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Control of the Action of Phospholipases A by "Vertical Compression" of the Substrate Monolayer

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Received January 6, 1987; Revised Manuscript Received April 21, 1987

ABSTRACT: Monolayers of *rac*-1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol at an air–water interface were "vertically compressed" by substituting an alkylated glass plate for air while maintaining a constant surface pressure of 15 mN m^{-1}. At this surface pressure the overlaying of the lipid film by the alkylated surface resulted in an average increase of 16 $Å^2$/molecule in the mean molecular area of those phospholipid molecules residing at the interface between water and the alkylated glass. Subsequently, the activities of phospholipases A1 and A2 toward the monolayers were measured both in the presence and in the absence of the support. While phospholipase A1 activity was increased 4-fold by the support, the activity of phospholipase A2 was reduced to 15% of the activity measured in the absence of the alkylated surface. These findings indicate that such a "vertical compression" of the monolayer is likely to induce a conformational change in the phospholipid molecules, which in turn would cause the above reciprocal changes in the activities of phospholipases A1 and A2. A molecular model accounting to these findings is presented.

Phospholipases A1 and A2 hydrolyze *sn*-1 and *sn*-2 fatty acyl ester bonds, respectively, of *sn*-3 phospholipids (de Haas et al., 1968). Phospholipase A2 (EC 3.1.1.4, PLA2)[1] is present in pancreatic tissue and juice and in snake and bee venoms [for a review, see Verheij et al. (1981)]. Intracellular PLA2s have been found in almost every type of cell studied (van den Bosch, 1980). PLA2s are the best characterized of the lipolytic enzymes, and their catalytic mechanisms, amino acid sequences, and X-ray crystallographic structures have been worked out (Verheij et al., 1981; Dijkstra et al., 1981a). Far less is known about the properties of phospholipases A1 [PLA1; for a review, see van den Bosch (1982)].

Phospholipases A belong to the group of esterases acting at lipid–water interfaces on water-insoluble substrates. Micellarization of the substrate strongly enhances the rate of catalysis (Verheij et al., 1981), and even 10^4 times higher activities are observed when the critical micellar concentration of the substrate phospholipid is exceeded (Wells, 1978). In spite of the efforts made, no general agreement exists on the molecular basis of this interfacial activation. The most attractive viewpoints are as follows: (a) the substrate theory,

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[1] Abbreviations: PLA, phospholipase A; $diC_{12}PG$, 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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which assumes changes in the substrate conformation to cause the enhanced activity of PLA2 [Wells, 1972, 1974, 1978; for a review, see Dennis et al. (1981)]; (b) the enzyme theory, which assumes that changes in the conformation of the enzyme molecule are responsible (Verger & de Haas,]973; Pieterson et al., 1974; van Dam-Mieras et al., 1975). Notably, these two possibilities are not mutually exclusive. It is generally accepted, however, that the physicochemical state of the substrate is one of the major determinants of PLA2 action.

We have forwarded evidence that the activities of PLA1 and PLA2 can be reciprocally regulated by electrostatically changing the physical state of the substrate (Träuble, 1977; Thuren et al., 1984; Kinnunen et al., 1985). Thus, PLA2 hydrolyzed more readily phospholipids in the so-called "kinked" conformation whereas PLA1 preferred phospholipids in the "extended" conformation. The latter state was induced by increasing the ionic strength of the aqueous phase.

In this study phospholipid monolayers were "vertically compressed" by an alkylated glass support. These monolayers were further used as substrates for pancreatic lipase (EC 3.1.1.3) acting as PLA1 (de Haas et al., 1965; Slotboom et al., 1970) and for pancreatic PLA2. These results provide further evidence that a change in the conformation of the substrate phospholipid could control the action of PLA.

Experimental Procedures

Synthetic 1,2-didodecanoyl-*sn*-glycero-3-phosphocholine was purchased from KSV Chemical Corp. (Valimotie 7, SF-00380 Helsinki, Finland). The corresponding phosphatidylglycerol ($diC_{12}PG$) was prepared by phospholipase D catalyzed transphosphatidylation (Comfurius & Zwaal, 1977). Upon thin-layer chromatography on silicic acid, no impurities were detected with chloroform/methanol/water/ammonia (90/55/5.5/5.5 v/v/v/v) as the solvent system.

Porcine pancreatic PLA2 was from Sigma 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). Purified porcine pancreatic lipase used as PLA1 was a generous gift from R. Verger. No contaminating PLA2 activity could be measured in this enzyme preparation by our fluorometric techniques (Thuren et al., 1983, 1985).

