Binding of Quinacrine to Acidic Phospholipids and Pancreatic Phospholipase A2. Effects on the Catalytic Activity of the Enzyme†

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ABSTRACT: Binding of quinacrine to phospholipids and porcine pancreatic phospholipase A2 (PLA2) was investigated using fluorescence resonance energy transfer, Langmuir films, assay for the enzymatic activity, and molecular modeling. No significant binding of this drug to the zwitterionic phosphatidylcholine was observed whereas a high affinity for acidic phospholipids was revealed by quenching of pyrene-labeled phospholipid analogues. Partial reversal of this binding was observed due to the addition of 4 mM $CaCl_2$. Quinacrine efficiently and independently of the lipid surface pressure penetrated into monolayers of phosphatidylglycerol while only a weak penetration into phosphatidylcholine films was evident. Quinacrine also bound to eosin-labeled PLA2, and the addition of 4 mM $CaCl_2$ reversed this interaction almost completely. In the presence of acidic phospholipids both the drug and the enzyme were attached to the lipid surface. Studies on the influence of quinacrine on the activity of PLA2 toward pyrene-labeled phospholipid analogues revealed that the hydrolysis of phosphatidylcholine was progressively reduced as a function of increasing [quinacrine]. At low [$CaCl_2$] and low quinacrine:lipid molar ratios ($<$1:5) quinacrine enhanced slightly the rate of hydrolysis of acidic phospholipids whereas at higher drug:lipid molar ratios ($>$1:2) an inhibition was observed. In the presence of 1 mM $CaCl_2$ quinacrine inhibited PLA2-catalyzed hydrolysis of phosphatidylglycerol only when the drug:lipid molar ratio exceeded 1:1. The presence of 4 mM $CaCl_2$ abolished nearly completely the inhibition with all the substrate analogues used. Our data suggest that the inhibition of PLA2 by quinacrine is due to its binding to the enzyme. This is supported also by molecular modeling which suggested a binding site for quinacrine close to the active site and Ca^{2+} binding site of the enzyme. Importantly, our data indicate that quinacrine binds avidly to acidic phospholipids and their presence may influence the drug-enzyme interaction and the inhibition of the enzyme action. Accordingly, presence of quinacrine may interfere also with other processes that require the presence of acidic lipids and/or Ca^{2+}, such as the function of the nicotinic acetylcholine receptor.

The catalytic activity of PLA2[1] (E.C. 3.1.1.4) has been proposed to represent the rate-limiting step in the formation of eicosanoids following the liberation of arachidonic acid from membrane phospholipids (*1*). These locally appearing, hormone-like molecules contribute to several physiological and pathological states such as inflammation, immune events, allergic reactions, and ischemia (*2*). A high degree of sequence analogy prevails between the secretory forms of PLA2 (*3*) whereas no homologous regions between the 87 kDa cytosolic PLA2 and the secretory PLA2s have been found (*4*, *5*). The thoroughly characterized porcine pancreatic enzyme has been claimed to represent a good model for in vitro studies (*6*). Data on the physiological role and regulation of PLA2s in cells is still fragmentary and inconclusive, mostly due to the fact that these studies rely heavily on the use of pharmacological reagents of often poorly defined specificity (*7*). The difficulties in the characterization of the inhibitory mechanisms of different molecules have been recently reviewed by Gelb et al. (*8*). The antimalarial drug quinacrine (Figure 1) is frequently employed as an in vivo inhibitor of PLA2, and attenuation of stimulated release of arachidonic acid in different cell lines has been reported (*9*). Accordingly, it has been widely used

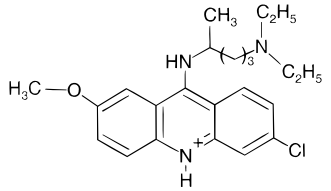

FIGURE 1: Structure of quinacrine.

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[1] Abbreviations: PLA2, porcine pancreatic phospholipase A2; PPHPG, 1-palmitoyl-2-[6-(pyren-1-yl)hexanoyl]-*sn*′-glycerophosphatidyl-*sn*′-*rac*′-glycerol; PPHPC, 1-palmitoyl-2-[6-(pyren-1-yl)hexanoyl]-*sn*′-glycero-3-phosphatidylcholine; C_{28}-O-PHPM, 1-octacosanyl-2-[6-(pyrene-1-yl)hexanoyl]-*sn*′-glycero-3-phosphatidylmethanol; egg PG, egg yolk phosphatidylglycerol; egg PC, egg yolk phosphatidylcholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol.

