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Evidence for the Control of the Action of Phospholipases A by the Physical State of the Substrate†

Tom Thuren, Petri Vainio, Jorma A. Virtanen, Pentti Somerharju, Kurt Blomqvist, and Paavo K. J. Kinnunen*

ABSTRACT: Fluorescence and monolayer techniques were used as complementary methods to study the effect of NaCl on (i) the conformation of phosphatidylglycerol present in vesicles or monolayers and on (ii) the activities of phospholipases A on these lipids. The mean molecular area in monolayers of 1,2-didodecanoyl-*sn*-glycero-3-phospho-*rac*-glycerol ($diC_{12}PG$) at an air/water interface was measured and at a surface pressure of 15 dyn cm^{-1} did expand from 63 to 76 Å molecule when the NaCl concentration was increased from 0 to 1.0 M, respectively. Simultaneously with the increase in the salt concentration up to 1.0 M pancreatic phospholipase A_2 (PLA_2) was inhibited by 95% whereas bovine milk lipoprotein lipase here employed as phospholipase A_1 (PLA_1) was activated. No PLA_1 activity could be measured in the absence of NaCl. The electrolyte had no effect on the penetration of these enzymes into a film of a nonhydrolyzable substrate analogue, 1,2-dihexadecyl-*sn*-glycero-3-phospho-*rac*-glycerol (diEPG). The NaCl-induced changes were further examined by using vesicles of 1,2-bis[(pyren-1-yl)butanoyl]-*sn*-glycero-3-phospho-*rac*-glycerol (diPBPG). The ratio of pyrene excimer to monomer fluorescence intensities (I_e/I_m) increased from 2.9 to 3.3 as the NaCl concentration was increased from 0 to 0.5 M. Hydrolysis of diPBPG by PLA_1 was enhanced by salt 2-fold whereas the activity of PLA_2 was inhibited by 90%. The salt-induced conformational changes in phosphatidylglycerol could be connected with the altered accessibilities of *sn*-1 and *sn*-2 ester bonds to enzymatic hydrolysis. Briefly, it can be assumed that phosphatidylglycerol at low ionic strength is in the so-called "kinked" conformation, the glycerol backbone extending parallel to the *sn*-1 acyl chain, whereas the *sn*-2 chain starts in its first methylene segments parallel to the surface and then bends to align the *sn*-1 chain. At high ionic strength the glycerol backbone should be parallel to the plane of the interface with both acyl chains starting perpendicular to the surface. In the latter conformation an increased molecular surface area for $diC_{12}PG$ monolayer is observed. In spite of the predicted salt-induced increased average spacing between the phospholipids, an enhanced rate of pyrene excimer formation in diPBPG vesicles is evident. The measured salt-induced increase in I_e/I_m of diPBPG is likely to be due to intramolecular excimer formation, thus reflecting the parallel and equal alignment of the (pyren-1-yl)butanoyl chains.

Phospholipases A_1 and A_2 (PLA_1 and PLA_2)[1] hydrolyze respectively the *sn*-1 and *sn*-2 fatty acyl ester bonds of the naturally occurring *sn*-3 phospholipids (de Haas, 1968). These enzymes are found in mammalian both extracellularly serving

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[1] Abbreviations: $diC_{12}PG$, 1,2-didodecanoyl-*sn*-glycero-3-phospho-*rac*-glycerol; diEPG, 1,2-dihexadecyl-*sn*-glycero-3-phospho-*rac*-glycerol; diPBPG, 1,2-bis[(pyren-1-yl)butanoyl]-*sn*-glycero-3-phospho-*rac*-glycerol; cmc, critical micellar concentration; ffa, free fatty acid; I_e, pyrene excimer emission intensity; I_m, pyrene monomer emission intensity; PLA, phospholipase A; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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digestive functions and intracellularly taking part in regulatory functions connected to, e.g., the generation of prostaglandins and metabolism of cell membranes (Verheij et al., 1981; van den Bosch, 1980). PLA_2 was first purified to homogeneity from pancreatic tissue (de Haas, 1968). PLA_2 is also found in snake and bee venoms (Verheij et al., 1981). Amino acid sequences of pancreatic PLA_2 from a variety of species are known. Recently, the sequence of the human enzyme has been established (Verheij et al., 1983). Likewise the amino acid sequences of PLA_2 from snake and bee venoms have been worked out [for a review, see Verheij et al. (1981)]. Crystal structure of bovine pancreatic PLA_2 has been elucidated at 1.7-Å resolution (Dijkstra et al., 1981a).

