Crystal Structure of a Complex Formed between a Snake Venom Phospholipase A2 and a Potent Peptide Inhibitor Phe-Leu-Ser-Tyr-Lys at 1.8 Å Resolution*

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Phospholipase A2 is an important enzyme involved in the production of prostaglandins and their related compounds causing inflammatory disorders. Among the several peptides tested, the peptide Phe-Leu-Ser-Tyr-Lys (FLSYK) showed the highest inhibition. The dissociation constant (Kd) for this peptide was calculated to be 3.57 ± 0.05 x 10^-9 M. In order to further improve the degree of inhibition of phospholipase A2, a complex between Russells viper snake venom phospholipase A2 and a peptide inhibitor FLSKYK was crystallized, and its structure was determined by crystallographic methods and refined to an R-factor of 0.205 at 1.8 Å resolution. The structure contains two crystallographically independent molecules of phospholipase A2 (molecules A and B) and a peptide molecule specifically bound to molecule A only. The two molecules formed an asymmetric dimer. The dimerization caused a modification in the binding site of molecule A. The overall conformations of molecules A and B were found to be generally similar except three regions i.e. the Trp-31-containing loop (residues 25–34), the β-wing consisting of two antiparallel β-strands (residues 74–85) and the C-terminal region (residues 119–133). Out of the above three, the most striking difference pertains to the conformation of Trp-31 in the two molecules. The orientation of Trp-31 in molecule A was suitable for the binding of FLSYK, while it disallowed the binding of peptide to molecule B. The structure of the complex clearly shows that the peptide is so placed in the binding site of molecule A that the side chain of its lysine residue interacted extensively with the enzyme and formed several hydrogen bonds in addition to a strong electrostatic interaction with critical Asp-49. The C-terminal carboxylic group of the peptide interacted with the catalytic residue His-48.

Phospholipase A2 (EC 3.1.1.4, PL A2) enzyme specifically cleaves the sn-2 acyl ester bond of phospholipids (1). These enzymes were first described as components of snake venoms and were later identified in mammals. PLA2s of mammalian origin were found in the pancreas (2), blood platelets, macrophages, and neutrophils (3, 4). These are also found in fluids derived from patients with inflammatory conditions (5, 6) and are induced in several cell types in response to inflammatory stimuli (4). It is understood that the concentrations of PLA2 enzymes in serum and tissues correlate with the disease severity in several immune-mediated inflammatory pathologies in humans. These are also associated with the onset of rheumatoid arthritis (7, 8) and septic shock (5, 9). Therefore, it is of great interest to develop specific inhibitors for each of these enzymes. Several crystal structures of the tetrahedral mimic inhibitors with an sn-2-phosphate, substituent, 1-1(α-tocyl-2-heptyl phosphonyl)-sn-glycero-3-phosphoethanolamine, complexed with secreted PLA2 from Chinese cobra venom (10), bee venom (11), and inflammatory exudates (12) have been reported. Also the crystal structure of an sn-2-acyl-amino analogue of phospholipids complexed with the engineered (without the residues of the surface 62–66) porcine PLA2 (13) has been determined. The crystal structures of the complexes of bovine pancreatic PLA2 with an inhibitor n-dodecyl-phosphorylcholine (14) and human synovial PLA2 with an acyl amino analogue of phospholipids (15) are also of some interest. Recently, a series of indole inhibitors of human secretary PLA2 were developed (16). Further, there is an example of a natural inhibition (17) where specific interactions from one molecule of a heterodimer acts as a natural inhibitor for the other. The synthetic peptides derived from the primary sequence of the PLA2 itself were also studied as specific inhibitors (18, 19). The kinetic studies (18, 19) support their high inhibitory potencies. These peptide inhibitors were stable against acyl esterase as well as phospholipase A1, C, and D. Therefore, they were suited for in vivo studies where phospholipids may be more readily metabolized. As part of the program on structure-based rational design of specific potent inhibitors using a model snake venom phospholipase A2, we have synthesized a number of peptides (20). One of these peptides Phe-Leu-Ser-Tyr-Lys (FLSKYK) was earlier reported to be binding to PLA2 (18, 19). It has been shown to be having a high potency to inhibit PLA2. To determine the mode of its binding and details of its interactions with PLA2, we have prepared its complex with phospholipase A2 in solution. The complex was crystallized and its detailed crystal structure is