Defatted bovine serum albumin was from Sigma and was purified to homogeneity by chromatography on Blue–Sepharose (Pharmacia) according to Travis et al. (1976).

Monolayer Experiments. All experiments were carried out with a KSV-2200 surface barostat (KSV-Chemical Corp.) installed in a laminar flow enclosure. Data were collected into a Sperry PC interfaced with the DFC data acquisition system electronics and were analyzed with the KSV Enzyme Kinetics software. Surface pressure was measured with the Wilhelmy technique using a platinum plate. The trough consisted of a magnetically stirred (250 rpm) reaction compartment (total volume 45 mL; total surface 37 cm^2), a compartment for the platinum plate, and a film reservoir (20 × 149 mm) (Figure 1). These compartments were connected by shallow and narrow channels over sandblasted glass slides. The reaction compartment was thermostated at 25 °C with a glass coil immersed into the subphase of the reaction compartment and connected to a circulating water bath. The lipid was spread from a chloroform solution. The aqueous subphase consisted of water of pH 7.0 purified with the Milli-RO/Milli-Q system (Millipore) (Thuren et al., 1986).

When the alkylated plate was used, the monolayer was initially spread at an air/water interface to a pressure of approximately 9 mN m^{-1}. A glass plate alkylated with octadecylsilane (von Tscharner & McConnell, 1981) and of a surface area of 37 cm^2 exactly covering the surface of the reaction compartment was then gently brought into contact with the phospholipid monolayer, (Figure 1). Thereafter, the surface pressure was increased to 15 mN m^{-1}, a value which was maintained by a movable barrier. In experiments without the support, the phospholipids were initially spread to form a monolayer at approximately 14 mN m^{-1} and then compressed and maintained at 15 mN m^{-1}.

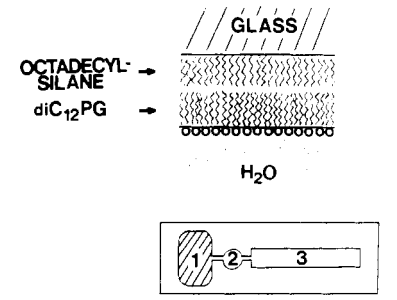

FIGURE 1: Monolayer trough and alkylated plate compressing a $diC_{12}PG$ monolayer. In the lower panel the numbering corresponds to (1) the reaction compartment, (2) the compartment to accommodate the platinum Wilhelmy plate, and (3) the film reservoir. See Experimental Procedures for further details.

The well-characterized pancreatic lipase was employed at PLA1 [for a review, see Verger (1984)]. This enzyme hydrolyzed $diC_{12}PG$ monolayers at approximately one-tenth of the rate of the pancreatic PLA2, in agreement with earlier results (Verger et al., 1977b).

With this lipid, optimal surface pressure ranges for pancreatic lipase and PLA2 are 18–19 mN m^{-1} and 20–22 mN m^{-1}, respectively (Verger et al., 1976). However, to allow comparison with our earlier experimental data recorded at 15 mN m^{-1} (Thuren et al., 1984; Kinnunen et al., 1985), this surface pressure was used also in the present study. Both pancreatic lipase and phospholipase A2 possess reasonably high activity at this lateral pressure (Verger et al., 1976; Thuren et al., 1984). Enzyme reactions were started by injecting 5 μg of the indicated enzyme with a microsyringe into the subphase underneath the film in the reaction compartment.

For a substrate underneath the alkylated support, kinetics for the PLA1 reaction could only be observed for a few minutes. However, linearity was achieved after the inclusion of 0.3 mg/mL (4.6 μM) albumin into the subphase to facilitate the removal of the reaction products from the film (Scow et al., 1979). No other effects by albumin on the system under study were evident.

