Accelerated Publications

Triggering of the Activity of Phospholipase A_2 by an Electric Field

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ABSTRACT: In this paper we show that the action of phospholipase A_2 can be triggered by applying an electric field across a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer residing between an alkylated silicon surface and water. When the silicon wafer served as a cathode, rapid activation of porcine pancreatic phospholipase was observed and did depend on the magnitude of the applied potential. The degree of activation was different for the pancreatic phospholipase A_2 and snake and bee venom enzymes. Maximally, a 7-fold activation of pancreatic phospholipase A_2 was observed when the applied potential was 75 V. The effective field over the lipid film could be estimated to be approximately 25–175 mV, i.e., in the range of membrane potentials found in cells. On the basis of these results, we suggest that changes in membrane potential might be an important factor in the regulation of the action of intracellular phospholipases A_2 in vivo.

Phospholipases A_2 (EC 3.1.1.4, PLA2)[1] hydrolyze the *sn*-2 fatty acyl ester bond of *sn*-3-phosphoglycerides producing free fatty acids and *sn*-2 lysophospholipids (van den Bosch, 1980). PLA2's are ubiquitous enzymes and have been discovered in every cell type studied so far (van den Bosch, 1980). Extracellularly, this enzyme is present in the pancreatic juice and in the venoms of snakes and anthropodes (Verheij et al., 1981). PLA2 is also found in seminal plasma, bronchoalveolar lavage, and rheumatoid synovial fluid (Offenstat et al., 1981; Pruzanski et al., 1985; Wurl & Kunze, 1985). Intracellular PLA2's take part in the cellular metabolism of membrane phospholipids. Liberation of arachidonic acid in the PLA2 reaction is currently believed to represent the rate-limiting step in the cascades of reactions leading to the formation of prostaglandins and leucotrienes (van den Bosch, 1980). These enzymes have also been shown to take part in inflammatory response, e.g., in rheumatoid synovial cartilage (van den Bosch, 1980; Vadas et al., 1981; Pruzanski et al., 1985). PLA2 has been suggested to participate in diverse cellular functions such as resealing of ruptured nerve membranes (Yawo & Kuno, 1983), chemotaxis, cytotoxicity, stimulus–secretion coupling, and cell differentiation (Vadas & Pruzanski, 1986). Extracellular PLA2 appears to be involved in the pathogenesis of pancreatitis (Creutzfeldt & Schmidt, 1970; Nevalainen, 1980; Thuren et al., 1985). Plasma PLA2 activity has been shown to be increased during the acute phase of schizophrenia (Gattaz et al., 1987).

The membrane-associated PLA2's are bound to their substrates, which necessitates stringent regulation of the expression of their catalytic activity. The mechanism(s) of regulation of the activity of PLA2 has (have) been subjected to intense research (van den Bosch, 1980). However, the mechanism(s) involved has (have) remained poorly understood. The discovery of specific inhibitory peptides (Blackwell et al., 1980; Hirata et al., 1980; di Rosa et al., 1984; Wallner et al., 1986) has given some insight into the control of intracellular PLA2's. There is experimental evidence that PLA2 associates with the steroid-induced nonenzymatic protein inhibitors lipocortins (Blackwell et al., 1980; Hirata et al., 1980; di Rosa et al., 1984; Wallner et al., 1986). However, a very recent report suggests that lipocortins would bind to membrane phospholipids and that their effect could be mediated via the substrate (Davidson et al., 1987). The exact role of the inhibition of PLA2 by lipocortins in the regulation of PLA2 activity is thus somewhat unclear. The properties of the substrate membrane, e.g., surface charge, bound polycations, and lateral packing density, have been shown to have an effect on the adsorption and action of PLAs (Dawson, 1969; Verger et al., 1973, 1976; Thuren et al., 1984, 1986). A connection between PLA2 activity and membrane energization state has been suggested (Parce et al., 1978; Bevers et al., 1978). Membrane depolarization in synaptic membranes has been shown to stimulate PLA2 activity (Baba et al., 1986).

In this paper we have studied the hydrolysis of $diC_{12}PG$ monolayers by PLA2 under an alkylated silicon support in an experimental setup where an electric field is applied over a $diC_{12}PG$ monolayer. The dependency on the magnitude of the applied potential and the effects of the field on the surface pressure of phospholipid monolayers are also reported.

Experimental Procedures

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

Porcine pancreatic PLA2 was from Boehringer Mannheim and appeared as a single Coomassie Brilliant Blue stained band

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[1] Abbreviations: PLA2, phospholipase A_2; $diC_{12}PG$, 1,2-didodecanoyl-*sn*-3-phosphoglycerol; EDTA, ethylenediaminetetraacetic acid.

