Plant Physiol. (1974) 54, 23–28

Lipase Activities in Castor Bean Endosperm during Germination[1]

Received for publication November 26, 1974 and in revised form February 19, 1974

SHOSHI MUTO,[2] AND HARRY BEEVERS
Division of Natural Sciences, University of California, Santa Cruz, California 95064

ABSTRACT

Two lipases were found in extracts from castor bean (*Ricinus communis* L.) endosperm. One, with optimal activity at pH 5.0 (acid lipase), was present in dry seeds and displayed high activity during the first 2 days of germination. The second, with an alkaline pH optimum (alkaline lipase), was particularly active during days 3 to 5. When total homogenates of endosperm were fractionated into fat layer, supernatant, and particulate fractions, the acid lipase was recovered in the fat layer, and the alkaline lipase was located primarily in the particulate fraction. Sucrose density gradient centrifugation showed that the alkaline lipase was located mainly in glyoxysomes, with some 30% of the activity in the endoplasmic reticulum. When glyoxysomes were broken by osmotic shock and exposed to KCl, which solubilizes most of the enzymes, the alkaline lipase remained particulate and was recovered with the glyoxysomal "ghosts" at equilibrium density 1.21 g/cm³ on the sucrose gradient. Association of the lipase with the glyoxysomal membrane was supported by the responses to detergents and to butanol. The alkaline lipase hydrolyzed only monosubstituted glycerols. The roles of the two lipases in lipid utilization during germination of castor bean are discussed.

During germination of castor bean, storage fats in the endosperm are converted to sucrose with high efficiency (1, 3). The primary stage in fat utilization is commonly supposed to be its hydrolytic fission to glycerol and free fatty acids under the action of lipase. However, the actual site of the lipolysis is not known. The evidence now available shows that activation of fatty acids, their oxidation to acetyl CoA, and the conversion of acetyl CoA to succinate occurs in glyoxysomes, and that conversion of succinate to oxaloacetate occurs in the mitochondria (4, 12). Since the long chain fatty acids do not accumulate (1, 3), lipolysis is conceivably an important control point in the over-all sequence of fat utilization.

It is well known that dry seeds of castor bean contain an acid lipase which has been well characterized by Ory and his colleagues (22–25) and others (21). In addition to the acid lipase, we found another lipase in germinating castor bean endosperm. In this paper we describe the change of activities of the two lipases during germination, and the intracellular distribution and characteristics of the enzymes.

[1] This work was supported by The Atomic Energy Commission Contract AT (04-3)-34.

[2] Present address: Institute of Applied Microbiology, University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

MATERIALS AND METHODS

Measurements of Lipase Activities during Germination. Seeds of castor bean (*Ricinus communis* L. var. Hale) weighing 375 mg ± 5% were selected and soaked in running tap water (16 C) for 16 hr. The end of this imbibition period was designated day zero of germination. Germination was carried out in moist vermiculite in darkness at 30 C. Endosperm tissue was carefully removed and the rest of the seedling was discarded. Sixteen endosperm halves were washed with distilled water and ground with 10 ml of grinding medium containing 0.4 M sucrose, 10 mM KCl, 1 mM EDTA, 10 mM dithiothreitol, 1 mM $MgCl_2$, and 165 mM Tricine-NaOH buffer (pH 7.5) for 3 min using a glass homogenizer, and were filtered through a layer of nylon cloth. The homogenate was centrifuged at 270*g* for 10 min. The pellet and residues on the nylon cloth were combined and rehomogenized with 3 ml of grinding medium for 3 min in the same homogenizer. The homogenizer was washed with 2 ml of grinding medium. After filtration, the homogenate was centrifuged at 270*g* for 10 min to remove cell debris. The two supernatants were combined and fractionated into fat layer, supernatant, and particulate fractions by centrifugation at 10,800*g* for 30 min. The fat layer and particulate fractions, suspended in grinding medium, and the supernatant fraction were each recentrifuged. The resulting fat layer, particulate fractions, and supernatant fractions were individually combined and resuspended in grinding medium.

Purification of Lipases. Alkaline lipase was purified from 4-day castor bean endosperm. Seventy-five endosperms were ground with 100 ml of grinding medium in a mortar and were filtered through a layer of nylon cloth. The crude homogenate was centrifuged at 270*g* for 10 min. The 270*g* supernatant obtained from this crude homogenate was centrifuged at 10,800*g* for 30 min. The crude particulate pellet was suspended in grinding medium and layered on a linear sucrose gradient composed of a 40-ml gradient from 33 to 60% (w/w) sucrose over a cushion of 10 ml of 60% (w/w) sucrose. All sucrose solutions contained 1 mM EDTA (pH 7.5). After centrifugation for 4 hr at 20,000 rpm in a Beckman L2-65B ultracentrifuge with Spinco rotor SW 25.2, the glyoxysomal fraction (2) was collected and diluted with the same volume of 2 M KCl containing 1 mM EDTA to extract the soluble glyoxysomal enzymes. After standing for 1 hr, the diluted glyoxysomal fraction was centrifuged for 30 min at 101,000*g*. The pellet was suspended in 1 mM Tricine-NaOH buffer (pH 7.5) containing 5 mM dithiothreitol.

