Short Communication

Lipase Activities in Scutella of Maize Lines Having Diverse Kernel Lipid Content

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ABSTRACT

The maize lines, Illinois High Oil, Illinois Low Oil, and their F₁ generations, contained about 18%, 0.5%, and 10%, respectively, of kernel lipids, which were present mostly in the scutella. We explored to see if the activities of gluconeogenic enzymes which appeared in postgerminative growth were proportional to the lipid content in each maize line. This proportionality was found to be valid in lipase, but the two glyoxysomal enzymes, catalase and isocitrate lyase, were the same in the three maize lines irrespective of the lipid content. The results suggest a difference in the genetic control of the gluconeogenic enzymes and a co-selection for high lipid content and high lipase activity through breeding.

The kernels of most maize lines contain 4% lipid by weight, localized mainly in the scutella. The lipid is used for gluconeogenesis to support the growth of the embryonic axis in postgerminative growth (9). Breeding maize for high or low lipid kernels has been successful in producing maize lines having kernels containing from 20% to less than 0.5% lipid (3). The lipid content is controlled by multigenic inheritance involving about 50 genes. A study was made to see if the change in lipid content through breeding was associated with a similar change in the enzymes of the gluconeogenic pathway in postgerminative growth. This was shown not to be the case for three glyoxysomal enzymes in two maize lines having a 40-fold difference in lipid content in their kernels (10). When the enzyme activities were calculated based on the amount of protein in the scutellum extracts, catalase and malate synthase were the same in the high and low lipid maize lines, and isocitrate lyase in the low lipid maize line was only 50% less than the high lipid maize line. Lipase is the first enzyme in the gluconeogenic pathway, and is associated with the membrane of the lipid bodies where the intracellular lipid is localized (5). Since the lipase is intimately linked to the lipid, both physically and metabolically, it is of interest to see if its activity is related to the content of lipid in the maize lines. We find this to be indeed the case, which is different from that of the glyoxysomal enzymes. The results suggest a difference in the genetic control of the gluconeogenic enzymes and a co-selection for high lipid content and high lipase activity through breeding.

MATERIALS AND METHODS

Plant Materials. The kernels of two maize (Zea mays L.) lines, Illinois High Oil (IHO, containing 18% lipid), were kindly provided by Dr. J. W. Dudley of the University of Illinois. The two lines were crossed (female IHO × male ILO) to yield kernels of the F₁ generation. The kernels were soaked in running tap water for 24 h, and allowed to germinate in moist vermiculite at 28°C in the dark. Seedlings of uniform size were collected at daily intervals, and the scutella were separated from the endosperm and embryonic axes. The scutella were homogenized in precooled grinding medium with a Willems Polytron (Brinkman Instruments) at speed 6 for 45 s. The grinding medium contained 0.15 M Tricine-KOH buffer (pH 7.5), 1 mM MgCl₂, 10 mM KCl, 1 mM EDTA, 2 mM DTT, and 0.6 M sucrose. The homogenate was filtered through a Nitex cloth with pore size 20 × 20 μm. The filtrate was used directly in various assays. Assays of enzyme activities were performed immediately, and were completed within 8 h after grinding.

Assays. Catalase activity was assayed spectrophotometrically at 24°C by the disappearance of H₂O₂ (4). Isocitrate lyase activity was assayed spectrophotometrically at 24°C with phenylhydrazine (4). Lipase activity was determined by a colorimetric method (5, 8). The 1-ml reaction mixture contained 0.1 M Tris-HCl buffer (pH 7.5), 5 mM DTT, 5 mM trilinolein, and enzyme preparation. Trilinolein was emulsified in 5% gum arabic (Specialty Chemical, Morristown, NJ) for 30 s at high speed with a Bronwill Biosonik IV ultrasonic generator (VWR Scientific, San Francisco, CA) fitted with a microprobe. The reaction was carried out at 34°C in a shaker-water bath, and the fatty acid contents in aliquots of the reaction mixture were measured. For each assay, two enzyme concentrations and five appropriate time intervals of sampling of the reaction mixture were carried out to ensure that linear kinetics were obtained. The activity of lipase was expressed as μmol fatty acid released per h.

