BBA 66247

THE ACID LIPASE OF CASTOR BEANS

POSITIONAL SPECIFICITY AND REACTION MECHANISM

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(Received September 24th, 1970)

SUMMARY

Hydrolysis of long-chain triglyceride catalysed by the acid lipase of castor beans proceeds *via* the intermediate formation of 1,2- and 1,3-diglyceride and 1- and 2-monoglyceride. The 1,3-diglyceride is formed *via* the reaction sequence:

1,2-Diglyceride $\rightleftharpoons$ 1-monoglyceride + fatty acid $\rightleftharpoons$ 1,3-diglyceride

No indication was found for any direct isomerization of the 1,2-di- and 2-monoglyceride during the reaction. The ester bonds of the 1,2-diglyceride are hydrolysed at similar rates, and the enzyme therefore has no positional specificity. The direct hydrolysis of 1- and 2-monoolein is also catalysed by the enzyme at similar rates.

INTRODUCTION

Hydrolysis of long-chain triglyceride catalysed by the acid lipase from castor beans is known to proceed to glycerol and fatty acids without appreciable accumulation of intermediate reaction products[1,2]. In this respect castor bean lipase is different from the lipase of pancreatic juice[3] and the lipase from *Rhizopus arrhizus*[4] which catalyse the hydrolysis of triglycerides with the accumulation of monoglyceride in the incubation. This accumulation is due to a high specificity of the triglyceride substrate for the primary ester bonds, as compared to the secondary ones.

The specificity of action of the castor bean lipase has not been agreed on. SAVARY *et al.*[2] reported that castor bean lipase was not specific for the position of the fatty acid in the triglyceride molecule, while such a specificity was claimed by ORY *et al.*[5]. These later authors believed that the cleavage of the 2-ester bond was preceded by an isomerization of the fatty acid to the 1-position that was favored by the high H^+ concentration of the incubation medium. The evidence for this conclusion was indirect, based on the finding that castor bean lipase did hydrolyse triglycerides to completion but did not catalyse the hydrolysis of the secondary ester bonds in 2,3-butane dioleate or 2-hexyloleate. Such a reaction course was favored by the recent finding of the appearance of an appreciable fraction of 1,3-diolein in the incubation

mixture during hydrolysis of triolein and 1,2-diolein with castor bean lipase[6]. As the stability of 1,2-diolein is high at the pH of the reaction[7], the conversion of the 1,2- to the 1,3-form must be an effect of the enzyme. The work presented in this report was undertaken to further study this reaction.

MATERIALS AND METHODS

Substrates

[^{3}H]Glyceryl trioleate was prepared from [2-^{3}H]glycerol (The Radiochemical Centre, Amersham) and oleoyl chloride, and purified by silica gel thin-layer chromatography to a purity of >99% by radioactivity. [1-^{14}C]1,3-Oleoyl-2-oleoyl glycerol was prepared as earlier described[8]. [^{3}H]Glyceryl 1,2-dioleate and [1-^{14}C]1-oleoyl-2-oleoyl glycerol were prepared from the trioleate by the action of pancreatic lipase followed by preparative thin-layer chromatography.

[^{3}H]Glycerol 2-monooleate was prepared by the action of pancreatic lipase on [^{3}H]glycerol trioleate. 320 μmoles of the trioleate was incubated in a 16-ml volume with 0.15 M phosphate buffer (pH 6.8) and 6 mM in sodium taurodeoxycholate, 500 mg DEAE-Sephadex (Pharmacia) and 40 mg lyophilized rat pancreatic juice powder for 1 h. The DEAE-Sephadex was used to bind the fatty acid liberated to drive the reaction toward monoglyceride. The glyceride recovered after extraction contained 82.9% 2-monoolein, 10.1% 1-monoolein, 5.9% diolein and 0.4% triolein and was used without further purification.

