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CHARACTERIZATION OF TWO MONOGLYCERIDE HYDROLYZING ENZYMES IN RAT ADIPOSE TISSUE

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SUMMARY

The present investigation has revealed the presence of two monoglyceride hydrolyzing enzymes in rat adipose tissue, tentatively designated as monoolein lipase and monolaurin lipase. These two enzymes can be differentiated on the basis of column chromatographic behavior, pH optimum, substrate preference, inhibition characteristics and heat sensitivity. The mobility of monolaurin lipase on Sephadex G-200 and gel electrophoresis is similar to that of a previously reported tributyrinase enzyme but can be differentiated from it on the basis of its inability to hydrolyze tributyrin and its relative insensitivity to F^- inhibition.

INTRODUCTION

In recent years, some emphasis has been placed on the elucidation of those processes involved in lipid mobilization. Much of this work has centered around lipoprotein lipase and the hormone-sensitive lipase, while relatively little has been done on the elucidation of the role of monoglyceride hydrolyzing enzymes in adipose tissue. VAUGHAN *et al.*[1] and KUPIECKI[2] have reported the presence of a specific monoglyceride lipase in rat adipose tissue, while WALLACH[3] has reported the presence of three monoglyceride hydrolyzing enzymes in rat skeletal muscle. The purpose of this paper is to present evidence for the presence of two enzymes in rat adipose tissue which have selectivity in hydrolyzing monoglycerides.

This investigation has also revealed that the behavior of one of the monoglyceride lipases tentatively called monolaurin lipase on Sephadex G-200 and gel electrophoresis is quite similar to a previously reported F^--sensitive tributyrinase[4]. This tributyrinase[5], which shows marked hydrolytic activity against tributyrin, also hydrolyzes monolaurin to the extent of 50% of its activity against tributyrin. However, further purification of the tributyrinase in our laboratory has revealed that this enzyme has high specific activity for tributyrin hydrolysis and an insignificant capacity to hydrolyze monolaurin (unpublished observations). Moreover, the monolaurin lipase shows marked hydrolytic activity against monolaurin and has no

Abbreviation: PCMB, *p*-chloromercuribenzoic acid.

capacity to hydrolyze tributyrin. In addition, the presently reported monoolein lipase was shown on the basis of several criteria to be different from these enzymes.

MATERIALS AND METHODS

Preparation of tissue extracts

Mesenteric, perirenal and parametrial adipose tissue was excised from decapitated female retired breeder Holtzman rats and homogenized with 2 vol. of ice-cold glass-distilled water containing 1 mM dithiothreitol and 1 mM EDTA in a Waring blender at maximal speed for 1 min. The homogenate was centrifuged at 100 000 × *g* at 0° for 5 min and the infranatant was filtered through gauze, centrifuged at 100 000 × *g* at 0° for 30 min and the particulate fraction discarded.

Preparation of $(NH_4)_2SO_4$ fractions

Enzyme grade $(NH_4)_2SO_4$ was added to the 100 000 × *g* supernatant to 0.60 saturation. After mechanical stirring for 30 min at 0°, the solution was centrifuged at 15 000 × *g* at 0° for 15 min and the resultant precipitate was taken up in 20 mM Tris buffer (pH 8.0). An alternate method in the preparation of $(NH_4)_2SO_4$ fractions consisted of making serial saturations of 0–0.50, 0.50–0.60 and 0.60–0.70 and the resultant precipitates after centrifugation were resuspended in 20 mM Tris buffer.

Column procedures

Gel filtration on Sephadex G-200 and anion-exchange chromatography on DEAE-cellulose were employed in further purification procedures. In the gel filtration procedure, the 0–0.60 $(NH_4)_2SO_4$ fraction was placed on a 1.5 cm × 80 cm column and eluted with 20 mM Tris buffer (pH 8.0) in 2-ml fractions at a flow rate of 6.0 ml/h at 0°. This stage of purification is hereafter designated as the Sephadex fraction.

