Medium-Chain versus Long-Chain Triacylglycerol Emulsion Hydrolysis by Lipoprotein Lipase and Hepatic Lipase: Implications for the Mechanisms of Lipase Action†

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ABSTRACT: To explore how enzyme affinities and enzyme activities regulate hydrolysis of water-insoluble substrates, we compared hydrolysis of phospholipid-stabilized emulsions of medium-chain (MCT) versus long-chain triacylglycerols (LCT). Because substrate solubility at the emulsion surface might modulate rates of hydrolysis, the ability of egg yolk phosphatidylcholine to solubilize MCT was examined by NMR spectroscopy. Chemical shift measurements showed that 11 mol % of [^{13}C]carbonyl enriched trioctanoin was incorporated into phospholipid vesicles as a surface component. Similar methods with [^{13}C]triolein showed a maximum solubility in phospholipid bilayers of 3 mol % (Hamilton & Small, 1981). Line widths of trioctanoin surface peaks were half that of LCT, and relaxation times, T_1, were also shorter for trioctanoin, showing greater mobility for MCT in phospholipid. In assessing the effects of these differences in solubility on lipolysis, we found that both purified bovine milk lipoprotein lipase and human hepatic lipase hydrolyzed MCT at rates at least 2-fold higher than for LCT. With increasing concentrations of MCT, saturation was not reached, indicating low affinities of lipase for MCT emulsions, but with LCT emulsion incubated with lipoprotein lipase, saturation was reached at relatively low concentration, demonstrating higher affinity of lipase for LCT emulsions. Differences in affinity were also demonstrated in mixed incubations where increasing amounts of LCT emulsion resulted in decreased hydrolysis of MCT emulsions. Increasing MCT emulsion amounts had little or no effect on LCT emulsion hydrolysis. These results suggest that despite lower enzyme affinity for MCT emulsions, shorter chain triacylglycerols are more readily hydrolyzed by lipoprotein and hepatic lipases than long-chain triacylglycerols because of greater MCT solubility and mobility at the emulsion–water interface.

Lipases catalyze main steps in the metabolism of plasma lipoproteins, but the factors which govern their action are poorly understood. These enzymes hydrolyze insoluble substrates, acting at phospholipid surfaces which emulsify or solubilize hydrophobic lipids (Brockman, 1984). The surface pool of substrate, which is small compared to the core pool, is probably the site of hydrolysis. The mechanisms of enzyme–substrate interactions are difficult to analyze because classical analysis requires conditions where all substrate molecules are equally accessible to the enzyme. It is clear, however, that two important factors which determine lipase actions are (1) the affinity of the enzymes for the substrate droplets and (2) the ability of the enzymes to hydrolyze the substrate lipid of the emulsion particle (Verger & de Haas, 1976).

The capability of weakly polar hydrophobic lipids to intercalate between phospholipids and assume a specific orientation with the carboxyl group proximal to the aqueous interface has been shown by recent nuclear magnetic resonance (NMR)[1] studies (Hamilton & Small, 1981, 1982; Hamilton, 1989; Hamilton et al., 1983). Long-chain triacylglycerol can be solubilized in phosphatidylcholine bilayers up to about 3 mol % (Hamilton & Small, 1981; Hamilton, 1989a). Presumably, a similar amount of long-chain triacylglycerol is present at the surface of phospholipid-stabilized emulsions and in lipoproteins. We speculated that the surface concentration and mobility of triacylglycerols might be major determinants of how lipases can attack them. To test this hypothesis, we have compared the behavior of medium-chain and long-chain triacylglycerols (MCT and LCT,[1] respectively) as substrates for lipases. First, the ability of egg yolk phosphatidylcholine bilayers to solubilize trioctanoin was determined by using ^{13}C NMR methods previously applied to long-chain triacylglycerols. We then monitored hydrolysis of emulsions of LCT and MCT separately, and of an emulsion where the two triacylglycerols were mixed before emulsification.

For our studies, we used two well-characterized lipases important to human lipoprotein metabolism: lipoprotein lipase and hepatic lipase. These enzymes are related and share a number of properties (Jackson, 1983; Garfinkel & Schotz, 1987). The recent cloning and analysis of their cDNA indicate that they have evolved from a common ancestral gene to fulfill

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[1] Abbreviations: MCT, medium-chain triacylglycerol; LCT, long-chain triacylglycerol; PC, phosphatidylcholine; T_1, spin–lattice relaxation time; NOE, nuclear Overhauser enhancement; NMR, nuclear magnetic resonance.

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different roles in lipoprotein metabolism (Wion et al., 1987; Kirchgesser et al., 1987; Persson et al., 1989). The two enzymes presumably have the same, or closely similar, active sites. They also have the same chemical and positional specificities. Yet, they demonstrate different behavior in some important aspects. For example, whereas lipoprotein lipase prefers large triacylglycerol-rich particles such as human VLDL as substrate carrier (Olivecrona & Bengtsson-Olivecrona, 1987), hepatic lipase has relatively greater affinity and activity on the smallest lipoprotein subclass, HDL (Jackson, 1983; Bengtsson & Olivecrona, 1980a). Another difference is that while lipoprotein lipase works more efficiently in the presence of an activator protein, apoprotein C-II (apo C-II) (Smith & Pownall, 1984), such activation is not required to enhance activity of hepatic lipase. Thus, studies with these two enzymes would allow differentiation between effects of different lipid properties on enzyme activity as compared to those contributed by nonlipid cofactors, e.g., apo C-II.

