# Lipolysis of Phospholipids in Model Cholesteryl Ester Rich Lipoproteins and Related Systems: Effect of Core and Surface Lipid Phase State†

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ABSTRACT: Porcine pancreatic phospholipase $A_2$ (PL $A_2$) was used as a probe to study the structure of phospholipid domains of dimyristoylphosphatidylcholine (DMPC) vesicles ± 2% cholesteryl oleate (CO), of discoidal structures formed by the interaction of apolipoprotein E (apoE) with these vesicles, and of large CO/DMPC microemulsion particles ± apoE. Results of phospholipid hydrolysis over a range of temperatures were compared with the thermal transitions of the lipid components of the particles as measured by differential scanning calorimetry. These studies revealed that DMPC vesicles were most susceptible to digestion at or near the transition temperature. A similar result was obtained with DMPC/apoE disks; however, these particles were hydrolyzed over a broader temperature range than the vesicles. DMPC/CO vesicles were resistant to hydrolysis at every temperature tested; however, discoidal structures formed by interaction of apoE with these vesicles were hydrolyzed maximally above their thermal transition. Large microemulsion particles of CO and DMPC were poor substrates for the enzyme at every temperature; binding of apoE to these particles improved the ability of PL $A_2$ to hydrolyze the phospholipid. These results suggest that the curvature of the surface, the presence of dissolved cholesteryl ester, and the binding of protein have profound effects on the temperature and breadth of the phospholipid phase transition and on the accessibility of the phospholipid to hydrolysis by PL $A_2$.

The mobility and structure of phospholipid domains in a variety of particle types have been examined by several physical techniques including NMR, EPR, and fluorescence spectroscopy (Sklar et al., 1977; Pownall et al., 1978; Barrett et al., 1969; Hubbell & McConnell, 1971; Brainard et al., 1984; Novosad et al., 1976; Krieger et al., 1980). These studies have provided useful information about the dynamics of phospholipid motion in vesicles, protein/phospholipid disks, lipoproteins, and microemulsion particles. However, the accessibility and reactivity of phospholipid molecules in various domains have not been examined and systematically compared. In the past, pancreatic phospholipase $A_2$, which is very sensitive to the structure of the phospholipid/water interface, has been used to determine the accessibility of phospholipid in vesicles and liposomes (Wilschut et al., 1979a,b; Goormaghtigh et al., 1981; de Haas et al., 1968). Unlike the snake venom phospholipases which are able to hydrolyze phospholipids in a wide variety of substrate particles, pancreatic phospholipase $A_2$ (PL $A_2$)[1] has strict substrate requirements in terms of the physical form of the phospholipid. Wilschut et al. (1979) have shown that the pancreatic enzyme will hydrolyze dimyristoylphosphatidylcholine (DMPC) liposomes only in the temperature region surrounding the gel → liquid-crystalline phase transition (15–26 °C). Hydrolysis of small, unilamellar DMPC vesicles occurs over a slightly larger temperature range; these particles are better substrates presumably because their highly curved surfaces induce defects in lipid packing which facilitate penetration of the lipase. Op den Kamp et al. (1975) found that mixtures of phospholipids which cocrystallize are maximally hydrolyzed at their coordinate transition temperature, while monotectic mixtures of phospholipids have hydrolysis maxima at the melting temperature of each component. Addition of cholesterol to DMPC liposomes decreases the cooperativity of the phospholipid thermal transition and inhibits hydrolysis, but does not alter the temperature dependence of the process. The action of PL $A_2$ on vesicles with other colipids and on other types of particles has not been examined.

Unlike cholesterol, the neutral cholesteryl esters can be incorporated into phospholipid vesicles at no more than 2–3% by weight (Hamilton & Small, 1982). Published data suggest that, in a bilayer, cholesteryl ester has a horseshoe-like conformation with the carbonyl group at the lipid/water interface and the steroid ring system and acyl chain extending back toward the center of the bilayer (Grover et al., 1979). Cholesteryl ester incorporated into vesicles has been shown to exchange rapidly between the inner and outer phospholipid leaflets (Mims et al., 1986a). The effect of dissolved cholesteryl ester on the accessibility of the phospholipid, however, has not been reported.