Acid lipase was purified from soaked beans by the method of Ory *et al.* (24).

Extraction and purification of lipases were carried out at 0 to 4 C.

Enzyme Assay. Two different assays for lipase were used. The fluorometric method of Guilbault and Hieserman (8) was used in the greater part of these studies with minor modifications. Fluorescence measurements were made with an Eppen-

dorf Fluorophotometer with excitation filter Hg 405/436 and emission filter 500 to 3000. The reaction mixture used routinely contained 83 mM glycine-NaOH buffer (pH 9.0), 5 mM dithiothreitol, 0.83 mM N-methylindoxylmyristate in ethylene glycol monomethyl ether (100 μl), and enzyme, in 3.0 ml. The reaction was started by addition of the substrate and the increase in fluorescence was recorded for 3 min.

A modified colorimetric method of Duncombe (7, 27) was used when the various glycerol esters were substrates. The free fatty acids produced were measured as copper soaps using sodium diethyldithiocarbamate. The reaction mixture contained in 1.0 ml: 83 mM glycine-NaOH (pH 9.5), 5 mM dithiothreitol, 10 mM substrate, and enzyme. The reaction was started by addition of substrate and stopped by addition of 1.0 ml of a mixture containing 0.9 M triethanolamine, 0.1 M acetic acid, and 5% cupric nitrate-3 H_2O. Emulsions of the substrate were prepared daily. For a typical emulsion preparation, 2.0 ml of 5% gum acacia and an aliquot of the chloroform solution containing 20 μmoles of substrate were emulsified for 2 min at 20 w without cooling in an Ultra Tip Model WW201 ultrasonic generator fitted with a microprobe. The emulsion was flushed with nitrogen gas at 40 C for 1 hr to remove all traces of chloroform.

N-Methylindoxylmyristate was obtained from Nutritional Biochemical Corporation, monostearin and 1,2-dipalmitin were obtained from Sigma and the other substrates from Nu-Chek Laboratories.

All other enzyme activities were assayed spectrophotometrically. The methods employed were those described in the literature as follows: catalase (19), isocitrate lyase (6), malate synthetase (10), cytochrome *c* oxidase (9), NADH-cytochrome *c* reductase (17).

All assays were carried out at room temperature (24 C).

Sucrose Density Gradient Centrifugation of the Total Homogenate. This was carried out following the procedure of Lord *et al.* (16) with slight modification. Twenty endosperm halves were homogenized by chopping for 15 min with a single razor blade in 10 ml of the grinding medium described above. The crude extract was filtered through two layers of nylon cloth, and cell debris was removed by centrifugation at 270*g* for 10 min. Four ml of supernatant were layered onto a sucrose density gradient consisting of 28 ml of sucrose solution increasing linearly in concentration from 33 to 60% (w/w) over a 2-ml cushion of 60% (w/w) sucrose, and topped with a 5-ml layer of 20% (w/w) sucrose. All sucrose solutions were prepared in 1 mM EDTA (pH 7.5). Gradients were centrifuged for 4 hr at 20,000 rpm in a SW 27 rotor in a Beckman L2-65B ultracentrifuge. After centrifugation, 0.6-ml fractions were collected with an ISCO density gradient fractionator Model 640. All steps were carried out at 0 to 4 C. The sucrose concentration of each 0.6-ml fraction from the gradient was measured with a Bausch and Lomb refractometer.

KCl, Deoxycholate, and Butanol Treatments. To 1.5 ml of the glyoxysome fraction separated from a crude particulate preparation, 1.5 ml of 0.05 M Tricine-NaOH buffer (pH 7.5) containing 5 mM dithiothreitol and the desired final concentration of KCl, deoxycholate, or butanol was added, and allowed to stand for 1 hr at 0 C. After centrifugation at 101,000*g* for 30 min, pellets were suspended in 1 ml of 0.05 M Tricine-NaOH buffer (pH 7.5) containing 5 mM dithiothreitol. Enzyme activities were assayed directly in the supernatants and suspended pellets.

Estimation of Lipid Content. Total lipid was extracted with chloroform-methanol according to Radin (26). Simple lipid and free fatty acid were separated from the total lipid by the method of Dittmer and Wells (5) using Silica AR CC-7 columns, and were weighed after removal of solvent.

Estimation of Protein. This was determined by the method of Lowry *et al.* (18) with the use of a calibration curve prepared for crystalline bovine serum albumin.

