Biochimica et Biophysica Acta, 1022 (1990) 146–154
Elsevier

BBAMEM 74752

Mixed monolayers of natural and polymeric phospholipids: structural characterization by physical and enzymatic methods

David W. Grainger [1], Anke Reichert [1], Helmut Ringsdorf [1], Christian Salesse [1], Diane E. Davies [2] and John B. Lloyd [2]

[1] *Institut für Organische Chemie, Universität Mainz, Mainz (F.R.G.) and* [2] *Department of Biological Sciences, University of Keele, Keele (U.K.)*

(Received 21 September 1989)

Key words: Phospholipase A_2; Monolayer; Isotherm; Isobar; Polymeric phospholipid mixture

This study has focused on physical characterization and enzymatic hydrolysis of mixed monolayers of a natural phospholipid substrate and a polymerizable phospholipid analogue. Such a mixed system presents the possibility to stabilize model biomembranes, vary the molecular environment within the layer through polymerization and simultaneously examine these influences on monolayer structure. Phospholipase A_2 was used here as a sensitive probe of the molecular environment within these mixed, polymerizable monolayers to complement information obtained from isotherm and isobar data. The results clearly show a strong influence of molecular environment on phospholipase A_2 activity, even if differences in the physical state of mixed monolayers are not detectable with isotherm and isobar measurements. Physical characterization indicated that both monomeric and polymeric mixed monolayers were phase-mixed. Enzyme hydrolysis, however, showed large differences in the ability of the enzyme to selectively hydrolyze the natural phosphatidylcholine component from the monomeric as opposed to the polymeric mixtures. This demonstrates a high sensitivity of phospholipase A_2 to distinguish subtle differences in molecular arrangement within mixed monolayers on a molecular level.

Introduction

Biomembrane interfaces are complex mixtures of lipids and proteins that form fluid, yet remarkably stable cellular barriers. Biomembrane models – planar bilayers, vesicles and monolayers – are often used in attempts to mimic, study and understand natural membrane architecture and function [1]. For the sake of simplicity, many studies to date have focused on characterizing pure phospholipid systems [2–4] and simple lipid mixtures [5–7] and therefore, barely approach the complexity in form and function present in natural cell membranes.

The use of phospholipases as probes of membrane structure is well documented [8–14]. Phospholipase A_2, a small, water-soluble enzyme that stereospecifically catalyzes hydrolysis of *sn*-2-acyl ester bonds in glycerophospholipids, has been typically utilized in monolayer and vesicle systems. Its ubiquitous nature and mounting physiological significance has made phospholipase A_2 the focus for research in a number of different fields – to an extent that it is now one of the best studied and characterized lipolytic enzymes (for recent reviews, see Refs. 14–19). Of more notable significance for biomembranes is that phospholipase A_2's hydrolytic action, although slightly active against dispersed monomeric phospholipid substrates, manifests its full activity only against organized interfaces such as monolayers, micelles and bilayers. For this reason, directing the action of the enzyme against model membranes is often the method of choice to probe membrane structure and organization.

Varying lipid components and their organization at interfaces in mixtures can modify the hydrolytic velocity of enzymatic lipolysis considerably [20–22]. Moreover, phospholipase A_2 activity can be influenced by structural factors operating within the substrate interface. These include physical properties such as lipid chain packing and surface lateral packing defects as well as lipid electrostatic and inherent chemical influences which contribute to the hydrolytic quality of

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DENPC, dienoylphosphatidylcholine.

Correspondence: H. Ringsdorf, Institut für Organische Chemie, Universität Mainz, Becher-Weg 18-20, 6500 Mainz, F.R.G.

the substrate interface. All of these properties – the physical state of the membrane as well as its composition – play significant roles in enzyme regulation, manifested by the high sensitivity of phospholipase A_2 action to differences in molecular environment [17,23–28]. Recently, influence of lipid monolayer physical state has been imaged visually [29]. Selective hydrolysis of solid, gel-phase lipid domains was observed, resulting in solid lipid domain destruction and subsequent formation of regular enzyme domains at the interface [29].

Nevertheless, in comparison to hydrolysis of other biomembrane systems such as vesicles and pure monolayers, mixed monolayer systems as model biomembranes have not been as well characterized using phospholipase A_2 hydrolysis [20–22]. Nearly all biomembrane models studied to date are concerned with kinetic variables or structural influences on hydrolytic action. Little information about enzyme selection and recognition in heterogeneous lipid mixtures in model systems has been forwarded. In fact, only one example of monolayer hydrolysis using mixtures of hydrolyzable and nonhydrolyzable substrate analogues is known [30].

Mixed systems of polymerizable and natural lipids provide an attractive method to stabilize model biomembranes [1,31–36] as well as to vary the molecular environment within the membrane through polymerization. Such a system is a suitable natural membrane analogue by combining stability through polymerization together with the inherent fluidity of the natural lipids. When phospholipase A_2 is injected into the subphase under monolayers containing a hydrolyzable phosphatidylcholine, the phospholipid is hydrolyzed to the corresponding lysophosphatidylcholine and a free fatty acid [14–19]. If the chain length is short enough, both hydrolytic products are sufficiently water-soluble that contraction of the monolayer under constant surface pressure can be measured as a function of time [13]. In mixed monolayers of natural phospholipids and nonhydrolyzable analogues, the amount of hydrolyzable phosphatidylcholine component, as well as the physical state of the monolayer (phase mixed or phase separated), are expected to influence both the kinetics and extent of monolayer hydrolysis [30]. Hydrolysis experiments on such mixed systems using phospholipase A_2 should complement other conventional characterization techniques to yield information on the structure and organization of polymerizable, mixed model membranes.

