Phase composition of lipoprotein SM/cholesterol/PtdCho affects FA specificity of $sPLA_2$s

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Abstract We have previously reported preferential release of polyunsaturated FAs during hydrolysis of lipoprotein phosphatidylcholine (PtdCho) by group X secretory phospholipase A_2 ($sPLA_2$) and preferential release of oligounsaturated FAs during hydrolysis of lipoprotein PtdCho by group V $sPLA_2$, but the mechanism of this selectivity has remained unknown. We now show that the rate and specificity of hydrolysis are affected by relative increases in endogenous SM and free cholesterol (FC) during the lipase digestion. The highest preference for arachidonate release from LDL and HDL by group X $sPLA_2$ was observed for residual SM/PtdCho molar ratio of 1.2 and 0.4, compared with the respective starting ratios of 0.4 and 0.2, as measured by liquid chromatography/electrospray ionization-mass spectrometry. Group V $sPLA_2$ showed preferential release of linoleate from LDL and HDL at SM/PtdCho ratio 1.5 and 0.6, respectively. We have attributed the change in FA specificity to segregation of molecular species of PtdCho and of $sPLA_2$s between disordered and ordered SM/FC/PtdCho lipid phases. The increases in SM and FC during digestion with group IIA $sPLA_2$ were more limited, and a preferential hydrolysis of any FAs was not observed. The significance of SM and FC SM and FC accumulation during $sPLA_2$ hydrolysis of lipoprotein PtdCho has been previously overlooked.—Kuksis, A. and W. Pruzanski. **Phase composition of lipoprotein SM/cholesterol/PtdCho affects FA specificity of $sPLA_2$s. *J. Lipid Res.* 2008.** 49: **2161–2168.**

Supplementary key words secretory phospholipase A_2 • phosphatidylcholine • sphingomyelin • phase diagrams • liquid chromatography-mass spectrometry

A preferential release of linoleate during hydrolysis of phosphatidylcholine (PtdCho) by group V secretory phospholipase A_2 ($sPLA_2$) was first reported in liposomal (1–3) and later in lipoprotein (4–6) incubations, but the mechanism of this selectivity has remained unknown. Early work (1, 2) attributed the selective enzyme activity to a difference in the physical state of substrate between the sonicate and the natural membranes that may contain a more optimum mixture of phospholipids. Alternatively, a preferential release of arachidonate by group V $sPLA_2$ exogenously added to cell cultures has been attributed to its combined action with cytosolic PLA_2 (7). Subsequent work with liposomes suggested a special role for ceramides (3) in regulation of group V $sPLA_2$ hydrolysis of plasma PtdCho, which, however, was difficult to reconcile with the apparent absence of sphingomyelinase activity from plasma lipoproteins (6, 8, 9). Likewise, the apparent preferential release of PtdCho arachidonate by group X $sPLA_2$ during liposomal (10, 11), lipoprotein (4–6), and cellular (10, 12) incubations has remained unexplained. In contrast, group IIA $sPLA_2$ apparently attacked liposomal and lipoprotein PtdCho without FA discrimination, although at a significantly lower rate than group V and group X $sPLA_2$s (3, 5, 9, 10, 12). Therefore, the true differences in the activity and apparent FA specificity of PtdCho hydrolysis by the $sPLA_2$s have remained obscure.

In other studies, a preferential release of arachidonate by group IIA $sPLA_2$ from phosphatidylethanolamine/phosphatidylserine (PtdEtn/PtdSer) micelles was observed (13, 14) in the presence of added SM/ceramide and was attributed to phase separation and exclusion of polyunsaturates from the ordered liquid phase formed under these conditions. The inhibitory effect of added SM upon enzyme activity was relieved by the addition of free cholesterol (FC) (14). Similarly, addition of SM/ceramide to PtdCho liposomes (3, 15) showed a clear preference for release of arachidonate by group X $sPLA_2$, although at reduced activity, whereas the addition of SM to the group V $sPLA_2$ digestion mixture inhibited the release of arachidonate (16), resulting in greater release of linoleate.

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Abbreviations: ESI, electrospray ionization; FC, free cholesterol; GroPCho, glycerophosphocholine; LC, liquid chromatography; MS, mass spectrometry; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; $sPLA_2$, secretory phospholipase A_2.

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Our study shows that the accumulation of endogenous SM and FC relative to PtdCho during $sPLA_2$ hydrolysis of lipoproteins affects the reaction rate similarly to SM added to microemulsions. It is postulated that the differential release of arachidonate and linoleate with progressive hydrolysis is related to a redistribution of PtdCho species and the $sPLA_2$s between the ordered and disordered lipid subphases. A lack of FA discrimination by group IIA $sPLA_2$ under present conditions was attributed to an inadequate formation of the ordered lipid phase due to limited hydrolysis of PtdCho.

MATERIALS AND METHODS

Materials

Human group IIA, V, and X $sPLA_2$s and plasma HDL and LDL were obtained as previously reported (6).

