Hydrolysis of Synthetic Triacylglycerols by Pancreatic and Lipoprotein Lipase[1]

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ABSTRACT

The stereochemical course of the hydrolysis of synthetic sn-glycerol-1-palmitate-2-oleate-3-linoleate, sn-glycerol-1,2-dipalmitate-3-oleate and their antipodes by pancreatic and milk lipoprotein lipase was investigated by thin layer and gas liquid chromatographies of the diacylglycerol intermediates. The enzymic hydrolyses were made with bile salts or lysolecithin in a 1:1 molar ratio to the substrate as emulsifiers and were limited to short time intervals which minimized isomerization and the reversal of lipolysis. In all instances, the products of hydrolysis by lipoprotein lipase contained a marked preponderance of the 2,3-diacylglycerols, while the composition of the diacylglycerol intermediates in the products of pancreatic lipase varied with the nature of the fatty acid in the 1 and 3 positions of the triacylglycerol molecule. Pancreatic lipase, but not lipoprotein lipase, gave a preferential release of unsaturated fatty acids. The above results are similar to those obtained with radioactive trioleoylglycerol and conventional stereospecific analyses and suggest that lipoprotein lipase may favor attack on the sn-1 position. It is hypothesized that the small amounts of the 1,2-diacylglycerols present may have arisen from a reversal of lipolysis also catalyzed by this enzyme.

INTRODUCTION

Most enzymes, including phospholipases, display a highly specific stereochemical course of action. The acylglycerol lipases appear unusual, since they are believed to hydrolyze enantiomeric acylglycerols at comparable rates. Thus, Tattrie, et al., (1) has shown that the palmitic and oleic acids in the 1 and 3 positions are released at equal rates during hydrolysis of 1,2-dipalmitoyl-3-oleoyl-sn-glycerol by pancreatic lipase. Similarly, Assmann, et al., (2) recently demonstrated that the unlabeled and labeled oleic acids in the 1 and 3 positions, respectively, of 1,2-dioleoyl-3-(9,10-^{3}H) oleoyl-sn-glycerol are released at about the same rates during hydrolysis by the lipases of postheparin plasma. Furthermore, Nilsson-Ehle, et al., (3) has shown that the lipoprotein lipase hydrolyzes the primary positions ahead of the secondary, as demonstrated earlier by Mattson and Beck (4) for pancreatic lipase. In addition, pancreatic lipase has been shown to yield ca. equimolar amounts of the 1,2- and 2,3-diacylglycerols from natural triacylglycerols and has been incorporated as a means of random generation of diacylglycerols in the original Brockerhoff method of stereospecific analysis (5).

On the other hand, stereospecific analyses of the diacylglycerol intermediates of lipoprotein lipase digestion (6) have indicated a significantly higher proportion of 2,3-diacyl-sn-glycerols than would have been anticipated from a purely random degradation. Since the diacylglycerols make up only a small proportion of the total digestion products of lipoprotein lipase, the results of the fatty acid and diacylglycerol intermediate analyses need not be in conflict. However, early studies by Karnovsky and Wolff (7) had shown that ca. 49% of the radioactivity originally present in the 1-^{14}C-glycerol of trioleoylglycerol may be recovered in the hydroxyl carbon of the diacylglycerols released by treatment with a clearing factor. Since this discrepancy could not be explained by isomerization or incomplete recoveries of enantiomers, it was decided to reinvestigate the problem using synthetic triacylglycerols which yield chromatographically distinguishable 1,2- and 2,3-diacylglycerols during enzyme digestion. The present studies with several pairs of mixed acid enantiomers confirmed our earlier claims (6) that the 2,3-diacylglycerols tend to accumulate in the digests of lipoprotein lipase, while the products of pancreatic lipase hydrolysis contained a preponderance of the more saturated diacylglycerols, regardless of their steric configuration.

MATERIALS AND METHODS

Reagents

Lecithin was isolated from total lipid extracts of egg yolk by preparative thin layer chromatography (TLC) (6). Lyso (1-acyl) lecithin was obtained from Sigma Chemical Co., St. Louis, Mo., and glycocholate from Pfaltz and

[1]Presented in part at the AOCS Fall Meeting, Ottawa, September 1972.

