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ARTICLE *in* BIOCHEMISTRY · JUNE 1991

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Bilayer Packing Stress and Defects in Mixed Dilinoleoylphosphatidylethanolamine and Palmitoyloleoylphosphatidylcholine and Their Susceptibility to Phospholipase A_2[†]

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Received May 25, 1990; Revised Manuscript Received January 3, 1991

ABSTRACT: The hydrolysis of mixed dilinoleoylphosphatidylethanolamine (DiLinPE) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) dispersions by porcine phospholipase A_2, under conditions leading to the bilayer-to-nonbilayer phase transition, has been studied. Two structurally distinct forms of the dispersions were used, multilamellar vesicles (MLV) and supercritical large unilamellar vesicles (SCLUV). In MLV, maximum free fatty acid was produced in dispersions containing 85 mol % DiLinPE. The peak in the fatty acid release is found at the onset of appearance of the nonbilayer defects reported earlier. DiLinPE was found to be preferentially hydrolyzed as compared to POPC. When cholesterol was added to the mixed DiLinPE/POPC MLV, the onset of the observable appearance of nonbilayer defects, the positions of the peaks for total hydrolysis, and the preferential hydrolysis of DiLinPE were all shifted toward lower DiLinPE concentrations. In SCLUV, where the appearance of nonbilayer structures is prevented by constraining the lipids in bilayer configuration, the hydrolysis by PLA_2 increases with increasing DiLinPE as predicted from the increase in the calculated monolayer bending energy. The results are interpreted to be related to the pretransition molecular-packing stress and defects at the onset of the bilayer-to-nonbilayer transition. Results indicate that the porcine pancreatic phospholipase A_2 activity is controlled by bilayer-packing stress, which may cause structural defects of the substrate, among other factors. Results also indicate a preferential localization of PE at stress-related defect regions.

Phospholipases play an important role in membrane phospholipid metabolism. In recent years, the regulation of their activities has been the focus of many investigations because of their putative role in signal transduction processes (Berridge & Irvine, 1984). Recently, phospholipase A_2 (PLA_2) has also been suggested to play a pivotal role in the repair of damage by lipid peroxidation (van Kruijk et al., 1987). The study of the regulatory mechanism of this enzyme is thus of much current interest.

The hydrolytic function of PLA_2 is believed to be based on a sequence of events involving binding and activation steps (Waite, 1985). The activation step is very sensitive to the physical state of the substrate. For instance, bilayers are poorer substrates than micelles. For bilayer substrates, the activation requires structural defects or fluctuations that culminate at the gel-to-fluid phase transition (Romers et al.,

[†] This work was supported by USPHS Grant GM-28120 from the National Institutes of Health.

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0006-2960/91/0430-4516$02.50/0

1987; Jain & Vaz, 1987; Noordam et al., 1982; Wilschut et al., 1978). The defects can also be induced in gel-phase bilayers by osmotic shock or by the high curvature in SUV (Menashe et al., 1986; Lichtenberg et al., 1986; Wilschut et al., 1978). Possible defects in bilayers composed of mixed long- and short-chain phospholipids are believed to contribute to the high susceptibility of these vesicles to PLA_2 (Gabriel et al., 1987).

Many biological membranes contain a high percentage of the so-called non-bilayer-preferring phospholipids. These lipids have a tendency to undergo a bilayer-to-inverted-hexagonal/cubic ($L_\alpha \rightarrow H_{II}/Q_{II}$) phase transition at physiological temperatures. The pretransition molecular-packing stress, which leads to bilayer structural defects, reaches a maximum at the onset of this transition (Gruner et al., 1986; Hui & Sen, 1989), and fluctuations culminate at the phase-transition region, as in the case of the gel-to-fluid phase transition. Bilayer defects occurring at the onset of the $L_\alpha \rightarrow H_{II}$ phase transition are more biologically relevant than those present during the $L_\beta \rightarrow L_\alpha$ phase transition, since the majority of biomembranes are in the L_α state at physiological conditions. A correlation between the propensity of the $L_\alpha \rightarrow H_{II}$ phase transition and the activities of some biological membranes has been reported earlier (Hui & Sen, 1989).

