Characteristics and Biosynthesis of Seed Lipases in Maize and Other Plant Species[1]

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Oilseed lipases from diverse plant species exhibit differences in their substrate specificity, pH for optimal activity, reactivity toward sulfhydryl reagents, hydrophobicity and subcellular location. Seed lipase from a certain plant species is relatively specific for the native triacylglycerols or triacylglycerols containing the major fatty acids of the storage triacylglycerols of the same species. This substrate specificity can be exploited in lipid biotechnology. In most seeds, with the known exception of castor bean, lipase activities are absent in ungerminated seeds and increase in postgermination. The biosynthesis of seed lipase has been studied only in maize. The maize enzyme is synthesized on free polyribosomes in postgermination. The newly synthesized enzyme is then transferred, without apparent co- or posttranslational modification, to the membrane of the lipid bodies.

Information on seed lipases is important in understanding their physiological roles, their action during seed storage, and their potential use in biochemistry and industry. In postgermination of oilseeds, the mobilization of oil reserves is essential in providing energy and carbon skeletons for embryonic growth. Lipase catalyzes the initial steps of lipid mobilization, and thus may be rate-controlling in germination and postgerminative growth. In agriculture, the crushing or storage of seeds may lead to an increase in lipolytic activity, resulting in an accumulation of free fatty acids. Further oxidation and degradation of these free fatty acids generate rancidity, which may render the oils unsuitable for human consumption. The removal of free fatty acids from dietary oils adds extra production costs. Also, the accumulation of free fatty acids during seed storage may cause a loss of seed vigor.

Currently, lipase represents about 3% of all enzymes used in industry. The uses of lipases to obtain fatty acids and glycerol from triacylglycerols eliminates the cost of energy in the traditional methods of hydrolysis under high temperature and pressure, and the mild enzymatic reaction generates fatty acids and glycerol that can be recovered more economically. Lipases also have been used in other industrial processes (1). Although lipases used in industry have been obtained from microbes, the unique substrate specificity of plant lipases, not found in microbes or mammalian systems, may be of special value in industrial utilization.

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PHYSIOLOGY AND CELL BIOLOGY OF SEED LIPASES

Oilseeds generally contain 20%-50% of their dry weight as storage triacylglycerols. In postgerminative growth, the storage triacylglycerols are rapidly mobilized to provide energy and carbon skeletons for the growth of the embryo. The triacylglycerols are localized in subcellular organelles called lipid bodies (oleosomes, oil bodies, spherosomes), which are bounded by a half-unit membrane (2). With only one well-documented exception (castor bean), lipase activity is absent in ungerminated seeds and increases rapidly in postgermination (3). Depending on the plant species, the lipase may be localized on the membrane of the lipid bodies or in other subcellular compartments. In those seeds where the lipase is associated with the membrane of the lipid bodies, the enzyme may be loosely (e.g. rapeseed, mustard seed) or tightly (e.g. castor bean and maize) associated with the organelles. In seeds where the lipase is found in subcellular compartments other than the lipid bodies, the enzymes still will have to come in contact with the membrane of the lipid bodies during catalysis. The fatty acids released by lipase activity are further metabolized in the glyoxysomes. The lipid bodies and glyoxysomes in vivo are in close proximity or direct physical contact with one another; presumably, this proximity could facilitate transport of fatty acids from the lipid bodies to the glyoxysomes.

SUBSTRATE SPECIFICITY OF SEED LIPASES

A distinct feature of seed lipases is their substrate specificity. Seed lipase from a certain plant species is relatively specific for the native triacylglycerols or triacylglyercols containing the major fatty acids of the storage triacylglycerols of that same species. As shown in Table 1, in general each seed lipase exhibits high activities with triacylglycerols containing the major storage fatty acids found within each respective plant species. Additional evidence of species-dependent substrate specificity of lipases comes from experimental results of the hydrolysis of mixtures of triacylglycerols (4). Such a specificity is of physiological significance. The findings from a direct comparison of triacylglycerols as lipase substrate negate the possibility that the lack of high lipase activity on triacylglyercols such as tristearin and tripalmitin is due only to the low solubility of these substrates. Furthermore, castor bean lipase and rapeseed lipase actually hydrolyze tristearin and tripalmitin at rates comparable with that of the more soluble triolein (Table 1). The relatively high activities of castor bean, rapeseed and maize lipases on tricaprin presumable are due in part to the physical nature of tricaprin, as in the case of the pancreatic lipase. However, this factor of physical properties is negligible in the case of elm lipase, which is active almost exclusively on tricaprin; such a specificity

