Journal on Applied Bioengineering, Vol.1, No.1, December 2007

Molecular Dynamics Study of Phospholipase A_2 - α-Tocopherol Complex

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Abstract

Inflammation is the complex biolog ical response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. Phospholipase A_2 (EC 3.1.1.4) is a key enzyme of the cascade mechanism involved in the production of pro-inflammatory compounds known as eicosanoids. The binding of Phospholipase A_2 (PLA_2) to membrane surfaces and the hydrolysis of phospholipids are thought to involve the formation of a hydrophobic channel into which a single substrate molecule diffuses before cleavage. The PLA_2 α-Tocopherol (α-TP) complex is the first evidence of inhibition of PLA_2 by Vitamin E (α-TP). PLA_2 contains two homologous chains in an asymmetric unit (Chain A & B). The crystal structure of α-TP bound Viper russelli PLA_2 with resolution 1.8 ? (PDBID: 1KPM) is available in the literature [1]. The feature to be considered here is the binding of vitamin E only in chain A. The reasons for the specificity in binding with chain A is the orientation of an aromatic ring (of W31), which is present in the gateway of hydrophobic channel that forms the active site of enzyme. Another reason may be presence of three water molecules in the active site of molecule B and not in molecule A. The torsion angles (φ, ψ) for the backbone of W31 in molecules A and B are -94°, -30° and -128°, 170° respectively [1]. The hypothesis assumed was that the change in conformation of binding site and the changes in Trp31 orientation are responsible for binding of ligand. The present work is focusing on molecular dynamic simulation of PLA2- α-TP complex. The resultant trajectory is probed for the evidence to prove the hypothesis.

Key words: Phospholipase A2, α-Tocopherol, Molecular Dynamics Simulation, Generalized Born Simulation, Amber.

I. INTRODUCTION

A. Structure of PLA_2

In general the structure of PLA_2s contains two crystallographically independent molecules, A and B, in the asymmetric unit. The hydrophobic channel of both chain A and B contain the His48 and Asp49 as key residues in binding. These residues have interaction with ligand in most of the PLA2 ligand complexes. Thus, H48 and D49 along with the residues W31 and L2 (aiding binding by opening channel) form the compact environment for ligand binding [1]. In addition to a characteristic catalytic site, PLA2 possesses several other functional sites which are responsible for various pharmacological properties. These sites are important since they differ greatly in various PLA2s. There are six such loop regions, which are held in different ways by common secondary structure motifs [3]. Apart from loops there are three helices (H1, H2 & H3) and two short helices (SH4 & SH5) and one β-wing with strands β1 & β2 in anti-parallel fashion.

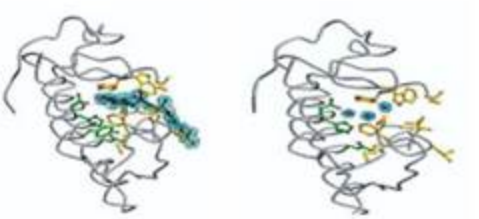

Fig 1a: PLA_2 αTP complex Fig 1b: Chain B+WAT

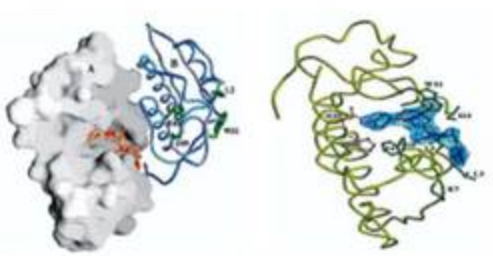

Fig 2a: PLA_2LAIYS(1JQ8) Fig 2b: Chain A with ligand

The structure taken for present work was *Viper russelli* PLA_2 complexed with vitamin E (PDBID: 1KPM, Fig. 2.2a and 2.2b). The superposition of molecules A and B shows an R.M.S. difference of 0.71 Å for the C_α

positions.