Using two different triacylglycerols in emulsion form and the two enzymes, we demonstrate here that the lipolytic rates with both lipases are substantially higher on MCT as compared to LCT emulsions. At the same time, the lipases show higher affinities for LCT-containing particles than for MCT emulsions. The mechanisms for these differences were explored with each emulsion separately, then by competitive experiments with both emulsions present simultaneously, and finally with emulsions in which the two triacylglycerols had been mixed before emulsification.

Materials and Methods

Emulsions. Emulsions were prepared with egg yolk phosphatidylcholine and soy or coconut oil triacylglycerol (supplied by B. Braun Melsungen AG, Melsungen, West Germany) to produce LCT and MCT emulsions, respectively.

The LCT oil used for these studies had a fatty acyl composition as follows (wt %): $C_{16:0}$, 10%; $C_{18:0}$, 4%; $C_{18:1}$, 23%; $C_{18:2}$, 55%; $C_{18:3}$, 6%; $C_{20:4}$ and others, 2%. The fatty acyl composition of MCT oil was as follows: C_6, 1%; C_8, 57%; C_{10}, 42%; C_{12}, 1%. Glycerol tri[1-^{14}C]palmitate (Amersham Buchler GmbH, Braunschweig, West Germany) was dissolved after prior evaporation of toluene in the LCT oil to give a specific radioactivity of 0.075 μCi/mg of LCT oil. Glycerol tri[2,3-^{3}H]octanoate (Nuclear Research Laboratories of the Negev, Beersheva, Israel), after evaporation from ethanol, was dissolved in MCT oil to give a specific radioactivity of 0.075 μCi/mg of MCT oil.

Labeled lipid emulsions were then prepared with the radiolabeled oils so that each 100 mL contained 20 g of MCT or LCt oil (i.e., 2000 mg/mL), 1.2 g of egg yolk phosphatidylcholine, 2.5 g of glycerol, double-distilled sterile water, pH 7.5, and 6 μCi/mL ^{14}C-labeled LCT or 5 μCi/mL ^{3}H-labeled MCT. In addition, a mixed MCT/LCT (1:1 w/w, 1:0.57 M/M) emulsion was prepared with 10 g of MCT oil and 10 g of LCT oil, blended together prior to addition of the other components (in the same ratios as described above) and sonication/homogenization procedures (described below). This latter emulsion contained 5 μCi of ^{14}C-labeled LCT/mL and 5 μCi of ^{3}H-labeled MCT/mL.

The emulsion lipid was mixed in double-distilled water (30 g of water, 20 g of oil) and dispersed by means of an Ultra-Turrax (Janke and Kunkel KG, Staufen, West Germany) for 20 min, and water was added to give a final volume of 100 mL and then dispersed for another 10 min. Subsequently, the dispersion was homogenized by ultrasound in a cooling cell with a Labsonic 2000 homogenizer (B. Braun Melsungen AG) for 10 min, at an energy input of 200 W. The emulsions were then sealed in 5-mL vials under N_2, and thereafter kept at 4 °C. Mean particle sizes determined by laser spectroscopy, for the emulsions used for these studies, were 0.29, 0.27, and 0.29 μm for the LCT, MCT, and blended MCT/LCT emulsions, respectively. While MCT/LCT and LCT emulsions prepared as described are known to maintain stable size properties for periods of over 12 months, such information was not available for MCT emulsions. Assessment of an aliquot of the MCT emulsions analyzed 12 months after preparation showed mean particle size remained at 0.27 μm by laser spectroscopy. After preparation, >99% of LCT and MCT appeared as single spots by thin-layer chromatography with >97% of all radiolabel coeluting with the unlabeled triacylglycerol.

Lipases and Enzyme Activity Assay. Bovine lipoprotein lipase (LPL) was isolated from skim milk by heparin–Sepharose affinity chromatography using methods detailed elsewhere (Bengtsson-Olivecrona & Olivecrona, 1985). The purified enzyme was stored frozen (−70 °C) in 10 mM Tris-HCl, 5 mM deoxycholate, and 0.1 mM lineoleate, at pH 8.5 at a concentration of 0.3 mg/mL. The activity of purified LPL was 300–400 units/mg of protein (1 unit = 1 μmole of fatty acid released/min at 25 °C). To facilitate pipetting of small amounts, stored LPL solution was diluted 1:10 in 0.19 M NaCl, pH 8.5, immediately before incubation with emulsions. Hepatic lipase was prepared from human post-heparin plasma by chromatography on heparin–Sepharose and N-desulfated acetylated heparin–Sepharose (Zaidan et al., 1985). Because hepatic lipase was not obtained as a homogeneous purified protein, activity was calculated per milliliter of enzyme solution; i.e., about 10.5 units/mL.

Apoprotein C-II. Apoprotein C-II (kindly provided by Dr. S. Eisenberg) was isolated after ethanol/ether delipidation of human VLDL apoproteins with ethanol/diethyl ether as previously described (Eisenberg et al., 1972) followed by chromatography of the pooled C-apoproteins on DEAE-cellulose in 6 M urea (Jackson & Holdsworth, 1986). Fractions containing apo C-II were pooled, desalted by dialysis against 0.005 M NH_4HCO_3, and then lyophilized.

