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GLUCOSE REGULATION OF ENZYME SYNTHESIS IN SUGAR CANE STEM TISSUE

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Abstract—The relative effectiveness of glucose, other sugars and sugar derivatives in repressing invertase synthesis in sugar cane storage tissue was compared. Pyranose and furanose ring forms evidently interact with different acceptor sites. Glucose appears to be active without modification and as the β anomer in the D-pyranose form. Rapid peroxidase synthesis was demonstrated concomitantly with invertase synthesis. Fructose repressed synthesis of both peroxidase and invertase, but glucose repressed only the latter. These and previous results indicate that glucose specifically mediates the destruction of m-RNA required for invertase synthesis.

INTRODUCTION

SYNTHESIS of the vacuolar acid invertase of immature storage parenchyma tissue of sugar cane is subject to product feedback repression by glucose and possibly fructose.[1–5] A number of other sugars have repressor activity but some sugars and polyhydric alcohols act as inducers. This paper reports further work on the specificity and mode of action of the glucose effect on invertase synthesis, and presents preliminary results on the induction of peroxidase synthesis in sugar cane stem tissue slices.

RESULTS AND DISCUSSION

Checks for Primary Activity as Repressors of Invertase Synthesis

In considering steric requirements for activity it is essential that substances added to the medium either not be altered by the tissue or the alterations be known, and that no secondary effects should occur. We have not considered results from substances which caused abnormal depression of respiration, discoloration of tissue, or leakage of stored sugar compared with tissues in water. Compounds which are quickly metabolized to the natural repressors, glucose or fructose, were readily detected by their ability to maintain the endogenous sugar pools.

Structural Requirements for Repressor Activity on Invertase Synthesis

Results for the effects of sugars in addition to those tested previously[6] are given in Table 1. Because of differences in the time course of invertase synthesis in different batches of tissue,[5] only a qualitative classification of compounds as repressors or inducers is possible, and is obtained by reference to tissues both in glucose and water.

[1] J. A. SACHER and K. T. GLASZIOU, *Biochem. Biophys. Res. Commun.* **8**, 280 (1962).
[2] J. A. SACHER, M. D. HATCH and K. T. GLASZIOU, *Physiol. Plantarum* **16**, 836 (1963).
[3] K. T. GLASZIOU and J. C. WALDRON, *Nature* **203**, 541 (1964).
[4] C. R. SLACK, *Australian J. Biol. Sci.* **18**, 781 (1965).
[5] K. T. GLASZIOU, J. C. WALDRON and T. A. BULL, *Plant Physiol.* **41**, 282 (1966).
[6] K. T. GLASZIOU and J. C. WALDRON, *Australian J. Biol. Sci.* **17**, 609 (1964).

TABLE 1. EFFECT OF SUGARS AND DERIVATIVES ON INVERTASE SYNTHESIS

Class	Compound	Invertase content relative to controls		Classification*
		Glucose	Water	
Hexoses and derivatives	D-Mannose	0·43	0·15	R
	3-Deoxy-D-glucose	0·88	0·52	R
	4-Deoxy-D-glucose	0·95	0·32	R
	Phenyl β-D-glucopyranoside	1·1	0·63	R
	D-Fructose	1·2	0·42	R
	L-Sorbose	1·7	0·45	R
	Methyl α-D-mannopyranoside	3·1	1·1	N
	6-Deoxy-L-mannose	3·8	1·2	N
	6-Deoxy-D-galactose	4·5	2·8	I
	6-Deoxy-L-galactose	5·4	3·1	I
	D-Galactose	6·6	1·3	N
	L-Mannose	11	1·4	N
Pentoses and derivatives	D-Arabinose	1·9	0·82	N
	Methyl-β-D-xylopyranoside	3·1	0·95	N
	2-Amino-D-lyxose	3·2	2·3	I
	2-Deoxy-D-ribose	3·6	2·4	I
	D-Xylose	3·7	0·9	N
	D-Ribose	5·3	0·7	N
	L-Xylose	8·4	1·6	I
	D-Lyxose	11	2·2	I
Tetroses	D-Erythrose	0·5	0·22	R

* R = Repressor; I = Inducer; N = No significant effect.