Interaction of excess apolipoprotein E (apoE) with DMPC vesicles of ~220-Å diameter produces smaller discoidal structures with a phospholipid:apoE ratio of 5.5:1 (w/w). In these particles, apoE is presumably oriented around the edge of a bilayer disk of DMPC. Thus while these particles are smaller than vesicles, their surface curvature is lower because they are flat. The enzymic reactivity of the phospholipids in these particles has not been described.

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[1] Abbreviations: PL $A_2$, porcine pancreatic phospholipase $A_2$; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TLC, thin-layer chromatography; CO, cholesteryl oleate; DMPC, dimyristoyl-L-α-phosphatidylcholine; DSC, differential scanning calorimetry; SUV, small unilamellar vesicles with a mean diameter of 218 Å (Laggner et al., 1979); MLV, multilamellar vesicles; ME, microemulsion of ~750 Å diameter containing DMPC and CO (Mims et al., 1986b).

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Recently, we have developed a stable microemulsion model of cholesteryl ester rich very low density lipoprotein which is composed of cholesteryl oleate and dymyristoylphosphatidylcholine in a 4:1 (w/w) ratio (Mims et al., 1986b). These large particles (~750 Å in diameter) have a nonpolar CO core surrounded by a surface monolayer of DMPC. Although EPR studies have demonstrated that small amounts of cholesteryl ester are dissolved in the microemulsion surface monolayer (Mims et al., 1986a), surface lipid domains in these particles undergo independent thermal transitions which are distinct from those of the isolated lipids. Microemulsion particles have also been combined with apoE to form a stable complex with a protein:phospholipid ratio of 11:1 (w/w) (Mims et al., 1986a). The accessibility of phospholipids on the surface of the microemulsion or the microemulsion/apoE recombinant has not yet been detailed.

In this study, we have investigated the interaction of porcine pancreatic phospholipase $A_2$ with vesicles of DMPC ± 2% CO, with discoidal structures formed by the interaction of apoE with vesicles, and with microemulsion particles ± apoE. Results from these experiments have been compared with the thermal transitions of the lipid components of the particles in order to gain insight into the effect of temperature on the structure and reactivity of the phospholipid.

## Materials and Methods

*Materials.* Porcine pancreatic phospholipase $A_2$ was purchased from Sigma and desalted by passage over a Bio-Gel P-4 column. The enzyme appeared as a single band on SDS–polyacrylamide gel electrophoresis (2–16%). The concentration of enzyme was determined with an $E^{1\%}$ of 13.0 at 280 nm. Cholesteryl oleate (Sigma) was judged >99% pure by TLC in hexane/diethyl ether/acetic acid (90:10:1). Dimyristoyl-L-$\alpha$-phosphatidylcholine (Sigma) was judged >99% pure by TLC in chloroform/methanol/water (65:35:4). DMPC $^3H$ labeled in the *sn*-2 acyl chain was prepared by acylation of lysoDMPC with myristic anhydride obtained from [7,8-$^3H$]myristic acid (New England Nuclear) (Patel et al., 1979). The standard buffer in all experiments was 0.1 M Tris–10 mM $CaCl_2$, pH. 7.5.

*Particle Preparation.* Vesicles were prepared by sonication of DMPC (±2% CO) in Tris buffer for 30 min at ~20% power in a Heat Systems sonifier equipped with a microtip. Vesicles containing CO were prepared by sonication at 55–60 °C, above the transition temperature (52 °C) of cholesteryl oleate (Hamilton & Small, 1982). Following sonication, samples were centrifuged at 25 °C to remove any titanium particles and multilamellar vesicles that might have been formed. Vesicles prepared in this way were maintained at 30 °C and used for experiments within 24 h. Cholesteryl ester content was determined by enzymatic test kit (Boehringer).

DMPC/apoE disks (±2% CO) were prepared by mixing 2 mL of vesicles (10 mg/mL) with 2 mL of apoE (5 mg/mL) and incubating for 2 h at room temperature. Complexes of DMPC and apoE were separated from free apoE by adjusting the density of the solution to 1.18 g/mL with KBr and centrifuging for 24 h at 35000 rpm in a Beckman SW 50.1 rotor. DMPC/apoE disks, which were concentrated at the top of the tube, were isolated and dialyzed against standard Tris buffer before use in experiments. Under these conditions, complexes with a phospholipid:protein ratio of 5.5:1 (w/w) were routinely obtained. Addition of more protein did not decrease this ratio.