The $L_\alpha \rightarrow H_{II}$ transition in a pure lipid such as phosphatidylethanolamine (PE) is generally a cooperative phenomenon that can be detected as an endothermic transition in differential scanning calorimetry (DSC) (Cullis & de Kruijff, 1979). In miscible binary lipid mixtures, the thermotropic transition from bilayer to H_{II} is generally difficult to detect by DSC, because the transition occurs over a wide temperature range. Within this transition range, the structure of the mixed lipids is complex; different types of structural defects can be identified by freeze-fracture electron microscopy (Boni & Hui, 1983; Verkleij, 1984). ^{31}P NMR measurements of lipids within the transition range show the appearance of an isotropic peak superimposed on an anisotropic bilayer-type or hexagonal-type spectrum (Hui et al., 1981a,b). In recent years there has been considerable interest in the bilayer-to-H_{II} transition zone, because it is thought that the presence of these intermediate defect structures in lipid bilayers and membranes affects a wide range of membrane phenomena including membrane fusion, protein insertion, and increased membrane activity (Hui et al., 1981a; Navarro et al., 1984; Hah et al., 1983; Bentz et al., 1985; Ellens et al., 1985, 1986; Cheng et al., 1986; Hui, 1987; Bryszewska & Epand, 1988; Epand & Bottega, 1988).

Cholesterol promotes the formation of H_{II} or Q_{II} phases when mixed with lipids with poor head-group hydration and with unsaturated acyl chains (Dekker et al., 1983; Tilcock et al., 1984; Cheng et al., 1986; Epand & Bottega, 1987). A mixed dispersion of DiLinPE/POPC (80:20 molar ratio) at 35 °C, though predominantly bilayer, is on the verge of the $L_\alpha \rightarrow H_{II}$ transition. The presence of 5% cholesterol is sufficient to trigger the formation of H_{II} structures, and the transition is complete at 15% cholesterol (unpublished data).

In this study, we aim to isolate the contribution of nonbilayer defects and/or the pretransition packing stress in the lipid substrates on the activation of PLA_2. We varied three pure lipid components, namely DiLinPE, POPC, and cholesterol, to shift the $L_\alpha \rightarrow H_{II}$ transition ranges of the mixtures with respect to the experimental temperature of 35 °C. Since the components remain the same, their variable ratio represents the temperature proximity to the phase transition. We used porcine pancreatic PLA_2, which is known to have preferential activity at defect regions, as a probe for the presence of lipid bilayer defect structures and/or pretransition packing stress, in mixed systems of PE, PC, and cholesterol.

Materials and Methods

Lipids. Dilinoleoylphosphatidylethanolamine (DiLinPE) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) were purchased from Avanti Polar Lipids (Birmingham, AL). The purity of all lipids used was checked by TLC and the actual concentration in chloroform solution determined from lipid phosphorus determination. The lipids, in chloroform, were mixed in predetermined molar ratios and stored at −80 °C prior to use. Cholesterol was purchased from Supelco (Bellefonte, PA).

Phospholipase A_2. Porcine pancreatic phospholipase A_2 was purchased from Boehringer Mannheim (Indianapolis, IN) and stored at 4 °C.

Preparation of Multilamellar Lipid Dispersions. A total of 5 μmol of the lipid mixture, in chloroform, was evaporated to dryness under a stream of dry nitrogen. The dried lipids were then placed under vacuum in a desiccator for at least 1 h to remove any remaining solvents. The dry lipids were then dispersed in unbuffered 0.1 M KCl and 1 mM $CaCl_2$ (pH 8.0) at 0 °C. The lipids were then dispersed by vortexing. The unbuffered solution of KCl and $CaCl_2$ was used so as to enable us to measure free fatty acid production by PLA_2 (see below). PLA_2 is optimally active at pH 8.0 in the presence of Ca^{2+}.