Enzyme assays

Enzyme digestions were performed a minimum of three times and were done in duplicate and triplicate, as described for acute-phase and control HDL, and LDL in the presence of 0.01% BSA, which was calculated to provide an excess of binding capacity for the liberated FAs and lysoPtdCho (7). Repeat solvent extractions were made with $CHCl_3$-MeOH (2:1, v/v) (6).

Liquid chromatography/electrospray ionization-mass spectrometry

Routine positive-ion liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) was performed as previously described (6, 17). Normal-phase HPLC separations of the total lipid extracts were performed using Spherisorb 3 μ columns (100 mm × 4.6 mm ID; Alltech Associates, Deerfield, IL) installed in a Hewlett-Packard Model 1060 liquid chromatograph connected to a Hewlett-Packard Model 5988B quadrupole mass spectrometer equipped with a nebulizer-assisted ESI interface (HP 59987A). The column was eluted with a linear gradient of 100% A ($CHCl_3$-MeOH-30% NH_4OH, 80:19.5:0.5 by vol) to 100% B ($CHCl_3$-MeOH-H_2O-30% NH_4OH, 60:34:5.5:0.5 by vol) for 14 min, followed by 100% B for 10 min at a flow rate of 1 ml/min. The capillary exit (Cap Ex) voltage was set at 150 V, with the electron multiplier at 1,795 V. Positive ESI spectra were obtained in the mass range 350–1,100. For fragmentation studies, Cap Ex voltage was raised to 300 V and the mass range lowered to 200–1,000. N_2 gas was used as both nebulizing gas (40 psi) and drying gas (60 psi, 270°C). Selected ion mass chromatograms were retrieved from the total ion spectra by computer. The molecular species of the PtdChos were identified on the basis of the mass provided by ESI-MS and the relative retention time (longer-chain species migrated ahead of the shorter-chain species) of the glycerophospholipids and SMs. Molecular species of PtdCho were quantified using endogenous d18:1/16:0 phosphocholine (*m/z* 703) as internal standard, whereas molecular species of SM were quantified using 15:0/15:0 glycerophosphocholine (GroPCho) (*m/z* 706) as internal standard at 50 nmol/mg of protein (8). The data were not corrected for differences in the ESI-MS response between polyunsaturated and oligounsaturated PtdChos. FC was determined by gas-liquid chromatography as the trimethylsilyl ether (6). The overall course of analysis is outlined in **Scheme 1**.

Phase diagrams

Tertiary phase diagrams of SM/FC/PtdCho were drawn and interpreted by reference to a standard text (18) and recent work (19).

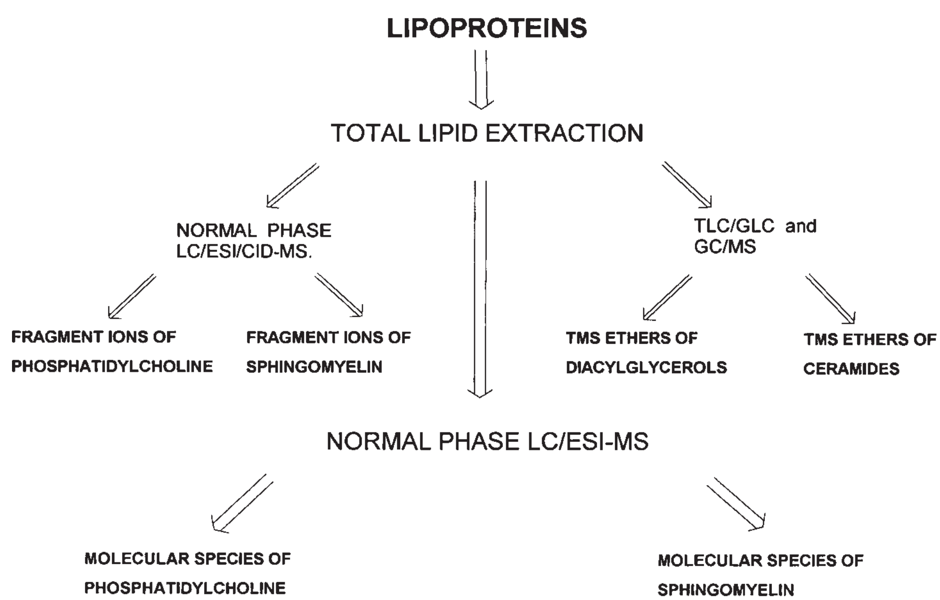
Statistics

Figures 3 and 4 represent the means ± SD of six replicate hydrolyses of LDL. The levels of PtdCho species in native and enzyme-digested lipoproteins were compared by a *t*-test (assuming unequal variances) using Microsoft Excel data analysis tools.