PLA_2 with penta-peptide (LAIYS) from *Daboia russelli pulchella* ($DPLA_2$, PDBID: 1JQ8) is another example to understand molecular association [6]. The figure 2a shows the association pattern of molecules A and B. The opening of the hydrophobic channel of molecule A is located at the interface, while that of molecule B is situated at the surface. Molecule A is shown in surface representation with the peptide (Fig. 2.3b) in the binding channel and molecule B is drawn in backbone mode. The hydrophobic channel in molecule B opens to the surface at Trp31 and Leu2 (Figure 2a) [2].

B.Molecular Dynamics Simulation:

Most of the biological macromolecules can exist in different conformations. Conformations of one molecule may vary in vacuum, in the liquid state, in various solutions, and in the crystal. In general, a population of conformations may exist in solution, and properties of interest (such as a biological activity) may be associated with one or a small number of them. These property specific conformations may not be the prevailing conformation in the Solid state, in solution, or in vacuum. It thus becomes necessary to calculate possible conformations and deduce which conformation is important for the property of interest.

The negative gradient of the potential energy evaluated from the force field yields the force. Using this force and the mass for each atom, Newton's equation of motion (F=ma) can be numerically integrated to compute the position of the atoms after a short time interval (typically of the order of 1 fs, 10^{-15} sec). By taking successive time steps, a time-dependent trajectory of all the atomic motions can be constructed.

Amber is designed to work with several simple types of force field, although it is most commonly used with parameterizations developed by Peter Kollman and his co-workers. The present work is based on 'Generalized Born' approach using amber package [21]. Essential features of this method are the solvation of solute using implicit water (implicit solvation) to the system instead of explicit one, the analytic GB model efficiently describes electrostatics of molecules in water environment, it represents the solvent implicitly as continuum with the dielectric properties of waters, and includes the charge screening effects of salt.

The present study is focusing on molecular dynamic simulation of PLA2- α-TP complex. The resultant trajectory is probed for the evidence to prove the hypotheses. The molecular dynamics simulation package Amber 8 is used. The trajectory was analyzed using ptraj & vmd software. The hypothesis was set that the change in conformation of binding site and Trp31 orientation is responsible for binding of ligand.

II. MATERIALS AND METHODS

In this study, MD was carried out in three different ways. First of all, barely Chain A is taken from the coordinate file (1KPM) by excising coordinates of all other molecules including crystallographic waters. Similarly, in the second, Chain A is taken along with its ligand molecule (complex). In the Final, Chain B is taken with three water molecules those present in the hydrophobic channel. All these three processes were performed using 'Amber Generalized Born Approach' [21].

A.Steps Involved in MD Process:

The molecular dynamics analysis includes the several steps. Preparation of ligand and proteins with reference to the preferred force field is the predominant. In the present study we used Amber99 force field (ff99) with General Amber Force Field (GAFF). In this step (esp., in amber) the force field for the ligand should be prepared using the preparatory programs as follows. As discussed earlier, three different simulations were performed separately. Since the solute of each dynamics is different in composition, the following steps are general and are not specific to all these three simulations.

B. Ligand Preparation:

In this step we used the Antechamber tools with Leap to create topology and coordinate files for α-TP. Since the amber force field does not contain force field for the ligand present in the complex, we are in need of creating it using the antechamber program. The antechamber program will automatically identify bond and atom types, judge atomic equivalence, generate residue topology files and find missing force filed parameters and supply reasonable suggestions. The parameter files are generated with bcc charge type, which will be used in xleap to define the ligand bound in PLA_2.