In the anion-exchange procedure, the 0.50–0.60 $(NH_4)_2SO_4$ fraction was placed on a column with a resin bed 4.5 mm × 5 cm, eluted with 20 mM phosphate buffer (pH 8.0) in 1.75-ml fractions at 0° at a flow rate of 0.5 ml/min. This stage of purification is hereafter designated as the DEAE-cellulose fraction.

Disc gel electrophoresis

Disc gel electrophoresis was carried out at 0° as previously reported[4]. The gels were either stained with Amido Schwarz dye or rapidly sliced into 2.2-mm slices which were assayed for monolaurin, tributyrin or monoolein hydrolyzing activity for 14 h at room temperature.

Lipase assay

Extracts and partially purified preparations were assayed by adding 0.1 ml of enzyme solution to a reaction mixture containing 10 μmoles of appropriate substrate, 20 μmoles of Tris buffer (pH 8.0) unless noted, and glass distilled water to a final volume of 1.0 ml. The assay mixture was incubated for 1 h at 37°. The reaction was terminated by the addition of 5 ml of Dole's extraction mixture and free fatty acid production was determined by the method of DOLE AND MEINERTZ[6].

Preparation of substrates

Sufficient substrate for 25 ml of 0.1 M emulsion was weighed, 0.3 ml of Triton X-100 was added and heated slightly to melt the substrate, 20 ml of warm 20 mM Tris buffer (pH 8.0) was added and the mixture was sonicated with a Branson sonifier cell disruptor Model S125 at a setting of 8 for 1 min. The resultant emulsion was diluted to a final volume of 25 ml with Tris buffer.

In several trials, highly purified monolaurin and monoolein were compared as substrates with the commercially available monoglycerides and were hydrolyzed to an identical extent.

Protein determination

Protein concentration of the enzyme solutions from the Sephadex G-200 columns was determined by absorption at 280 mμ. In the DEAE-cellulose procedure, protein was determined by the method of LOWRY *et al.*[7].

Materials

The materials used in this study were obtained as follows: 1-monolaurin, C.P. Hall Co. of Illinois, Chicago; 1-monoolein, Calbiochem, Los Angeles, Calif.; 1-monopalmitin, 1-monostearin, methyl laurate, methyl myristate, methyl palmitate, methyl stearate, methyl linoleate, tributyrin, tricaproin, tricaprylin, trilaurin, trimyristin, tripalmitin, tristearin, triolein, trilinolein and Trizma Base, Sigma Chemical Co., St. Louis, Mo.; 1-monobutyrin and methyl butyrate, Eastman Organic Chemicals, Rochester, N.Y.; 1-monomyristin, Miami Valley Labs, Cincinnati, Ohio; 1-monolinolein, Mann Research, New York City, N.Y.; methyl oleate, General Biochemicals, Chagrin Falls, Ohio; NaF, sodium phosphate and disodium EDTA, Fisher Scientific Co., Chicago, Ill.; dithiothreitol, Nutritional Biochemical Corp., Cleveland, Ohio; *p*-chloromercuribenzoic acid, Aldrich Chemical Co., Milwaukee, Wisc.; materials for disc gel electrophoresis, Canal Industrial Corp., Bethesda, Md.; Sephadex G-200, Pharmacia Fine Chemicals, Inc., New Market, N.J.; Triton X-100, Rohm and Haas, Philadelphia, Pa.; Cellex-D (DEAE-cellulose), Bio-Rad Labs, Richmond, Calif.; bovine albumin powder (Fraction V), Armour Pharmaceutical Co., Chicago, Ill. All other reagents were of the highest purity commercially available.

RESULTS

Assay of crude homogenate

In the assay system, there is no detectable hydrolysis of endogenous substrate. Assay of the crude homogenate with monolaurin and monoolein as added substrates in the presence and absence of *p*-chloromercuribenzoic acid (PCMB) and NaF indicates that monoolein hydrolysis is strongly inhibited by 0.1 mM PCMB (80%) and unaffected by 1.0 mM NaF while monolaurin hydrolysis is slightly inhibited by both PCMB (30%) and NaF (10%).

