

## Implications of a Consensus Recognition Site for Phosphatidylcholine Separate from the Active Site in Cobra Venom Phospholipases A<sub>2</sub><sup>†</sup>

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**ABSTRACT:** A model structure of *Naja naja kaouthia* cobra venom phospholipase A<sub>2</sub> has been constructed by utilizing molecular modeling techniques. Analysis of the model and available biochemical data reveal the presence in this enzyme of a putative recognition site for choline derivatives in loop 57–70 made up of residues Trp-61, Tyr-63, Phe-64, and Lys-65, together with Glu-55. The magnitude and shape of the electrostatic potential in this binding site are approximately 80% similar to that in the McPC603 antibody binding site specifically recognizing phosphocholine. Docking studies indicate that the recognition site we now describe and the phosphocholine head of an *n*-alkylphosphocholine molecule are complementary both sterically and electronically, mainly due to anion–cation and cation– $\pi$  interactions. Moreover, binding enthalpies of *n*-heptylphosphocholine to this site are found to parallel the catalytic rate of pancreatic, mutant pancreatic, and cobra venom phospholipase A<sub>2</sub> enzymes acting on dihexanoylphosphatidylcholine micelles, suggesting that it behaves as an activator site. This proposal is in keeping with the “dual phospholipid” model put forward to account for the phenomenon of interfacial activation. This novel site is also shown to be able to discriminate choline derivatives from ethanolamine derivatives, in accord with experimental data. On the basis of the results obtained, two functions are assigned to this putative activator site: (i) desolvation of the lipid–enzyme interface, particularly the surroundings of tyrosine at position 69 (Tyr-63), and (ii) opening of the entrance to the active site by means of a conformational change of Tyr-63 whose  $\chi_2$  angle rotates nearly 60°.

The lipolytic enzyme phospholipase A<sub>2</sub> (PLA<sub>2</sub>) splits the 2-acyl bond of L-1,2-diacylphosphatides in a calcium dependent, stereospecific, and stereoselective reaction (Dennis, 1983). Release of fatty acids, arachidonic acid in particular, provides the substrate required for the synthesis of eicosanoids, involved in pathophysiological processes such as inflammation, platelet aggregation, and acute hypersensitivity reactions (Mobilio & Marshall, 1989). Thus, modulation of PLA<sub>2</sub> activity is a current pharmacological goal, and there is a great interest in developing specific inhibitors for this enzyme in order to prevent, or suppress, the consequences of PLA<sub>2</sub> action in certain chronic inflammatory conditions, such as rheumatoid arthritis and asthma. To this end, a clear understanding of the interaction of PLA<sub>2</sub> with substrates is required.

On the other hand, PLA<sub>2</sub> is probably one of the best studied enzymes catalyzing a chemical reaction at the lipid–water interface (Volwerk & de Haas, 1981; Dennis, 1983; Jain & Berg, 1989). For most lipolytic enzymes, including PLA<sub>2</sub>, the hydrolytic activity rate on aggregated forms of the substrate (micelles, mixed micelles, monolayers, and bilayers) is much higher than the activity on soluble substrates that are monomolecularly dispersed in solution. This phenomenon is known as “interfacial activation” and is not yet completely understood even though several models have been suggested in order to account for it (Barlow et al., 1988): (1) an “interfacial recognition site” model, according to which a conformational change takes place in the enzyme at the interface; (2) a “dual phospholipid” model in which an activator phospholipid accelerates the rate of hydrolysis of another phospholipid upon binding to a specific activator site; and (3) a “substrate effect”

model in which the restrictions induced in the substrate upon packing lead to a productive encounter with the active center.

PLA<sub>2</sub>s are found both intracellularly and extracellularly. Among the extracellular PLA<sub>2</sub>s, the best known families are the pancreatic and cobra venom enzymes which are both type I PLA<sub>2</sub>s, of which the best characterized ones appear to act in monomeric form (Scott et al., 1990). Yet two major differences make them distinct: (i) in structural terms, part of the surface loop comprising positions 57–70 is deleted in cobra venom PLA<sub>2</sub>s (Davidson & Dennis, 1990); (ii) in kinetic terms, pancreatic PLA<sub>2</sub>s are activated by negatively charged substrates whereas cobra venom PLA<sub>2</sub>s are activated by substrates containing phosphocholine (Kuipers et al., 1989a; Yuan et al., 1990; Noel et al., 1990). By means of protein engineering and X-ray crystallographic techniques (Kuipers et al., 1989a), it has been shown that removal of residues 62–66 from the surface loop 57–70 of porcine pancreas PLA<sub>2</sub> increases the catalytic activity rate on choline derivatives and decreases this rate on negatively charged substrates. In the present work we advance a structural explanation to account for this fact based on a model according to which cobra venom PLA<sub>2</sub>s, in addition to the active site, do have another binding site for the choline head of choline derivatives on this surface loop, thus lending further support to the dual phospholipid model.

### MATERIALS AND METHODS

**Force Field and General Modeling Parameters.** In our calculations we followed a molecular mechanics approach to build up the molecular models and refine their energy, making use of the AMBER suite of programs (Seibel et al., 1989) and considering all the atoms explicitly. The necessary parameters for both the enzymes and the solvent were taken from the AMBER database. The atoms of phospholipid molecules and derivatives were assigned the van der Waals and hydrogen-

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