RESULTS

Evidence for inhibition of $sPLA_2$s

We investigated the $sPLA_2$ activity over a wide range of enzyme concentrations [0.025, 0.05, 0.1, 1.0, and 2.5 μg/mg



Scheme 1. Overall course of analysis of lipoprotein lipids.

of lipoprotein (1 mg/ml)] and different incubation times (15, 30, 60, 240, 480, and 1,440 min) and showed that under these conditions, a complete hydrolysis of lipoprotein PtdCho is not achieved with group IIA, group V, or group X $sPLA_2$. Figures 1 and 2 demonstrate that the leveling off in enzymatic activity and increase in FA specificity is related to a relative accumulation of SM (increase in molar SM/PtdCho ratio) in the digestion medium. **Figure 1** shows the time course of differential disappearance of 16:0/18:2 and 16:0/20:4 GroPCho during hydrolysis of HDL_3 with group X (Fig. 1A) and group V (Fig. 1B) $sPLA_2$s over a period of 8 h at 1 μg/ml enzyme. The leveling off in the enzyme activity begins after destruction of 40–60% of the PtdCho, when the molar SM/PtdCho ratio has reached about 0.6. At this time (about 2 h), the hydrolysis of 16:0/18:2 GroPCho proceeds at much higher rate than the hydrolysis of 16:0/20:4 GroPCho, with destruction of both species leveling off further after about 4 h. **Figure 2** shows the time course of differential disappearance of 16:0/18:2 and 16:0/20:4 GroPCho during hydrolysis of LDL with

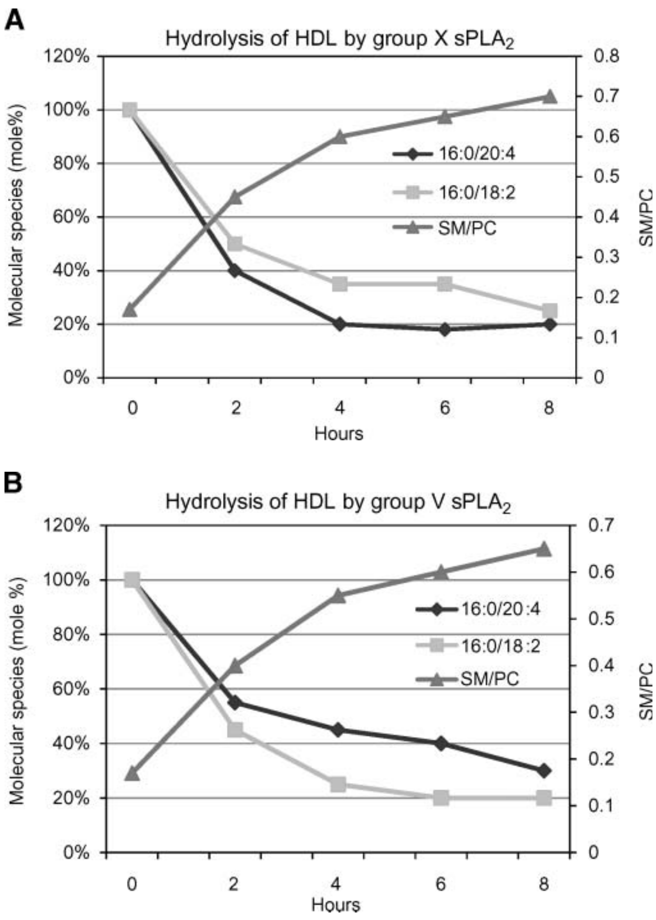


Fig. 1. Time course of differential disappearance of 16:0/18:2 and 16:0/20:4 glycerophosphocholine (GroPCho) in relation to SM/phosphatidylcholine (PtdCho) ratio during hydrolysis of HDL_3 by group X (A) and group V (B) secretory phospholipase A_2s ($sPLA_2$s) over a period of 1–8 h at 1 μg/ml of enzyme. Changes in glycerophospholipid concentration were estimated by normal-phase liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) using d18:1/16:0 (*m/z* 703) as internal standard.

