Gibberellic acid-stimulated α -amylase secretion and phospholipid metabolism in wheat aleurone tissue

R. B. MIRBAHAR* and D. L. LAIDMAN

Department of Biochemistry and Soil Science, University College of North Wales, Bangor,
Gwynedd LL57 2UW, Wales, U.K.

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1. Turnovers of [^{14}C]glycerol-labelled phospholipids in wheat aleurone tissue have been measured by using a pulse-decay technique. The most metabolically active phospholipids were phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidylglycerol. 2. Gibberellic acid action on the tissue led to breakdown of phosphatidylcholine and stimulated turnover of the other phosphatides concomitant with the secretion of α -amylase from the tissue. After pulse-labelling of the aleurone tissue with [^{14}C]glycerol, radioactivity was lost from the phospholipids and appeared quantitatively in triacylglycerols, suggesting a stoichiometric metabolism of the former into the latter. Although 1,2-diacylglycerol is an expected intermediate in such a conversion, the patterns of radioactivity in diacylglycerols gave no indication of this. 3. Several aspects of the response of aleurone tissue to gibberellic acid resemble the responses of exocytotic animal tissues to external agonists. In particular, our results and previous reports in the literature suggest that endomembrane flow, exocytosis, phosphatidylinositol turnover and a requirement of Ca^{2+} for enzyme secretion are common to both plant and animal systems. Although considerable differences also exist between the two, the similarities are sufficient to warrant further consideration that plants and animals might have conserved a similar hormone response–secretion mechanism.

During germination of cereal seeds the aleurone tissue synthesizes and secretes hydrolytic enzymes, including α -amylase, into the starchy endosperm (Filner *et al.*, 1969). Both synthesis and secretion of α -amylase are induced by the hormone gibberellic acid, which emanates from the embryo. De-embryoed seeds or isolated aleurone layers thus provide a convenient experimental system for the study of gibberellic acid-induced α -amylase synthesis. The enzyme is synthesized at the rough endoplasmic reticulum and the available evidence indicates that it is secreted via a vesicular route terminating in exocytosis at the plasma membrane (Chrispeels, 1976; Tomos & Laidman, 1979). Evidence includes the facts that α -amylase isolated from aleurone tissue is lysosomal (Gibson & Paleg, 1972; Firn, 1975) and endomembrane flow from the endoplasmic reticulum into vesicles is induced by gibberellic acid (Colborne *et al.*, 1976).

Involvement of the endomembrane system in the synthesis and secretion of α -amylase has led several groups of workers to study phospholipid metabolism in the aleurone tissue (see Laidman, 1982). Although the early evidence was contradictory, it is now clearly understood that phospholipids are synthesized and endoplasmic reticulum is formed during the first day of germination. Only the imbibition of water is needed to initiate these processes and gibberellic acid is not involved. Gibberellic acid might, however, stimulate the formation of some additional endoplasmic reticulum over and above that formed in control tissue incubated without the hormone (Colborne *et al.*, 1976; Jones, 1980).

Although gibberellic acid is not required for the synthesis of endoplasmic reticulum, some doubts remain regarding its effect on membrane lipid turnover. In barley, large increases have been observed in the incorporation of [$\text{Me-}^{14}\text{C}$]choline and [^{32}P]P_i into microsomal lipids 5–10 h after hormone application (Evins & Varner, 1971; Koehler & Varner, 1973). Incorporations of [^{14}C]acetate and [^3H]glycerol were not affected (Firn &

Abbreviations used: GA, gibberellic acid; SIC units, starch-I₂ colour units (see Briggs, 1967).

* Present address: Department of Agricultural Chemistry, Agricultural University of Sind, Tandojam, Pakistan.

Kende, 1974). Similar studies on wheat failed, however, to show a hormone effect on either choline, glycerol or P_i incorporation (Varty & Laidman, 1976). In the present work we have therefore re-examined the situation in wheat aleurone tissue using a pulse-decay technique. We have also re-analysed changes in phospholipid levels and hormone-induced α -amylase production so that temporal relationships between them and phospholipid turnover can be identified.