Results and Discussion

As the alkylated glass surface was substituted for air over a monolayer of $diC_{12}PG$, an immediate increment in the surface pressure was evident. For instance, when the support (37 cm^2) was made to contact the monolayer (total film area 66.8 cm^2) at a surface pressure of 15 mN m^{-1}, the surface pressure increased by 6.5 mN m^{-1} up to 21.5 mN m^{-1}. When the phospholipid film was then relaxed back to 15 mN m^{-1}, it was observed that the additional surface area required was approximately 7.5 cm^2. We assume expansion of the film, i.e., increase in the mean area/phospholipid, to take place only in those molecules underneath the support. It could thus be calculated that the mean molecular area of the molecules underneath the support was increased to 80.2 $Å^2$/molecule. In comparison, the mean molecular area of $diC_{12}PG$ molecules was 64 $Å^2$/molecule in a free monolayer at an air–water interface. This increment of 16.2 $Å^2$ caused by the support is of similar magnitude as the electrostatically induced (Träuble,

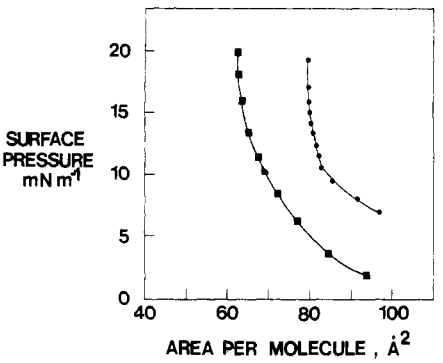

FIGURE 2: Comparison of the compression isotherms of $diC_{12}PG$ at free air–water interface (■) and under an alkylated support (Fdt). $DiC_{12}PG$ monolayers were spread to different initial surface pressures. Thereafter, the support was made to contact the monolayer, and the film was allowed to equilibrate for 8–10 min. The monolayer was then relaxed back to the initial surface pressure value, and the mean molecular area for the molecules underneath the support was calculated from $A_p/x = (A_1A_p)/[n(A_1 - A_2 + A_p)]$, where A_p is the area of the alkylated support, A_1 and A_2 are the surface areas before and after the alkylated plate was made to contact the monolayer, respectively, x is the number of molecules underneath the support, and n is the total number of lipid molecules. Temperature was 25 °C. The subphase was purified water of pH 7.0.

1983) increase of 13 Å^2 in the mean molecular area of $diC_{12}PG$ molecules at 15 mN m^{-1} reported by us earlier (Thuren et al., 1984) and suggests a conformational change in the phospholipids in a monolayer to be caused by a vertical compression of the film by the support. This procedure was repeated at different initial surface pressures. These data are plotted as a compression isotherm in Figure 2, which for comparison illustrates also the compression isotherm for $diC_{12}PG$ at a free air–water interface. The increment in the mean molecular area at 6 mN m^{-1} was 23.0 Å^2 and reached a minimum of 13.3 Å^2 at 10 mN m^{-1}. At surface pressures exceeding 12 mN m^{-1}, the increment in the mean molecular area of $diC_{12}PG$ was 16.5 Å^2 and remained nearly constant.

The effects of the alkylated support on the hydrolysis of the $diC_{12}PG$ monolayers by PLA1 and PLA2 are shown in Figure 3. The activity of PLA2 toward the substrate monolayer decreased by 85% when the lipid resided at the interface between the alkylated glass and water. Under similar conditions the activity of PLA1 was enhanced approximately 4-fold by the support compared to the activity measured with the substrate at a free air–water interface.

In the presence of 1 M NaCl, the activity of PLA2 toward the $diC_{12}PG$ monolayer at the air–water interface was 0.89 × 10^{14} molecules hydrolyzed min^{-1} and toward the same monolayer under an alkylated plate was 0.93 × 10^{14} molecules hydrolyzed min^{-1}. The activities of PLA1 against a $diC_{12}PG$ monolayer in the presence of 1 M NaCl were 5.87 × 10^{13} and 6.75 × 10^{13} molecules hydrolyzed min^{-1}, without and with the alkylated support, respectively.

While it cannot be concluded at this stage if the observed reciprocal changes in the activities of PLA1 and PLA2 do reflect differences in the penetration of these enzymes into the supported lipid, these results are consistent with our previous model on the reciprocal regulation of phospholipases A1 and A2 by the substrate conformation (Thuren et al., 1984; Kinnunen et al., 1985). Also, the relative changes in the PLA activities agree with our previous data on NaCl-induced effects. More support on our previous model comes from experiments on the effects of alkylated plate in the presence of molar NaCl. The results are consistent with findings on salt-induced inhibition of PLA2 and activation of PLA1. The plate could not inhibit or activate PLA2 in the presence of NaCl. In the

