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Degradation of Dilauroylphosphatidylcholine by Phospholipase A_2 in Monolayers Containing Glycosphingolipids†

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ABSTRACT: The ability of phospholipase A_2 from porcine pancreas to degrade all of the available dilauroylphosphatidylcholine in mixed monolayers with galactocerebroside, sulfatide, or ganglioside GM1 was investigated at different constant surface pressures. Under the conditions used the interfacial glycosphingolipid composition was continuously enriched as the enzyme action proceeded. The total percentage of phospholipid degradation depends on the surface pressure and on the type of glycosphingolipid. The presence of sulfatide activates the enzyme while galactocerebroside and ganglioside GM1 are inhibitory. The extent of phospholipid hydrolysis is independent of the effect of glycosphingolipids on the enzyme velocity. This is so when the latter is measured either in conditions of constant glycosphingolipid composition and zero-order kinetics [Bianco, I. D., Fidelio, G. D., & Maggio, B. (1989) *Biochem. J. 258*, 95–99] or under variable surface composition as in the present work. The modulation of phospholipase A_2 activity by glycosphingolipids operates at two independent levels. One controls the rate of enzyme activity, and the other modulates the total extent of substrate degradation. This depends on the initial interaction of the enzyme with the interface. The glycosphingolipid effect on the activity is different depending on whether the enzyme has access to the substrate from the subphase or is already adsorbed to the lipid interface.

The intermolecular organization and lipid composition have a profound influence on the ability of phospholipases to degrade the phospholipid substrate (Dawson, 1969; Pieroni & Verger, 1979; Brokmann, 1984; Laboda et al., 1986; Verger & Pieroni, 1986; Bell & Biltonen, 1989a,b; Jain, 1990). We have recently shown that neutral and negatively charged GSLs[1] in mixed monolayers with dlPC and dlPA modify remarkably the activity of porcine pancreatic PLA_2 and *Clostridium perfringens* phospholipase C (Bianco et al., 1989, 1990). Conditions of zero-order kinetics were employed in those studies by supplying pure substrate from a reservoir to replace the phospholipid degraded according to the technique introduced by Verger and de Haas (1973). The enzyme velocity depends critically on the surface pressure and the proportions and the type of GSLs (Bianco et al., 1989, 1990). Sulf activates both phospholipases irrespective of its proportions in the film while GalCer leads to inhibition or activation depending on its molar fraction. The presence of gangliosides in relatively low proportions in the monolayer inhibits reversibly the enzyme activity. GSLs (below their critical micellar concentration but still in a molar ratio above 10:1 with respect to the enzyme) or their constituent carbohydrate moieties in the subphase do not affect the catalytic activity of PLA_2 or PLC against films of pure phospholipid (Bianco et al., 1989, 1990).

During our previous studies the question arose as to whether an enzyme with a modified rate of activity could nevertheless degrade all of the substrate available as the proportion of GSLs increased in the monolayer. We have addressed this question with films kept at constant surface pressure but whose composition becomes continuously enriched in GSLs. We found that the total extent of dlPC degradation depends on the surface pressure and on the type of GSLs but is independent of the initial rate of enzyme activity. The modulation by GSLs of the PLA_2 activity operates at two different levels, apparently independent of each other; one is related to the velocity and the other to the extent of enzyme action. The effect of GSLs depends on whether the enzyme has access to the substrate from the subphase or is already adsorbed to the interface.

Materials and Methods

Porcine pancreas PLA_2 and dlPC were from Sigma Chemical Co. (St. Louis, MO). Over 95% of the protein ran as a

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[1] Abbreviations: dlPC (dilauroylphosphatidylcholine), didodecanoyl-*sn*-glycero-3-phosphocholine; dlPA (dilauroylphosphatidic acid), didodecanoyl-*sn*-glycero-3-phosphoric acid; dpPC (dipalmitoylphosphatidylcholine), dihexadecanoyl-*sn*-glycero-3-phosphocholine; PLA_2, porcine pancreas phospholipase A_2; Cer (ceramide), *N*-acylsphingosine; GalCer (galactocerebroside), Galβ1–1′Cer; Sulf (sulfatide), Gal(3-sulfate)β1–1′Cer; GM1 (monosialoganglioside), Galβ1–3GalNAcβ1–4Gal(3–2αNeuAc)1–4Glcβ1–1′Cer; GSLs, glycosphingolipids.