[1-^{14}C]1-Oleoyl glycerol was prepared from isopropylidene glycerol and the acid chloride. [9,10-^{3}H$_2$]2-Oleyl glycerol was prepared as previously described[3]. [9,10-^{3}H$_2$]1-Oleyl glycerol was prepared from isopropylidene glycerol and [9,10-^{3}H$_2$]-oleyl sulfonate, the product purified by preparative thin-layer chromatography to a radiopurity of >98%.

Enzyme

Castor bean lipase was kindly supplied by Dr. Robert Ory, U.S. Department of Agriculture, Southern Utilization Research and Development Division, New Orleans.

Incubations

The substrate, usually 128 μmoles, was evaporated to dryness in a 50-ml glass-stoppered test tube and 16 ml 0.01 M phosphate buffer (pH 6.8), and the amount of enzyme to be used was added. The mixture was sonified for 1 min to obtain a homogeneous emulsion using a Branson Sonifier at the maximum power. At this pH the enzyme is inactive and 2-ml samples of the emulsion was transferred to 20 ml glass-stoppered test tubes. The reaction was started by the addition of 1 ml 0.15 M sodium acetate buffer (pH 4.0) and the samples incubated with shaking for the times described. The reaction was stopped by adding 12 ml of a mixture of ethanol–ethyl ether–heptane (1:1:1, by vol.) to the test tube.

Extraction and separation

The lipids were extracted with two additional upper phases, and the lipids evaporated to dryness at reduced pressure at a temperature below 20°. The lipids

from each incubation representing usually 16 μmoles were dissolved in 500 μl heptane, 100 μl were taken directly to a counting vial and 100 μl were each put on one silica gel G thin-layer plate 20 cm × 20 cm and one boric acid-impregnated silica gel H plate. The silica gel plate was developed, using as solvent a mixture of acetic acid–methanol–ethyl ether–hexane (1:1.5:10:45, by vol.), and the boric acid-impregnated plate with a mixture of acetone–chloroform (4:96, by vol.)[9]. The silica acid plate was used for the isolation of the tri-, di- and monoglyceride fractions, the boric acid plate for the 1- and 2-monoglyceride and the 1,2- and 1,3-diglyceride. In order to get a better separation of the 1,2-diglyceride and the free fatty acids in some cases, a third silica gel plate was run and the diglyceride spot scraped off, eluted with chloroform and run on a boric acid-impregnated plate.

In order to determine the extent of isomerization of the di- and monoglyceride fractions possibly taking place during the experimental conditions, the following experiment was undertaken. [1-^{14}C]1,3-Oleoyl-2-oleoyl [^{3}H]glycerol (8 μmoles/ml) was sonicated with a 0.01 M phosphate buffer (pH 8.0). Purified pancreatic lipase was added, and the sample incubated for 30 min at 25°. Aliquots in duplicate were extracted and carried through the total analytical procedure described above to obtain the di- and monoglyceride isomers. Other aliquots were added with their volume of 0.15 M sodium acetate buffer (pH 4.0), incubated for another 30 min, extracted and analysed. The figures for per cent 2-monoglyceride in the monoglyceride fraction were, respectively, 94.4, 94.9 and 94.4, 94.8 before and after the pH 4.0 incubation. For the 1,2-diglyceride the corresponding per cent figures were 98.2 and 98.3 before and 98.3 and 98.5 after the pH 4.0 incubation. The ^{14}C/^{3}H ratio in the mono- and diglyceride fractions were 1.00 and 1.01, respectively, when the ratio in the original triolein was set to 1.00. The results show that incubation at pH 4.0 does not effect any isomerization of the mono- or diglyceride fraction furthermore, if it is assumed that pancreatic lipase catalyses the formation of the 2-mono- and 1,2-diglyceride, little isomerization takes place during the analytical procedures. The slight isomerization of the 2-monoolein may, to a large extent, be caused by the alkaline pH of the incubation medium used with pancreatic lipase[10].