Preparation of Supercritical Large Unilamellar Lipid Dispersions. One possible source of artifacts measuring PLA_2 activity in MLV is the surface accessibility. In order to avoid this possible artifact, we developed a method of preparing and maintaining large unilamellar vesicle dispersions of lipids containing high percentages of DiLinPE. Dried lipid mixtures were dispersed in 0.1 M KCl and 1 mM $CaCl_2$ at pH 10.0. The lipid dispersion was vortexed vigorously and then extruded through 0.4-μm filter (Nuclepore, Pleasanton, CA) under high pressure (200 psi) applied from a cylinder of dry nitrogen. The extruded vesicles were centrifuged at 15 000 rpm in a Sorvall SS34 rotor for 15 min. The pellet was collected and resuspended in 0.1 M KCl and 1 mM $CaCl_2$ at pH 8.0. The final vesicle preparation was analyzed for the PE/PC ratio and lipid phosphorus-to-cholesterol ratio and also examined by negative-stain and freeze-fracture electron microscopy. These vesicle are labeled "supercritical" as under normal conditions identical lipid dispersions do not form stable unilamellar bilayers but form a wide array of different phases like MLV with lipidic particles, Q_{II} phases, and H_{II}, depending on the ratio of PE to PC. The pH jump, used in this method, traps these vesicles in a unilamellar phase. We must, however, emphasize at this point that there is currently no known method to determine whether or not these vesicles are symmetric with respect to lipid composition. The commonly used methods for labeling the amine group of PE are not useful due to the leakiness of these vesicles. The SCLUV so prepared can be reverted to the MLV and a nonlamellar form by a sudden drop of pH.

Lipid Hydrolysis by Phospholipase A_2. The lipid dispersions were incubated in a water bath at 35 °C for 10 min and then 3 units (3.5 ng of protein) of PLA_2 was added to the lipid dispersion, and the dispersion was incubated for another 15 min at 35 °C. The reaction was stopped by adding 0.5 mL of 0.2 M EDTA.

Extraction and Analysis of Free Fatty Acids. Following hydrolysis by PLA_2 the total lipids were extracted from the dispersion by chloroform/methanol (2:1 v/v). The organic layer was separated and dried over anhydrous sodium sulfate. The free fatty acids produced by the hydrolysis of the lipids

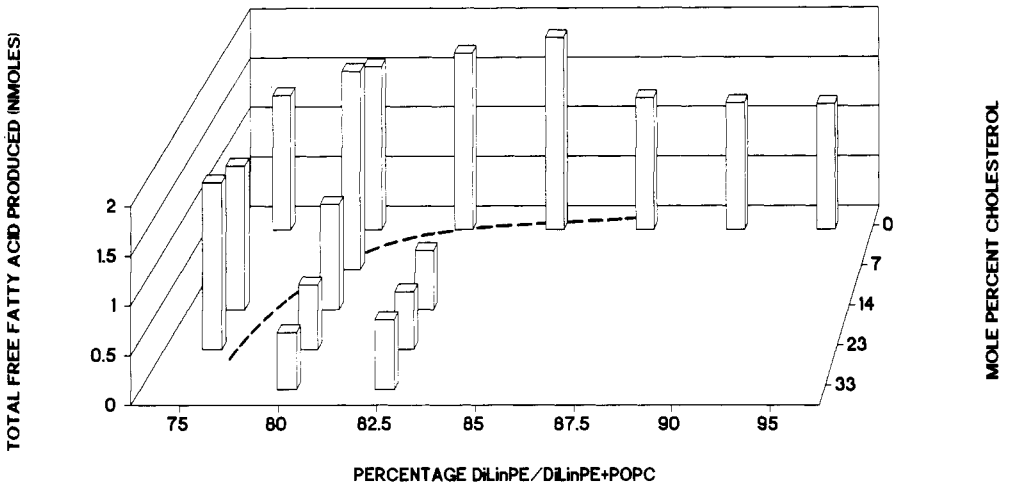

FIGURE 1: 3D plot of the total free fatty acid produced by PLA_2 in MLV dispersions of varying ratios of DiLinPE/POPC and with added cholesterol. The phase boundary between the bilayer and inverted phases is shown by the dashed line.