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single band on SDS–PAGE. No surface-active compounds were detected in the enzyme preparation when injected into the subphase at a concentration 10-fold higher than that routinely used in the assays. No changes of surface tension or surface potential were detected in clean interfaces when the surface area was reduced to about 10% of the initial value. Purification and sources of GSLs were as reported previously (Maggio et al., 1978a, 1985b). The apparatus, troughs, techniques, and reproducibility for the monolayer experiments were described in detail elsewhere (Maggio et al., 1978a; Bianco et al., 1989, 1990). Briefly, mixed monolayers of GSLs–dlPC were spread (5–20 μL, depending on the film) from premixed solutions (approximately 1 mM) in the desired proportions made in chloroform–methanol (2:1) for mixtures of dlPC with GalCer or Sulf and chloroform–methanol–water (2:1:0.15) for dlPC–GM1. After 5 min the monolayer was compressed at constant rate (routine speed was 3.6 $cm^2 \cdot min^{-1}$). GSLs do not desorb from the interface in these conditions (Maggio et al., 1978a,b). This was thoroughly controlled as previously described in detail (Fidelio et al., 1986a,b; Bianco & Maggio, 1989). This involves controls of possible variations of the surface pressure and surface potential at different periods of time after the original interface is swept clean and of several compression and decompression cycles. The isotherms are reproducible within ± 0.3 nm^2 and ± 10 mV after several compression and decompression cycles at different rates and at different times after spreading. Two to three runs were averaged for each experiment. For the zero-order kinetic experiments (Bianco et al., 1989, 1990) a constant GSL–dlPC composition is maintained in the monolayer. This is done by spreading a mixed GSLs–dlPC film over the enzyme compartment and a *pure* dlPC monolayer, at the same surface pressure, on the reservoir compartment. The extent of hydrolysis of dlPC by PLA_2 when the proportion of GSLs in the film was continuously increased is assessed in a similar trough. However, in this case the *same* mixed monolayer is spread on *both* the surface of the reaction compartment and the reservoir. The proportion of GSLs at the interface over the enzyme compartment continuously increases as a result of the enzymatic activity. The kinetics will obviously not be zero order, except for a short period of relative substrate excess after the initial lag time (see Figure 1 for more details). Ideally, the enzyme (either with an enhanced or diminished velocity) should continue to degrade all the dlPC available. The constancy of the surface composition on the reservoir compartment is monitored by measuring the surface potential. This parameter is very sensitive to the magnitude and type of surface dipoles and permits detection of less than 3% variation of the monolayer composition (Bianco et al., 1989, 1990; Bianco & Maggio, 1989; Maggio et al., 1978a,b). The results are given as a percentage of dlPC degraded on the reaction compartment as a function of time after injection of the enzyme into the subphase. The initial number of molecules of dlPC on the reaction compartment is taken as 100%. The percentage of dlPC degraded corresponds to the difference between this value and the percentage of dlPC remaining at the surface of the reaction compartment; this is calculated from the measured area at each time. Mixed monolayers of dlPC with GalCer or Sulf show ideal mixing behavior (Bianco & Maggio, 1989). For these films the only factor to take into account in the calculations is the dilution of the substrate by the GSLs, weighted by their corresponding molecular areas at the surface pressure employed. On the other hand, mixed films of dlPC with GM1 show nonideal behavior; negative deviations of mean molecular area between 15 and 20%, compared to ideally mixed films, occur at surface pressures between 5 and 15