Materials and methods

Soft-grained winter wheat, cultivar Flanders, was used in batches of 30 seeds weighing 1.7 g. Their embryos were removed and the de-embryoed seeds were sterilized in dilute NaOCl (0.15% available chlorine) under reduced pressure (Hall & Laidman, 1968). All subsequent treatments were carried out under aseptic conditions. The de-embryoed seeds were incubated in the dark at 25°C (Doig *et al.*, 1975). The incubation medium contained chloramphenicol (10 µg/ml) $\pm$ 1 µM-GA. For phospholipid turnover measurements, de-embryoed seeds were first incubated for 24 h in medium to which 10 µCi of [U- 14 C]glycerol (sp. radioactivity 37 Ci/mol) was added. They were then washed several times with medium containing unlabelled 10 mM-glycerol, followed by a final wash in sterile distilled water. The labelled, de-embryoed seeds were then re-incubated for up to a further 96 h in medium containing chloramphenicol (10 µg/ml) $\pm$ 1 µM-GA but no glycerol.

α -Amylase activity was determined on whole de-embryoed seeds (aleurone layers plus starchy endosperm) by the method of Briggs (1967). For the extraction of lipids, de-embryoed seeds were dissected to obtain their aleurone layers. The aleurone layers were washed in distilled water and extracted with hot water-saturated butan-1-ol. The crude lipid extract was purified by partition chromatography on Sephadex G-25. Total lipid phosphorus was determined on portions taken from this purified extract. The extract was then fractionated into neutral lipids, glycolipids and phospholipids by silicic acid column chromatography. These methods have been described in detail previously (Colborne & Laidman, 1975; Varty & Laidman, 1976).

Neutral lipids from silicic acid column chromatography were fractionated further on columns of neutral alumina deactivated to grade IV. Deactivated Al_2O_3 (5 g), suspended in light petroleum (b.p. 40–60°C), was packed in a glass column (int. diameter 1 cm) (Hall & Laidman, 1968). Neutral lipids were applied to the column in light petroleum and the hydrocarbons and sterol esters were eluted with 20 ml of light petroleum. The column was then eluted sequentially with 25 ml of 15% diethyl ether in

light petroleum (triacylglycerols), 30 ml of 50% diethyl ether in light petroleum (sterols and diacylglycerols) and 20 ml of diethyl ether (non-esterified fatty acids). Efficiency of separation was monitored by t.l.c. on silica-gel G. The developing solvent for triacylglycerols was light petroleum (b.p. 40–60°C)/diethyl ether (17:3, v/v) and that for diacylglycerols was light petroleum (b.p. 40–60°C)/diethyl ether/ethanol/acetic acid (250:250:10:1, by vol.). The latter system separated 1,2-diacylglycerols from 1,3-diacylglycerols. Authentic trioleoylglycerol, 1,2-dioleoylglycerol and 1,3-dioleoylglycerol were used to help locate the glyceride lipids. The same t.l.c. systems were also used to purify the glyceride lipids from Al_2O_3 column chromatography before determination of their radioactivities. Phospholipids from silicic acid column chromatography were separated into individual phosphatide classes by two-dimensional t.l.c. on silica-gel G (Colborne & Laidman, 1975). Lipids on developed chromatograms were located by staining lightly with I_2 vapour, and radioactivity was located by a chromatogram spark chamber (Birchover Instruments, Letchworth, Herts., U.K.). Phospholipids were determined by measuring P_i and radioactivity was determined by scintillation spectrometry as previously described (Varty & Laidman, 1976).

[U- 14 C]Glycerol was obtained from Amersham International (Amersham, Bucks., U.K.). Authentic diacylglycerols and triacylglycerol were obtained from Sigma Chemical Co. All other materials were obtained from sources previously specified (Colborne & Laidman, 1975).

Results

Stimulation of α -amylase secretion and phospholipid breakdown by gibberellic acid

Fig. 1(a) shows the time course of α -amylase production in de-embryoed seeds incubated with gibberellic acid. There was a lag of 18–24 h, after which α -amylase activity increased rapidly. Enzyme production did not appear to be complete even after 120 h when the experiment was terminated. More than 90% of the enzyme activity was located in the starchy endosperm (results not shown). This distribution illustrates the rapid secretion of the enzyme from the aleurone tissue where it is synthesized. Only low amylolytic activity appeared in control tissue incubated without gibberellic acid.

Total phospholipid levels in the aleurone tissue are shown in Fig. 1(b). During the first 24 h, phospholipid levels increased by about 30%. This increase was independent of the presence of gibberellic acid. The levels continued to increase slowly in the control tissue for the next 72 h of incubation. In hormone-treated tissue, however, the level fell after

Fig. 1. Time course of gibberellic acid-stimulated α -amylase secretion (a) and phospholipid breakdown (b) ●, Control tissue incubated without gibberellic acid; ○, tissue incubated with $1\mu\text{M}$ gibberellic acid. Values in (a) are averages from two determinations and those in (b) are averages for the numbers of determinations indicated in parentheses. The vertical bars indicate standard deviations.