The goal of the present study is to characterize the mixing behavior of natural, hydrolyzable lecithins and a synthetic polymerizable phospholipid analogue in monolayers. Comparison of isothermal compressions and isobaric expansions of these mixed systems with hydrolysis by phospholipase A_2 provides a contrast of the sensitivity of each technique in assessing the molecular environment within the monolayer. The influence of the proportion of different monolayer components as well as polymerization on enzyme recognition and hydrolysis in mixed monolayers is more dramatic than observed from the other physical characterization methods, indicating an enhanced effect of monolayer organization on phospholipase A_2 as a membrane probe.

Materials and Methods

Materials. Phospholipase A_2 (*Naja naja*, cobra venom; activity 935 U/mg protein) and L-α-dimyristoylphosphatidylcholine (DMPC; > 99% purity) were purchased from Sigma. DMPC showed a single spot by TLC analysis (chloroform/methanol/water (65 : 25 : 4) as eluting solvent). The polymerizable phospholipid analogue, dienoylphosphatidylcholine (DENPC) containing C-18 alkyl chains, each with butadiene groups conjugated to the glycerol backbone ester linkage in the acyl 1 and acyl 2 positions [1,32,34], was synthesized by extending the methods of Patel et al. [37].

Isotonic Tris buffer (pH 8.9, 10 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, chloroform-washed) was prepared by dissolution of salts in 10-times concentration in acid-cleaned glass flasks and diluted with Millipore water to final concentration for subphases for each monolayer experiment. Water for buffer was thrice distilled and then passed through a Millipore filtration apparatus (> 18 Mohm resistivity).

Phospholipid monolayers were spread from chloroform solutions (0.2–0.5 mg/ml). For mixed monolayers, appropriate volumes of equimolar stock solutions of the pure lipids were premixed and spread.

Phospholipase A_2 was dissolved from the supplier's bottle with buffer and the solution further diluted with buffer to a concentration of 0.33 mg/100 ml. Aliquots of 6 ml were frozen in polypropylene vials at −22 °C and thawed ambiently before use.

Prepolymerized DENPC for monolayer measurements. For isotherm studies of mixed systems containing polymeric DENPC and DMPC in various ratios, vesicles of monomeric DENPC were prepared by 10 min sonication of the pure monomeric lipid in water using a Branson sonifier (model B 15 P, probe type). The vesicle suspension was then polymerized under continuous stirring by irradiation in quartz cuvettes with an ultraviolet lamp for about 90 min under argon at a distance of about 1 cm. The main portion of the radiation dose was contributed by the 254 nm line with an exposure of about 5.0 mW/cm^2. Polymerization was continued until the absorption band of the DENPC butadiene double-bond at 254 nm had vanished. Subsequently, the polymerized vesicles were freeze-dried and the resulting, uncrosslinked solid prepolymer was recovered for preparation of spreading solutions. Molecular weight, determined using GPC (solvent: $CHCl_3$, room temperature, columns: PL gel, 10^4 and 10^3 Å) was approx. $3.8 \cdot 10^5$ using polystyrene standards. It is known from

the literature that photopolymerization of DENPC in vesicles leads to a polymer, which is soluble in organic solvents, suggesting that it is not crosslinked [38]. In contrast to this photoinitiated polymerization of DENPC, polymerization with AIBN and its water-soluble analogue in vesicles leads to a polymer which is not soluble in any organic solvent and is therefore certainly crosslinked. This difference may be due to the special conformation of the lipid chains in vesicles. [39].

Isotherm measurements. Measurements of surface pressure/area diagrams were carried out with a previously described Langmuir film balance [40]. Film balance measurements were controlled through an interfaced personal computer using a home-designed software program. The trough was completely thermostated and encloses a built-in low-pressure mercury lamp (Gränzel) installed 50 mm above the monolayer, allowing direct polymerization of the monolayer at various temperatures. A continuous stream of nitrogen through the enclosed trough was maintained during polymerization. Isotherms of the mixed systems were all measured at 5 °C on buffer subphases with a compression time of 30 min (3.9 Å^2/molecule per min).

Isobar measurements. Isobaric measurements of monolayer phase transitions over a temperature range from 2 to 30 °C were carried out over buffer subphases with another type of computer-controlled film balance [40]; in this case the trough is equipped with a Wilhelmy pressure detection system. A constant surface pressure of 20 mN/m was used in all cases.

Enzymatic hydrolysis of mixed monolayers by phospholipase A_2. The experimental method, schematically presented in Fig. 1, allows characterization of the hydrolytic activity of the enzyme in monolayers. The Langmuir film balance used for the isotherm measurements was used also for these enzyme hydrolysis experiments. Mixtures of DMPC and monomeric DENPC lipid solutions were spread on the buffer subphase at 37 °C and, after compressing the film slowly to a surface pressure of 10 mN/m, were allowed to remain under these conditions for 50 min. Diluted enzyme (5 ml, 16.5 μg) was injected slowly into the subphase under the monolayer from behind the barrier to start hydrolysis. Change in monolayer area was recorded as a function of time at a constant surface pressure of 10 mN/m. The decrease in surface area as lipid hydrolysis products were solubilized from the monolayer into the subphase over time at constant surface pressure [13] was plotted.

For studies on mixtures containing polymerized DENPC, the monomeric lipid solutions were spread, the film was compressed to 10 mN/m and allowed to remain under these conditions for 10 min. The film was subsequently irradiated with the film balance ultraviolet lamp (intensity: 2.2 mW/cm^2, main radiation dose contributed by the 254 nm line) for 30 min. Before and during the ultraviolet irradiation, the internal compartment of the equipment was flushed with nitrogen. The polymeric film was then left for 10 min before introduction of phospholipase A_2 to start the hydrolysis reaction as described above.