Protein content was determined by the Bradford method (1), using BSA (fraction V) as the standard. In the assay of lipid content, an aliquot of the filtrate was extracted with a chloroform-heptane-methanol mixture (4/3/2, v/v/v), and the acyl ester bonds in the organic solvent fraction were determined by the Dittmer and Wells method (2). Trilinolein was used as the standard, and the lipid content was expressed as μmol equivalent of trilinolein. Phospholipid content was determined by Dittmer and Wells method (2).

Lipid bodies were isolated from scutellum homogenates by repeated flotation centrifugation as described (5). They were photographed by Normarski differential-interference/contrast microscopy. Four fields on each slide of a preparation from one maize line were photographed. Each negative was projected onto a screen by a slide projector, and the sizes of about 200 lipid bodies from each maize line were measured.

RESULTS AND DISCUSSION

Two maize lines, IHO which contains 18% kernel lipid, and ILO which contains less than 0.5% kernel lipid (3), and the F₁...
generation (female IHO × male ILO) which contains about 10% kernel lipid, were used. The difference in the kernel lipid content of the maize lines is due partly to the size of the kernel and the scutella (3). IHO have relatively smaller kernels but larger scutella than the ILO. In addition, we observed that the ILO kernel germinated (protrusion of the embryonic axis) about 1 d slower than the IHO kernel. In spite of these differences among the three maize lines, there were consistent patterns of change in the fresh weight, lipid, and protein content in the scutella during the course of seedling growth (Fig. 1). Whereas the fresh weight and protein remained about the same in the first 6 d, the lipid started to disappear after the 2nd d.

We studied the pattern of appearance of the activities of lipase and two glyoxysomal enzymes (catalase and isocitrate lyase) during the first 6 d of seedling growth when lipolysis was most active. Since the sizes of the scutella in the three maize lines were very different (see fresh weight in Fig. 1), we compared their enzyme activities based on the amount of protein in the scutellum. It should be noted that the protein content remained about the same during the first 6 d of seedling growth (Fig. 1). During the course of seedling growth, catalase and isocitrate lyase activities increased to the same extent in IHO, ILO, and F1 (Fig. 2).

![Fig. 1](image1.png)

**Fig. 1.** Changes in fresh weight, lipid, and protein of the scutella of seedlings of the maize lines Illinois High Oil (H), Illinois Low Oil (L), and their F1 generation. The amounts are expressed on a per scutellum basis.

![Fig. 2](image2.png)

**Fig. 2.** Changes in the activities of catalase, isocitrate lyase, and lipase in the scutellum extracts of seedlings of the maize lines Illinois High Oil (H), Illinois Low Oil (L), and their F1 generations. The amounts are expressed on per mg protein in the scutellum extracts. Thus, the activities of these two glyoxysomal enzymes are similar in the scutella of the three maize lines irrespective of the lipid content. The results are quite consistent with the earlier findings that the activities of three glyoxysomal enzymes (catalase, malate synthase, and isocitrate lyase) were similar in IHO and ILO (10). Nevertheless, we observed no substantial difference (instead of a 50% reduction in ILO) in isocitrate lyase activity between IHO and ILO; no explanation for the discrepancy can be offered at this time.

In sharp contrast, lipase activity increased to a much higher amount in IHO, and only slightly in ILO, during seedling growth (Fig. 2). The pattern is clearly different from that of catalase and isocitrate lyase. The total lipase activity peaked slightly after those of catalase and isocitrate lyase in seedling growth. This is consistent with our observation on normal maize lines having 4% lipid in the kernel (MO 17 and EK 3732-F12), and is presumably due to the remnant of lipase in the cells after the lipid bodies had been consumed (5, 6). When the lipase activity was calculated based on the amount of lipid in the scutella of
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Fig. 3. Changes in the activities of lipase in the scutellum extracts of seedlings of the maize lines Illinois High Oil (H), Illinois Low Oil (L), and their F1 generation. The amounts are expressed on per µmol of trilinolein in the ungerminated kernels.