Incubation of Emulsions with Lipases. Incubations were carried out in 0.2 M Tris-HCl buffer, pH 8.4, containing 0.08 M NaCl, 2 IU of heparin/mL (Evans Medical Ltd., Liverpool, U.K.), and 4% albumin (bovine, fatty acid free, Sigma). The amounts and type of lipid emulsion were varied, as indicated in the figure legends. In some experiments with higher amounts of triacylglycerol, the amount of albumin was increased so that even if 50% hydrolysis occurred the molar ratio of released free fatty acid to albumin would not exceed 3:1. In incubations with lipoprotein lipase, apolipoprotein C-II was added in a weight proportion of 1:40 to emulsion triacylglycerols. Incubation was at 37 °C for 30 min, unless otherwise stated. All assays were performed in duplicate. Hydrolysis was terminated by addition of the incubation mixture to solvent, and free fatty acids were determined (using double-radiolabel counting techniques when necessary) according to the method of Belfrage and Vaughan (1969).

To determine if release of radiolabeled fatty acid represented total triacylglycerol hydrolysis, free fatty acid was quantitated in initial experiments. For LCT incubations, the content of free fatty acid was measured by radiochemical assay (Ho, 1970) in extracts prepared by Dole's method (Dole, 1956). For MCT, release of medium-chain fatty acids was assayed by the NEFA C Kit (Biochemical Diagnostics, Inc., Ed.). Hydrolysis of [^{14}C]tripalmitin and [^{3}H]trioctanoin correlated linearly with actual hydrolysis as measured by total long-chain fatty acid and medium-chain fatty acid release, respectively, with r values

for each of 0.987 and 0.999, respectively, over ranges of triacylglycerol hydrolysis of 1–95%.

NMR: MCT Solubility in Phospholipid. For these experiments, egg yolk phosphatidylcholine (PC) was obtained from Lipid Products (Nutly, England) and 99% [1-^{13}C]-trioctanoin from Cambridge Isotopes (Woburn, MA). Purity (>99%) was verified by thin-layer chromatography and ^{13}C NMR spectroscopy. A spectrum of [1-^{13}C]trioctanoin in C^2HCl_3 showed only two carbonyl resonances, assigned to the β (*sn*-2) chain and the α (*sn*-1 and *sn*-3) chains.

Vesicles with [1-^{13}C]trioctanoin and egg PC were prepared essentially as described for egg PC/triolein vesicles (Hamilton & Small, 1981). After NMR analysis, samples were analyzed for composition and purity. The composition determined chemically agreed (±5%) with the starting composition. Some samples showed traces of unesterified fatty acid and lysolecithin. These samples were prepared again under the same conditions and analyzed by NMR. No (<1%) lipid contaminants were found by thin-layer chromatography, and the NMR spectra were essentially identical with those obtained for samples with traces of fatty acid and lysolecithin.

Fourier-transform NMR spectra were obtained at 50.3 MHz with a Bruker WP200 spectrometer as described (Hamilton & Small, 1981). Internal ^2H_2O was used as a lock and shim signal. Spin–lattice relaxation times (T_1) were measured by using a fast inversion recovery trchnique (Canet et al., 1975) and calculated by using a three-parameter exponential fitting routine (Sass & Ziessow, 1977) provided in the Bruker program. Nuclear Overhauser enhancement (NOE) was measured as the ratio of integrated intensities with broad-band decoupling and with inverse-gated decoupling (maximum NOE = 3.0) by the method of Opella (Opella et all, 1976). Chemical shifts and line widths were measured digitally, and the applied line broadening was subtracted from the reported line-width results. The fatty acyl terminal methyl resonance at 14.10 ppm was used as an internal chemical shift reference (Hamilton et al., 1974). NMR peak intensities were measured by the Aspect integration program to an estimated accuracy of 10%. Sample temperature was controlled (±1 °C) with the Bruker B-VT-1000 variable-temperature unit and measured by removal of the sample from the probe and insertion of a thin thermocouple.

Results

An important factor modulating hydrolysis rates of different triacylglycerols might be their solubility in the phospholipid emulsified at the emulsion particle surface. We examined this possibility using NMR spectroscopy to measure the solubility of MCT in phospholipid bilayers. Results from systems with increasing amounts of [^{13}C]carbonyl-enriched trioctanoin with egg phosphatidylcholine (Figure 1) showed that the carbonyl peaks of MCT were shifted downfield from those of neat MCT (172.1 and 171.8 ppm, respectively), demonstrating an orientation of the carbonyl carbons to the aqueous interface (Hamilton & Small, 1981). These peaks increased in intensity with increasing trioctanoin up to 10.5 mol % trioctanoin, at which point small peaks representing trioctanoin in an oil phase appeared. The intensity of the signals from the oil peaks increased with further addition of the MCT, while those of the MCT solubilized by phospholipid remained constant. The solubility of triolein and tripalmitin in egg yolk or dipalmitoylphosphatidylcholine determined by similar methods showed maximum incorporation of about 3 mol % before a separate oil phase appeared (Hamilton & Small, 1981; Hamilton, 1989a). While the solubilities in the bilayer vesicles may not be quantitatively identical with that in the emulsion