Microemulsion particles were prepared by sonication and column chromatography (Mims et al., 1986b) and dialyzed against standard buffer before use in experiments. Microemulsion/apoE complexes were prepared by incubation of 3 mL of microemulsion (3 mg/mL DMPC) with 1 mL of apoE (5 mg/mL) at 31 °C for 2 h. The particles were separated from free apoE by centrifugation for 24 h at 35000 rpm in an SW 50.1 rotor (Mims et al., 1986a). Complexes prepared in this way had a phospholipid:protein ratio of 11:1.

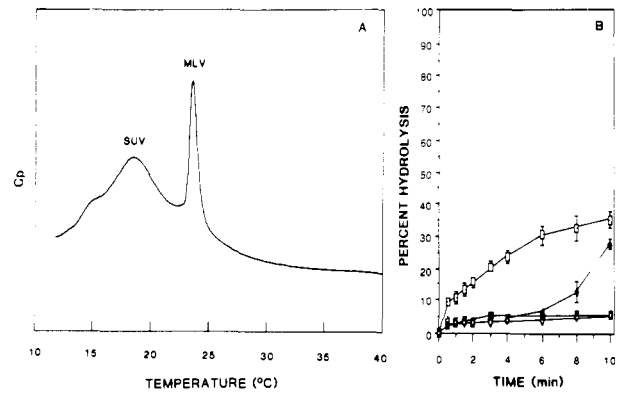


FIGURE 1: (A) Heat capacity versus temperature plots for a mixture of small unilamellar (SUV) and large multilamellar (MLV) vesicles of DMPC. (B) Time course for hydrolysis of SUV of DMPC by PL $A_2$ at (⊡) 19, (♦) 25, (■) 37, and (◇) 50 °C. Data represent the average of duplicate determinations.

*Assay Conditions.* The PL $A_2$ reaction mixture consisted of 1 mL of $^3H$-labeled substrate particles diluted to 1 mM DMPC. Temperature was controlled with a water bath; all samples were equilibrated for at least 10 min at the desired temperature. Ten microliters (2.3 μg) of PL $A_2$ was added to start the reaction. Aliquots were removed over a 10-min period and injected into test tubes containing 100 μL of 100 mM EDTA to stop the reaction. All assays were performed in duplicate at each temperature.

Free fatty acids were separated from DMPC and lysophosphatidylcholine via a Dole extraction (Dole & Meinerz, 1960). An aliquot of the heptane phase (containing $^3H$-labeled fatty acid) was chromatographed over a disposable silica gel minicolumn, and the eluate was collected in scintillation vials. The column was washed twice with 0.5 mL of ethyl ether and the fractions were counted (Van den Bosch & Aarsman, 1979).

*Differential Scanning Calorimetry.* Differential scanning calorimetry (DSC) was performed on all particles with a Microcal MC-2 scanning calorimeter. All data were collected as heating scans at a heating rate of 30 °C/h.

## Results

Small unilamellar vesicles (SUV) of DMPC have a main thermal transition at 18.4 °C (Figure 1A). A small shoulder occurring at 15.4 °C represents the pretransition of DMPC SUVs. Even after gel filtration chromatography, the SUV preparation contains a detectable amount of larger multilamellar vesicles (MLV transition temperature = 23.4 °C) whose proportion increases as the vesicle preparation ages or is heated and cooled repeatedly during DSC.

Hydrolysis of DMPC in vesicles at their transition temperature, ~19 °C, was rapid up to almost 35% hydrolysis (Figure 1B). At 25 °C, there was an initial lag period of 4 min, followed by an acceleration of activity. At 37 and 50 °C, well above the DMPC vesicle transition, less than 6% of the total phospholipid was hydrolyzed over the 10-min assay period.

