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GENERATION OF α-AMYLASE IN GERMINATING *HORDEUM DISTICHON*

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Abstract—The scutellar tissue of germinating barley synthesises significant amounts of α-amylase. The endogenous level of sugars probably limits the initial formation of α-amylase mainly by repressing the supply of gibberellins to the aleurone layer.

IN CONTRAST to separated aleurone layers barley embryos make more α-amylase (approx. ×10) when supplied with a mixture of amino acids (casein hydrolysate) than when they are not, and they are not dependent entirely on exogenous gibberellins,[1,2] Further, gibberellic acid approximately doubles the yield of enzyme from embryos, if amino acids are present.[2] Small concentrations of sugars (e.g. 5 mM) significantly depress enzyme production in isolated embryos, even in the presence of amino acids and GA_3.[2] This is not the case with aleurone layers.[2]

The addition of GA_3 to germinating grain approximately doubles the amount of α-amylase found in the embryo; therefore *in vivo* nutrients do not limit enzyme production. After 6 days germination (malting conditions), around 15% of the α-amylase found in the grain is derived from the embryo. About half of this is retained in the embryo, and half is released into the endosperm.[2] It has been suggested that the major part of the α-amylase is produced in isolated embryos by adherent aleurone tissue, and not in the embryonic tissue of the scutellum.[3] However, the dissected embryos were not supplied with amino acids[3] and so enzyme production by the scuteller tissue would have been limited by this lack.

Using an improved assay[4] it has been confirmed that GA_3 added to whole grains increased the level of α-amylase found in the embryo.[5] The enzyme was located in the scutellum, together with any attached aleurone tissue, and not the embryonic axis.[6] To re-evaluate whether the enzyme is generated in scutellar tissue, rings of material were cut from the edges of the scutella of decorticated grains of Proctor barley, taking precautions to maintain sterility.[7] Thus the edge of each scutellum together with any adhering tissue, and some aleurone with underlying starchy endosperm was removed, and called the peelings. Each 'pared' grain was divided into two by a transverse cut, parallel to the inner face of the scutellum, through the starchy endosperm. Thus a 'plug' of embryonic tissue, retaining the axis and about half of the scutellar epithelium, was obtained free of aleurone, but with some

[1] BRIGGS, D. E. (1963) *J. Inst. Brewing* **69,** 13.
[2] BRIGGS, D. E. (1964) *J. Inst. Brewing* **70,** 14.
[3] MACLEOD, A. M. and PALMER, G. H. (1966) *J. Inst. Brewing* **72,** 580.
[4] BRIGGS, D. E. (1967) *J. Inst. Brewing* **73,** 361.
[5] BRIGGS, D. E. (1968) *Phytochemistry* **7,** 531.
[6] BRIGGS, D. E. (1968) *Phytochemistry* **7,** 513.
[7] GROAT, J. I. and BRIGGS, D. E. (1969) *Phytochemistry* **8,** 1615.

attached starchy endosperm. The bulk of the grain, which remained, comprised most of the starchy endosperm together with about 90% of the aleurone layer. All these preparations were incubated with GA_3. Other grains, divided into two by transverse cuts, were also incubated with GA_3 as controls. The embryonic 'plug' was incubated with amino acids and made significant quantities of α-amylase (Table 1). As the total enzyme produced at different times by the separated parts (released into the medium or retained), approximately equalled that made by transected grains the dissection process did not greatly damage the enzyme forming tissues. Estimation of the proportion of tissues in the different separated parts, by inspection of scanning electron micrographs, indicated that the 'peelings' contained about 50% of the scutellar epithelium and 10% of the aleurone layer. Thus, of the total α-amylase (18.2 S.I.C. units) produced by the peelings about 6 enzyme units came from the scutellar tissue (equal to the enzyme produced by the embryonic 'plug'), and 12 units from the aleurone (about 10% of that produced by the aleurone with starchy endosperm). Thus in these conditions the true embryonic scutellum made about 10% of the total α-amylase. It is not possible to say that adhering aleurone tissue makes no contribution to enzyme production by whole, dissected embryos. It has recently been shown that the initial supply of enzymes which catalyse the breakdown of the cell walls of the starchy endosperm also comes from the scutellum.[8]

TABLE 1. α-AMYLASE PRODUCED BY DISSECTED GRAINS INCUBATED IN LIQUID MEDIUM (5 ml/flask) CONTAINING GA_3 (1 μg/ml). THE INCUBATION MEDIUM WAS CHANGED AFTER 48 hr*

	α-Amylase (S.I.C./fraction from 1 grain)			
	Medium 0–48 hr	Medium 48–72 hr	Grain part 0–72 hr	Total (all fractions)
Embryonic axis + scutellum (Edges peeled) + little starchy endosperm. Protein hydrolysate added (1·5 mg/l.) (15 grain parts/flask)	2·1	2·5	1·6	6·2
Aleurone peelings and edges of scutellum (5 grain parts/flask)	4·8	4·4	9·0	18·2
Remainder of aleurone, and starchy endosperm (5 grain parts/flask)	37·0	53·5	33·6	124·1
Control samples, halved grains (5/flask)	44·0	60·4	44·6	149·0

* Results—means of triplicates.

Low levels of sugars (5 mM) depress α-amylase production by isolated embryos even in the presence of added amino acids and GA_3.[2] Sugars also repress gibberellin synthesis in the barley scutellum.[9] Further, high concentrations of sugars (0·2–0·4 M) depress enzyme

[8] BRIGGS, D. E. (1972) *Planta* **108**, 351.
[9] RADLEY, M. (1969) *Planta* **86**, 218.

formation by aleurones incubated with GA_3.[10] Thus sugars, formed from the endosperm reserves, may repress gibberellin formation and enzyme formation in the embryo and aleurone.

