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Effect of Surface Composition on Triolein Hydrolysis in Phospholipid Vesicles and Microemulsions by a Purified Acid Lipase†

Robert E. Burrier‡ and Peter Brecher*

ABSTRACT: Sonicated dispersions of egg yolk phosphatidylcholine and triolein as vesicles and microemulsions have been used as substrates for the assay of a purified acid lipase. Previous studies have also shown that triolein localized in the surface phase of emulsions is the preferred substrate. In this study, we examined enzyme activity following several surface modifications using both vesicles and microemulsions. When the acidic phospholipids phosphatidylserine and phosphatidic acid were incorporated into both vesicles and microemulsions at up to 10 mol % of the total phospholipid, a dose-dependent reduction in the apparent K_m was observed. Using the vesicles as substrate, a dose-dependent decrease in V_{max} was also observed. Agarose gel electrophoresis was used to verify suspected changes in net particle charge. Analogous inclusion of phosphatidylethanolamine, sphingomyelin, or cholesterol did not affect kinetic parameters. Addition of oleic acid to sonication mixtures produced vesicles with a decreased apparent K_m and V_{max}, but triolein hydrolysis in microemulsions was not significantly altered. Triolein-containing vesicles prepared by using dimyristoyl- or dipalmitoylphosphatidylcholine were hydrolyzed maximally at the gel ↔ liquid-crystalline transition temperatures of the appropriate phospholipid. Differential scanning calorimetry was used to verify the temperatures of transition in these vesicles. The results indicate that acid lipase activity is influenced by the charge or physical state of the surface phase of model substrates and suggest that degradation of core components of naturally occurring substrates such as lipoprotein may be influenced by chemical changes on the surface of these particles.

Acid lipase is a lysosomal enzyme involved in the intracellular degradation of triglycerides and cholesteryl esters associated with lipoproteins. Studies on the mechanism of action for the enzyme have been hampered by methodological problems including low yields, instability of the purified enzyme, and poorly characterized substrate preparations. In a previous study (Burrier & Brecher, 1983), we isolated a form of an acid lipase from rat liver using a modification of a procedure for purification of the human enzyme (Warner et al., 1981). The purified enzyme preparation was characterized by using miceoemulsions and vesicles containing triolein as substrates. Both substrate types were readily hydrolyzed by the enzyme, and kinetic parameters suggested that triolein was preferentially hydrolyzed when incorporated into the phospholipid surface layer.

The action of lipases on membrane bilayers or microemulsions is thought to involve an initial binding to the enzyme to the lipid surface followed by localization of the substrate molecule to the active site where hydrolysis then occurs. It was suggested that alterations in the composition, charge, or structure of the substrate cause changes in "the quality of the interface", thus affecting the ability of the enzyme to interact with the substrate (Verger, 1980). Previous studies using lipases which act on cholesteryl esters or triglycerides have utilized several types of substrates including lipid monolayers (Demel et al., 1982; Pieroni & Verger, 1979), phospholipid vesicles (Brecher et al., 1976), and micelles or emulsions containing detergents or emulsifiers (Mahadevan & Tappel, 1968; Brown & Sgoutas, 1982).

The present work examines the activity of the rat liver acid lipase toward triolein-containing microemulsions and vesicles prepared with egg yolk lecithin in which the surface phase of the particles has been altered. The effect on enzymatic activity of the incorporation of the acidic phospholipids, phosphatidylethanolamines, sphingomyelin, cholesterol, and oleic acid into both substrate types was investigated. Triolein-containing vesicles of either dimyristoyl- or dipalmitoylphosphatidylcholine also were characterized and used as substrates at temperatures below and above the transition temperatures.

Materials and Methods

Materials. Tri[1-^{14}C]oleoylglycerol (99.8 mCi/mmol), [1-^{14}C]oleic acid (54.5 mCi/mmol), and [*2-palmitoyl*-9,10-^{3}H]dipalmitoylphosphatidylcholine (60 Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA. Liquiscint was purchased from National Diagnostics, Som-

† From the Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118. *Received December 27, 1983.* This work was supported by a grant from the U.S. Public Health Service (HL 25157).