RESULTS

Changes in Lipid Content of the Endosperm during Early Growth. As shown in Figure 1, the fresh weight of endosperm tissue increases slowly during the first 2 days of germination and then more rapidly, until at day 6, it is roughly four times that of the dry seed. After day 7, when the root shoot axis is some 15 cm in length, the endosperm liquefies and the contents are completely absorbed by the expanding cotyledons. The total lipid of the endosperm remains unchanged until, beginning at day 3, a sharp decline sets in and by day 7 it has almost completely disappeared (Fig. 1). As shown, simple (neutral) lipid comprise essentially all of the lipid, at least until day 5. Only after this time is any appreciable fraction not accounted for by neutral lipid; analyses for free fatty acid showed only negligible amounts until they accounted for 5 to 10% of the total lipid at days 6 and 7.

Changes in Lipase Activities during Germination. Experiments with crude homogenates showed that two separate lipases could be distinguished: one with an acid pH optimum which was present in the dry seed and with high activity during the first 2 days of germination (Ory's enzyme), and a second with an alkaline pH optimum which was particularly active during days 3 to 5. The changes in these enzyme activities during the 7-day period are shown in Figure 2a. When lipase was assayed at pH 7.0, activity was very low throughout the germination period.

Intracellular Location of Lipase Activities. The data in Figure 2, b and c, show that when the crude extract was separated into fat layer, supernatant, and particulate fractions, essentially all of the lipase activity at pH 5.0 was recovered in the fat fraction. On the other hand, activity at pH 9.0 was low throughout the period in the fat component and, particularly in the peak 3 to 5 day interval, it was found predominantly in the particulate fraction. The relationship between pH and the activities of the two enzymes is shown in Figures 3 and 4. The enzyme in the fat layer shows optimal activity at pH 5.0 and that in the particulate fraction displays a sharp optimum at pH 9.0. Neither enzyme shows appreciable activity at the optimum pH for the other. The relationship between substrate concentration and the activities of the two lipases is shown in Figures 5 and 6. The K*m* values of acid and alkaline lipases for N-methylindoxylmyristate were 1.67 and 0.23 mM, respectively.

Association of Alkaline Lipase Activity with Glyoxysomes. When the total homogenate from 4-day endosperm tissue was centrifuged on the sucrose gradient (see "Materials and Methods"), the data in Figure 7 were obtained. On such gradients, previous work (16) has shown that glyoxysomes, mitochondria, and the endoplasmic reticulum are cleanly separated. The distribution of the marker enzymes from these components, respectively, isocitrate lyase, cytochrome *c* oxidase, and NADH-cytochrome *c* reductase, are shown in the upper part of Figure 7. The sharp peaks at equilibrium densities 1.24 g/cm^3, 1.19 g/cm^3, and 1.12 g/cm^3 show that good separation had been achieved; malate synthetase, a second marker for glyoxysomes, was recovered primarily at the same density as isocitrate lyase. The activity of alkaline lipase and the protein profile across the gradient is shown in the lower part of Figure 7. Virtually no activity was present in those fractions at the top of the gradient representing soluble proteins and those

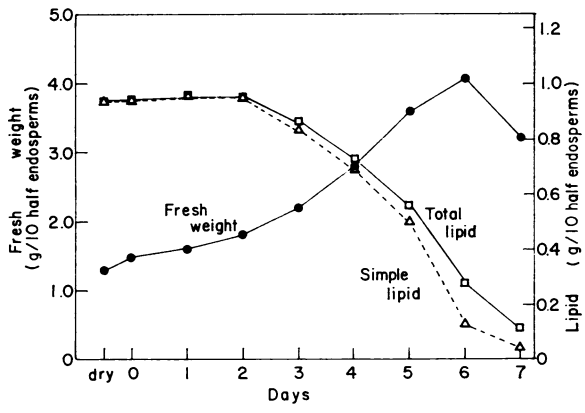

FIG. 1. Change in lipid content of castor bean endosperm during early growth. □: total lipid; △: simple lipid; ●: fresh weight.

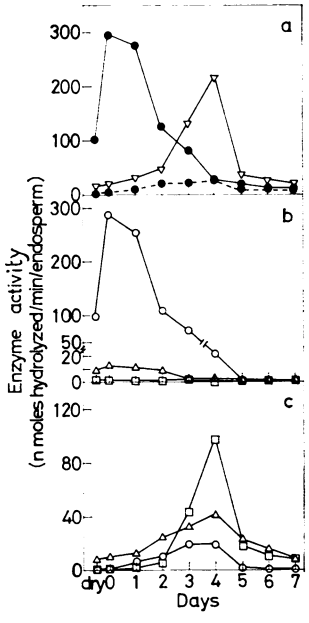

FIG. 2. Changes in lipase activities in castor bean endosperm during germination. (a) Total activity (fat layer + particulate + supernatant) at pH 5.0 (●——●), 7.0 (●- - -●) and 9.0 (▽——▽). Activity in fat layer (○), particulate (□) and supernatant (△) at pH 5.0 (b) and pH 9.0 (c). The enzyme activity was measured by the fluorometric method.