24h and at 120h it was about half of that observed at 24h. These data agree closely with our previous results (Varty & Laidman, 1976) obtained from a different cultivar. The response pattern is, therefore, probably characteristic of wheat generally. Comparison of the graphs in Fig. 1(b) with those in Fig. 1(a) shows the coincidence of phospholipid breakdown with α -amylase secretion.

Incorporation and turnover of [U - ^{14}C]glycerol in phospholipids, diacylglycerols and triacylglycerols

We used the 24h lag period before the appearance of α -amylase activity, and when phospholipids

were accumulating, to pulse-label the aleurone tissue with [U - ^{14}C]glycerol. This period also corresponds to the time when endoplasmic reticulum is rapidly synthesized (Colborne *et al.*, 1976). During this time radioactivity was incorporated more rapidly into phospholipids (Fig. 2a) than into triacylglycerols (Fig. 2b), supporting the suggestion that phospholipids can act as precursors of triacylglycerols in plants (Slack *et al.*, 1978). Incorporation into 1,2- and 1,3-diacylglycerols (Figs. 2c and 2d) was much less than into triacylglycerols. Up until 18h in the pulse-labelling period, gibberellic acid had no effect on precursor-incorporation. Between 18 and 24h, precursor incorporation into the total lipid fraction continued equally in both the control and the hormone-treated tissues. Within this general trend, however, there was a sudden and apparent cessation of incorporation into the phospholipid fraction in the hormone-treated tissue but not in the control. Further, the loss of phospholipid radioactivity compared with the control was exactly balanced by an increase in radioactivity in triacylglycerols in the hormone-treated tissue compared with the control. Gibberellic acid had no effect on the levels of radioactivity in the diacylglycerols.

During the decay incubation that followed the pulse-labelling, radioactivity disappeared from the phospholipids in both control and hormone-treated tissue, reappearing in triacylglycerols (Figs. 2a and 2b). The conversion of radioactivity from phospholipids into triacylglycerol was quantitative up to 96h, after which time there was an overall loss of radioactivity from the system (Table 1). The conversion was considerably faster in the hormone-treated tissue than in the control tissue. In contrast, gibberellic acid had no effect on the decay of radioactivity from the diacylglycerols (Figs. 2c and 2d).

Table 2 shows results from the analyses of individual phosphatides. Four major phosphatides (phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidylglycerol) accounted for most of the observed metabolism. All four phosphatides increased in concentration during the 24h pulse-labelling period and they all actively incorporated labelled glycerol. Phosphatidylserine, lysophosphatidylcholine, phosphatidic acid and the *N*-acyl phosphatides incorporated very small quantities of radioactivity (results not shown). Up until 18h, incubation with gibberellic acid did not affect the quantities of radioactivity in the phosphatides. Between 18 and 24h, however, it inhibited the apparent incorporation into phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. The lost radioactivity from these phosphatides accounted for that lost from the total phospholipid fraction into the triacylglycerols during the same period (see Fig. 2). Phosphatidylcholine

Fig. 2. Incorporation and turnover of [^{14}C]glycerol in total phospholipids (a), triacylglycerols (b), 1,2-diacylglycerols (c) and 1,3-diacylglycerols (d)

●, Control tissue incubated without gibberellic acid; ○, tissue incubated with $1\text{ }\mu\text{M}$ -gibberellic acid. Each value is the average from two determinations.

Table 1. Changes in transfer of radioactivity from total phospholipids to triacylglycerols
De-embryoned seeds were pulse-labelled with [$\text{U-}^{14}\text{C}$]glycerol for 24 h and then incubated for a further 96 h. Incubations were carried out either in the absence or in the presence of $1\text{ }\mu\text{M}$ -gibberellic acid (see the Materials and methods section for details). Each value is the average from two determinations.