TABLE 1

Hydrolysis of Various Triacylglycerols by Lipases from Various Sources[a,b]

Substrate	Relative activity (%)							
	Porcine pancreas	Castor bean	Maize	Rapeseed	Erucic acid-free rapeseed	Elm	Mustard	Palm
Tricaprin	207	43	127	89	—	100	—	100
Trilaurin	92	60	0	31	—	4	—	60
Trimyristin	50	26	0	92	—	3	—	15
Tripalmitin	5	46	0	27	51	0	39	35
Tristearin	0	62	0	36	89	0	40	—
Triolein	97	55	38	44	138	4	96	—
Trilinolein	100	57	100	89	116	6	89	—
Triricinolein	53	100	0	83	—	0	—	—
Tribehenin	—	—	0	16	—	0	—	—
Trierucin	92	36	45	100	100	0	100	—

[a]From Huang (1987).

[b]Lipases from different seed species were selected for comparison because of their contents of special fatty acids in storage triacylglycerols: castor bean (90% ricinoleic acid), maize (50% linoleic acid and 30% oleic acid), rapeseed and mustant (65% of equal amounts of erucic acid, oleic acid, and linoleic acid), elm seed (60% capric acid), and palm (50% lauric acid in kernel).

should be due to inherited structural properties of the enzyme.

The above pattern of fatty acyl specificity also was observed on diacylglycerols, monoacylglycerols and fatty acyl 4-methylumbelliferone, although the pattern is less distinctive (4). The seed lipases were inactive on lecithins. The gradual loss in the fatty acyl specificity of the seed lipases from tri- to di- to monoacylglycerols may be of physiological significance. Each storage triacylglycerol molecule generally is not comprised of only the major fatty acid but rather of the major fatty acid and other fatty acids in the same molecule. Thus, after the lipase has hydrolyzed the major fatty acid from a triacylglycerol molecule, it still should have high capacity to hydrolyze the remaining diacylglycerol and monoacylglycerol containing the other fatty acids.

Both maize and rapeseed lipases were more active on tri-, than on di- or monolinolein (4). Castor bean lipase was active equally on tri-, di- and monolinolein, as has been reported (5). These relative activities are different from those of pancreatic lipase, which is more active on tri- than dilinolein, and is rather inactive on monolinolein.

In general, the seed lipases release linoleic acid from both primary and secondary positions from trilinolein (Fig. 1). As judged from the kinetics of hydrolysis of rac-glyceryl-2,3-stearate-1-oleate and rac-glyceryl-1,3-stearate-2-oleate (4), and of trilinolein and dilinolein (Fig. 1), maize lipase exerts some degree of preference in releasing fatty acid from the primary position of a triacylglycerol. At the primary position, maize lipase is more active on oleyl ester than stearyl ester.

BIOSYNTHESIS OF MAIZE LIPASE

The classical studies of castor bean lipase have been well-documented (8). The only plant lipase that has been purified to homogeneity and its biosynthesis studied is the enzyme from maize. In the scutella of maize, lipase activity is absent in ungerminated seeds and

