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CHANGES IN STARCH-BOUND LYSOPHOSPHOLIPIDS AND LYSOPHOSPHOLIPASE IN GERMINATING GLACIER AND HI AMYLOSE GLACIER BARLEY VARIETIES

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; germination; starch-lipid; lysophosphatidylcholine; lysophospholipase; amylose; inclusion complex.

Abstract—Total starch, amylose content and amylose-included lipid phosphorus and lysophosphatidylcholine (LPC) were measured in normal Glacier (G) and Hi Amylose Glacier (HA) barley varieties during germination. From days three to six, alkaline and acidic lysophospholipase (LPL) activities in the starchy endosperm were measured and the distribution of these activities between a soluble and particulate form determined. During germination the amylose content of the starches increases as the total starch levels decline. The starch-bound LPC and lipid phosphorus disappear at the same rate between days three and six in both barley varieties, indicating no discrimination among the different lipid-included amylose population for degradation. However, both lipid phosphorus and LPC disappear more rapidly in the G than in the HA variety. This is presumably due to the slightly larger content of LPC per mg amylose of the G than of the HA variety, equivalent to 134 and 150 anhydroglucose residues per lipid molecule in G and HA, respectively. There is no increase in starch-bound lipid phosphorus or LPC expressed as nmol of phosphorus or LPC per mg amylose as amylose content declines, indicating no selective resistance of lipid-included amylose to degradation. The alkaline and acidic LPC activities in each variety increase 2–4-fold between days four and five. In both varieties *ca* 30% of the acidic LPL and *ca* 50–60% of the alkaline LPL is particulate from days three to six. No correlation can be made between the content of amylose or amylose-included lipid and particulate LPL activity. However, the possibility that particulate LPL activity is associated with specific populations of residual amylose-included lipid molecules cannot be excluded.

INTRODUCTION

Starch-bound lysophosphatidylcholine (LPC), present as an inclusion complex in the amylose component of barley starch, disappears as germination progresses [1]. The lipid does not accumulate and is degraded by a lysophospholipase (LPL) which appears in the scutellum, aleurone and starchy endosperm [2, 3]. The enzyme activity, which is enhanced by gibberellic acid in barley half seeds and isolated aleurone layers [3], appears in a soluble and a particulate form and has activity over a broad pH range with a peak activity near pH 8 and an inflection near pH 5. The particulate activity of the starchy endosperm appears to be associated with the starch fraction and can be solubilized by salt extraction (unpublished observation). The transformation of a soluble starch synthetase to a particulate form in the presence of amylose, first observed by Akazawa and Murata [4], suggested to us that differences might be observed between the two varieties of barley, Glacier (G) and Hi Amylose Glacier (HA), with respect not only to the relative amounts of soluble and particulate LPL, but also to the level of starch lipids and to their metabolism. This communication presents the results of experiments examining such possible differences between these two varieties of barley.

RESULTS AND DISCUSSION

The mobilization of starch and the decline in its amylose component during germination of the HA and G barley varieties is shown in Figs. 1a and 1b. In each case, the convergence of the starch and amylose data points as germination progresses illustrates the increasing proportion of amylose in the residual starch and confirms the previously observed increase in the amylose–amylopectin ratio which accompanies the mobilization of starch in cereal grains [5]. The accessibility for attack by β-amylase of the large number of non-reducing ends of each amylopectin molecule compared with the single non-reducing end of an amylose molecule is clearly an important factor contributing to the increasing amylose–amylopectin ratio. We have also suggested [2] that the presence of inclusion lipids in the amylose component of the starch may hinder attack by this enzyme, thereby contributing to the increasing amylose composition of the starch.

The two G barleys used in this study differ in the amylose content of their starches. As the starch-bound lipid phosphorus is in the form of an inclusion complex with the amylose component of the starch [6], different metabolic profiles of starch mobilization and starch lipid metabolism during germination might be anticipated. Figures 1a and 1b show that substantial metabolism of the starch reserves occurs between days three and six of germination; consequently, starch-bound lipid phosphorus and LPC were measured at 1 day intervals in seeds which had imbibed water for 1 day and had germinated for 3–6 days. The data are shown in Table 1.