Serial $(NH_4)_2SO_4$ fractions

Serial $(NH_4)_2SO_4$ fractionation was carried out in an attempt to separate the monoolein, monolaurin and F^--sensitive tributyrinase enzymes for further purification procedures. Table I shows the results of assay of the various $(NH_4)_2SO_4$

TABLE I

ASSAY OF $(NH_4)_2SO_4$ FRACTIONS

Assay procedure described under MATERIALS AND METHODS. Incubation time was 60 min at 37°. n = 5. Figures in parentheses are percent inhibition.

$(NH_4)_2SO_4$ fraction	*Substrate*	*Specific activity (mean ± S.E.) (μequiv. free fatty acids per mg protein per h)*		
			+0.1 mM PCMB	*+1 mM NaF*
0–0.50 satn.	Monolaurin	10.84 ± 2.38	4.15 ± 1.44 (61.7)	9.60 ± 2.52 (11.4)
	Monoolein	17.44 ± 3.32	1.50 ± 0.79 (91.4)	17.11 ± 3.73 (1.9)
	Tributyrin	13.66 ± 4.87*	10.04 ± 2.88 (26.5)	3.75 ± 1.42 (72.5)
0.50–0.60 satn.	Monolaurin	14.92 ± 2.30	11.95 ± 2.17 (19.9)	14.47 ± 1.91 (3.0)
	Monoolein	18.77 ± 3.22	3.39 ± 0.57 (81.9)	19.29 ± 4.30 (0)
	Tributyrin	52.90 ± 9.65	55.09 ± 11.11 (0)	15.04 ± 5.34 (71.6)
0.60–0.70 satn.	Monolaurin	12.59 ± 2.31	10.93 ± 2.28 (13.2)	9.10 ± 1.91 (27.7)
	Monoolein	4.20 ± 1.20	1.86 ± 0.36 (55.7)	2.34 ± 0.83 (44.3)
	Tributyrin	41.92 ± 6.80	45.97 ± 8.09 (0)*	6.39 ± 1.11 (84.8)

* $n = 4$.

fractions with monolaurin, monoolein and tributyrin as substrates in the presence and absence of PCMB and NaF. In the 0–0.50 saturated fraction, monoolein hydrolysis is strongly inhibited by PCMB with only a slight inhibition by NaF. PCMB is inhibitory to monolaurin and tributyrin hydrolysis, while NaF which is virtually noninhibitory to monolaurin and monoolein hydrolysis, strongly inhibits the hydrolysis of tributyrin. In the 0.50–0.60 saturated fraction, monoolein hydrolysis is still present and is strongly inhibited by PCMB. The specific activity against monolaurin and tributyrin are increased in this fraction. Both PCMB and NaF inhibition of monolaurin hydrolysis is greatly decreased; the activity toward tributyrin is not inhibited by PCMB but greatly inhibited by NaF. In the 0.60–0.70 saturated fraction,

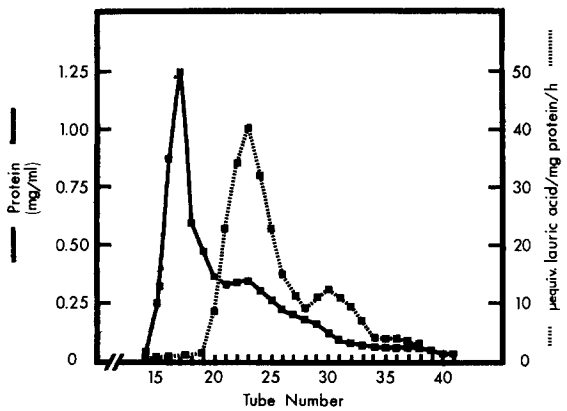


Fig. 1. Gel filtration of the 0–0.60 $(NH_4)_2SO_4$ fraction on Sephadex G-200. Experimental procedure is described under MATERIALS AND METHODS. ■—■, protein (absorption at 280 mμ); ■— —■, monolaurin lipase activity. Fractions were 2 ml each, and 0.1 ml from each fraction was assayed for monolaurin lipase activity. Incubation time was 60 min at 37°.

TABLE II

SUMMARY OF A TYPICAL MONOLAURIN LIPASE PURIFICATION WITH SEPHADEX G-200

Assay procedure described under MATERIALS AND METHODS. Incubation time was 60 min at 37°.

