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## Phospholipases A<sub>2</sub> Protein Structure and Natural Products Interactions in Development of New Pharmaceuticals

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### 1. Introduction

Phospholipases are enzymes that hydrolyze phospholipids into fatty acids and other lipophilic substances. There are four main classes, named A, B, C and D, which are distinguished by the type of reaction they catalyze. The phospholipase A<sub>2</sub> (PLA<sub>2</sub>), EC 3.1.1.4, are enzymes that release fatty acids from the second carbon group of glycerol. These enzymes are common in many living organisms and can be found both intra and extracellularly, which are referred to as secretory PLA<sub>2</sub> (sPLA<sub>2</sub>) and cytosolic PLA<sub>2</sub> (cPLA<sub>2</sub>), respectively (Burke & Dennis, 2009; Dennis, 1997; Diz Filho et al., 2009; Schaloske & Dennis, 2006). The enzymatic action of cPLA<sub>2</sub> on lipids, allows the formation of a compound that acts as mediators of inflammatory diseases (Chakraborti, 2003; Dennis, 1994). Furthermore sPLA<sub>2</sub> is divided in 5 different groups among them group I (sPLA<sub>2</sub> from mammalian pancreas) and group II (sPLA<sub>2</sub> from venom snake) are the most studied since they are also involved in pathological processes (Fonteh et al., 2000). The focus of this chapter is on the structure and interaction of snake venom sPLA<sub>2</sub> with natural compounds. These sPLA<sub>2</sub> also present pharmacological activities such as: neurotoxicity, cardiotoxicity, myotoxicity, edema formation and hemorrhagic effects (Kini Chan, 1999; Valentin & Lambeau, 2000). Due to the implication of these enzymes in the inflammatory process, several studies have been proposed in order to find new compounds able to inhibit the action of sPLA<sub>2</sub>.

## 2. Structural features and biological activities of sPLA2

Enzymatic reactions promoted by sPLA2 happen in a lipid-aqueous interface and the phospholipase activity is more efficient on substrates such as monolayers, bilayers, micelles, membranes and vesicles with monomolecular dispersed soluble substrates. This phenomenon has been termed "interfacial activation" and includes "inter-facial-binding" enzyme and 'activation' steps (Berg et al., 2001; Chakraborti, 2003; Burke & Dennis, 2009). The proposed mechanism of sPLA2 involves the side chain of Asp99 and His48 and a molecule of water (Scott, 1994). According to the mechanism, a proton in the position 3 of the imidazole ring from His48 is bound to the carboxylic group of Asp99, and rotation is prevented, this way the nitrogen (position 1) is able to attack the molecule of water releasing a molecule of hydroxyl. The hydroxyl through a nucleophilic attack, to tie up the carbon from ester group substrate and an intermediate is formed. Afterwards, the oxygen from ester group attacks the proton (position 1) of imidazole ring to produce a lysophospholipid alcohol and a double bond between carbon and oxygen of ester group is remade and then occurs the hydrolysis of acyl-ester sn-2 releasing two products: a phospholipid and a fatty acid (Verheij et al., 1980).

They are known several isoforms of sPLA2 all of them have a conserved His/Asp catalytic dyad and a Ca<sup>2+</sup>-binding loop. sPLA2-IB, a pancreatic sPLA2, is characterized by a N-terminal pro-peptide whose proteolytic removal gives rise to a functional enzyme, besides the presence of a Cys11-Cys77 disulfide bonds (group I-specific disulfide), which is a unique bond of pancreas sPLA2s. The group II subfamily (IIA, IIC, IID, IIE and IIF) is characterized by the absence of the pro-peptide and the presence of Cys 49 in the C-terminal extension (group II-specific disulfide). sPLA2-IIF has a long C-terminal extension, which is pro-rich. sPLA2-V is evolutionarily close to the group II subfamily, but has no group II-specific disulfide and no C-5 terminal extension. sPLA2-X has properties of both groups I and II, since it has an N-terminal pro-peptide and both groups I and II-specific disulphide bonds. sPLA2-III is unique considering the central domain sPLA2, which is more similar to bee venom PLA2 than to group I / II / V / X sPLA2s, that is flanked by unique and highly cationic N-terminal and C-terminal domains. C-terminal domain is removed to produce a unique domain, the mature sPLA2 form. The group XII contains two isoforms collection, XIIa and XIIb, whose general structures (except for the catalytic domain and Ca<sup>2+</sup>-binding site) do not show any homology with other sPLA2s. The catalytic site is replaced by Leu in sPLA2-XIIb, indicating that this enzyme has no catalytic active (Murakami et al., 2010; Murakami et al., 2011).