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1 The abbreviations used are: PLA 2, phospholipase A 2; Fmoc, fluorenylmethoxy carbonyl; DLS, dynamic light scattering; r.m.s., root mean-square; DAAD, Deutsche Akademische Ausdienst.
Crystal Structure of the PLA₂-FLSK Complex

Data Collection—The crystals of the complex were stabilized in 15% glycerol as a cryoprotectant for data collection at low temperature. A single crystal was mounted in a nylon loop and frozen in a flash of nitrogen stream at 100 K. The crystals diffracted to 1.8 Å resolution. The x-ray intensity data were collected on the EMBL beamline X-11 at DESY, Hamburg with \( \lambda = 0.98 \) Å using MAR 345 imaging plate scanner with 1° rotation for each image. The data were processed using DENZO and SCALEPACK program packages (26). The results of the data collection and processing are given in Table I.

Structure Determination and Refinement—The structure was obtained by molecular replacement method using the model of PLA₂ determined earlier at 1.9 Å resolution (24). The structure contains two molecules designated as A and B. The refinement was carried out using the program CNS (27). In each step \( 2F_o - F_c \) and \( F_c - F_o \) maps were calculated to improve the structure in the density maps using the program O (28). The resolution was extended stepwise from 3.0 to 1.8 Å. Several cycles of positional refinement with restrained individual B-factor refinement and rounds of 3000 K simulated annealing allowed the correct tracing of flexible loops where the conformations were found to be considerably different from the initial model. At the end of this stage, the R-factor dropped to 0.227 while R-free came down to 0.271. Both Fourier \( 2F_o - F_c \) and difference Fourier \( F_c - F_o \) maps computed at this stage clearly indicated the presence of the peptide FLSKY in the binding site of molecule A (Fig. 1). On the other hand, there was no continuous density in the binding regions of molecule B. The presence of three clearly separated spherical densities were interpreted as water molecules (Fig. 2). Since, the presence of FLSKY in molecule A and its absence in molecule B were unexpected results, the \( 2F_o - F_c \) and \( F_c - F_o \) maps were calculated at various cutoffs at different stages of refinement, but it did not indicate the presence of peptide FLSKY in molecule B. A further round of rigid body refinement and several rounds of positional and restrained individual B-factor refinements were subsequently performed for both molecules with the peptide FLSKY bound to molecule A in the binding region. At the end of this stage, some missing side chains appeared more clearly, and the FLSKY electron density in molecule A improved further. Four significant peaks above 3 \( \sigma \) were identified as acetate ions (Fig. 2). The final coordinates have been deposited in the Protein Data Bank with accession code 1JQ9.

RESULTS AND DISCUSSION

Inhibition of PLA₂ by FLSKY—The purified samples of PLA₂ indicated a molecular size of 14 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). However, the results of DLS showed that the mean hydrodynamic radius \( \langle R_h \rangle \) of PLA₂ in the protein concentration ranging from 0.05 to 20 mg/ml was 2.54 nm. The molecular mass corresponding to

| Table I | Crystallographic data for the complex formed between PLA₂ and the pentapeptide FLSKY |
|---------|----------------------------------|
| Space group | C222₁ |
| Unit-cell dimensions (Å) | |
| a | 76.13 |
| b | 89.16 |
| c | 77.55 |
| \( V_m (Å^3/Đa) \) | 2.51 |
| Solvent content (%) | 51 |
| Z | 16 |
| Resolution range (Å) | 20.0–1.8 |
| Number of measured reflections | 22959 |
| Number of unique reflections | 94.2 |
| Overall completeness of data (20.0–1.8 Å) (%) | 98.0 |
| Overall \( R_{merge} \) (%) | 4.0 |
| Overall \( R_{free} \) (%) | 9.0 |
| \( R_{merge} \) in the highest resolution shell (1.86–1.80 Å) (%) | 4.0 |
| \( R_{free} \) in the highest resolution shell (1.86–1.80 Å) (%) | 3.2 |

EXPERIMENTAL PROCEDURES

Synthesis of Peptide FLSKY—The peptide was synthesized using an automated solid phase peptide synthesizer PS3 (Rainin). The resin used was Fmoc-Lys-Wang resin and solvent used for synthesis was dimethylformamide (DMF).