The starch-bound lipid phosphorus (μmol/seed, Table

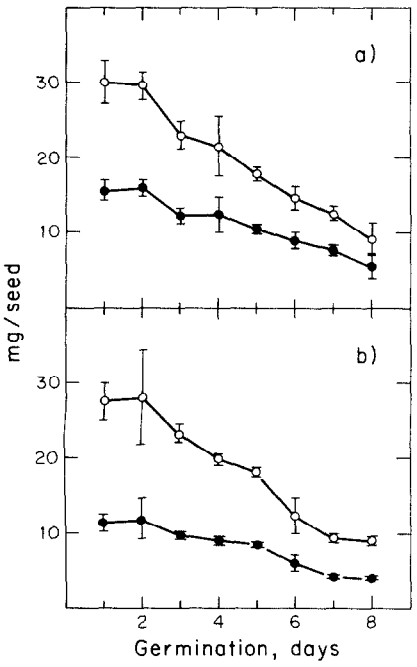

Fig. 1. Starch (○) and amylose (●) contents of the starchy endosperms of germinating HA (a) and G (b) varieties of barley. The methods used for the measurement of the polysaccharides are described in the Experimental. Each data point represents the average of at least duplicate measurements made on duplicate groups of 10 seeds each throughout the germination interval shown.

1) appears to suffer a sharper decline during the 3–6 day germination period in the G than in the HA variety; *ca* 50% loss in G and 32% in HA. These are the same percentage losses shown in the starch-bound LPC column for the respective barleys over this germination interval. This is consistent with the earlier observation [2] that there is no discrimination by degrading enzymes for amylose molecules included with LPC than for amylose molecules included with any other lysophospholipid.

Comparisons of Figs. 1a and 1b reveal that the overall rate of degradation of amylose in the two varieties between the third and sixth days of germination is essentially the same. The explanation for the increased rate of disappearance of lipid phosphorus in the G variety is found in Table 1 in which the lipid phosphorus is expressed as mg of amylose. Clearly, although the G variety has a lower amylose content, there is more included lipid per amylose molecule and, therefore, a greater loss of included lipid upon degradation of the polysaccharide. From Table 1, over the 4 days of germination, the average bound lipid phosphorus is 46 and 41 nmol/mg amylose for the G and HA varieties, respectively. Assuming the total lipid phosphorus to be present in a form similar to LPC, these values represent 134 and 150 anhydroglucose residues for each included lipid for the G and HA barleys, respectively. These values fall within the range of 100–200 calculated by Acker for the included lipids of the cereal starches [6].

Evidently, the presence of included lipid in the amylose does not affect its rate of degradation. Were the lipid to exert an inhibitory effect, the lipid phosphorus per mg amylose would increase during germination. The data in Table 1 suggest, therefore, that molecules of amylose included with the lysophospholipids are no more resistant or susceptible to degradation than amylose molecules not included with lipid. If the capping of the non-reducing ends of included amylose by the polar head groups of lysophospholipids were to inhibit attack by β-amylase, then it is likely that the degradation of included amylose occurs from attack by α-amylase.

Alkaline and acidic LPL activities dramatically increase in the starchy endosperm of both the HA and G barley varieties between the fourth and fifth days of germination as shown in Fig. 2. The proportion of each activity which is associated with the particulate fraction is shown in Table 2. It is clear that the alkaline activity is associated more with the particulate fraction than is the acidic activity. It is also evident that no direct correlation can be made between bound enzyme activity and either amylose or starch lipid content; the amount of LPL activity associated with the particulate fraction is increasing during this 3–6 day germination period and at the same time both the amylose and included lipid contents of the starches are decreasing (Fig. 1 and Table 2). This does not