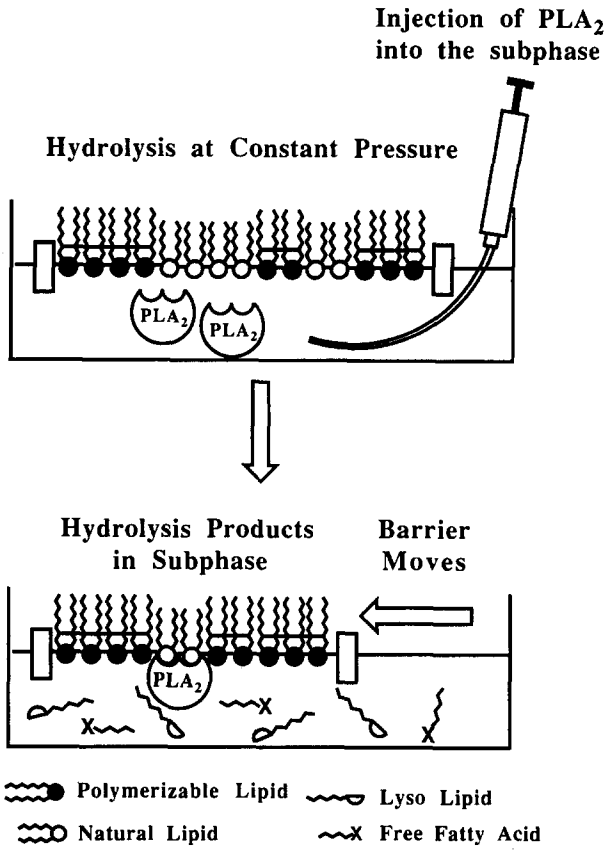

Fig. 1. Schematic representation of enzymatic hydrolysis of the phosphatidylcholine substrate, DMPC, in mixed monolayers with polymeric lipids. Lipid monolayer is maintained at constant pressure and subjected to enzyme hydrolysis. Hydrolytic products are solubilized from the layer into the subphase and the barrier moves, reducing the area. Changes in area are plotted versus time (see Table I and Fig. 8).

Results and Discussion

Mixing behavior of natural phosphatidylcholines with polymerizable lipids

Mixed monolayers of DMPC and monomeric DENPC. Surface pressure/area isotherms of mixed monolayers composed of DMPC and monomeric DENPC are shown in Fig. 2. Single-component systems show phase transitions: for DMPC between 9 and 12 mN/m and for DENPC between 14 and 17 mN/m. These two isotherms of the pure compounds correspond to those previously described [41,42], although small deviations from these results may be attributed to different subphases with different pH values and ionic strengths. In the case of phase separated systems, the phase transitions of both components would be observed at their respective surface pressures in the isotherm of each mixture. Every two-component system isotherm would,

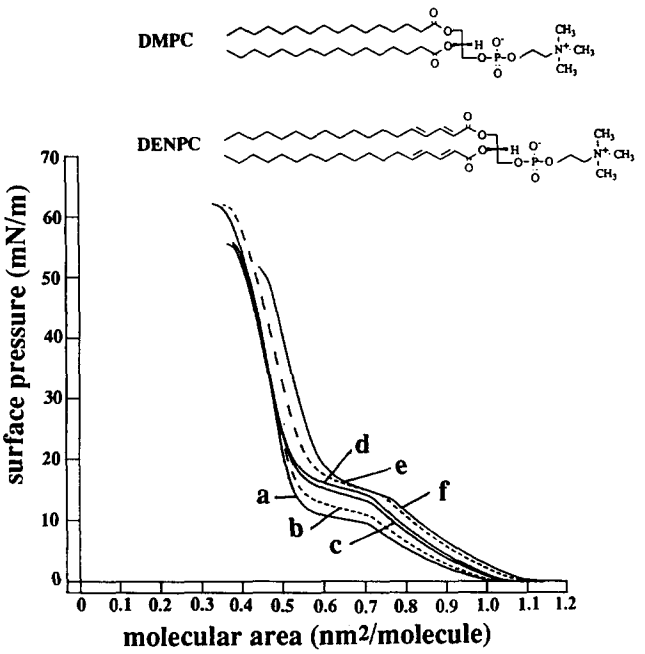

Fig. 2. Surface pressure/area isotherms of mixtures of DMPC and monomeric DENPC, x = molar percentage of monomeric DENPC, a: $x = 0\%$; b: $x = 20\%$; c: $x = 40\%$; d: $x = 60\%$; e: $x = 80\%$; f: $x = 100\%$; $T = 5\,^{\circ}C$; subphase: Tris buffer (pH 8.9); (see Materials and Methods for experimental conditions).

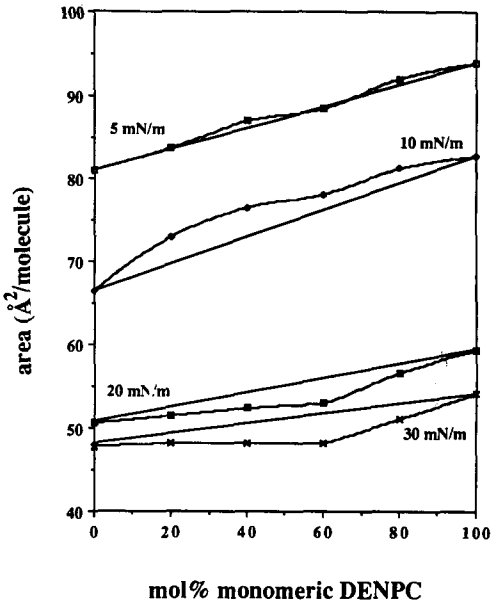

Fig. 3. Monolayer mixing diagram for mixtures of DMPC and monomeric DENPC at various surface pressures as a function of monomeric DENPC content.

therefore, show at least two phase-transition regions. However, all mixed monolayers show only one transition region with the respective surface pressures of the transition onset (slope discontinuity) increasing continuously with increasing mole fraction of DENPC in the mixture. This indicates homogeneous phase mixing of the two compounds in the binary system [42–45].