Bauer, Acetochemical Co., Flushing, N.Y. Mixed bile salts were purchased from Difco Chemicals, Detroit, Mich. Trisil/Bisacetamide (BSA) was from Pierce Chemical Co., Rockford, Ill. The stigmasterol and tridecanoylglycerol used as internal standards in the gas liquid chromatography (GLC) analyses were, respectively, from Applied Science Laboratories, State College, Pa., and Eastman Organic Chemicals, Rochester, N.Y.

Pancreatic lipase was obtained from General Biochemicals, Chagrin Falls, Ohio, while the bovine milk lipoprotein lipase was saved from an earlier preparation (6) held at -20 C.

Substrates

The synthetic triacylglycerol substrates, sn-glycerol-1-palmitate-2-oleate-3-linoleate (A) and its antipode (B) were prepared by reacting the appropriate C_{16} and C_{18} mixed acid diacylglycerols with the corresponding fatty acid chloride in pyridine (8, 9). The resulting triacylglycerol was isolated by silicic acid column chromatography and the triacylglycerols were resolved further from minor amounts of free fatty acids and mono- and diacylglycerols by TLC in a neutral lipid solvent system (8). The synthetic substrates, sn-glycerol-1-oleate-2,3-dipalmitate (C) and its antipode (D) were obtained from Supelco, Bellefonte, Pa., and (carboxyl-^{14}C) glycerol trioleate (15.2 mC/mM) was purchased from ICN Chemical and Radioisotope Division, Irvine, Calif. The commercial products were checked for purity by chromatography. Furthermore, each enantiomer of the synthetic triacylglycerols was subjected to Grignard degradation, and the 1,2(2,3)- and 1,3-diacylglycerols were resolved by GLC on polar and nonpolar columns (10). In each case, only the anticipated molecular species in the appropriate proportions were found which attested to the success of the syntheses and the probability of the correctness of the stated structures.

Enzyme Hydrolyses

Hydrolyses with lipoprotein lipase were performed using two types of substrate emulsions. In one, 20 mg (20 mM) triacylglycerol was mixed with 12 mg dry glycocholic acid and then emulsified with 2 ml 0.1 M Tris buffer at pH 8.0. Aliquots of the emulsion were activated at 37 C for 30 min with normal rat serum (which contained ca. 1 mg/ml lecithin). In the other, 12 mM triacylglycerol was sonicated in a glass tube with 6.5 mg (12 mM) lysolecithin in either saline or Tris buffer. Aliquots of these emulsions were activated with rat serum which had been delipidated (11) and extracted in the ammonium BSA buffer of the assay system. In some instances, the activation period was reduced to 5 min. In all cases the activated substrate was treated with lipoprotein lipase, as described previously (6). Saline was substituted for the enzyme solution in the blank controls. Total lipids extracted from the hydrolysates between 0-20 min were separated into the component neutral lipids by TLC on borate treated Silica Gel G and the 1,2(2,3)-diacylglycerols recovered free from contamination with the 1,3-isomers. GLC also permitted analyses of aliquots of the total lipid extracts without TLC separation.

Hydrolyses with pancreatic lipase were carried out according to Luddy, et al., (12) with 20 mg aliquots of triacylglycerols in a Vortex mixer for 1.5 min at 40 C, without the addition of organic solvent. The mixed 1,2(2,3)-diacylglycerols released were separated from the total lipid extracts and their proportional composition determined by argentation TLC and GLC.

Analyses of Diacylglycerols

The synthetic triacylglycerol substrates had been selected to yield 1,2- and 2,3-diacylglycerols of differing mol wt and degree of unsaturation. Thus, the 1,2-diacylglycerols from compound A had a carbon number of 34 which readily is resolved from carbon number 36 of the 2,3-diacylglycerol by GLC. Since the 1,2-diacylglycerols released from compound A had only 1 double bond and the 2,3-diacylglycerol had 3 double bonds, the 2 isomeric diacylglycerols could be resolved readily by argentation TLC. For the diacylglycerol end products of compound B, the reverse situation is found, and the same methods are, therefore, applicable. The 1,2- and 2,3-diacylglycerols of compounds C and D are distinguishable chromatographically by separation of the carbon numbers 32 and 34, and saturated and monounsaturated species of diacylglycerols.