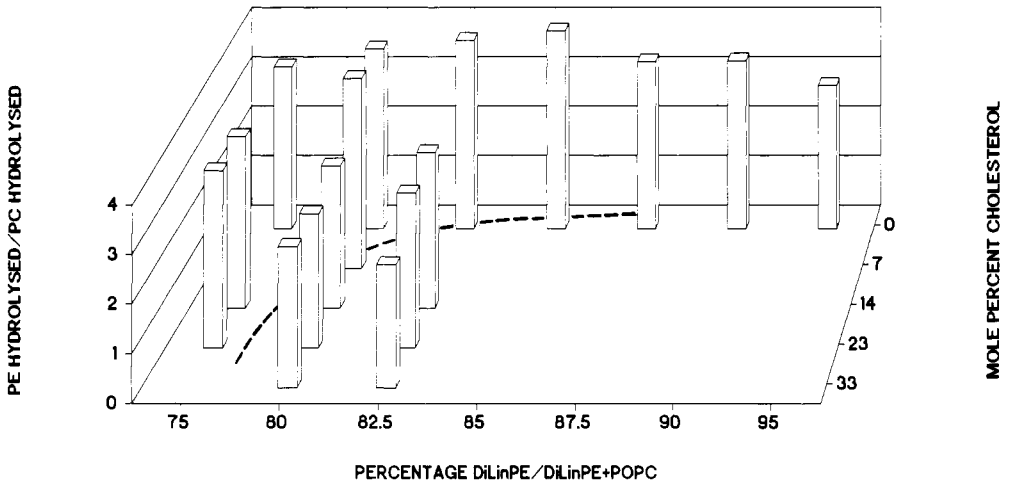

FIGURE 2: Preferential hydrolysis of PE in MLV dispersions of DiLinPE/POPC with and without added cholesterol. The calculation of the preferential hydrolysis of PE is described in the text. The phase boundary is indicated by the dashed line.

by PLA_2 were separated by thin-layer chromatography (TLC) on silica gel. Petroleum ether/diethylether/acetic acid (80:20:1 v/v) was used as the developing solvent. The free fatty acid spot was scraped off the TLC plate, and methyl esters of the fatty acids were prepared by using BF_3 (Sen et al., 1981). The fatty acid methyl esters were extracted with petroleum ether and analyzed on a Perkin-Elmer Model 900 gas chromatograph (GC) with a column of 3% SP2310/2% SP2300 coated on 100/120 Chromsorb W (Supelco). The GC run was under isothermal conditions with the oven temperature at 195 °C. An internal standard (pentadecanoic acid) was added to the lipid dispersions before the lipids were extracted following hydrolysis. The fatty acids produced were quantitated from the GC peaks by using the pentadecanoic acid peak as the standard.

The amounts of linoleic acid (18:2) and oleic acid (18:1) produced are respectively equivalent to the amounts of DiLinPE and POPC hydrolyzed by PLA_2. The ratio of 18:2 produced to 18:1 produced divided by ratio of DiLinPE to POPC in the initial dispersion gives a measure of the preferential hydrolysis of DiLinPE as compared to POPC.

Kinetics of Lipid Hydrolysis by Phospholipase A_2. The initial rate of lipid hydrolysis by PLA_2 was measured by using a pH meter with a recorder output. (These experiments were performed in a closed nitrogen environment to prevent atmospheric carbon dioxide from interfering with the pH measurements.) The reaction was allowed to proceed for 5 min. The rate of lipid hydrolysis was measured from the slope of the recorder trace after the first 15 s. The first 15 s of the trace, after the addition of PLA_2, were not used due to possible incomplete-mixing artifacts.

Freeze-Fracture Electron Microscopy: Samples for freeze fracture were sandwiched between thin copper sheets and quick frozen in liquid propane maintained at liquid-nitrogen temperature. Freeze-fracture replicas were prepared with a modified Polaron freeze-fracture instrument and examined in a Hitachi H600 electron microscope. Details of the methods are given in Boni and Hui (1983).

Results

Figure 1 is a 3D bar graph of the total fatty acid produced from lipid hydrolyzed by PLA_2 in an MLV dispersion containing varying ratios of DiLinPE and POPC with and without varying amounts of cholesterol. The onset of the lamellar-to-inverted-hexagonal phase transitions of these mixtures have been determined previously (Boni & Hui, 1983; Cheng et al., 1985, 1986; and unpublished results). The onset boundary at pH 8.0 is indicated by the dashed lines in Figures 1 and 2. In the absence of cholesterol, the total fatty acid produced has a maximum at 85% DiLinPE. When cholesterol was added to the 75:25 (DiLinPE/POPC) mixture, the total fatty acid produced increased with an increase in added cholesterol. When cholesterol was added to the mixture of 80:20 DiLinPE/POPC, there was initially, at 7% cholesterol, an in-