Table 1. Loss of starch lipid phosphorus and LPC from G and HA varieties during germination

	Starch lipid							
	Phosphorus				LPC			
Germination (days)	μmol/seed G	μmol/seed HA	nmol/mg amylose G	nmol/mg amylose HA	μmol/seed G	μmol/seed HA	nmol/mg amylose G	nmol/mg amylose HA
1	0.50	0.50	43	32	0.38	0.38	33	25
3	0.50	0.53	50	41	0.36	0.34	36	28
4	0.44	0.50	48	34	0.28	0.33	31	26
5	0.32	0.44	38	40	0.23	0.27	27	26
6	0.26	0.36	50	43	0.18	0.23	30	26

The lipid phosphorus determinations were made as described in the Experimental. Each value is the average of at least two measurements, each of which is reproducible to within ± 10% of the average. The values for mg amylose were the averages used in Fig. 1.

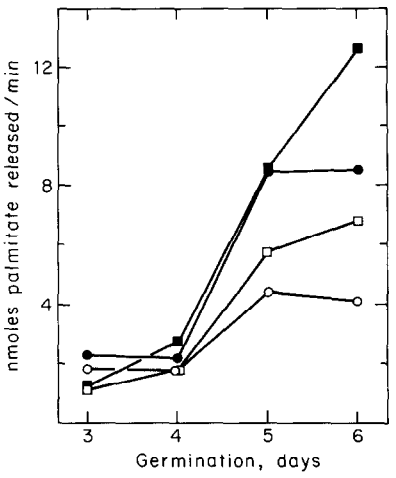

Fig. 2. Lysophospholipase activities per seed of the starchy endosperms of barley. Normal Glacier: alkaline (■) and acidic (□) activities; HA: alkaline (●) and acidic (○). Isolation and measurements of the lysophospholipase activities are described in the Experimental.

Table 2. Percentage of total LPL activities associated with the starch fraction in G and HA varieties germinated for three to six days

	Total LPL associated with the particulate fraction (%)			
Germination (days)	3	4	5	6
Alkaline HA	43	54	57 ± 4	47
G	48	64	61 ± 7	65
Acidic HA	30	32	36 ± 4	25
G	32	32	31 ± 1	32

The acidic and alkaline LPL activities were measured on the crude supernatant obtained from a 4000 *g* centrifugation of a homogenate of the starchy endosperm of the two barley varieties. The pellet fraction was resuspended and the LPL activities assayed as described in the Experimental. The values represent the percentage of the total LPL activity in the starchy endosperm that is associated with the 4000 *g* pellet. With the exception of day five which was run on duplicate batches of germinated seeds, the remaining values are single determinations.

preclude the possibility that the enzyme is binding to a specific population of amylose or lipid-included amylose molecules. However, as it is the polar head group of the included lipid which apparently distinguishes one amylose population from another, then it seems reasonable to assume that, if the particulate enzyme is associated with starch, it is the lipid-included amylose that may be the site of binding.

EXPERIMENTAL

Materials. Seeds of *Hordeum vulgare* L. cvs Glacier and Hi Amylose Glacier 1970 crops were kindly supplied by Dr. Robert F. Eslik, Montana State University. The average seed wt of the HA variety was 48 mg and that of the normal variety (G), 52 mg. Unlabelled 1-palmitoyl LPC and wheat starch were from Sigma. 1-[1-^{14}C]Palmitoyl LPC (5 mCi/mmol) was from Amersham, U.K. Potato amylose was a product of the U.S. Biochemical Corporation, Cleveland, Ohio.

Seed germination. The seeds were germinated in the dark by total immersion in H_2O for 24 hr and then transferred to moist paper towels in loosely covered dishes at 20–23° for specified times. The seeds were rinsed with H_2O each day.