GLC

Aliquots of 1,2(2,3)-diacylglycerols in the hydrolysates of either pancreatic or lipoprotein lipase were reduced to dryness and trimethylsilylated with an excess of Trisil/BSA at room temperature overnight. Separations based upon carbon number were made on a Beckman GC-4 gas chromatograph equipped with stainless steel columns (50 cm x 2 mm inside diameter) packed with 3% OV-1 on 100-120 mesh Gas Chrom Q. The temperature was programed from 150-350 C in 16 min. Peak areas were estimated by an Infotronics electronic peak area integrator. The peaks were identified and quantitated in relation to tridecanoylglycerol or

TABLE I

Products of Digestion by Lipoprotein Lipase of Tri-(-^{14}C)-Oleoylglycerol Emulsified with Different Agents

Emulsifier	Molar ratio[a] with triolein[b]	Incubation min	Triglyceride digestion moles %	Products DG moles %	Products MG moles %	Products FFA moles %
Sodium glycocholate	1.2	15	15.9	2.6	11.4	31.5
Egg yolk extract	0.8	10	34.4	3.9	38.4	66.3
		20	46.2	3.6	22.5	108.9
Lecithin[d]	0.2	10	5.9	2.2	3.9	9.5
		20	9.6	2.7	3.7	16.7
	0.8	10	28.0	5.9	21.0	51.3
		20	33.5	6.5	23.1	64.8
Lyso (1-acyl) lecithin[d]	0.8	10	32.9	4.2	24.6	66.0
		20	52.5	4.2	29.7	119.7
Lyso (1-acyl) lecithin, no serum activation	0.8	10	6.2	3.6	2.4	9.3
		20	11.2	5.7	5.4	16.8

[a]Molar ratio for lecithin equivalent except for sodium glycocholate.

[b]The substrate consisted of 12 or 20 mg unlabeled triolein in addition to the tri-(1-^{14}C)-oleoylglycerol.

[c]DG = diglycerides, MG = monoglycerides, and FFA = free fatty acids.

[d]Single determinations. Other values are means of duplicate determinations.

stigmasterol used as internal standard.

The diacylglycerols released from the synthetic triacylglycerols by Grignard degradation were resolved and quantitated by an F&M biomedical gas chromatograph equipped with glass columns (180 cm x 3 mm inside diameter) containing 3% SILAR 5 CP on 100-120 mesh Gas Chrom Q, as described by Myher and Kuksis (10).

Argentation TLC

For argentation TLC suitable aliquots of the mixed 1,2(2,3)-diacylglycerols were acetylated with 5 μC 1-^{14}C-acetic anhydride in pyridine. The labeled diacylglycerol acetates were purified by TLC in a neutral lipid solvent system and the monoene and triene fractions resolved by argentation TLC on Silica Gel G containing 20% silver nitrate using 1.2% methanol in chloroform as the developing solvent. To facilitate the location of the bands of labeled acetates, unlabeled carrier was added in the form of acetylated diacylglycerols isolated from a pancreatic lipase digest of commercial trioleoylglycerol. The radioactivity of the bands was estimated following elution with chloroform in a Mark 1 6894 series Nuclear Chicago liquid scintillation system with external quenching.

RESULTS

Selection of Emulsifier

Since lipoprotein lipase is active only with emulsions of triacylglycerols, a surface active material is required as an emulsifier. In previous work (6), this need was met by egg yolk lipids, which were rich in phosphatidylcholine and phosphatidylethanolamine. To simplify the mass analyses of the intermediates, purified lecithin, lysolecithin, and bile salts were considered as potential emulsifiers of simple chemical structure. Figure 1 shows the effect of the

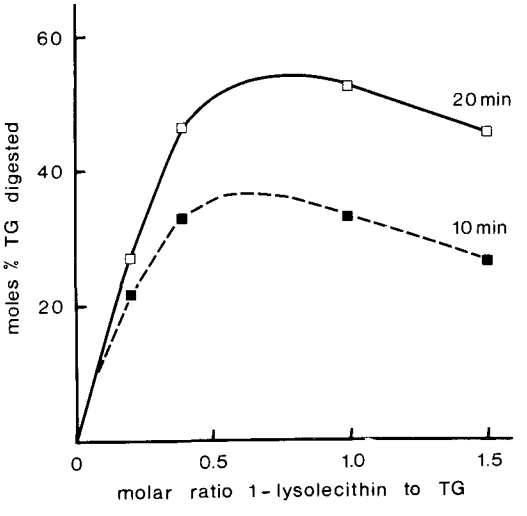

FIG. 1. Effect of 1-lysolecithin upon hydrolysis of trioleoylglycerol. Tri-(1-^{14}C)-oleoylglycerol was added for quantitation. TG = triglycerides.