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in studies on signal transduction, blood clotting, ischemia, hypertension, and inflammation. Quinacrine is classified as a nonspecific inhibitor, and it appears to be able to inhibit both the cytosolic and the secretory PLA2s. In distinction, the tight-binding competitive inhibitors designed for the secretory PLA2s do not inhibit the cytosolic enzyme (*8*). Quinacrine has also been shown to inhibit the *Torpedo* nicotinic acetylcholine receptor (*9*, *10*), and the binding site for the drug has been suggested to be in contact with the lipid bilayer (*11*). Additionally, in clinical practice quinacrine has been implicated as an important agent for nonsurgical female sterilization in undeveloped countries (*12*).

The present study was undertaken in order to elucidate the interaction of quinacrine with phospholipids and the determinants of the inhibition by the drug of porcine pancreatic PLA2 in vitro. As substrates, we used fluorescent phospholipid analogues developed for the sensitive and specific measurement of PLA2 activity (*13*, *14*). Spectroscopic properties of quinacrine and eosin-labeled PLA2 allowed us to study the interactions between each component in mixtures of the drug, enzyme, different phospholipids, and Ca^{2+}. We also utilized molecular modeling, which has recently proven to be a powerful tool in designing structure-based inhibitors of PLA2 (*15*). Combination of these methods allows for the delineation of the molecular level interactions in a test system composed of three or more components and imitating in vivo conditions.

MATERIALS AND METHODS

Reagents. The pyrene-labeled phospholipids 1-palmitoyl-2-[6-(pyren-1-yl)hexanoyl]-*sn*-glycero-3-phosphocholine and -phospho-*sn*′-*rac*′-glycerol (PPHPC and PPHPG) were made by total organic synthesis and were from Kibron Inc. (Helsinki, Finland), while 1-octacosanyl-2-[6-(pyrene-1-yl)-hexanoyl]-*sn*′-glycero-3- phosphomethanol (C28-O-PHPM) was from Molecular Probes, Inc. (Eugene, OR). Egg yolk phosphatidylglycerol (egg PG) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were from Sigma. Porcine pancreatic phospholipase A2 was from Boehringer Mannheim and when indicated was labeled at 1:1 molar ratio with eosin:isothiocyanate according to Nargessi and Smith (*16*). Quinacrine and other reagent grade materials were from Sigma.

Binding of Quinacrine to Liposomes. Fluorescence measurements were carried out with a Kontron SFM 23 spectrofluorometer equipped with a magnetically stirred thermostated cuvette compartment and fixed 10 nm slits for both excitation and emission beams. For pyrene excitation and emission, wavelengths of 343 and 480 nm, respectively, were employed. Liposomes were prepared by rapidly injecting the indicated lipid mixtures dissolved in ethanol into the indicated buffer solution (*17*). The spectral overlap of quinacrine absorption and pyrene excimer emission spectra allows for use of the former as energy transfer acceptors to assess the binding of this drug to pyrene−lipid-containing liposomes. To yield the indicated final drug concentrations 10 μL samples of 10 μM quinacrine were added to the liposome solutions while the consequent fluorescence intensity changes due to energy transfer were recorded.

Monolayer Experiments. Penetration of quinacrine into lipid monolayers was monitored using a magnetically stirred circular well (surface area 3 cm^2, volume 0.4 mL). Surface pressure was monitored with a μTrough S tensiometer (Kibron Inc., Helsinki, Finland). Data were collected and analyzed using FilmWare, a dedicated software provided by Kibron Inc. The aqueous subphase was 20 mM Tris, pH 7.4, containing the indicated $CaCl_2$ concentrations. All experiments were performed at room temperature (approximately 20 °C). The lipid was spread on the air−buffer interface with a microsyringe from a chloroform solution. Prior to the injection of quinacrine through the lipid monolayer into the subphase, the monolayers were allowed to stabilize for 15 min. Collapse pressures of DMPG and DMPC monolayers were approximately 55 and 58 mN/m, respectively.

Binding of Quinacrine to Eosin-Labeled PLA2. When measuring quenching of quinacrine fluorescence by eosin-labeled PLA2, the excitation and emission wavelengths were 425 and 500 nm, respectively. As above, eosin absorption and quinacrine emission spectra were used to study the binding of the drug to the eosin-labeled enzyme. Similarly, 10−50 μL samples of 23 μM eosin-labeled PLA2 were added to 1 μM quinacrine solution in the absence or presence of unlabeled liposomes. All the fluorescence spectroscopy experiments were performed at low concentrations of pyrene-labeled lipids, quinacrine, and eosin-labeled PLA2 so as to result in an insignificant inner filter effect (maximally <1% of the initial emission).