removed from organelles during preparation. Apart from a small peak of enzyme activity in the endoplasmic reticulum region, it is clear from the sharp peak at density 1.24 g/cm^3 that the bulk of the alkaline lipase coincides in distribution with that of isocitrate lyase and malate synthetase. Thus, the alkaline lipase is specifically present in the glyoxysomes. Endosperm extracts from 3- and 5-day castor bean seedlings showed essentially the same distribution pattern of the alkaline lipase activity on the sucrose gradient as that in Figure 7 (data not shown).

Intracellular location of the alkaline lipase was surveyed in castor bean tissues other than endosperm, with the aid of the sucrose gradient centrifugation. Roots and cotyledons from dark grown 4-day seedlings, and cotyledons and leaves from green plants grown for 2 weeks in light were examined. The activity of alkaline lipase was located in the endoplasmic reticulum region as a sharp peak and slight activity was present at the top of gradient in all of these tissues (data not shown).

When intact glyoxysomes are recovered from the gradient in 54% sucrose and diluted, most of the enzyme proteins (*e.g.*, isocitrate lyase) are solubilized and on recentrifugation in the sucrose gradient, the glyoxysomal membranes (ghosts) with a few associated enzymes, *e.g.*, malate synthetase, are recovered at density 1.21 to 1.22 g/cm^3 (11). To find whether lipase was present in the matrix or in the membrane, glyoxysomes were

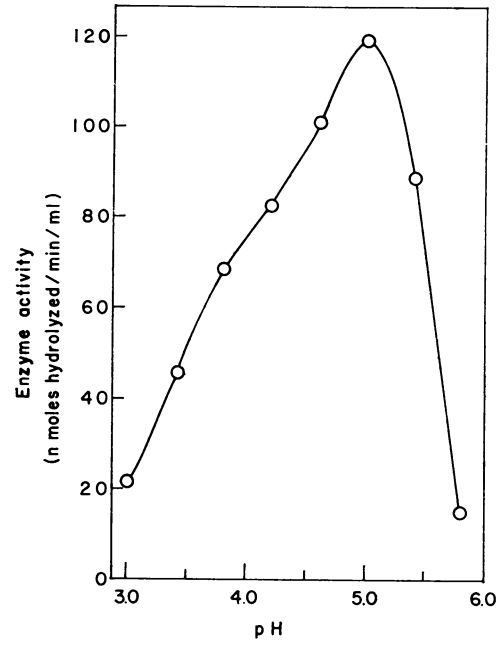

FIG. 3. Activity of the acid lipase as a function of pH. The activity was assayed fluorometrically. Reaction mixtures contained 83 mM citrate buffer, 5 mM dithiothreitol, 0.83 mM N-methylindoxyl-myristate and 100 μl of enzyme, in 3.0 ml.

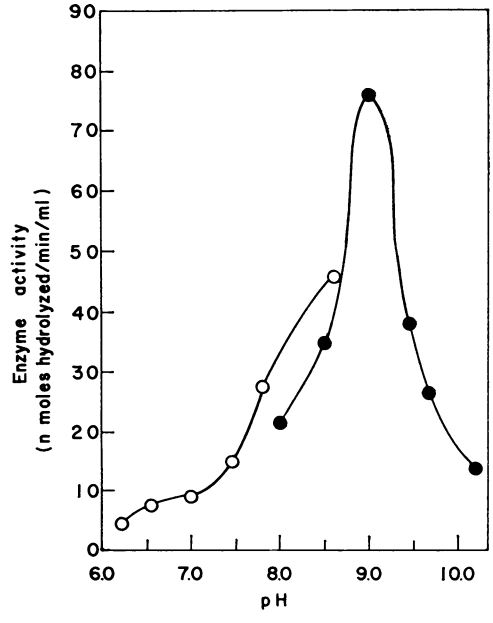

FIG. 4. Activity of the alkaline lipase as a function of pH. The activity was assayed fluorometrically. Reaction mixtures as in Figure 3. Buffers used: ○: tris-HCl; ●: glycine-NaOH.