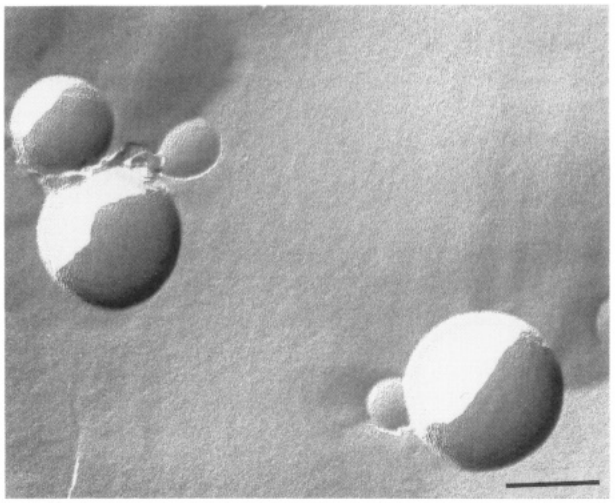

FIGURE 3: Freeze-fracture electron micrograph of DiLinPE/POPC (90:10 molar ratio) SCLUV dispersion at pH 7.0 and quenched from 20 °C. Scale bar = 200 nm.

crease in the total fatty acid produced, followed by a decrease at higher cholesterol content. If cholesterol was added to the 82.5:17.5 (DiLinPE/POPC) mixture, then the addition of cholesterol resulted in a decrease in lipid hydrolysis.

DiLinPE showed a higher susceptibility to PLA_2 as compared to POPC. The preferential hydrolysis of DiLinPE by PLA_2 also showed a dependence on the lipid ratio. In Figure 2, the ratio of hydrolyzed DiLinPE to hydrolyzed POPC (normalized to the initial ratio of DiLinPE to POPC in the dispersion) is plotted against the DiLinPE/POPC ratio in the dispersion with and without added cholesterol. The ratio of hydrolyzed DiLinPE to hydrolyzed POPC shows a peak at 85% DiLinPE, the same ratio as that for the total hydrolysis. With cholesterol added to the 75:25 mixture of DiLinPE/POPC, there is a small increase in the ratio of DiLinPE/POPC hydrolysis with increasing cholesterol. When cholesterol was added to 80:20 mixed DiLinPE/POPC, there was a peak in the ratio of DiLinPE/POPC hydrolyzed, at 7% cholesterol, followed by a decrease in the ratio at higher cholesterol. Cholesterol caused a decrease in the hydrolysis ratio when added to 82.5:17.5 DiLinPE/POPC. The ratio of the hydrolysis of the two lipids, DiLinPE and POPC, thus mirrors the total hydrolysis by PLA_2.

In order to distinguish the molecular-packing effect from morphological effects (surface accessibility) in the phase-transition range, similar experiments were carried out with SCLUV instead of MLV. An electron micrograph of a freeze-fracture replica of a SCLUV preparation of a 90:10 DiLinPE/POPC mixture is shown in Figure 3. Under normal conditions, i.e., in MLV dispersions, this lipid mixture shows lipidic particles and hexagonal structures, whereas in our SCLUV preparations only smooth vesicles were found. The PE:PC ratio and phospholipid-to-cholesterol ratio in these vesicles were found to be identical with the ratio in the original dispersion. The vesicles in the SCLUV preparations of all the lipid mixtures studied were of generally uniform size and were unilamellar.

The kinetics of the hydrolysis of lipids by PLA_2 were determined by measuring the change of the pH (δpH) of unbuffered lipid dispersions. The initial rate of hydrolysis of MLV of DiLinPE/POPC by PLA_2 is shown in Figure 4. The plot is similar to that in Figure 1 in that there is a peak in the activity at around 80% DiLinPE. However, if the hydrolysis is measured in SCLUV, there is no peak; instead, the initial rate of lipid hydrolysis increases linearly with increasing