C. Protein Preparation:

There is a possibility for the defects in initial protein structures, such as, missing side chains, missing residues, improper atom type, modified residues etc., These should be found and fixed before using it for the analysis. Apart from this, the PDB file does not contain bond information. Disulfide bond (-S-S-) is important in protein conformation and we create explicitly using xleap program. For all the three dynamics, 'chain A', 'complex' and 'chain B with active site waters', were prepared. Both the chains of PLA_2 complex contain seven disulfide bonds and those will be

created manually. In addition to that, the command 'set default PBradii bondi' is used, because, the GB charge-charge interaction (smoothing function) depends upon atomic radii and inter-atomic distance. Finally, the topology and initial coordinate files were saved with out solvating the system.

D. Energy Minimization:

To relax the initial structure from internal strain, we used energy minimization technique. In practice, few cycles of 'steepest descent' followed by extensive 'conjugate gradient' method is used for getting accurate low energy conformation. The process control file was set with the total of 500 cycles (200 cycles of former and 300 cycles of latter one), salt concentration of 0.2 M, cutoff of 10 Å and with modified GB model.

E. Equilibration:

The equilibration step is to heat and equilibrate our three systems. We run this 20ps of equilibration with 1 fs time step for all 3 of our systems (using the final structure from the minimization as our input structure). To achieve the effective equilibration, we allowed the process for 2000 cycles with integration time step 1fs (0.001 ps), using SHAKE algorithm to constrain the bonds to H atoms, removed translational and rotational motion at every 1000 cycle (desired for GB model) and the temperature was slowly raised frm 0K to 300K.

F.Production Run:

In molecular dynamics simulation, we disable periodicity and set control file according to implicit solvent procedure using Berendsen temperature coupling. We also set the initial and final temperatures to 300K which means that our system's temperature should remain around 300 K. For all three dynamics we run a total of 2,000,000 steps each with a 1 fs time step giving simulation lengths of 2 ns (2000000 x 1fs).

G.Trajectory Analysis:

The output file, which will be generated as a result of each run of sander program (for energy minimization, equilibration and production), contains the value of all force field terms. The perl program called 'process_mdout.pl' is used to extract the information contained in the output file (of equilibration and production run) to analyze using XMGR. The trajectory can be represented in the form of different graphs as described in discussion.

The changes in the orientation of W31 can be monitored by plotting the conformational angles (Phi, Psi, Chi1 and Chi2) against the time (in pico second). The plots were generated using the SIRIUS and XMGR software packages and the movie can be generated using SIRIUS and VMD [18]. The whole dynamics processes including energy minimization and equilibration was run in P4 (3.14GHz) processor in RedHat (EL4) workstation and it took about 9 days for 1ns.

III.RESULTS & DISCUSSION

The comparative molecular dynamics must reveal the dynamic nature of these three substructures of PLA_2 taken for analysis. Hence graphs were plotted from trajectory and corresponding snapshots were taken for interpretation.

Energy minimization of all the structures was performed. The favourable least energy was attained as a result of 200 cycles of steepest descent, followed by 300 cycles of conjugate gradient minimization techniques. Since the system to support GB simulation, the equilibration and production run were carried out with the non periodic boundary using Berendsen temperature coupling. Figure 3 shows the temperature profile during equilibration process. The temperature was raised form the 0K and reached the equilibrium (300K) at about 8ps and stabilized thereafter.

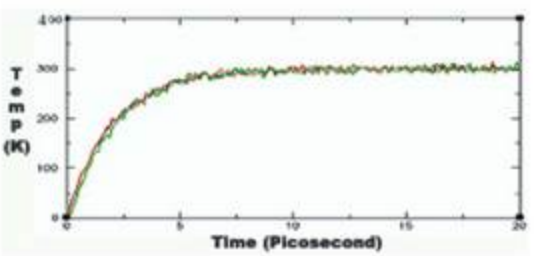

Figure 3: Temp Vs Time Plot Chain A (black), complex (red) and B with water (green)