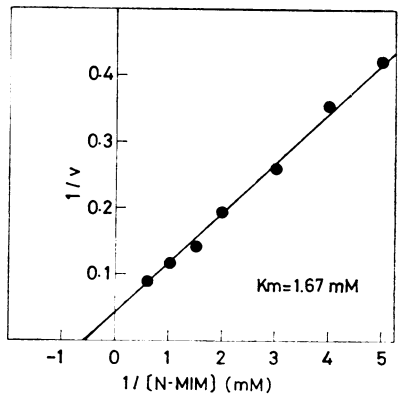

FIG. 5. Activity of the acid lipase as a function of substrate concentration (Lineweaver-Burk plot). The activity was assayed fluorometrically at pH 5.0 (83 mM citrate buffer).

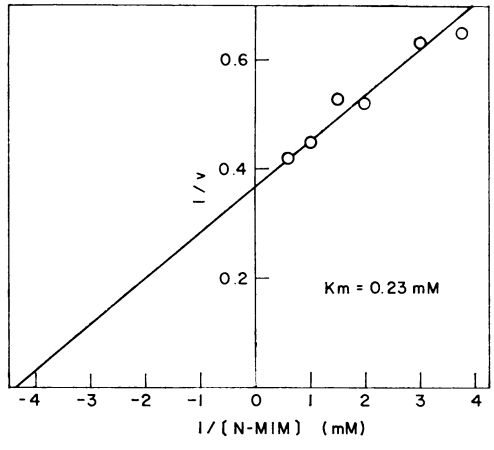

FIG. 6. Activity of the alkaline lipase as a function of substrate concentration (Lineweaver-Burk plot). The activity was assayed fluorometrically at pH 9.0 in 83 mM glycine-NaOH.

diluted and recentrifuged and the results are shown in Figure 8. As shown, much of the protein which previously had sedimented at 1.24 g/cm^3 was solubilized by this treatment and appeared at the top of the gradient, and most of the isocitrate lyase appeared in this region, as expected (11). However, none of the alkaline lipase was solubilized and all was recovered at the new density 1.21 to 1.22 g/cm^3, completely overlapping the distribution of malate synthetase. Thus, the lipase is clearly associated with the glyoxysomal membrane.

The data in Figure 9 establish that the tightness of this association is considerably greater than that of other enzymes which have been examined (11). Thus exposure of glyoxysomal membranes to 0.2 M KCl solubilizes most of the malate synthetase but at this salt concentration the lipase activity was virtually unaffected. Even at salt concentration greater than 0.9 M, more than 80% of the lipase remained attached to the membranes.

In the hope of solubilizing the enzyme for further purification, the glyoxysomes were treated with deoxycholate and butanol of various concentrations. The results were disappointing. Those concentrations (0.1% deoxycholate, 10% butanol) which solubilized part of the lipase also led to considerable inactivation of the enzyme.

Properties of the Lipase in Purified Glyoxysomal Membranes. Problems of solubilization and stability prompted the use of the KCl-treated glyoxysomes (purified membranes) as a source of lipase for further investigation of enzyme properties. The major enrichment of lipase (some 20-fold) occurred during the preparation of the crude particulate fraction, and this was accompanied by a 50% loss in total enzyme activity. Further loss of lipase occurred during separation of the glyoxysomes and KCl treatment, so that no improvement of specific activity occurred during these stages of membrane purification.

As shown in Table I, a variety of detergents have strong inhibitory effects on the enzyme. Response to metal ions and possible inhibitors are given in Table II. Mg^{2+} and Ca^{2+} both brought about 80% inhibition of enzyme activity at 1 mM and the inhibition was reversed by EDTA. However, K^+ and Na^+ were without effect. *p*-Chloromercuribenzoate inhibited almost

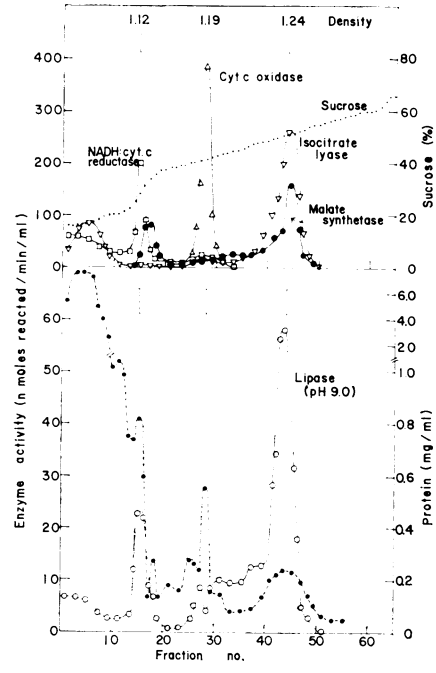

FIG. 7. Enzyme distribution after separation of crude extract from castor bean endosperm on a sucrose density gradient. □: NADH: cytochrome *c* reductase; △: cytochrome *c* oxidase; ▽: isocitrate lyase; ●: malate synthetase; ○: lipase at pH 9.0; ●---●: protein; ····: sucrose concentration. Equilibrium densities of the three main peaks are indicated by vertical lines.