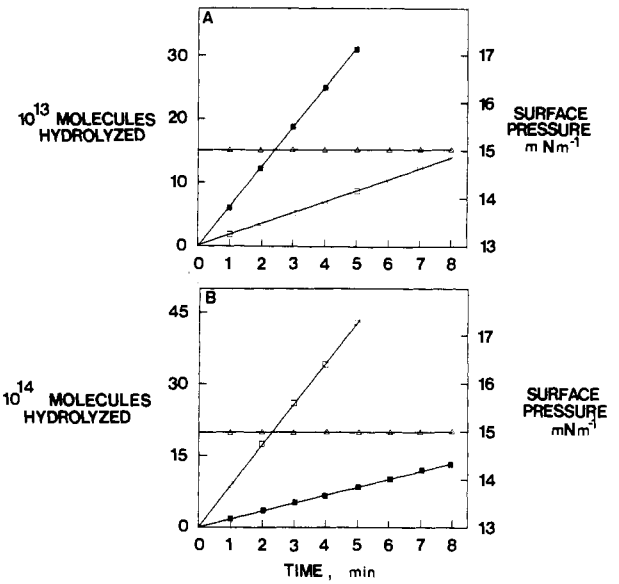

FIGURE 3: Hydrolysis of $diC_{12}PG$ monolayer at 15 mN m^{-1} by pancreatic lipase (panel A) and by pancreatic phospholipase A2 (panel B) in the absence (□) and in the presence (■) of the alkylated glass plate. The surface pressure trace at 15 mN m^{-1} is illustrated with (△). The activities of the pancreatic lipase with and without the support were 6.34 × 10^{13} and 1.68 × 10^{13} molecules hydrolyzed min^{-1}, respectively. For the pancreatic phospholipase A2, the corresponding values were 1.67 × 10^{14} and 11.50 × 10^{14} molecules hydrolyzed min^{-1}. See Experimental Procedures for further details.

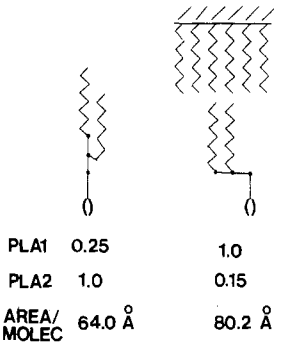

FIGURE 4: A schematic model for the suggested phospholipid conformations in free and supported monolayers at a surface pressure of 15 mN m^{-1} and a summary of the experimental findings supporting this model. On the left side is illustrated a possible conformation for a $diC_{12}PG$ molecule at a free air–water interface and the observed mean molecular area. The right-hand side shows a proposal for the conformation of $diC_{12}PG$ at an interface between water and the alkylated surface together with its calculated mean molecular area. Also shown are the measured relative activities of PLA1 and PLA2 against both types of substrates.

presence of NaCl, PLA1 was activated 15% by the plate. This can be explained to be due to the plate changing the conformation of $diC_{12}PG$ molecules and NaCl increasing the degree of deprotonation of $diC_{12}PG$ (Träuble, 1977).

In contrast to our results, high salt has been shown with a titrimetric assay to enhance the action of PLA2 toward short-chain lecithins (de Haas et al., 1971). This observation could not be verified with KCl, and instead, V_{max} was found to decrease above the cmc of the substrate (Wells, 1974). In other assay systems, NaCl concentrations of 0–0.3 M were found to have no effect on PLA2 activity toward short-chain lecithins (Zografi et al., 1971).

A molecular model accounting for these results is illustrated in Figure 4. At an air–water interface at 15 mN m^{-1}, $diC_{12}PG$ would adapt the "kinked" conformation in which the *sn*-2 acyl chain starts perpendicular to the glycerol backbone and after the first two methylene segments bends to align the glycerol

backbone and the *sn*-1 acyl chain. At the interface between the alkylated glass and water, the molecules in the monolayer should favor the "extended" conformation where both acyl chains are equally aligned and both start perpendicular to the glycerol backbone. The change from the kinked to the extended conformation should thus result in an increment in the mean molecular area of phospholipids. Of the lipolytic enzymes, PLA2 would hydrolyze more readily the substrate in the kinked conformation in agreement with the X-ray crystallographic data on the active site of PLA2 (Dijkstra et al., 1981a,b) whereas PLA1 would prefer phospholipids in the extended state.

As far as we know this is the first report on a direct mechanical effect to influence the action of phospholipases A on monolayers. It should probably also be noted that these kinds of model systems show somewhat closer resemblance to bilayers than free phospholipid monolayers at an air–water interface.

ACKNOWLEDGMENTS

We thank Dr. R. Verger, Marseille, France, for providing us with the purified porcine pancreatic lipase.

Registry No. PLA1, 9043-29-2; PLA2, 9001-84-7; $diC_{12}PG$, 63644-55-3.

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