Assays for Phospholipase A2. PLA2 action on the substrate analogues used results in liberation of pyrenehexanoic acid into the aqueous phase. The direct assays with diacyl substrate analogues were performed in 2.0 mL of 20 mM Tris-HCl, pH 7.4, using magnetically stirred cuvettes. The assays were calibrated by the addition of free 6-(pyren-1-yl)hexanoic acid in the absence of enzyme. The $CaCl_2$ concentration was varied as indicated, and the substrate concentration was 5 μM. The progress of hydrolysis was monitored by the intensity of pyrene monomer fluorescence at 400 nm vs time. The excitation wavelength was 344 nm. In the presence of 1 mM $CaCl_2$ the specific activities of PLA2 with the lipids used were as reported previously (*18*). In the end-point assay used with C28-O-PHPM the assay medium consisted of 31.5 μM substrate in 20 mM Tris-HCl, pH 7.4, in a total volume 200 μL. The method has been described previously (*13*) except for the inclusion of 0.1 μg/mL oleic acid which was included as a carrier in the organic phase consisting of chloroform−methanol−heptane (1.25:1.41:1.00, by volume). Each data point represents the mean from two experiments with an average error of <15%. In the experiments performed in the absence of added $CaCl_2$ the residual Ca^{2+} concentration was approximately 50 nM (*14*). In all experiments a complete loss of activity was seen due to the inclusion of excess (0.1 mM) EDTA.

Molecular Modeling, Site Selection, and Docking. All computations were done on an IRIS 4D workstation (Silicon Graphics, Mountain View, CA). *Naja naja atra* (E.C. 3.1.1.4, 1POB) was used as a model because of availability of the 1.5 Å resolution X-ray structure for this enzyme complexed with a transition state analogue (*19*). Coordinates for PLA2 were retrieved from the Brookhaven Protein Data Bank (*20*). The structure of quinacrine was minimized with the AMBER potential function.

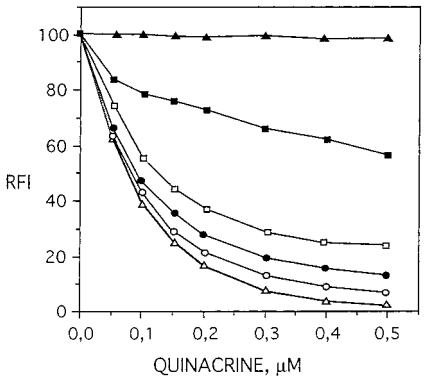

FIGURE 2: Quenching of pyrene excimer fluorescence as a function of quinacrine concentration. (▲) is 5 μM PPHPC and (△) is 5 μM PPHPG in the presence of 0.1 mM EDTA. With PPHPG the content of $CaCl_2$ was 100 μM (○), 500 μM (●), 1 mM (□), and 4 mM (■). The buffer was 20 mM Tris-HCl, pH 7.4, and the temperature 25 °C.

DOCK 3.5 (*21*) was used to explore the active site of PLA2 with quinacrine. Spheres were originally generated using SPHGEN on a molecular surface calculated by the program MS (*22*) using a 1.4 Å radius probe. The solvent-accessible surface of PLA2 was first searched for concave regions and each cavity characterized as a set of overlapping spheres. Potential binding sites on PLA2 were identified, and the energies for docking of quinacrine in different conformations were calculated using two clusters with 52 spheres (*23*). Over 2.2 million orientations were screened. For scoring the orientations we used a cubic grid at $^1/_3$ Å resolution with the internal distance tolerance set to 1.5 Å, and AMBER minimization was conducted essentially as described (*24*). Ten angstrom cutoff was used for nonbonded interactions with a distance-dependent dielectric function of 4.5*R*, minimizing each system for 500 cycles. The quality for the fit of quinacrine to PLA2 was judged by a simplified molecular mechanics force-field energy. PLA2 was visualized by the MidasPlus computer graphics program (Molecular Design Institute, UCSF). PLA2 was represented as a RIBBON model, and ball-and-stick representation was used for quinacrine in the docked position.