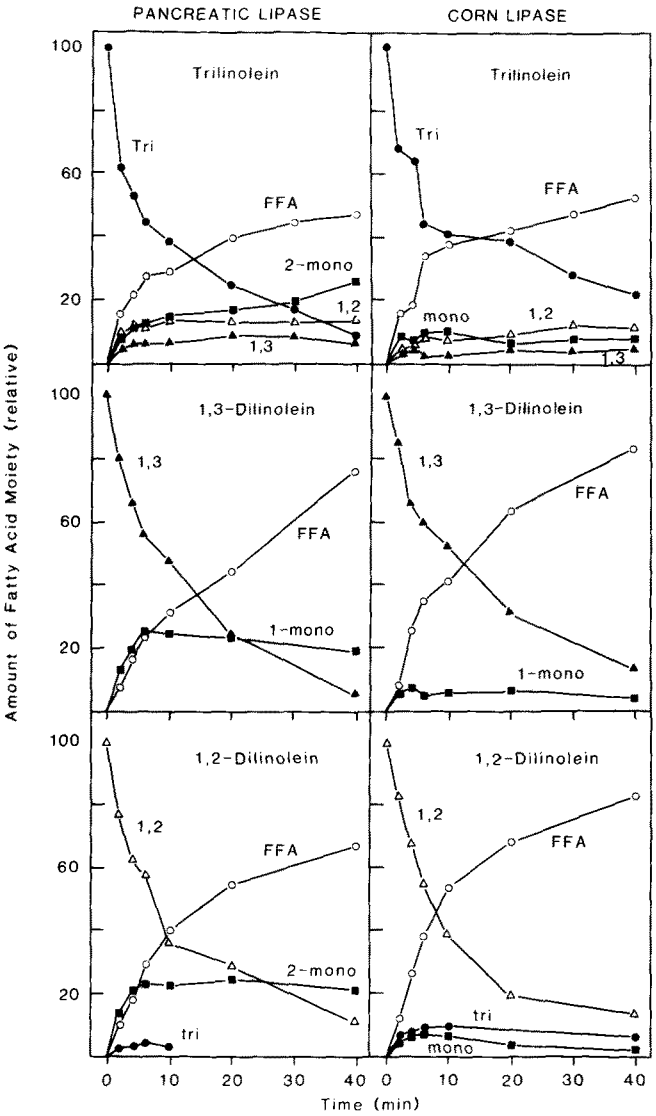

FIG. 1. Time courses or hydrolysis of trilinolein, 1,3-dilinolein, and 1,2-dilinolein by pancreatic lipase and maize lipase. The release of different products by the lipases on each substrate is shown.

increases in postgermination (6). At the peak stage of lipolysis, about 60% of the lipase activity can be recovered in the lipid body fraction after flotation centrifugation. The lipase is tightly bound to the lipid bodies, and it resists solubilization by repeated washing with buffers or NaCl solution. The lipase has been purified 272-fold to apparent homogeneity (7). The enzyme in sodium deoxycholate has an approximate Mr of 270,000 by sucrose gradient centrifugation and Mr of 65,000 by SDS-polyacrylamide gel electrophoresis. The amino acid composition as well as a biphasic partition using Triton-X 114 reveals the enzyme to be a hydrophobic protein.

The biosynthesis of the maize lipase has been pursued using monospecific rabbit antibodies raised against the purified maize lipase (9). Using an in vitro protein synthesis system, the mRNA for the lipase has been detected in germinated but not in maturing seeds. The in vitro and in vivo synthesized lipase exhibit the same Mr (65,000) by SDS-polyacrylamide gel electrophoresis, and thus there is no apparent co- or posttranslational processing of the lipase. The enzyme is synthesized by mRNA extracted from free and not bound polyribosomes. Apparently, after its synthesis the lipase will attach itself specifically to the membrane of the lipid bodies and not other cell organelles.

The maize lipase activity starts to appear two days after imbibition, concomitant with the decrease in total lipids (9). The activity reaches a maximum at about day 5-6. The rise and peaking of lipase activity at the initial stage of seedling growth parallel those of catalase and isocitrate lyase, two enzymes known to be involved in gluconeogenesis from lipids. However, after reaching the maximum at day 5-6, lipase activity remains almost unaltered from day 5 to 10, whereas catalase and isocitrate lyase activities drop off rapidly. Apparently, either lipase is continuously being synthesized or, more likely, the old lipase is not being degraded.

At the peak stage of lipolysis (day 5-6), about 60% of the lipase activities can be recovered in the isolated membrane of the lipid bodies, and the remaining activity (presumably representing nascent enzyme and enzyme derived from consumed lipid bodies) is present in the 10,000 g pellet, 120,000 g pellet and 120,000 g supernatant (9). As the seedling grows beyond day 5, the proportion of lipase in the lipid bodies decreases (concomitant with the decrease in triacylglycerols), whereas it increases in the 10,000 g pellet, 120,000 g pellet and 120,000 g supernatant. Because there is no change in the total lipase activity from day 5-10, it is likely that there is a transfer of the membrane of the consumed lipid bodies together with the lipase to a fragile compartment in the cell, presumably the vacuoles. Electron microscopic observation does shown a physical connection between the membrane of degrading lipid bodies and the membrane of enlarging cell vacuoles. During the fusion, the lipid body membrane should rearrange itself to form a double phospholipid layer.

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