Radioactivity assay

After indication by iodine the spots on the thin-layer plates were scraped off into counting vials and the radioactivity was determined using an automatic Tri-Carb Scintillation spectrometer. Free [^{3}H]glycerol was determined by taking 1-ml aliquots of the lower phases after extraction of the lipids from the incubations.

Calculations

In most of the experiments to be described, tri- or 1,2-diglyceride was used labeled in the glycerol part with ^{3}H and in the fatty acid in the 2-position with ^{14}C. The results are given as the ^{14}C/^{3}H ratio related to a figure of 1.00 for the original tri- or diglyceride.

RESULTS

Experiments with [1-^{14}C]1,3-oleoyl-2-oleoyl [^{3}H]glycerol

When incubated with castor bean lipase, triolein was hydrolysed with low inter-

TABLE I

MOLAR COMPOSITION OF THE REACTION MIXTURE FORMED WHEN TRIOLEIN WAS INCUBATED WITH CASTOR BEAN LIPASE AT pH 4.0

Substrate emulsified in the buffer at a concentration of 4 μmoles/ml. Enzyme concentration, 1 mg/ml.

Incubation time (min)	*Triolein*	*Diolein*	*1,3-Isomer (%)*	*Monolein*	*1-Isomer (%)*	*Glycerol*
1	88.5	5.0	34.2	2.2	63.0	2.8
3	74.2	8.3	25.5	4.4	65.8	11.7
5	61.7	9.3	25.7	4.9	72.2	23.1
8	54.6	10.8	25.8	4.3	65.2	30.4
16	32.2	8.7	26.1	4.0	62.7	48.7
32	27.2	10.8	35.5	3.1	61.6	56.2

mediate accumulation of di- or monoglyceride (Table I). The molar per cent of diglyceride at most reached figures of 10%, that of monoglyceride less than 5%. Glycerol appeared in the incubation almost linearly related to the time of incubation.

The composition of the di- and monoglycerides isolated during the course of the hydrolysis is also given in Table I and shows that 25–35% of the diglyceride fraction was of the 1,3-configuration and 62–72% of the monoglyceride fraction of the 1-configuration.

When the radioactivities of the different glyceride fractions were related to a $^{14}C/^{3}H$ ratio of the original triglyceride of 1.00, the results seen in Table II were obtained.

The ratio of the triglyceride fraction increased from 1.00 to 1.54 after 32 min incubation. The 1,2-diolein fractions showed a ratio between 0.98 and 1.15. The 1,3-diolein fraction started at around 0.35 and slowly increased to 0.54. The activity in the monoglyceride fraction was in all samples low, and this was especially the case for the 2-monoglyceride. In all samples with significant activities, however, the $^{14}C/^{3}H$ ratio for the 1-monoglyceride was much less than that of the 2-monoglyceride.

The relative increase of ^{14}C activity in the tri- and 1,2-diglyceride fraction can

TABLE II

$^{14}C/^{3}H$ RATIO OF THE GLYCERIDE FRACTIONS OBTAINED IN THE EXPERIMENT GIVEN IN TABLE I

[^{3}H]Glyceryl [1-^{14}C]2-oleoyl trioleate was used as substrate. The radioactivity ratio of the fractions have been related to a $^{14}C/^{3}H$ ratio of 1.00 in the triolein substrate at zero time.

Incubation time (min)	*Triolein*	*Diolein*		*Monoolein*	
		1,2-	*1,3-*	*1-*	*2-*
1	1.02	1.11	0.35	—	—
3	1.11	1.02	0.36	0.14	—
5	1.24	1.00	0.39	0.14	0.67
8	1.35	0.98	0.42	0.20	0.77
16	1.48	1.11	0.50	0.27	0.93
32	1.54	1.15	0.54	0.33	1.07

TABLE III

% MOLAR COMPOSITION OF THE REACTION MIXTURE FORMED WHEN 1,2-DIOLEIN WAS INCUBATED WITH CASTOR BEAN LIPASE AT pH 4.0

Substrate emulsified in the buffer at a concentration of 4 μmoles/ml. Enzyme concentration, 0.5 mg/ml.