Table I. Characteristics of Lipid Bodies Isolated from the Scutella of Kernels of Maize Lines Illinois High Oil (IHO), Illinois Low Oil (ILO), and Their F1 Generation

<table>
<thead>
<tr>
<th></th>
<th>IHO</th>
<th>F1</th>
<th>ILO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (diameter in µm)</td>
<td>1.31</td>
<td>1.24</td>
<td>1.09</td>
</tr>
<tr>
<td>SE</td>
<td>0.47</td>
<td>0.57</td>
<td>0.39</td>
</tr>
<tr>
<td>Protein/total lipid</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Phospholipid/total lipid</td>
<td>0.08</td>
<td>0.04</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The mean size of lipid bodies of IHO is larger than that of ILO at a 0.01 level of significance.

In ungerminated kernel, it became very similar in IHO, ILO, and the F1 (Fig. 3). Thus, the lipase activities in the three maize lines are proportional to their respective lipid content. In all studies, the contents of lipid, protein, and enzyme activities in the F1 were intermediate between those in IHO and ILO.

Lipid bodies isolated from the scutella of ungerminated kernels of the three maize line were compared. There was no substantial difference in the size, protein content, and phospholipid content among the three maize lines (Table I). The lipid bodies from IHO were only slightly, although of statistical significance, larger than those from ILO. This slight difference in size was reflected in the lesser content of protein and phospholipid per unit lipid in the lipid bodies from IHO (i.e., lesser membrane surface area per unit lipid). By calculation, the total lipid bodies from IHO had total surface area (where the lipase is localized) per unit lipid only 19% less than those from ILO. This minor difference is relatively unimportant in view of the enormous difference of lipid content among the maize lines. In addition, the lipid bodies from IHO, ILO, and the F1 all contained the same major protein components of mol wt 51,000, 22,000, and 21,000 in SDS-PAGE (data not shown). Similar to the normal maize lines (5), 50 to 70% of the total lipase activity in the scutellum extracts of 4-d-old seedlings of the three maize lines could be recovered in the isolated lipid bodies. Thus, the lipase activity in the three maize lines correlated with the lipid content or approximately with the number of lipid bodies.

The results suggest that the lipase of the lipid bodies and the enzymes of the glyoxysomes are under separate genetic control. A noncoordinate expression of their activities occurs in the high and low lipid maize lines. Selection for high lipid in maize lines apparently also selects for high lipase activity but not the subsequent gluconeogenic enzymes. The mechanism of coordinate selection for both high lipid and high lipase activity is unknown. There are about 50 genes for the expression of high lipid in IHO (3), and they are expressed only in kernel formation and not in seedling growth. Yet, lipase activity is absent in the forming and ungerminated kernel, and appears only after germination (postgerminative growth). It is unlikely that the genes for high lipid content are tightly linked to the lipase gene(s). It is possible that the high lipid genes and the lipase genes are both expressed in kernel formation, but the latter expression results in the production of pro-lipase protein or m-RNA, which is processed to active lipase in postgerminative growth. Alternatively, the lipase is synthesized or degraded in postgerminative growth in proportion to the availability of substrate and thus metabolic need. Whatever the mechanism is, it is different from the control of expression of the glyoxysomal enzymes. In the present study, we used catalase and isocitrate lyase to represent the glyoxysomal enzymes. It should be noted that in Anise cell culture, the glyoxysomes together with the enzymes of the β-oxidation and glyoxylate cycle can be coordinately induced by the addition of acetate to the growth medium (7).

Acknowledgment.—We thank Dr. J. W. Dudley for the supply of kernels of Illinois High Oil, Illinois Low Oil, and other maize lines; Dr. L. Knapp and Dr. M. Dewey for assistance in microscopy; and Dr. M. Felder for valuable suggestions.

LITERATURE CITED

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Corrections

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Michael J. Muhitch and John S. Fletcher. Isolation and Identification of the Phenols of Paul's Scarlet Rose Stems and Stem-Derived Suspension Cultures.
The term 'gallotannins' should be replaced by 'galloyl esters of flavan-3-ols'.

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Shue-mei Wang, Yon-hui Lin, and Anthony H. C. Huang. Lipase Activities in Scutella of Maize Lines Having Diverse Kernel Lipid Content.
Page 837, column 1, last line should read: Illinois High Oil (IHO, containing 18% lipid) and Illinois Low Oil (ILO, containing less than 0.5% lipid) were kindly provided . . .