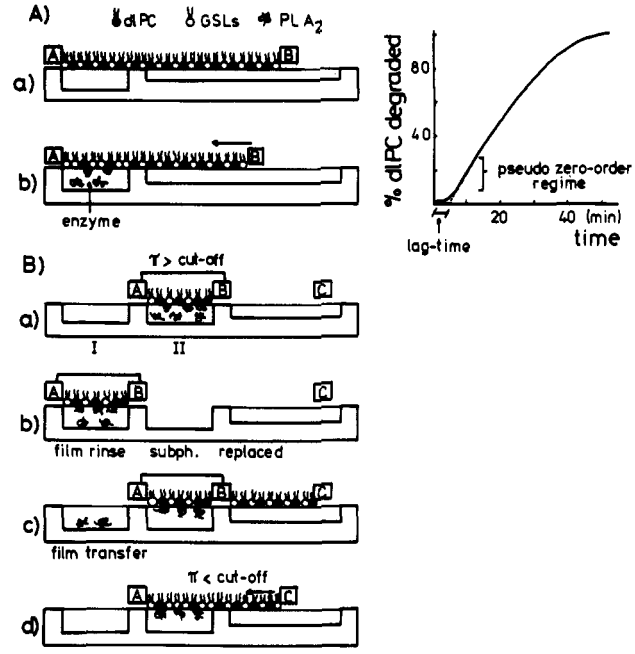

FIGURE 1: Conditions used to study PLA_2 activity at constant pressure and continuously variable surface composition. (A) No film transfer. (a) The same mixed monolayer of dlPC and GSLs is spread on the reaction compartment (surface area 16.15 cm^2; volume 20 mL) and the reservoir (surface area 48.45 cm^2; volume 30 mL) separated by a slit (5 mm × 5 mm × 1 mm). The surface pressure is adjusted with barrier B. (b) The enzyme is injected into the subphase of the reaction compartment (under magnetic stirring, 100–150 rpm, with a small rod, 5 mm × 1 mm), and the reaction is recorded through the area reduction automatically compensated by the surface barostat driving barrier B. (B) Film transfer. (a) At time zero, the enzyme is injected into the subphase of compartment II beneath a film of dlPC–GSLs at a surface pressure (adjusted with barrier B) above the cutoff point (no enzymatic activity; Bianco et al., 1989, 1990). (b) After a time (with subphase stirring) the monolayer is transferred at constant pressure between two syncronous barriers (A and B) over a fresh subphase without enzyme in compartment I and rinsed. The subphase in compartment II is removed; after washing, it is filled with fresh subphase without enzyme. (c) The film is transferred back onto compartment II. A monolayer of the same composition is spread on the reservoir and is adjusted at the same pressure. (d) Barrier B is removed, the surface pressure is rapidly changed (below the cutoff point) with barrier C, and the reaction is recorded. The graph on the right shows the general time course for dlPC degradation (Figures 2–5 show the actual experiments). After a lag time, the enzyme velocity becomes constant (pseudo zero order) due to the relative substrate excess for a short period of time; after this, the reaction rate decreases and deviates from the apparent zero-order regime due to continuous substrate depletion. For film-transfer experiments, no lag time is found due to the prior adsorption of the enzyme.

$mN \cdot m^{-1}$ (Bianco & Maggio, 1989). The proportion of dlPC degraded may be underestimated in this case. The monolayer technique does not allow unambiguous interpretation about which of the components is mostly responsible for the change in molecular area. As described in detail elsewhere (Bianco et al., 1989) the deviations can be taken into account by ascribing the deviation in area to GM1 only, distributed between dlPC and GM1 according to their molar ratio in the film, or to dlPC only. The latter case would lead to the larger possible underestimation of the actual percentage of dlPC degraded. A correction factor taking into account the above negative deviations was therefore applied to calculate the percentage of dlPC degradation. According to the above negative deviations in mean molecular areas, the uncertainty that would arise by not using this correction would amount to between 10 and 15% of dlPC degraded, depending on the surface pressure and molar proportions of GM1.