Incubation time (min)	*Triolein*	*Diolein*	*1,3-Isomer (%)*	*Monoolein*	*1-Isomer (%)*	*Glycerol*
0	0.6	97.4	0.9	2.0	—	0
1	13.0	60.8	16.0	19.4	48.6	6.5
2	14.4	48.6	—	22.5	44.8	14.1
3	14.6	42.3	27.8	20.6	42.1	21.3
5	13.8	35.8	35.8	17.0	41.0	32.5

only be explained by an exchange of the primary ester bonds by fatty acids containing ^{14}C activity. This exchanges must have been obtained by hydrolysis of fatty acid at the 2-position of the original triglyceride. The low ratio $^{14}C/^{3}H$ of the 1,3-di- and 1-monoglyceride would indicate that these species are not derived directly from the 1,2- or 2-monoglyceride by isomerization.

Experiments with [1-^{14}C]1-oleoyl-2-oleoyl [^{3}H]glycerol

In the experiments given in Table III, one-third of the glycerol originally present as diolein has been liberated at the longest incubation time. Increasing amounts of 1,3-diolein appeared in the incubation with time. The monoolein formed was 41–49% made up of the 1-isomer. The $^{14}C/^{3}H$ ratios of the different glyceride fractions in relation to that of the original 1,2-diolein (= 1.00) are given in Table IV. The 1,2-diolein increased its ratio from the starting value of 1.00. The 1,3-diolein that appeared had figures between 0.58 and 0.65. The 2-monoolein was in the range of 0.93–0.97 and the 1-monoolein went from 0.17 to 0.28. The triolein fraction had $^{14}C/^{3}H$ figures higher than any of the other fractions. As the 1,3-diolein appearing had an activity ratio definitely lower than the 1,2-diolein, it cannot be formed directly from this by isomerization. Nor can the 1-monoolein have been formed directly from the 2-monoglyceride by isomerization.

TABLE IV

$^{14}C/^{3}H$ RATIO OF THE GLYCERIDE FRACTIONS OBTAINED IN THE EXPERIMENT PRESENTED IN TABLE III

[1-^{14}C]1-Oleoyl-2-oleoyl [^{3}H]glycerol was used as substrate. The radioactivity ratio of the fractions has been related to a $^{14}C/^{3}H$ ratio of 1.00 in the 1,2-diolein substrate used.

Incubation time (min)	*Triolein*	*Diolein*		*Monoolein*	
		1,2-	*1,3-*	*1-*	*2-*
0	—	1.00	—	—	—
1	1.36	1.08	0.65	0.17	0.93
2	1.34	1.18	0.55	0.22	0.97
3	1.29	1.19	0.59	0.25	0.93
5	1.36	1.30	0.58	0.28	0.96

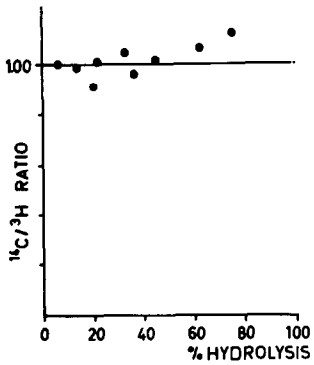

Fig. 1. $^{14}C/^{3}H$ ratio of the total glyceride mixture remaining in the incubation when [1-^{14}C]1-oleoyl-2-oleoyl [^{3}H]glycerol ($^{14}C/^{3}H$ ratio = 1.00) was hydrolysed with castor bean lipase, plotted against % fatty acid liberated (= % hydrolysis).

The $^{14}C/^{3}H$ ratio of the total glyceride fraction in these experiments was close to that of the original 1,2-diolein, indicating that the fatty acids originally in the 1- and 2-position were hydrolysed at similar rates (Fig. 1).