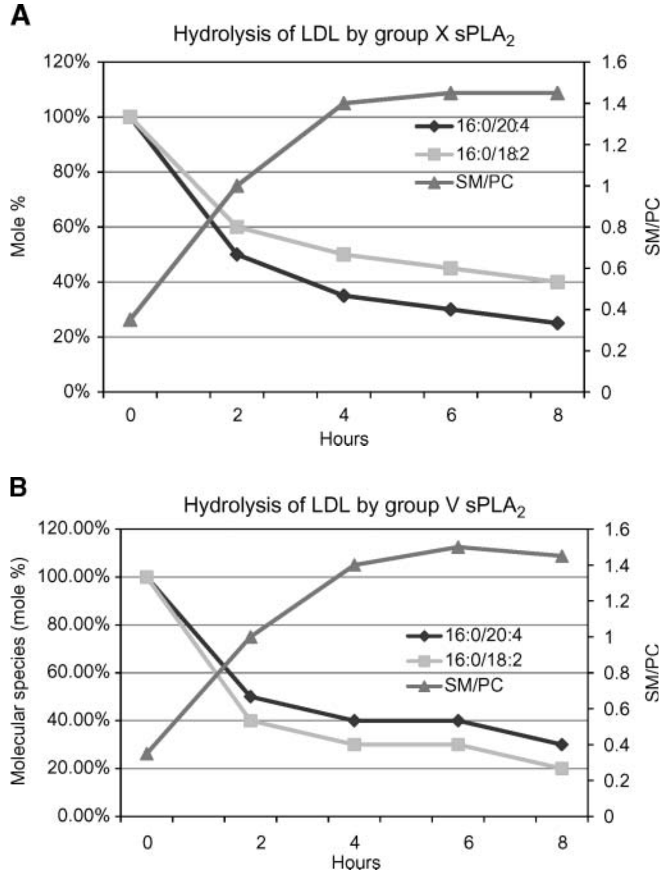


Fig. 2. Time course of differential disappearance of 16:0/18:2 and 16:0/20:4 GroPCho in relation to SM/PtdCho ratio during hydrolysis of LDL by group X (A) and group V (B) $sPLA_2$s over a period of 1–8 h at 1 μg/ml of enzyme. Changes in glycerophospholipid concentration were estimated by normal-phase LC/ESI-MS using d18:1/16:0 (*m/z* 703) as internal standard.

group X (Fig. 2A) and group V (Fig. 2B) $sPLA_2$s over a period of 8 h at 1 μg/ml of the enzyme. Noticeable leveling off in the enzyme activity begins after hydrolysis of 30–40% of the PtdCho, when the molar SM/PtdCho ratio has reached about 0.6. Previously, a leveling off in hydrolysis of PtdCho had been observed for group IIA $sPLA_2$ at about 20% hydrolysis of normal and acute-phase HDL (8). In LDL, the activity of group IIA $sPLA_2$ leveled off after about 30% of the PtdCho had been hydrolyzed and the SM/PtdCho molar ratio had reached about 0.6.

Apparent absence of enzyme specificity during initial digestion

There was little evidence of a preferential attack on arachidonate or linoleate species during the initial stages (0.5 h to 1 h) of hydrolysis of PtdCho LDL or HDL by group X and group V $sPLA_2$s, respectively. **Figure 3** shows only slight change in the molecular species of residual PtdCho following 1 h of incubation of LDL with group V and group X $sPLA_2$s (1 μg/mg protein, 20–30% hydrolysis) when expressed in mol% of total. Because the values for the 18:0/18:2 and 18:0/20:4 as well as the 16:0/18:1 and 16:0/22:5 species show small differences between

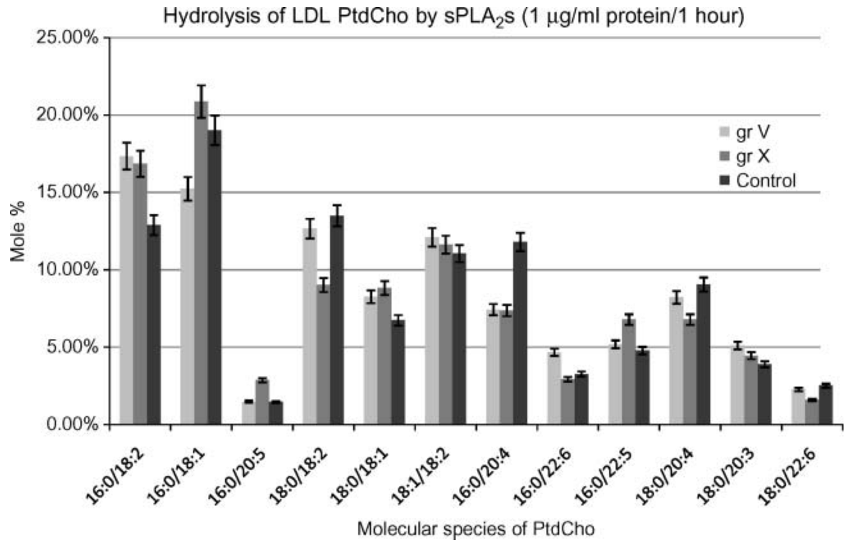


Fig. 3. Absence of significant changes in molecular species of residual PtdCho following a 60 min incubation of HDL_3 (1 mg/ml) with 0.1 to 2.5 μg/ml protein (less than 30% hydrolysis). The values are means ± SD of six replicate hydrolyses. Analytical conditions were as indicated in the legend to Fig. 1.

group V and group X enzymes, the possibility of marginal intrinsic FA specificity cannot be completely excluded. In both instances, the composition of the residual PtdCho was closely similar to that of the 0 h control. Likewise, there was no evidence of a preferential hydrolysis of any PtdCho species by group IIA $sPLA_2$ under these conditions. The starting PtdChos employed in this study appear rich in monounsaturates and low in diunsaturates, when compared with our previous reports (8, 17) as well as to literature data (20). High dietary oleate, which is well known to increase oleate and decrease linoleate in plasma PtdCho, as well as an uncorrected instrument response may have contributed to the observed 18:1/18:2 ratios. Koivusalo et al. (21) have reported that the instrument response varies with sample load, and that at 10 pmol/μl, the polyunsaturated species give 40% higher intensity than the fully saturated ones. The present lipoprotein preparations were the same as those employed in our original study of $sPLA_2$ specificity (6), which had shown starting compositions for HDL and LDL PtdChos comparable to those reported in the present study.