In the first step, Fmoc-Lys-Wang resin was deprotected by 20% piperidine in DMF to form H-N-Lys-Wang resin. The uronium salt 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in the presence of the base N-methylmorpholine (NMM) activated the amino acid to form an active ester of Fmoc-Tyr-OH. This was coupled with H₂N-Lys-Wang resin to get Fmoc-Tyr-Lys-Wang resin. The resin was cleaved from the peptide with trifluoroacetic acid. The peptide was purified by reverse phase chromatography on C₁₈ PepRPC column (1.6 × 10 cm, Amersham Biosciences). The purity of the peptide was confirmed by determining the sequence of amino acids in the peptide with the protein sequencer PPSQ-21A (Shimadzu, Japan).

Inhibition and Binding Studies of PLA₂ and FLSKY—The purified enzyme was used for inhibition and binding studies. The inhibitory assays with 1:0.5, 1:1, 1:10, and 1:20 molar ratios of enzyme and the peptide FLSKY were carried out. 1.5 \( \mu \)M enzyme was incubated separately with 0.750, 1.0, 15.0, and 30.0 \( \mu \)M of inhibitor peptide FLSKY in 20 mM sodium cacodylate buffer, pH 7.0 at 25 °C for 12 h. In these experiments, the 1:2-dithio analogue of diheptanoyl phosphatidylcholine having a CMC value of 4 × 10⁻³ M (22) was used as a substrate (PLA₂ assay kit, Cayman Chemicals, MI). The substrate 1:2-dithio analogue of diheptanoyl phosphatidylcholine was added to a final concentration of 1 mM. Upon hydrolysis of the thiol ester bond at the sn-2 position by PLA₂, free thiols were liberated, which were detected using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) at 412 nm. The control reaction was carried out in the absence of inhibitor.

The binding data were analyzed for saturation binding and Scatchard analysis using GraphPad PRISM software (GraphPad software, Inc. San Diego, CA 92121, www.graphpad.com). The nonlinear regression analysis was used to calculate the dissociation constant.

Dynamic Light Scattering (DLS)—As reported earlier (23, 24) two molecules of PLA₂ were found to be associated in the crystalline state and were expected to be in association in solution as well. To establish this assumption, the experiments on size distribution of PLA₂ molecules were performed with DLS. The data were generated with a DLS system (Dierks & Partner, Hamburg, Germany) and analyzed using Schulte software (25). The sample solutions were prepared in sodium cacodylate buffer (20 mM, pH 6.5) made with Milli Q water. To eliminate dust and other large particles, all samples were further filtered on 0.1 \( \mu \)m polyvinylidene difluoride filters (Millipore) prior to measurements. PLA₂ concentrations were varied from 0.05 to 20 mg/ml at a constant temperature. Samples were measured into the flow cell (50 μl) and illuminated by a 25 milliwatt, 600 nm solid state laser. The data were collected in five replicates for each measurement.

Crystallization—The crystals of the complex were obtained by co-crystallization of protein with peptide FLSKY. 15 mg of purified and lyophilized PLA₂ was dissolved in 1 ml of 20 mM sodium cacodylate buffer at pH 6.5. Peptide FLSKY was added to make the protein/peptide molar ratio 1:10. This solution was incubated for 24 h, and then 20 μl drops of the above mixture were set in the hanging drop vapor diffusion method with reservoir containing 1.4 M ammonium sulfate and 3% dioxane in the same buffer. Well shaped rectangular crystals of dimensions up to 0.5 × 0.4 × 0.3 mm² were obtained at 25 °C within 2 weeks.

reported here. The structure clearly shows that the peptide binds to PLA₂ through its hydrophobic channel and forms a number of interactions with the protein molecule including the active site residues His-48 and Asp-49. It also shows that the placement of the inhibitor with respect to PLA₂ enzyme in the complex is very different than that reported earlier by modeling studies (19). Hence, the nature and number of interactions between the inhibitor and the PLA₂ differ greatly in the two studies.