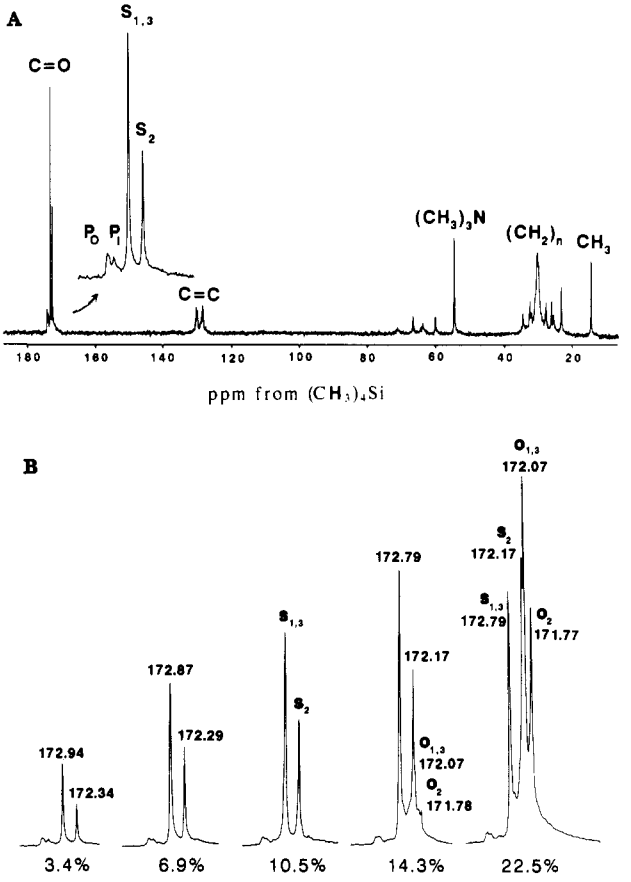

FIGURE 1: ^{13}C NMR spectra of trioctanoin in egg phosphatidylcholine (PC) vesicles. (A) Proton-decoupled ^{13}C Fourier-transform NMR spectrum at 35 °C of sonicated 2% [1-^{13}C]trioctanoin/98% egg PC at 50 mg of egg PC/mL. The spectrum was recorded at 50.3 MHz after 2000 accumulations with a 200 ppm spectral width, 32 768 time domain points, a recycle time of 8.64 s, and a line broadening of 1.0 Hz. The carbonyl region is shown in the inset, and peaks from the [^{13}C]carboxyl-enriched trioctanoin are designated $S_{1,3}$ for α-chain "surface" carbonyls and S_2 for the β-chain "surface" carbonyl. Phospholipid peaks are designated as follows: CH_3, fatty acyl terminal methyl; CH_2 groups, fatty acyl methylenes; $(CH_3)_3N$, choline methyls; C=C, fatty acyl olefinic carbons; P_o, PC carbonyls on the outside of the bilayer; P_i, PC carbonyls inside of the bilayer. (B) Carbonyl region of the ^{13}C NMR spectrum at 35 °C of sonicated [1-^{13}C]-trioctanoin/egg PC at different starting compositions, as indicated by mole percent trioctanoin. Spectral conditions are the same as those in (A). The egg PC concentration was 40–50 mg/mL. The line width of the $S_{1,3}$ and S_2 peaks was 3.5 ± 1 Hz. The chemical shift of the $O_{1,3}$ peak was 172.07 ppm and the O_2 peak 171.77 ppm. These peaks represent trioctanoin in an oil phase. The chemical shift of the $S_{1,3}$ and S_2 peaks decreased slightly with increasing mole percent trioctanoin from 172.94 ppm ($S_{1,3}$) and 172.34 ppm (S_2) for 3% trioctanoin to 172.79 ppm and 172.17 ppm for 10.5% and 14.3% trioctanoin. As can be seen, the peak intensities of the trioctanoin peaks increase relative to the PC carbonyl peaks (small peaks downfield from the trioctanoin peaks) with increasing trioctanoin, but the surface trioctanoin peaks reach a limiting intensity corresponding to 10.5% trioctanoin.

surface, there clearly is a potential for more MCT (possibly 3–4 times) to be present at the emulsion phospholipid surface as compared to LCT.

The mobility of triacylglycerol molecules in a phospholipid surface can be assessed by NMR measurements of T_1, NOE, and line width, which reflect the motions of the particular carbon. For long-chain triacylglycerols in phospholipid bilayers, these quantities (measured for the carbonyl carbon) are similar for both lipid components, indicating similar mobilities of the triacylglycerol and phospholipid, a conclusion reached also from ^{2}H NMR studies of deuterated triolein in phospholipids (Gorrissen et al., 1982). The NOE for the