Preferential hydrolysis of polyunsaturated PtdChos

Figure 4 contrasts the composition of the molecular species of residual PtdChos following 4 h hydrolysis of LDL by group IIA, V, and X $sPLA_2$s at 2.5 μg/mg protein. The differences in composition of molecular species of residual and control PtdCho are obvious and are not restricted to 16:0/18:2- and 16:0/20:4-containing PtdCho, as already pointed out (4, 6). Although group X $sPLA_2$ attacked preferentially the 20:4-containing PtdCho species, group V $sPLA_2$ favored the 18:2- and 18:1-containing PtdCho species. Despite a 30% hydrolysis of LDL PtdCho by group IIA $sPLA_2$, there was only a limited change in the proportions of the molecular species, on the basis of which it was concluded that this enzyme does not exhibit FA specificity under these conditions. It is possible that some of the discrepancy in hydrolysis of the molecular species at the later times (beyond 4 h) of hydrolysis was due to a gradual depletion of the preferred substrate.

Absence of hydrolysis of SM

There was no detectable change in the absolute SM content after 4 h of incubation of HDL, HDL_3, and LDL with group IIA, V, and X $sPLA_2$s at 2.5 μg/mg protein. There was also no significant alteration in the composition of molecular species of SM during incubation of plasma LDL and HDL with group V and X $sPLA_2$s at 1 μg/ml protein over a period of 1–24 h (data not shown). The composition of the molecular species of SM from the HDL fractions was similar to that reported earlier (17) for normal and acute-phase HDL, whereas the molecular species of SM of LDL were closely similar to those reported earlier (22) on the basis of GC-MS analyses of the silyl ethers of the corresponding ceramides.

Redistribution of residual lipids

During digestion of lipoprotein PtdCho by $sPLA_2$s, the residual lipids would be expected to undergo a redistribution and a phase separation. FFAs and lysoPtdCho would be removed from the lipid phase by the added bovine albumin, as shown by reisolation of LDL particles after a honey bee PLA_2 digestion (23). As a result of PtdCho hydrolysis, therefore, the lipid-phase monolayer of the lipoprotein would approach a three-component system of progressively increasing immiscibility, which would obey the phase rules of Gibbs (18, 19). **Table 1** gives the lipid-phase composition of HDL and LDL before and after 75% hydrolysis of PtdCho by group X and group V $sPLA_2$s (2.5 μg/ml protein, 4 h). The original total HDL lipid, recalculated following FFA and lysoPtdCho binding to BSA, consisted of about 12 mol% SM, 19 mol% FC, and

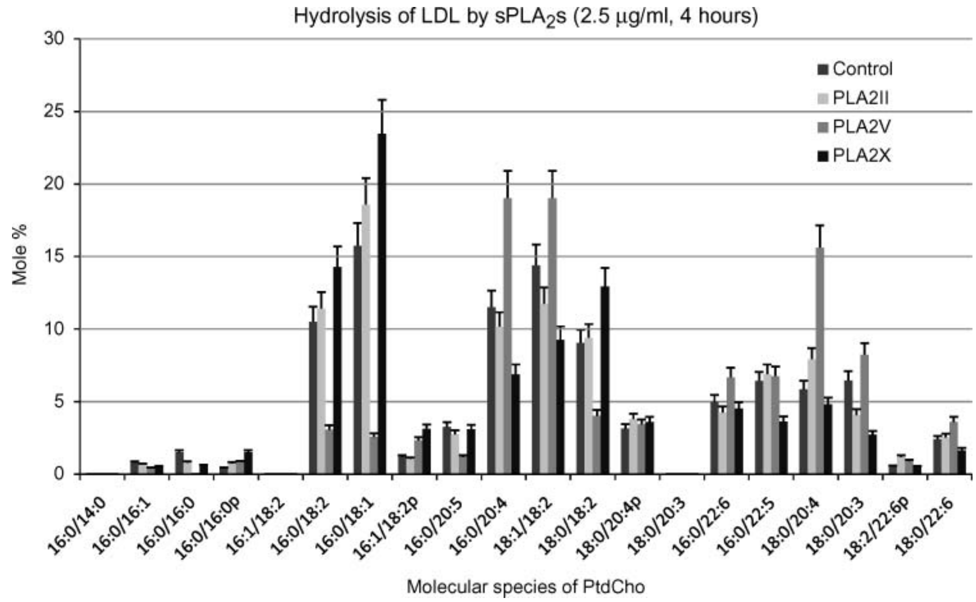


Fig. 4. Maximum changes in molecular species of residual PtdCho following 4 h hydrolysis of LDL (1 mg/ml) by group IIA, V, and X sPLA$_2$s at 2.5 μg/ml protein. The values are means ± SD of six replicate hydrolyses. Analytical conditions were as indicated in the legend to Fig. 1.