Plots of the mean areas per molecule versus mole fraction of DENPC at different surface pressures reveal only small deviations from ideality and the additivity rule (Fig. 3), suggesting also miscibility of the compounds in the monolayer, but with little interaction between the molecules of each or of different species. In such phase diagrams, a linear relationship between molecular area and monolayer composition would represent either ideal mixing or perfect phase separation, while any deviation from linearity is indicative of intermolecular interactions between the two lipids [44]. The conclusion from the isotherm data is that these binary mixtures are not detectably phase separated. This is confirmed by isobar measurements.

Isobars of monolayers comprised of pure DMPC, pure DENPC and the 1:1 molar mixture of DENPC monomer and DMPC are shown in Fig. 4. Both pure compounds show defined phase-transition temperatures: DMPC at 10 °C and DENPC at 6 °C. The phase transition for DMPC corresponds to a slightly higher value previously documented for DMPC on water subphases [46] with the deviation due possibly to a buffer subphase. That for DENPC is identical to that previously described [47]. Phase separated systems would show both phase transition temperatures in the isobars of a mixed system. However, the curve of the 1:1 mixture indicates that this binary system is phase-mixed, because it shows only one phase transition temperature in between the transitions of the pure compounds.

Mixed monolayers of DMPC and polymerized DENPC. Surface pressure/area isotherms of these mixed monolayers are shown in Fig. 5. In this system, only the isotherm of pure DMPC has a phase transition region. Polymerization of DENPC causes loss of its phase transition (compare Fig. 2). Because the system exists in only one phase state (no phase transition) and because of the small area per molecule at the monolayer collapse point (0.5 nm^2/molecule), it is hypothesized that po-

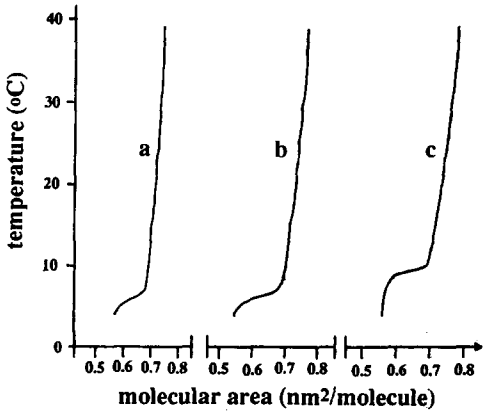

Fig. 4. Monolayer isobars of monomeric DENPC (a), DMPC (c) and the 1:1 mixture of DMPC and monomeric DENPC (b); lateral surface pressure = 20 mN/m;subphase: Tris buffer (pH 8.9); (see Materials and Methods for experimental conditions).

lymerized DENPC monolayers exist in only a type of solid analogue state, due to the rigid and inflexible polymer backbone which reduces lateral mobility within the film. In the mixed monolayers, the detectable phase transition region in the mixed isotherms becomes increasingly ill-defined as the proportion of polymerized DENPC increases; in the mixed system of DENPC/DMPC in a molar ratio of 8 : 2, it has vanished. Because of this, a firm conclusion regarding phase separation or phase mixing is difficult. However, a speculation would be that DMPC and polymerized DENPC interact strongly and positively in monolayers and are not compelled to phase-separate. It is unlikely, however, that ideal phase mixing can be achieved in such a two-dimensionally constrained system. Ideal phase mixing is probably prevented by the fact that in the polymerized system each DMPC molecule can interact only with DENPC polymer coils and not with each single DENPC molecule as in the monomer mixtures. Headgroup interactions could remain the same in both systems, but the hydrophobic acyl chains in the polymer coils are linked together and are not free to associate with DMPC. The DENPC polymer backbone, therefore, presents a completely different molecular environment than the monomeric DENPC mixtures with DMPC. That these differences can be explained by microphase separation is further supported by evidence below.

The corresponding area/composition curves (Fig. 6) for the polymeric DENPC/DMPC mixtures show more

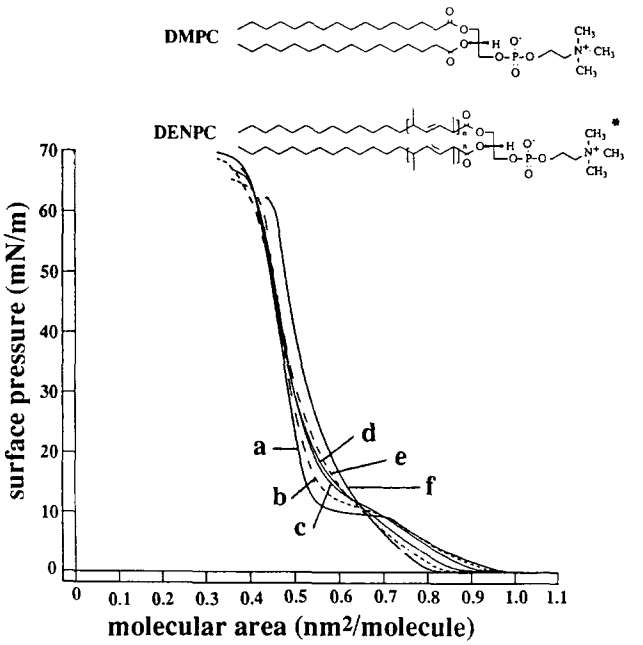

Fig. 5. Surface pressure/area isotherms for mixtures of DMPC and polymeric DENPC, x = molar percentage of polymeric DENPC based on polymer repeat unit, a: $x = 0\%$; b: $x = 20\%$; c: $x = 40\%$; d: $x = 60\%$; e: $x = 80\%$; f: $x = 100\%$; $T = 5\,^{\circ}C$; subphase: Tris buffer (pH 8.9) (see Materials and Methods for experimental conditions). * Schematic representation of the polymer.