‡ Present address: Department of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

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merville, NJ. Bio-Gel A-15m was obtained from Bio-Rad Laboratories, Richmond, CA, and Blue Dextran 2000 was obtained from Pharmacia Fine Chemicals, Piscataway, NJ. Egg yolk phosphatidylcholine (grade 1) was purchased from Lipid Products, Surrey, U.K., and all other lipids were obtained from Sigma Chemical Co., St. Louis, MO. Agarose electrophoresis supplies were purchased from Corning Medical, Palo Alto, CA. All other chemicals were of the highest reagent grade available.

Acid Lipase Purification. The acid lipase was purified from rat liver as described previously (Burrier & Brecher, 1983). The data reported in these studies were obtained from a single enzyme preparation, although similar results were obtained with other enzyme preparations.

Preparation of Vesicles and Microemulsions. Vesicles and microemulsions were prepared by cosonicating aqueous dispersions of phospholipid and triolein as described previously (Burrier & Brecher, 1983). The designated amounts of labeled and unlabeled lipid were dissolved in chloroform/methanol (2:1 v/v), evaporated under a stream of nitrogen, dessiccated "in vacuo" for 30 min, and resuspended in 7 mL of a solution containing 0.1 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane (Tris),[1] pH 7.4, and 0.02% sodium azide. All vesicle preparations were prepared such that the final concentrations were 38 mM phospholipid and 0.54 mM triolein. Unless specifically indicated, egg yolk phosphatidylcholine (EYPC, assumed M_r 787) was the phospholipid used. Other preparations contained a portion of the phospholipid as bovine brain phosphatidylserine (PS, assumed M_r 786), bovine brain sphingomyelin (SM, assumed M_r 750), egg yolk phosphatidylethanolamine (PE, assumed M_r 745), and egg yolk phosphatidic acid (PA, assumed M_r 701). Where designated, cholesterol and oleic acid were added to the preparations. The microemulsion was prepared by mixing equimolar amounts of phospholipid and triolein such that after resuspension in 7 mL of the aqueous buffer, the concentration of each lipid was 14.1 mM. Microemulsions were also prepared with compositional alterations similar to those described for the vesicles.

The resulting suspensions were then sonicated continuously under a nitrogen atmosphere with a Branson W-350 sonicator fitted with a standard 0.5-in. horn at a power setting of about 3.5 which delivered 100–135 W. Samples were contained in a glass-jacketed sonication cell with a spherical bottom and dimensions of 1.8 × 7 cm. The temperature of the sonicating sample was monitored by a thermocouple (Omega Engineering Inc., Model 199 TC, Stamford, CT) and maintained between 37 and 42 °C by water flow through the glass jacket. For vesicle preparation, sonication time was 20–30 min, and the microemulsions were sonicated for 1 h. The resulting opalescent mixtures were then centrifuged in a Beckman 50 Ti rotor at 40000 rpm for 1 h at 5 °C. From the ultracentrifuge tube, the upper 1 mL was removed, and 4 mL of the remaining infranatant was then transferred to another tube and centrifuged at 40000 rpm in a Beckman SW 60 Ti rotor for 16 h at 5 °C. Vesicles were obtained from the lower 1 mL, and the microemulsions were collected in the upper 1-mL fraction of the appropriate centrifuge tube.

Preparation of Dimyristoyl- and Dipalmitoylcholine Vesicles. Vesicles either containing or lacking triolein were prepared. Sonication was performed so as to maintain the temperature inside the sonication cell approximately 5 °C above the gel ↔ liquid-crystal transition (T_m) of the phospholipid. Following sonication, the vesicle preparations were centrifuged for 1 h at 40000 rpm in a Beckman Ti 50 rotor previously equilibrated and maintained at 10 °C above the T_m to remove titanium fragments as well as large multilamellar liposomes.

Gel Filtration. Sonicated preparations of vesicles and microemulsions were applied to a Bio-Gel A-15m column (2.6 × 28 cm) which was preequilibrated with a solution containing 0.1 M NaCl and 0.01 M Tris, pH 7.4. Samples were eluted at ambient temperature (22–25 °C) with the preequilibration buffer at a flow rate of 24 mL/h. The void volume and total volumes were determined with Blue Dextran 2000 and tritiated water, respectively. Eluted fractions were analyzed for radioactivity by using Liquiscint as the scintillation cocktail and a Packard 300CD scintillation counter programmed to distinguish between ^3H and ^{14}C.