Experiment with [^{3}H]glycerol 2-monooleate

The substrate used was up to 93% composed of monoolein with minor amounts of di- and triolein. The monoolein fraction was up to 89.2% of the 2-form at the start of the experiment. The results are given in Table V and show that glycerol appeared

TABLE V

% MOLAR COMPOSITION OF THE REACTION MIXTURE WHEN 2-MONOOLEIN (4 μmoles/ml) WAS INCUBATED WITH CASTOR BEAN LIPASE (0.75 mg/ml)

Incubation time (min)	*Triolein*	*Diolein*	*1,3-Isomer (%)*	*Monoolein*	*1-Isomer (%)*	*Glycerol*
0	0.4	5.9	2.9	93.0	10.8	0.8
3	0.6	10.2	7.0	72.3	10.8	17.0
6	0.6	10.9	8.1	58.4	11.1	30.0
9	0.6	12.8	7.8	48.9	10.7	37.7
15	0.7	16.2	8.5	42.6	11.5	40.5
30	1.0	13.9	8.3	24.8	13.0	60.3
60	1.0	11.7	8.2	16.5	15.0	70.8

early and that after 60 min incubation, 70% of the glyceride had been hydrolysed. The composition of the monoolein fraction remained almost constant during the course of the reaction with a mean of 88.2% 2-isomer. Diglyceride is formed, the 1,2-isomer making up as a mean, 92.7% of the whole fraction. Minor amounts triolein also appear in the incubations.

The results of this experiment show that 2-monoolein is hydrolysed by the castor bean lipase, and the rate of hydrolysis of the 2- and 1-isomers is rather similar as their reactive proportions are not changed during the reaction.

TABLE VI

MOLAR COMPOSITION OF THE REACTION MIXTURE WHEN 1-MONOOLEIN (4 μmoles/ml) AND OLEIC ACID (8 μmoles/ml) WAS INCUBATED WITH CASTOR BEAN LIPASE (0.5 mg/ml)

Incubation time (min)	*Triolein*	*Diolein*	*1,3-Isomer (%)*	*Monoolein*	*1-Isomer (%)*	*Glycerol*
0	0	0.7	—	96.3	99.7	3.0
3	0	5.9	81.2	88.1	99.5	6.0
6	0	10.1	79.8	78.9	99.2	11.0
9	0	14.4	78.7	69.9	98.7	15.7
18	0.3	20.8	80.5	51.9	98.5	26.9
36	0.9	22.5	78.8	34.9	97.1	41.7

Experiment with [1-^{14}C]1-oleoyl glycerol

When fatty acid-labeled 1-monoolein was incubated with castor bean lipase in the presence of 2 moles of oleic acid per mole monoolein, the latter was hydrolysed and free glycerol was formed, but diolein and traces of triolein also appeared (Table VI). The diolein fraction was up to around 80% of the 1,3-configuration. The amount of 2-monoolein in the monoglyceride fraction was not over 1%. These experiments show that the 1,3-diolein was formed directly from the 1-monoolein by acylation and that acylation also can occur at the 2-position, although the reaction favors formation of the 1,3-isomer.

Incubation of 1- and 2-glycerol monoether and fatty acid with castor bean lipase

In these experiments labeled 1- or 2-oleyl ethers were incubated with 2 moles of oleic acid for long periods of time to obtain the composition of the equilibrium mixture. The results of the experiment with the 1-monoether are given in Table VII. After 2 h incubation the concentration of the components is practically constant. The equilibrium mixture from the 1-monoether contains mono-, di- and tri-compounds in the approximate relationship, 50:40:10. With the 2-monoether the same relationship was 15:50:35. The di-compound from the 1-ether was made up to 80% by the 1-ether 3-ester compound. The equilibrium thus favors the acylation of the outer position of the 1-ether by a factor of 4.