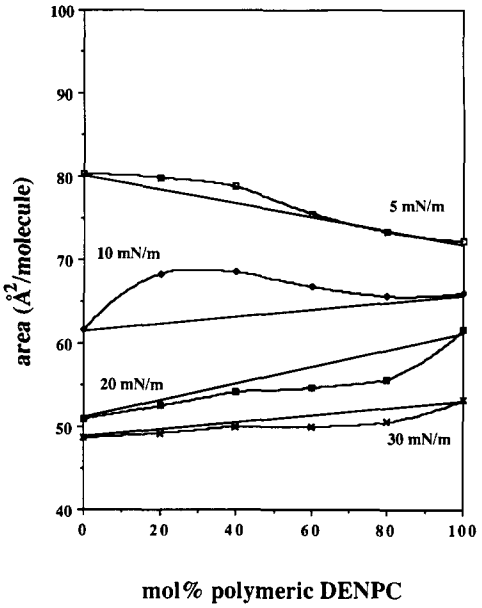

Fig. 6. Monolayer mixing diagram for mixtures of DMPC and polymeric DENPC at various surface pressures as a function of polymeric DENPC content (mol% based on polymer repeat unit).

deviation from the addivity rule compared to the monomeric mixed system (compare Fig. 3), especially at a surface pressure of 10 mN/m, at which further enzymatic characterization (described below) was investigated. This again suggests miscibility of the compounds in the monolayer but with a positive deviation from ideal mixing due to the presence of polymer chains. Such positive deviation indicates some sort of repulsive interaction, in this case a structural incompatibility in the mixture caused by local hindrance of free motion by polymer segments. This may indicate that DENPC polymerization induces a type of microphase separation, or more accurately, structuring between the polymer and the natural lecithin in the monolayer on a local, molecular scale difficult to detect as phase separation, but promoting a localized nonideal mixing. Interaction between molecules of DMPC and polymerized DENPC could take the form of localized groups of DMPC lipids isolated from one another by chains (but not large chain aggregates) of DENPC polymers. These small-scale effects would lead to extensive nonideality of mixing within the monolayer, but on such a localized scale that it essentially dissimilar to phase separation and cannot be detected as such.

The isobars of the two pure lipids and a 1 : 1 molar mixture are shown in Fig. 7. As expected, the pure polymerized compound shows no phase transition, while DMPC shows a well-defined one. The phase transition temperature of the 1 : 1 mixture is not very sharp but, because it does not correspond to that for pure DMPC, suggests a degree of interaction of the natural and the polymerized lipid as dicussed above.

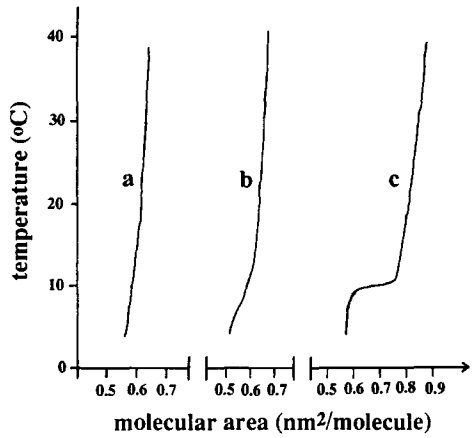

Fig. 7. Monolayer isobars of polymeric DENPC (a), DMPC (c) and the 1:1 mixture of DMPC and polymeric DENPC (b); lateral surface pressure = 20 mN/m; subphase: Tris buffer (pH 8.9) (see Materials and Methods for experimental conditions).

Taken together, these data suggest that the polymerized mixed monolayer system, like the monomeric monolayer, is phase-mixed but with greater deviation from ideality, indicative of monolayer structural inhomogeneity on a localized scale due to two-dimensional DENPC polymer coils. Evidence from the following section supports isotherm and isobar data showing differences in molecular organization between monomeric and polymeric binary mixtures using enzymatic hydrolysis as a monolayer structural probe.

Enzymatic hydrolysis of DMPC in mixed monolayers

Initially, DENPC was shown to remain inert against the action of phospholipase A_2 in both monomeric and polymeric states. In DENPC monolayers, whether monomeric or polymerized, no contraction of the film was observed. This is in agreement with the inability of phospholipase A_2 to hydrolyze small DENPC vesicles [1,47] as well as giant liposomes (unpublished results). The very slow hydrolysis of monomeric DENPC vesicles, described by Tsuchida et. al. [48] is not a contradiction to this result, because very small amounts of hydrolytic products of DENPC will not destabilize liposomes. Furthermore, it is known [17,25] that one requirement for the hydrolyzability of phospholipid molecules by phospholipase A_2 is chain flexibility. This requirement is certainly not very well fulfilled for the butadiene-containing DENPC molecule with its rigid conjugated double bond acyl chains next to the ester linkage. This might explain the very slow hydrolysis rate mentioned by Tsuchida et. al [48].