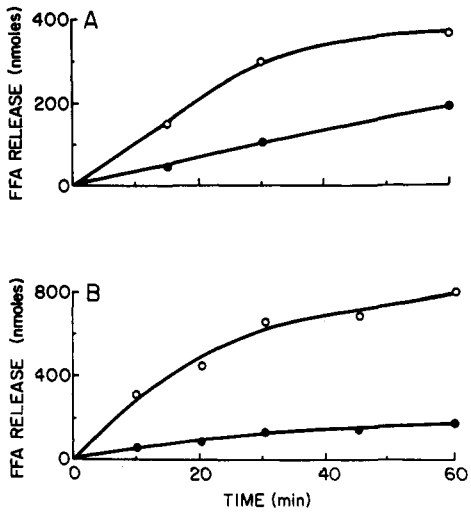

FIGURE 2: Hydrolysis of MCT and LCT emulsions with increasing time of incubation. (A) Incubation of 200 nmol of MCT (○) or 200 nmol of LCT (●) with 0.025 μL of lipoprotein lipase for 30 min at 37 °C in an incubation volume of 200 μL. (B) Incubations of 350 nmol of MCT (○) or 350 nmol of LCT (●) with 0.5 μL of hepatic lipase for 30 min at 37 °C in an incubation volume of 200 μL. Results are the mean of two separate parallel experiments.

trioctanoin surface carbonyl peaks (10.5 mol % in egg PC) was 1.8, a value similar to that for long-chain triacylglycerol, while the T_1 was ~2.6 s for the $S_{1,3}$ and S_2 peaks in a 3.4% trioctanoin sample, somewhat longer than for triolein, ~2.0 s (Hamilton & Small, 1981). The line width of the surface peaks of trioctanoin (3–4 Hz) was ~$^1/_2$ the value for long-chain triacylglycerols. Since line width is often the most sensitivie measure of anisotropic motions which would occur when triacylglycerol molecules are oriented in phospholipid, the NMR results suggest that MCT has more mobility than LCT at the surface.

Increased MCT mobility and solubility at the emulsion surface could be important mechanisms allowing for more rapid hydrolysis of MCT as compared to LCT in triacylglycerol emulsions. When the action of the lipases on LCT and MCT emulsions was compared under a variety of conditions, it was consistently found that hydrolysis was more rapid for MCT. Figure 2 shows time curves for hydrolysis of the two emulsions with lipoprotein lipase (Figure 2A) and hepatic lipase (Figure 2B). MCT hydrolysis with both enzymes exceeded that of LCT by a factor of 2 or more at any given time point, and this difference was maintained even at high degrees of lipolysis.

The difference between rates of hydrolysis of MCT and LCT emulsions was maintained over a 6-fold or more range of increasing substrate concentration with both enzymes, as illustrated in Figure 3 for lipoprotein lipase. Compared to LCT, MCT emulsion again demonstrated high lipolytic rates, but saturation hydrolysis was not reached experimentally, indicating a low affinity of the enzyme for the MCT emulsions. For the LCT emulsion, saturation was reached at relatively low concentrations, suggesting a higher affinity of the enzyme for the LCT emulsion.

To probe further the differences in enzyme affinity between the two triacylglycerol emulsions, we determined how the presence of one would affect hydrolysis of the other. With either enzyme, when the amount of MCT emulsion was held constant, proportional increases in the amount of LCT emulsions led to progressive decreases in MCT hydrolysis and increases in LCT hydrolysis (Figure 4A,C). In contrast, when the amount of LCT emulsion was held constant and MCT emulsion was added, medium-chain fatty acid release increased

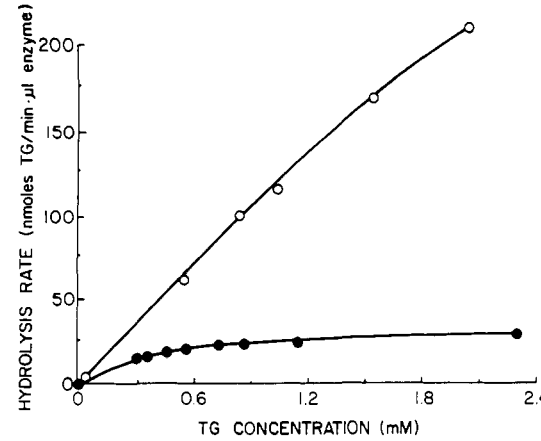

FIGURE 3: Velocities of lipoprotein lipase hydrolysis of MCT (○) and LCT (●) emulsions with increasing substrate concentrations. Increasing substrate concentrations were achieved by increasing the emulsion concentrations in the incubation mixtures. Incubations were performed at 37 °C in volumes of 200 μL for 30 min with 0.1 μL of enzyme. Each point represents the mean of two separate parallel experiments.

substantially and in proportion to the amount of MCT emulsion, but there was only a minimal decrease in LCT hydrolysis (Figure 4B,D). These results suggest that in mixtures of the MCT and LCT emulsions, higher affinity for the LCT-containing particles results in partitioning of the lipase away from MCT emulsion with consequently diminished MCT hydrolysis.

To determine if these apparent differences in enzyme affinities would also affect MCT hydrolysis in emulsions where MCT and LCT were "competing" in the same emulsion particle, we compared two different physical mixtures of MCT and LCT: (a) MCT and LCT oils blended (at 1:1 weight ratios) and then emulsified together to produce emulsions containing both MCT and LCT in the same particle (MCT/LCT emulsions); and (b) mixtures where MCT emulsion particles and LCT emulsion particles were prepared separately but then mixed together (at 1:1 weight ratios) in the incubation mixture (MCT + LCT emulsions). At any given time point (Figure 5) or enzyme concentration (data not shown), the blended MCT/LCT emulsions showed higher MCT hydrolysis than the mixed MCT + LCT emulsions; this difference was maintained also at relatively high degrees of lipolysis. Contrariwise, LCT hydrolysis was less efficient in MCT/LCT emulsions relative to the separate MCT + LCT emulsions.