Fraction	*Protein (mg/ml)*	*Specific activity**	*Fold purification*	*Total activity***	*Recovery (%)*
Homogenate	8.64	1.52		123 256	
10 000 × *g* infranatant	3.72	3.22	2.12	71 100	57.7
100 000 × *g* supernatant	2.26	4.38	2.88	54 442	44.2
$(NH_4)_2SO_4$ 0.60 satn.	3.88	5.99	3.94	12 104	9.8
Sephadex G-200 peak	0.13	60.48	39.79		

* Specific activity expressed as μequiv. of lauric acid released per mg of protein per h.

** Total activity expressed as the product of μl of titrant per 0.1 ml of enzyme solution and total volume of the fraction.

the decrease in monoolein hydroysis is accompanied by a decrease in PCMB inhibition and an increase in NaF inhibition. PCMB is noninhibitory on tributyrin hydrolysis while NaF is highly inhibitory. It is quite evident from this table that the three enzyme activities are present in all the $(NH_4)_2SO_4$ fractions, but the 0–0.50 saturated fraction is most suitable for further studies of monoolein lipase, and the 0.50–0.60 fraction for those of monolaurin lipase.

Purification of monolaurin lipase

Gel filtration on Sephadex G-200 of the 0–0.60 $(NH_4)_2SO_4$ fraction yielded two protein peaks, as shown in Fig. 1. Upon assaying the collected fractions, it was found that monolaurin hydrolyzing activity was confined to the second peak; when monoolein was used as substrate, no hydrolysis was evident.

Table II represents a summary of a typical monolaurin lipase purification utilizing Sephadex G-200. Although the specific activity of this enzyme increases in all of the procedural steps, the greatest increase is realized with gel filtration. Although not presented in the table, all of the monolaurin lipase activity in the $(NH_4)_2SO_4$

TABLE III

SUMMARY OF A TYPICAL MONOLAURIN LIPASE PURIFICATION WITH DEAE-CELLULOSE

Assay procedure described under MATERIALS AND METHODS. Incubation time was 60 min at 37°.

Fraction	*Protein (mg/ml)*	*Specific activity**	*Fold purification*	*Total activity***	*Recovery (%)*
Homogenate	3.39	5.80		88 020	
10 000 × *g* infranatant	1.73	11.08	1.91	42 750	48.6
100 000 × *g* supernatant	1.35	13.50	2.33	39 498	44.9
$(NH_4)_2SO_4$ 0.50–0.60 satn.	1.59	15.46	2.67	7 334	8.3
DEAE-cellulose	0.08	47.88	8.26		

* Specific activity expressed as μequiv. of lauric acid released per mg of protein per h.

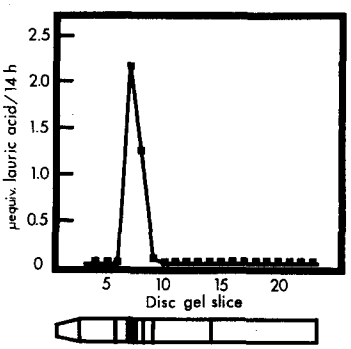
** Total activity expressed as the product of μl of titrant per 0.1 ml of enzyme solution and total volume of the fraction.

fraction is recovered from the gel filtration procedure. 10% of the total activity in the original homogenate is recovered at the end of the four steps of the procedure.

The second procedure utilized in increasing the specific activity of monolaurin lipase consisted of placing the 0.50–0.60 $(NH_4)_2SO_4$ fraction on DEAE-cellulose and collecting two 1.75-ml fractions. The first fraction contained both monolaurin lipase and tributyrinase activities, while the second fraction contained only monolaurin lipase activity. Both fractions were unable to hydrolyze monoolein when used as substrate. Table III represents a summary of monolaurin lipase purification utilizing the second fraction from DEAE-cellulose. As with Sephadex G-200, all the monolaurin lipase activity in the $(NH_4)_2SO_4$ fraction can be recovered in the two 1.75-ml fractions (60% in first fraction, 40% in the second). This method does not yield as great an increase in specific activity as does the Sephadex G-200 procedure, but is more rapid and convenient.