Discoidal structures formed by the interaction of DMPC SUV with apoE had a broad thermal transition centered at ~25 °C (Figure 2A). At 19 °C, ~6 °C below the midpoint of the thermal transition, hydrolysis was characterized by an initial lag of 4 min followed by a modest acceleration in activity

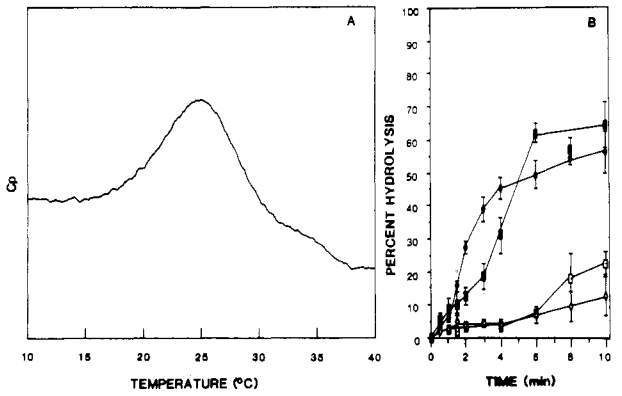


FIGURE 2: (A) Heat capacity versus temperature plots for discoidal complexes of apoE and DMPC. (B) Time course for hydrolysis of DMPC by PL $A_2$ at (⊡) 19, (♦) 25, (■) 37, and (◊) 50 °C. Data represent the average of duplicate determinations.

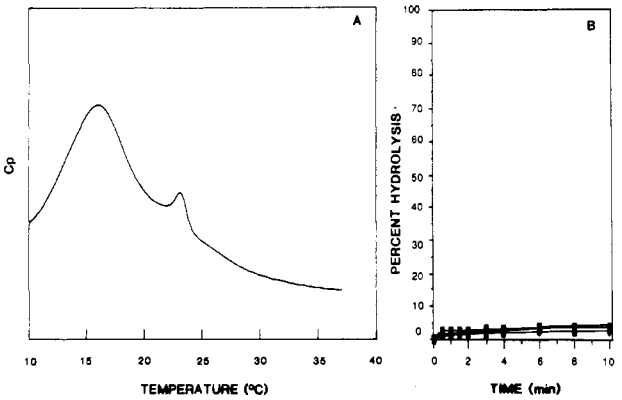


FIGURE 3: (A) Heat capacity versus temperature plots for SUV of DMPC containing 2% CO. (B) Time course for hydrolysis of DMPC/CO vesicles at (●) 16, (⊡) 19, (♦) 25, (■) 37, and (◊) 50 °C. Data represent the average of duplicate determinations.

(Figure 2B). At 25 and 37 °C, at the transition temperature and 12 °C above it, respectively, there was no lag, and hydrolysis proceeded to more than 50% during the 10-min incubation. At 50 °C, well above the transition, there was a lag phase followed by a slow rise in activity.

Including 2% CO in the DMPC vesicle broadened the transition only slightly but lowered the transition temperature from 18.4 to 15.9 °C (Figure 3A). In addition, the CO-containing vesicles were more stable and resistant to fusion and formation of multilamellar vesicles as evidenced by the smaller MLV peak at ~23 °C, which remained approximately constant in size throughout repeated heating and cooling of the sample. Figure 3B shows that DMPC/CO vesicles were resistant to PL $A_2$ at every temperature tested, demonstrating no more than 5% hydrolysis over the 10-min incubation period.

Incubation of apoE with DMPC/CO vesicles also appeared to produce disk-like structures as judged by their calorimetric behavior (Figure 4A) and their chromatographic elution profiles (data not shown). Like the DMPC/apoE complex, these disks exhibited a broad thermal transition centered at about 25 °C. Unlike DMPC/apoE disks, however, at 19 and 25 °C the DMPC/CO/apoE disks were fairly resistant to the action of PL $A_2$ (Figure 4B). Above the phospholipid thermal transition, at 37 and 50 °C, there was a lag phase of 2–3 min followed by accelerated activity.

Large CO/DMPC microemulsion particles exhibited two thermal transitions. The lower temperature transition occurred at about 28 °C and has been shown previously to correspond to a structural transition of the surface phospholipids. The higher temperature thermal transition occurred at ~44 °C

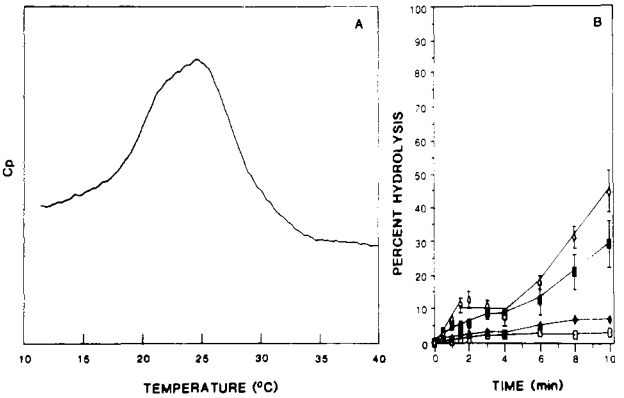


FIGURE 4: (A) Heat capacity versus temperature plots for discoidal complexes of apoE and DMPC/CO vesicles. (B) Time course for hydrolysis of DMPC/CO/apoE disks by PL $A_2$ at (⊡) 19, (♦) 25, (■) 37, and (◊) 50 °C. Data represent the average of duplicate determinations.