Measurement of starch and amylose. Starch was measured according to the procedure of ref. [7]. For each germination interval the starchy endosperms were separated from duplicate groups of 10 seeds. Each endosperm sample was macerated in 10 mM Pi buffer (pH 7) with a pestle and mortar. The starch pellet produced by centrifugation of the resulting suspension at 800 *g* for 5 min was washed repeatedly with 80% EtOH. The washed starch was digested with 52% $HClO_4$ and the starch content measured by the anthrone–H_2SO_4 method described in ref. [8]. The amylose content of the starches was determined according to the method of ref. [9]. An aliquot of the $HClO_4$ soln containing 0.1 mg of each starch sample was diluted to 4.95 ml with H_2O. An I_2–KI soln (0.05 ml) was added and after 30 min the *A* at 660 nm was measured. The blank was starch-free $HClO_4$. The I_2–KI soln was made up of 250 mg I_2 and 2.5 g KI in 125 ml H_2O. A standard curve for the measurement was made using mixtures of known composition of potato amylose and amylopectin. The latter was isolated from wheat starch after removal of amylose according to ref. [10]. Measurements of starch and amylose were made in duplicate on each starch sample.

Isolation and measurement of starch-bound lipid phosphorus and LPC. Duplicate batches of 50 seeds at specific germination intervals were used. The procedure used for the isolation of starch-bound lipid was that described in ref. [1]. Total organic phosphorus was measured on the processed lipid according to the method of ref. [11]. LPC-P was measured on the LPC zone scraped from the TLC plates after separation of an aliquot of the processed lipid according to the procedure described in ref. [1].

Isolation and assay of LPL of the starchy endosperm. Batches of the two barleys were germinated for 3–6 days. Duplicate groups, each of nine seeds, were used for the measurements. The starchy endosperm was removed from the seeds and macerated with 6 ml ice-cold Pi buffer (10 mM, pH 7) with a pestle and mortar. The suspension was spun at 4000 *g* for 5 min. The pellet was resuspended in the Pi buffer to give a total vol. of 3 ml. Both the supernatant and the pellet suspension were assayed for LPL activity according to the method of ref. [2]. LPC (12 000 cpm, 200 nmol) was evaporated in a 15 ml centrifuge tube. The substrate was dissolved in 100 mM Tris–HCl (pH 8, 0.8 ml) for the alkaline LPL assay, and in 100 mM NaOAc (pH 5, 0.6 ml) for the acidic assay. Appropriate amounts of either supernatant or pellet suspension were added to give final vols. of 1.0 ml. The assays were run at 25° and were stopped with 6 ml heptane–*iso*-PrOH–0.5 M H_2SO_4 (60:40:1). One half of the heptane extract was counted using a scintillation counting fluor composed of 4 g PPO in 1 l. toluene–Triton X-100–H_2O (6:3:1).

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REFERENCES

1. Baisted, D. J. (1981) *Phytochemistry* **20**, 985.
2. Baisted, D. J. and Stroud, F. (1982) *Phytochemistry* **21**, 29.
3. Baisted, D. J. and Stroud, F. (1982) *Phytochemistry* **21**, 2619.

4. Akazawa, T. and Murata, T. (1965) *Biochem. Biophys. Res. Commun.* **19**, 21 (1965).
5. Fukui, T. and Nikumi, Z. (1974) *J. Biochem. (Tokyo)* **43**, 33.
6. Acker, L. (1977) *Fette, Seifen, Anstrichm.* **79**, 1.
7. McCready, R. M., Guggolz, J., Silviera, V. and Owens, H.S., (1950) *Analyt. Chem.* **22**, 1156.
8. Spiro, R. G. (1966) *Methods in Enzymology* (Neufeld, E. F. and Ginsburg, V., eds.) Vol. 8, p. 4. Academic Press, New York.
9. McCready, R. M. and Hassid, W. (1943) *J. Am. Chem. Soc.* 1154.
10. Banks, W. and Greenwood, C. T. (1975) *Starch and Its Components* p. 13. Edinburgh University Press, Edinburgh.
11. Bartlett, G. R. (1951) *J. Biol. Chem.* **234**, 466.