Far less is known about the structural and functional properties of PLA_1. This is probably due to the fact that they are less abundant, their molecular weights are higher, and they are generally rather labile enzymes (van den Bosch, 1982).

PLAs belong to esterases hydrolyzing water-insoluble substrates. Although PLA_2 can hydrolyze monomeric substrate molecules, substrates present in an organized lipid–water interface are attacked at a much higher rate (Roholt & Schlamowitz, 1961; de Haas et al., 1968; Wells, 1972). Thus, even 10^4 times higher activities can be observed upon increasing the substrate concentration above the cmc of the substrate (Wells, 1978). This characteristic kinetic property of PLA_2 is best observed in an enzyme activity vs. substrate concentration curve and is not shared by its zymogen prophospholipase A_2 which can only act on monomeric phospholipids (de Haas et al., 1971; Pieterson et al., 1974). Prophospholipase A_2 obeys Michaelis–Menten kinetics (Pieterson et al., 1974) as does the PLA_1 activity of bovine milk lipoprotein lipase (Shinomiuya & Jackson, 1983).

The molecular mechanism(s) of interfacial activation of PLA_2 has been a subject of intensive studies. In spite of the efforts made there is no conclusive unambiguous explanation for this phenomenon. At present two viewpoints appear most attractive: (a) One is the "substrate theory", which assumes that the substrate molecules upon binding to an interface undergo a change in their conformation, rendering the *sn*-2 ester bond more susceptible for the action of PLA_2 [Wells, 1972, 1974, 1978; for a review, see Dennis et al. (1981)]. (b) The other viewpoint is the "enzyme theory", which assumes a conformational change to occur in the enzyme upon adsorption to the lipid–water interface (Verger et al., 1973; Pieterson et al., 1974; van Dam-Mieras et al., 1975). These two alternatives need not be mutually exclusive. The validity of the substrate hypothesis can only be estimated after the physicochemical properties of the aggregated substrate are fully understood. Verger et al. have suggested a set of interfacial parameters describing the "quality" of the interface and regulating the rate of PLA_2-catalyzed hydrolysis by inducing a conformational change in the enzyme (Zografi et al., 1971, Verger et al., 1980). Thus, it is generally accepted that the physicochemical state of the substrate—the quality of the interface—plays an important role in determining the catalytic activity of PLA_2.

The purpose of the present study was to explore the possible role of the conformational changes of the substrate responsible for controlling the action phospholipases A. Monolayer and fluorescence techniques were used to observe NaCl-induced changes in the conformation of phosphatidylglycerol, and the measured physical parameters are compared to the accessibilities of *sn*-1 and *sn*-2 ester bonds to the action of PLA_1 and PLA_2, respectively. Phosphatidylglycerol is avidly hydrolyzed by both of these enzymes, and optimal conditions including surface pressure have been established (Verger et al., 1976, Vainio et al., 1983a). Notably phosphatidylcholines could not be used as monolayers of this lipid have been observed to be for unknown reasons nonhydrolyzable by both lipoprotein lipase and pancreatic lipase (Pieroni & Verger, 1979; Jackson et al., 1979; Vainio et al., 1983b).