2-g lots of tissue were incubated at 30° with the compounds indicated at a concentration of 0·06 M and harvested after 10–12 hr. Invertase assays were made on extracts from the tissues and compared with the enzyme level for tissue treated with water or 0·06 M D-glucose.

In relating structure and activity, key importance is attached to the effects of D-glucose, D-galactose, D- and L-mannose, 2-amino-D-glucose, and phenyl β-D-glucopyranoside. Previous results show that 2-amino-D-glucose gives virtually equivalent effects on invertase synthesis to D-glucose. The carbon chain of ^{14}C-labelled 2-amino-D-glucose was not fragmented or converted to either glucose or fructose.[6] Currently we found that this sugar did not affect respiration, confirming that it has primary activity.

Phenyl β-D-glucopyranoside is effective as a repressor (Table 1), does not affect respiration appreciably and does not maintain the endogenous sugar pool. By these criteria it has primary activity, and therefore the pyranose ring structure must be an active configuration. The relative inactivity of methyl α-D-glucopyranoside,[6] and methyl α-D-mannopyranoside indicates that only the β anomer may be active.

D-Mannose is a better repressor than D-glucose, but L-mannose is an inducer. D-Mannose and D-glucose differ in orientation only at the C-2 position. However D-galactose, which does not repress invertase synthesis (Table 1), differs from D-glucose only at the C-4 position. Since 4-deoxy-D-glucose is a good repressor (Table 1), we conclude that a hydroxyl group at

C-4 is unnecessary, but if present, must not be orientated as in D-galactose. The sketetal structure for repressor activity is shown in Fig. 1 (*a*).

In our standard test procedure both fructose and sucrose are classified as repressors.[6] Since both maintain the endogenous glucose pool, alternative techniques were necessary to reveal primary activity. Short-term time course experiments showed that sucrose as such has little or no repressor activity, and may in fact be an inducer. Similar and other more elaborate experiments did not reveal any consistent difference between D-glucose and D-fructose activity on immature tissue, but Hatch and Cillekens (unpublished) have found that fructose is a much more effective repressor of invertase synthesis in mature stem tissues than glucose.

In formulating a working hypothesis relating structure and activity of repressors we have considered the following alternatives.

1. Substances having repressor activity interact with the active site of a single macromolecular cell constituent. Only the pyranose forms of sugars can have activity, but with the restrictions indicated in Fig. 1 (*a*).

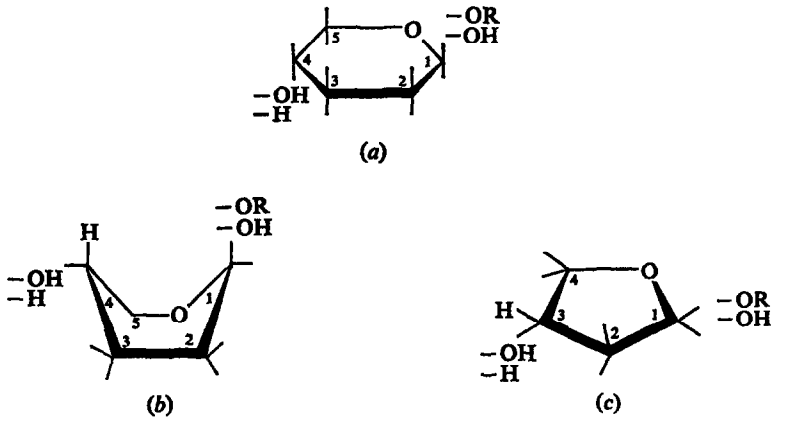

FIG. 1. STRUCTURAL CONFORMATION AND REPRESSOR ACTIVITY.

(*a*) Pyranose sugars should have no hydroxyl group above the plane of the ring at the C-4 position and pyranosides should be the β anomers; (*b*) Boat form of (*a*); (*c*) The ring oxygen, oxygen at C-1 and hydrogen at C-3 are spatially superimposable with the ring oxygen, oxygen at C-1 and hydrogen at C-4 of (*b*). Hence these two structures may give a three-point fit to the same active site.

2. As for (1) except that both pyranose and furanose ring forms can fit the same active site.

3. The pyranose and furanose ring forms fit two different active sites to give repressor activity.

A corollary for each of these alternatives is that badly fitting substances may competitively exclude endogenous repressor sugars from the active site and give apparent induction.