The sPLA2 compose a superfamily of hydrolases, which can be divided into calcium depended and independent and can be classified mainly based on sequence homology and the position of disulfide bonds. Recently, a large number of new enzymes that hydrolysis glycerophospholipids in the sn-2 position have been characterized and classified into three main groups and several subgroups were extended. There is structural similarity between sPLA2 and that its catalytic domain is characterized by a three-tier architecture employing a being saved the Asp catalytic dyad instead of the classical catalytic triad, these similarities and other structural and biochemical evidences suggest that sPLA2 evolved a common ancestral gene. The information gathered on the physiological action, structure and functioning of these enzymes suggest that in addition to its complex distribution, these enzymes may have different regulatory mechanisms and functional roles (Schaloske, 2006; Murakami et al., 2011).

The sPLA2 molecules are well characterized and established as important enzymes. Evidences show that some of these sPLA2 are involved in the release of arachidonic acid from cellular phospholipids for the biosynthesis of eicosanoids in the inflammatory process (Lambeau, 2008; Lättig et al., 2007; Murakami et al., 1997; Oliveira et al., 2008; Toyama et al., 2011; ValentinLambeau, 2000). Although several experimental data show that sPLA2 may also modulate the activity of cPLA2 and that this modulation may be mediated through specific cell receptors for sPLA2, there is still a discrete mechanism elucidation of the molecular events generated by the interaction of sPLA2 with their receptors (Fonteh et al., 2000; Boilard et al., 2010).

Experimental evidence shows that certain sPLA2 Group IIA, V, X can have many different pro-atherogenic properties in the blood vessel walls. This pro-atherogenic activity depends on the generation of pro-inflammatory lipid mediators such as prostaglandins, thromboxanes, leukotrienes, and lysophospholipids; hydrolysis of low-density lipoprotein (LDL) and conversion of them into more pro-atherogenic particles, besides promote multiple inflammatory processes in various artery wall cells (Boyanovsky et al., 2010; Divchev, 2008; Hanasaki, 2002; Jayaraman et al., 2011; Murakami, 2003; Oestvang & Johansen, 2006; Rosengren et al., 2006; Webb, 2005). Scientific evidence supports the importance of sPLA2 in physiological and pathological processes.

Due to the large variability of the structure and function of sPLA2, they can be considered the most important class of phospholipases. Therefore this important class of enzymes is promising as a therapeutic target for the development of new drugs sPLA2 inhibitors which may be used in the treatment and control of cellular processes, such as acute inflammation.

## 2.1 Natural compounds as inhibitors of sPLA2

Due to the role of sPLA2 in the inflammatory process, there is interest in pharmacological inhibitors of sPLA2 from natural products isolated from plants, algae and other sources. Polyphenols constitute one of the most abundant and widely distributed plant secondary metabolites, present in plants that are commonly consumed in the diet including various grains, vegetables, fruits, extra virgin olive oil, red wine and tea (Garcia-Salas et al., 2010; Yang et al., 2006).

Polyphenols have been reported to have a wide range of biological activities. Many studies show that the beneficial effects of phenolic compounds and suggests its role as a promising therapeutic tools in several acute and chronic diseases. They are extensively metabolized *in vivo* and several studies have focused on the interaction of polyphenols with intracellular proteins involved in vital signalling pathways to cell function. One important anti-inflammatory mechanism played by polyphenols is the inhibition of eicosanoid generating enzymes, including PLA2, cyclooxygenase and lipoxygenase, thereby reducing the concentration of prostanoids and leukotrienes. Arachidonic acid (AA) is released from membrane phospholipids by PLA2 cleavage, which can be metabolized by cyclooxygenases (COX) into prostaglandins (PGs) and thromboxane A2 (TXA2), or by lipoxygenases (LOX) to hydroperoxyeicosatetraenoic acids (HPETE), hydroxyeicosatetraenoic acids (HETES) and leukotrienes (Lts) (Blanchard et al., 1998; Kim et al., 2008; Tanaka, 1995; Terracciano et al., 2006).