Materials and Methods

Lipids. 1,2-Bis[(pyren-1-yl)butanoyl]-*sn*-glycerol-3-phosphocholine, 1,2-didodecanoyl-*sn*-glycero-3-phosphocholine, and 1,2-dihexadecyl-*sn*-glycero-3-phosphoglycerol (diEPG) were purchased from KSV-Chemicals Oy (Valimotie 7, Helsinki 38, Finland). Corresponding phosphatidylglycerols were prepared by phospholipase D catalyzed transphosphatidylation (Comfurius & Zwaal, 1977). No impurities were detected upon thin-layer chromatography on silicic acid using chloroform/methanol/water/ammonia (90/55/5.5/5.5 v/v/v/v) as as the solvent system. Critical micellar concentration (cmc) of diPBPG was obtained from the break occurring at 3 μM lipid concentration vs. pyrene I_e/I_m. This value is in good agreement with the cmc of 7.3 μM reported for the corresponding phosphatidylcholine (Hendrickson & Rauk, 1981).

Enzymes. Porcine pancreatic PLA_2 was from Sigma (specific activity approximately 600 μmol of free fatty acid min^{-1} mg^{-1}) and appeared as a single Coomassie brilliant blue stained band upon gel electrophoresis in 12% polyacrylamide in the presence of sodium dodecyl sulfate (Laemmli, 1970). Porcine pancreatic prophospholipase A_2 was a generous gift from Drs. A. H. Slotboom and G. H. de Haas, Utrecht, The Netherlands. Lipoprotein lipase was used as PLA_1 (Scow & Egelrud, 1976) and was purified from bovine skimmed milk (Kinnunen, 1977). Experiments with lipoprotein lipase (PLA_1) were performed in the absence of its specific activator apolipoprotein CII, which enhances both the triacylglycerol-hydrolyzing and phospholipid-hydrolyzing activity of lipoprotein lipase (Havel et al., 1973; Kinnunen et al., 1977; Groot et al., 1978; Vainio et al., 1983b).

Fluorescence Measurements. Vesicles of diPBPG were prepared by transferring 382.5 μg (450 nmol) of this lipid in toluene/ethanol solution (1/1 v/v) into a conical glass test tube. After removal of the solvent under a stream of nitrogen, the lipid was hydrated with 1.5 mL of 20 mM Tris-HCl buffer, pH 7.4, and sonicated for 1 min on an ice-water bath with a Branson sonifier-cell disruptor equipped with a microtip. The sonicated lipid was kept on ice. Of this solution 0.1 mL (corresponding 30 nmol of diPBPG) was taken into 1.9 mL of the above Tris buffer to yield 15 μM lipid. Intensities of the pyrene excimer (I_e) and monomer (I_m) emission of diPBPG were recorded with a SLM-4800S spectrofluorometer using magnetically stirred four-window quartz by cuvettes thermostated to 37 °C with a circulating waterbath. Prior to each measurement the cuvette with its contents was allowed to equilibrate for 5 min. Excitation wavelength was 345 nm, and emission wavelengths were 400 and 480 nm for I_m and I_e, respectively. Three readings were taken by using a signal average of 100. A spectral band-pass of 4 nm was used for both exciting and emitting light.

Hydrolysis of 15 μM diPBPG by PLA_1 and PLA_2 was measured with a Kontron SFM 23 spectrofluorometer equipped with a chart recorder. The instrument has fixed slits for both exciting and emitting light with a spectral band width of 10 nm. The reaction was monitored by following the increase in the intensity of the monomer emission at 400 nm as a function of time (Hendrickson & Rauk, 1981; Thuren et al., 1983). Addition of 0.1 μg of PLA_2, 1.0 μg of prophospholipase