69 mol% PtdCho, whereas that of the original LDL consisted of about 15 mol% SM, 44 mol% FC, and 41 mol% PtdCho. The recalculated composition of the residual lipid of HDL following a 75% hydrolysis by group X sPLA$_2$ (2.5 μg/ml protein, 4 h) was 23 mol% SM, 40 mol% FC, and 37 mol% PtdCho. The recalculated composition of residual lipid after 4 h hydrolysis of LDL with group X sPLA$_2$ (2.5 μg/ml protein) was 22 mol% SM, 64 mol% FC, and 15 mol% PtdCho. Thus, the different lipoprotein subclasses possessed different SM/PtdCho starting ratios, which increased with time of digestion, and different FC/SM ratios, which remained constant.

Figure 5 shows a ternary phase diagram of SM/FC/PtdCho along with the demarcation lines obtained for various experimentally determined phase boundaries reported at a temperature of 34°C, as redrawn from Pokorny et al (19). The synthetic PtdCho and the pig brain SM employed previously (19) to construct the ternary phase diagram provide a good representation of the residual PtdCho and SM of plasma lipoproteins. An important property of the triangular diagram is the significance of a straight line joining an apex to a point on the opposite edge. Any point along such a line represents a composition that becomes progressively richer or poorer in the apex component, respectively, as it approaches or moves away from the apex, but the ratio of the components making up the binary mixture on the opposite edge remains the same. Therefore, to represent the changing composition

TABLE 1. Lipid phase composition of HDL and LDL before and after 75% hydrolysis of PtdCho with group X and V sPLA$_2$s (2.5 μg/mg protein, 4 h incubation)

	HDL			LDL	
	Control	75% lysis		Control	75% lysis
FC	19.3 ± 1	40.3 ± 2	FC	43.7 ± 2	63.5 ± 3
SM	11.8 ± 1	23.2 ± 1	SM	14.6 ± 1	22.2 ± 1
PtdCho	68.8 ± 3	37.4 ± 2	PtdCho	41.6 ± 2	15.1 ± 1

PtdCho, phosphatidylcholine. Results are given as mean ± SD (n = 6), estimated on the basis of a complete binding of any FFAs and lysoPtdCho by the fat-free 0.01% BSA added to the incubation medium. Lipid analyses were performed as described in Materials and Methods.

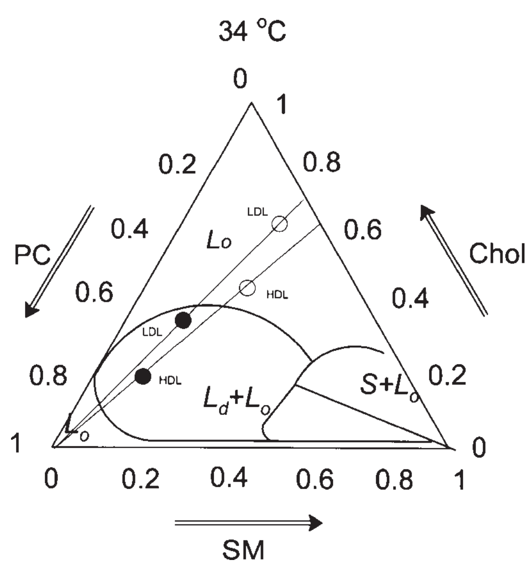


Fig. 5. Ternary phase diagram of SM/free cholesterol (FC)/PtdCho along with the demarcation lines obtained for various experimentally determined phase boundaries at 34°C [redrawn with modification from Fig. 10 of (19)]. The original SM/FC/PtdCho proportions of LDL and HDL confine them to the *Ld+Lo* phase (closed circles), whereas the respective residual proportions (Table 1) relocate them to the *Lo* phase (open circles).

of the system as PtdCho is depleted, it is only necessary to draw the line from the apex PtdCho to the point representing the initial FC/SM ratios of the binary system (1.6 and 3.0 for HDL and LDL, respectively). Any ternary system formed by depleting PtdCho lies at some point on the separate lines for LDL and HDL. As shown in Fig. 5, the SM/FC/PtdCho composition of native LDL and HDL places them within the liquid-ordered plus liquid-disordered (L_d+L_o) region, whereas that of the corresponding residual lipoproteins locates them in the liquid-ordered (l_o) phase. Such a segregation of the native and residual lipoprotein lipids would be expected to be accompanied by a preferential association of the oligoenoic species of PtdCho with the L_o and the polyunsaturated species with the L_d+L_o phase, in keeping with suggestions of previous workers (1, 13, 14, 24, 25). A hypothetical basis for the resulting $sPLA_2$ FA specificity is discussed below.