Tissue treatment	Incubation period (h)	$10^{-3} \times \text{Radioactivity (d.p.m./30 seeds)}$		
		Phospholipids	Triacylglycerols	Total
Without gibberellic acid	24	122	43	165
	48	99	68	167
	72	89	71	160
	96	80	83	163
	120	74	79	153
With gibberellic acid	24	100	64	164
	48	78	98	176
	72	63	106	169
	96	45	109	154
	120	22	117	139

accounted for most (approx. 80%) of the change. Although the incorporation of radioactivity into phosphatidylcholine was reduced by gibberellic acid,

the concentration of the phosphatide was not affected between 18 and 24 h.

During the decay incubation two trends were

Table 2. *Effects of gibberellic acid on phosphatide levels and the turnover of [^{14}C]glycerol in phosphatides*
De-embryoed seeds were pulse-labelled with [$\text{U-}^{14}\text{C}$]glycerol for 24 h and then incubated for a further 96 h. Incubations were carried out either in the absence or in the presence of $1\text{ }\mu\text{m}$ -gibberellic acid (GA; see the Materials and methods section for details). Each value is the average from two determinations

Incubation period	Phosphatide level ($\mu\text{mol}/30$ seeds)		$10^{-3} \times$ Radioactivity (d.p.m./30 seeds)		$10^{-3} \times$ Sp. radioactivity (d.p.m./ μmol)	
	–GA	+GA	–GA	+GA	–GA	+GA
Phosphatidylcholine						
12	0.54	0.54	40.8	40.8	—	—
18	—	—	54.7	55.9	—	—
24	0.61	0.63	72.6	53.7	119	85
48	0.67	0.63	53.4	37.1	80	59
72	0.73	0.56	46.4	28.7	64	51
96	0.73	0.48	40.7	19.0	56	40
120	0.74	0.40	35.2	10.4	48	26
Phosphatidylinositol						
12	0.23	0.23	7.5	7.5	—	—
18	—	—	12.7	13.1	—	—
24	0.25	0.24	18.0	16.3	72	68
48	0.24	0.24	18.0	15.0	75	63
72	0.23	0.22	16.0	12.8	70	58
96	0.25	0.24	15.7	9.3	63	39
120	0.25	0.23	15.5	4.0	62	17
Phosphatidylethanolamine						
12	0.10	0.10	4.7	4.9	—	—
18	—	—	8.5	9.1	—	—
24	0.13	0.14	13.4	11.6	103	83
48	0.15	0.16	14.2	11.1	95	69
72	0.17	0.15	13.2	9.4	78	62
96	0.17	0.16	12.3	8.6	72	54
120	0.18	0.14	11.6	4.0	64	29
Phosphatidyl glycerol						
12	0.05	0.05	5.1	5.2	—	—
18	—	—	11.2	11.2	—	—
24	0.05	0.05	13.3	13.9	270	280
48	0.06	0.05	7.3	6.2	120	130
72	0.06	0.06	5.7	4.5	95	80
96	0.06	0.06	5.2	2.7	83	45
120	0.07	0.05	4.6	1.1	71	20

observed, illustrated by phosphatidylcholine and phosphatidylinositol respectively. Phosphatidylcholine concentration in the control tissue increased slowly, whereas its radioactivity declined, indicating turnover of the phosphatide. In the hormone-treated tissue, its concentration decreased, whereas its radioactivity decreased more rapidly (specific radioactivity also decreased more rapidly), indicating an increased turnover and overall breakdown. In contrast, the phosphatidylinositol concentration did not change during the decay incubation and it was unaffected by gibberellic acid. Its radioactivity, and therefore its specific radioactivity, declined very slowly in the control tissue but rapidly in the presence of gibberellic acid. Thus, gibberellic acid caused a turnover of phosphatidylinositol without affecting its pool size significantly. The concentrations of phosphatidylethanolamine and phosphatidylglycerol increased marginally through the incubation and they were not affected significantly by gibberellic acid. Phosphatidylethanolamine turnover resembled that of phosphatidylinositol, being slow in the control and rapid in the hormone-treated tissue. Turnover of phosphatidylglycerol was already fairly rapid in the control but it was also increased in the presence of gibberellic acid; it resembled phosphatidylcholine in this respect.