TABLE II

Products of Digestion by Lipoprotein Lipase of Tri-(-^{14}C)-Oleoylglycerol Activated with Delipidated Serum

Activation[a] min	Incubation min	Triglyceride digestion moles %	Products DG moles %	MG moles %	FFA moles %
5	2	9.9	2.4	9.9	15.2
	10	44.6	4.5	39.3	85.8
10	2	8.5	1.8	8.1	14.1
	10	32.7	3.8	27.9	62.4

[a]The substrate consisted of 12 mg unlabeled triolein in addition to the tri-(1-^{14}C) oleoylglycerol.

[b]DG = diglycerides, MG = monoglycerides, and FFA = free fatty acids.

proportion of lysolecithin to substrate on the rate of hydrolysis by lipoprotein lipase. The optimum ratio was ca. mole to mole, which was higher than that reported by other workers (13, 14). This may have been due to the fact that the lysolecithin in these experiments was added with a delipidated serum. Similar results were obtained with lecithin and total egg yolk lipids. Lecithin in a molar ratio of 0.2, a level considered suitable by others, (13, 14) was not satisfactory under our conditions. Sodium glycocholate was a much less effective emulsifier than either the lecithins or egg yolk lipids.

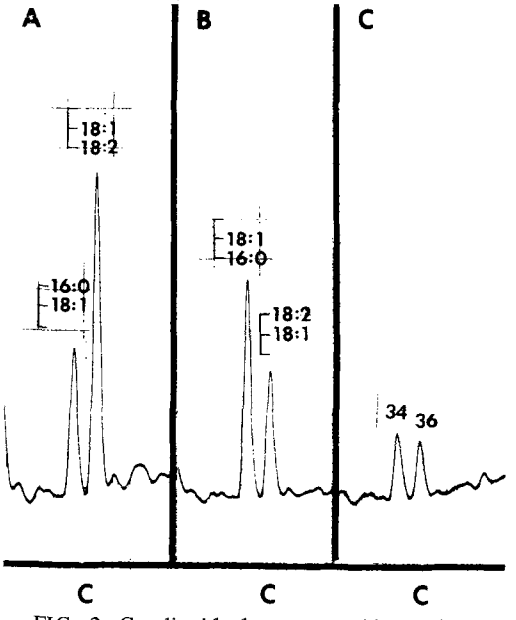

FIG. 2. Gas liquid chromatographic elution patterns of 1,2- and 2,3-diacylglycerols from lipoprotein lipase digestion of triacylglycerols A and B. C represents a pooled control without addition of enzyme. The temperature was programed from 150-350 C in 16 min. Major peaks, representing carbon numbers 34 and 36 were eluted at 290 and 300 C respectively.

Table I lists the various emulsifiers tried and gives the yields of monoacyl- and diacylglycerols and free fatty acids, as well as the estimates of the overall extent of digestion at 10-20 min of incubation, as quantitated by tri- (1-^{14}C)-oleoylglycerol as substrate. With the need for phospholipid filled by lysolecithin, delipidated serum extracted in buffer could be introduced into the reaction medium without loss of capacity for activation. Table I confirms our previous observation that the omission of the activation reduced the triacylglycerol hydrolysis by 80-90%. Table II shows that the period of preactivation of substrate could be shortened to as little as 5 min without reducing the enzyme activity. There was little difference in the levels of diacylglycerol accumulated in the medium with the different emulsifiers, namely an average 5-6 moles % of the total digestion products. Since the proportion of the enantiomers obtained might vary with the composition of the incubation medium, we examined the diacylglycerols collected with both glycocholate and lysolecithin as the emulsifiers.