Among the polyphenolic compounds, flavonoids are the most common in human diet. Studies have shown that the ability of sPLA2 inhibition by flavonoids is related to structural features, such as: the 5-hydroxyl group and the double bond and the double bonded oxygen in the ring oxana, and that the groups in the 3'-hydroxyl and 4' -position are necessary for the selective inhibition of sPLA2 (Lindahl, 1997). However, the exact mechanism by which flavonoids inhibit sPLA2 remains unclear. Iglesias et al. (2005) showed that morin modifies the secondary structure of sPLA2 from *Crotalus durissus cascavella* venom, but did not significantly affect its pharmacological activity.

Flavonoids and other natural compounds isolated from plants have shown promising results in the development of anti-inflammatory candidates, because they may interact with key enzymes such as PLA2, COX and LOX. In this chapter is presented an overview of general techniques that can be used to measure the effectiveness of new sPLA2 inhibitory compounds, including techniques for analyzing proteins in tandem using HPLC detectors in combination with circular dichroism, fluorescence, UV-Vis and other techniques such as mass spectrometry and molecular docking. The sPLA2 isolated from *Crotalus durissus* and *Bothrops jararacussu* are the two main models used by our research group as molecular targets to study mechanisms of different anti-inflammatory compounds, because they are abundant in snake venom and structurally well characterized with atomic coordinates deposited in protein data banks (Chioato & Ward, 2003 Oliveira, Fonseca, Antunes et al., 2008; Lomonte et al., 2003; Nunes et al., 2009; Soares et al., 2003).

Furthermore, integrated biochemical-pharmacological techniques such as paw and skin edema, mast cell degranulation and myotoxicity are shown here. Finally, techniques for measuring enzyme activity using specific substrates for sPLA2 are also explained.

## **2.2. Experimental procedures to evaluate interactions between sPLA2 and natural compounds**

### **2.2.1 Snake venom sPLA2: a model for molecular target of NC**

sPLA2 used for assessing potential inhibitors from natural compounds in our research group are usually isolated from *Crotalus durissus terrificus* and *Bothrops jararacussu* venoms. The first sPLA2 was purified by crystallization in 1938 by Slotta and Fraenkel-Conrat from the venom of *Crotalus terrificus* (*Crotalus durissus terrificus*) and has been one of the catalytically active sPLA2 molecules better characterized in terms of cellular interaction and an example of cooperation between different venom components, because when sPLA2 is associated with crotapotin, the pharmacological and biochemical properties change (Nunes, Zychar, Della-Casa et al., 2009). The sPLA2 from *Bothrops jararacussu* venom has been studied since the 80's and the great interest in this poison is the presence of a non-catalytically active sPLA2 (BthTX-I), which represents approximately 28% of dried whole venom. In addition, the whole venom of *Bothrops jararacussu* has approximately 7 to 8% of catalytically active sPLA2 (BthTX-II) (Lomonte, Angulo, Santamaria, 2003). These two sPLA2 have already been well characterized in terms of their pharmacological, physiological, biochemical and structural properties, highlighting good crystallographic data deposited in proteins data banks. The fractioning of these two venoms have been determined and maximized to obtain the maximum achievement of sPLA2 (Chioato, 2003).

For the isolation and purification of sPLA2 from the venom of *Crotalus durissus terrificus*, two chromatographic steps are done. First, venom of *Crotalus durissus terrificus* is fractionated on a molecular exclusion HPLC column, which allows the purification of the major toxin groups: convulxin (CLC, ~ 85kDa), gyroxin (Gyr, ~ 50 kDa), crotoxin (Crtx, ~ 35kDa) and crotamine (Crot, ~ 10kDa). The crotoxin fraction is then subjected to a new chromatographic run on a reversed-phase HPLC column, isolating the catalytically active sPLA2.

*Bothrops jararacussu* venom has two types of sPLA2, the catalytically active (D49) and catalytically inactive (K49) sPLA2s. Both proteins account for approximately 35% of dried venom. Again, two chromatographic steps are performed to obtain these sPLA2s. The initial step comprises the fractionation of whole venom on a column of Cation Exchange Chromatography (CEC), which allows to obtain both catalytically active sPLA2 (D49, BhtTx-II) and catalytically inactive sPLA2 (K49, BhtTX-II). These two fractions are then subjected to a new chromatographic run using a reverse phase HPLC column. All sPLA2 obtained from both venoms of *Crotalus durissus terrificus* and *Bothrops jararacussu* are examined to characterize its enzymatic activity and molecular weight by MALDI-TOF mass spectrometry (Fig. 1).