RESULTS

Binding of Quinacrine to Phospholipids. Binding of quinacrine to vesicles composed of 5 μM PPHPG and PPHPC was monitored by measuring the extent of resonance energy transfer from pyrene to quinacrine (Figure 2). The fluorescence from the acidic PPHPG is very efficiently quenched by increasing quinacrine concentrations, thus revealing the association of the drug with the liposomes. In the presence of 0.1 mM EDTA 0.5 μM quinacrine quenched more than 95% of the pyrene fluorescence. Quinacrine also bound with high affinity to C28-O-PHPM, an acidic alkylacyl phospholipid derivative. In contrast, no binding of the drug to the zwitterionic PPHPC was observed. Increasing [$CaCl_2$] diminished the binding of the drug to PPHPG and C28-O-PHPM (Figure 3) and the dissociation of the drug from C28-O-PHPM occurred at lower [$CaCl_2$] than from PPHPG. Essentially similar binding of the drug to the acidic lipids was observed in the presence of the residual calcium needed for the PLA2 activity measurements (see below) as in the presence of 0.1 mM EDTA.

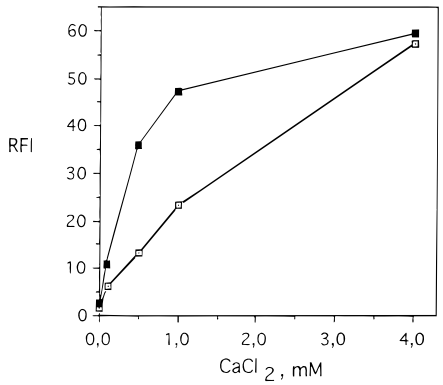

FIGURE 3: Reversal of the quenching by 0.35 μM quinacrine of the pyrene excimer fluorescence as a function of increasing $CaCl_2$ concentration. (⊡) is 5 μM PPHPG and (■) is 5 μM C28-O-PHPM. The experimental conditions were as described in Figure 1.

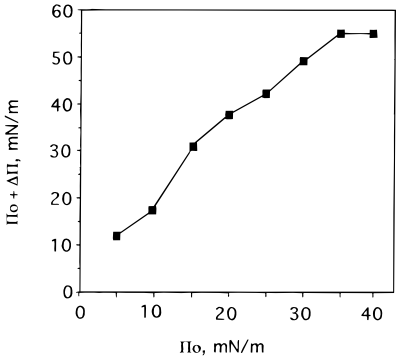

FIGURE 4: Penetration of 20 μM quinacrine into a DMPG monolayer as revealed by a change in the surface pressure ($\Delta\Pi$) as a function of the initial surface pressure (Π_0). The subphase was 20 mM Tris-HCl, pH 7.4, and the temperature 25 °C.

Lipid monolayers were used to further study the association of quinacrine with lipids. In accordance with the results of the fluorescence quenching measurements, only an insignificant or no association of 20 μM quinacrine with the DMPC monolayer was observed. Completely different behavior was observed for DMPG monolayers as illustrated in Figure 4 by the change in surface pressure ($\pi_0 + \Delta\pi$) due to penetration of 20 μM quinacrine into a DMPG monolayer. A large increase in π was evident almost immediately after the addition of quinacrine into the subphase. The increase in π was not inversely proportional to the initial surface pressure as has been observed by us for many other drugs and proteins (*25*). Instead, the surface area occupied by the drug due to penetration appeared to be almost independent of the lipid-packing density, and at high initial π's the penetration of the drug resulted in bigger changes in π and ended at approximately 55 mN/m, probably due to a collapse of the DMPG film. Importantly, in the absence of the lipids no changes in π were detected due to the addition of 20 μM quinacrine into the subphase.

Binding of Quinacrine to PLA2. Using eosin-labeled catalytically active PLA2, we then studied the interaction between quinacrine and the enzyme. In Figure 5 is depicted the quenching of 1 μM quinacrine fluorescence due to the interaction with the eosin-labeled enzyme. Addition of 4 mM $CaCl_2$ reversed by large this association. We then tested the effect of lipids on the drug−enzyme interaction. The presence of 20 μM DMPC liposomes had no influence on the interaction between the drug and the labeled enzyme. In