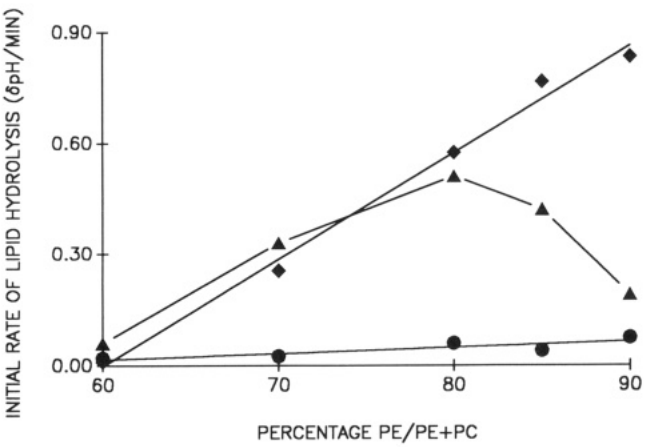

FIGURE 4: Kinetics of lipid hydrolysis by PLA_2 in mixed PE/PC dispersions. (▲) MLV dispersions of DiLinPE/POPC; (◆) SCLUV dispersions of DiLinPE/POPC and (●) SCLUV dispersions of egg PE/POPC.

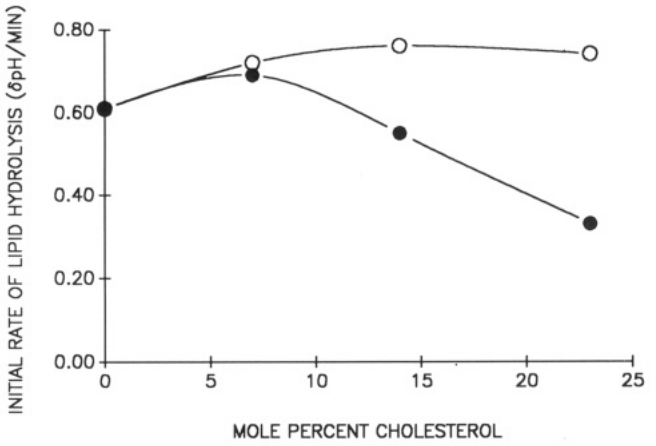

FIGURE 5: Initial rate of lipid hydrolysis in SCLUV (○) and in MLV (●) dispersions of DiLinPE/POPC plotted as a function of cholesterol concentration.

DiLinPE content (Figure 4). The experimentally measured initial rate of lipid hydrolysis thus follows the increase of the bending energy of the system provided that the dispersion is maintained in the bilayer phase. To further test our hypothesis that it is the bending energy rather than the chemical nature of the lipid head group, we prepared extruded LUV of egg PE/POPC (egg PE is a transphosphatidylated product from egg PC) in exactly the same manner as used to prepare SCLUV of DiLinPE/POPC. These vesicles are, however, different from the former in one critical parameter. The $L_\alpha \rightarrow H_{II}$ transition of the egg PE we used in these measurements was at 65 °C, and thus these vesicles are far removed from supercritical state and have much lower bending energy. The plot of the initial rate of hydrolysis in egg PE/POPC LUV is also shown in Figure 4. The hydrolysis rate is very low as expected from the small bending energy of the system.

We next asked the question of whether we can replace DiLinPE with cholesterol in SCLUV and still see the same increase in the hydrolysis by PLA_2. Figure 5 shows a plot of the initial rate of lipid hydrolysis by PLA_2 in DiLinPE/POPC (80:20) containing varying amounts of cholesterol. The plot shows that addition of cholesterol increases the susceptibility of the lipid to PLA_2 to about 14 mol % cholesterol. Above 14 mol % cholesterol the rate of hydrolysis levels off. This leveling off of lipid hydrolysis could be due to the relatively large amount of cholesterol in the bilayer, which prevents the formation of contiguous area of phospholipid molecules as