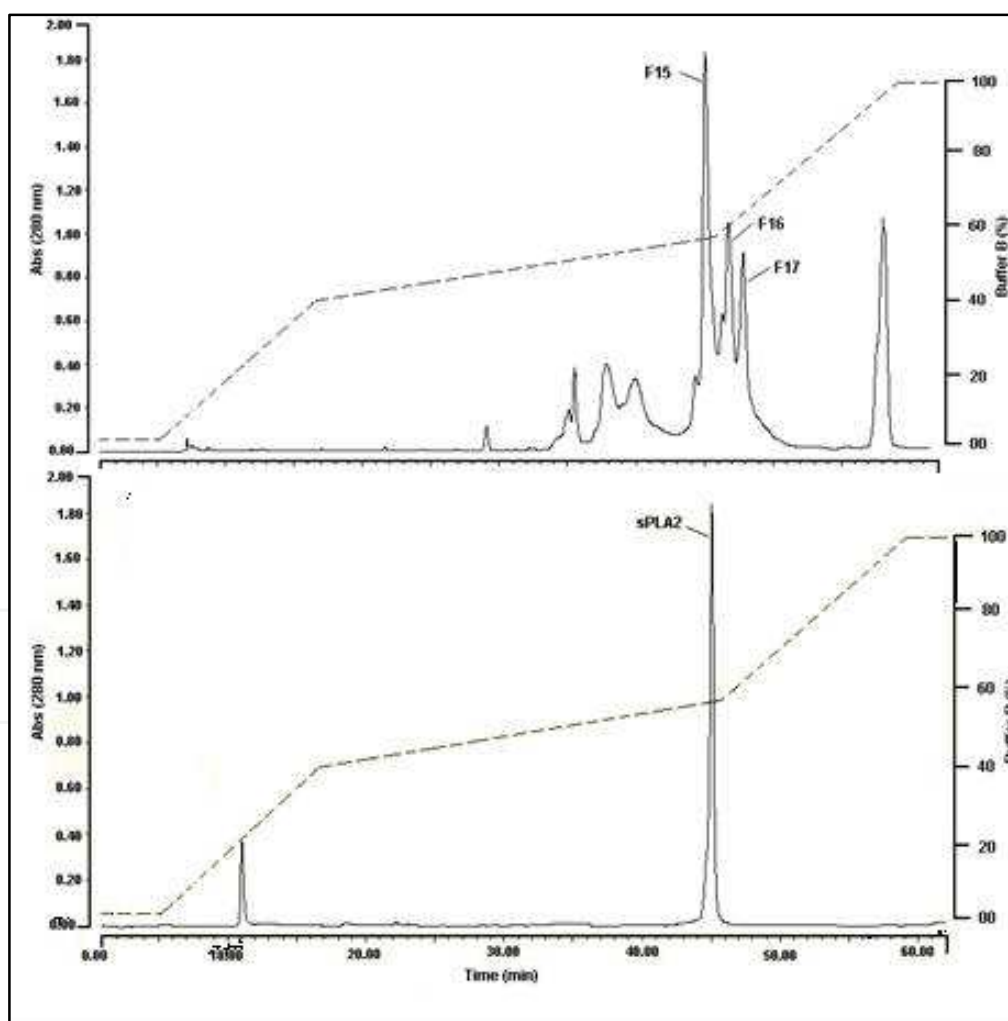


Fig. 1. Profile of purification of the whole venom of *Crotalus durissus terrificus* (Cotrim et al., 2010).

### 2.2.2 Natural compounds (bioassay-guided fractioning of secondary metabolites)

Isolation of natural compounds (NC) with specific pharmacological activities requires a bioassay-guided fractioning which usually uses crude extracts and polarity/chromatographic fractioning to find active fractions for the specific pharmacological effects wanted (Militao et al., 2007). Herein, sPLA2 and specific substrate 4-nitro-3-octanoyloxy-benzoic acid (4NOB3A) were used to screening samples with inhibitory properties (Cotrim, De Oliveira, Diz Filho et al., 2010). Briefly, crude extract is tested and if it inhibited the substrate cleavage by the enzyme, this extract is subjected to different extractions with increasing polarity solvents. Then, these new fractions are tested and those with inhibitory properties subjected to chromatographic techniques to isolate secondary metabolites according to their chemical classes as described by (Proença Da Cunha Roque, 2009).