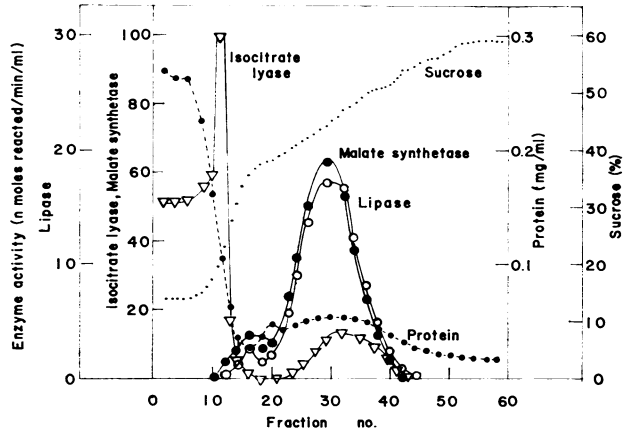

FIG. 8. Enzyme distribution on the sucrose gradient after exposing the glyoxysome fraction from Figure 7 to osmotic shock (see text). Symbols are as in Fig. 7.

completely at 0.1 mM and this inhibition was reversed by subsequent addition of dithiothreitol. The pH optimum of the enzyme in the purified membrane fraction was about 9.5 when monopalmitin was used as the substrate.

The substrate specificity of the enzyme is shown in Table III. Out of the various palmityl glycerol esters, only monosubstituted forms were hydrolyzed. The alkaline lipase thus seems to be a monoglyceride lipase of the kind described previously from rat adipose tissue (14, 15, 27) and skeletal muscle (29). As shown in Table III, the nature of the fatty acid in the monosubstituted ester has a marked effect on lipase activities. The rate of hydrolysis of monopalmitin (measured by production of free fatty acid) was close to that observed for the hydrolysis of the model substrate N-methylindoxymyristate (fluorometric assay). Furthermore, the kinetics of the activity on monopalmitin are similar to those for N-methylindoxylmyristate; the K*m* was 0.27 mM.

In Table IV, the substrate specificity of the acid lipase, purified from ungerminated beans by Ory's method (24), is shown. Although the monosubstituted derivatives are hydrolyzed most rapidly, it is clear that the acid lipase attacks the tri- and disubstituted glycerols.

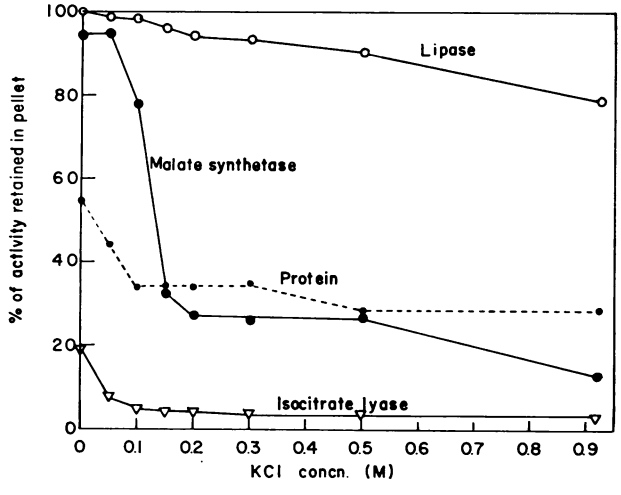

FIG. 9. Effect of dilution and increasing KCl concentration on the solubility of enzymes and protein in the glyoxysomes. Samples of glyoxysomes recovered in 54% sucrose were diluted 2:1 with water and the indicated concentrations of KCl, and were centrifuged. Activities in the pellet and supernatant were measured to determine the percentage of activity retained in the pellet.

Table I. *Effects of Detergents on activity of the Lipase in Glyoxysomal Membranes*

Activity was assayed fluorometrically, with N-methylindoxylmyristate as substrate, at pH 9.0.

Detergent	Conc	Relative activity
	%	
None		100
Deoxycholate	0.2	2
	0.1	7
	0.02	75
	0.01	96
Digitonin	0.03	0
	0.01	12
Triton X-100	0.02	1
	0.01	2
Tween 20	0.02	0
	0.01	3

Table II. *Effects of Metal Ions and p-Chloromercuribenzoate (PCMB) on the Activity of Purified Alkaline Lipase*

Addition	Relative Activity
	%
None	100
$CaCl_2$ (1 mM)	21
$CaCl_2$ (1 mM) + EDTA (1 mM)	112
$MgCl_2$ (1 mM)	18
$MgCl_2$ (1 mM) + EDTA (1 mM)	110
NaCl (1 mM)	107
KCl (1 mM)	104
EDTA (1 mM)	120
PCMB (0.1 mM)	5
Dithiothreitol (5 mM)	106
PCMB (0.1 mM) + Dithiothreitol (5 mM)	105