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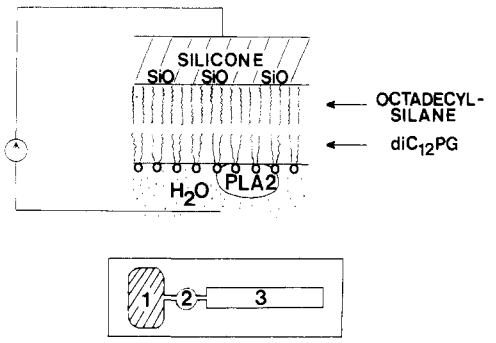

FIGURE 1: Schematic illustration of the phospholipid monolayer underneath an alkylated n-type silicon plate of a specific resistance of 3 ohm cm^{-1} and an area of 37 cm^2 in contact with the phospholipid monolayer.

upon gel electrophoresis in 12% polyacrylamide in the presence of sodium dodecyl sulfate (Laemmli, 1970). Snake venom (*Crotalus atrox*) and bee venom PLA2's were from Sigma. Rat liver mitochondrial PLA2 was a generous gift from Professor Henk van den Bosch, Utrecht, The Netherlands.

Monolayer Experiments. A KSV 2200 AFC monolayer system installed in a laminar flow enclosure was used. Data were collected into a Sperry PC and analyzed with KSV Enzyme Kinetics software. The experimental arrangement is schematically illustrated in Figure 1. In the hydrolysis experiments a Verger–de Haas zero-order trough with an alkylated (von Tscharner & McConnell, 1981) semiconductor support of total area 37 cm^2 exactly covering the reaction compartment was employed (Thuren et al., 1987). A platinum wire immersed in the aqueous subphase of the reaction compartment was used as the counterelectrode. Connection to the Pt wire was made through a small hole separate from the film compartment. A monolayer of $diC_{12}PG$ was spread on a free air–water interface from a chloroform solution. The lipid was allowed to settle for 8–10 min before the semiconductor plate was substituted for air over the film in the reaction compartment. After the alkylated support was allowed to overlay the monolayer, the surface pressure was adjusted to 15 mN m^{-1} and was maintained at this value during the experiment. After the adjustment of the surface pressure, a dc potential was applied between the semiconductor and the counterelectrode. In accordance with the well-known insulating properties of lipid films, potentials up to approximately 75 V were accompanied by currents in the range of 0–30 nA only. Increasing the voltage further, however, caused a breakdown of resistance.

The subphase consisted of Milli-RO/Milli-Q (Millipore) purified water, pH 7.0. The temperature was maintained at 25 °C with a glass coil immersed into the reaction compartment and connected to a circulating water bath. The subphase of the reaction compartment was magnetically stirred at a rate of 250 rpm. Enzyme reactions were started by injecting 5 μg of enzyme with a microsyringe into the subphase underneath the film in the reaction compartment. PLA2 from porcine pancreas was used as a model enzyme due to its stability and well-characterized properties (Verheij et al., 1981).

Results and Discussion

The effect of an electric field on PLA2-catalyzed hydrolysis of the $diC_{12}PG$ monolayer under an alkylated support is illustrated in Figure 2. When the silicon support served as a cathode, pancreatic PLA2 was activated 4-fold by a field of −25 V (Figure 2). By reversing the polarity this activation could be abolished to a level of 1.5-fold the control activity. Reversing the polarity again caused reactivation of PLA2.

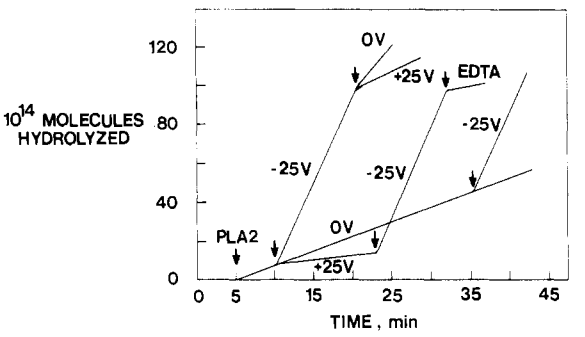

FIGURE 2: Hydrolysis by PLA2 of a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer residing between an alkylated support and water. The surface pressure was 15 mN m^{-1}. Five micrograms of purified porcine PLA2 (Boehringer Mannheim) was used. Arrows indicate the application of potential or the inclusion of 12 mM EDTA, as shown.