### 2.2.3 Treatment of sPLA2 with NC

The development of this kind of protocol is important to create an adequate environment for molecular interaction between sPLA2 and NC, and allow the isolation of sPLA2 chemically treated, without any kind of interference. In some cases, it is possible to obtain also the modified NC. The incubation of sPLA2 with NC (mol:mol), followed procedures described by (Iglesias, Aparicio, Rodrigues-Simioni et al., 2005). Natural compounds were dissolved in dimethyl sulphoxide (DMSO) at maximum concentration of 1%. Purified sPLA2 (1.5 mg, 100 nmol/mL) was dissolved in 1000  $\mu$ l of water and after complete homogenization, 10  $\mu$ l of NC solution (100 nmol) was added and the samples were warmed for 30 minutes in a water bath at 37 °C. Samples of 200  $\mu$ l of this mixture were loaded onto a preparative reverse phase HPLC column to separate the modified sPLA2 (sPLA2:NC) from NC. Samples were eluted using a discontinuous buffer gradient (66.6% of acetonitrile in TFA 0.1%) at a constant flow rate of 2.0 mL/min. The chromatographic run was monitored at A280 nm.

After purification of chemically treated sPLA2 with NC (sPLA2: NC), assessments on the ability of NC to inhibit sPLA2 are carried out. Most of the NC are coloured substances that can interfere with the measurements of inhibition of enzymatic activity, since the reaction product, of sPLA2 with 4N3OBA, absorbs at 405 - 425 nm. This substrate was tested and optimized for simple microplate assay for PLA2 in human serum. Using this substrate, PLA2 activity levels were similar to that measured with the previously characterized chromogenic phospholipid substrate 1,2-bis heptanoylthioglycerophosphocholine, which is one of the substrates routinely used to determine the activity enzymatic of PLA2. The use of NOB substrate introduces some technical advantages over the use of 1,2-bis heptanoylthioglycerophosphocholine, in terms of one step product quantification as well as its stability. The evaluation of residual enzyme activity of sPLA2 is a standard procedure to check the ability of NC to reduce the enzymatic activity of these enzymes. The protocol for measuring the activity of the enzyme following protocols described by (Rigden et al., 2003) and modified by (Toyama et al., 2000) or the 96-well plate, using 4N3OBA as substrate is follow described.

Enzymatic activity is expressed as the initial velocity of the reaction ( $V_0$ ) and is calculated from the increase in absorbance after 30 min of experiment. All assays are performed using  $n = 12$  and absorbance at 425 nm are measured using a SpectraMax 340 multi-wells plate

reader (Molecular Devices, Sunnyvale, CA). After the addition of sPLA2 native or treated with NC (20 mg), the reaction mixture is incubated for 30 min at 37°C and absorbance is read at 10 min intervals. Moreover, using this procedure is possible to estimate the minimum inhibitory concentration of NC on the enzymatic activity of sPLA2 in this way can be assessed in a comparative inhibitory activity of different NC.

In addition, sPLA2 treated with NC allows us to estimate structural changes induced by their interaction. The detection of fluorescence from either specific molecular probes or the inherent fluorescence of molecules allows the investigation of the interactions between of sPLA2 and NC. Monitoring the intrinsic fluorescence of tryptophan (Trp) is used to estimate protein unfolding, but this technique is not efficient when the NC absorbs the same wavelength that the Trp.