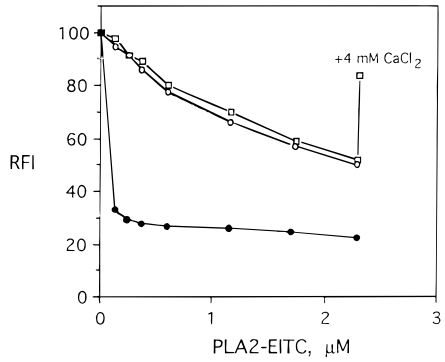

FIGURE 5: Quenching of 1 μM quinacrine fluorescence by eosin-labeled PLA2 in the presence and absence of lipids. (⊡) is no added lipid, (○) is 20 μM DMPC, and (●) is 20 μM egg PG. Experimental conditions were as described in Figure 1.

identical conditions the presence of 20 μM egg PG liposomes resulted in the most efficient quenching of the quinacrine fluorescence by the eosin-labeled enzyme, indicating that the drug and the enzyme were both concentrated onto the surface of the liposomes. Addition of 4 mM $CaCl_2$ to this suspension resulted in nonspecific aggregation of the liposomes and difficulties for interpretation of the data. The same experiment conducted in the presence of 0.1 mM EDTA revealed also efficient quenching; however, less quenching was observed than in the presence of the residual [$CaCl_2$] of approximately 50 nM (data not shown).

Effects of Quinacrine on PLA2-Catalyzed Hydrolysis of Pyrene-Labeled Phospholipids. In both the absence and presence of added $CaCl_2$ the addition of low concentrations of quinacrine (<0.5 μM) resulted in a slight enhancement of the PLA2-catalyzed hydrolysis of 5 μM PPHPG (Figure 6, panel A). At higher quinacrine concentrations this activation was followed by a progressive inhibition of the hydrolysis. This inhibition was not observed in the presence of 4 mM $CaCl_2$. Notably, at 20 μM quinacrine the presence of 4 mM $CaCl_2$ resulted in more than 50-fold higher activity of PLA2 than observed in the absence of added $CaCl_2$. In the absence of the drug the activation of PLA2 by 4 mM $CaCl_2$ was approximately 4-fold. Similar results were obtained with the alkylacyl substrate analogue, C28-O-PHPM (Figure 6, panel B). However, in the presence of 4 mM $CaCl_2$ no activation of the enzyme by low quantities of quinacrine was observed.

Results obtained with PPHPC differed from those obtained with the acidic lipids. No activation at low quinacrine concentrations was observed, and inhibition of the hydrolysis of PPHPC was observed both in the absence of added calcium and in the presence of 4 mM $CaCl_2$ (Figure 7). Quinacrine had no observable effect on the lag time between the addition of the enzyme and the start of the hydrolysis.

In the presence of 1 mM $CaCl_2$ we tested whether the drug:lipid molar ratio had an impact on the inhibition of PLA2 by the drug (Figure 8). At all the tested PPHPG concentrations (2.5−20 μM) the inhibition of PLA2 by quinacrine was observed only if the drug concentration stoichimetrically exceeded the lipid concentration. Completely different results were obtained with PPHPC. Independently of the lipid concentration 10 μM or higher quinacrine concentration was needed for the inhibition of PLA2, and the quinacrine:PPHPC molar ratio played no role in the inhibition of the enzyme activity.

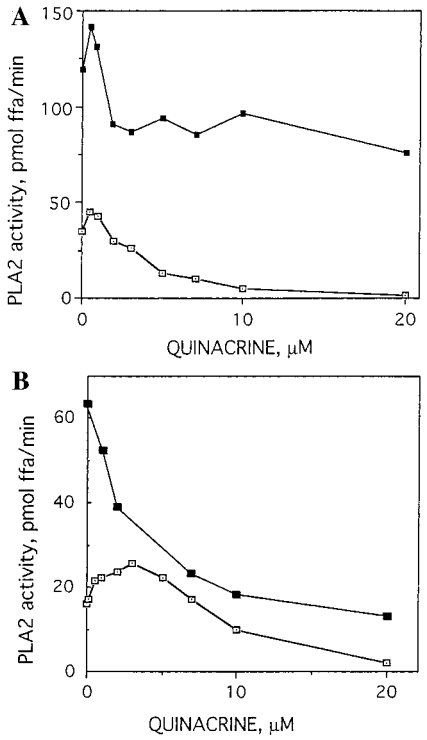

FIGURE 6: Effect of quinacrine on the PLA2-catalyzed hydrolysis of 5 μM PPHPG (panel A) and 31.5 μM C28-O-PHPM (panel B) in the absence of exogenous $CaCl_2$ (⊡) and in the presence of 4 mM $CaCl_2$ (■). Reactions were started by the addition of 100 and 200 ng of PLA2 for PPHPG and C28-O-PHPM, respectively. The buffer was 20 mM Tris-HCl, pH 7.4, and the temperature 25 °C.