During the decay incubation, the decline of radioactivity appeared to be exponential, at least in some of the phosphatides. This suggested that first-order decay kinetics might exist. We investigated this possibility by plotting logarithms of radioactivity against time as shown in Fig. 3. In the case of the control tissue, the data for each phosphatide gave a straight line and half-life values could be conveniently calculated. Phosphatidylcholine

Fig. 3. Semi-logarithmic plots of the decay of radioactivity from phosphatidylcholine (a), phosphatidylinositol (b), phosphatidylethanolamine (c) and phosphatidylglycerol (d)

●, Control tissue incubated without gibberellic acid; ○, tissue incubated with $1\mu\text{M}$ -gibberellic acid. Each value is the average from two determinations. Turnover half-life values are indicated in parentheses. The 48–120h values were taken from Table 2 and the 144h values were taken from original results not shown in Table 2.

ine and phosphatidylglycerol had similar half-lives, which were considerably shorter than those of phosphatidylinositol or phosphatidylethanolamine. In the hormone-treated tissue, however, turnover appeared to take place in two phases, a relatively slow one between 48 and 96h followed by a faster turnover between 96 and 144h. Turnover during both phases was considerably faster than that in the control tissue. In the first phase phosphatidylcholine and phosphatidylglycerol again had shorter half-lives than phosphatidylinositol and phosphatidylethanolamine, but in the second phase all of the phosphatides had similar and very short half-lives.

Discussion

In preliminary experiments we found that $[^{14}\text{C}]$ -glycerol had clear advantages over $[\text{Me-}^{14}\text{C}]$ choline

or $[^{32}\text{P}]\text{P}_i$ as a label in studies of phospholipid metabolism in aleurone tissue. In particular, radioactive incorporation was conveniently confined to the pulse-labelling period and little further incorporation occurred into total lipids during the following decay incubation. Estimations of free $[^{14}\text{C}]$ glycerol remaining in the tissue at the end of the pulse-labelling period (results not shown) supported the view that most of the precursor had been either incorporated or washed from the tissue before the decay incubation was started. Also, preliminary experiments showed that $[^{14}\text{C}]$ glycerol was almost exclusively incorporated into the glycerol moiety of the lipid glycerides (97% recovered as glycerol or glycerol 3-phosphate after hydrolysis), so that metabolic conversions between glyceride classes could be followed. The data for phosphatidylglycerol could, however, have been compromised by

incorporation into the non-acylated glycerol moiety of the molecule, although this was not detected in degradation studies on the total phospholipid fraction (results not shown).

Data for the turnover of phospholipids in plants are scarce. The turnover half-life of [^{14}C]choline in phosphatidylcholine in onion stem was found to be 43 h (Morré, 1970), and that in germinating wheat embryos was 35–45 h (McDonnell *et al.*, 1982). The turnover half-life of [^3H]glycerol in various phosphatides in dormant *Avena fatua* embryos varied from 26 to 56 h (Cuming & Osborne, 1978). Our data from aleurone tissue are comparable with these values, at least in the case of the hormone-treated tissue.

The hormone-stimulated transfer of radioactivity from phospholipids to triacylglycerols could be interpreted as a redirection of biosynthesis away from the former into the latter. The quantitative nature of the exchange, especially during the decay incubation when gross incorporation of glycerol had ceased, leads us, however, to prefer the alternative possibility; that is, the stoichiometric metabolism of the phospholipids into triacylglycerols. At first sight, 1,2-diacylglycerol would be an expected intermediate in the metabolism, but the patterns of radioactivity in the diacylglycerols gave no indication of this. Diacylglycerol could still be an intermediate, however, since the rate of metabolism of phospholipid to diacylglycerol might be slow compared with the rate of metabolism of diacylglycerol to triacylglycerol, so that radioactivity does not accumulate in the diacylglycerol fraction. Regarding the mechanism of the metabolism, the aleurone tissue contains a very active phospholipase D (Colborne & Laidman, 1975). There is no evidence, however, that it is involved in phospholipid metabolism in this system. Alternatively, phosphatide-specific phosphodiesterases, similar to the phosphatidylinositol phosphodiesterase found in celery (Irvine *et al.*, 1980), might be involved. This enzymology is clearly an important aspect awaiting investigation.