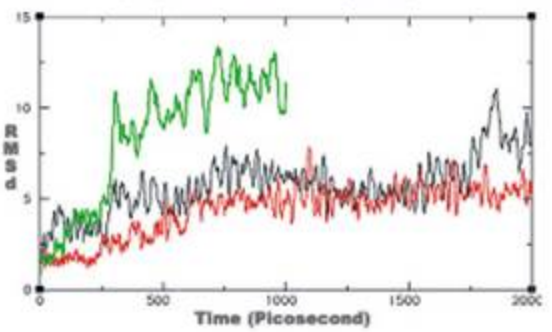

Figure 4: Backbone RMSd of Chain A (black), complex (red) and B with water (green)

The Fig.4 shows the more conformational fluctuation in Chain-B, and least in complex. From this

observation we can conclude that the more fluctuation in chain B disrupts the hydrophobic channel formation. This is very much needed for the ligand binding. More importantly, the less conformational changes observed in the complex than ligand free Chain A can also been seen. Hydrophobic channel filled by the ligand leads to the conformational stability in complex. In this case, less mobility Helix nearby the channel and the anti-parallel beta sheet and favourable mobility in the W31 can be clearly observed. The mobility of the same residue and other secondary structural elements is more in the case of ligand free Chain A of Phospholipase A_2

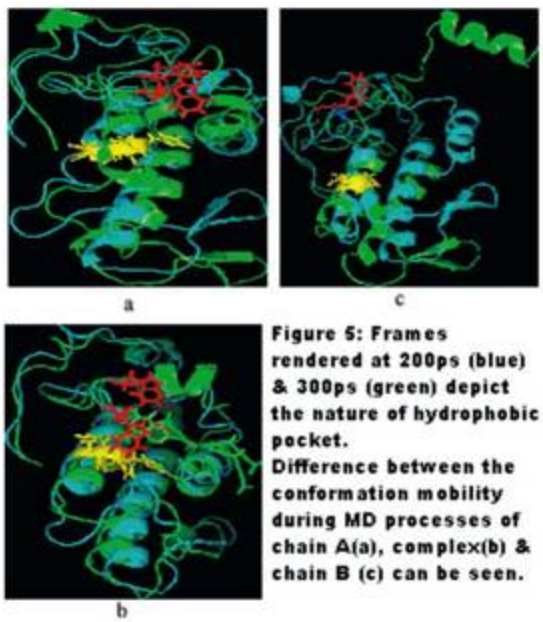

Figure 5: Frames rendered at 200ps (blue) & 300ps (green) depict the nature of hydrophobic pocket. Difference between the conformation mobility during MD processes of chain A(a), complex(b) & chain B (c) can be seen.

The above pictures give us an idea about the difference between the conformation mobility during MD process. The helix (H1), beta sheet and W31 (red) are important elements to be noted. Fluctuation is drastic in chain B especially in H1, slight chain A and very less in complex.

A. Analysis of Dihedral Angles (φ, ψ, χ1 & χ2):

The entire conformation of the protein can be described by these angles of internal rotation. Geometrically, the main chain of a protein is a succession points in space (Phi, Psi and Omega). The side chain conformations are also described by the angles of internal rotation. Different side chains have different numbers of degrees of freedom. The side chain conformational angles are denoted by χ_1 & χ_2 In the present study, the W31 orientations were observed using these entire angles together. All the dihedral angles were calculated for whole simulation data, using the ptraj and plotted using xmgr tool. The following igures show graph (Angle Vs Time) for all the dihedral angles for each of three dynamics simulations separately.