Data were collected in five replicates for each measurement and analyzed using a nonlinear regression analysis program with reservoir containing 1.4 M ammonium sulfate and 3% dioxane in the same buffer. Well shaped rectangular crystals of dimensions up to 0.5 × 0.4 × 0.3 mm² were obtained at 25 °C within 2 weeks.
this hydrodynamic radius was estimated to be 28 kDa (29, 30). The polydispersity values in these estimations were below 15% of the average radius, indicating that all the PLA2 molecules existed in the dimeric form in solution (Fig. 3a). It may be mentioned here that the approximate radius calculated from the crystal structure for the monomer and dimer were 1.1 nm and 2.2 nm, respectively. Furthermore, the gel filtration profile also indicated the molecular weight of 28 kDa. Thus, the above results clearly show that the two molecules of PLA2 exist in association in the solution state.

The percentage inhibition values of PLA2 by peptide FLSYK at different molar ratios of 1:0.5, 1:1, 1:10, and 1:20 of enzyme to peptide FLSYK were estimated. At all these concentrations of the peptide FLSYK the levels of enzyme inhibition were found to be lower than 50%. The higher molar concentrations of FLSYK with respect to the enzyme PLA2 were expected to show more than 50% levels of inhibition. However, the inhibition of the enzyme did not exceed the level of 50% (Fig. 3b). The data presented in Fig. 3b indicated that the peptide FLSYK interacted only with 50% of the PLA2 molecules. It may be mentioned here that the calculated Bmax obtained from the above binding studies was 0.56 nmol, which also corresponded to the 50% binding of the enzyme molecules. As seen from Fig 3b, it is clear that the peptide binds to one PLA2 molecule of the homodimer. On adding the micellar substrates, the portion of the second molecule of the homodimer may sit against the substrate vesicles with an attached FLYSK to the first molecule. Then in the absence of the inhibitor, in the micellar environment the two molecules separate from each other, and each binds to the phospholipid interface to produce its catalytic effects. The enzyme kinetics shows a complex behavior and hence cannot be described in terms of standard parameters. In this case, the calculated value of the dissociation constant (Kd) was found to be 3.57 ± 0.05 × 10⁻¹⁰ M.

Quality of the Model—The final model consists of two asymmetrically associated PLA2 molecules, one peptide molecule bound to one PLA2 molecule, four acetate ions, and 299 water molecules. The refinement of the final model converged to an R-factor of 20.5% for all data without a cutoff in the resolution range of 20.0–1.8 Å excluding 5% randomly distributed reflections assigned to calculate Rfree (22.5%). In the Ramachandran plot (31), 93.3% of the residues were in the most allowed regions and the remaining 6.7% in the additionally allowed parts of the map (Table II). The structure of PLA2 is based on the well defined electron density for both molecules. The peptide FLSYK was clearly defined in molecule A with an excellent electron density (Fig. 1). The corresponding region in molecule B contained only three water molecules (Fig. 2). The dispersion precision indicator (32, 33) estimates an average root mean-square (r.m.s.) coordinate error of 0.14 Å. The overall G-factor calculated by PROCHECK (34, 35) as a measure of stereochemical quality of model is 0.04.

Molecular Association—The gel filtration profile and the DLS scattering studies had indicated the association of two molecules in solution. Similarly the crystal structure of the complex contains two crystallographically independent molecules A and B in the form of an asymmetric dimer. The overall size of the dimer in the crystals was found to be −48.4 × 30.7 × 20.4 Å³ and the total interface area between the two molecules

**Fig. 1.** The (Fo−Fc) map contoured at 2.5σ showing the electron density for peptide FLSYK in molecule A. This figure was drawn with BOBSCRIPT (46) and RASTER3D (47).