Fig. 8 shows the resulting hydrolysis curves for the monomeric and the polymerized DENPC binary mixtures with DMPC. In monolayers containing DMPC, injection of phospholipase A_2 into the subphase caused a decrease in monolayer area at constant pressure. The extent of this decrease increases with an increase in the proportion of DMPC. If it is assumed that decreases in monolayer area correspond to extents of hydrolysis of DMPC in the layer, it is possible to compare the proportion of DMPC in the starting monolayers with the extent to which these are subsequently hydrolyzed in monolayers of different composition. Table I shows that, in monolayers containing polymerized DENPC, DMPC was quantitatively removed by the action of phospholipase A_2. That is, mixed monolayers containing 25 mol% DMPC demonstrated a 25% reduction in area after hydrolysis; monolayers of 50 mol% DMPC a 50% reduction in area, and so on, indicating complete recognition and removal of available DMPC by enzymatic hydrolysis.

With unpolymerized monolayers, hydrolysis stopped well before complete removal of DMPC. Specifically, mixed monolayers containing 75 mol% DMPC showed only 59% decrease in area; monolayers having 50 mol%

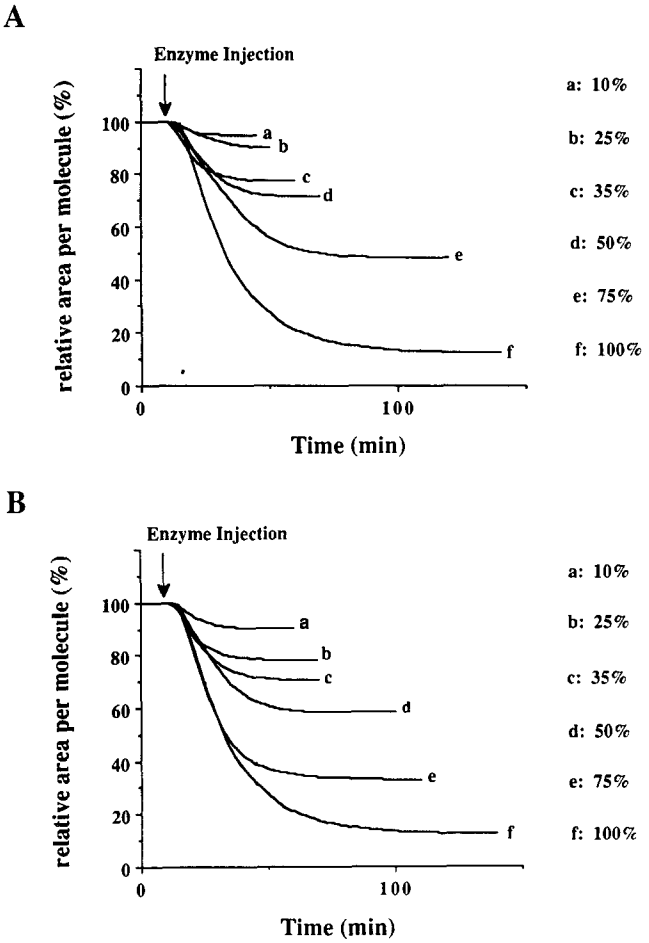

Fig. 8. Relative area contraction of monomeric and polymerized mixed monolayers during enzymatic hydrolysis; (A) mixed system: monomeric DENPC and DMPC at different molar ratios; (B) mixed system: polymeric DENPC and DMPC at different molar ratios; lateral surface pressure = 10 mN/m; $T = 37\,^{\circ}$C, subphase: Tris buffer (pH 8.9) (see Materials and Methods for experimental details and Fig. 1 for a schematic representation). Percentages on the right refer to DMPC.

TABLE I

Results of enzymatic hydrolysis of mixed monolayers

Monolayer hydrolysis experiments conducted at constant surface pressure (10 mN/m) over buffer subphase at 37 °C. Phospholipase A_2 solutions in buffer (5 ml, 16.5 μg) were carefully injected under the monolayer from behind the barrier. Hydrolysis was monitored over time by following the decrease in monolayer surface area at constant pressure as DMPC hydrolytic end-products were solubilized into the subphase (see text for further details, Fig. 1 for schematic representation and Fig. 8 for corresponding hydrolysis curves).

(A) Monolayer mixtures of DMPC and monomeric DENPC

DMPC:DENPC starting ratio	Decrease in area (%)	Decrease in area (%) normalized [a]	% of DMPC in final monolayer [b]
100: 0	89	100	0
75:25	53	59	39 (16:25) [c]
50:50	28	32	26 (18:50) [c]
35:65	23	26	26 (9:65) [c]
25:75	10	11	16 (14:75) [c]
10:90	5	6	4 (4:90) [c]

(B) Monolayer mixtures of DMPC and polymeric DENPC

DMPC:DENPC starting ratio	Decrease in area (%)	Decrease in area (%) normalized [a]	% of DMPC in final monolayer [b]
100: 0	89	100	0
75:25	68	77	0
50:50	43	48	4 (2:50) [c]
35:65	29	33	3 (2:65) [c]
25:75	22	25	0
10:90	10	11	0

[a] Assuming decrease in area of pure DMPC monolayers as 100%.
[b] Calculation assumes that decrease in area reflects the extent of hydrolysis of DMPC.
[c] Final ratio of DMPC/DENPC in the monolayer to compare with starting ratio.

DMPC, only 32% decrease in area; monolayers having 35 mol% DMPC, only 26% reduction in area, and so on as detailed in Table I. The smaller the proportion of DMPC in the starting monolayer, the greater is the deviation from what might be expected to be removed by enzyme hydrolysis. For example, in monolayers containing 25 mol% DMPC, less than half of it is hydrolyzed by the enzyme (11% reduction in area). Furthermore, there was always some degree of hydrolysis, even when the starting proportion of DMPC was well less than that at which hydrolysis had stopped in other monolayers rich in DMPC.