Lipolysis of Synthetic Triacylglycerols

The structures of the diacylglycerol intermediates were determined on the basis of the known structure of the original triacylglycerols and the GLC and argentation TLC properties of the 1,2(2,3)-diacylglycerols recovered from borate TLC. Figure 2 shows the GLC elution patterns recorded for the 1,2(2,3)-diacylglycerols from the lipoprotein lipase digestion of triacylglycerols A and B, incubated in the presence of sodium glycocholate as the emulsifier. In both instances, major peaks are seen for carbon numbers 34 and 36. The larger peak in each case, however, represents the 2,3-diacylglycerol. A calculation of the peak areas reveals that the enzyme yielded ca. the same ratio of

TABLE III

Diacylglycerol Composition of Hydrolysate during Digestion of Synthetic Triacylglycerols by Lipoprotein Lipase

Substrate	Emulsifier	Argentation chromatography 1,2-[a]	Argentation chromatography 2,3-[a]	Gas liquid chromatography 1,2-[b]	Gas liquid chromatography 2,3-[b]
A		%	%	%	%
sn-glycerol-1-palmitate-2-oleate-3-linoleate	Glycocholate	21.3	78.7	34.9	65.1
		35.5	64.5	29.9	70.1
				31.4[c]	68.6[c]
	Lysolecithin	23.1	76.9	20.3	79.7
		27.2	72.8	23.8	76.2
B					
sn-glycerol-1-linoleate-2-oleate-3-palmitate	Glycocholate	20.7	79.3	23.9	76.1
		25.2	74.8	30.0	70.0
				35.5[c]	64.5[c]
	Lysolecithin	22.7	77.3	17.5	82.5
		22.0	78.0	24.8	75.2
C					
sn-glycerol-1-oleate-2,3-dipalmitate	Lysolecithin			24.1	75.9
D					
sn-glycerol-1,2-dipalmitate-3-oleate	Lysolecithin			34.6	65.4

[a]Means of duplicate determinations of diacylglycerol fractions as monoenes and trienes.

[b]Means of duplicate determinations of diacylglycerol fractions as carbon numbers 32, 34, or 36.

[c]The source of the lipoprotein lipase in these two experiments was rat postheparin plasma. In all other cases, the source was bovine skim milk.

1,2- to 2,3-diacylglycerols in each case. Table III gives the results of analyses of the diacylglycerol composition of the hydrolysates under other conditions of degradation which comprised the effect of different emulsifying agents, as well as the consequences of using delipidated or fresh serum. This table also includes analyses of diacylglycerol products when the source of the lipoprotein lipase was rat post heparin plasma (6) instead of bovine skim milk. In all instances, a preferential accumulation of the 2,3-diacylglycerols was observed with the AB as well as the CD pairs of isomeric triacylglycerols. The average ratio of 2,3- to 1,2-diacylglycerols was 75 to 25. Only small amounts of 1,3-diacylglycerols were recovered from any of the incubation mixtures under the present experimental conditions.

Since the 1,2- and 2,3-diacylglycerols released from the A and B triacylglycerols also differed in the degree of unsaturation, they could be separated by argentation TLC. The results of these analyses are included in Table III. In all instances, the proportions for the 1,2- and 2,3-diacylglycerols agreed closely with those based upon GLC analyses of the corresponding products of lipolysis. Since the analyses by carbon number and by the number of double bonds are not affected by possible isomerization, because the corresponding isomers would overlap in these analytical systems, both sets of results are not only consistent but experimentally sound. As pointed out above, there was very little isomerization observed under the present conditions. It would, therefore, appear that lipoprotein lipase preferentially attacks position 1 of the sn-glycerol, regardless of whether it is occupied by palmitic, linoleic, or oleic acids. These results fully agree with previous findings based upon stereospecific analyses of the 1,2 (2,3)-diacylglycerols released from trioleoylglycerol by lipoprotein lipase under comparable conditions (6).