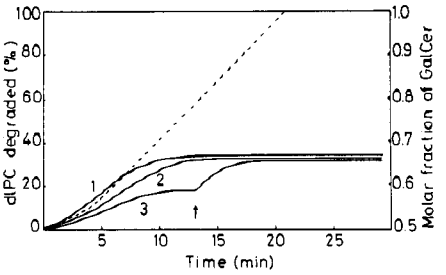

FIGURE 2: Effect of GalCer on the extent of dlPC degradation by PLA_2. The percent of dlPC degraded is shown as a function of time. At zero time 0.050 (1) or 0.025 μg/mL (2) PLA_2 is injected under a monolayer of GalCer–dlPC (initial molar fraction of GalCer of 0.5) at 15 mN·m^{-1}. The effect of changing the surface pressure from 5 to 15 mN·m^{-1} (arrow) after the plateau level is reached is shown (3) for PLA_2 at 0.025 μg/mL. The dashed line corresponds to the extent of dlPC hydrolysis of a film of pure dlPC at 15 mN·m^{-1} by 0.025 μg/mL PLA_2.

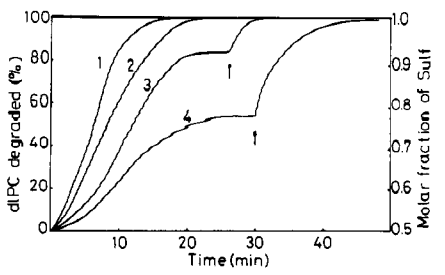

FIGURE 3: Effect of Sulf on the extent of dlPC degradation by PLA_2. The percent of dlPC degraded is shown after injection of 0.050 (1) or 0.025 μg/mL (2) PLA_2 under a monolayer of Sulf–dlPC (initial molar fraction of Sulf of 0.5) at 15 mN·m^{-1}. The effect of changing the surface pressure from 10 (3) or 5 (4) to 15 mN·m^{-1} (arrows) after the plateau level is reached for PLA_2 at 0.025 μg/mL is also shown.

Experiments of monolayer transfer (Rietsch et al., 1977) were done as described recently (Bianco et al., 1990). Mixed films of dlPC and GM1 (2:1) at 15 mN·m^{-1} (a pressure at which no enzyme activity is detected; Bianco et al., 1989) were employed. Ten minutes after injection of the enzyme into the subphase, the monolayer was transferred (enclosed between two syncronous barriers to keep the surface pressure constant at 15 mN·m^{-1}) onto another subphase without PLA_2 and rinsed. The film was kept for 8 min on the subphase without enzyme and transferred back, at 15 mN·m^{-1}, to the inital compartment filled with a fresh subphase, without enzyme. After this, the film pressure was rapidly brought to 5 mN·m^{-1}, and the extent of dlPC degradation was recorded (see also Figure 1).

Results

The dashed line in Figure 2 corresponds to the kinetics of the PLA_2 activity toward a film of pure dlPC at 15 mN·m^{-1}. After a lag time of 2.5 ± 0.4 min (average of three experiments) the enzyme activity proceeds with a constant velocity of (2.4 ± 0.2) × 10^{14} molecules·min^{-1}·cm^{-2}. The kinetics is zero order in this case because of the continuous supply of pure dlPC from the reservoir. After about 21 min an amount of dlPC equivalent to that initially present at the interface of the reaction compartment has been degraded and replaced, at constant pressure, by substrate molecules from the reservoir.

In a film consisting initially of a mixed monolayer of GalCer–dlPC (1:1) the percentage of dlPC degraded at 15 mN·m^{-1} reaches only about 34% after 14 min and remains constant thereafter (Figure 2). In the range of enzyme concentration employed, the portion of constant enzyme velocity after the lag time is linearly proportional to the amount of enzyme in the subphase, with rates similar to those obtained under zero-order kinetics (Bianco et al., 1989, 1990). The temporary pseudo-zero-order kinetics is due to the relatively high substrate excess during this period. Figure 2 shows that the maximum percentage of dlPC degraded in the presence of GalCer does not depend on the initial rate of activity or enzyme concentration but it is very sensitive to the surface pressure. The amount of dlPC degraded at 5 mN·m^{-1} shows a plateau reaching only about 17% after 10 min (Figure 2, curve 3). If, after this plateau level is reached, the surface pressure is rapidly (within 20 s) increased to 15 mN·m^{-1}, the enzyme starts to degrade the phospholipid with no measurable lag time. A new plateau is obtained after about 8 min with almost the same value of dlPC degradation as when the surface pressure is set from the beginning at 15 mN·m^{-1} (Figure 2). This also indicates that the inability of the enzyme to degrade more than about 17% of the dlPC available at 5 mN·m^{-1} is not due to an irreversible inactivation by GalCer.