This interesting result can be rationalized when considering possible influences of hydrolytic end-products in the monolayer – namely, free fatty acid and lysolipid – on enzyme interaction with the substrate. Starting mixtures of DMPC and DENPC would be expected to demonstrate different degrees of hydrolysis than other mixed monolayers containing the same amounts of DMPC and DENPC mixed with fatty acids and lysolipids. The influence of such hydrolytic end-products on phospholipase A_2 action has already been documented in micelles and vesicles [49–51] as well as in monolayers [29]. However, why these end-product effects are seen in the monomeric mixtures and not in the polymeric mixtures remains unclear. Recent evidence for phase separation of critical concentrations of hydrolytic end-products induced by phospholipase A_2 hydrolysis of phospholipid vesicles provides one suitable explanation [52]. Possibly, fatty acid produced by hydrolysis of DMPC in mixed polymerized monolayers may not be able to phase-separate because of the physical hindrance and barrier created by the DENPC polymer backbone. In contrast, after critical degrees of hydrolysis, fatty acid could easily phase-separate in mixed monomeric monolayers and interact with the enzyme to inhibit further hydrolysis.

A high sensitivity of phospholipase A_2 to differences in the molecular environment of the DMPC in the monolayer – differences that other techniques cannot clearly discern – serves to explain different hydrolytic responses seen between polymerized and monomeric mixed monolayers. These differences could reflect localized phase separation between DMPC and polymerized DENPC. Existence of a non-ideally mixed system could mean that, in these mixed monolayers, nanometer-scale islands of liquid analogue phase DMPC exist surrounded by the two-dimensional coils of polymerized solid analogue DENPC. On a molecular scale, the environment for the enzyme in the monolayer would be quite different from that in an ideally mixed system, but in a strict sense, not phase-separated. This type of microphase separation has already been described in liquid crystal polymer mixtures [53]. Groups of DMPC are locked in by constrained DENPC polymer chains. Because there is no chemical basis for DMPC to phase separate from polymeric DENPC, the physical incompatibility with the polymeric chain results in small DMPC molecular pockets interacting nonideally in isolated structures. From the enzyme's perspective, this allows easier recognition and more complete hydrolysis of DMPC from the polymer binary mixture. In monolayer mixtures containing monomeric DENPC where near-ideal mixing is supposed, every DMPC molecule would be in close proximity to molecules of DENPC, constituting an environment in which hydrolysis of DMPC would be hindered or even prohibited. Only upon binding could the enzyme differentiate substrate from analogue. This process would naturally slow or even prevent monolayer hydrolysis.

It is known already that phospholipase A_2 activity is sensitive to structural factors – head group structure, charge and packing density – which contribute to the physical state of the interfacial region [17,20,29,54]. That these factors would be changed by the possible molecular association and build-up of hydrolytic end products within the intact monolayer is certain. Association and phase separation of these products only in mixed monomeric monolayers and not in polymerized monolayer mixtures would create two drastically different molecular environments in the two systems. Isothermal and isobaric measurements indicating phase-mixing characterize the state of both the unhydrolyzed monomeric and polymerized mixed monolayers, while enzymatic hydrolysis indicates different responses to their respective hydrolytic destruction. In this way, phospholipase A_2 hydrolysis yields additional, complementary information on the different molecular environment within mixed monolayers not detectable by isothermal and isobaric methods.

Acknowledgements

J.B.L. and D.E.D. thank the Science and Engineering Research Council for grants in support of this work. D.W.G. appreciates a Postdoctoral Research Fellowship from the Alexander von Humboldt Foundation. C.S. was supported through a Postdoctoral Research Fellowship from the Natural Sciences and Engineering Council of Canada. Research in Mainz is supported by a BMFT Grant for Ultrathin Films (03M4008F1).

