2. REVIEW OF LITERATURE

Phospholipase A2 is one of the enzyme found in Snake venom. It plays crucial roles in diverse cellular responses, including phospholipid digestion and metabolism, host defense and signal transduction (Murakami et al 1997). Many researchers did their study using this compound for its characteristics, prediction, function and phylogenetic analysis.

2.1 Visual Basic

Visual Basic (VB) is the third-generation event-driven programming language and integrated development environment (IDE) from Microsoft for its COM programming model. VB is also considered a relatively easy to learn and use programming language, because of its graphical development features and BASIC heritage (Gary Cornell 2005).

Visual Basic was derived from BASIC and enables the rapid application development (RAD) of graphical user interface (GUI) applications, access to databases using Data Access Objects, Remote Data Objects, or ActiveX Data Objects, and creation of ActiveX controls and objects. Scripting languages such as VBA and VBScript are syntactically similar to Visual Basic, but perform differently. A programmer can put together an application using the components provided with Visual Basic itself. Programs written in Visual Basic can also use the Windows API, but doing so requires external function declarations (Michael Halvorson 2008).
Language features

Like the BASIC programming language, Visual Basic was designed to be easily learned and used by beginner programmers. The language not only allows programmers to create simple GUI applications, but can also develop complex applications. Programming in VB is a combination of visually arranging components or controls on a form, specifying attributes and actions of those components, and writing additional lines of code for more functionality. Since default attributes and actions are defined for the components, a simple program can be created without the programmer having to write many lines of code. Performance problems were experienced by earlier versions, but with faster computers and native code compilation this has become less of an issue. Although programs can be compiled into native code executables from version 5 onwards, they still require the presence of runtime libraries of approximately 1 MB in size. This runtime is included by default in Windows 2000 and later, but for earlier versions of Windows like 95/98/NT it must be distributed together with the executable. Forms are created using drag-and-drop techniques. A tool is used to place controls (e.g., text boxes, buttons, etc.) on the form (window). Controls have attributes and event handlers associated with them. Default values are provided when the control is created, but may be changed by the programmer. Many attribute values can be modified during run time based on user actions or changes in the environment, providing a dynamic application. For example, code can be inserted into the form resize event handler to reposition a control so that it remains centered on the form, expands to fill up the form, etc. By inserting code into the event handler for a keypress in a text box, the program
can automatically translate the case of the text being entered, or even prevent certain characters from being inserted (Paul Deitel et al. 2008).

Visual Basic can create executables (EXE files), ActiveX controls, or DLL files, but is primarily used to develop Windows applications and to interface database systems. Dialog boxes with less functionality can be used to provide pop-up capabilities. Controls provide the basic functionality of the application, while programmers can insert additional logic within the appropriate event handlers. For example, a drop-down combination box will automatically display its list and allow the user to select any element. An event handler is called when an item is selected, which can then execute additional code created by the programmer to perform some action based on which element was selected, such as populating a related list. Alternatively, a Visual Basic component can have no user interface, and instead provide ActiveX objects to other programs via Component Object Model (COM). This allows for server-side processing or an add-in module (Evangelos Petroutsos 2008).

The language is garbage collected using reference counting, has a large library of utility objects, and has basic object oriented support. Since the more common components are included in the default project template, the programmer seldom needs to specify additional libraries. Unlike many other programming languages, Visual Basic is generally not case sensitive, although it will transform keywords into a standard case configuration and force the case of variable names to conform to the case of the entry within the symbol table. String comparisons are case sensitive by default, but can be made case insensitive if so desired.
The Visual Basic compiler is shared with other Visual Studio languages (C, C++), but restrictions in the IDE do not allow the creation of some targets (Windows model DLLs) and threading models (Francesco Balena 2005).