Disc gel electrophoresis

The relative purity of the second protein peak obtained by gel filtration with Sephadex G-200 which exhibited monolaurin hydrolytic activity was determined by disc gel electrophoresis. Fig. 2 schematically illustrates the presence of five protein bands in this enzyme preparation (right hand side of figure is the anode). Unstained gels were sliced and incubated at room temperature over night with monolaurin and



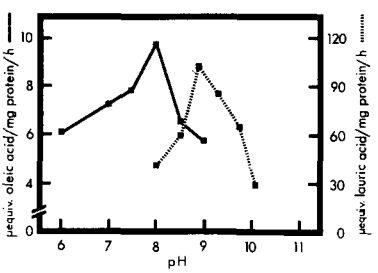


Fig. 2. Disc gel electrophoresis (Sephadex G-200 fraction) and assay of monolaurin lipase activity. Experimental procedure described under MATERIALS AND METHODS. Sephadex G-200 fraction (0.05 mg protein per 0.1 ml) was added. Unstained gels were sliced and incubated with monolaurin for 14 h at room temperature.

Fig. 3. pH optimum of monolaurin lipase (DEAE-cellulose fraction) and monoolein lipase (0–0.50 $(NH_4)_2SO_4$ fraction). Assays for monolaurin lipase activity were carried out in 100 mM histidine buffer; those for monoolein lipase activity in 60 mM phosphate or Tris buffers. Incubation time was 60 min at 37°.

upon assay it was found that the protein associated with the major (second) band was active in hydrolyzing monolaurin. Although not represented in this figure, tributyrin and monoolein hydrolysis did not occur with these gel slices.

Characteristics of monolaurin lipase

Effect of incubation time on hydrolysis of monolaurin. In order to determine the optimal incubation time for monolaurin hydrolysis, time-course studies were carried

out which indicated that the production of lauric acid from monolaurin is linear with time for 60 min at 37°.

pH optimum. The assays for pH optimum were carried out using three different buffers: histidine (pH 8.0–10.1), Tris (pH 7.5–9.0) and phosphate (pH 4.5–7.0). The activity of the enzyme is similar in Tris and histidine buffers in the pH 7.5–9.0 range; with phosphate buffer, slight monolaurin hydrolysis was observed in the pH 4.5–7.0 range. Fig. 3 shows that this enzyme has maximum activity near pH 8.9 in 100 mM histidine buffer.

Substrate specificity. Fig. 4 represents the result of assays of the activity of monolaurin lipase against various substrates. Three classes of compounds were employed: monoglycerides, triglycerides and methyl esters. The enzyme showed the greatest activity against the methyl esters, followed by the 1-monoglycerides and triglycerides. In the methyl ester series, methyl laurate was hydrolyzed greater than methyl butyrate; other tested esters were not hydrolyzed. In the monoglyceride series, the enzyme exhibited a high specificity for monolaurin; monobutyrin was hydrolyzed approx. 30% as actively while other monoglycerides were not hydrolyzed. In the triglyceride series, only tricaproin was hydrolyzed and that to the extent of approx. 60% of the activity seen with monolaurin.

Effect of inhibitors. A number of inhibitors known to be inhibitory to other lipases were tested on the monolaurin lipase activity obtained from DEAE-cellulose. Insulin (1 munit/ml) and albumin (4%) were without effect; 1 mM NaF inhibited approx. 15%; 1 mM physostigmine produced approx. 27% inhibition, and 0.1 mM PCMB inhibited the enzyme about 20%. Monolaurin lipase activity obtained from the Sephadex G-200 procedure was inhibited approx. 90% by 1 mM malathion. 1 mM phentolamine was noninhibitory to monolaurin lipase from either source.

Heat stability. A Sephadex G-200 fraction containing monolaurin lipase activity retained full lipolytic activity after heating to 60° for 5 min in a water bath and shock cooling.