At constant surface pressure, PLA_2 exhibits a lower rate of activity against monolayers of dlPC–GalCer in equimolar proportions compared with a film of pure dlPC; if the molar fraction of GalCer is higher than 0.5, the enzyme velocity increases (Bianco et al., 1989). Therefore, it could be expected that an increase of the proportion of GalCer above the 1:1 ratio with respect to dlPC would have facilitated further degradation of the phospholipid. However, this effect is not observed, and even at the optimum pressure of 15 mN·m^{-1} [see Bianco et al. (1989)], the enzyme cannot degrade more than about 34% of dlPC in the presence of GalCer. The composition of the film at this plateau level corresponds to a molar fraction of GalCer of about 0.66 (Figure 2).

We have reported previously that in monolayers containing Sulf at a molar ratio of 0.5 or above the enzyme exhibits a greater velocity than that toward pure dlPC under zero-order kinetics (Bianco et al., 1989, 1990). However, under the conditions of variable composition employed in this work the enzyme cannot degrade more than about 54% (at 5 mN·m^{-1}) or 84% (at 10 mN·m^{-1}) of the available dlPC even if the molar fraction of Sulf has progressively increased to 0.77 and 0.92, respectively (Figure 3, curves 3 and 4). Similar to the effects of GalCer, the maximum amount of dlPC degraded depends on the surface pressure. At 15 mN·m^{-1} the percentage of substrate degraded in films containing Sulf reaches 100%, and the velocity depends on the amount of enzyme (Figure 3, curves 1 and 2). As above, the arrest of the enzyme action at lower pressures is not due to irreversible inhibition (at least at 5 and 10 mN·m^{-1}). The activity is immediately restored without lag time if the surface pressure is rapidly brought to 15 mN·m^{-1}. The degradation of dlPC reaches 100% in about 5–10 min after the pressure change (Figure 3, curves 3 and 4).

The zero-order PLA_2 activity against monolayers containing relatively low proportions of ganglioside GM1 is markedly decreased or totally inhibited, depending on the ganglioside molar fraction (Bianco et al., 1989, 1990). However, in spite of the diminished enzyme velocity, all of the available dlPC in the mixed film with GM1 at 10 mN·m^{-1} is degraded after about 35 min under conditions of variable composition (Figure 4, curves 1 and 2). After 15 min the proportion of dlPC degraded at 10 mN·m^{-1} is between 50 and 60% (depending on the amount of enzyme). This corresponds to a change of the molar fraction of GM1 in the film from 0.33 (the initial proportion) to about 0.70. We emphasize that *no enzyme activity* is detected when PLA_2 is injected beneath a film containing, *initially*, a proportion of GM1 greater than 0.5 (Bianco et al., 1989, 1990). However, a further 50% of dlPC can be degraded if the enzyme interacted with the film when

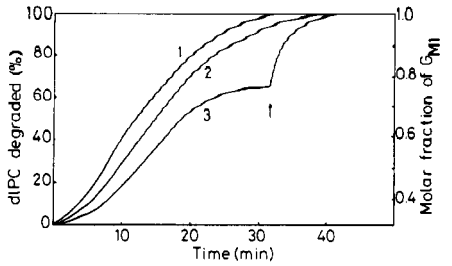

FIGURE 4: Effect of GM1 on the extent of dlPC degradation by PLA_2. The percent of dlPC degraded is shown as a function of time after injection of the enzyme (time zero) at 0.050 (1) or 0.025 μg/mL (2) beneath a monolayer of GM1–dlPC (initial molar fraction of GM1 of 0.33) at 10 mN·m^{-1}. The effect of changing the surface pressure from 5 (3) to 10 mN·m^{-1} (arrow) after the plateau level is reached for PLA_2 at 0.025 μg/mL is also shown.

the initial molar fraction of ganglioside was 0.33 (Figure 4). Once degradation started, the subsequent increase of the ganglioside proportions does not stop the enzyme action. Again, the plateau level of dlPC degradation depends, in addition, on the surface pressure. Only about 64% of the dlPC can be hydrolyzed at 5 mN·m^{-1}. An immediate increase in activity is obtained if the surface pressure is increased to 10 mN·m^{-1}; complete degradation of dlPC on the reaction compartment is reached after 10 min (Figure 4, curve 3).