2.2 Computational Bioinformatics

To many pre-genomic biologists, computational bioinformatics seems like an oxymoron. The traditional biology curriculum was heavily weighted toward the qualitative humanitics, while advanced numerical, programming, and computerized visualization techniques were the purview of engineers and physicists. Bioinformatics is conceptualizing biology in terms of molecules and applying informatics techniques derived from disciplines such as applied maths, computer science and statistics to understand and organize the information associated with these molecules on a large scale. In short, bioinformatics is a management informations system for molecular biology and has many practical applications. The term bioinformatics can be considered to mean information technology applied to the management and analysis of biological data. This has implications in diverse areas, ranging from artificial intelligence and robotics and genome analysis. In the context of genome initiatives the term originally applied to the computational manipulation and analysis of biological sequence data (DNA and/or protein). However in view of the recent rapid accumulation of available protein structures, the term now tends also to be used to accept the manipulation and analysis of three dimensional structural data (Bryan Bergeron 2003).
2.3 Phospholipase A2 Sequences

The nucleotide sequences of two cDNAs and four genes encoding Trimeresurus gramineus venom gland phospholipase A2 (PLA2) isozymes were determined and compared internally and externally with those encoding Trimeresurus flavoviridis venom gland PLA2 isozymes. It was revealed that the protein-coding regions are much more diversified than the 5' and 3' untranslated regions (UTRs) and the introns except for the signal peptide domain. The evolutionary trees derived from the combined sequences of the 5' and 3' UTRs and the signal peptide domain of cDNAs were in accord with the consequences from taxonomy. In contrast, the evolutionary trees from the mature protein-coding region sequences of cDNAs and from the amino acid sequences showed random patterns. Estimations of nucleotide divergence of genes and the phylogenetic analysis reveal that snake venom group IJ PLA2 isozyme genes have been evolving under adaptive pressure to acquire new physiological activities (Ogawa et al 1996)

A phospholipase A2 (PLA2) of basic nature (pI 8.5) was isolated from the venom of Amami-Oshima T. flavoviridis. Its amino acid sequence determined by the ordinary procedures was completely in accord with that predicted from the nucleotide sequence of the cDNA previously cloned from Amami-Oshima T. flavoviridis venom gland, which was named PLA-B'. It consists of 122 amino acid residues and has aspartate at position 49. Prediction of physiological activities of some PLA2s was made based on their location in the phylogenetic tree. Relationship of divergence of PLA2s
via accelerated evolution followed by less rapid mutation and physiological activities (Chijiwa et al. 2005)

Ammodytin L is a myotoxic Ser49 phospholipase A2 (PLA2) homologue, which is tissue-specifically expressed in the venom glands of Vipera ammodytes. The complete DNA sequence of the gene and its 5′ and 3′ flanking regions has been determined. The gene consists of five exons separated by four introns. Comparative analysis of the ammodytin L and ammodytoxin C genes shows that all intron and flanking sequences are considerably more conserved (93-97%) than the mature protein-coding exons. The amplification of Bov-B LINEs in snakes may have occurred before the divergence of the Viperinae and Crotalinae subfamilies. Due to its wide distribution in Viperidae snakes it may be a valuable phylogenetic marker. The neighbor-joining phylogenetic tree shows two clusters of truncated Bov-B LINE, a Bovidae and a snake cluster, indicating an early horizontal transfer of this transposable element (Kordis et al. 1997)

In order to better understand the digestive physiology and phylogeny of the pancreatic serine proteases of teleosts, they cloned trypsin, chymotrypsin and elastase from flounder (Paralichthys olivaceus). Fifty phage plaques randomly chosen from a flounder pancreatic cDNA library were found to contain three species of trypsin, two species of chymotrypsin and four species of elastase. cDNAs of two species of carboxypeptidase A, one carboxypeptidase B and lipase were also obtained. In total, 23 out of 24 digestive enzyme cDNAs were those of proteolytic enzymes. Such a high ratio
of proteolytic enzyme cDNA in the pancreas may reflect the carnivorous feeding habits of flounder. A phylogenetic comparison of the peptide sequences of flounder enzymes with those of other teleosts and mammals suggested that duplication of trypsin, chymotrypsin and elastase occurred before the divergence of the ray finned fish. It is also hypothesized that functional descendants of both duplicated genes of elastase exist in the teleosts and mammals, whereas only one of the genes of trypsin and chymotrypsin gave rise to the functional descendants in the teleosts but not in the mammals (Suzuki et al. 2002).