Table III. *Hydrolysis of Various Substrates by the Purified Alkaline Lipase, Assayed by the Colorimetric Method*

Experiment	Substrates	Fatty Acid Produced
		nmoles/min·ml
1	Tripalmitin	0
	1,2-Dipalmitin	0
	1,3-Dipalmitin	0
	1-Monopalmitin	9.2
	2-Monopalmitin	8.3
2[1]	Monolaurin	48.5
	Monomyristin	21.9
	Monopalmitin	20.0
	Monostearin	10.6
	Monoarachidin	1.5
3[2]	N-Methylindoxylmyristate	21.3

[1] Monoglycerides are natural equilibrium mixtures of 1- and 2-substituted glycerides.

[2] Fluorometric assay.

Table IV. *Hydrolysis of Various Substrates by the Purified Acid Lipase*

Substrates	Fatty Acid Produced
	nmoles/min·ml
Tripalmitin	220
1,3-Dipalmitin	200
1-Monopalmitin	506
2-Monopalmitin	491
Monolaurin[1]	860
Monomyristin[1]	901
Monostearin[1]	186
Monoarachidin	143

[1] Natural equilibrium mixture of 1- and 2-substituted glycerol.

DISCUSSION

The data show that two different lipases are present in the endosperm of castor bean during the period of germination and early growth. The acid lipase, which can hydrolyze tri-, di-, and monoglycerides is most active at an early stage when storage

fats are not being utilized. On the other hand, the activity of the alkaline lipase increases when fat is being utilized and declines as the fat is depleted. However, the latter enzyme hydrolyzes only monoglycerides and thus by itself cannot account for the breakdown of fats which temporally coincides with its highest activity during germination. It seems rather that the functioning of the glyoxysomal enzyme must depend on the prior action of the acid lipase, although this enzyme is declining in activity at 3 days when fat utilization begins. It seems quite clear from the fact that free fatty acids do not accumulate during the germination process that the acid lipase does not attack the storage fat prior to day 3, when the alkaline lipase activity in the glyoxysomes becomes appreciable.

As shown by Ory *et al.* (23) the acid lipase is retained in the fat layer obtained after homogenization and centrifugation of ungerminated seeds, and histochemical investigations have shown that it is associated with the membranes of the spherosomes within which this fat is contained (25). Vigil (28) showed that spherosomes decreased in size and ultimately disappeared from endosperm cells during germination. The data presented here which show that an acid lipase is recovered in the fat layer (spherosomes) throughout germination support these previous results and suggest that the decline in the activity after day 3 coincides with the decomposition of the spherosome membrane.

The alkaline lipase is also associated with membranes, namely those of the glyoxysomes and, to a minor degree, those of the endoplasmic reticulum. A recentrifugation experiment showed that the enzyme activity in the endoplasmic reticulum is not artificially derived from glyoxysomes during isolation, and in other tissues which do not contain glyoxysomes, alkaline lipase activity is found only in the endoplasmic reticulum. It is not clear whether, in the endosperm, the alkaline lipases in the endoplasmic reticulum and glyoxysomes are identical but they do have the same optimal pH and K*m* for N-methylindoxyl-myristate. The association of part of the activity with the endoplasmic reticulum may be related to the observations showing that this is the site of origin of the glyoxysomal membrane (13).

We are still far from a definitive understanding of the process whereby long chain fatty acids are released in a controlled manner from storage fat in the spherosomes and transferred to the glyoxysomes where activation and β-oxidation occur. From the available evidence it must be presumed that the acid lipase of the spherosomes is responsible for at least the initial phases of hydrolysis, although it is not clear how hydrolysis is held in check for the 0 to 3 day period when this enzyme shows its highest activity. The movement of free fatty acids or monoglycerides from the site of their production in spherosome membrane to the glyoxysome may be facilitated by the close juxtaposition of these organelles which is revealed by electron-micrographs (20, 28). There is no proof at present that the monoglyceride lipase that we have described in the glyoxysomal membrane is actually functional during the conversion of storage fat to carbohydrate, since the acid lipase in the spherosomes could bring about complete hydrolysis. Nevertheless, the relationship between the developmental behavior of the monoglyceride lipase and the timing of fat breakdown, and the advantageous location of this enzyme in a membrane interposed between the site of origin of monoglycerides and the site of fatty acid utilization are intriguing and suggest that the enzyme has significance beyond its pragmatic role as a very useful marker for the glyoxysomal membrane.

Acknowledgment—We thank Dr. W. E. Domingo, Baker Castor Oil Company, Plainview, Texas for kindly supplying the castor bean seeds.