Circular Dichroism (CD) spectroscopy is a powerful technique that is more sensitive to evaluate the molecular interactions between NC and sPLA2. CD spectroscopy is an extremely valuable technique for the study of the secondary structural components of proteins, such as  $\alpha$ -helix and  $\beta$ -sheet. In this technique, circularly polarized light interacts differently with chiral centers and is absorbed. CD is characterized as a very fast way for obtaining information about protein structural integrity, conformational changes and folding-unfolding process (Corrêa, 2009). Recently, such method has been used for the evaluation of the sPLA2 treatment with different NC, mainly flavonoids. (Cotrim, De Oliveira, Diz Filho et al., 2010; De Oliveira et al., 2009; Iglesias, Aparicio, Rodrigues-Simioni et al., 2005; Santos et al., 2011; Toyama, Diz Filho, Cavada et al., 2011). In these studies native and modified PLA2 with different flavonoids (naringin, quercetin and morin) were prepared in 10 mM sodium phosphate buffer pH 7.4. Samples were transferred to quartz cuvettes with an optical path length of 1 mm. CD spectra in the wavelength range 185-300 nm were acquired in-house on a J720 spectropolarimeter (Jasco Corp., Japan) using a bandwidth of 1 nm and a response between 1-4 s. Scans were accumulated for each sample and all spectra were corrected by subtraction of buffer blanks. Comparison of spectra was performed after concentration and optical path length normalization. Results from CD revealed significant changes in the secondary structure composition of sPLA2 after treatment with flavonoids. In order to correlate CD spectral information and sPLA2 structure, homology modeling and secondary structure prediction using SWISS-MODEL (Schwede et al., 2003) and PSIPRED (Mcguffin et al., 2000) web servers, respectively, were performed. This evaluation suggested that CD signal alteration was consequence of modifications of mainly helical components, which in sPLA2 are responsible for forming the hydrophobic channel (Arni, 1996).

#### 2.2.4 Mass spectroscopy

Mass Spectrometry (MS) can also be used for the evaluation of sPLA2 modifications induced by NC treatment, as was previously done to naringin and quercetin (Cotrim, De Oliveira, Diz Filho et al., 2010; Santos, Toyama, Oliveira et al., 2011). This technique can be applied to measure the mass-to-charge ratio of native and NC-treated PLA2. In these studies, mass measurements are performed through matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) using a Voyager-DE PRO MALDI-TOF mass spectrometer (Applied Biosystems). Samples of one microliter are mixed with 2  $\mu$ L of the matrix  $\alpha$ -cyano-4-hydroxycinnamic acid, 50% acetonitrile, and 0.1% TFA (v/v). The matrix is prepared with



30% acetonitrile and 0.1% TFA (v/v). Ion masses are determined under the following conditions: accelerate voltage 25 kV, the laser operated at 2890  $\mu\text{J}/\text{cm}^2$ , delay of 300 nanoseconds, and in the linear analysis mode. Results from MALDI-TOF mass spectrometry displayed an increasing in sPLA2 mass-to-charge ratio after NC treatment in comparison with the native protein if chemical stable complexes are formed. Our data shows that chemical shift observed for naringin treatment was discrete; therefore, modifications on the PLA2 structure as consequence of this flavonoid action did not indicate an attachment of such specie, similarly to the results found in a previous study of sPLA2 treated with umbelliferone (Toyama, Diz Filho, Cavada et al., 2011).

### 2.2.5 Molecular docking

When the target protein has its crystal structure elucidated and deposited in databases online, these data can be used to perform a virtual screening of possibly active compounds which bind in specific regions of protein surface. For this purpose, the structural optimization of the natural compounds are initially achieved using the quantum chemical AM1 method (Tang et al., 1999) implemented in the BioMedCache program with default values for the convergence criteria. Docking calculations are performed with the GOLD 4.0 program (Jones et al., 1997) to obtain the in silico affinity of natural compounds to the target proteins, herein, sPLA2 from snake venoms. These PLA2 structures were taken from the RCSB Protein Data Bank [PDB]. Usually, if the chains are homologous and the active sites (docking regions) are not close in the dimeric form, only one chain is chosen for calculations. Docking calculations are performed to consider the flexibility of the natural compound ligand in such a way that torsions were considered active during the calculation. The active site is defined as all atoms within a 10 Å radius from His48, an important residue according to the literature (Scott, 1994; Scott et al., 1990). When the docking is performed in Asp49 PLA2s, calculation maintaining the  $\text{Ca}^{2+}$  ion and the coordination water molecule is also done (Fig. 2; Fig. 3).