Differential Scanning Calorimetry. Calorimetry experiments were performed on a DSC-2 (Perkin-Elmer) instrument at a full-scale sensitivity of 0.2 mcal/s. Cooling rates of 2.5 and 5.0 °C/min were used, and transition temperatures were corrected to a rate of 0 °C/min. Samples (75 μL) containing 2–3 mg of lipid were hermetically sealed in stainless-steel sample pans (Perkin-Elmer), and 75 μL of 0.1 M NaCl, 0.01 M Tris, pH 7.4, and 0.02% sodium azide was used as a reference.

Agarose Gel Electrophoresis. Agarose gel electrophoresis was performed by using the Corning agarose gel electrophoresis system on preformed gels containing 65 mM barbital buffer, pH 8.6, and 0.035% EDTA. Sample aliquots (1–2 μL) were applied to each of eight lanes and electrophoresed at 90 V for 35 min by using the same buffer in the electrode wells. Gels for electrophoresis at an acid pH were prepared by dialysis against 4 L of 50 mM sodium acetate, pH 4.4, overnight. Electrophoresis was performed as described above, using the acetate buffer in the electrode wells. Gels were dried in a 60 °C oven and stained with Fat Red 7B.

Assay for Lipase Activity. Assays for acid lipase activity using vesicles or microemulsions as substrates were performed as previously described (Burrier & Brecher, 1983). Briefly, assays were performed in a buffer containing 50 mM sodium acetate, pH 4.4, 2.5 mM 2-mercaptoethanol, and 0.5 mM EDTA to which the designated amount of labeled substrate and 10 μL of the purified enzyme were added. The final reaction volume was 250 μL. Twenty-minute incubations were routinely performed at 37 °C. Assays involving the triolein containing DMPC and DPPC vesicles were preincubated 5 min at the appropriate temperature prior to the addition of enzyme. Incubations were terminated by the addition of 3.5 mL of benzene/chloroform/methanol (1.0:0.5:1.2 v/v) containing unlabeled oleic acid as carrier. A 0.6-mL aliquot of 0.3 M NaOH was added, and the resulting suspension was mixed and then centrifuged at 1000*g* for 10 min to separate the phases. The free fatty acid was then quantitated by counting a 1-mL aliquot of the upper phase in 10 mL of Liquiscint. Under these conditions, 89% of the free fatty acid partitioned into the upper phase. All measurements were made in duplicate and verified by replicate experiments. Acid lipase activity is expressed relative to the volume of the purified enzyme preparation rather than protein due to difficulty in obtaining accurate protein determinations (Burrier & Brecher, 1983). However, the protein concentration of the enzyme used in these studies was judged to be less than 10 μg/mL by assay (Lowry et al., 1951) and Coomassie Blue staining on poly-

[1] Abbreviations: EYPC, egg yolk phosphatidylcholine; PS, phosphatidylserine; PA, phosphatidic acid; PE, phosphatidylethanolamine; SM, sphingomyelin; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; TO, triolein.

