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Structure of acidic phospholipase A_2 for the venom of *Agkistrodon halys blomhoffii* at 2.8 Å resolution

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SUMMARY. The crystal structure of acidic phospholipase A_2 from the venom of *Agkistrodon halys blomhoffii* has been determined by molecular replacement methods based on the known structure of *Crotalus atrox* PLA_2, a same group II enzyme. The overall structures, except the calcium-binding regions, are very similar to each other. A calcium ion is pentagonally ligated to two carboxylate oxygen atoms of Asp-49 and each carbonyl oxygen atoms of Tyr-28 ,Gly-30 and Ala-31. A reason why the former enzyme functions as monomeric form, while the latter one does as dimer, could be presumed by the structural comparison of these calcium-binding regions. Although Gly-32 is usually participated as a ligand in the coordination with calcium ion in group I PLA_2, it is characteristically replaced to Ala-31 in the present structure, and thus the coordination geometry of calcium ion is rather different from the usually observed one.

Phospholipase A_2 (PLA_2) [EC 3.1.1.4] are Ca^{2+}-requiring esterases that catalyze specifically the hydrolysis of the fatty acid ester bond at position 2 of 1,2-diacyl-3-*sn*-phosphoglycerides and are closely concerned

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with the inflammatory response through the release of arachidonic acid from the phospholipids in the plasma membrane (1,2). Thus, it is of special importance for developing the medically usable inhibitors that can controll the biological activities of PLA_2 to elucidate their catalytic mechanisms at the atomic level.

According to the characteristic of primary amino acid sequences, PLA_2 are grouped into two types, *i.e.*, groups I and II (Fig.1) (3). Although their sequential homologies are relatively high to one another, they exhibit major functional differences in the biological actions (4,5). In order to study the catalytic mechanism of enzyme, the X-ray structual analysis is a useful approach. It is interesting to note that while several group I PLA_2 [bovine (6), porcine pancreatic (7) and cobra venom PLA_2 (8)] have been subjected to X-ray structural analyses, the example is very few concerning the group II PLA_2 (9), irrespective of its biological significance. Thus, we have been studying the crystal structure of acidic PLA_2 including calcium ions, from the venom of *Agkistrodon halys*

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          1                   10                  20                  30
AHB       S L M Q F E T L I M K I A G - R S G I W Y Y G S Y G C Y C G A G G Q
CA        * * V * * * * * * * * * * * - * * * L L W * S A * * * * * * W * * H
BOVINE    A * W * * N G M * K C K I P S S E P L L D P N N * * * * * * L * * S

          35                  45                  55                  65
AHB       G R P Q D A S D R C C F V H D C C Y G K A T - - G - C - - - - - D P K
CA        * L * * * * T * * * * * * * * * * * * * * - - D - * - - - - - N * *
BOVINE    * T * V * D L * * * * Q T * * N * * R Q * K K L D S * K V L V D N * Y

          70                  80                  90                  100
AHB       L D V Y T Y T E E N G A I V C G G - D D P C K K Q I C E C D K D A A I
CA        T V S * * * S * * * * E * I * * * - * * * * G H * * * * * * A * * *
BOVINE    T N N * S * S C S * N E * T * S S E N N A * E A F * * N * * R N * * *

          105                 115                 125
AHB       C F R D N I D T Y D N K Y W F F P A - K N C Q E E S E P C
CA        * * * * * * P S * * * * * * L * * P - * D * R * * P * * *
BOVINE    * * S K V - P - * N K E H K N L D - K * * *
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Fig. 1. Comparision of the amino acid sequences of extracellular PLA_2. AHB, CA and BOVINE represent *Agikistrodon halys blomhoffii* venom, *Crotalus atrox* venom and bovine pancreatic PLA_2, respectively. Each amino acid is indicated by one-letter symbol. Asterisks show the same residues as *ahb*PLA_2.

blomhoffii (*ahb*PLA_2) (10). This enzyme belongs to a group II PLA_2 consisting of 122 single polypeptide (pI=4.0) and characteristically reveals its biological activity as a monomer form (11), whereas many other PLA_2 function as dimer. This paper deals with the first crystal structure of *ahb*PLA_2 and the structural comparison with *Crotalus atrox* PLA_2 (*ca*PLA_2), a functionally related group II enzyme; the crystal structure of *ca*PLA_2 has been analysed as the calcium-free dimer structure (9).

MATERIALS AND METHODS

Crystallization and data collection.