With film transfer, we investigated more directly if the enzyme could interact with the film when no activity was detected. In film-transfer experiments (Figure 5, lines 1 and 2) the profile for the time course of the enzyme action is similar to that found without transferring the film (Figure 4). In different experiments (including pure films of dlPC to control the level of enzyme activity remaining after transfer; see dashed line in Figure 5) the values of enzyme velocity and plateau levels were about 30–40% and 10–20% lower, respectively, than for experiments without film transfer. This is due to inevitable losses during transfer and/or to less protein interacting with the film at the initially higher surface pressure (Bianco et al., 1989, 1990; Fidelio et al., 1986c; Pattus et al., 1979). Nevertheless, the results clearly show that, even when no activity is detected against a film of dlPC–GM1 at 15 mN·m^{-1}, the enzyme is capable of adsorbing to the interface, it remains associated to the film during transfer and rinsing, it regains activity at a suitable surface pressure, and it can degrade a percentage of dlPC similar to that found in the experiments without film transfer.

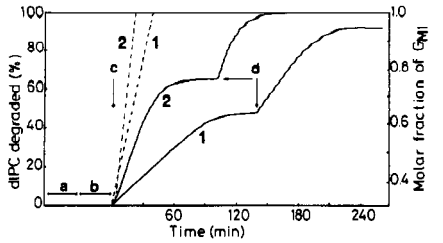

FIGURE 5: Experiments of film transfer. (Curve 1) At time zero 0.025 μg/mL enzyme is injected beneath a monolayer of GM1–dlPC (initial molar fraction of GM1 of 0.33). The surface pressure is maintained at 15 mN·m^{-1} (above the cutoff pressure; Bianco et al., 1989) for 10 min (a). Subsequently, the film was transferred at the same constant surface pressure onto another compartment, rinsed over a subphase without enzyme, and brought back to the initial compartment filled with enzyme-free subphase (b). The arrows at (c) and (d) mark the changes of surface pressure to 5 and 10 mN·m^{-1}, respectively. The results are similar if the change of surface pressure is done immediately after step a, without bringing the monolayer back to the initial compartment (this eliminates the rinsing step but allows for desorption). The activity against a film of dlPC–GM1 of the same composition but without transfer (step b not performed) is shown as curve 2 (see also Figure 4). Dashed line 1 shows a control experiment with a film of pure dlPC in the presence of 0.025 μg/mL enzyme: the film was (a) at an initial surface pressure of 22 mN·m^{-1} (above the cutoff pressure; Bianco et al., 1989), (b) it was transferred and rinsed at this pressure, and (c) the pressure was changed to 5 mN·m^{-1}. The control of this reaction, without film transfer (step b not performed), is shown as dashed line 2 (see also Figure 2). The time scale in the abscissa barely allows detection of the presence of a lag time of about 3–5 min [see also Figures 2 and 4 and Bianco et al. (1989, 1990)] in both curves 2 but not in curves 1 in the actual experiments.