Figure 6 compares MCT hydrolysis to LCT hydrolysis in the two different types of mixed emulsions. In the blended emulsions, the increase of MCT hydrolysis when compared to increasing degrees of LCT hydrolysis was about 2-fold. In the MCT + LCT emulsion, however, the increase in MCT hydrolysis as a function of increasing LCT hydrolysis was only about 0.5. These results demonstrate that in blended emulsions of MCT/LCT, MCT is the preferred substrate for lipase and that this results in relatively slower hydrolysis of LCT. On the other hand, in mixtures of MCT emulsions and LCT emulsions, the higher affinity of lipase for LCT particles effectively reduces MCT hydrolysis.

Discussion

The present study shows that shorter chain triacylglycerols are more soluble and have higher mobility in phospholipid/water interfaces than LCT. This is associated with more rapid hydrolysis of MCT, but a decrease in lipase affinities for MCT emulsions compared to LCT emulsions.

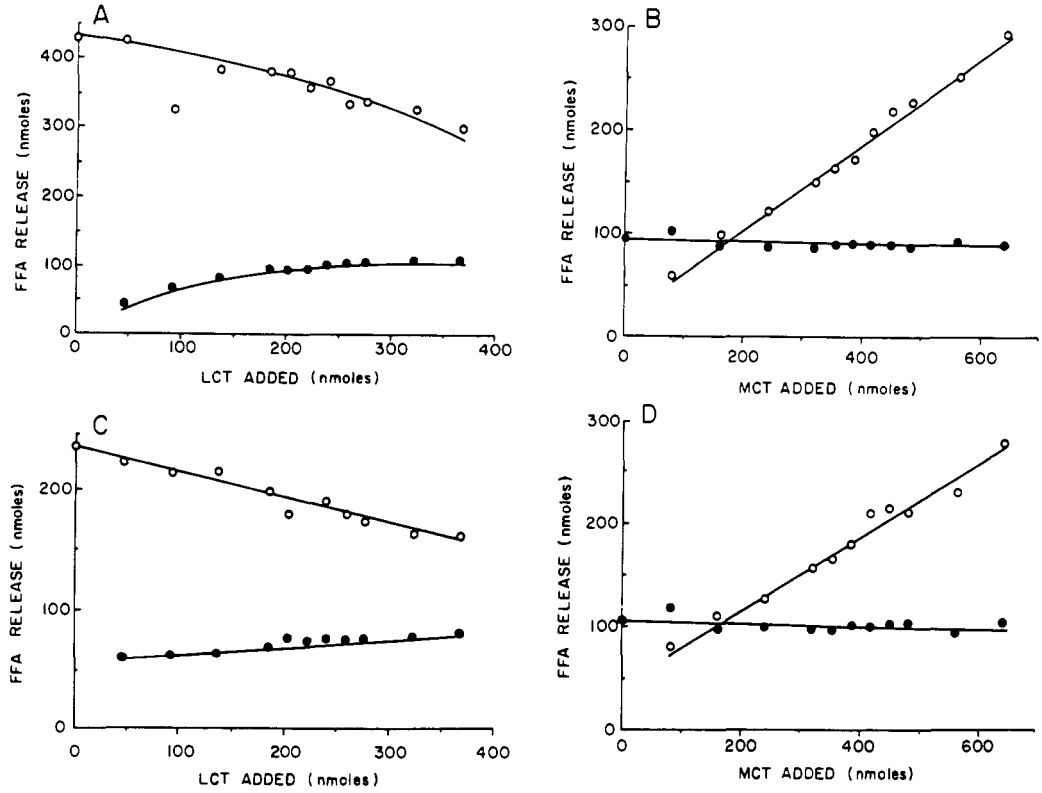

FIGURE 4: Competitive hydrolysis of MCT and LCT emulsions. (Top) Incubations with lipoprotein lipase (0.01 μL). (A) Hydrolysis of MCT (○) and LCT (●) in incubations where the amount of MCT was kept constant at 183 nmol and the amount of LCT was increased as shown. (B) Hydrolysis of MCT and LCT when the amount of LCT was kept constant at 240 nmol and the amount of MCT increased as shown. (Bottom) Incubation with 4 μL of hepatic lipase where (C) MCT was kept constant and (D) LCT was kept constant at the same amounts as in (A) and (B). All incubations were for 30 min at 37 °C in incubation volumes of 200 μL.

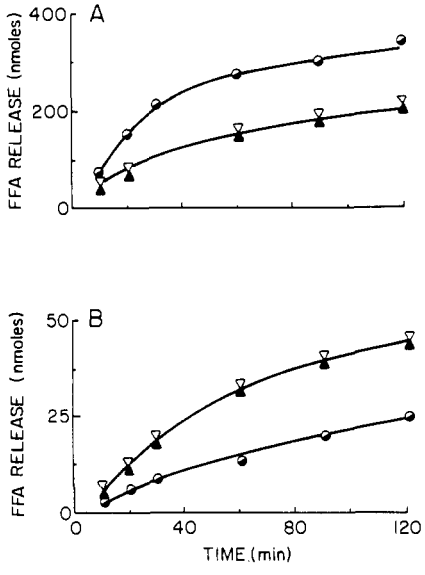

FIGURE 5: Hydrolysis of MCT and LCT over time in emulsion mixtures where MCT and LCT were blended together in the same emulsion particles [MCT/LCT (◒)] at weight ratios of 1:1 or were in separate particles of each emulsion [MCT + LCT (▽/▲)] at weight ratios of 1:1. (A) FFA release from MCT. (B) FFA release from LCT. In each incubation, 200 μg of total triacylglycerol was incubated with 0.025 μL of lipoprotein lipase at 37 °C for 30 min in a 200-μL volume. Results are the mean of two separate parallel experiments.