Details of purification and crystallization of *ahb*PLA_2 were already descrived in a previous paper (10). The lyophilized PLA_2 was dissolved in 50mM Tris-HCl buffer (pH 8.0), 10mM $CaCl_2$ to give a final concentration of 20mg/ml. Single crystals were obtained from the sitting drop method by vapor diffusion from the sample solution containing 18% 2-methyl-2,4-pentanediol (MPD) which was equilibrated against 65% MPD buffer. The crystals belong to hexagonal, space group $P6_122$ or $P6_522$, with cell dimensions $a=b=64.36$, $c=172.41$ Å.

X-Ray diffraction data were collected with a Rigaku automated four-circle diffractometer mounted on a rotating anode generator using Cu-Kα radiation. A total of 7256 independent reflections up to 2.7 Å resolution were collected by the ω-scan method.

Rotation and translation search

The structure was solved by the molecular replacement method. Since the amino acid sequences of *ahb*PLA_2 and *ca*PLA_2 shown in Fig.1 are highly homologous (about 80% homology), the Cα atomic coordinates of the latter enzyme were used as a model structure, where the non-homologous residues were replaced with glycine. The search model was placed in an artificial unit cell ($P1$, $a=b=c=80$ Å, $\alpha=\beta=\gamma=90^o$).

A variety of rotation and translation searches were performed using the program package MERLOT (12). The correct solution of the rotation search was obtained with the resolution range of 6 - 3.5 A and a radius of integration of 20 Å, The highest peak ($\alpha=34.17$, $\beta=57.0$, $\gamma=40.0^o$) was at 3.71σ above the mean value, while the second one was at 3.08σ. Using the model thus oriented, the translation search was then performed. The translation function was calculated for both the enantiomorphic space

groups $P6_122$ and $P6_522$. The possible Harker sections showed a single major peak for $P6_122$, but not for $P6_522$. Thus, the former was decided to be the space group of present crystal. The refined final parameters of the rotation and translation functions were α=38.63, β=59.25, γ=41.05°, x=0.319, y=0.779 and z=0.805. The R-value at this stage was 0.531 using the resolution range of 10 - 2.8Å.

Refinement

The model obtained by the molecular replacement was refined with the program XPLOR (13) using a combination of simulated annealing and conventional restrained refinement methods. Successive difference Fourier syntheses and consequent model fittings were carried out using the FRODO program (14) on an IRIS 3000 interactive computer graphic system. The structure of *ahb*PLA_2 including 35 solvent molecules gives a present R-value of 0.278 for the 4931 reflections at the resolution range of 8 - 2.8Å.

RESULTS AND DISCUSSION

As is shown in Fig.2, the overall three-dimensional structure of *ahb*PLA_2 molecule is similar to that of *ca*PLA_2. Although a useful comparison of *ca*PLA_2 structure must await further refinement of *ahb*PLA_2, several features are noteworthy at this stage. The structure of monomer is dominated by three helices of 1-12, 41-54 and 90-109 residues. The axes of the latter two are nearly parallel and are linked together by two of the seven disulfide bonds of the molecule. When the Cα atoms of *ahb*PLA_2 is optimally superimposed on those of *ca*PLA_2, the

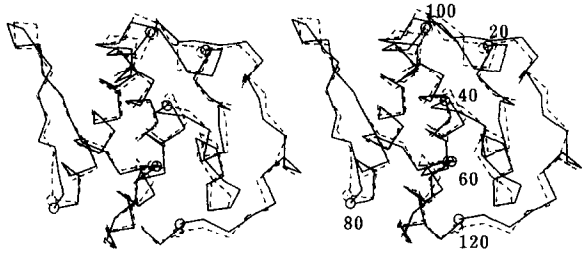

Fig. 2. Superposition of the Cα chain foldings of *ahb*PLA_2 (solid line) and *ca*PLA_2 (broken line).

root-mean-square deviation between the corresponding Cα-carbon atoms was 0.73 Å. In spite of the similarity of these two overall structures, however, a major difference is observed in the calcium-bindig region between *ahb*-and *ca*PLA_2.