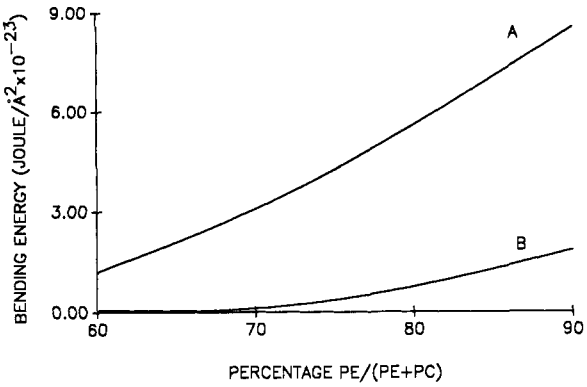

FIGURE 6: Calculated monolayer bending energy for mixed (A) DiLinPE/POPC and (B) egg PE/POPC plotted as a function of PE content. The calculations are according to Hui and Sen (1989).

substrates for PLA_2. Binding of the dimer to the bilayer is a prerequisite for rapid hydrolysis by PLA_2, as suggested by Biltonen and co-workers (Romero et al., 1987; Gheriani-Gruszka et al., 1988; Bell & Biltonen, 1989). When the initial rate of hydrolysis is measured in DiLinPE/POPC MLV containing cholesterol, there is again a peak in the plot at 7 mol % cholesterol. Thus, in MLV systems, measuring the total hydrolysis after 15 min and measuring the rate of hydrolysis by δpH yield similar results.

Discussion

The term "defects" has been used in the literature for a wide variety of structural forms and lateral molecular-packing states found in lipid dispersions (Some workers use the term "structural fluctuations" instead of the term "defects" that we have adopted). "Bilayer defects" can be best defined as those arising from imperfections in molecular packing such as the region between structural domains. An example of this kind of defect is one formed when a lipid bilayer undergoes the $L_\beta \rightarrow L_\alpha$ transition. Structural defects are also expected to exist in a lipid dispersion at the onset of bilayer-to-nonbilayer transition because of the packing stress due to the increasing bending energy. These defects are not readily observable by electron microscopy, but there is indirect evidence that suggests their existence (Bentz et al., 1985; Ellens et al., 1986a,b; Hui, 1987; Hui & Sen, 1989). These defects arise due to the increase in the monolayer spontaneous curvature as a lipid bilayer approaches the H_{II} phase (Gruner et al., 1986). The amount of defects is expected to correlate with the propensity of the lipid to express nonbilayer structures. When high-curvature nonbilayer structures like lipidic particles or H_{II} phase appear, the built-up bending energy is relaxed, and the defects diminish. These kind of pretransition stress-related defects, rather than the high-curvature structures such as lipidic particles, are believed to play a major role in membrane activities.

Mixed dispersions of PE and PC have been examined in the past by freeze-fracture electron microscopy, NMR, and X-ray diffraction (Boni & Hui, 1983). It was found that in dispersions of 10–25% POPC with DiLinPE the $L_\alpha \rightarrow H_{II}$ transition was broad (5–15% POPC at 35 °C) and freeze-fracture replicas prepared from dispersions within this transition range showed the presence of large numbers of nonbilayer structures. The addition of cholesterol promotes the formation of H_{II} even in mixtures containing 20% POPC, which is normally in the bilayer state at 35 °C. The cholesterol-induced phase-transition range covers from 5% to 15% cholesterol for this particular PE/PC mixture (unpublished data). The results from our present study show that the PE/PC ratio that shows peak susceptibility to PLA_2 coincides with the onset and not the center of the transition range (Boni & Hui, 1983), where nonbilayer structures are maximal. The same holds for PE/PC/cholesterol mixtures. It is known that the susceptibility of PC bilayers to PLA_2 exhibits a biphasic response to temperature as the bilayers undergo a $L_\beta \rightarrow L_\alpha$ transition (Noordam et al., 1982), and it was suggested that lipid bilayers are more susceptible to PLA_2 when bilayer defects or structural fluctuations are present (Wilschut et al., 1978; Romero et al., 1987). It is believed that the activation of PLA_2 is hinged on the availability of defects in the molecular packing of the bilayer. The principal driving force for the $L_\alpha \rightarrow H_{II}$ transition is the bending force of each monolayer, in a manner similar to the role of the lateral-expansion force in the $L_\beta \rightarrow L_\alpha$ phase transition. The bending stress is the strongest at the onset of the $L_\alpha \rightarrow H_{II}$ transition (Hui & Sen, 1989), and the (spatial) defects and (temporal) fluctuations are expected to be at the maximum under this condition. This defect-dependent susceptibility of lipid bilayers to PLA_2 is expected to peak at the onset of the $L_\alpha \rightarrow H_{II}$ transition. When nonbilayer structures predominate, the bending stress is relaxed, and the bilayer-packing defects as well as the susceptibility to PLA_2 diminish as expected.