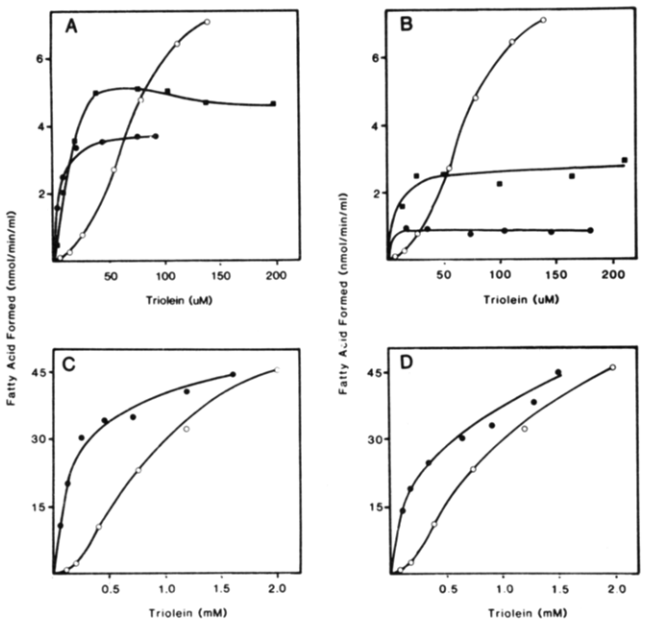

FIGURE 1: Effect of substrate concentration on enzymatic activity using vesicles and microemulsions containing small amounts of PA or PS. (A) Effect of PS on triolein hydrolysis in vesicles. (B) Effect of PA on triolein hydrolysis in vesicles. (C) Effect of PS on triolein hydrolysis in microemulsions. (D) Effect of PA on triolein hydrolysis in microemulsions. Substrates containing only EYPC as phospholipid (○); substrates containing 5 (■) or 10 mol % (●) of the phospholipid as either PA or PS. For reaction conditions, see Materials and Methods.

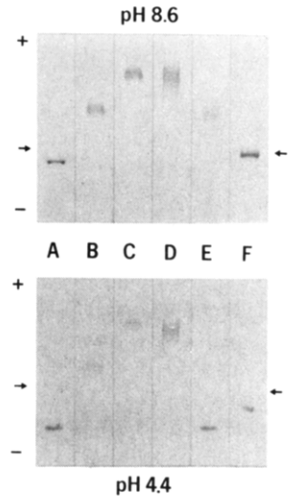

FIGURE 2: Agarose gel electrophoresis of vesicles and microemulsions. Aliquots of each substrate preparation were subjected to electrophoresis at pH 8.6 (top) or pH 4.4 (bottom). Lane A, vesicle containing only EYPC and triolein; lane B, vesicles containing 5 mol % of the phospholipid as PA; lane C, vesicles containing 10 mol % of the phospholipid as PA; lane D, vesicles containing 10 mol % of the phospholipid as PS; lane E, vesicles containing an additional 10 mol % of oleic acid relative to phospholipid; lane F, microemulsions containing only EYPC and triolein. The origin is marked with the arrows.

acrylamide gel electrophoresis.

Analytical Procedures. Following preparation, each of the vesicle and microemulsion preparations was subjected to chemical analysis to verify composition. Total phospholipid was measured by the method of Bartlett (1959) with a factor of 25 used to estimate phospholipid from the phosphorus measured. Cholesterol was measured by the method of Rudel & Morris (1973), and triglycerides were assayed by the method of Fletcher (1968). Lipid extracts were prepared by the method of Folch et al. (1957). Thin-layer chromatography was performed on precoated silica gel G plates (Applied Science Inc., State College, PA). For resolution of the major lipid classes, hexane/diethyl ether/acetic acid (70:30:1 v/v) was used as a developing solvent, and individual phospholipid classes were separated with chloroform/methanol/acetic acid/water (50:30:8:4 v/v) as the developing solvent.

Results

The hydrolysis of triolein in vesicles and microemulsions containing small amounts of PS and PA is shown in Figure 1. For comparison, substrate particles containing only EYPC and triolein were prepared and assayed similarly. Although saturation was not observed with EYPL/triolein vesicles at the substrate concentration shown in Figure 1A,B, other experiments indicated the V_{max} to be 8 nmol min^{-1} mL^{-1}. Substitution of 5 or 10 mol % of the phospholipid as PS caused at least a 10-fold reduction in the substrate concentration required to achieve the half-maximal rate (Figure 1A). The maximal velocities observed also were decreased in a dose-dependent manner when PS was contained in the vesicles.

The effect of PA incorporation into vesicles on triolein hydrolysis is similar to that observed with PS-containing vesicles (Figure 1B). Decreases in both the substrate concentration required to reach saturation and the maximal velocity were observed. The decrease in activity also was dependent on the amount of PA contained in the vesicles. The incorporation of 10 mol % of PA or PS into triolein-containing microemulsions caused a shift of the apparent K_m when compared to the microemulsion lacking acidic phospholipids (Figure 1C,D). No change in V_{max} was observed as a result of adding PA or PS to the microemulsions.