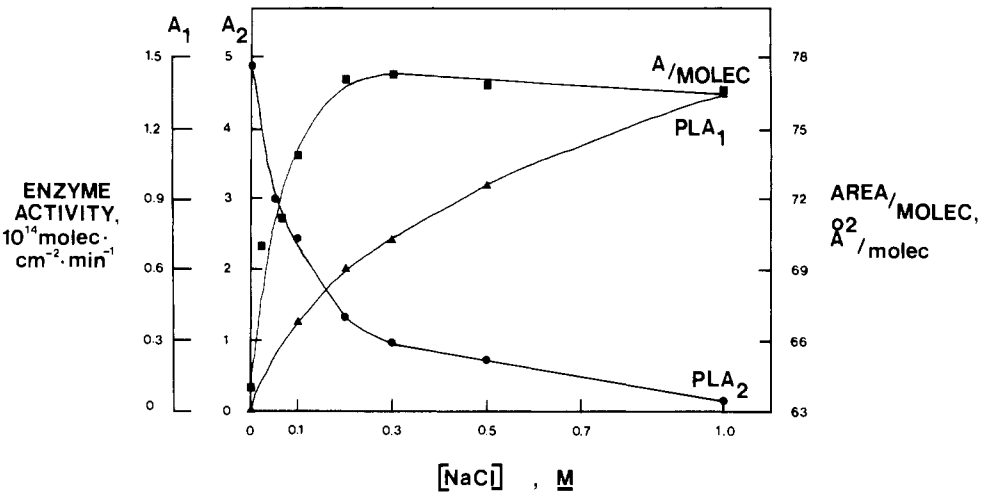


FIGURE 1: Activities of phospholipases A and the mean molecular area of $diC_{12}PG$ film spread on an air/water interface as a function of NaCl concentration. (●) Pancreatic PLA_2 activity, (▲) bovine milk PLA_1 activity, and (■) mean molecular area of $diC_{12}PG$. The enzymatic hydrolysis of monolayers was determined at a constant surface pressure of 15 dyn cm^{-1}. The values for area per molecule at this surface pressure were calculated from compression isotherms. See Materials and Methods for experimental details.

A_2, or 5.0 μg of PLA_1 started the reaction. Calibration of the assay was done by subsequent additions of 20-pmol aliquots of pyren-1-ylbutanoic acid to the reaction mixture in the absence of the enzyme to a final concentration of 50 nM while recording the increase in I_m. Results are expressed as the quantity of free fatty acids (ffa) released per minute.

Monolayer Experiments. All experiments were carried out in a thermostated metallic box at 25 ± 0.5 °C; the aqueous subphase of the reaction compartment was thermostated to 25 ± 0.5 °C with an immersed glass coil and agitated with a magnetic stirrer turning at 250 rpm. Before each experiment the trough was carefully cleaned with ethanol and rinsed with tap water and finally with distilled water.

Compression isotherms were obtained with a rectangular Teflon trough (169 × 280 nm) by using a constant compression velocity of 79.4 cm^2 min^{-1}. The subphase consisted of quartz-distilled water containing the indicated concentrations of NaCl with pH adjusted to 7.4 by adding HCl.

Surface barostat method with a mobile Teflon barrier was used to determine the rates of hydrolysis of $diC_{12}PG$ monolayers by PLA_1 and PLA_2 at a constant pressure (Verger & de Haas, 1973). The trough consisted of a reaction compartment (total volume 210 mL; total surface 124.5 cm^2) and a reservoir (169 × 280 mm) essentially as described by Verger and de Haas.

Monolayers of $diC_{12}PG$ were spread from chloroform solution of this lipid with an Agla micrometer syringe (Wellcome) on a subphase of 20 mM Tris-HCl, pH 7.4, containing the indicated amounts of NaCl. Enzymatic reactions were performed at a constant surface pressure of 15 dyn cm^{-1} which was chosen as it is the optimal surface pressure for PLA_1 (Vainio et al., 1983a) while also PLA_2 is capable of hydrolyzing $diC_{12}PG$ films at this surface pressure, the optimum being at 20–22 dyn cm^{-1} (Verger et al., 1976). Reactions were started with an injection of 1.0 μg of PLA_2 or 5.0 μg of PLA_1. Results are expressed as the number of molecules hydrolyzed cm^{-2} min^{-1}.

