A unique structural feature of a phospholipase A_2 is probed by molecular dynamics

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The unusual catalytic network, revealed by the crystal structure of one of the two phospholipases A_2 (PLA_2) from the venom of the crotalid A.p.piscivorus *has been probed using molecular dynamics. The catalytic network has been remodeled to a conformation similar to that found in all other PLA_2, and the modeled structure has been submitted to energy minimization and molecular dynamics simulation, to explore the conformational space of the network. The calculations have yielded a large reorganization of the catalytic network, which gets a conformation close to that of the crystal structure. These results suggest that the unusual catalytic network observed in the studied PLA_2 is a structural feature of the protein and not a crystal artifact.*

Keywords: molecular dynamics, molecular mechanics, molecular modeling, phospholipase A_2

INTRODUCTION

The comparison of the two X-ray structures of phospholipases A_2 (PLA_2) from pancreatic bovine (Brookhaven Code 3bp2) and from *C.atrox* venom (BC 1pp2) has shown a striking homologous core which could be expected to be preserved in other PLA_2[1]. One of the common features is the catalytic site, composed of highly conserved residues: HIS 48, TYR 52, TYR 73, ASP 99, and the N-terminus amino group. These residues form an hydrogen-bond network called the *catalytic network*. Another common feature is the presence of a so-called calcium-ion binding loop for which the backbone carbonyl oxygens O-28, O-30 and O-32 are ligands of the calcium ion, as well as the carboxylic group of the invariant ASP 49. Although the calcium ion was not revealed in the three-dimensional (3D) structure of the dimeric PLA_2 from *C.atrox* venom, its role in the stability of the molecule has been confirmed by an extensive molecular dynamics study.[2]

In the venom of the crotalid *A.p.piscivorus*, two monomeric PLA_2 have been identified. Their primary sequences are very similar to each other and to the sequence of *C.atrox*. Nevertheless, one of these PLA_2 is unusual in that the invariant residue ASP 49 is replaced with a lysine (Figure 1).[3,4] This PLA_2, known as App–K49, has long been regarded as active in a calcium free environment.[5] This property could be related to a structural feature, as it was at first conceivable that LYS 49 could replace calcium ion to stabilize the calcium binding loop.[5] The X-ray structure of App–K49 confirms this structural role since LYS 49 interacts with the backbone carbonyl oxygens of residues 28, 30, and 32, thus contributing to the stability of the molecule in the absence of calcium.[6] The X-ray study also reveals an unique structural feature in the active site of App–K49. Indeed TYR 52, which is usually involved in the hydrogen bond network, is rotated away from the catalytic system, while HIS 68, which replaces a highly conserved proline, is positioned to form an hydrogen bond with ASP 99, thus replacing the contribution of TYR 52 to the catalytic network (Figure 2a). Consequently, the 3D conformation of the loop that contains the catalytic network is different from that observed in *C.atrox*, although the sequences are very similar in this region.

It has recently been demonstrated that App–K49 was inactive, even in the presence of calcium.[7] This raises the question of whether the 3D structure, and the peculiar catalytic network noticed by Scott et al., could explain the inactivity of the protein. The first step in this investigation is to check whether the observation that TYR 52 is replaced with HIS 68 in the catalytic network may be due to a crystal artifact. Therefore, we have used molecular modeling and dynamics techniques to examine the stability of the crystal structure around the catalytic network.

MATERIALS AND METHODS

Interactive model building and molecular dynamics (MD) trajectory visualization were performed on an Evans & Sutherland PS390 interactive graphics display using the program FRODO.[8] Molecular mechanics and dynamics calculations were accomplished on a STAR ST-100 array processor (con-