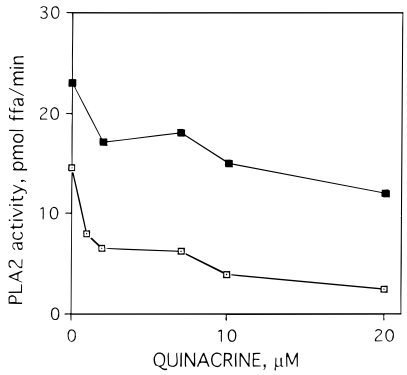

FIGURE 7: Effect of quinacrine on the PLA2-catalyzed hydrolysis of 5 μM PPHPC in the absence of exogenous $CaCl_2$ (⊡) and in the presence of 4 mM $CaCl_2$ (■). Reactions were started by the addition of 1 μg of PLA2. The experimental conditions were as described in Figure 5.

Molecular Modeling of the Quinacrine Binding to PLA2. In Figure 9 is illustrated the fit of quinacrine to PLA2 which produced a calculated free energy gain of −51.9 kJ/mol. Although the values for free energies produced by DOCK 3.5 are suggestive only, this result implies a good match between quinacrine and the accommodating site on PLA2. Accordingly, the calculated free energy for the second best fit was only −22.9 kJ/mol. In the lowest energy site the aromatic ring system of quinacrine occupies a hydrophobic cleft formed by residues Leu 2, Phe 5, Trp 18, Trp 19, Ile 9, and Phe 47. Interestingly, this site is located close to the opening of the hydrophobic groove (formed by Leu-2, Ile-9, and Trp-19) where the substrate is bound (*19*). In addition, the bound quinacrine resides in the vicinity of the Ca^{2+}

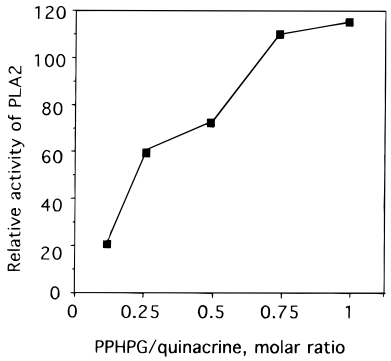

FIGURE 8: Relative activity of PLA2 as a function of quinacrine:PPHPG molar ratio in the presence of 1 mM $CaCl_2$. 100% refers to PLA2 activity in the absence of quinacrine. Reactions were started by the addition of 100 ng of PLA2. The experimental conditions were as described in Figure 5.

binding site. The aliphatic side chain of quinacrine is accommodated on residues Asp 23 and Arg 30.

DISCUSSION

The inhibition of phospholipid hydrolysis by quinacrine has been suggested to be due to nonspecific interference of quinacrine with the substrate interface and perturbation of the protein−lipid interaction (*26*). The present results from fluorescence resonance energy transfer measurements indicate that quinacrine interacts very weakly or not at all with phosphatidylcholine (Figure 2), and at similar conditions the drug avidly binds to the enzyme (Figure 5). Lack of interaction between phosphatidylcholine and the drug has also been suggested by studies using differential scanning calorimetry (*27*). A weak association of the drug with dipalmitoylphosphatidylcholine at high drug:lipid molar ratios has been observed by nuclear magnetic resonance (*28*). According to our data it seems likely that the inhibition of phosphatidylcholine hydrolysis by PLA2 is due to drug−enzyme interaction. The inhibition could be at least partly due to a competition of the Ca^{2+} binding site of the enzyme since addition of 4 mM $CaCl_2$ partially reversed the drug−enzyme interaction (Figure 4) and reversed by large the inhibition of the PPHPC hydrolysis by the drug (Figure 7). Our results do not exclude the possibility that the enzyme−interface complex could be modulated by the drug as has been suggested (*6*).