Analysis of the substrate preparations by agarose gel electrophoresis is shown in Figure 2. Electrophoretic migration of the vesicles and microemulsions was assessed at pH 8.6 and at pH 4.4, the latter value being the pH of the assay. Triolein-containing EYPC vesicles (lane A) or microemulsions (lane F) migrated slightly toward the cathode at either pH. Substitution of 5 mol % by PA of EYPC (lane B) caused the vesicles to migrate toward the anode at both pHs to form a single band, indicating particle homogeneity with respect to lipid composition and the incorporation of a net negative charge into the vesicles. This effect was more pronounced using 10 mol % of PA (lane C). dose-dependent changes also were obtained when PS-containing vesicles were analyzed by electrophoresis (lane D). Similar changes in electrophoretic mobility were observed by using microemulsions with corresponding polar lipid compositions except that overall migrational distances were slightly less than those of vesicles.

Gel filtration using Bio-Gel A-15m was performed by using the vesicles and microemulsions containing acidic phospholipid. Elution profiles appeared identical with those previously reported for the particles lacking the acidic phospholipid (Burrier & Brecher, 1983). A broad peak containing greater than 90% of the lipid elutes at a volume corresponding to a particle diameter of about 220 Å for vesicles while the microemulsions eluted at slightly larger volumes, indicating slightly greater particle diameter (220–280 Å). The elution profiles indicated that incorporation of acidic phospholipid does not change the size or homogeneity of either particle type.

Additional experiments were performed to determine if other phospholipids caused alterations in kinetic parameters. Vesicles and microemulsions were prepared containing 5 and 10 mol % of the phospholipid as PE. Plots of velocity vs. substrate concentration revealed no significant differences when compared to particles lacking PE. In a similar manner, SM was

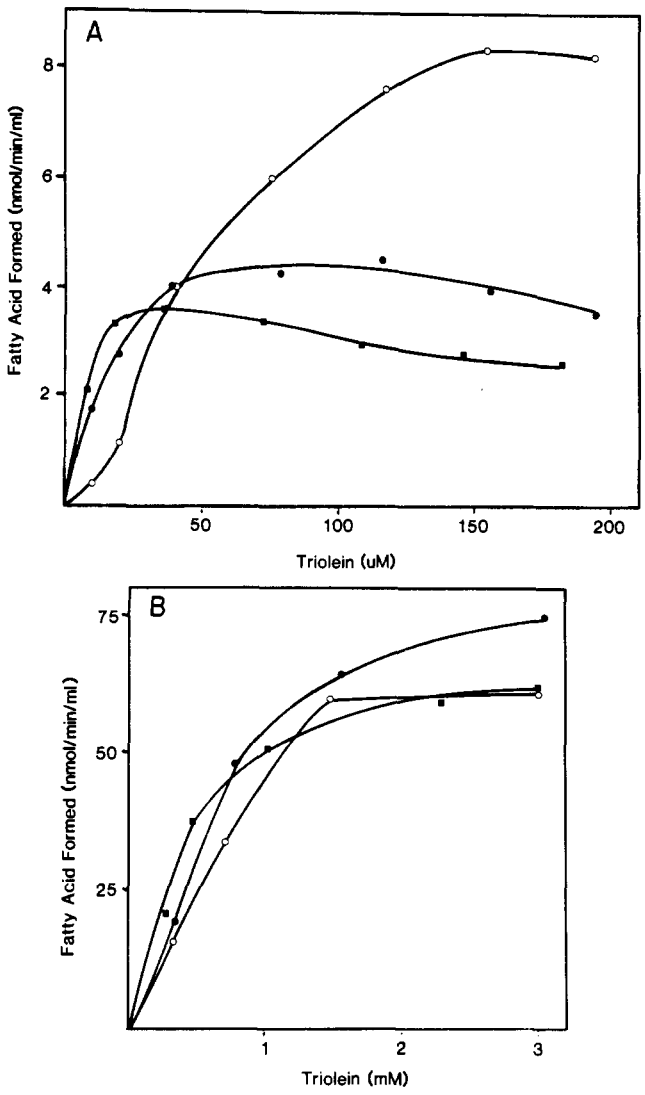

FIGURE 3: Effect of the oleic acid incorporation into vesicles and microemulsions on enzyme activity. (A) Effect of oleic acid incorporation into vesicles. (B) Effect of oleic acid incorporation into microemulsions. Substrates containing only EYPC and triolein (○); substrates containing 10 (●) or 20 mol % (■) of additional oleic acid relative to EYPC. All incubations were performed as described in the text, and the averages of duplicates are reported.

substituted for up to 20 mol % of the phospholipid, and again no significant changes in the kinetic parameters were observed. Cholesterol was incorporated into both substrates at concentrations up to 20 mol % of the phospholipid, and no differences were observed in kinetic plots. Electrophoresis of particles containing cholesterol, PE, and SM was also performed, and electrophoretic migrations did not differ from those for the control EYPC particles.