Penetration experiments were performed by using cylindrical wells (volume 8.0 mL; surface 6.16 cm^2) drilled in Teflon. Monolayers of diEPG were spread from chloroform solution to initial surface pressure of 8, 12, or 15 dyn cm^{-1}. The aqueous subphase consisted of 20 mM Tris-HCl, pH 7.4, and the given NaCl concentrations. The enzyme was injected under the diEPG monolayer, thereafter monitoring the increase in the surface pressure for 30 min (MacRitchie, 1968); either 5.0 μg of PLA_2 or 1.0 μg of PLA_1 was used.

Results

Hydrolysis of $diC_{12}PG$ Monolayers by PLAs. Effects of ionic strength on the expression of the catalytic activity of PLA_1 and PLA_2 toward $diC_{12}PG$ monolayers are illustrated in Figure 1. Increasing the salt concentration from 0.0 to 1.0 M inhibited PLA_2 by 95%. In contrast PLA_1 was activated by salt. No PLA_1 activity could be detected in the absence of salt.

In order to obtain a semiquantitative estimate on the effect of NaCl on the binding of PLA to substrate monolayers, penetration of the enzymes into a film of a nonhydrolyzable substrate analogue diEPG was determined (Figure 2). Concentrations of NaCl up to 0.5 M had only insignificant effect on the binding of the two enzymes to the film.

Hydrolysis of diPBPG Vesicles by PLAs. The rates of hydrolysis of diPBPG vesicles by PLA vs. NaCl concentration are shown in Figure 3. Increasing the salt concentration to 0.5 M inhibited pancreatic PLA_2 by approximately 90%, whereas the activity of PLA_1 was increased 2-fold.

Under identical conditions the electrolyte exerted no effect on the activity of $proPLA_2$. The specific activity of the zymogen was 2% of that of the active enzyme when the latter was measured in the absence of NaCl (data not shown).

Evidence for NaCl-Induced Conformational Changes in Phosphatidylglycerol. In contrast to the DLVO theory, lowering the concentration of NaCl in bulk condenses monolayers of phosphatidylglycerol (Tocanne et al., 1974a,b) and phosphatidylserine at an air/water interface (Phillips et al., 1975). In the present study this was confirmed for phosphatidylglycerol. Increasing the NaCl concentration from 0 to 1.0 M increased at 15 dyn cm^{-1} the limiting area per phospholipid molecule from 63 to 76 Å (Figure 1). An increase of 0.9 Å in the average intermolecular spacing in monolayers could be calculated. This observation cannot be due to dissociation of residual Ca^{2+} from the film by NaCl as essentially similar results were obtained in the presence of a bulk phase containing 5.0 mM EDTA. On the other hand, when diPBPG vesicles were used, 0.5 M salt increased the I_e/I_m of pyrene fluorescence from 2.9 to 3.3 (Figure 3).

Discussion

Sodium chloride has been found to enhance the activity of PLA_2 toward micellar phosphatidylcholine (de Haas et al., 1971). By use of the monolayer technique, the independence