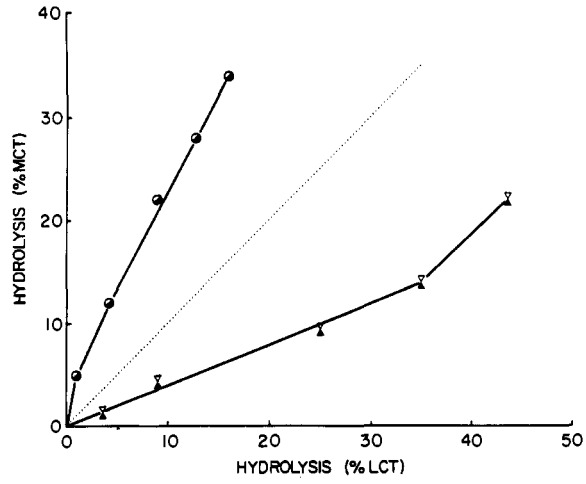

FIGURE 6: Percent hydrolysis of MCT as a function of LCT hydrolysis in emulsion mixtures prepared by two different methods of mixing. (◒) represents mixtures of MCT/LCT blended together and (▽/▲) mixtures of MCT and LCT emulsion particles mixed together at 1:1 weight ratios. Incubations were performed with 0.025 μL of lipoprotein lipase at 37 °C for 30 min in a 200-μL volume. The dotted line represents a line where the percent MCT hydrolysis would equal the percent LCT hydrolysis. Results are taken from an experiment performed in duplicate where the different emulsion mixtures were studied with increasing incubation times from 10 to 60 min.

The NMR results indicate that the glyceryl regions of trioctanoin and long-chain triacylglycerols have similar orientations and conformations in the surface. This is presumably the region in the molecule that the lipase engages directly. The methylene groups beyond C8 in LCT likely affect surface accessibility and mobility of the glycerol–carbonyl region of the molecule by decreasing the maximum incorporation into the surface and anchoring the molecule more firmly. In addition, the presence of MCT compared to LCT in the phospholipid surface likely resulted in differences in the properties of the interface as may be inferred from decreasing chemical shifts of the MCT carboxyl peaks at higher surface concentrations, showing a small decrease in hydration, a result not seen for LCT (Hamilton & Small, 1981; Hamilton, 1989a).

The increase in mobility of surface-located MCT compared to LCT may also be significant. The NMR results—T_1, NOE, and line-width measurements—show a net increase in the MCT molecular motions, the various modes of which cannot be simply resolved. Factors other than substrate surface concentration and mobility may also contribute to a higher rate of MCT hydrolysis. Trioctanoin monolayers (at an air–water interface, with no phospholipid present) have shown a higher rate of hydrolysis on a per molecule basis than those of triolein (Smith, 1972). Although such systems are not directly comparable to ours, a common factor which could influence hydrolysis rate is the faster rate of desorption of products from the MCT substrate surface. The equilibration of octanoic acid between phospholipid bilayers and albumin occurs $\sim 10^4$ times faster than that of oleic acid (Hamilton, 1989b).

Another possibility to account for higher rates of MCT hydrolysis is that product inhibition by released long-chain fatty acids may have slowed LCT hydrolysis. This consideration was essentially ruled out because the amounts of albumin in our incubation mixtures were such that long-chain fatty acid to albumin molar ratios rarely exceeded 2–3 (after hydrolysis) and never exceeded 6. At these molar ratios, released fatty acids do not accumulate at the emulsion surface (Spooner et al., 1988) nor form complexes with lipases, or slow lipolysis (Bengtsson & Olivecrona, 1980b). In lipolytic systems similar to those used herein, released free fatty acids will decrease lipase activity only when the molar ratio of fatty acids to albumin exceeds 6–7 (Scow & Olivecrona, 1977). Moreover, the higher lipolytic rates of MCT compared to LCT were always apparent at the lowest substrate concentrations, and at early points in time curve experiments, i.e., under conditions where product accumulation was still minimal.

[^{3}H]Trioctanoin was hydrolyzed more rapidly than [^{14}C]-tripalmitin, both when the MCT and LCT emulsions were incubated separately with the lipases and when MCT and LCT were blended in the same emulsion. Since trioctanoin has a surface orientation similar to long-chain triacylglycerol, the higher rate of hydrolysis likely occurs because trioctanoin is present at a higher surface concentration rather than because of possible subtle differences in surface conformation. This implies that the enzyme at the interface of a LCT droplet is working below substrate saturation (Brockman, 1984; Olivecrona & Bengtsson-Olivecrona, 1987), whereas the higher solubility of trioctanoin in the surface results in higher active-site occupation.