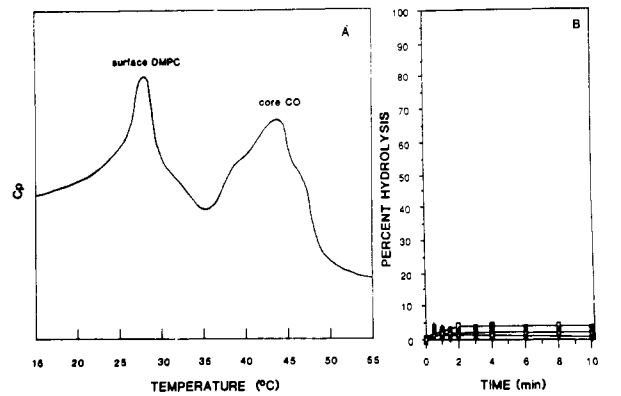


FIGURE 5: Heat capacity versus temperature plots for large CO/DMPC microemulsion particles. (B) Time course for hydrolysis of CO/DMPC microemulsion by PL $A_2$ at (⊡) 19, (♦) 25, (■) 37, and (◊) 50 °C. Data represents the average of duplicate determinations.

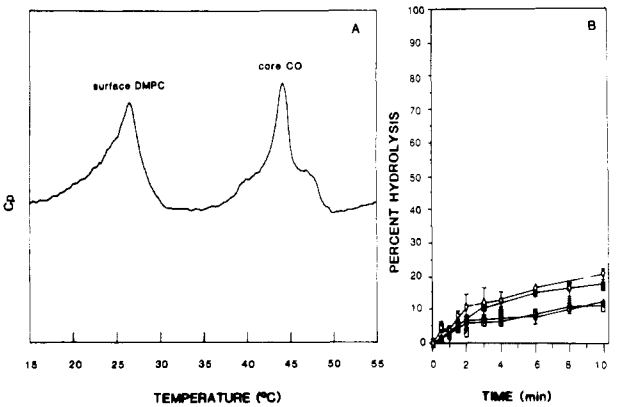


FIGURE 6: Heat capacity versus temperature plots for the apoE–microemulsion complex. (B) Time course for hydrolysis of the apoE–microemulsion complex by PL $A_2$ at (⊡) 19, (♦) 25, (■) 37, and (◊) 50 °C. Data represent the average of duplicate determinations.

and has been shown to reflect a structural transition of the core cholesteryl esters (Figure 5A) (Mims et al., 1986a). DMPC on the microemulsion surface was resistant to hydrolysis by PL $A_2$ at every temperature tested, i.e., 19, 25, 37, and 50 °C (Figure 5B).

Binding of apoE to the microemulsion surface did not appear to alter the midpoint of the core or surface lipid thermal transitions (Figure 6A). However, the microemulsion particles were more stable with apoE bound to their surface. Interestingly, the higher temperature transition appeared to be composed of two or more overlapping thermal transitions.

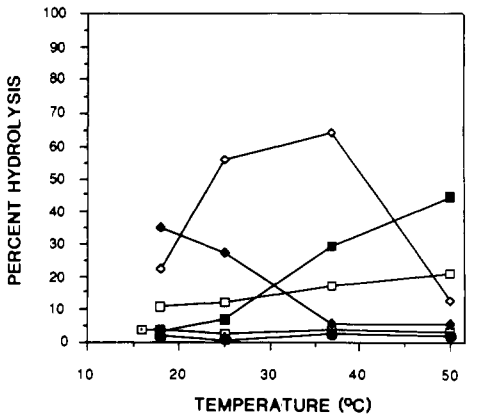


FIGURE 7: Percentage of total DMPC hydrolyzed by PL $A_2$ after 10 min over the temperature range 16–50 °C for (♦) DMPC SUV, (◇) discoidal complexes of apoE and DMPC, (⊡) SUV of DMPC containing 2% CO, (■) discoidal complexes resulting from admixture of apoE and DMPC/CO vesicles, (●) large CO/DMPC microemulsion particles, and (□) apoE–microemulsion complex.