Two phospholipases A2 (PLA2s) were purified from the venom of Trimeresurus flavoviridis (Crotalinae) inhabiting Tokunoshima island, Japan, and named PLA-A and PLA-B in the order of elution on a cation-exchange column. Lipolytic activities of PLA-A and PLA-B toward mixed micelles and liposomes were substantially lower than that of PLA2 (an [Asp49]PLA2) which had been isolated from the same venom. Both PLA-A and PLA-B consisted of 122 amino acids and contained aspartate at position 49. The sequence around position 79 which constitutes a beta-turn segment seems to be crucial for edema-inducing activity. Phylogenetic tree of Tokunoshima T. flavoviridis venom PLA2 isozymes indicated that PLA-B and PL-X' diverged from PLA2 after branching of [Asp49]PLA2 forms and [Lys49]PLA2 forms (Yamaguchi et al. 2001)

The nucleotide sequences of 13 cDNAs encoding group II phospholipases A2 (PLA2s), which are from viperidae snake venoms and from mammalian sources, were aligned and analyzed by phylogenetic trees constructed using various components of the
sequences. The evolutionary trees derived from the combined sequences of the untranslated (5' and 3') region and the signal peptide region of cDNAs were in accord with the consequences from taxonomy. In contrast, the evolutionary trees from the mature protein-coding region sequences of cDNAs and from the amino acid sequences showed random patterns. These observations indicated that the mature protein-coding region has evolved through a process differently from the untranslated and signal peptide regions. The trees built from the nucleotide differences at each of three positions of codons in the mature protein-coding region suggested that snake-venom-gland PLA2 genes have evolved via a process different from mammalian PLA2 genes. So the present phylogenetic analysis together with the estimation of nucleotide divergence of cDNAs provides further evidence that snake-venom-group II PLA2 isozyme genes have evolved by accelerated evolution to gain diverse physiological activities (Ogawa et al. 1995).

A novel phospholipase A2 (PLA2) with Asn at its site 49 was purified from the snake venom of Protobothrops mucrosquamatus by using SP-Sephadex C25, Superdex 75, Heparin-Sepharose (FF) and HPLC reverse-phage C18 chromatography and designated as TM-N49. Phylogenetic analysis found that that TM-N49 combined with two phospholipase A2s from Trimeresurus stjeinegeri, TsR6 and CTs-R6 cluster into one group. Structural and functional analysis indicated that these phospholipase A2s are distinct from the other subgroups (D49 PLA2, S49 PLA2 and K49 PLA2) and represent a unique subgroup of snake venom group II PLA2, named N49 PLA2 subgroup (Wei et al. 2006).
To explore the venom diversity of Asian pit vipers, they investigated the structure and function of venom phospholipase A2 (PLA2) derived from two primitive tree vipers Trimeresurus puniceus and Trimeresurus borneensis. They purified six novel PLA2s from T. puniceus venom and another three from T. borneensis venom. A phylogenetic tree based on the amino-acid sequences of 17 K49-PLA2s from Asian pit viper venoms illustrates close relationships among the Trimeresurus species and intergeneric segregations. Basic D49-PLA2s with a unique Gly6 substitution were also purified from both venoms. They showed edema-inducing and anticoagulating activities. It is notable that acidic PLA2s from both venoms inhibited blood coagulation rather than platelet aggregation, and this inhibition was only partially dependent on enzyme activity. These results contribute to their understanding of the evolution of Trimeresurus pit vipers and the structure-function relationships between various subtypes of crotalid venom PLA2 (Wang et al. 2005).