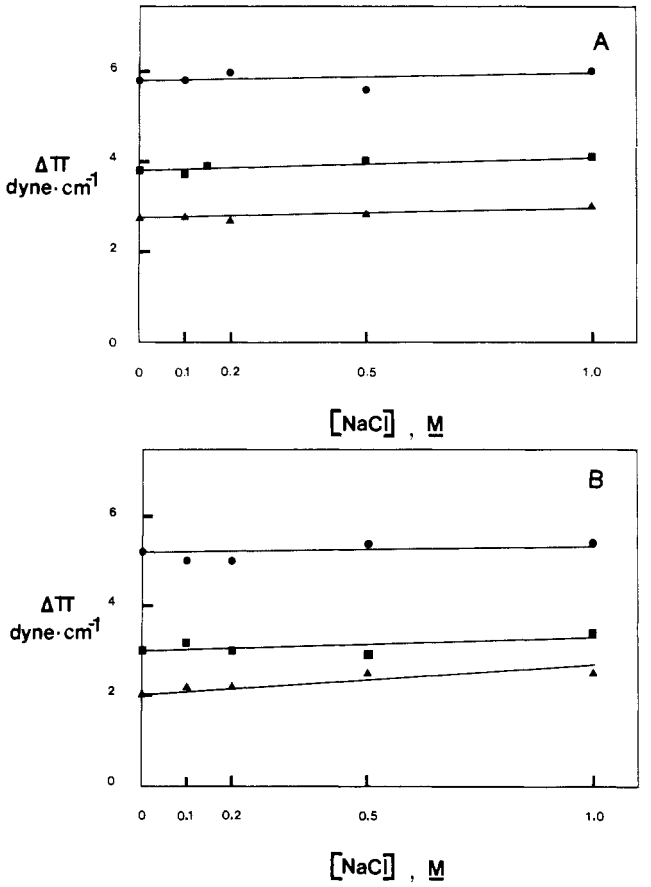


FIGURE 2: Penetration of phospholipases A in the diEPG monolayer. (A) pancreatic PLA_2; (B) bovine milk PLA_2. Increase in the surface pressure was recorded 30 min after injecting the enzyme under a monolayer of diEPG spread at different initial surface pressures of 8 (●), 12 (■) and 15 dyn cm^{-1} (▲). $\Delta\pi$ is the difference between the initial surface pressure and the surface pressure 30 min after the enzyme injection. The amounts of PLA_1 and PLA_2 used were 1.0 and 5.0 μg, respectively.

of the hydrolysis of phosphocholines by PLA_2 from the salt concentration in the range 0.0–0.3 M has been reported (Zografi et al., 1971). Wells has studied the influence of KCl on the kinetic parameters of the PLA_2 reaction and found that V_{max} is lowered except at concentrations of dihexanoyllecithin below 25 mM where V_{max} is increased in the presence of 1 M KCl (Wells, 1974). The effect of NaCl on the activity of PLA_2 thus appears to depend on the chemical nature of the substrate as well as on the quality of the substrate interface. In the present study two independent experimental techniques were used to determine the effect of NaCl on the accessibilities of *sn*-1 and *sn*-2 ester bonds of phosphatidylglycerol to the hydrolytic action of PLA_1 and PLA_2, respectively. The significant qualitative finding of the present study was the inhibition of PLA_2 and activation of PLA_1 by salt. NaCl had no effect on the binding of the two enzymes to a monolayer of nonhydrolyzable substrate analogue diEPG (Figure 2). This result suggests the salt-induced variations in the PLA activities to be unlikely explained by the electrolyte changing the degree of association of the enzymes with the substrate interface. However, in this experiment a diether phospholipid had to be used. The physicochemical differences between the diester and diether lipids may have an effect on their association with PLA. In accordance the interpretation of this experiment has to be taken as tentative only.

Other possible reasons for the inhibition of hydrolysis of phosphatidylglycerol by PLA_2 could be (i) dissociation of the mandatory Ca^{2+} from the enzyme active site by NaCl or (ii) direct inhibitory effect by NaCl on the chemical catalytic mechanism. We did not observe any effect by NaCl on pro-PLA_2. Due to the identical structure of the active sites of PLA_2 and proPLA_2, both (i) and (ii) are improbable. As proPLA_2 acts on monomeric phospholipids only this result further suggests that the monomer–vesicle partition of diPBPG is insignificantly altered by the electrolyte. Both (i) and (ii) are inconsistent with the observation that NaCl activates hydrolysis of short-chain phosphatidylcholine by PLA_2 at least up to a concentration of 4 M (de Haas et al., 1971). The active site of lipoprotein lipase here employed as PLA_1 is insensitive to the electrolyte concentration in the absence of the apolipoprotein CII activator (Fielding & Fielding, 1976). It is compelling to conclude that the effects of NaCl on the hydrolysis of phosphatidylglycerol by PLA_2 are due to NaCl-induced changes in the conformation of the phospholipid.