TABLE VII

MOLAR COMPOSITION OF THE REACTION MIXTURE WHEN 1-OLEYL GLYCEROL (4 μmoles/ml) AND OLEIC ACID (8 μmoles) WERE INCUBATED WITH CASTOR BEAN LIPASE (0.5 mg/ml)

Incubation time (h)	*Monoether diglyceride*	*Monoether monoglyceride*	*1,3-Isomer (%)*	*Monoether*
0	0.2	4.3	—	95.5
1	5.3	40.9	79.9	53.8
2	6.3	40.8	77.9	53.0
4	7.6	40.7	78.2	51.8
8	8.5	41.3	80.1	50.2
16	8.7	41.7	80.1	49.7
32	9.0	41.2	75.8	49.8

DISCUSSION

In agreement with previous results[1,2] the present experiments show that triolein is hydrolysed by castor bean lipase to glycerol and fatty acid with low levels of intermediate di- and monoglyceride formation. In agreement with recent findings from this laboratory[6] the diglyceride fraction formed during the reaction was found to contain appreciable quantities of the 1,3-isomer. In addition it was found that the 1-mono-isomer made up 60–70% of the monoglyceride fraction. As the castor bean enzyme has been shown not to hydrolyse a fatty acid in the 2-position from a 1,3-diether 2-ester glycerol[6], the diglyceride primarily formed could be expected to be of the 1,2-configuration. Furthermore as the incubation and analytical procedures do not lead to isomerization the formation of the 1,3-diglyceride must be catalysed by the enzyme. One possible reaction sequence would be a direct isomerication of the 1,2-diglyceride to the 1,3-diglyceride. Such a reaction course was tested in the present experiments by the use of the triolein and 1,2-diolein substrates labeled in the 2-position with [^{14}C]oleic acid. The reasoning was a sfollows; if the fatty acid in the 2-position was directly isomerized to the 1-position the $^{14}C/^{3}H$ ratio should be the same in the 1,2- and the 1,3-diolein. This was not found to be the case. As seen in Tables II and IV and Fig. 1, the radioactivity ratio in the 1,3-isomer was always definitely lower than that of the 1,2-isomer.

Several other facts appear from the results. The $^{14}C/^{3}H$ ratio increased during the course of incubation for the tri- and 1,2-diolein fractions. This could only be explained by an exchange of the fatty acids in the 1- and 3-position of the glyceride with [^{14}C]oleic acid originally present in the 2-position of the glyceride. Experiments with 1,2-diolein and 1-monoolein showed that the enzyme catalyses the acylation of the di- and monoglyceride to form tri- and diglyceride, respectively. The results further show that the fatty acids of 1,2-diolein are hydrolysed at similar rates but that the re-esterification of the 1-monoolein favors the formation of the 1,3-isomer by a

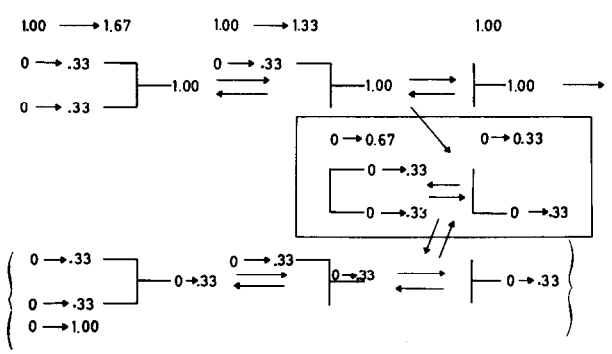

Fig. 2. Schematic presentation of the hydrolysis of a long-chain triglyceride by castor bena lipase. The reaction starts in the upper left part with a triglyceride with a labeled fatty acid in the two position with a specific activity of 1.00. Due to hydrolysis to glycerol and fatty acid, the specific activity of the free acid pool increases from 0 to 0.33 and a continous exchange of the primary ester bonds with the free fatty acid pool increases the total content of radioactivity of the triglyceride from 1.00 to 1.67, the 1,2-diglyceride from 1.00 to 1.33, *etc.* The lower part of the curve represents a pathway resulting from the formation of 1,2-diglyceride by acylation of a 1-monoglyceride. To simplify the scheme no free fatty acids have been included.