According to the first hypothesis, D-arabinose, D-lyxose, 2-deoxy-D-ribose, D-ribose, D-lyxosamine, and D-xylose should all be repressors but only D-ribose and D-arabinose show slight signs of activity (Table 1, and Glasziou and Waldron[6]). Lefevre and Marshall[7] noted anomalies in respect of the carrier-complex dissociation constants of pentose sugars for the human red blood cell sugar transport system, and suggested these may be partially due to higher proportions of furanose and straight-chain structures. A similar argument cannot be applied to sugar-mediated repression of invertase synthesis since methyl β-D-xylopyranoside,

[7] P. G. LEFEVRE and J. K. MARSHALL, *Am. J. Physiol.* **194**, 333 (1958).

which is homomorphous with β-D-glucopyranosides and should be a strong repressor, is without activity (Table 1). Other anomalies which do not conform with hypothesis 1 are the repressor activities of D-erythrose which can form only a furanose ring, and L-sorbose which in the pyranose form has the hydroxyl group at the C-4 position orientated as in D-galacto-pyranose. Studies with ^{14}C-L-sorbose showed that its activity was not due to conversion to glucose or fructose.

Using Courtauld atomic models spatially equivalent three-point fit structures were constructed for both ring forms. The points were at C-4, the ring oxygen, and the hydroxyl

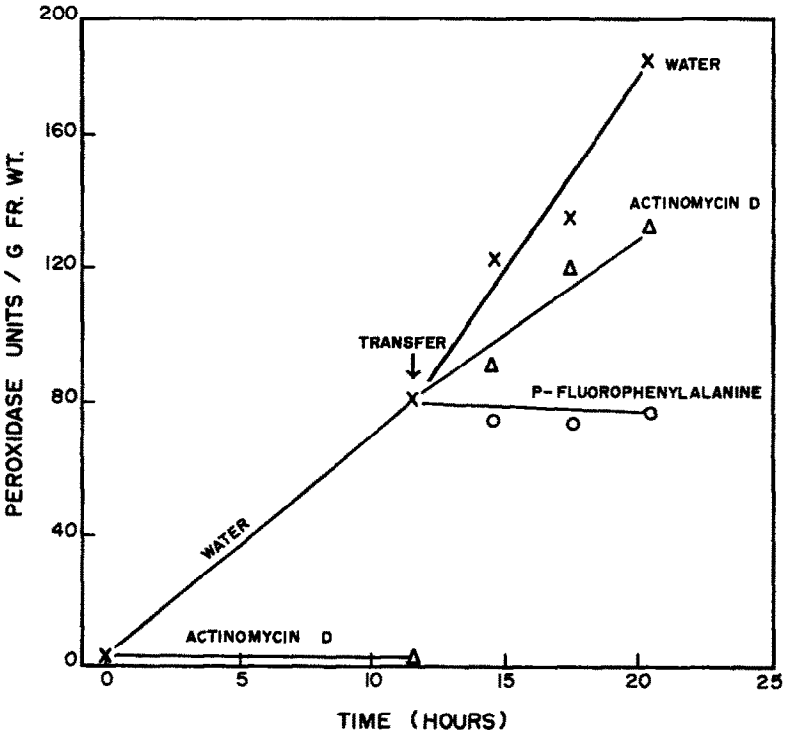

FIG. 2. EFFECT OF 8×10^{-6} M ACTINOMYCIN D AND 2×10^{-3} M *p*-FLUOROPHENYLALANINE ON PEROXIDASE SYNTHESIS.

Tissues were cut from immature internodes, 2-g lots washed and placed in water or actinomycin D for 11·5 hr at 30°. Water-treated tissues were then transferred to actinomycin D, *p*-fluorophenylalanine, or water. Solutions were changed at 2-hr intervals. Harvest and assay detail as for Table 2.

oxygen at C-1 of the β-anomer of a pyranose ring in a boat form. The equivalent points for the furanose ring were at positions C-1, C-3, and the ring oxygen (Fig. 1(*b*), (*c*)). The activities of L-sorbose and D-erythrose can be accounted for in the furanose forms, but the pentoses present difficulties, and particularly the inactivity of methyl β-D-xylopyranoside.