Table I

| particle | $T_m$ (°C) | $\Delta T_{1/2}$ (°C) | enthalpy (cal/g) |
|---|---|---|---|
| DMPC vesicles (unilamellar) | 18.4 | 5.0 | 4.3 |
| DMPC/apoE disks | 25.4 | 7.6 | 5.0 |
| DMPC/2% CO vesicles (unilamellar) | 15.9 | 6.4 | 4.1 |
| DMPC/CO/apoE disks | 24.6 | 7.7 | 4.9 |
| CO/DMPC microemulsion | 27.8 (DMPC) | 3.6 | |
| | 44.4 (CO) | | 0.8 |
| CO/DMPC/apoE microemulsion | 27.0 (DMPC) | 4.0 | |
| | 44.0 (CO) | | 0.7 |

When they were subjected to the action of PL $A_2$ at 19 and 25 °C, about 10% of the surface DMPC was hydrolyzed over the 10-min incubation (Figure 6B). At 37 °C, ~12 °C above the transition of the surface, and approaching that of the core, almost 20% hydrolysis occurred over 10 min. At 50 °C, which is ~23 and 6 °C above the surface and core transitions, respectively, ~20% hydrolysis occurred during the incubation period.

Figure 7 compares the total hydrolysis at 10 min of the six different particle types over the temperature range studied. Calorimetric and phase transition data for the six particles studied are summarized in Table I.

## Discussion

Lipolysis by PL $A_2$ requires that the enzyme insert into the phospholipid monolayer at the particle surface. Early studies on the activity of the enzyme toward vesicles demonstrated that phospholipid hydrolysis was maximal at temperatures slightly below or above the phase transition where solid and liquid phospholipid domains could exist (Op den Kamp et al., 1975). It was suggested that boundaries between these domains formed defects into which PL $A_2$ could insert. In the present work, PL $A_2$ was used to examine the organization of a series of different phospholipid domains. Together with our thermal data, the lipolysis studies reflected the importance of a number of factors on the structure and accessibility of phospholipid domains. A general feature seen in the lipolytic time courses of DMPC vesicles and DMPC/apoE disks ± 2% CO was a distinct lag phase followed by an acceleration in hydrolysis at temperatures considerably above or below the thermal transitions of these particles. This lag has been observed previously and attributed variously (i) to a time-dependent insertion of the enzyme into the lipid/water interface (Verger & de Haas, 1976), (ii) to a product-induced change in the $T_m$, causing the bilayer to reach thermal transition at the reaction temperature (Op den Kamp et al., 1974), or (iii) to a slow, product-dependent enzyme desorption (Tinker & Wei, 1979). Recent studies using phospholipid vesicles indicate that the first alternative, slow insertion of the enzyme into the lipid surface in regions of local defects, is probably the correct one for the pancreatic enzyme (Menashe et al., 1981). Surface curvature is known to affect packing of phospholipids, with highly curved surfaces producing more defects and thus more hydrolysis by lipases. Thus, small unilamellar vesicles have been reported to be better substrates for PL $A_2$ than MLV. Our results suggest that for DMPC vesicles the effect of surface curvature is limited to the temperature region immediately surrounding the thermal transition. Above the temperature of the gel → liquid-crystalline phase transition, there exist few defects which allow penetration of the enzyme into the vesicle surface.