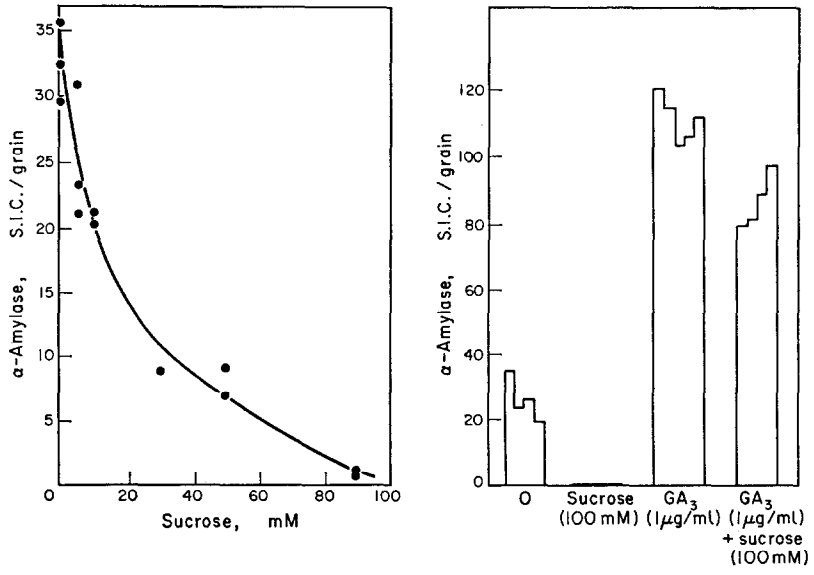

FIG. 1. THE EFFECT OF SUCROSE AND GIBBERELLIC ACID ON ENZYME PRODUCTION BY DECORTICATED BARLEY GRAINS BISECTED INTO GERM AND APICAL PORTIONS.
The graph illustrates the repression of enzyme production by increasing levels of sucrose. The histogram indicates the enzyme levels attained in incubations with no additions, or with fixed concentrations of sucrose or GA_3 added separately or together. Bisected grains (8/flask) were cultivated for 46 hr at 25° in liquid, (1 ml; 0·5 mM $CaSO_4$), with or without sucrose or GA_3.

As the sugars are generated largely by starch hydrolysis catalysed *inter alia*, by α-amylase a regulatory feed back loop controlling enzyme formation may exist. In malting grain endogenous gibberellins clearly limit the rate of formation of α-amylase.[7] The rise in gibberellin content occurs at a time when the sugar levels in the grain are known to be declining, and the subsequent fall occurs when the sugars are being replenished by endosperm hydrolysis,[7,11] which is consistent with the idea that sugars regulate gibberellin levels *in vivo*. However, in grain grown on a moist substratum at 25°, the rise and fall in the rate of net α-amylase increase *precedes* the rise and fall in gibberellin content.[7] As α-amylase destruction goes on in grain germinated in this fashion[5,6,12] the peak rate of increase in α-amylase content must preceed the maximal rate of synthesis. Further the presence of gibberellin in the grain is no indication that it has access to the aleurone layer. The addition of GA_3 to the grain, when the peak amount of α-amylase is present, accelerates the subsequent decline in α-amylase content.[6] At this time the endosperm is partly liquefied and contains a high level of soluble sugars.[6,10] Aleurone isolated from the grain at this stage is still able to make enzyme in response to GA_3, so *in situ* enzyme synthesis may be prevented by the high osmotic pressure caused by the sugars,[10] or the rate of enzyme destruction may match the rate of synthesis.[5,6,12]

[10] JONES, R. L. and ARMSTRONG, J. E. (1971) *Plant Physiol.* **48**, 137.
[11] POLLOCK, J. R. A. (1962) in *Barley and Malt* (COOK, A. H., ed.), p. 303, Academic Press, London.
[12] BRIGGS, D. E. (1968) *Phytochemistry* **7**, 539.

While the formation of α-amylase in the scutellum will probably be prevented early on by low concentrations of sugars it is of interest to know if the quantitatively more important enzyme formation in the aleurone is prevented primarily by; (a) sugars stopping gibberellins reaching the aleurone layer, or (b) by the high osmotic pressure caused by the sugars preventing the aleurone responding to gibberellins. Separated embryos and endosperms were cultured at 25° in a minimum volume of liquid which served to distribute 'endogenous' and added substances between the grain parts. Increasing quantities of sucrose in the medium progressively depressed the amount of α-amylase formed until, at 0·1 M, no enzyme was produced (Fig. 1, left). Embryo growth was unimpaired. Incubations were also carried out with and without additions of sucrose and GA_3 (Fig. 1, right). Again sucrose added alone prevented enzyme formation. When sucrose was added with GA_3 enzyme formation was reduced but not entirely prevented compared to incubations supplemented with GA_3 only, so grain tissues were able to form enzyme when gibberellin was present. Thus the sucrose must have suppressed the supply of endogenous gibberellins. However, the depression caused by sucrose in GA_3 incubations was greater than would be expected from inhibiting only enzyme formation in the embryo. Thus it appears that low levels of sugar prevent enzyme formation in embryos, at higher levels suppress the gibberellin supply, and at higher levels still depress the ability of the aleurone to respond to gibberellins. It would seem the most important initial restriction on enzyme production caused by sugars is likely to be the restriction of gibberellin production in grain germinated under any conditions. Later, in grain grown at 25 with water, the osmotic pressure in the endosperm may limit the response of the aleurone and or enzyme destruction may equal or exceed enzyme synthesis to explain the apparent lack of response to exogenous gibberellins.

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