**Fig. 2.** The difference (Fo−Fc) map showing the presence of three water molecules in the hydrophobic channel of molecule B. The contours were drawn at 2.5σ. This figure was drawn with BOBSCRIPT (46) and RASTER3D (47).
is ~1299 Å². In the dimer the entrance to the hydrophobic channel of molecule A opens into the interface whereas that of molecule B has its opening to the surface. The overall conformations of molecules A and B were found to be essentially similar. The r.m.s. shift for the C\(^{\text{α}}\) positions in the two molecules was found to be 0.8 Å. Three regions which showed significantly larger variations contained the Trp-31-containing loop (residues 25–34), the \(\beta\)-wing consisting of two antiparallel \(\beta\)-strands (residues 74–85) and the C-terminal residues 119–133. However, the most striking difference was observed in the orientations of Trp-31. The values of torsion angles \(\varphi, \psi, \chi^1\), and \(\chi^2\) for Trp-31 in molecules A and B were –109°, –19°, –166°, 78° and –129°, 172°, –57°, –86°, respectively. In the dimer, three weak intermolecular hydrogen bonds: (A) Leu-2 N ••• (B) Lys-131 O = 3.4 Å; (A) Leu-3 N ••• (B) Lys-131 = 3.4 Å; and (A) Leu-111 O ••• (B) Gln-108 N32 = 3.1 Å are formed. There are additional hydrogen bonds through structured water molecules. The interface has a high concentration of hydrophobic residues producing a good number of hydrophobic interactions between the two molecules but the most important interactions were observed involving Trp-31 of molecule A, which formed van der Waals contacts with Arg-43, Phe-46, and Val-47 of molecule B. These interactions determined the orientation of the side chain of Trp-31 in molecule A.

**Binding of Peptide FLSYK to PLA\(_2\)**—The electron density for the peptide in molecule A was exceptionally clear (Fig. 1), which allowed a detailed description of the interactions between PLA\(_2\) and the peptide FLSYK. As seen from Fig. 4, the peptide is completely buried in the pocket. Two key hydrogen bonds involving His-48 and Asp-49 with peptide are also indicated by dotted lines. The electron density for the peptide in molecule A was exceptionally clear (Fig. 1), which allowed a detailed description of the interactions between PLA\(_2\) and the peptide FLSYK. As seen from Fig. 4, the peptide is completely buried in the pocket. Two key hydrogen bonds involving His-48 and Asp-49 with peptide are also indicated by dotted lines. The electron density for the peptide in molecule A was exceptionally clear (Fig. 1), which allowed a detailed description of the interactions between PLA\(_2\) and the peptide FLSYK. As seen from Fig. 4, the peptide is completely buried in the pocket. 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Two key hydrogen bonds involving His-48 and Asp-49 with peptide are also indicated by dotted lines.
and a large number of van der Waals interactions (data not shown). It is noteworthy that the lysine of the peptide interacts with the enzyme rather extensively (Fig. 6). It also nullifies the charge effect of Asp-49, which plays a major role in stabilizing substrate during catalytic mechanism (36). Furthermore, the peptide also forms important hydrogen bonds with the carbonyl oxygen atoms of Tyr-28, Gly-30, and Gly-32 of the Trp-31-containing loop in a manner similar to the coordinating interactions provided by calcium ion if present in the structure. Yet another important interaction involving peptide carboxylic group and His-48 Nε curtails the ability of His-48, to extract a proton from crucial conserved water molecule for initiating the catalytic mechanism (36). It may be noted that the critical water molecule, which bridges His-48 to Asp-49 in the native structure is absent in the present complex. It has, apparently been displaced by the peptide FLSYK.