Hypothesis 3 has the disadvantage of increased complexity but is supported by experiments (Hatch and Cillekens, unpublished) on mature tissue in which fructose but not glucose repressed invertase synthesis. The same minimum structural requirement is assumed for the pyranose ring (Fig. 1(*a*)), with the additional restriction that a carbinol group at C-5 orientated as in D-aldohexoses is essential for activity. This means that ketohexoses, aldopentoses and

aldotetroses can have no activity as repressors at the active site for pyranose ring action, but may have inducer properties through competitive effects with endogeneous repressor (glucose). The requirement for the carbinol group also accounts for the lack of repressor activity of 6-deoxy-L-galactose (Table 1).

To account for the repressor activities of D-fructose, L-sorbose, and D-erythrose we assume

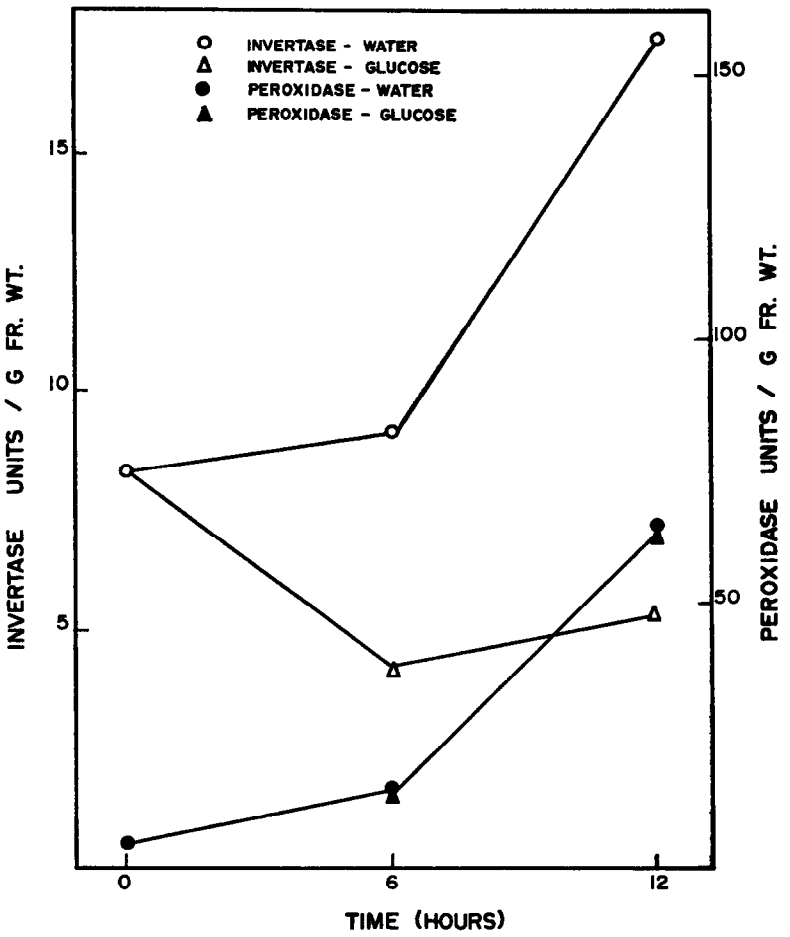

FIG. 3. EFFECT OF GLUCOSE ON INVERTASE AND PEROXIDASE SYNTHESIS.

Tissues were cut, washed and placed in 0·12 M glucose or water at 30°, solutions being changed at 2-hr intervals. Harvest and peroxidase assays as for Table 2. Invertase assays were on extracts dialysed against distilled water.

the furanose acceptor has no specific requirements for the presence of a carbinol group at the C-1 position nor in respect of the presence or orientation of a carbinol group at C-4 of the furanose ring. The pentoses would have potential repressor activity in the furanose forms and inducer activity in the pyranose forms.

Hypothesis 3 accounts for the independent effects of glucose and fructose in mature tissue, the inactivity of methyl β-D-xylopyranoside, and predicts the observed anomalous behaviour of the pentoses, hence is the preferred hypothesis.

Specificity of Glucose and Fructose Effects

Recently we found that cane peroxidase behaves in a similar manner to invertase in that it is synthesized rapidly when freshly cut tissues are placed in water. Synthesis was completely repressed by actinomycin D added immediately after cutting, but not if added during the phase of rapid synthesis (Fig. 2), results which are consistent with the hypothesis that m-RNA synthesis is required for initiation of peroxidase synthesis. In contrast to invertase which turns over rapidly,[5] no significant loss of peroxidase occurred when enzyme synthesis was interrupted with inhibitors such as *p*-fluorophenylalanine (Fig. 2).