Several aspects of the gibberellic acid response in aleurone tissue resemble the response of secretory animal tissues to external agonists (see Michell, 1975; Hawthorne, 1982). Thus, the aleurone tissue has an exocytotic function, secreting hydrolases, including α -amylase, in response to hormone (Filner *et al.*, 1969). The secretory mechanism involves vesicular transport within the cell (Gibson & Paleg, 1972; Firn, 1975) leading to exocytosis through the plasmalemma (see Tomos & Laidman, 1979; Laidman, 1980). The synthesis and secretion of α -amylase requires Ca^{2+} (Goodwin & Carr, 1972). Gibberellic acid action also leads to the release of mineral ions, including K^+ and Ca^{2+} , from aleurone tissue (Eastwood & Laidman, 1971). This response

is superficially similar to the muscarinic, cholinergic and α -adrenergic stimulation of K^+ and amylase secretion in rat parotid gland (see Michell, 1975). In animals, emphasis has been placed on the importance of Ca^{2+} gating through the plasmalemma and the possible role of Ca^{2+} as a second messenger in the response to agonists (Michell, 1975; Kirk *et al.*, 1981). In contrast with the animal system, however, Ca^{2+} transport in the aleurone cell appears to be in the opposite direction, taking place from the storage aleurone grains to the outside of the cell. Hormone-induced Ca^{2+} mobilization from the aleurone grains could, nevertheless, lead to elevated Ca^{2+} levels in the cytosol, and the possibility exists that Ca^{2+} is a second messenger in the gibberellic acid response.

Ca^{2+} gating in stimulated exocytotic animal tissues is closely associated with increased phosphatidylinositol turnover. Our present study has shown that gibberellic acid action leads to increased phosphatidylinositol turnover taking place over the same time scale as α -amylase secretion from the aleurone tissue. A similar temporal relationship between phosphatidylinositol turnover and enzyme secretion has been observed recently in rabbit neutrophils (Cockcroft *et al.*, 1981). Cockcroft *et al.* (1981) also showed that Ca^{2+} gating leads to phosphatidylinositol turnover, rather than phosphatidylinositol turnover leading to Ca^{2+} gating, as previously thought. Although the response of the aleurone tissue to its agonist is much slower and more prolonged than the animal systems, our present data and those of Eastwood & Laidman (1971) are also consistent with a Ca^{2+} -gating-stimulated phosphatidylinositol turnover sequence.

Despite the similarities, there are also some major differences between phospholipid turnover in aleurone tissue and that in animals. In aleurone tissue, phosphatidylinositol is metabolized irreversibly into triacylglycerol, whereas in the animal system it is metabolized into 1,2-diacylglycerol, which is re-incorporated into phosphatidylinositol in a closed cycle. Also, the stimulation of phospholipid metabolism is not restricted to phosphatidylinositol as it is in the animal system. Turnover of phosphatidylethanolamine and phosphatidylserine is also stimulated after gibberellic acid action. The situation with respect to phosphatidylcholine is even more complex. Hormone action first causes a turnover, whereas in the longer term it leads to an overall breakdown of this phosphatide. The enhanced turnover, which accompanies the first appearance of secreted α -amylase activity, recalls the earlier reports that gibberellic acid induces phosphatidylcholine synthesis and endoplasmic-reticulum formation during the lag phase leading to α -amylase synthesis (Evins & Varner, 1971; Koehler & Varner, 1973; Johnson & Kende, 1971; Ben-Tal

& Varner, 1974). Although several of the experiments on which that view was based have been largely discounted for technical reasons (Laidman, 1980), the view itself can now be seen to contain some substance and deserves further re-examination. The early phosphatidylcholine turnover is possibly an aspect of endomembrane reorganisation rather than synthesis associated with the initiation of α -amylase synthesis and secretion. Gibberellic acid action over longer periods is known to lead to phospholipid breakdown (Varty & Laidman, 1976; Firn & Kende, 1974). Our present experiments confirm this and they have shown that the breakdown is almost entirely accounted for by the breakdown of phosphatidylcholine occurring concomitantly with the continued synthesis and secretion of α -amylase during the second and third days after hormone application. This particular phenomenon must be treated with some caution, however, since phospholipid breakdown does not occur in the aleurone tissue of whole germinating seeds (Varty & Laidman, 1976). The action of gibberellic acid is therefore different from that of the embryo in this respect and it contrasts with their similar induction of α -amylase synthesis. We have reported previously (Varty & Laidman, 1976) that diffusates from wheat embryos contain a factor(s) that attenuates the gibberellic acid action on phospholipid breakdown, although not inhibiting the induction of α -amylase synthesis. The nature of the factor and its mode of action are not known.

Although the response of the aleurone tissue to gibberellic acid differs in several respects compared with the responses of exocytotic animal tissues to their agonists, several similarities also exist. It is possible that the differences between the plant and the animal systems reflect a diversity in a basic mechanism that has been conserved in both kingdoms. In our view, the aleurone tissue system warrants further investigation to prove or disprove this proposal.

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