The venom of the spitting cobra, Naja naja sputatrix contains highly potent alpha-neurotoxins (NTXs) in addition to phospholipase A2 (PLA2) and cardiotoxin (CTX). This study reports the complete characterization of three genes that are responsible for the synthesis of three isoforms of alpha-NTX in the venom of a single spitting cobra. DNA amplification by long-distance polymerase chain reaction (LD-PCR) and genome walking have provided information on the gene structure including their promoter and 5’ and 3’ UTRs. The high percentage of similarity observed among the NTX gene isoforms of N. n. sputatrix as well as with the alpha-NTX and kappa-
NTX genes from other land snakes suggests that the NTX gene has probably evolved from a common ancestral gene (Afifiyan et al. 1999).

The flexibility of individual amino acid side chains of pancreatic phospholipase A2 in aqueous and micellar solutions was studied with deuterium nuclear magnetic resonance (2H NMR). Bovine pancreatic phospholipase A2 was selectively deuterated at the aromatic ring systems of Trp-3 and Phe-5 and porcine pancreatic phospholipase A2 at Trp-3 only. Solid-state 2H NMR spectra of the lyophilized enzymes exhibited quadrupole splittings on the order of 130 kHz, indicating almost complete immobilization of the aromatic ring systems. Addition of a micellar solution of oleoylphosphocholine had no influence on the motional freedom of the tryptophyl residue but approximately doubled the correlation time of the phenyl ring, indicating an increase of the effective volume of the tumbling particle due to lipid-protein interaction. A different behavior was observed for the Trp-3 residue of porcine phospholipase A2 (Allegrini et al. 1985).

Phospholipases A2 (PLA2) is widely distributed in nature and is well characterized proteins with respect to their catalytic and pharmacological activities. A wealth of structural information has recently become available both from X-ray diffraction and NMR studies, and although a detailed model of the catalytic mechanism of PLA2 has been proposed, the structural bases of other aspects of PLA2 function, such as interfacial activation and venom PLA2 pharmacological activities, are still
under debate. An appreciation of the PLA2 protein structure will yield new insights with regard to these activities (Arni et al. 1996).

Indian cobra (Naja naja naja) venom is reported to contain multiple forms of phospholipase A2. Only a couple of them have been isolated and characterized. A lethal phospholipase A2 (NN-IVb1-PLA2) from Naja naja naja venom has been purified in three steps involving CM-Sephadex C-25, Sephadex G-50 and rechromatography on CM-Sephadex C-25 columns. It induces neurotoxic symptoms in the experimental mice and is devoid of myotoxic, anticoagulant, edema inducing and direct hemolytic activities (Bhat et al. 1991).

Reptile venoms exhibit a wide diversity of phospholipase A2 forms when examined by electrophoretic and chromatographic techniques which separate on the basis of net charge. In principle, diversity in charge among the enzyme forms could result from two types of structural modifications: (i) pretranslational modifications, such as differences in amino acid sequences; (ii) post-translational modifications, such as partial proteolysis or hydrolysis of amide functions of asparagine and glutamine residues. Some types of modifications alter both charge and molecular weight. Examination of a variety of snake and lizard venoms using this technique revealed the presence of multiple molecular weight forms of labeled enzymes, but the extent of diversity was less than that observed with the same venoms using separation of phospholipase electrophoretic variants on the basis of charge. The results are consistent
with diversity in reptile venom phospholipase A2 forms being derived from differences in both molecular weight and charge (DuBourdieu et al. 1987).

The sequences of 32 phospholipases A, were systematically compared on the basis of polypeptide chain length and similarity at selected amino acid positions around the active site. Two difference matrices were constructed and the various groupings present in the data were expressed in dendrogram form. The two methods of comparison yielded different results, and this is seen as a consequence of separate aspects of phospholipase evolution being highlighted in each case. The Asian Elapids seem to have active-site vicinities which are closer to those in the mammalian pancreatic phospholipases. The relevance of the classifications to structure/activity relationships (especially j-neurotoxicity) and phospholipase evolution is discussed (Mark Dufton et al. 1983).