In SCLUV where the appearance of nonbilayer structures is prevented by constraining the lipids in a bilayer structure, the bending energy cannot be relaxed through nonbilayer structures and should result in continuous increase in bending stress with DiLinPE. The calculated monolayer bending energy with increasing PE content does indeed show an almost linear increase in bending energy with DiLinPE, in the range studied (Figure 6). [The calculation is based on the formulation described by Hui and Sen (1989).] Consequently, the susceptibility to PLA_2 should increase with increase in DiLinPE content. It is reassuring to observe that the initial hydrolysis rates in mixed dispersions of DiLinPE/POPC and egg PE/POPC (Figure 4) closely resemble their calculated monolayer bending energy (Figure 6). Since PLA_2 activity also depends on other factors, in addition to the monolayer bending energy, the only correlation expected between the two, is in the general trend rather than the actual numerical values.

In mixtures with defect structures, PE appears to be more susceptible to PLA_2 than PC. This difference in susceptibility could be due to the difference in hydration of PE and PC [PE binds 8 water molecules while PC binds ~30 (Sen & Hui, 1988), as has been suggested by other workers (Jain & Vaz, 1987)]. Since in the presence of defects there is a 4-fold difference in susceptibility to PLA_2 between DiLinPE and POPC at the peak composition, the results can be interpreted in either of two ways: (1) there is more PE at the defect regions or (2) the difference in hydration of the two lipids plays a role only when there are defect structures present. It is not possible, at present, to say definitely which of the two possibilities is the reason for the greater hydrolysis of PE. The first possibility fits the concept that PE clusters cause local concentration of packing stress. However, in existing nonbilayer structures such as lipidic particles, PC and PE are miscible within the NMR time scale (Vasilenko et al., 1982; Boni & Hui, 1983). The substrate susceptibility to PLA_2 is thus determined more by the pretransitional packing stress and related packing defects, as suggested by earlier analysis (Hui & Sen, 1989), rather than the existence of nonbilayer structures.

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A Conformational Switch Is Associated with Receptor Affinity in Peptides Derived from the CD4-Binding Domain of gp120 from HIV I†

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Received September 28, 1990; Revised Manuscript Received February 8, 1991

ABSTRACT: A 15-residue region within the CD4-binding domain of gp120 from HIV I was identified with use of folding algorithms as conserving the potential for forming a particular secondary structure throughout 11 sequenced HIV strains. The region chosen has a potential for forming both β-sheet and α-helix; the helical form would be amphipathic with the five hydrophobic residues all totally or functionally conserved. Five peptides were synthesized corresponding to this region in strain LAV and the strain most highly divergent from it in primary structure (Z3) plus three additional peptides with critical substitutions in the LAV sequence. The conformation of these five peptides was examined under various conditions with circular dichroism, and the results were compared with the ability of each peptide to bind to a CD4-expressing strain of HeLa cells (HeLa T4). In solution, the unmodified peptides exhibit a bistable structure, existing as β-sheet in dilute buffer and converting to α-helix under more apolar conditions. The transition is reversible and sharp, occurring at a particular point in the polar/apolar gradient with virtually no intermediate state. The ability to undergo this bistable flip is closely associated with binding ability, amino acid substitutions that eliminate binding ability also eliminating the switch, and vice versa. The transition thus may reflect conformational changes occurring in this region of gp120 as it binds to the CD4 receptor.

The high degree of variability in the envelope protein gp120 of HIV I is well-known (Hahn et al., 1985; Wong-Staal et al., 1985) and constitutes one of the strongest difficulties facing the development of an effective antiserum. The problem is increased by the fact that the majority of the predicted antigenic epitopes of gp120 coincide with regions of high sequence variability (Modrow et al., 1987). The principal neutralizing determinant has in fact been found to differ by as much as 50% among viral isolates (Myers et al., 1989). An alternative

† This work was supported by a grant from the Bundesgesundheitsamt.
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0006-2960/91/0430-4521$02.50/0