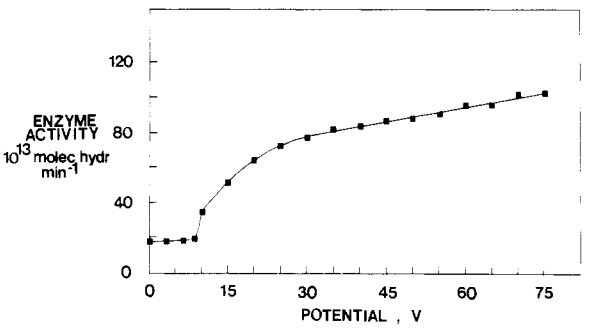

FIGURE 3: Activation of the hydrolysis of a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer by PLA2 as a function of the applied potential. Experimental conditions as in Figure 2.

This procedure could be repeated several times. If the potential was removed after triggering of the PLA2 activity, the rate of PLA2 reaction decreased to 2.5 times the original velocity. If the support was used directly as an anode at +25 V without first applying a negative potential, the activity of PLA2 was inhibited to 65% of the initial enzyme velocity.

We then investigated the dependency of the degree of catalytic rate enhancement on the potential (Figure 3). No activation was observed below −10 V whereas potentials exceeding this magnitude activated PLA2 approximately 2.0-fold. Increasing the voltage to −75 V caused a 7-fold activation. The hydrolysis of the $diC_{12}PG$ monolayer by PLA2 in the presence and absence of the field was linearly dependent on the amount (in the range of 1–10 μg) of the enzyme (data not shown).

PLA2's isolated from different sources responded differently to the field. Under identical conditions snake venom (*C. atrox*) PLA2 was activated 2.5-fold and bee venom PLA2 only 1.5-fold by a potential of −25 V (Table I). The activities of all PLA2's studied were reduced by varying degrees by a field of +25 V (Table I). As to the mechanism(s) of the field-induced triggering of PLA2 activity described above, several possibilities remain to be considered. PLA2 has an absolute requirement for calcium. Accordingly, changes in the concentration of intracellular calcium have been suggested to regulate the action of PLA2 (van den Bosch, 1980). Therefore, the above results could be explained by a field-induced increase in the effective calcium ion concentration in the Gouy–Chapman layer adjacent to the lipid film. However, similar activation as well as maximal activity was observed at 1 μM and 4 mM $CaCl_2$ in the subphase.

EDTA completely abolished PLA2 activity when added prior to the application of potential. For reasons unknown, EDTA only partially inhibited PLA2 activity when included in the subphase after the potential induced activation. If

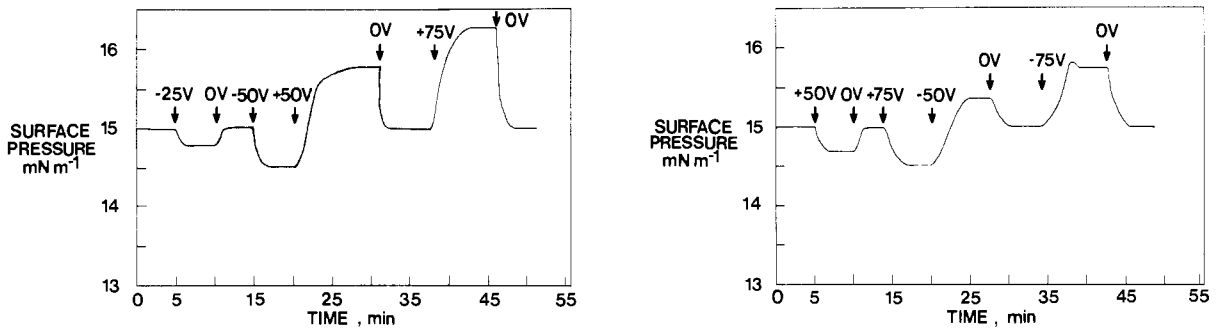

FIGURE 4: (Left panel) Electric field induced changes in the surface pressure of a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer on pure water. A rectangular Teflon trough (200 mm × 100 mm) was used. The surface area of the silicon support was 37 cm^2. Otherwise the system was similar to that described under Experimental Procedures. The field was turned on and off repeatedly while the surface pressure was recorded. The initial surface pressure was 15 mN m^{-1}. Arrows show the application of the indicated voltages. (Right panel) Electric field induced changes in the surface pressure of a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer on 150 mM NaCl.