Activation of thio-PE hydrolysis by didecanoylphosphatidylcholine (PC) was found to be a function of the surface concentration of activator rather than bulk concentration. Its presence did not affect the initial binding of enzyme to phospholipid in the micelle surface as determined kinetically. After initial binding of enzyme to the surface, the activation appears to be due to enzyme-lipid binding in the surface. Activation does not appear to affect the affinity of the enzyme for phospholipid substrate, but rather affects the catalytic efficiency of the enzyme as characterized by the value of $V_{m\infty}$. The monomeric phospholipid dibutyryl-PC, when used as an activator at 57 mM (bulk concentration), also showed effects of surface dilution with
Triton X-100, which would not be expected unless the lipid is incorporated into the micelles to some extent at these high concentrations. A thiol ester analog of phosphatidylcholine, thio-PC, was less effective than didecanoyl-PC as an activator, but appeared to be more effective than decylphosphoryl-choline. A conformational change of the enzyme upon binding of the activator, after enzyme is bound to substrate at the interface, is discussed as a possible mechanism for this activation (Stewart Hendrickson et al. 1984)

A toxic component (AgTx) from the venom of Agkistrodon halys (Pallas) was isolated using DEAE-cellulose DE11 and CM-Sephadex C50 column chromatography and finally purified to homogeneity by FPLC on a MonoQ column. The toxin is a neutral (pI 6.9) single chain polypeptide with a mol. wt of 14,000 and an amino acid composition (123 residues) roughly similar to that of notexin. AgTx was found to have phospholipase A2 activity which was dependent on calcium and stimulated by sodium deoxycholate. The toxin caused efflux of 2-deoxy-(1-3H)-glucose-6-phosphate (a cell membrane integrity probe) as well as of [3H]acetylcholine from rat brain synaptosomes. No cell membrane damage was induced by AgTx on cultured N1E 115 neuroblastoma cells and chick myotube cultures. The LD50 ws 150 micrograms/kg (i.p.) in mice. The main symptom observed was respiratory paralysis. The results obtained show that AgTx can be classified as a toxic phospholipase A2 with a presynaptic site of action (Jiang et al. 1987).
A major phospholipase A2 (VRV PL-VIIIa) which constitutes 24% of the whole Vipera russelli venom was purified to homogeneity by CM-Sephadex C-25 column chromatography followed by gel filtration on Sephadex G-50. VRV PL-VIIIa is a basic protein with a molecular weight of 11,800 by SDS-PAGE. This enzyme contributes 45% of the total PLA2 activity of the venom, but it is least toxic compared to other purified basic PLA2 enzymes prepared from V. russelli venom. The LD50 value (i.p.) of VRV PL-VIIIa is 5.3 mg/kg body wt. It shows neurotoxic symptoms and damages vital organs such as lung, liver and kidney at LD50 doses. It induces myonecrosis when injected i.m. into the thigh muscle of mice and edema when injected into the foot pads (Kasturi et al. 1989)

Snake venom phospholipase A2 enzymes induce a wide variety of pathological symptoms in animals, despite sharing a common catalytic activity and similar structural features with nontoxic mammalian pancreatic enzymes. A hypothetical model is described to explain how specific pharmacological effects, such as presynaptic neurotoxicity, cardiotoxicity, myotoxicity, anticoagulant and platelet effects are exhibited by venom PLA2 enzymes. The model is an effort to elucidate many controversial and contradictory observations which have previously been difficult to interpret. The essential feature of the model is the targeting of venom PLA2 enzymes to the specific tissue or cell due to their affinity towards specific proteins, rather than lipid domains. After the initial binding, PLA2 enzymes induce various pharmacological effects by mechanisms which are either dependent or independent of their enzymatic
activity. The model and its predicted target proteins thus provide a new focus for toxin research (Kini et al 1989).