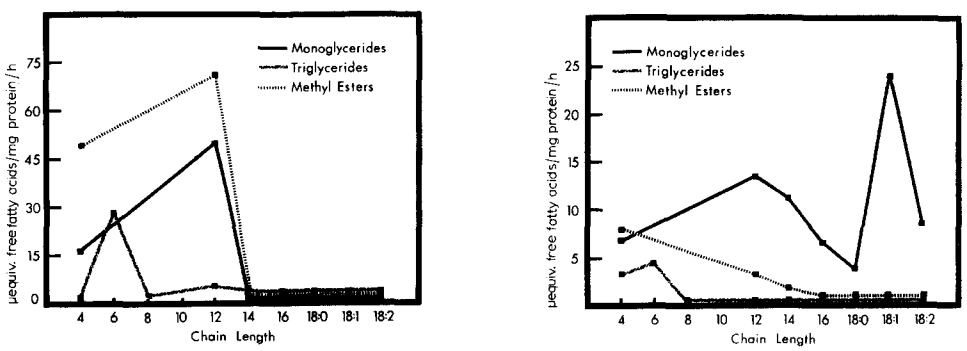


Fig. 4. Hydrolysis of esters of fatty acids of various chain lengths by monolaurin lipase (DEAE-cellulose fraction). 1-Monoglycerides, triglycerides and methyl esters were used. Assays were carried out in 100 mM histidine buffer at pH 8.9. Incubation time was 60 min at 37°. Chain length refers to the number of carbon atoms in the saturated or unsaturated fatty acid component.

Fig. 5. Hydrolysis of esters of fatty acids of various chain lengths by monoolein lipase (0–0.50 $(NH_4)_2SO_4$ fraction). 1-Monoglycerides, triglycerides and methyl esters were used. Assays were carried out in Tris buffer at pH 8.0. Chain length refers to the number of carbon atoms in the saturated or unsaturated fatty acid component.

Purification of monoolein lipase

Monoolein lipase has proved to be very labile and procedures utilizing Sephadex or Sepharose columns produced preparations lacking hydrolytic activity. Attempts to prevent this loss of activity by keeping dithiothreitol (1 mM) in contact with the enzyme throughout the procedure were unsuccessful. Elution of DEAE-cellulose and Dowex AG-50 columns with pH and salt gradients was effective in retaining some monoolein lipase activity but was ineffective in separating this enzyme from the monolaurin lipase. Disc gel electrophoresis also inactivated the enzyme. Hence, the following results were obtained using the 0–0.50 saturated $(NH_4)_2SO_4$ fraction.

Characteristics of monoolein lipase

Effect of incubation time on hydrolysis of monoolein. A time-course study on the monoolein lipase activity was performed and it indicated that hydrolysis of monoolein is linear for 60 min at 37°.

pH optimum. The assays for pH optimum were carried out using phosphate buffer at pH 6.0, 7.0 and 7.5 and Tris buffer at pH 8.0, 8.5 and 9.0. The activity of the enzyme is similar in both buffers at the same pH. The results shown in Fig. 3 indicate that monoolein lipase has maximum activity near pH 8.0.

Substrate specificity. Fig. 5 shows the results of assays of the 0–0.50 $(NH_4)_2SO_4$ fraction against various substrates. Three classes of compounds were used: monoglycerides, triglycerides and methyl esters. The greatest activity was seen against the 1-monoglycerides followed by the methyl esters and triglycerides. There appears to be no direct relationship between chain length and hydrolytic activity since the monoglycerides are hydrolyzed in the following order: monoolein, monolaurin, monomyristin, monolinolein, monobutyrin, monopalmitin and monostearin. In the methyl ester series, methyl butyrate was hydrolyzed to the extent of approx. 30% of that activity seen with monoolein; little or no activity was seen with the other methyl esters. Of the triglycerides tested, tributyrin and tricaproin were hydrolyzed at approx. 20% of the rate of hydrolysis seen with monoolein; no hydrolysis was evident with the other tested triglycerides.

Effect of inhibitors. Since monoolein lipase has not been obtained in a more purified state, inhibition characteristics cannot be presented in complete form. Preliminary studies indicate that the hydrolysis of monoolein is inhibited approx. 18% by 1 mM physostigmine and 12% by 1 munit/ml insulin. The hydrolysis of monoolein is inhibited 80–90% by 0.1 mM PCMB and 50–60% by 0.01 mM PCMB.