It may be emphasized that the peptide FLSYK forms a larger number of interactions with PLÅ2 as compared with those observed in other complexes formed between PLÅ2 and synthetic/natural inhibitors reported earlier (10, 11, 13–16, 37–41) making it to be the strongest inhibitor known so far against PLÅ2. Its dissociation constant, \( K_d \), was found to be \( 3.57 \times 10^{-9} \) M. Recently, molecular modeling studies showing the binding of FLSYK to human sPLÅ2-IIA were also reported (19). However, as compared with the docking model, the experimental data reported here show very different interactions between the enzyme and the peptide FLSYK. In order to distinguish peptide residues from the protein residues, hereafter the peptide residues will be represented with a \( P \) in parentheses. In the docking model, main chain atoms of Phe (P) residue were implicated to be interacting with three carbons of the side chain of Arg-7 of PLÅ2, whereas in the present study, the backbone Phe O (P) forms a hydrogen bond with OW-4 while Phe N (P) forms two hydrogen bonds with Lys-7 N and Leu-3 O. The majority of the interactions with the aromatic ring of Phe (P) are with Lys-7, Leu-10, and Leu-17 while the model studies had indicated it to be interacting with Tyr-73 to Phe-75 region. Furthermore, Leu O (P) forms a hydrogen bond with Ile-19 N while no such interaction was indicated by the model studies. Ser N (P) forms a hydrogen bond with Gly-6 N while Tyr OH (P) forms a hydrogen bond with OW-64. The side chain of Tyr (P) forms several van der Waals contacts with Leu-2, Gly-30, and Trp-31 of molecule A. As the Tyr (P) is located at the interface of the asymmetric dimer, it also forms a number of van der Waals contacts with Arg-43, Phe-46, and Val-47 of molecule B. The side chain of Leu (P) interacts with Ala-18 and Ile-19 while Ser Oγ (P) forms an intramolecular hydrogen bond with Tyr O
tions observed in the present complex underline the signif-

ications of the Trp-31 loop (residues: 28–34) in native PLA2 and in complexes with inhibitors (A) FLSYK, (B) α-tocopherol, (C) Aristolochic acid and (D) LAIYS

| Protein residues | Torsion angle | Native structure 1YB2 | (A) L1Q8 | (B) 1KPM | (C) 1FVO | (D) 1JQ8 |
|------------------|---------------|-----------------------|----------|----------|----------|----------|
| Tyr-28           | φ             | −114                  | −115     | −114     | −117     | −115     |
|                  | χ1            | −67                   | −68      | −66      | −66      | −66      |
| Cys-29           | φ             | −86                   | −92      | −89      | −87      | −91      |
|                  | χ1            | 78                    | 87       | 77       | 90       | 75       |
| Gly-30           | φ             | −179                  | −129     | −141     | −156     | −143     |
|                  | χ1            | −174                  | −166     | −66      | −61      | −55      |
| Trp-31           | φ             | −109                  | −109     | −96      | −75      | −94      |
|                  | χ1            | −174                  | −166     | −66      | −61      | −55      |
| Gly-32           | φ             | −78                   | −59      | −65      | −52      | −49      |
|                  | χ1            | −126                  | −179     | 179      | 169      | 163      |
| Gly-33           | φ             | 138                   | 180      | 173      | 170      | 153      |
|                  | χ1            | 118                   | 47       | 64       | 62       | 51       |
| Lys-34           | φ             | −142                  | −148     | −145     | −147     | −163     |
|                  | ψ             | 173                   | 163      | 156      | 162      | 168      |

(P) to stabilize the structure of the peptide. The most significant interactions observed with Lys (P) are not even indicated by the docking model. Lys N (P) forms a hydrogen bond with Gly-30 O while Nε C interacts with carboxylic group of Asp-49, Tyr-28 O, Gly-30 O, and Gly-32 O. The carboxylic group of Lys (P) interacts with His-48 Nδ1. As compared with the interactions indicated by the modeling studies, the interactions observed between the peptide FLSYK and PLA2 in the present crystal structure are far more extensive and remarkably different, thus showing the weakness of the modeling studies. The modeling studies have failed to highlight the critical interactions with active site residues His-48 and Asp-49 involving lysine of the peptide molecule. In view of this, the results of theoretical investigations may be used with caution and experimental studies may often be essential to arrive at final design goals.