Manoalide, an unusual nonsteroidal sesterterpenoid recently isolated from sponge, antagonizes phorbol-induced inflammation but not that induced by arachidonic acid, suggesting that manoalide acts prior to the cyclooxygenase step in prostaglandin synthesis, possibly by inhibiting phospholipase A%. They studied the inhibitory effect of manoalide on a homogeneous preparation of phospholipase Az from cobra venom. Manoalide is now shown to react irreversibly with lysine residues in the enzyme. Surprisingly, the cobra venom phospholipase normally acts poorly on phosphatidylethanolamine as substrate, but after reaction with manoalide, the enzyme is somewhat more active to ward this substrate rather than being inhibited. This suggests that a lysine residue may be important in understanding the substrate specificity of phospholipid Az. (Dominique Lombardo et al. 1984).

PLA2 provides precursors for generation of eicosanoids, such as prostaglandins (PGa) and leukotrienes (LTs), when the cleaved fatty acid is arachidonic acid, platelet-activating factor (PAF) when the sn-1 position of the phosphatidylcholine contains an alkyl ether linkage and some bioactive lysophospholipids, such as lysophosphatidic acid (lysoPA). As overproduction of these lipid mediators causes inflammation and tissue disorders, it is extremely important to understand the mechanisms regulating the expression and functions of PLA2. Recent advances in molecular and cellular biology have enabled us to understand the molecular nature, possible function, and regulation of a variety of PLA2 isozymes. Mammalian tissues and cells generally contain more than
one enzyme, each of which is regulated independently and exerts distinct functions. Here mammalian PLA2s classified into there large groups, namely, secretory (sPLA2), cytosolic (cPLA2), and Ca(2+)-independent PLA2s, on the basis of their enzymatic properties and structures and focus on the general understanding of the possible regulatory functions of each PLA2 isozyme. In particular, the roles of type II sPLA2 and cPLA2 in lipid mediator generation are discussed (Murakami et al. 1997)

The N and C terminals and tyrosine-phosphorylating site of the middle-sized tumor antigen of polyoma virus were chemically synthesized. The sequences of these peptides were Met-Asp-Arg-Val-Leu-Ser-Arg-Ala-Asp-Lys (N-MT), Met-Leu-Phe-Ile-Leu-Ile-Lys-Arg-Ser-Arg-His-Phe (C-MT), and Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu (MT-Tyr), respectively. Among these peptides, the C-MT peptide inhibited phospholipase A2 (EC 3.1.1.4), phospholipase C (EC 3.1.4.3), and phospholipase D (EC 3.1.4.4). In addition, phosphatidylinositol-specific phospholipase C (EC 3.1.4.10) was also inhibited by this peptide. Inhibition of phospholipase A2 by the C-MT peptide was reversed by low concentrations of sodium deoxycholate but not by Triton X-100 or Nonidet P40, nonionic detergents. These detergents and the modification of acyl groups altered the micellar state of phospholipids. These results, taken together, suggest that the binding of the C-MT peptide near the low-affinity Ca2+ binding sites modifies the interaction of phospholipid substrates with the active center of phospholipase A2 (Notsu et al. 1985)
Phospholipase A2 from cobra venom (Naja naja naja), which acts poorly on phosphatidylethanolamine (PE) in mixed micelles, is activated toward PE by the monomeric phospholipid dibutyrylphosphatidylcholine (dibutyryl-PC) which is an, even poorer substrate. Phosphorus-31 nuclear magnetic resonance spectroscopy was employed to show that only PE is hydrolyzed in mixtures of PE and dibutyryl-PC of various concentrations. The activation shows saturation behavior, and the fully activated enzyme hydrolyzes PE at a rate similar to its optimal substrate PC containing long chain fatty acid groups. The hydrolysis of the monomeric dibutyryl-PC can also be stimulated by SPH in mixed micelles. This reaction shows no effect of detergent. Several models are considered to explain these observations, and it is suggested that the enzyme has two types of functional sites: an activator site and a catalytic site (Pluckthun et al. 1985).