Heat stability. An enzyme preparation active in hydrolyzing monoolein was heated to 60° in a water bath for 5 min and then shock cooled. Such treatment resulted in complete inactivation of the enzyme.

DISCUSSION

Present concepts of the sequence of reactions in triglyceride breakdown indicates a role for monoglyceride lipases in the complete hydrolysis of triglyceride by various tissues. At present, there are three reports of monoglyceride lipases[1–3]. There seems to be little doubt that in skeletal muscle more than one enzyme exists which has as at least a portion of its function, the hydrolysis of monoglycerides. The present investigation extends to adipose tissue the probability that there are at least two

monoglyceride hydrolyzing enzymes which we have tentatively designated as monolaurin lipase and monoolein lipase.

A step by step comparison of these enzymes in adipose tissue with those found by other investigators in adipose and other tissues would be rather fruitless because of differences in experimental design, in animal and tissue sources, and in methodology. However, some broad similarities and differences are present and may be examined productively.

With regard to the monolaurin lipase, the substrate preference, the pH optimum, inhibition characteristics and stability to heat are similar in the adipose tissue and in the skeletal muscle preparations[3]. Some minor differences in substrate preference are seen such as the skeletal muscle enzyme having little activity against monobutyrin while the adipose tissue enzyme has about 30% of its monolaurin activity against this substrate. In addition, the skeletal muscle enzyme has some hydrolytic activity against tributyrin which is not observed in the adipose tissue enzyme. The latter observation may be related to the recent findings in our laboratory that a F^--sensitive tributyrinase[4] and the monolaurin lipase have quite similar molecular weights and charge properties based upon Sephadex filtration and disc electrophoresis. Previous reports[5] indicated that this F^--sensitive tributyrinase preparation had the ability to hydrolyze monolaurin to the extent of 50% of its tributyrin activity. As previously mentioned, further purification of the tributyrinase in our laboratory has resulted in a preparation which actively hydrolyzes tributyrin with insignificant activity towards monolaurin. Based upon this information and the data presented herein, it appears that some of the previously reported characteristics of the F^--sensitive tributyrinase may be attributed to the combined action of a tributyrinase and the monolaurin lipase, and in addition this may explain the tributyrin hydrolyzing activity of the skeletal muscle enzyme.

The inhibition of the skeletal muscle monolaurin lipase by albumin was not observed in the adipose tissue enzyme. A possible explanation for this may lie in the observation of VAUGHAN AND STEINBERG[1,8] that albumin from different suppliers had inhibitory properties on adipose tissue homogenate lipolytic activity.

On balance, the monolaurin lipase from adipose tissue reported herein appears to be very similar to the one from skeletal muscle with the minor differences probably explicable on the basis of different tissue sources for the enzyme.

The role of monolaurin lipase in lipid metabolism is uncertain at this time. On the basis of its activity against the soluble methyl butyrate, one might suspect it to be more of an esterase than a true lipase (by definition of DESNUELLE[9]). However, the higher activity noted against the insoluble substrates (and hence its action at the oil–water interphase) more properly classifies the enzyme as a lipase.

With regard to the monoolein lipase, the substrate specificity, the pH optimum and the inhibition by PCMB makes it possible that the monoglyceride lipase described by VAUGHAN *et al.*[1] and by KUPIECKI[2] are similar to the one reported here. Minor differences in pH optima and in substrate preference between these enzymes are probably due to the rather poor state of purification so far obtained for monoolein lipase.

The function of monoolein lipase in lipid mobilization is not entirely clear but since oleic, palmitic and linoleic acids are known components of adipose tissue triglycerides, it is rather easy to envision an important physiological role for the enzyme.

It should be recalled that while substrates labeled as 1-monoglycerides were used in these studies, some isomerization to the 2-position may occur during substrate preparation and assay therefore having an influence on the apparent substrate specificity. As such, 2-monoglycerides may serve as better substrate for the above described monoglyceride lipases. Further investigation is needed to determine this possibility.

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