DISCUSSION

Inhibition of $sPLA_2$ activity

Direct digestion of synthetic PtdCho by $sPLA_2$s is inhibited by the released FFAs and lysoPtdCho. This effect is not observed during digestion in the presence of albumin or apolipoproteins, where the proteins bind the FFAs and lysoPtdCho (26). The FFAs bind to BSA in a molar ratio of 3:1 via the high-affinity FA binding sites (26), whereas lysoPtdCho is bound in the molar ratio of 5:1 via the bilirubin binding sites (27). In our studies, a gradual inhibition of the activity of all three $sPLA_2$s took place with relative accumulation of endogenous SM and FC in relation to the hydrolyzed PtdCho, which was particularly noticeable with group V and group X $sPLA_2$, in agreement with earlier work (4, 6, 8). Previously, inhibition of group V $sPLA_2$ had been observed as a result of exogenous addition of SM to the liposomal substrate of the enzyme (3). Later, it was pointed out that the inhibition is specifically directed against 16:0/20:4 GroPCho, with the 18:1 and 18:2 GroPCho being the major species hydrolyzed (16). The amount of SM needed to initiate the inhibition (16) was closely similar to that resulting from the relative increase in endogenous SM due to a 4 h hydrolysis of HDL PtdCho by group V $sPLA_2$, i.e., 16% versus 20%, respectively.

Likewise, there was evidence for leveling off in the rate of hydrolysis of PtdCho by group X $sPLA_2$ (6) at about 75% of total hydrolysis, at which time the relative increase in the proportion of the endogenous SM had reached about 25%. This coincides with the greatest inhibition of PtdCho hydrolysis by group X $sPLA_2$ detected following addition of about 30% SM to the PtdCho liposomes (16). In earlier work (13, 14) with group IIA $sPLA_2$ and cytosolic PLA_2 in PtdCho/PtdEtn/PtdSer micelles, it was found that addition of SM to disperse PtdCho substrate inhibited the hydrolytic activity of both PLA_2s by 20–30%. It was observed (14) that FC relieved the inhibitory effect of SM on group IIA $sPLA_2$, which was thought to be a result of a specific sequestration of the enzyme apart from the glycerophospholipid substrate, or phase-separated domains of SM.

FA specificity

Several laboratories have advanced the idea that the FA specificity of $sPLA_2$s might be a result of a phase separation of the lipids in the digestion medium, but no direct evidence of this has been presented. The influence of the physical state of the medium upon $sPLA_2$ activity was first proposed (1, 2) as an explanation for the observed preferential hydrolysis of 18:2 GroPCho by group V $sPLA_2$. The fact that 20:4 was not a good substrate for group V $sPLA_2$ suggested that the 20:4 GroPCho species became excluded from the enzyme active center. The physico-chemical state may also have influenced the outcome of experiments involving additions of SM to incubations of group V and group X $sPLA_2$s with PtdCho micelles. Thus, incorporation of SM into PtdCho liposomes was shown (3, 15, 16) to inhibit preferentially the hydrolysis of 16:0/20:4 GroPCho by group V $sPLA_2$, allowing the 18:2 hydrolysis to proceed. Hydrolysis of liposomal SM by sphingomyelinase C resulted in group X $sPLA_2$ activation and loss of its arachidonate preference.

In the present study, we have determined the changes in the proportion of residual PtdCho to SM and FC in the reaction medium during incubation of plasma lipoproteins with $sPLA_2$s. We used the ternary phase diagram of brain of SM/FC/PtdCho reported by Pokorny et al. (19) to specify the location of the native and hydrolyzed lipoprotein lipid mixtures among the *Lo* and *Ld*+*Lo* subphases. Pokorny et al. (19) had established the subphase domains based on the results obtained with carboxyfluorescein efflux kinetics induced by an amphipathic peptide (δ-lysin) and by differential scanning calorimetry. The diagram is qualitatively similar to those proposed for other ternary mixtures containing FC, SM, and PtdCho (28–30). The PtdCho/SM/FC ratios of native HDL and LDL would place them in the L_d+L_o region, which is also compatible with the location of polypeptides, such as δ-lysin (19). Thus, it may be assumed that initially, the $sPLA_2$s would be located, along with the HDL and LDL lipids, in the L_d+L_o region. With increased hydrolysis of PtdCho, progressively more of the L_o phase would be formed and the molecular species of the residual PtdCho would be redistributed, with the more-saturated oligoenoic species being transferred to the L_o phase, whereas the polyunsaturated species would be retained in the *Ld*+*Lo* lipid phase. It has been suggested (31) that the organization of the l_o and L_d+L_o phases would be highly dynamic and the segregated L_o domains should be at rapid equilibrium with monomeric SM and FC dissolved in the bulk PtdCho, with rapid exchange between the two phases. Thus, Group X $sPLA_2$, due to its association with the L_d+L_o lipid phase, would release more polyunsaturated than oligounsaturated PtdCho because of the exclusion of the former species from the L_o lipid phase.

