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Accumulation of Free Ricinoleic Acid in Germinating Castor Bean Endosperm[1]

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ABSTRACT

Lipids from the endosperm of germinating castor bean (*Ricinus communis* var. Hale) were separated by thin layer chromatography and quantitated by gas chromatography. During the later stages of lipid breakdown (4-6 days germination at 30 C), several lipid classes were found in addition to the storage triglycerides, which are triricinoleins for the most part. One was identified as free ricinoleic acid, the proportion of which increased as germination progressed. After 6 days germination, ricinoleic acid comprised more than 30% of the total lipid. The appearance of this fatty acid implies that lipase activity (lipolysis) is not strictly coordinated with β oxidation in this tissue.

There is little change in the storage lipid content of castor bean endosperm during the early stages of germination (13) although an active acid lipase is associated with the storage lipid bodies, or spherosomes (17). The net disappearance of the storage lipid, consisting mainly of triricinolein, occurs later, coincidentally with the development of glyoxysomal alkaline lipase, β oxidation, and the glyoxylate cycle enzymes (12, 13). It has been supposed that the storage lipid is converted to sucrose without the accumulation of free ricinoleic acid (1, 3, 11, 13). The possible occurrence of free ricinoleate has not been examined directly.

MATERIALS AND METHODS

Castor beans (*Ricinus communis* L. var. Hale) were imbibed, germinated for varying periods, and the endosperms homogenized in the usual manner (13). The storage lipid was separated from the homogenate by centrifugation (13). A sample of the storage lipid equivalent to about one bean was extracted in chloroform-methanol (8). Alternatively, whole endosperms (10 g) were extracted directly in hot isopropyl alcohol and chloroform to minimize lipase activity (4). Extracts were stored at −15 C.

Lipid classes were separated on thin layer plates obtained from E. Merck (0.25-mm silica gel 60 on glass). Lipid extract samples equivalent to 0.4 mg (dry beans) to 40 mg (6-day germinated) endosperm were applied and developed with benzene-diethyl ether-ethanol (100:30:2, v/v). After development, the lipids were detected with I_2 vapor, the silica gel containing each lipid scraped up, sucked into a Pasteur pipette plugged with glass wool, eluted with chloroform-methanol (1:1, v/v), taken to dryness, and subjected to methanolysis (6).

The fatty acid methyl esters were analyzed on a column (60 cm × 5 mm) containing 3% SE-30 on 100/120 mesh Gas-chrom Q at 200 C, 35 cm³/min He flow in a Beckman GC 65 with flame ionization detector (250 C). The output of this gas chromatograph was quantitated electronically (Isco 950) relative to a constant amount of internal standard (heptadecanoate, C17) which had been included in the methanolysis reagent (6).

RESULTS

The hydroxyl group of ricinoleic acid imparts a polar character to the storage lipids of castor bean such that ricinoleate and glycerides containing it are separated from unhydroxylated fatty acids and their glycerides in TLC (Fig. 1) (11, 14). Triricinolein (TR_3), the most prominent component in castor bean, is the most polar triglyceride. Triglycerides containing two ricinoleates and one unhydroxylated acyl moiety are less polar (TR_2). Triglycerides of two or three unhydroxylated acyl moieties (TR_1 and TG) are least polar and are found close to the solvent front.

In addition to the triglycerides, free ricinoleic acid (R) was observed in castor bean extracts at all stages of germination, the proportion increasing in the latter stages, 4 to 6 days (Fig. 1). Free ricinoleic acid is very polar especially when the thin layer solvent mixture contains no acetic acid. In the presence of acetic acid, free ricinoleate is not separated from triricinolein. This may explain why other investigators have not observed free ricinoleic acid.

After 6 days germination when most of the storage lipid has been degraded (11, 13) several new lipid classes become apparent (Fig. 1). None were identified. The lipid spot designated UD, located between ricinoleic acid and triricinolein in TLC, may represent diricinolein since it contains mostly ricinoleate but does not correspond to the free acid or any of the known triglycerides.