The amino acid sequence of calcium-binding region (residues 25 - 35) is highly conserved among the PLA_2 family. From the viewpoint of crystal structures containing calcium ions, the calcium-binding environment is well defined for group I PLA_2. This is in contrast with group II PLA_2, where the calcium-binding mode is so far little known. The present result provides an example of the possible geometry for calcium-binding ligands in group II PLA_2 structure. As is shown in Fig.3(a), the crystal structure of *ahb*PLA_2 contains one calcium ion which is pentagonally coordinated to two carboxylate oxygens of Asp-49 and each carbonyl oxygens of Tyr-28 ,Gly-30 and Ala-31. On the other hand, the same region of *ca*PLA_2 structure (containing no calcium ion) is shown in Fig.3(b), where the carboxylate oxygen of Asp-49 forms a hydrogen bond with the Lys-69 side chain of a symmetry-related neighboring PLA_2, thus contributing to the stabilization of PLA_2 dimer formation.

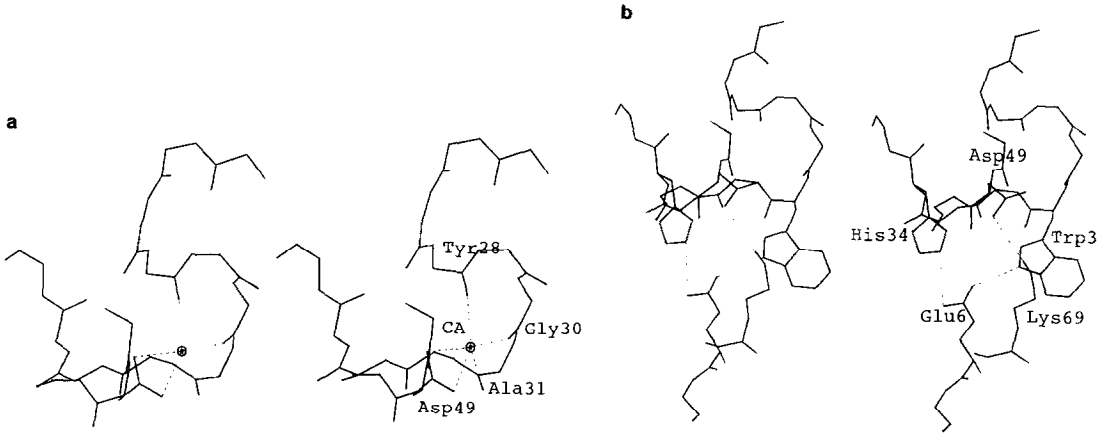

Fig. 3. Stereoscopic views of the calcium binding rigions in *ahb*PLA_2 (a) and *ca*PLA_2 (b). The broken lines in (a) show the ligation between the calcium ion and ligands. The Asp-49 , Trp-31 and His-34 residues in (b) form the hydrogen bonds with the Lys-69 and Glu-6 residues on the neighboring PLA_2.

As is obvious from Fig.3(b), the dimer structure of *ca*PLA2 is further stabilized by the hydrogen bonds between the NHs of Trp-31 and His-34 and Os of Glu-6 side chain. It is characteristic that the *ahb*PLA2 functions as monomer form, while the active forms of *ca*PLA2 and other group II PLA2 are dimer. A reason why *ahb*PLA2 acts as monomer form could be presumed from the comparison of Fig.3 (a) and (b). The substitution of Ala-31 and Gln-34 in *ahb* PLA2 for the corresponding Trp and His residues in *ca* PLA2 is inconvenient for the dimer formation under such a nearly same three-dimensional structure. This would reflect the fact that the calcium ion is necessary for the preparation of *ahb*PLA2 stable crystals, while this is not necessarily true in the case of *ca*PLA2.

The calcium coordinations of Asp-49 ,Tyr-28 and Gly-30 in *ahb*PLA2 are in a conventional manner and are also commonly observed in other group I PLA2. However, the Ala-31 is characteristically participated in the coordination to the calcium ion, while the Gly-32 is usually used as a ligand for the calcium coordination in group I PLA2. This difference could characterize the calcium-binding mode of *ahb*PLA2. Very recently, the structure of human nonpancreatic secretory PLA2 (15), a related group II enzyme involving calcium ion, was reported, in which the calcium binding environment was rather similar to the group I PLA2. Therefore, the characteristic of calcium-binding mode observed in the *ahb*PLA2 may not be common in other group II PLA2.

On the other hand, the conserved residues of His-48, Tyr-52, Tyr-73 and Asp-99 form a catalytically active hydrogen-bonded network and no notable difference is observed as compared with other structures of groups I and II PLA2. Similarly, the essentially same structure is conserved at the N-terminal region which plays the important role for the recognition of lipid-water interface. Since X-ray diffraction ability and space group of the present crystal limit further structural refinement, attempts at improving the crystal quality are now in progress.

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