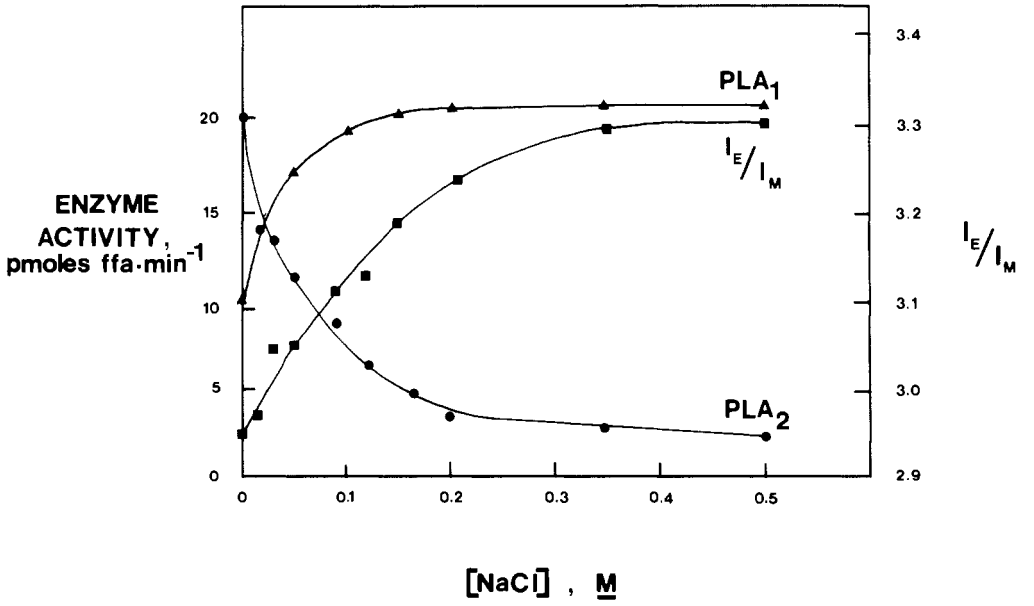
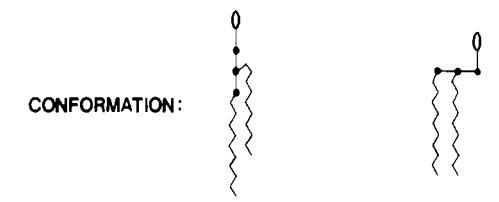


FIGURE 3: Effect of NaCl on the activities of phospholipase A and on the pyrene fluorescence of diPBPG. (●) pancreatic PLA_2 activity, (▲) bovine milk PLA_1 activity, and (■) the ratio of excimer to monomer emission intensities, I_e/I_m. The final concentration of diPBPG was 15 μM, the amount of PLA_1 was 1.0 μg, and the amount of PLA_2 was 0.1 μg. The total volume in the cuvette was 2.0 mL and consisted of 20 mM Tris-HCl buffer, pH 7.4, and appropriate amounts of NaCl.



CONFORMATION:		
di C_{12}PG		
[NaCl], M	0.0	1.0
AREA/MOLEC, Å^2	64.0	76.5
PLA ACTIVITY		
A_1	0.0	1.0
A_2	1.0	0.03
$(PB)_2$PG		
[NaCl], M	0.0	0.5
I_E/I_M	2.9	3.3
PLA ACTIVITY		
A_1	0.46	1.0
A_2	1.0	0.1

FIGURE 4: Schematic model relating the experimental results with the suggested changes in the phospholipid conformation controlling PLA action.