Discussion

Three modes of reversible inhibition of the interfacial catalysis of PLA_2 against bilayer vesicles have been postulated (Jain et al., 1989). Briefly, in mode 1 the inhibitor at the interface competes with the substrate for binding to the catalytic site of the adsorbed enzyme. In mode 2 the inhibition is brought about by changes of the physical organization of the interface that promote enzyme desorption. In mode 3, a soluble inhibitor prevents enzyme binding to the interface. In monolayers, the effect of gangliosides apparently does not fit into the above mechanisms for the following reasons: (a) Gangliosides or GalCer (Sulf does not apply here because it activates the enzyme) do not compete with the substrate for the catalytic site because they lack the region of acyl ester groups. If the constituent oligosaccharide moieties of GSLs (i.e., sialic acid and carbohydrates) or the lipids as monomers (below their critical micellar concentration but still in molar ratios over 10:1 with respect to PLA_2) are preincubated with the enzyme in the subphase, they do not alter the enzyme activity against a film of pure dlPC (Bianco et al., 1989, 1990). These results are not compatible with mode 1. (b) The enzyme associates and remains with the ganglioside-containing monolayer during film transfer and rinsing, and it regains activity at a suitable surface pressure; this is against mode 2 (see Figures 4 and 5). (c) Gangliosides do not prevent enzyme adsorption (see Figures 4 and 5) necessary for mode 3. PLA_2 can hydrolyze all the substrate present in the outer monolayer of vesicles containing substrate and inhibitor (Upreti & Jain, 1980). This is not in keeping with the results found for films with GalCer, but it may be compatible with the effects of gangliosides.

Changes of the intermolecular organization and interfacial accessibility to the phospholipid acyl ester region or impairment of an earlier essential activation step is involved in the modulatory effect of GSLs. The rate at which the enzyme can effectively degrade dlPC is markedly dependent on the GSL composition and the surface pressure (Bianco et al., 1989, 1990). However, once the enzyme associates to the interface, its sensitivity to the intermolecular packing and lipid composition changes dramatically. The velocity may be either increased or decreased, but the enzyme can still degrade dlPC even after the proportion of GSLs exceeded the amount that, if present initially, would have permitted a very different activity. This shows that there are at least two independent levels at which the modulatory action of GSLs is exerted. One appears related to the initial activation step or interaction of PLA_2 with the interface; another controls the molecular organization or availability of substrate (or enzyme) for the expression of activity after the surface association. Also, our results imply that there is a very low exchange, if any, between the enzyme in the subphase and the adsorbed enzyme. Therefore, a large number of catalytic cycles are performed by the enzyme without desorption. This is in agreement with previous conclusions of Pattus et al. (1979) and Jain et al. (1986).

Several molecular details on the complex interfacial modulation of phospholipase activity have been unraveled by

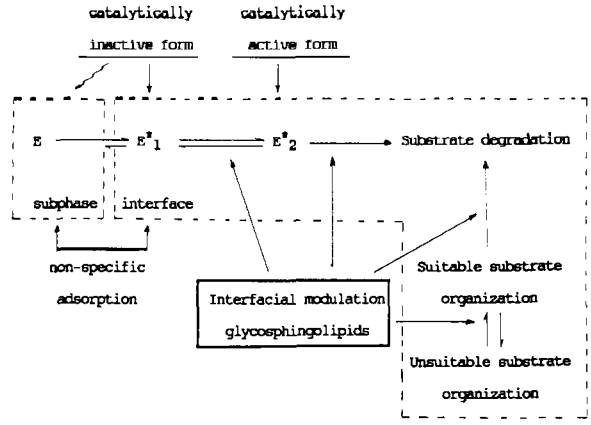

FIGURE 6: Illustration of possible levels at which the modulation of PLA_2 by glycosphingolipids may occur.

different groups of investigators (Bell & Biltonen, 1989a,b; Jain & Berg, 1989; Jain & Vaz, 1987; Jain et al., 1986, 1989; Lichtenberg et al., 1986; Menashe et al., 1986; Pieterson et al., 1974; Roberts et al., 1977; Romero et al., 1987; Upreti & Jain, 1980; Verger et al., 1973; Verger & Pieroni, 1986). Our results are compatible with the above mechanisms based on the existence of two independent steps.

The scheme in Figure 6 may help illustrate the effects of GSLs observed. When the enzyme is adsorbed to a GSLs-containing interface, it may reach state E^*_1. In this state it could remain inactive but associated with the surface for a relatively long time without appreciable desorption or exchange with the soluble enzyme (E) (see Figure 5). If the surface organization becomes favorable, the enzyme may reach state E^*_2 and express catalytic activity. The GSLs do not impair the interaction of the enzyme with the lipid interface. However, they lead to an intermolecular organization at the surface that may interfere (GalCer, gangliosides) or facilitate (Sulf) a subsequent and necessary activation step or access to the substrate acyl ester region. This depends on the surface pressure and composition. Our results are compatible with the scheme in Figure 6 if the interfacial requirements for the expression of a particular level of catalytic activity for state E^*_2 are different from those required to reach state E^*_1.