Substrates containing 10 and 20 mol % of oleic acid were prepared and used to determine the effect of this reaction product on enzyme activity. As shown in Figure 3A, the oleic acid containing vesicles caused both a dose-dependent decrease in the maximal velocity observed and an increase in activity at low substrate concentrations, an effect analogous to that observed with the negatively charged phospholipids. When microemulsions were used as substrate, addition of oleic acid did not cause any appreciable change in triolein hydrolysis (Figure 3B).

The addition of oleic acid to both vesicles and microemulsions causes a net negative charge to be imparted on the particles as determined by electrophoresis performed at pH 8.6 (Figure 2, lane E). However, when electrophoresis was

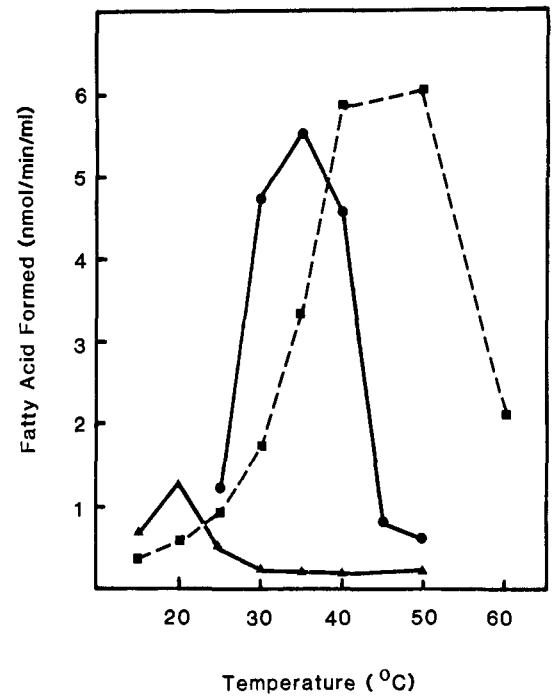

FIGURE 4: Dependence of triolein hydrolysis on temperature in egg yolk dipalmitoyl- and dimyristoylphosphatidylcholine vesicles (70:1 phospholipid:triolein molar ratio). Each of the vesicle preparations was added to incubation tubes and preincubated at the designated temperature for 5 min prior to the addition of enzyme. EYPC/TO vesicles (■) were assayed at 77 μM triolein, DMPC/TO vesicles (▲) were assayed at 77 μM triolein, and DPPC/TO vesicles (●) were assayed at 55 μM triolein.

performed at pH 4.4, the fatty acid containing particles migrated similarly to particles containing only EYPC and triolein. This indicates that the pK_a for a fatty acid in a layer of phospholipid lies between pH 4.4 and 8.6. Ptak et al. (1980) have estimated the pK_a to be between 7.2 and 7.4 in sonicated bilayers of EYPC containing 1–15 mol % of fatty acid. These results suggest that the kinetic alteration observed when oleic acid is incorporated into vesicles may not be due to a charge effect.

To examine the effect of saturated phospholipids on triolein hydrolysis, triolein-containing DPPC and DMPC vesicles were prepared as substrates and assayed at constant substrate concentrations as a function of temperature (Figure 4). Also shown for comparison is the temperature–activity profile for triolein-containing EYPC vesicles, which was similar to that reported previously (Burrier & Brecher, 1983). When DPPC-containing vesicles were assayed under similar conditions, a peak of activity was observed at about 35 °C, and DMPC-containing vesicles had maximal activity at 20 °C, although enzymatic activity was relatively less at thjis temperature. This suggested that enzymatic activity was greatest at temperatures close to the phase transitions of DMPC or DPPC.