To account for the greater hydrolysis of the oligoenoic species of PtdCho by group V $sPLA_2$, it is proposed that this enzyme is also transferred to the L_o phase, or at least that it works preferentially at the interface between the L_o and L_o+L_d lipid phases, where it would hydrolyze disproportionately higher amounts of the oligoenoic species. A preferential binding to the L_d+Lo phase appears to be a general feature of amphipathic peptides (19, 32, 33), but there have been no studies on the relative distribution of the sPLA$_2$s among the different regions of the ternary phase diagram of SM/FC/PtdCho. There is evidence, however, for a greater binding of group V than group X sPLA$_2$ to cell membranes, which may also apply to liposomes, as discussed below.

Role of membrane and enzyme structure

It has been pointed out that the characteristic differences between group IIA and group V sPLA$_2$ in hydrolyzing lipoprotein phospholipids appear to be due to the presence of tryptophan residues, especially Trp31, and a glycine residue, Gly53, in the interfacial binding region of group V sPLA$_2$, which are absent in group IIA sPLA$_2$ (34, 35). Such structural dissimilarity increases the ability of group V sPLA$_2$ to penetrate PtdCho monolayers, and explains why human group V sPLA$_2$, but not group IIA sPLA$_2$, can hydrolyze this major phospholipid in outer plasma membranes in mammalian cells and lipoproteins (3, 36, 37). Studies of tryptophan analogs have shown that a balance between overall hydrophobicity and unfavorable entropy for membrane insertion causes these residues to insert only to the glycerol region of the bilayer (38, 39), as opposed to the interpolation between the hydrocarbon chains of the lipids. Studies on human group IIA sPLA$_2$ in which one or more surface residues were mutated to tryptophan, have generated variants that exhibit activity similar to human group V sPLA$_2$ on dioleoyl GroPCho membranes (40). In contrast to group V sPLA$_2$, human group X sPLA$_2$, which is most active on zwitterionic surfaces, has no interfacial tryptophan residues (41), although it has the same number of interfacial aromatic residues as human group IIA sPLA$_2$. Interestingly, a report in which an expanded definition of the interfacial binding surface was utilized demonstrated that a mutation of tryptophan 67 to alanine in human group X sPLA$_2$ altered binding to dioleoyl GroPSer/dioleoylGroPCho vesicles (11).

Role of cholesterol

The inhibition of group IIA sPLA$_2$ by SM in a readily hydrolysable PtdEtn/PtdSer model mixture is promptly relieved by FC (13, 14). The addition of FC to SM liposomes with a 1:1 stoichiometry relieves completely the inhibition of sPLA$_2$ exerted by SM. In native plasma lipoproteins, the FC in the lipid monolayer is coordinated to the large excess of PtdCho and the minor proportion of SM. The earlier-held belief of a specific interaction between FC and SM has been recently challenged, and evidence has been presented for a lack of any specific interaction between FC and SM, although a hydrophobic matching condition may be responsible for the colocalization of SM and FC in biomembranes (42). During the digestion of plasma PtdCho by group V and group X sPLA$_2$s, the enzymatic activity levels off as the relative proportion of SM increases to 20–30% of the residual lipid. At this time, the mol% of FC in HDL exceeds that of SM 2-fold, but in LDL 3-fold, clearly sufficient to relieve completely any inhibition of group IIA sPLA$_2$ by SM (see above). The proportion of the residual PtdCho in the HDL was more than double that of residual PtdCho in LDL, at 75% overall lysis. The fact that significant inhibition of group V and group X sPLA$_2$s remained must reflect differences in the phase separation of the residual lipids in the presence of apoproteins and in the interaction of the sPLA$_2$s with the SM and FC under these conditions. It would, therefore, be of interest to determine the sPLA$_2$ specificity in other instances (20, 22) in which SM/FC/PtdCho ratios are characterized, respectively, by excessive presence of SM (Niemann-Pick disease) and SM + FC (hypercholesterolemia and atherosclerosis).

Role of apoprotein and neutral ester composition

The present experiments were not controlled for differences in apoprotein composition or alterations in exchangeable apoproteins. Likewise, the effect on enzyme activity and phase separation was not assessed for the cholesteryl ester, triacylglycerol, and α-tocopherol (a total of 1%) that may have been present in the surface monolayer during the sPLA$_2$ hydrolysis of PtdCho. It was assumed that the lipoprotein particles remained intact and that the phase changes took place in the surface lipid monolayers and were not influenced by the presence of minor amounts of neutral lipid esters or tocopherol.

CONCLUSION

The present study demonstrates that the endogenous enrichment of plasma lipoproteins with SM influences the activity of group V and group X sPLA$_2$s, which is comparable to that of exogenously added SM. Our findings obtained during HDL and LDL hydrolysis provide quantitative results, which can be used for construction of ternary phase diagrams and a physico-chemical interpretation of the results. The present results anticipate the possibility that similar physico-chemical effects may influence the activity and FA specificity of the sPLA$_2$s in cell membranes and tissues, but a complete absence of any intrinsic enzymatic specificity was not excluded.

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