Sequence analysis of this enzyme reveals the expected strong homology with the phospholipases Az a and /3 from the closely related species Crotalus adamanteus. The C. atrox enzyme has 122 residues with seven disulfide bridges. Cleavage with cyanogen bromide at the single methionine (position 10) yields two fragments, an NH2-terminal decapeptide and a 112-residue carboxyl-terminal fragment. Neither the individual purified fragments nor a reconstituted mixture of the two are enzymatically active and the COOH-terminal fragment, which presumably contains much of the native structure of the enzyme, is a monomer in solution, unlike the native enzyme dimer. Akylation of Met-10 by reaction with iodoacetamide at pH 2.6 yields a positively charged sulfonium derivative that is a dimer with specific enzyme activity approximately 150% that of phospholipase toward lecithin and 8.6 times that of phospholipase toward phosphatidic
acid. This activation is proposed to result from an electrostatic interaction between the positively charged sulfonium group and the substrate. The NH$_2$ terminal 15-residue peptide forms a stable monolayer at the air-water interface with a collapse pressure of 15 dynes/cm. The area occupied per amino acid residue at the interface is 23.6 Å$^2$ indicating that the peptide assumes a relatively compact (presumably an amphipathic, α-helical) conformation. These findings, taken as a whole, suggest that the NH$_2$-terminal region of phospholipases A$_2$ plays an important mechanistic role in catalysis and that it constitutes a surface-active component of the catalytic site. It may, as well, be of central importance in maintaining the stability of phospholipase dimers, both in solution and at the interface (Anne Randolph et al. 1982)

The amino acid sequence of ammodytoxin A, the most toxic presynaptically active phospholipase A$_2$ isolated from Vipera ammodytes ammodytes venom, was determined. The primary structure was deduced from peptides obtained by Staphylococcus aureus proteinase and trypsin digestion of reduced and carboxymethylated protein and from the automated Edman degradation of the N-terminal part of the non-reduced molecule. According to the sequence, the enzyme classifies to the subgroup IIA of the phospholipase A$_2$ family of enzymes. The location of basic residues believed to be responsible for the toxic activity of presynaptically active phospholipases differs substantially from those in the highly toxic enzymes of other subgroups. Comparison of the sequence with sequences of other snake venom enzymes indicates that the toxic site(s) may not be the same in all subgroups of presynaptically active phospholipases (Ritonja et al. 1985)
Two acidic phospholipases A have been purified from the venom of common sea snake (Enhydrina schistosa). The two preparations were shown to be homogeneous by polyacrylamide gel electrophoresis and ion-exchange chromatography. The enzymes were shown to be specific for the 'two' position of egg yolk lecithin. The molecular weight of both enzymes determined by gel filtration chromatography and SDS-polyacrylamide gel electrophoresis was approx. 14,000. Both enzymes were non-lethal. Amino acid composition data indicated high contents of aspartic acid, glycine and alanine in both enzymes (Tan 1982).

The two major phospholipase A2 enzymes (OHPLA-DE1 and OHPLA-DE2) of king cobra (Ophiophagus hannah) venom have been purified to electrophoretic homogeneity. The isoelectric points of OHPLA-DE1 and OHPLA-DE2 were 3.81 and 3.89, respectively and the Mws were 14,000 and 15,000, respectively, as estimated by Sephadex G-75 gel filtration chromatography; and 14,000 as estimated by SDS-PAGE. The enzymes were not lethal to mice at a dosage of 10 micrograms/g body wt by i.v. route. Both phospholipase A2 enzymes, however, exhibited moderate edema-inducing and anti-coagulant activities. Bromophenacylation of the enzymes reduced the enzymatic activity drastically but did not affect the edema-inducing activity of the enzymes (Tan et al. 1990).
2.4 Algorithms for Phylogenetic analysis