Small differences in the composition of the interface can have marked effects on the binding of lipases and related proteins. For example, small amounts of fatty acids markedly enhance the binding of pancreatic colipase (Borgstrom, 1980) and of lipid exchange proteins (Tall et al., 1984) to phospholipid–triacylglycerol droplets. Pancreatic phospholipase A_2 activity on phosphatidylcholine vesicles with surface-loaded cholesteryl ester showed a marked decrease compared with pure phospholipid vesicles, an effect suggested to be mediated by decreased binding of the enzyme to surfaces containing cholesteryl ester (Mims & Morrisett, 1988).

Both lipoprotein lipase and hepatic lipase displayed higher affinity for the LCT than for the MCT emulsion. This conclusion comes from the dependence of reaction velocity on substrate concentration, when the LCT and MCT emulsions were presented to the enzyme separately, and from the marked preference for LCT hydrolysis when mixtures of the two emulsions were used. There were no marked differences in particle size distribution between the two emulsions. Hence, the observed differences in apparent affinity must reflect differences in composition and physical properties of the interfaces. At the same time, the greater surface concentration of MCT at the emulsion surface more than compensates for the decreased affinity, yielding faster lipolysis of MCT.

Verger and others have pointed out that the activity expressed by a lipase depends on the "quality" of the interface in which the substrate molecules are located (Brockman, 1984; Verger & de Haas, 1976). In the present study, hydrolysis proceeded quite differently when the two triacylglycerols were presented in separate droplets or blended in the same droplets. In the blended droplets, trioctanoin hydrolysis was faster than LCT, presumably because trioctanoin was the dominant triacylglycerol at the interface. In contrast, when the triacylglycerols were presented in separate droplets, LCT hydrolysis was favored, presumably because the enzyme preferred to bind to the LCT droplets. General theories on lipase action have suggested that these enzymes probably have lipid binding sites separate from the active sites (Brockman, 1984; Verger & de Haas, 1976). Our results fit this conceptual framework in illustrating that the interface can determine the reaction in two different ways—one is the affinity of the lipase for the interface while the other is the accessibility of individual substrate molecules to the lipase.

Registry No. Lipoprotein lipase, 9004-02-8; lipase, 9001-62-1; trioctanoin, 538-23-8.

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Interactions between Bovine Myelin Basic Protein and Zwitterionic Lysophospholipids†

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ABSTRACT: The binding of myelin basic protein to lysolauroylphosphatidylcholine (lysoLPC) and lysolauroylphosphatidylethanolamine was investigated at neutral pH using gel partition chromatography and equilibrium dialysis at 20 and 37 °C. The results show that the protein–lysolipid interactions are highly cooperative and that the free lysolipid concentration at which the binding commences is markedly influenced by both the chemical structure of the lysolipids and the temperature. The binding begins just below the critical micelle concentration for both lysolipids, which suggests that the forces governing micellization and the binding are similar. Circular dichroism (CD) spectroscopy was used to follow changes in the conformation of the protein caused by lysomyristoylphosphatidylcholine and lysoLPC. The CD results indicate that lysolipid association with the protein commences below the critical micelle concentration and continues above this concentration. Mechanisms for the lysolipid–protein interaction, which are consistent with the binding and CD data, are discussed.

Myelin basic protein (MBP)[1] is an extrinsic membrane protein which is believed to play a major role in the compaction and stabilization of central nervous system myelin through protein–protein and protein–lipid interactions (Smith, 1977; Braun, 1977; Readhead et al., 1987). However, as yet, there is no clear evidence of the mechanism by which MBP stabilizes myelin. The complex multilamellar structure of the myelin membrane limits the strategies which can be employed and complicates the interpretation of experiments designed to examine the interaction between MBP and other molecules in its native environment. Thus, investigations of this nature have largely been confined to in vitro studies using purified components.

The binding of zwitterionic lysolipids to MBP has been examined by a number of groups, using techniques such as nuclear magnetic resonance (NMR) (Littlemore & Ledeen, 1979; Smith, 1982a; Hughes et al., 1982; Mendz et al., 1984), circular dichroism spectroscopy (Anthony & Moscarello, 1971; Keniry & Smith, 1981; Mendz et al., 1984), ultracentrifugation (Smith, 1982b; Mendz et al., 1988), and electron microscopy (Mendz et al., 1988). These studies have revealed many aspects of lysolipid–MBP interactions, including regions of the protein which may be involved in the binding and the nature of the induced protein conformational changes.

Isotherms for the binding of lysomyristoylphosphatidylcholine (lysoMPC) to MBP have previously been measured by using a recycling gel partition technique (Gow et al., 1987). In the present study, we have extended our examination of zwitterionic amphiphile–MBP interactions to evaluate the dependence of this binding upon temperature, acyl chain length, and headgroup size of the lysolipid. Circular dichroism (CD) experiments were used to determine whether binding occurred above the critical micelle concentration (cmc) of the lysolipid as has been shown for other MBP–lysolipid interactions (Mendz et al., 1984). These results have enabled us to comment on the mechanism of the association of micelle-

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[1] Abbreviations: CD, circular dichroism; cmc, critical micelle concentration; DLPE, dilauroylphosphatidylethanolamine; DPC, dodecylphosphocholine; lysoLPC, lysolauroylphosphatidylcholine; lysoMPC, lysomyristoylphosphatidylcholine; lysoLPE, lysolauroylphosphatidylethanolamine; MBP, myelin basic protein; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

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