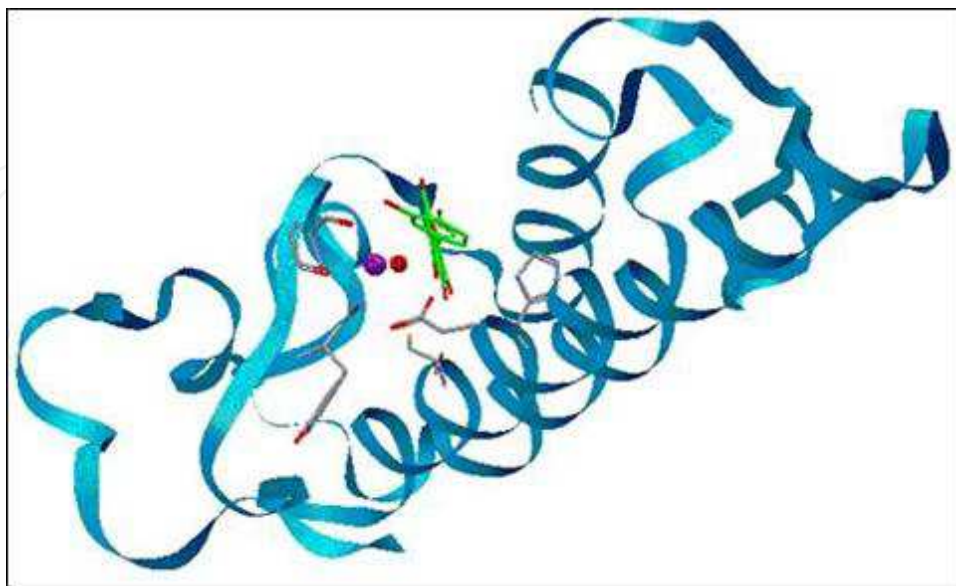


Fig. 2. Analysis of the interaction of a natural compound with phospholipase A2 by docking technique (Cotrim, De Oliveira, Diz Filho et al., 2010).

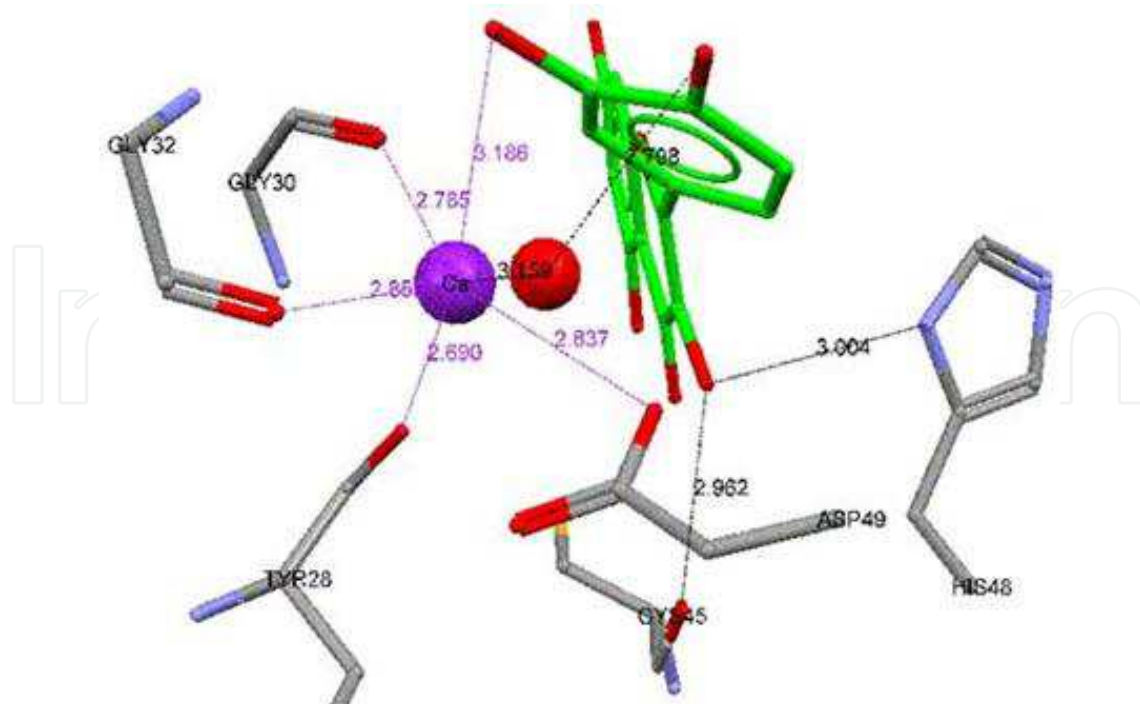


Fig. 3. Analysis of the interaction of a natural compound with phospholipase A2 by docking technique (Cotrim, De Oliveira, Diz Filho et al., 2010).