Figure 3 shows the proportions of the 1,2- and 2,3-diacylglycerols derived from the synthetic A and B triacylglycerols by hydrolysis with pancreatic lipase. It is seen that the proportion of the 1,2-isomer exceeds that of the 2,3-isomer for triacylglycerol A, while the opposite result is obtained for B. This suggests that pancreatic lipase favors the release of linoleate over palmitate, regardless of the type of primary position involved. Table IV gives the results of other analyses of pancreatic digests obtained with triacylglycerols A,B,C, and D. In all cases, the unsaturated fatty acid was prefer-

TABLE IV

Diacylglycerol Composition of Hydrolysate during Digestion of Synthetic Triacylglycerols by Pancreatic Lipase

Substrate	Argentation chromatography		Gas liquid chromatography	
	1,2-[a]	2,3-[a]	1,2-[b]	2,3-[b]
	%	%	%	%
sn-Glycerol-1-palmitate-2-oleate-3-linoleate	59.7	40.3	60.3	39.7
	59.6	40.4	61.5	38.5
sn-Glycerol-1-linoleate-2-oleate-3-palmitate	43.0	57.0	41.8	58.2
	45.9	54.1	45.7	54.4
sn-Glycerol-1-oleate-2,3-dipalmitate			35.1	64.9
sn-Glycerol-1,2-dipalmitate-3-oleate			66.7	33.3

[a]Diacylglycerol fractions were determined as monoenes and trienes.

[b]Diacylglycerol fractions were determined as carbon numbers 32, 34, or 36.

entially released. Furthermore, the GLC findings were reproduced faithfully in the results derived by argentation TLC of the diacylglycerols from the corresponding incubations of triacylglycerols A and B.

DISCUSSION

Hydrolysis with Lipoprotein Lipase

The results of the chromatographic studies of the diacylglycerols released from synthetic triacylglycerols coincide with those obtained previously by stereospecific analyses of the hydrolysis products of radioactive trioleoylglycerol. In contrast to the previous methods, the present assays are more rapid and much more direct, especially the GLC technique, and less likely to be subject to error due to involved analytical manipulations. The accumulation of the 2,3-diacyl-sn-glycerols during lipoprotein lipase hydrolyses must, therefore, be considered real and deserves further discussion.

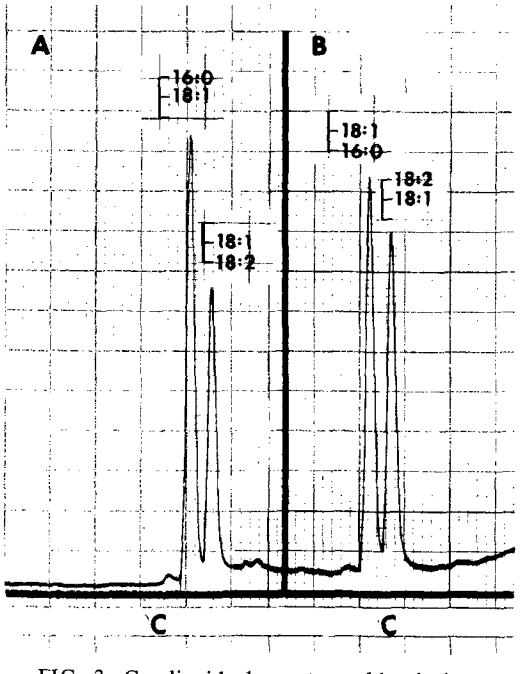

FIG. 3. Gas liquid chromatographic elution patterns of 1,2- and 2,3-diacylglycerols derived from synthetic A and B triacylglycerols by pancreatic lipase hydrolysis. Temperature was programed for 150-350 C in 16 min and major peaks appeared at 290 and 300 C.

It should be emphasized that such a disproportionation of the composition of a relatively minor intermediate is fully compatible with a largely random release of large amounts of fatty acids from the 1 and 3 positions of the triacylglycerol molecule, as observed by Assmann, et al., (2) for postheparin plasma lipase and Jensen (15) for milk lipase. The accumulation of the 2,3-diacylglycerols in a small total population of diacylglycerols also is not excluded by the results of Nilsson-Ehle, et al., (3), who showed that the primary positions of triacylglycerols are attacked by lipoprotein lipase ahead of the secondary position.