Changes in the substrate conformation have been suggested to regulate the action of PLA_2 (Wells, 1972, 1974; Dennis et al., 1979; Dennis, 1981). This hypothesis rests on ^1H and ^{13}C NMR studies of phospholipid micelles or mixed micelles of long-chain phosphatidylcholine with detergents (Roberts et al., 1978; Burns & Roberts, 1980; DeBony & Dennis, 1981) which revealed the α-methylenic protons of *sn*-1 acyl chain to be magnetically nonequivalent to those of the *sn*-2 acyl chain. No differentiation between *sn*-1 and *sn*-2 acyl chains is observed in monomeric phospholipids, and it has been proposed that a change in phospholipid conformation occurs upon phospholipid aggregation (Roberts et al., 1978; Burns & Roberts, 1980; Dennis et al., 1979, 1981). As shown by X-ray diffraction of lipid crystals (Hitchcock et al., 1974; Pearson & Pascher, 1979), by deuterium NMR, and by neutron diffraction of lipid multilayers (Oldfield et al., 1978; Seelig & Browning, 1978; Buldt et al., 1978), the *sn*-1 acyl chains extend parallel to the three carbons of the glycerol backbone and perpendicular to the interface while the *sn*-2 acyl chain starts parallel to the interface and then bends to parallel the *sn*-1 acyl chain. This conformation of phospholipids has been nominated as "kinked".

Results obtained in the present study suggest salt-induced conformational changes in phosphatidylglycerol to be related to the altered accessibilities of *sn*-1 and *sn*-2 ester bonds to enzymatic hydrolysis (Figures 1 and 3). The fluorescent phospholipid diPBPG can give rise to both intramolecular and intermolecular pyrene excimer fluorescence. Increasing the NaCl concentration caused an increase in I_e/I_m of diPBPG vesicles (Figure 3). NaCl increased the mean intermolecular distance of $diC_{12}PG$ in monolayers by 0.9 Å. Analgous behavior of diPBPG in vesicles should lead to decreased intermolecular excimer formation. The electrolyte-induced increase in I_e/I_m of diPBPG should therefore reflect the increased rate of intramolecular excimer formation which more than compensates for the decreased intermolecular process. This is in accordance with our preliminary results with liposomes of a monopyrene derivative, 1-palmitoyl-2-[(pyren-1-yl)hexanoyl]-*sn*-glycero-3-phospho-*rac*-glycerol, which can only give rise to the intermolecular excimer. For this lipid, the increase in NaCl causes the I_e/I_m to decrease as expected from an increase in the distance between the pyrene moieties (T. Thuren, J. A. Virtanen, and P. K. J. Kinnunen, unpublished results). Therefore, the salt-induced increase in the intramolecular excimer fluorescence of diPBPG is likely to require a change in the alignment of the acyl chains (Kinnunen & Virtanen, 1982).

We constructed a schematic model to explain the observations of the present study (Figure 4). In the absence of salt the conformation of the phospholipid should be kinked. In this conformation the area per molecule of $diC_{12}PG$ at 15 dyn cm^{-1} is 63 Å, and the I_e/I_m for diPBPG is 2.9. In this conformation phosphatidylglycerol is avidly hydrolyzed by PLA_2 whereas it does not provide a good substrate for PLA_1. It should be noted that according to the X-ray crystal structure of bovine pancreatic PLA_2, the three-dimensional structure of this enzyme is such that it can only accommodate a phospholipid into the active site when the substrate is in the kinked conformation (Dijkstra et al., 1981b). In the presence of salt the conformation of phosphatidylglycerol is altered. The mean molecular area of $diC_{12}PG$ at 15 dyn cm^{-1} is increased to 76 Å, and I_e/I_m of diPBPG is 3.3. Simultaneously, the action of PLA_2 is inhibited, and the activity of PLA_1 is enhanced. The glycerol backbone is now assumed to lie parallel to the plane of the interface, and both acyl chains should start perpendicular to the surface. In this conformation the lipid does not fit into the active site of PLA_2 but can be acted upon by PLA_1. Quantitatively the responses observed in the two systems used, monolayers of $diC_{12}PG$ and vesicles of diPBPG, differ. This is not surprising as the packing constraints for the lipids in monolayers must be different from those in vesicles. Also, saturation of the effects as a function of NaCl is distinct. Therefore, the proposed model should be considered essentially as a qualitative explanation for the experimental data.

Although the model proposed by us evidently lends support for the hypothesis of interfacial activation of PLA_2 to be due to altered conformation of substrate, the possibility still exists that proper conformation of the phospholipid is required to induce a conformational change in the enzyme, thus leading to enhanced catalytic rates.

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