Although the physical properties of small unilamellar vesicles of DMPC and DPPC have been described previously (Melchior & Steim, 1976), studies were performed to verify the existence and temperature of the gel ↔ liquid-crystal transition in the presence of small amounts of triolein. Figure 5 shows the differential scanning calorimetry cooling curves of vesicles synthesized and maintained at least 10 °C above the T_m. Vesicles lacking triolein exhibited transitions at predicted temperatures (37.5 and 18 °C for DPPC and DMPC, respectively) with fairly symmetric exothermic peaks, suggesting purity of the small unilamellar vesicles. The in-

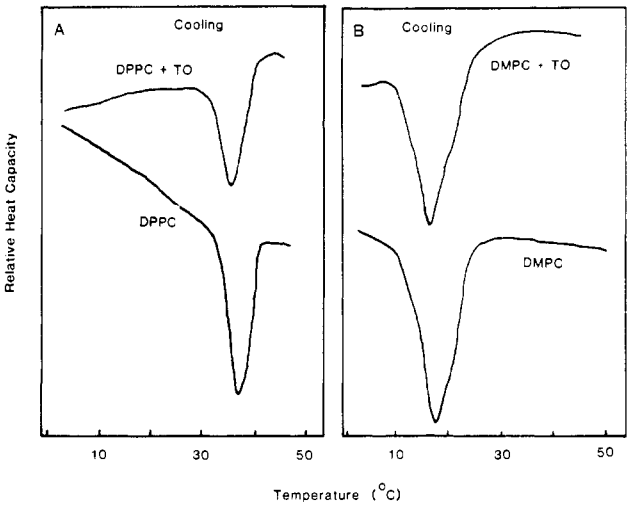

FIGURE 5: Differential scanning calorimetry cooling curves for vesicles of DMPC and DPPC either lacking or containing triolein (70:1 phospholipid:triolein molar ratio). (A) DPPC vesicles with and without triolein (TO) having peak transitions occurring at 37.5 and 36.0 °C, respectively. (B) DMPC vesicles with and without triolein having peak transitions at 18 and 17 °C, respectively. Tracings shown have been corrected to a cooling rate of zero.

corporation of triolein into the vesicles (phospholipid:triolein molar ratio of 70:1) had the effect of lowering the T_m to 36 and 17 °C for DPPC and DMPC, respectively. Once the vesicles were cooled below the T_m, aggregation of particles occurred such that another transition was observed as a shoulder on the peak at the higher temperature, indicating the presence of larger liposomes (data not shown) as have been described previously (Melchior & Steim, 1976).

Discussion

This study was undertaken to help elucidate the mechanism of acid lipase action. Although the detailed mechanism for any lipase is not yet known, the study of acid lipase may be advantageous since this enzyme does not appear to require a cofactor. Native substrates for this enzyme include lipoproteins internalized within the lysosome prior to degradation. Therefore, vesicles and microemulsions were used as model substrates because they contain properties similar to those of a lipoprotein interface. Such substrates allow experimental control of both particle molecular composition and particle number in each assay. In addition, comparisons between vesicle and microemulsion experiments are useful in determining the influence of the oil phase core region of the microemulsion on lipolysis. In vesicles containing phospholipids and triglycerides, the triglycerides are oriented with the polar glycerol portion toward the aqueous environment and the acyl chains perpendicular to the vesicle surface (Hamilton & Small, 1981). The microemulsion is a particle of approximately similar size containing a monolayer of phospholipid surrounding the core oil phase of triglyceride. Interspersed in the phospholipid monolayer are small (2.5–4.0 mol %) amounts of triglyceride, presumably in an orientation similar to triglyceride in vesicles (Miller & Small, 1982). Movement of triglycerides from core to surface appears to be a rapid process, and therefore, although only a small portion of the triglyceride in a microemulsion is surface oriented at a given time, all of the triglyceride in the particle may become accessible to the surface phase.