The apparent conflict between our results and those obtained by Karnovsky and Wolff (7) may be due to differences in the experimental conditions, although both analyses followed a comparable degree of overall hydrolysis. Karnovsky and Wolff conducted their experiments under the conditions of Borgstrom which had been shown to lead to a significant lipase catalyzed reversal of lipolysis. The present short term digestions would have afforded less opportunity for random reesterification of the 2-monoacylglycerols to the 1,2- and 2,3-diacylglycerols. Nevertheless, the presence of 25%

total X-1,2-diacylglycerol as 1,2-diacylglycerol could be accounted for by partial random reversal of lipolysis from 2-monoacylglycerol, with the 2,3-diacylglycerol the exclusive original diacylglycerol product of hydrolysis. Nilsson-Ehle, et al., (3) demonstrated the acylation of 2-monoacylglycerols to diacylglycerols by milk lipase.

The possibility remains, however, that some 1,2-diacylglycerol could be formed as the result of enzyme attack on the sn-3 position of the triacylglycerol, since Greten, et al., (16) has shown hydrolysis of 1,2-dialkyl-3-acyl-sn-glycerols by lipoprotein lipase. Nilsson-Ehle, et al., (17) claims that such degradation takes place at much lower rates than for triacylglycerols and, therefore, may not adequately indicate specificity. Alternatively, a disproportionation of the intermediate 1,2- and 2,3-diacylglycerol mixture could have occurred as a result of unequal rates of further hydrolysis of diacylglycerols. Using equimolar mixtures of enantiomeric diacylglycerols, we have shown elsewhere (unpublished results) that toward the end of hydrolysis, there is a tendency for the 2,3-diacylglycerols to accumulate in the incubation medium. The observed differences were not sufficient to account for the marked preponderance of the 2,3-isomers seen during triacylglycerol digestion.

Hydrolysis with Pancreatic Lipase

In agreement with previous work (1), the hydrolysis of synthetic triacylglycerols by pancreatic lipase did not show any significant specificity for either of the primary positions. However, on the basis of the composition of the diacylglycerol intermediates, the pancreatic lipase appeared to attack the unsaturated fatty acids in marked preference to the saturated acids, in both 1 and 3 positions of the triacylglycerol molecule. Other studies (18) have shown that the preferential release of unsaturated acids by pancreatic lipase depends upon the interfacial conditions of the digestion medium and the overall structure of the substrate. Saturated and unsaturated acids are released at ca. equal rates in the presence of organic solvents and elevated temperature (12). Reesterification of 2-monoacylglycerols to form diacylglycerols has been demonstrated experimentally for pancreatic lipase in the absence of albumin and calcium (19).

Comparative Studies of Pancreatic and Lipoprotein Lipase

Despite earlier claims to the contrary (2,3,7,15), pancreatic and lipoprotein lipases appear to possess different specificities. It is clear that both enzymes attack the primary positions preferentially, if not exclusively. However, lipoprotein lipase appears to prefer attack on the sn-1 position, while pancreatic lipase does not seem to differentiate between the primary positions of the triacylglycerol molecule. Moreover, the need for bile salts is essential for pancreatic lipase activity, (15) while they tend to inhibit lipoprotein lipase (20) which is activated best by lysolecithin (13). It appears that both enzymes catalyze the reversal of lipolysis but not necessarily to the same extent or under the same conditions. Furthermore, most preparations of lipoprotein lipase appear to contain more than one lipolytic activity (21). Thus, Egelrud and Olivecrona (22) showed that purified bovine lipoprotein lipase had rather low substrate specificity which could be modified by different types of emulsifiers. Greten, et al., (16) showed that the triacylglycerol lipase was distinct from a phospholipase A_1 as seen from different sensitivities to heat and inhibitors. It is, therefore, possible that the presence of these enzyme activities may have obscured the true stereochemical course of the reaction of the triacylglycerol lipase.

In summary, the present results would suggest that a stereospecific attack on acylglycerols by conventional preparations of lipoprotein lipase remains a strong possibility. Further studies with purified enzymes in the presence of appropriately labeled hydrolysis products in combination with stereospecific analyses may be required to clarify this problem.

ACKNOWLEDGMENTS

These studies were supported by grants from the Medical Research Council of Canada, the Ontario Heart Foundation, and the Banting and Best Department of Medical Research.

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[Received January 29, 1974]