieces; other roots were cut into discs 5 mm in diameter, 1 mm thick; all tissues were incubated for 20 hr. ‡ The figures in parentheses represent the inhibition (%) of invertase activity relative to that of the activity of samples incubated in H_2O.

tuber and bulb initiation [24, 25]. The concentration of cytokinins in the tissues may reach a peak when cell division is most active and decline afterwards [26].

From the preceding considerations it may be suggested that the cellular levels of both GA's and cytokinins are much higher in the young carrot root than in the mature tuberous root. Conversely, sugar content is low in the non-tuberized root and rapidly increases from the early stages of tuber development to reach a high value in the mature root [3, 6]. It thus appears that significant changes in sugar and hormone contents occur during growth of the carrot root. Such changes in the content of endogenous sugars and hormones could explain why in roots of different age exogenously applied sugars and hormones affect differently the development of acid invertase activity.

EXPERIMENTAL

Material. Mature storage roots, of unspecified variety, were bought locally. Carrots cv. Nantes were grown in white sand, watered 3 × week with a nutrients soln and kept on a 13 hr photoperiod, as previously described [6].

Tissue incubation. Up to 5 g of root tissue were incubated at 25° in 500 ml of the appropriate soln through which filtered air was bubbling; solns were changed every 12 hr. GA was dissolved in 0.1 ml of EtOH and dispersed in 1 l. with H_2O. Kinetin was dissolved in 0.3 ml of 1 N HCl, dispersed in *ca* 600 ml H_2O neutralized with 1 N NaOH and the vol. adjusted to 1 l.

Extraction and assay of invertase. After incubation the tissues were homogenized in a mortar with Na_2HPO_4–citric acid buffer of ionic strength 0.4 and pH 7.5; invertase activity was determined at pH 4.5 and 30° in the unfractionated homogenate after dialysis (see [6]).

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REFERENCES

1. Hatch, M. D. and Glasziou, K. T. (1973) *Plant Physiol.* **38,** 344.
2. Hawker, J. S. (1969) *Phytochemistry* **8,** 9.
3. Ricardo, C. P. P. and ap Rees, T. (1970) *Phytochemistry* **9,** 239.
4. Maclachlan, G. A., Datko, A. H., Rollit, J. and Stokes, E. (1970) *Phytochemistry* **9,** 1023.
5. Lyne, R. L. and ap Rees, T. (1971) *Phytochemistry* **10,** 2593.
6. Ricardo, C. P. P. and Sovia, D. (1974) *Planta* **118,** 43.
7. Glasziou, K. T. and Waldron, J. C. (1964) *Australian J. Biol. Sci.* **17,** 609.
8. Vaughan, D. and MacDonald, I. R. (1967) *Plant Physiol.* **42,** 456.
9. Wray, J. L. and Brice, R. E. (1973) *Phytochemistry* **12,** 1917.
10. Flood, A. E., Price, R. and Rowe, H. F. (1973) *Phytochemistry* **12,** 1005.
11. Ricardo, C. P. P., ap Rees, T. and Fuller, W. A. (1972) *Phytochemistry* **11,** 2435.
12. Edelman, J. and Hanson, A. D. (1971) *Planta* **101,** 122.
13. Copping, L. G. and Street, H. E. (1972) *Physiol. Plantarum* **26,** 346.
14. Klis, F. M., Groot, C. de and Verwer, R. (1974) *Physiol. Plantarum* **30,** 334.
15. Glasziou, K. T. (1969) *Ann. Rev. Plant Physiol.* **20,** 63.
16. Bradshaw, M. J. and Edelman, J. (1969) *J. Exp. Botany* **20,** 87.
17. Marcus, A. (1971) *Ann. Rev. Plant Physiol.* **22,** 313.
18. Rappaport, L. and Sachs, M. (1967) *Nature* **214,** 1149.
19. Railton, I. D. (1974) *Planta* **120,** 197.
20. Balz, H. P. (1966) *Planta* **70,** 207.
21. Jolivet, E. (1969) *Ann. Physiol. Vég.* **11,** 265.
22. Garrod, J. F. (1974) *J. Exp. Botany.* **25,** 945.
23. Kende, H. (1971) *Int. Rev. Cytol.* **31,** 301.
24. Smith, O. E. and Palmer, C. E. (1970) *Physiol. Plantarum* **23,** 599.
25. Ginsburg, C. and Ziv, M. (1973) *Ann. Botany* **37,** 219.
26. Mackenzie, I. A. and Street, H. E. (1972) *New Phytologist* **71,** 621.