Table I: Effect of an Electric Field on PLA2's Isolated from Porcine Pancreas, *C. atrox* Venom, Bee Venom, and Rat Liver Mitochondria[a]

	potential[b]		
PLA2 source	0 V	−25 V	+25 V
porcine pancreatic	1.8×10^{14} (100)[c]	8.9×10^{14} (501)	2.6×10^{13} (15)
porcine pancreatic + 150 mM NaCl	0.8×10^{14} (100)	3.4×10^{14} (404)	2.4×10^{13} (30)
snake venom (*C. atrox*)	1.4×10^{13} (100)	3.3×10^{13} (237)	1.5×10^{12} (11)
bee venom	0.8×10^{13} (100)	1.4×10^{13} (169)	1.4×10^{11} (1.7)
rat liver mitochondria	3.5×10^{12} (100)	3.4×10^{12} (97)	1.6×10^{11} (4.7)

[a] Conditions are as described under Experimental Procedures. Five micrograms of PLA2 was used in each experiment. PLA2 activities are expressed as molecules hydrolyzed per minute and are the means of duplicate or triplicate experiments. [b] A negative sign refers to a cathodal and a positive sign to an anodal support. [c] Values in parentheses are in percent.

EDTA was added after triggering the PLA2 activity by the field, the enzyme activity was inhibited only by 65% (Figure 2).

Electrostatically induced changes in the conformation of substrate phospholipids have been shown to influence the action of PLA2 (Thuren et al., 1984; Träuble, 1983; Kinnunen & Virtanen, 1986). Figure 4 shows field-induced changes in the surface pressure of a 1,2-didodecanoyl-*sn*-3-phosphoglycerol monolayer. A negative potential (cathodal silicon) decreases the mean molecular area of phospholipids. However, the voltage required (−20 V) to observe a decrement in surface pressure was higher than the voltage required (−10 V) for PLA2 activation. It could be calculated that the mean molecular area of 1,2-didodecanoyl-*sn*-3-phosphoglycerol molecules under the support was decreased by 6.7 $Å^2$ when a potential of −50 V was applied across the lipid monolayer (Thuren et al., 1987). Reversing the field direction resulted in reciprocal changes in surface pressure.

If a negative potential was applied with 150 mM NaCl in the subphase, an increment in the surface pressure was observed. The opposite was true for a positive field, which decreased the surface pressure (Figure 4). The effects of salt on the potential-induced changes in the surface pressure are readily explained by electrostatics (Träuble, 1983; Kinnunen & Virtanen, 1986). In the absence of NaCl a cathodal support should cause a reduction in the surface pressure due to a repulsion between the acidic phospholipid polar head group and the wafer, thus shifting the lipid conformation toward "kinked". The opposite should be true for an anodal support. In the presence of salt, however, a negative field causes an increase in the effective Na^+ concentration in the Gouy–Chapman layer, which further leads to deprotonation of the phosphoglycerol head group due to screening. Subsequently, lateral repulsion of the negatively charged head groups shifts the lipid conformation toward "extended". A reciprocal effect should be evident with a positive potential in accordance with our observations.

In spite of the field-induced reciprocal changes on surface pressure, an activation of PLA2 by a negative potential was observed in both the presence and absence of NaCl (Table I). Therefore, electrostatically induced changes in the substrate conformation are unlikely to explain the observed potential-dependent changes in PLA2 activity. The remaining possibilities are field-induced alterations in the conformation and film penetration as well as orientation of PLA2. However, we do not have techniques available at present to approach these questions experimentally.

The results summarized above do show that an electric field can trigger the action of PLA2 toward a phospholipid monolayer. Assuming a thickness of approximately 0.8–1.0 μm for the insulating SiO layer and using 10 and 3.6 as the dielectric constants for the lipid and silicon, respectively, it follows that the effective potential was only 0.25% of the applied voltage. Accordingly, the potential over the lipid region can be estimated to be in the range of 25–175 mV. This corresponds reasonably well to values generally measured for potentials across cellular membranes.

The present results imply that the action of PLA2 in vivo could also be regulated by changes in membrane potential. Yet, there is no a priori reason to believe that this could not be extended to concern phospholipases with specificities different from A_2, e.g., phospholipase C. Importantly, the present findings suggest that there would be no need for a direct chemical (enzymatic) cascade, but it could be speculated that at some point a change in membrane potential could be responsible. Notably, a number of important cellular phenomena involve changes in membrane potential and are connected to the action of phospholipases. Insulin binding to its receptor, for instance, changes the membrane potential and eventually leads to the phosphatidylinositol response (Michell, 1975; Moore, 1983).

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