### 2.2.6 Small Angle X-ray Scattering (SAXS)

Small Angle X-ray Scattering (SAXS) is a very useful technique for analysing shape and size of macromolecules in solution (Svergun, Koch, 2002). Using SAXS, structural information about tertiary and quaternary structures of proteins at the nanometer level can be recovered from the elastic scattering of X-rays by the sample. Such methodology has been also employed in the study of structural modifications induced in PLA2 by its treatment with different flavonoids, especially naringin (Santos, Toyama, Oliveira et al., 2011). SAXS measurements were carried out at the D02A-SAXS2 beamline of the Brazilian Synchrotron Light Laboratory (LNLS) (Kellermann et al., 1997). Data collection was performed at a sample-to-detector distance of 985.7 mm using a MAR CCD detector (MAR Research) with X-rays at a wavelength of 1.488 Å, to cover  $q$  ( $q = 4\pi\sin\theta/\lambda$ ) values ranging from 0.013 to 0.34 Å<sup>-1</sup>, with samples in different concentrations. X-ray scattering intensities were reduced to a 1D profile using the Fit2D program (Hammersley et al., 1996). The several frames collected for each sample (native and naringin-treated PLA2) were inspected and averaged with the program PRIMUS (Konarev et al., 2003). GNOM program (Svergun, 1992) was used to calculate distance distribution functions,  $p(r)$ , and radius of gyration,  $R_g$ , by the indirect Fourier transform method.  $R_g$  values were also obtained employing Guinier analysis (Guinier, 1955). The degree of protein globularization was evaluated by the so-called Kratky plot ( $Iq^2 \times q$ ) (Putnam et al., 2007). Molecular weight of proteins was estimated using the web tool "SAXS MoW" (Fischer et al., 2010). Low resolution models were recovered from the scattering curves through the ab initio procedure embedded in the program GASBOR (Svergun et al., 2001). Final envelopes were obtained by spatial average of 50 independent models with the program DAMAVER (Konarev, Volkov, Sokolova et al., 2003). Interpretation of native and naringin-treated PLA2 SAXS models were performed by the

superposition of crystallographic models of several PLA2 found in the Protein Data Bank (PDB) using the program SUPCOMB (Kozin, 2001). The smallest discrepancy (Chi) calculated by the program CRY SOL (Svergun et al., 1995) between experimental data and PLA2 PDB models was achieved to the dimer composed of chains A and E of agkistrodotoxin (PDB ID 1BJJ) (Tang, Zhou, Lin, 1999).

According to our results, SAXS models displayed a dimeric elongated shape, but completely different from two previous reports published regarding PLA2 SAXS studies (Arni et al., 1999) (Murakami et al., 2007). The most remarkable result derived from our work corresponds to a conformational change observed for the PLA2 dimer after naringin treatment. SAXS envelope obtained for naringin-treated PLA2 exhibited a clear bending in comparison with the models for native PLA2 that has been attributed to the action of naringin in the dimerization interface of this protein (Santos, Toyama, Oliveira et al., 2011).

### 3. Conclusion

One of the biggest challenges for pharmacology is to find molecules that are able to interact with specific sPLA2 because the side effects are often related to low-affinity inhibitors of the proteins. The screening of inhibitors by in vitro enzyme activity inhibition enables a quick approach to molecules of interest, which will be assessed for their efficiency by techniques of molecular interaction as SAX and molecular docking.

### 4. Acknowledgment

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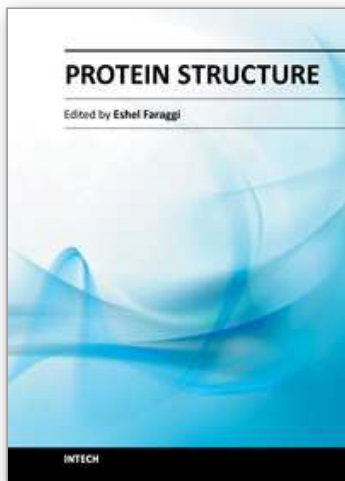
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Since the dawn of recorded history, and probably even before, men and women have been grasping at the mechanisms by which they themselves exist. Only relatively recently, did this grasp yield anything of substance, and only within the last several decades did the proteins play a pivotal role in this existence. In this expose on the topic of protein structure some of the current issues in this scientific field are discussed. The aim is that a non-expert can gain some appreciation for the intricacies involved, and in the current state of affairs. The expert meanwhile, we hope, can gain a deeper understanding of the topic.

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