Various surface phenomena elicited by the presence of GSLs (Maggio et al., 1985, 1987, 1988a) provide some insights as to the possible molecular basis for the effects observed. Thus, lateral defects due to packing incompatibilities (Bianco & Maggio, 1989; Maggio et al., 1978a,b) or coexistence of different surface domains (Maggio et al., 1985b, 1987, 1988a) are present when the proportion of substrate is high. These inhomogeneities are known to facilitate phospholipase activity (Lichtenberg et al., 1986; Menashe et al., 1986; Romero et al., 1987; Upreti & Jain, 1980). The system becomes more homogeneous as the proportion of phospholipid decreases (Maggio et al., 1985b). In addition, it has been shown that interfaces enriched in gangliosides are more polar and hydrated (Bianco et al., 1988; Bach et al., 1982; Fidelio et al., 1986a,b; Montich et al., 1985, 1988). This would also interfere with the dehydration step required for the enzyme to interact with the lipid interface (Jain & Vaz, 1987). Concomitantly, the activity in the presence of ganglioside gradually stops at low proportions of phospholipid.

The oligosaccharide chain of gangliosides protrudes into the aqueous phase, and the negatively charged sialosyl residues are at least 1 nm away from the interface (Maggio et al., 1978a, 1985, 1988; McDaniel et al., 1986). If bound to this region, the enzyme may be in a difficult position to reach the phospholipid ester bonds to be cleaved. In addition, gangliosides show thermodynamically favorable interactions, tighter packing, and strong dipolar interactions with phosphatidylcholine (Maggio et al., 1978b, 1980; Bianco & Maggio, 1989). This probably interferes with the enzyme action. However, only the enzyme velocity is affected, and the ganglioside effect is not due to irreversible substrate sequestering or enzyme inactivation. It is evident that various intermolecular variables regulate the enzyme action. Their combination and resultant effect cannot be generalized for all systems on similar bases. Different surface phenomena occur in the presence of Sulf, and the enzyme becomes more active against these films. The negatively charged polar head group is shorter in Sulf than in gangliosides (Maggio et al., 1978a, 1983). This can facilitate the enzyme action as described for other negatively charged amphiphiles with small polar head groups (Pieterson et al., 1974; Upreti & Jain, 1980). Also, different from those of gangliosides, the interactions of Sulf with the phospholipid are not thermodynamically favored (Bianco & Maggio, 1989). The unfavorable interactions with Sulf are implicitly associated with increased surface defects leading to enhanced pressure and molecular area fluctuations [see Phillips et al. (1975)]. This should facilitate an increased enzyme activity.

On the other hand, the electrostatic field across the interface affects remarkably and reversibly the PLA_2 activity. The enzyme is more active when the interface is hyperpolarized while its activity diminishes in depolarized interfaces (Thuren et al., 1987). The dipolar component of the oligosaccharide chain decreases the resultant dipole moment of the ganglioside molecules (Maggio et al., 1978a, 1985a, 1988; Beitinger et al., 1989). As a consequence, the mixed interface with dlPC is locally depolarized at the molecular level compared to the pure substrate (Bianco & Maggio, 1989; Maggio et al., 1978a; Beitinger et al., 1989). At least qualitatively, the variations of phospholipase activity are in keeping with the changes of dipolar properties and surface organization induced by GSLs. Recent findings on the penetration ability of monoacylated PLA_2 mutants into phospholipid monolayers (van der Wiele et al., 1988) and the interfacial activation of *Agkistrodon piscivorus piscivorus* PLA_2 by autoacylation (Cho et al., 1988) suggest additional levels for the modulation of the PLA_2 activity by GSLs.

Registry No. PLA_2, 9001-84-7; dlPC, 18194-25-7; GalCer, 85305-88-0; GM1, 37758-47-7.

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