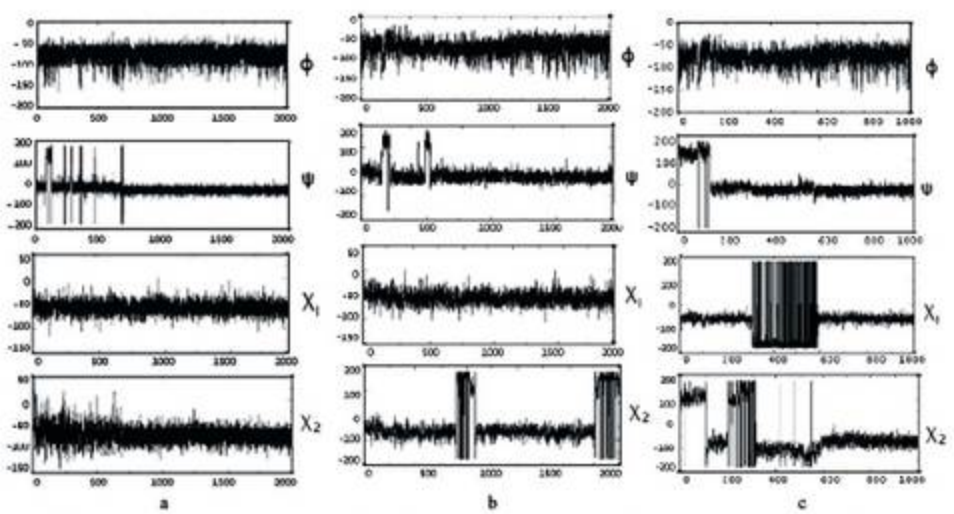

Figure 6: Dihedral Angles of W31 (Y- axis) in Chain A (a), Complex (b) and Chain B (c)

between -180 to +180 degrees with some in ~50 ps intervals. In the case of Chain B, there is a change in initial steps of 100ps, and it was stabilized thereafter at around -25 degrees (Figure 6c). As expected, the fluctuation is less in complex than the chain A. This is because of the hydrophobic channel filled by the ligand. Hence we confirm that the mobility of the W31-residue also favours the ligand binding. The side chain angles (χ1 and χ2) do not reveal any favourable pattern of mobility. There is a constant fluctuation Chi1 angle in Chain A and in complex, but unreasonable fluctuation observed in chain B. Similarly the χ2 angle is stable in chain A. But, the angles changed more rapidly between +180 to -180 in the middle and later stage of complex, whereas in Chain B these fluctuated in the initial 250ps. In order to conclude, there is an opening and re-closing in W31 residue due to the changes Psi angles. This is already proposed in the literature [1].

IV. SUMMARY

As discussed earlier, the mobility of the side chain is described by chi1 and chi2 angles, whereas, that of the residue is described by phi and psi angles. The graphs shown in figure 6 were plotted for the residue W31 present in all the three models taken for the analysis. The flip-flop nature of the W31 can be confirmed by comparing these plots. The Phi angle profile has almost the same pattern in all the simulations. But, the Psi angles, in chain A (Figure 6a), constantly shuttle

The crystal structure of α-TP bound Vipera russelli PLA2 at 1.8 ? resolution is taken for the present study. The feature considered here is the binding of α-TP in chain A only. The reasons for the specificity in binding with chain A is the orientation of an aromatic ring (of W31), which is present in the gateway of hydrophobic channel that forms the active site of the enzyme. Another reason may be the presence of three water molecules in the active site of molecule B and not in molecule A. The torsion angles (φ, ψ) for backbone of W31 in molecules A and B are -94°, -30° and -128°, 170° respectively [1].

From the above, we can infer the exact role of W31 in ligand binding. The fluctuations observed (esp., in Psi angle) throughout the simulation are more informative. Moreover, the active site conformation in all the three dynamics output reveals the role of helices around the active site. These helices are form more stable architecture for ligand binding. This is observed only in the case of Chain A and complex (Figure 5a & 5b) and the helix assembly is absent in the Chain B (Figure 5c).

Apart from the W31 orientation, we can see the movement of the N-terminal helix and the anti-parallel β-sheet. The distance between these two elements is continuously increasing after 1ns simulation, and the separation is less in Chain A and complex. But the separation increases very rapidly in the case of Chain B. This might be another reason for the formation of compact binding site along with W31 in chain A.

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