In contrast to phosphatidylcholine our data reveal quinacrine to bind avidly to acidic phospholipids (Figures 2 and 4). Increasing concentrations of $CaCl_2$ reversed by large this interaction. No K_d values for quinacrine binding could be calculated since quenching of fluorescence intensity is dependent on the orientation of the chromophores, on magnitude of the overlapping of the spectra, and on the distance of the chromophores in reciprocal to sixth power, and thus the resonance energy transfer data cannot be analyzed in a quantitative manner. At low [Ca^{2+}] both quinacrine and the eosin-labeled enzyme were bound to egg PG liposomes as revealed by a very efficient resonance energy transfer probably due to high local concentration of the drug and the enzyme on the surface of the liposome (Figure 5). With PPHPG the inhibition of PLA2 by quinacrine in the presence of 1 mM $CaCl_2$ was dependent on the drug:lipid molar ratio (Figure 8). More specifically, inhibition was observed only when [drug] > [lipid], i.e., when the drug:lipid molar ratio exceeded 1:1, which indicates that the affinity of the drug for PPHPG is higher than for PLA2 and that there is complex formation between the drug and the lipid at 1:1 molar stoichiometry. At lower drug:lipid molar ratios binding of the drug to the membrane did not result in decreased PLA2 activity, indicating that the enzyme−interface complex is not significantly disturbed due to the presence of the drug in the membrane. This also indicates that the membrane-bound drug is not able to bind to the enzyme. In this respect it is of interest that Jain has shown that the binding of PLA2 to acidic phospholipids is practically irreversible (*6*). Complex formation by the drug and phosphatidylglycerol is supported also by our results obtained with DMPG monolayers at different surface pressures. For a nonspecific penetration of an amphiphilic molecule into a monolayer, the change in surface pressure is in general inversely proportional to the increasing initial surface pressure. For quinacrine, in contrast, the penetration was not dependent on the packing density of the lipids, and the change in the surface pressure at high initial pressures due to addition of 20 μM quinarine suggested a 1:1 complex formation between the drug and the lipid. To this end, we have also performed monolayer experiments using lower concentrations of quinacrine. The binding of quinacrine to acidic PLs was evident at very low concentrations (<1 μM). The conditions for the monolayer experiments were selected so as to allow more direct comparison with the data using the conditions where PLA2 inhibition occurs and where the binding to monolayers could be more explicitly explained due to saturation of the binding. Thus, above 20 μM quinacrine no further change in surface pressure could be observed due to addition of more quinacrine.

At the suboptimal conditions used the presence of 4 mM $CaCl_2$ resulted in an approximately 4-fold increase in the hydrolysis of 5 μM PPHPG by PLA2. In the presence of 20 μM quinacrine the hydrolysis of PPHPG was more than 50-fold enhanced by 4 mM $CaCl_2$ (Figure 6, panel A). This large difference was presumably due to reversal of the drug−enzyme interaction by Ca^{2+} (Figure 5) and/or exclusion of the drug from the membrane surface (Figure 3). To this end, reversal of the inhibition by quinacrine of PLA2 by increasing [Ca^{2+}] in platelets has been reported (*29*). Ca^{2+} has also been reported to modulate the inhibition by quinacrine of the binding of israpidine to L-type voltage-operated calcium ion channels in rat cardiac membranes (*30*). Interpretation of the effects of Ca^{2+} is difficult in general and also in our in vitro experiments for several reasons. The presence of Ca^{2+} at the active site of the PLA2 is an absolute requirement for its catalytic activity, and there is also another binding site for this cation (*31*). In addition to binding to PLA2 Ca^{2+} has a high affinity for acidic phospholipids (*32*). Already at the suboptimal conditions employed Ca^{2+} causes significant activation of PLA2 and changes the electrostatic properties of the membrane.

Molecular docking algorithms such as DOCK have been used to find inhibitors for thymidylate synthase and HIV proteases with good success (*21*, *24*). We used this approach to explore the possibility of quinacrine binding sites on the surface of PLA2 indicated by our experimental results. Results from molecular modeling provided evidence for a site for quinacrine residing in the opening of the phospholipid