The major storage lipid classes were characterized by their fatty acid content as shown in Table I. The major lipid spot (TR_3) contained only ricinoleate. TR_2 contained about two-thirds ricinoleate. The unidentified lipid (UD) consisted mainly of ricinoleate. The spot corresponding to the free ricinoleic acid standard was also comprised of mostly ricinoleate with small quantities of C18 and C16 fatty acids. In samples from 6-day germinated seed this spot contained only ricinoleic acid. Phospholipids contained fatty acids of the C16 and C18 series but no ricinoleate, as previously reported (7).

Free ricinoleic acid and its glycerides were further characterized by gas chromatography of alcohol acetate derivatives. Glycerides were hydrolyzed and the fatty acids converted to alcohols with $LiAlH_4$ (4). The alcohols, including glycerol, were then acetylated (4). These acetate esters were chromatographed under the same conditions as the methyl esters. The lipid identified as triricinolein (TR_3) was found to contain only glycerol and ricinoleate. No glycerol was detected in the ricinoleic acid spot (R).

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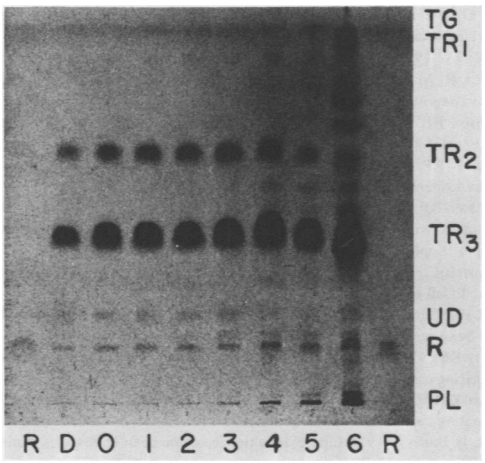

FIG. 1. Lipid components of castor bean endosperm separated by TLC. Whole endosperms were extracted with hot isopropyl alcohol and chloroform (4). The samples were equivalent to 0.4 mg dry seed (D), 0.8 mg imbibed seed (O), 0.8 mg endosperm germinated at 30 C for 1 day, 1.2 mg 2-day endosperm, 1.6 mg 3-day, 10 mg 4-day, 20 mg 5-day, and 40 mg 6-day endosperm. Triglyceride (TG) ran near the solvent front, triglyceride containing one ricinolein (TR_1) just behind followed by triglyceride containing two ricinoleins (TR_2), triricinolein (TR_3), an unknown lipid containing ricinoleate (UD), free ricinoleic acid (R), and phospholipid (PL) at the origin. Authentic ricinoleic acid was run on the extreme right and left sides of the thin layer plate.

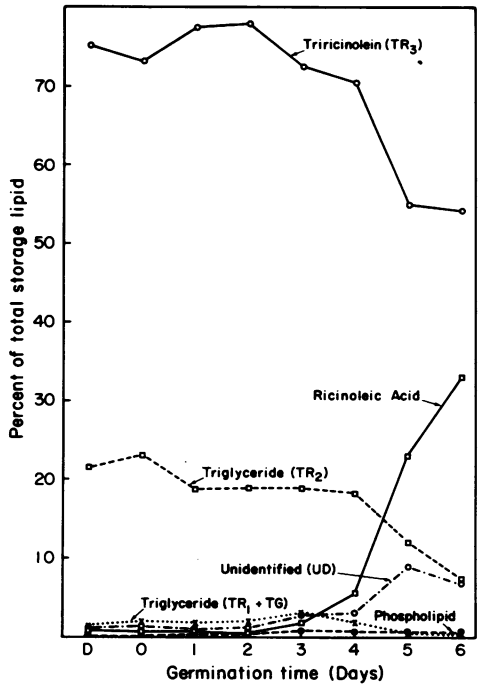

FIG. 2. Changes in storage lipid components during germination of castor bean endosperm. Storage lipid was obtained by centrifuging homogenates of endosperms from dry seed (D), imbibed seed (O), and seed germinated at 30 C for 1 to 6 days (13). Samples of the storage lipid were extracted with chloroform-methanol (8), the lipid classes separated (Fig. 1) by TLC and quantitated by gas chromatography (see under "Materials and Methods"). The lipid classes (TR_3, TR_2, TR_1, TG, and UD) correspond to those shown in Figure 1.