References

1 Ringsdorf, H., Schlarb, B. and Venzmer, J. (1988) Angew. Chem. Int. Ed. Engl. 27, 113–158.
2 Hupfer, B. and Ringsdorf, H. (1985) in Surface and Interfacial Aspects of Biomedical Polymers: Vol. 1, Surface Chemistry and Physics (Andrade, J.D., ed.), pp. 77–103, Plenum Press, New York.
3 Fahey, D. and Small, D.M. (1988) Langmuir 4, 589–594.
4 Balthasar, D.M., Cadenhead, D.A., Lewis, R.N.A. and McElhaney, R.N. (1988) Langmuir 4, 180–186.
5 Albrecht, O., Prass, W. and Ringsdorf, H. (1986) Eur. Biophys. J. 14, 97–102.
6 Heckl, W.M., Cadenhead, D.A. and Möhwald, H. (1988) Langmuir 4, 1352–1358.
7 Robert, S., Tancrède, P., Salesse, C. and Leblanc, R.M. (1983) Biochim. Biophys. Acta 730, 217–225.
8 Pattus, F., Slotboom, A.J. and De Haas, G.H. (1979) Biochemistry 13, 2698–2702.
9 Op den Kamp, J.A.F., De Gier, J. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 345, 253–256.
10 Kensil, C.R. and Dennis, E.A. (1985) Lipids 20, 80–83.
11 Verger, R., Rietsch, J., Pattus, F., Ferrato, F., Pieroni, G., De Haas, G.H. and Desnuelle, P. (1978) in Enzymes of Lipid Metabolism (Gatt, S., Freysz, L. and Mandel, P., eds.), pp. 79–94, Plenum Press, New York.
12 Verger, R. and De Haas, G.H. (1976) Annu. Rev. Biophys. Bioeng. 5, 77–117.
13 Verger, R. and Pattus, F. (1982) Chem. Phys. Lipids 30, 189–227.
14 Verheij, H.M., Slotboom, A.J. and De Haas, G.H. (1981) Rev. Physiol. Biochem. Pharmacol. 91, 91–203.
15 Van den Bosch, H. (1980) Biochim. Biophys, Acta 604, 191–246.
16 Dennis, E.A. (1983) in The Enzymes, 3rd Edn., Vol. 16 (Boyer, P.D., ed.), pp. 307–353, Academic Press, New York.
17 Achari, A., Scott, D., Barlow, P., Vidal, J.C., Otwinowski, Z., Brunie, S., and Sigler, P.B. (1987) Cold Spring Harbor Symp. Quant. Biol. 52, 441–452.
18 Slotboom, A.J., Verheij, H.M. and De Haas, G.H. (1982) in Phospholipids (Hawthorne, J.H. and Ansell, G.B., eds.), pp. 359–434, Elsevier, Amsterdam.
19 Drenth, J., Dijkstra, B.W. and Renetseder, R. (1987) in Biological Macromolecules and Assemblies, Vol. 3 – Active Sites of Enzymes (Jurnak, F.A. and McPherson, A., eds.), pp. 287–312, Wiley, New York.
20 Alsina, A., Valls, O., Pieroni, G., Verger, R. and Garcia, S. (1983) Coll. Polymer Sci. 261, 923–928.
21 Gheriani-Gruszka, N., Almog, S., Biltonen, R.L. and Lichtenberg, D. (1987) J. Biol. Chem. 263, 11808–11813.
22 Alsina, A., Garcia, M.D., Valls, O. and Garcia, S. (1985) Coll. Surf. 14, 277–283.
23 Thuren, T., Vainio, P., Virtanen, J.A., Somerharju, P., Blomquist, K. and Kinnunen, P.K.J. (1984) Biochemistry 23, 5129–5134.
24 Israelachvili, J.N., Marcelja, S. and Horn, G. (1980) Q. Rev. Biophys. 13 (2), 121–200.
25 Barlow, P.N., Vidal, J.-C., Lister, M.D., Dennis, E.A. and Sigler, P.B. (1988) J. Biol. Chem. 263, 12954–12958.
26 Lichtenberg, D., Romero, G., Menashe, M. and Biltonen, R.L. (1986) J. Biol. Chem. 261, 5328–5340.
27 Romero, G., Thompson, K. and Biltonen, R.L. (1987) J. Biol. Chem. 262, 13476–13482.
28 Sundler, R. (1984) Chem. Phys. Lipids 34, 153–161.
29 Grainger, D.W., Reichert, A., Ringsdorf, H. and Salesse, C. (1989) FEBS Lett. 252, 73–82.
30 Büschl, R., Hupfer, B. and Ringsdorf, H. (1982) Makromol. Chem. Rapid Commun. 3, 589–596.
31 Büschl, R., Gaµb, H., Ringsdorf, H. and Sackmann, E. (1984) Biophys. J. 45, 725–731.
32 Ohno, H., Takeoka, S., Iwai, H. and Tsuchida, E. (1989) Macromolecules 22, 61–66.
33 Ringsdorf, H., Schlarb, B., Tyminski, P.N. and O'Brien, D.F. (1988) Macromolecules 21, 671–677.
34 Bader, H., Dorn, K., Hupfer, B. and Ringsdorf, H. (1985) Adv. Polym. Sci. 64, 1–62.
35 Regen, S. (1987) in Liposomes: From Biophysics to Therapeutics (Ostro, M.J., ed.), pp. 73–108, Dekker, New York.
36 Leaver, J., Alonso, A., Durrani, A.A. and Chapman, D. (1983) Biochim. Biophys. Acta 727, 327–335.
37 Patel, K.M., Morisett, J.D. and Sparrow, J.J. (1979) J. Lipid Res. 20, 11099–11106.
38 Ohno, H., Yoshitaka, O. and Tsuchida, E. (1986) Macromolecules 20, 929–933.
39 Ohno, H., Takeoka, S., Hidetaka, I. and Tsuchida, E. (1988) Macromolecules 21, 319–322.
40 Albrecht, O. (1983) Thin Solid Films 99, 227–234.
41 Hupfer, H., Ringsdorf, H. and Schupp, H. (1981) Makromol. Chem. 182, 247–253.
42 Albrecht, O., Gruler, H. and Sackmann, E. (1978) J. Phys. 39, 301–313.
43 Albrecht, O., Gruler, H. and Sackmann, E. (1981) J. Coll. Int. Sci. 79, 319–338.
44 Crisp, D.J. (1949) in Surface Chemistry, pp. 17–35, Butterworths London.
45 Phillips, M.C. and Chapman, D. (1986) Biochim. Biophys. Acta 163, 301–313.
46 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44.

47 Büschl, R. (1986) Ph. D. thesis, University of Mainz.
48 Hasegawa, E., Eshima, K., Matsushita, Y.-I., Nishide, H. and Tsuchida, E. (1986) Biochim. Biophys. Acta 862, 235–242.
49 Plückthun, A. and Dennis, E.D. (1985) J. Biol. Chem. 260, 11099–11106.
50 Jain, M.K. and Jahagirdar, D.V. (1984) Biochim. Biophys. Acta 814, 313–318.
51 Jain, M.K. and Berg, O. (1989) Biochim. Biophys. Acta 1002, 127–156.
52 Jain, M.K., Yu, B.-Z. and Kozubek, A. (1989) Biochim. Biophys. Acta 980, 23–32.
53 Westphal, S., Diele, S., Mädicke, A. and Kuschel, F. (1988) Makromol. Chem. Rapid Commun. 9, 489–493.
54 Thuren, T. (1988) FEBS Lett. 229, 95–99.