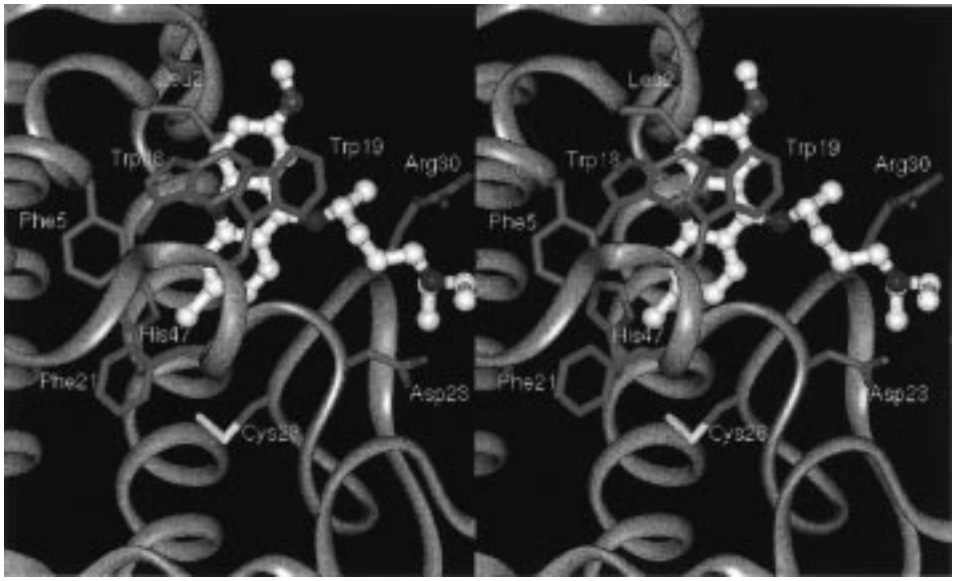

FIGURE 9: Computer-generated view of the PLA2−quinacrine complex, obtained by MidasPlus. PLA2 is visualized as a stereopair in ball-and-stick representation (quinacrine shown as white), and the hydrophobic residues of the enzyme contributing to the association are indicated. The position of quinacrine was obtained using DOCK 3.5 (see text for further details).

substrate accommodating hydrophobic groove and in the vicinity of the calcium binding site. The free energy gain estimated for the association of quinacrine to this site was −51.9 kJ/mol. The model generated by DOCK is in agreement with our results, and the location of the lowest energy site could, in theory, explain the effects of quinacrine on PLA2 activity.

The effects of quinacrine on the hydrolysis of acidic lipids did not correlate to the actual concentration of quinacrine but rather to the quinacrine:lipid molar ratio (Figures 8 and Figure 6). These data suggest that in the presence of acidic phospholipids the drug binds to the lipids instead of the enzyme. Interestingly, in interleukin I stimulated synoviocytes a slight activation by quinacrine of the hydrolysis of phosphatidylinositol has been reported while the hydrolysis of phosphatidylcholine and -ethanolamine was inhibited (*33*). Comparison of the different acidic alkylacyl and diacyl phospholipids revealed different effects of $CaCl_2$ on the binding of the drug and on the catalytic activity of the enzyme. These differences are probably due to headgroup-dependent Ca^{2+} specificity and/or the lack of the carbonyl moiety of C28-O-PHPM. At low drug concentrations there was an activation of the hydrolysis of C28-O-PHPM and PPHPG while at higher quinacrine contents an inhibition was evident. Closely related behavior has been reported for a local anesthetic dibucaine using mitochondrial PLA2 and phosphatidylethanolamine as substrates (*34*). We have previously shown polyamines, platelet activating factor, adriamycin, phorbol esters, and sphingosine to influence the activity of PLA2 due to interactions of these compounds with the phospholipid substrates (*13*, *17*, *25*, *35*, *36*). The wide structural variance of the above compounds may all reflect a change in some common physical property of the substrate, such as surface potential, thus leading to changes in enzyme activity (*17*, *35*, *37*, *38*). The observed small activation by quinacrine of the hydrolysis of acidic phospholipids by PLA2 is likely to result from the modulation of the phospholipid membrane surface. In addition to the requirement for Ca^{2+} it is noteworthy that the catalytic activity and binding of PLA2 to the substrate are also highly dependent on the physicochemical properties of the interface. For instance, the activity of PLA2 is highest toward phospholipids at the phase transition temperature (*39*). In addition, we have shown that the action of PLA2 can be triggered by electric fields imposed across a substrate monolayer (*40*) as well as by altered lipid packing upon osmotic stretching of the liposome substrates (*41*).

The present in vitro results suggest that quinacrine inhibits the PLA2-catalyzed hydrolysis of phospholipids by binding to the enzyme and possibly by competing for the Ca^{2+} binding site of the enzyme. The presence of acidic phospholipids may also influence the inhibition of PLA2 by this drug. These results clearly indicate that the use of quinacrine as a specific inhibitor of PLA2 warrants caution. The combination of molecular modeling data and the information obtained by fluorescence spectroscopy and monolayer experiments and the effects of quinacrine in the presence of different kinds of substrate molecules and $CaCl_2$ give us further understanding of the complexity of the regulation of PLA2 action.

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