Figure 2 shows the changes in the several storage lipid components relative to each other during the progress of germination. This representation does not show the depletion in the absolute amount of lipid which is known to occur rapidly after 2 days germination (13). The proportions of triricinolein (TR_3) and other triglycerides (TR_2, TR_1, TG) all decrease relative to the total after 2 days germination. At the same time there is an increase in the relative amounts of free ricinoleic acid and an unidentified lipid containing ricinoleate (UD). This lipid may be a diglyceride. There is also a slight increase in phospholipid (PL) although the amount of phospholipid, representing organelle membranes, is small in comparison to the amount of storage lipid.

DISCUSSION

Each of the steps necessary for the ultimate conversion of stored triglyceride to sucrose has been described in castor bean (2). However, an orderly sequence of events has yet to be described. Although maximal lipolytic activity is manifest immediately after imbibition, the net disappearance of lipid begins only after 2 days germination (13).

One study indicates a net synthesis of lipid prior to 2 days (10). In these early stages of germination there is an active lipid synthetic system capable of converting acetate to diglyceride which appears in the lipid bodies. The newly synthesized diglycerides contain no ricinoleate (6). However, this activity does not significantly alter the composition of the storage lipid (11).

The net disappearance of lipid coincides with the development of an alkaline monoglyceride lipase which is a component of the glyoxysome membrane (13). This lipase achieves maximum activity along with the glyoxysome enzymes, β oxidation (12), and the glyoxylate cycle (9, 13). Although apparently specific for monoglyceride, this enzyme may exhibit broader capacities with the physiological substrates, glycerides containing ricinoleate. The acid lipase of the spherosomes has the ability to hydrolyze a variety of substrates depending on the acyl composition (16).

The accumulation of free ricinoleic acid and perhaps other products of lipid breakdown suggests that lipolysis and β oxidation are not completely coordinated. Such accumulation might be expected between the time of maximum lipolysis and maximum β oxidation, that is between 2 and 4 days. An increasing proportion of the free fatty acid began to appear after 3 days. However, this trend continued through the period of maximum β oxidation and beyond. The comparison of the data presented here with the measurements of total lipid (13) shows that the absolute amount of ricinoleic acid may increase from about 30 mg/10 endosperm halves to as much as 500 mg after 5 days.

The accumulation of free ricinoleic acid after 5 to 6 days may be the result of hydrolysis of a residual amount of storage lipid in the absence of a well organized system of β oxidation. At this stage of germination the endosperm has become partially senescent (18), and the activities of β oxidation (12) and the glyoxyl-

Table I. Fatty acid content of lipid classes from castor bean endosperm after 1 day germination.

The lipid classes were separated as shown in Fig. 1, and analyzed by gas chromatography on SE 30 which did not resolve saturated fatty acids from unsaturates. Thus, for example, C18 includes stearate, oleate, linoleate and linolenate.

Lipid Class	Abbreviation	Ricinoleate	C18	C16	C20-22
		percent fatty acid			
Triricinolein	TR_3	100.0	nd[1]	nd	nd
Triglyceride, two ricinolein	TR_2	69.0	27.4	3.6	nd
Triglyceride, one or no ricinolein	TR_1,TG	45.1	40.3	7.6	7.0
Unidentified	UD	97.3	2.7	nd	nd
Free ricinoleic acid	R	85.6	9.8	4.6	nd
Phospholipid	PL	nd	64.9	35.1	nd

[1] not detected

ate cycle have diminished (9). Also, the lipase activity has declined along with the total amount of lipid (13).

The acid lipase of castor bean is capable of converting triricinolein to glycerol and free ricinoleic acid with little accumulation of diglyceride or monoglyceride (15). Thus, an accumulation of free glycerol might also be expected to accompany the ricinoleic acid. This has been reported (5).

Further studies are required to understand the coupling of lipolysis in the spherosomes to lipolysis and β oxidation in the glyoxysomes. It is not known whether the glyoxysomes utilize triricinolein, diricinolein, monoricinolein, or the free fatty acids as physiological substrate, although isolated glyoxysomes are capable of activating and oxidizing free fatty acid (2). Since the lipases may function as acyl transferases coordination of lipolysis and β oxidation may depend on the direct transfer of acyl groups from storage triglycerides to glyceride components of the glyoxysome membrane.

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