LITERATURE CITED

1. Beevers, H. 1961. Metabolic production of sucrose from fat. Nature 191: 433–436.
2. Breidenbach, R. W., A. Kahn, and H. Beevers. 1968. Characterization of glyoxysomes from castor bean endosperm. Plant Physiol. 43: 705–713.
3. Canvin, D. T. and H. Beevers. 1961. Sucrose synthesis from acetate in the germinating castor bean: kinetics and pathway. J. Biol. Chem. 236: 988–995.
4. Cooper, T. G. and H. Beevers. 1969. β-Oxidation in glyoxysomes from castor bean endosperm. J. Biol. Chem. 244: 3514–3520.
5. Dittmer, J. C. and M. A. Wells. 1969. Quantitative and qualitative analysis of lipid and lipid components. Methods Enzymol. 14: 482–530.
6. Dixon, G. H. and H. L. Kornberg. 1959. Assay methods for the key enzymes of the glyoxylate cycle. Biochem. J. 72: 3P.
7. Duncombe, W. G. 1962. The colorimetric determinaton of long-chain fatty acids in the 0.05–0.5 μmole range. Biochem. J. 83: 6P.
8. Guilbault, G. G. and J. Hieserman. 1969. Fluorometric substrate for sulfatase and lipase. Anal. Chem. 41: 2006–2009.
9. Hackett, D. P. 1964. Enzymes of terminal respiration. *In:* H. F. Linskens, B. D. Sanwall, and M. V. Tracey, eds., Modern Methods of Plant Analysis, Vol. 7. Springer-Verlag, Berlin. pp. 646–694.
10. Hock, B. and H. Beevers. 1966. Development and decline of glyoxylate-cycle enzymes in watermelon seedlings (*Citrullus vulgaris* Schrad.). Effects of dactinomycin and cycloheximide. Z. Pflanzenphysiol. 55: 405–414.
11. Huang, A. H. C. and H. Beevers. 1973. Localization of enzymes within microbodies. J. Cell. Biol. 58: 379–389.
12. Hutton, D. and P. K. Stumpf. 1969. Characterization of the β-oxidation systems from maturing and germinating castor bean seeds. Plant Physiol. 44: 508–516.
13. Kagawa, T., J. M. Lord, and H. Beevers. 1972. The origin and turnover of organelle membranes in castor bean endosperm. Plant Physiol. 51: 61–65.
14. Katocs, A. S., Jr., D. N. Calvert, and J. J. Lech. 1971. Characterization of two monoglyceride hydrolyzing enzymes in rat adipose tissue. Biochim. Biophys. Acta 229: 608–617.
15. Kupiecki, F. P. 1966. Partial purification of monoglyceride lipase from adipose tissue. J. Lipid. Res. 7: 230–235.
16. Lord, J. M., T. Kagawa, and H. Beevers. 1972. Intracellular distribution of enzymes of the cytidine diphosphate choline pathway in castor bean endosperm. Proc. Nat. Acad. Sci. U.S.A. 69: 2429–2432.
17. Lord, J. M., T. Kagawa, T. S. Moore, and H. Beevers. 1973. Endoplasmic reticulum as the site of lecithin formaton in castor bean endosperm. J. Cell Biol. 57: 659–667.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
19. Lück, H. 1965. Catalase. *In:* H. U. Bergmeyer, ed., Methods in Enzymatic Analysis. Academic Press, New York. pp. 885–894.
20. Mollenhauer, H. H. and C. Totten. 1970. Studies on seeds. V. Microbodies, glyoxysomes, and ricinosomes of castor bean endosperm. Plant Physiol. 46: 794–799.
21. Noma, A., and B. Borgström. 1971. The acid lipase of castor beans. Positional specificity and reaction mechanism. Biochim. Biophys. Acta 227: 106–115.
22. Ory, R. L. 1969. Acid lipase of the castor bean. Lipids 4: 177–185.
23. Ory, R. L., A. J. St. Angelo, and A. M. Altschul. 1960. Castor bean lipase. action on its endogenous substrate. J. Lipid Res. 1: 208–213.
24. Ory, R. L., A. J. St. Angelo, and A. M. Altschul. 1962. The acid lipase of the castor bean. Properties and substrate specificity. J. Lipid Res. 3: 99–105.
25. Ory, R. L., L. Y. Yatsu, and H. W. Kircher. 1968. Association of lipase activity with spherosomes of *Ricinus communis*. Arch. Biochem. Biophys. 264: 255–264.
26. Radin, N. S. 1969. Preparation of lipid extracts. Methods Enzymol. 14: 245–254.
27. Vaughan, M., J. E. Berger, and D. Steinberg. 1964. Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. J. Biol. Chem. 239: 401–409.
28. Vigil, E. L. 1970. Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. J. Cell Biol. 46: 435–454.
29. Wallach, D. P. 1968. Isolation and characterization of four lipolytic preparations from rat skeletal muscle. J. Lipid Res. 9: 200–206.