A major difference between the behaviour of invertase and peroxidase was that glucose gave no repression of peroxidase synthesis (Fig. 3; Table 2). However, fructose which represses invertase synthesis, also reduced peroxidase synthesis by about 50 per cent (Table 2).

TABLE 2. EFFECT OF GLUCOSE AND FRUCTOSE ON PEROXIDASE SYNTHESIS

Treatment	Peroxidase units/g fr. wt.
Fresh tissue	4
Water	94
0·12 M Fructose	45
0·24 M Fructose	49
0·12 M Glucose	80
0·24 M Glucose	98

Tissue was cut from immature internodes and harvested immediately or 2-g lots incubated for 14 hr at 30° in the solutions shown, the solutions being changed at 2-hourly intervals. At harvest, tissues were washed, ground, squeezed through muslin, and the extracts dialysed for 24 hr at 3° against 10 mM phosphate buffer, pH 7, prior to peroxidase assay.

In freshly prepared stem tissue extracts of both peripheral and internal tissue from intercalary meristems, zones of rapid stem elongation, and fully expanded internodes, the peroxidase level ranged from two to six units per gram tissue. In mature internodal tissue placed in water for 12 hr the peroxidase level increased by 4-fold, and in immature tissue by as much as 30-fold. This rapid attainment of unphysiological levels of peroxidase may indicate the loss of endogenous diffusible repressors of peroxidase synthesis when tissues are placed in water.

Previously we have reported that glucose prevented invertase synthesis under conditions where actinomycin D had little or no effect, indicating that glucose does not regulate m-RNA synthesis. However, when tissues were transferred to water following a glucose treatment, actinomycin D completely repressed the onset of invertase synthesis, indicating that m-RNA required for invertase synthesis was destroyed during the glucose treatment. Glucose did not affect the rate of breakdown of invertase.[5] Our present results showing that peroxidase synthesis continued unabated in the presence of glucose demonstrates that the glucose effect on invertase is not due to altered levels or effectiveness of substrates, ribosomes, or enzymes which are common to the general process of enzyme synthesis. This observation is in accord-

ance with the hypothesis that elevated glucose levels result in the destruction of m-RNA specifically required for invertase synthesis.[5]

MATERIALS AND METHODS

Preparation, Incubation and Extraction of Tissue, and Invertase and Peroxidase Assays

Immature internodal tissue from the zone of rapid cell elongation was cut, washed, weighed into 2-g lots and incubated in 5 ml solution at 30°. The solutions were removed and renewed at 1–3 hourly intervals. The tissue was harvested after 10–12 hr, washed, and ground with the aid of a little sand in a chilled mortar and pestle. The juice was squeezed through muslin. For invertase assays 1·0 ml aliquots were dialysed at 3° against distilled water and assayed as previously.[5] For peroxidase assays, dialysis was carried out against three changes of 10 mM phosphate buffer, pH 7·0.

The peroxidase assay system contained 10 mM phosphate buffer, pH 7·0, 0·33 mM guiacol, 0·27 mM H_2O_2 in a volume of 3·0 ml.[8] A unit of peroxidase activity gave an absorbancy change of 0·1 at 470 nm in 100 sec at 22°.

Freedom from Micro-organisms

That peroxidase synthesis was due to the plant tissue only was checked by the centrifugation method described previously,[5] and by comparison of starch gel electrophoresis zymogram patterns of fresh and water-incubated tissue (Gayler and Glasziou, unpublished).

Chemicals

3-Deoxy-D-glucose and 4-deoxy-D-glucose were gifts from Mr. R. A. Kitchen, C.S.R. Research Laboratories, Sydney. All other sugars were obtained from Nutritional Biochemicals Corporation. Actinomycin D was obtained from Merck, Sharp and Dohme, and *p*-fluorophenylalanine from Sigma Chemical Co.

[8] B. CHANCE and A. C. MAEHLY, In *Methods in Enzymology* (Edited by S. P. COLOWICK and N. O. KAPLAN), Vol. 2, p. 764. Academic Press, New York (1955).