factor of four. The results obtained best agree with the reaction sequence given in Fig. 2. When triolein is the substrate, the fatty acid first removed is in the 1- or 3-position leading to 1,2-diolein. In this respect the enzyme can be said to have a positional specificity. The 1,2-diolein then is further hydrolysed with no positional specificity to the 1- and 2-monoglyceride. This leads to an increasing content of [^{14}C]acid in the free fatty acid fraction which is used for the reacylation of essentially the primary ester bonds of the remaining glycerides. This results in the formation of 1,3-diglyceride, both the fatty acids of which have an activity similar to that of the free fatty acid pool. The 1,3-diglyceride thus will be formed *via* the sequence:

1,2-Diglyceride $\rightleftharpoons$ 1-monoglyceride + fatty acid $\rightleftharpoons$ 1,3-diglyceride

The monoglyceride formed from the 1,2-diglyceride is also reacylated back to 1,2-diolein. The relative specific activity of the free fatty acid pool will increase from 0 to 0.33 if the radioactivity of the 2-fatty acid of the original triglyceride is set to 1.00. In the case when triolein was the substrate the ^{14}C/^{3}H ratio of the triolein would therefore approach 1.67, and that of the 1,2-diolein, 1.33, The fact that the 1-monoglyceride to some extent, as evidenced by the experiments with 1-monoolein or the ether, is acylated also in the 2-position (lower part of the figure) would tend in the long run to randomize the labeled fatty acid and would explain why the experimental figures for ^{14}C/^{3}H ratio of the tri- and 1,2-diolein never reach the values expected from the reaction sequence given in the upper part of the curve. A similar scheme can also be derived from the experiments in which the substrate was 1,2-diolein. The diolein experiments were undertaken in order to study a less complicated reaction and to obtain larger quantities of the monoglycerides for a more accurate determination of their activity ratio. The data obtained largely agree with those of the triolein experiments and clearly show the difference between the activity ratio of the 1,2- and 1,3-diglyceride and the 2- and 1-monoglyceride. As discussed earlier, the first step of the hydrolysis of the triglyceride leads to the formation of 1,2-diglyceride. In this step the enzyme therefore can be said to be specific for the primary ester bond, in line with the results of ORY *et al.*[5] that the fatty acids first released from the triglyceride are from the 1- and 3-position. The fatty acids from the 1,2-diolein are, however, split by the castor bean lipase at similar rates, and the enzyme therefore has no positional specificity at this level. In this respect the activity of the enzyme is different from pancreatic lipase[3] and the lipase of *Rhizopus arrhizus*[10] which have a high relative specificity for the ester bond in the 1-position of 1,2-diglyceride. The finding that the castor bean lipase did not hydrolyse at any appreciable rate the secondary ester bonds of 2,3-butane dioleate or 2-hexyl oleate[5] cannot be taken as an indication of the specificity of this enzyme against glyceride ester bonds, as the latter have different reactivities due to the influence of the neighbouring hydroxy groups.

Isomerization from the 2-position to the 1-position is shown by the isotope experiments not to be of any importance under the conditions of our experiments. The results from the experiments in which 2-monoolein was used as substrate therefore definitely demonstrate the direct hydrolysis of 2-monoolein by castor bean lipase at a rate similar to that of the 1-isomer.

Due to the irreversible hydrolysis of the last ester bond to glycerol and fatty acid, the reaction will finally go to completion. With a lipid-soluble acceptor for the fatty acid–enzyme complex, such as the monoglycerol ethers, the reaction will go to

equilibrium, the equilibrium mixture containing approximately equal proportions of mono-ether and acylated products. These results indicate that an acyl–enzyme intermediate is formed which transfers the acyl to groups other than water and that lipase most likely functions as a hydrophobic acyl group transferase. In this respect the function of the castor bean lipase is similar to that earlier demonstrated for pancreatic lipase[3] and the lipase from *Rhizopus arrhizus*[10].

ACKNOWLEDGMENT

Financial support has been obtained from the Swedish Medical Research Council grant B70-13X-71-06B.

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