Lot of algorithms is used to construct phylogenetic trees. Some of them are Neighbor Joing method and UPGMA method. Neighbor-joining is a bottom-up clustering method used for the creation of phylogenetic trees. Usually used for trees based on DNA or protein sequence data, the algorithm requires knowledge of the distance between each pair of taxa (e.g. species or sequences) in the tree. Neighbor-joining is based on the minimum evolution criterion for phylogenetic trees, i.e. the topology that gives the least total branch length is preferred at each step of the algorithm. However, neighbor-joining may not find the true tree topology with least total branch length because it is a greedy algorithm that constructs the tree in a step-wise fashion. Even though it is sub-optimal in this sense, it has been extensively tested and usually finds a tree that is quite close to the optimal tree. The main virtue of neighbor-joining is its efficiency. That is, neighbor-joining is a polynomial time algorithm. It can be used on very large data sets for which other means of phylogenetic analysis (e.g. minimum evolution, maximum parsimony, maximum likelihood) are computationally prohibitive. Unlike the UPGMA algorithm for phylogenetic tree reconstruction, neighbor-joining does not assume that all lineages evolve at the same rate (molecular clock hypothesis) and produces an unrooted tree. UPGMA (Unweighted Pair Group Method with Arithmetic mean) is a simple bottom-up data clustering method used in bioinformatics for the creation of phylogenetic trees. The input data is a collection of objects with their pairwise distances and the output is a rooted tree.
(dendrogram). It is sometimes used for creating rooted phylogenetic trees under the assumption of a constant evolutionary rate. Initially, each object is in its own cluster. At each step, the nearest two clusters are combined into a higher-level cluster. The distance between any two clusters A and B is taken to be the average of all distances between pairs of objects a in A and b in B (Atteson 1999 and Saitou et al. 1987). The following papers are studied for this research work.

A new method called the neighbor-joining method is proposed for reconstructing phylogenetic trees from evolutionary distance data. The principle of this method is to find pairs of operational taxonomic units (OTUs [=neighbors]) that minimize the total branch length at each stage of clustering of OTUs starting with a star like tree. The branch lengths as well as the topology of a parsimonious tree can quickly be obtained by using this method. Using computer simulation, They studied the efficiency of this method in obtaining the correct unrooted tree in comparison with that of five other tree-making methods: the unweighted pair group method of analysis, Far-r-is’s method, Sattath and Tversky’s method, Li’s method, and Tateno et al.’s modified Fan-is method. The new, neighbor-joining method and Sattath and Tversky’s method are shown to be generally better than the other methods (Saitou et al. 1987).

In our research work Phylogenetic trees are constructed using the protein sequences with PI (isoelectic point) and MW (Molecular weight). These two parameters are already used to construct 3D phylogenetic trees (Milner et al. 2003). The following paper is studied for this decision.
Lens crystallins are highly conserved tissue specific proteins. Crystallins from eight vertebrates were compared on the basis of their Isoelectric point and molecular weight. The phylogenetic trees constructed and tested by bootstrap and fixed in 3D space by multidimensional scaling (Milner et al. 2003).

In our research work Phylogenetic trees are constructed using the nucleotide sequences with Codon impact factor(CIP). This parameter is already used to construct phylogenetic trees(Sumarajit et al.). The following paper is studied for this decision.

They analysed forty seven chloroplast genes of the large subunit of RuBisCo, from the algal order Ecotocarpales, sourced from GenBank. Codon usage weighted by the nucleotide base bias defines the score called Codon Impact Parameter. This score is used to obtain phylogenetic relations amongst the 47 Ectocarpales (Smarajit Das et al. 2005).