Comparison of the Structures of PLA2 in the Present Complex and in Other Complexes (PDB Codes: 1KPM, 1FVO, 1JQ8)—

The structure of PLA2 from Daboia russelli pulchella has been studied in its native form (24) and in the complexes with natural substances such as α-tocopherol (39) and aristolochic acid (40), and with a designed peptide inhibitor Leu-Ala-Ile-Lys. The root mean square shift for all the atoms in loop 28–34 is 1.1 Å. The corresponding values for the complexes with α-tocopherol, aristolochic acid and LAIYS were found to be 1.9, 1.7, and 1.9 Å, respectively. A comparison of the torsion angles in the same loop (Table III) involving five structures clearly shows a similarity between the structures of the native PLA2 and its complex with FLSYK whereas the other three structures (39–41) resemble each other well but differ remarkably with the former (Fig. 7). The fact that the conformation of the binding site in the complex with FLSYK is similar to that in the native state is indicative of an excellent complementarity between the structures of the peptide FLSYK and the binding site of PLA2. This was reflected in the form of a very low dissociation constant, KD at 3.57 ± 0.05 × 10−9 M for the PLA2–FLSYK complex. The corresponding dissociation constants for the complexes with α-tocopherol (39), aristolochic acid (40), and LAIYS (41) were found to be 1.63 ± 0.04 × 10−6 M, 1.37 ± 0.05 × 10−6 M, and 9.74 ± 0.03 × 10−7 M, respectively.

CONCLUSIONS

The present studies confirm that the PLA2 from Daboia russelli pulchella exists as a dimer both in the crystalline as well as in solution states. It may be mentioned here that native structure of this enzyme was also found to form an asymmetric homodimer (24). Also it is noteworthy that the PLA2 enzymes of snake venom origin have been often found in the multimeric forms, whereby their activities get regulated by the manner their subunits are associated. At times, as a result of multimerization, the access to active site residues may be physically blocked whereas at other times it might induce changes in the conformation of the binding site making it suitable/unsuitable for simple binding and might require a strong ligand to induce a suitable conformation for its activity. Generally, the multimerization enhances the enzyme stability, though often at the expense of its activity. In the present case, however, the conformationally suitable site is located at the interface of the two molecules as the peptide FLSYK is bound to molecule A of the PLA2 enzyme at the interface of the asymmetric homodimer. The failure of binding of FLSYK to the exposed site in molecule B indicates that the conformation of the binding site in molecule B was unsuitable for the binding of the inhibitor. On adding the micellar substrate to this complex, the B portion of the homodimer sits against the substrate vesicles with an

ent complex does not change appreciably (Fig. 7, Table III). The root mean square shift for all the atoms in loop 28–34 between the present complex and the native structure was 1.1 Å. The corres
attached molecule A-FLSK. Then in the absence of the inhibitor and in the micellar environment, molecules A and B presumably separate from each other, and each binds to the phospholipid interface and generates catalytic effects. Apparently, the dimensions of the interface are smaller than the dimensions of the micellar substrates. Hence, it is expected that the aggregated substrates are required to dissociate the dimer before binding to molecule A. On the other hand, the binding site of molecule B is inherently unsuitable and hence the substrate must induce an appropriate conformational change before binding to molecule B. These two steps involving dissociation of molecules A and B and induction of suitable conformation in molecule B might account for the lower rate of enzyme activity reported in the PLA2 from Daboia russelli pulchella as compared with PLA2 molecules from other species (42–44). The results of these studies also support the fact that the PLA2s show higher activities against aggregated substrates than that observed with monodispersed molecule (45).

Finally, the present model complex has provided a lead peptide for a further structure-based design of specific inhibitors. It may be mentioned here that the inhibitors of PLA2 enzymes reported so far had primarily exploited the presence of a terminal OH group providing the two key interactions with His-48 (36). The present result that the inhibitors of PLA2 enzymes may be useful for the development of specific inhibitors of PLA2.

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