In contrast to vesicles, DMPC/apoE disks exhibited a broad thermal transition centered at 25 °C, and their phospholipids were hydrolyzed over a wide temperature range. The increase in $T_m$ from 19 °C for the vesicles to 25 °C for the disks probably reflects the essentially flat surface of the bilayer disk compared to the highly curved vesicle, while the greater breadth of the transition (reflecting decreased molecular cooperativity) indicates the coexistence of solid and liquid phospholipid domains over a greater temperature range in these particles. Near the $T_m$, more than 50% of the substrate was hydrolyzed in these particles compared to 35% for DMPC vesicles at their $T_m$. Increased hydrolysis of disk phospholipids near the transition temperature may reflect exposure of both sides of the bilayer to the enzyme, increasing the effective substrate concentration relative to that in unilamellar vesicles where only one side of the bilayer is exposed. Although decreased surface curvature of the disks relative to that of vesicles would suggest tighter molecular packing which might lead (as in the case of MLV) to decreased hydrolysis, PL $A_2$ was surprisingly active on these particles. Lipolysis at temperatures well above and below the center of the thermal transition might be explained by interaction of phospholipids with apoE at the perimeter of the disk, producing a discontinuity in the lipid matrix and permitting insertion of PL $A_2$. The existence of an annular layer of lipid surrounding membrane proteins has been proposed by a number of investigators (Jost et al., 1973; Brotherus et al., 1981; Vanderkooi et al., 1972) and could explain enhanced hydrolysis of the disk phospholipids at temperatures removed from the thermal transition. If phospholipid molecules adjacent to apoE interact with the protein in a manner that alters their order compared to that of the remaining lipid molecules, defects could be created that allow penetration of the enzyme into the disk surface. Thus in spite of their lack of curvature, lipid packing defects could exist in the DMPC/apoE disks, which would explain their efficacy as substrates for PL $A_2$.

Although vesicles containing DMPC and 2% CO showed a distinct thermal transition at ~16 °C, PL $A_2$ showed no activity toward them in the assay. The effect of CO partitioned into the DMPC domain on the thermal behavior of these vesicles is reminiscent of the effect of some drugs and anesthetics on phospholipid bilayers. Lipid-soluble drugs such as short-chain alkanes, *n*-alkanols, local anesthetics, and phenothiazine tranquilizers both broaden and lower the $T_m$ of dipalmitoylphosphatidylcholine liposomes (Mountcastle et al., 1978). In addition, these drugs have been shown to have an antihemolytic effect which correlates with the increased

stability of DMPC vesicles containing CO (Roth & Seeman, 1971). It has been suggested that when incorporated into a bilayer, these drugs increase the fluidity of the membrane and reduce the size of cooperative lipid clusters (Jain et al., 1975). This theory may also explain the effect of CO on DMPC vesicles; dissolved CO may serve to fluidize the vesicle bilayer and decrease the degree of cooperative interaction between lipid molecules. A decrease in the size of gel and liquid-crystalline lipid clusters below a critical value might prevent surface defects of sufficient dimensions for enzyme insertion. Alternatively, the CO molecules themselves may fill surface defects and prevent penetration of the enzyme. Whatever the mechanism, inclusion of this small amount of CO in the vesicle alters the $T_m$, but not the enthalpy of the transition. This alteration is reflected in the apparent inability of PL $A_2$ to penetrate the vesicle surface and cause hydrolysis (Figure 3B). Incubations performed for 30 min indicated that significant hydrolysis eventually took place at 16 °C, but not at the higher temperatures. Thus, the lack of hydrolysis at 16 °C may reflect a long lag period rather than truly inhibited hydrolysis. These results suggest that neutral cholesteryl esters have appreciably different effects on the thermal behavior of DMPC vesicles compared to those of cholesterol.

Like DMPC/apoE disks, DMPC/CO/apoE disks also exhibited a broad thermal transition centered at ~25 °C; however, significant hydrolysis occurred only at temperatures above the thermal transition of these particles and only following an initial lag phase. This behavior suggests that the interaction of apoE with the liquid-crystalline phospholipid induces defects which permit penetration of PL $A_2$. The role played by cholesteryl ester in these disks is unclear; however, the behavior of PL $A_2$ toward these particles is clearly different from its activity toward DMPC/apoE disks. Significant hydrolysis occurs at every temperature studied for the DMPC/apoE disk (Figure 2B) but only at 37 and 50 °C for DMPC/apoE disks containing CO, suggesting that CO plays an important role in filling or reducing the size of surface defects in disks as well as in vesicles.

Large microemulsions of DMPC and CO exhibit two thermal transitions; the DMPC surface monolayer melts at ~27 °C, while the CO core melts at ~44 °C. The phospholipid transition in these particles is narrower and occurs at a considerably higher temperature (about 9 °C) than that of DMPC SUV. It is also at least 2 °C above that of the multilamellar vesicle transition. Although the increase in $T_m$ for these particles might in part reflect their smaller radius of curvature, an increase in the DMPC $T_m$ was also noted by Ginsburg et al. (1982) for smaller (~220-Å diameter) DMPC/CO microemulsion particles. Thus, the increase cannot be attributed solely to a decrease in surface curvature or to an increase in size relative to small unilamellar vesicles. It is more likely that the increased $T_m$ reflects the monolayer structure of the microemulsion surface and the interaction of its phospholipids with cholesteryl esters in the core. Interdigitation of the surface lipids with the higher melting core lipids might stabilize the surface transition relative to the bilayer.