An initial step in the action of lipases involves the adsorption of the enzyme to the substrate surface. Isoelectric focusing has shown that the acid lipase has a pI of 6.1 (Brown & Sgoutas, 1980), and therefore, substrates with a net negative charge at pH 4.4 may exhibit ionic interactions. Early work on purified rat liver acid lipase preparations revealed that incorporation of PS and cardiolipin into Triton X-100 containing substrates stimulated enzyme activity greater than 2-fold (Teng & Kaplan, 1974). We prepared vesicles and microemulsions with small amounts of PS or PA to determine the kinetic effects of negatively charged lipid. Sigmoid-shaped velocity vs. substrate curves were obtained when control EYPC/TO-containing microemulsions and vesicles were used as substrates. Such kinetic data were previously observed and are probably due to the small amount of Triton X-100 which was required for enzyme purification and storage. Vesicular substrates containing PS or PA caused a decrease in the V_{max} and an increase in the apparent affinity of the enzyme toward the vesicles. To establish the net charge of substrates prepared, a microelectrophoretic method utilizing 1 μL of substrate was developed. Using this method, it was demonstrated that incorporation of either PS or PA actually did impart a net negative charge to the particles. Several other surface constituents common to biological systems including sphingomyelin, PE, and cholesterol were also incorporated into the model substrates and found to change neither the net particle charge nor the kinetic parameters. In contrast, using a monolayer-contained substrate, Demel et al. (1982) have demonstrated that sphingomyelin inhibits lipoprotein lipase activity greater than 50% when included at concentrations of less than 20 mol %.

Increased enzyme affinity could limit enzymatic hydrolysis to only the substrate particle onto which the enzyme initially was bound. As the triolein concentration in the vesicles become less upon hydrolysis, substrate concentrations relative to the enzyme on that particle would become limiting. The kinetic data would then show a decreased V_{max}, and the value of the apparent K_m would be altered. When microemulsions containing the acidic phospholipids were compared to control microemulsions, an increased affinity of the enzyme toward the negatively charged vesicles was observed, but no decrease in V_{max} was observed. In the particles, the core of triolein may prevent substrate concentrations from becoming limiting if core triolein would rapidly diffuse to the surface. Such an interpretation emphasizes the role of core phase lipid and the use of microemulsions as model substrates.

When fatty acids were included in the vesicular substrates, a decrease in the apparent K_m was observed. In this case, as in the case of the negatively charged vesicles, increased enzyme affinity may cause triolein to become concentration limited on some particles and be responsible for the decreased V_{max}. Analysis of these substrates by electrophoresis showed that the net charge was similar to that of the control EYPC vesicles. Fatty acid containing microemulsions did not alter the V_{max} or K_m. However, at the pH used for assay, the fatty acid exists primarily in the protonated form and may reside in the core region of these particles where it is not accessible to the surface. The ability of oleic acid to alter kinetic parameters in vesicles may be related to the presence of excess reaction product or an alteration in the substrate surface conformation which allows for increased enzyme affinity.

The effect of temperature on lipase action must account for both the intrinsic activity of the enzyme and the conformation or physical state of the substrate surface. An approximation of the intrinsic thermal activity of the acid lipase was obtained by using triolein-containing EYPC vesicles which remain in the liquid-crystalline state at all temperatures studied. Vesicles prepared by using DMPC and DPPC were hydrolyzed max-

imally at temperatures corresponding to the phase transition of the phospholipid. Similar results were obtained by using phospholipase A_2 preparations from pig pancreas (Op den Kamp et al., 1975), bee venom (Upreti & Jain, 1980), and cobra venom (Kensil & Dennis, 1979). Similar findings for cholesteryl esterase acty of a rat liver lysosomal fraction were reported (Lundberg et al., 1979). Optimal activity may occur at temperatures near the phase transition because at such temperatures an alteration in the packing of lipid molecules can cause defects in the surface of the particle where the enzyme may act more readily (Op den Kamp et al., 1975; Upreti & Jain, 1980). The lack of enzymatic activity above the phase transition using substrates with saturated phosphatidylcholines suggests that the fatty acid heterogeneity of EYPC substrates may provide a substrate surface with a favorable conformation for enzymatic activity.

Our data demonstrate that the surface composition of the substrates used was important for determining the rate of triglyceride hydrolysis by an acid lipase. Microemulsions may be particularly useful as models for lipoprotein particles although the presence of apoprotein on naturally occurring lipoproteins obviously must be considered. Procedures for the incorporation of apoproteins into triglyceride microemulsions (Tajimo et al., 1983) and apoprotein B into cholesteryl ester microemulsions (Walsh et al., 1982) have been reported recently. The use of such particles should facilitate future studies to assess the influence of specific apoproteins on lipase activity.

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