The lack of phospholipid hydrolysis at any temperature in microemulsion (Figure 5B) may in part be a reflection of the decreased surface curvature in these large particles which results in closer packing of the surface lipids. Hydrolysis of DMPC in the closely packed large multilamellar vesicles shows a sharper temperature dependence than in unilamellar vesicles, but significant hydrolysis does occur in the temperature region surrounding the multilamellar vesicle transition (Op den Kamp et al., 1975). Thus, tighter packing of the phospholipid at the microemulsion surface cannot account totally for the lack of hydrolysis. Interaction of the surface phospholipids with the core cholesteryl esters could also affect the availability of phospholipid for hydrolysis; however, EPR studies have indicated little difference between the order of the phospholipid acyl chains in the vesicle bilayer and that in the surface monolayer of the microemulsion (Mims et al., 1986a). We have shown previously that as much as 12% cholesteryl ester may be partitioned into the surface of the microemulsion particle (Mims et al., 1986a) and that the exchange of CO between the core and surface is quite rapid. Thus, it appears that CO from the microemulsion core may move to the surface to fill defects which occur, preventing PL $A_2$ from inserting into the monolayer and initiating hydrolysis. The conformation of surface-oriented cholesteryl ester in vesicles and microemulsion particles is not entirely clear; however, a horseshoe configuration with the carbonyl extending toward the more apolar center of the bilayer or core of the particle has been suggested (Grover et al., 1979; Mims et al., 1986a).

Binding of apoE to the microemulsion to form a stable complex had little effect on the $T_m$ or the cooperativity of the DMPC surface monolayer transition (Figures 5A and 6A). Binding of apoE, however, stabilized these particles and made their surface monolayers more accessible to the enzyme. Lipolysis studies indicated that the enzyme was able to insert into the monolayer below the surface $T_m$, but hydrolysis was increased at higher temperatures where DMPC was in the liquid-crystalline state. The transition of the core at ~44 °C had little effect on the extent of surface hydrolysis. Interestingly, no lag phase was observed at any temperature for these particles. Since no albumin was present in the incubation mixture, lysophosphatidylcholine and fatty acid generated by the action of the enzyme probably remained at the particle surface (Wilschut et al., 1979a,b). Other authors have suggested that accumulation of hydrolysis products in the vesicle bilayer formed further defects which accelerated enzyme insertion and hydrolysis (Op den Kamp et al., 1974). This did not appear to be the case with the apoE–microemulsion complex, however, since hydrolysis did not accelerate over the 10-min incubation. This may reflect closer surface packing in the monolayer and/or filling of defects by cholesteryl ester.

In general, these studies suggest that the accessibility of phospholipid in surfaces is influenced by a number of factors which include the transition temperature of the phospholipid domain, surface curvature, the presence of dissolved cholesteryl ester, and protein binding. These findings may have considerable biological significance when related to the plasma lipoproteins. The data suggest that the presence of cholesteryl ester in a lipoprotein surface might alter the accessibility of the surface lipids and that the apoproteins may have a role in increasing the accessibility of the surface to lipolytic enzymes and transfer proteins. Most of the plasma lipolytic enzymes bind and insert into the surfaces of the lipoproteins. Lipoprotein lipase, for instance, acts at the surface of the large triglyceride rich/cholesteryl ester poor VLDL to produce the smaller, cholesteryl ester rich/triglyceride poor LDL particle which does not undergo further catabolism in the plasma compartment. These studies suggest that the presence of protein on the surface and the paucity of cholesteryl ester in the core of VLDL might produce a surface monolayer which is accessible to lipoprotein lipase. In contrast, the VLDL of hypertriglyceridemic patients contains much more cholesteryl ester and is poorly metabolized to LDL, perhaps because the increased cholesteryl ester content reduces the accessibility of

surface-oriented triglyceride to the lipase. The effect of the physical state of core and surface domains of lipoproteins on their intracellular lipolysis is not known. Studies to examine this question using lysosomal enzymes are in progress.

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