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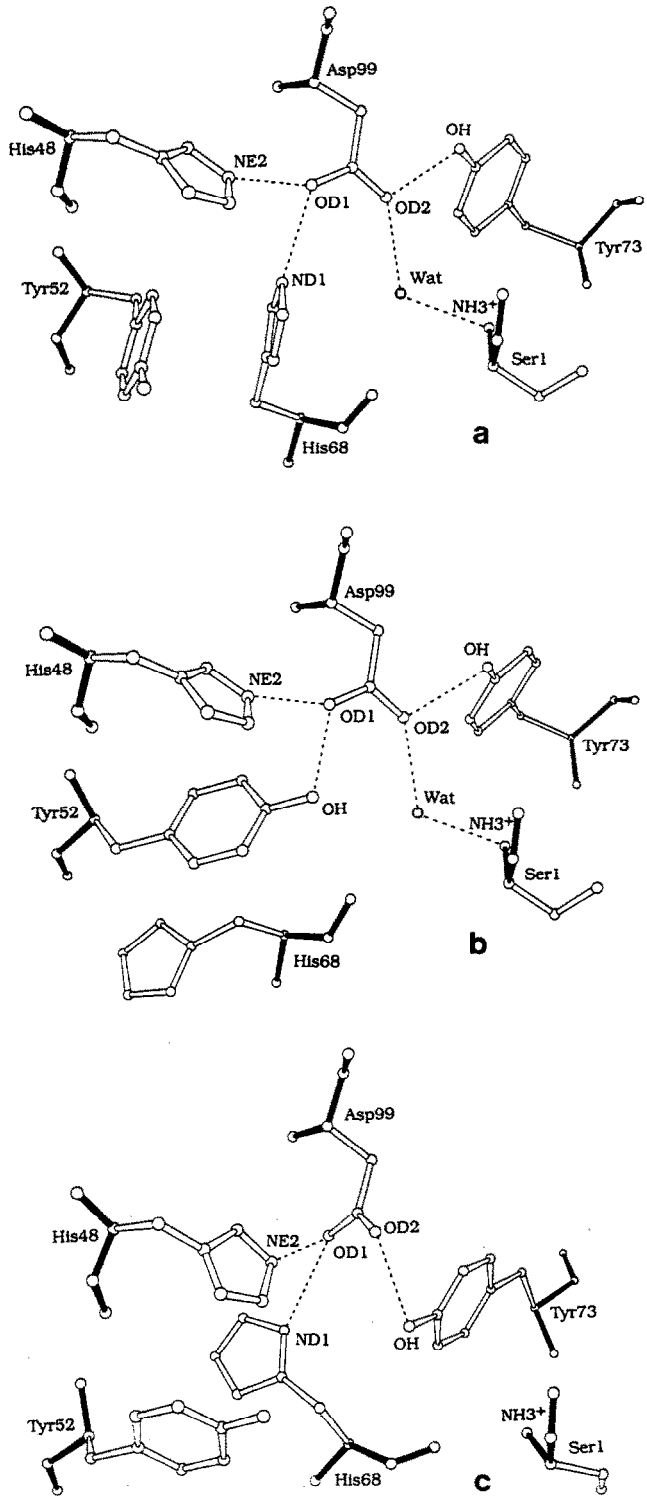
	48	49	50	51	52	53	54	55	56	59	61	67	68	69	70	71	72	73	74	75	76
C.atrox	H	D	C	C	Y	G	K	A	T	D	C	N	P	K	T	V	S	Y	T	Y	S
App-K49	H	K	C	C	Y	K	K	L	T	D	C	N	H	K	T	D	R	Y	S	Y	S

Figure 1. Comparison of the sequences of C.atrox *and App–K49 PLA_2, in the region 48–76; in App–K49 PLA_2, the invariable ASP 49 is replaced with a lysine and PRO 68 is replaced with a histidine. The sequences are numbered according to Renetseder* et al.; *this explains that residues 57–58, 61 and 62–66 are missing.*



nected to a VAX 11/780 minicomputer), using CHARMm version 21.[9]

The peptide 52–74, which contains the catalytic network of the PLA_2 (except HIS 48), was remodeled in the App–K49 structure as closely as possible to the conformation of the same peptide in the *C.atrox* PLA_2. This peptide has indeed very similar sequences in both structures (Figure 1), the main difference being the replacement of PRO 68 with an histidine in the App–K49 sequence. In the remodeled APP 49 structure, TYR 52 is therefore involved in the hydrogen bond network and HIS 68 is almost 10 Å away from ASP 99 (Figure 2b). We have thus willingly rebuilt the conventional scheme observed in all PLA_2, which differs from the unusual scheme noticed in the App–K49 crystal structure. This modeled structure of App–K49 was subsequently energy minimized and a 100 ps molecular dynamics simulation was achieved. A protocol similar to the one developed for the three published crystal structures of PLA_2 (pancreatic bovine,[10,11] pancreatic pig,[12] and *C.atrox* venom[13]) was used. Although this protocol has been described,[2] it will be briefly summarized.

In the use of CHARMm, only the polar hydrogens were considered and the histidines were doubly protonated. Because the calculations were performed *in vacuo*, the effects of the solvent in damping the electrostatic forces were modeled with a distance-dependent dielectric; sigmoid cutoff functions were applied to all external energy terms, which were zeroed between 6.5 Å and 7.5 Å. The overestimation of the electrostatic interactions was cured according to the method described by Mouawad et al.:[14] The charge densities of the atoms exposed to the solvent (except those of the main chain) were lowered by as much as 70% when the atoms were not involved in H-bond interactions and up to 30% otherwise. In addition, when the most polar residues (ASN, ASP, ARG, GLN, GLU and LYS) were more than 30% exposed to the solvent, the charge densities of the atoms

Figure 2. a, Active site of the crystal structure of App–K49; just like in the other crystal structures of PLA_2, a water molecules forms an hydrogen bond between ASP 99 and the amino group of the N-terminus; b, active site modeled to be superimposable on that of C.atrox; *particularly, the hydroxyl of TYR 52 forms an hydrogen bond with ASP 99; c, active site after the dynamics simulation; HIS 48 has formed an hydrogen bond with ASP 99 while TYR 52 has been turned over, extending towards the surface of the molecule. It is noteworthy that, as the calculations were performed in vacuum, the hydroxyl of TYR 73 replaces the water molecule that is observed in the crystal structure to form an hydrogen bond between ASP 99 and the amino group of the N-terminus.*

which did not form H bonds with atoms other than those of the main chain were set to zero. Particularly, the charge densities on the atoms of the hydroxyl group of TYR 52 were kept unchanged at $-0.35e$ for the oxygen and $0.25e$ for the hydrogen, while the charges densities on the atoms of the imino groups of HIS 68, originally $-0.30e$ for the nitrogens and $0.35e$ for the hydrogens, were reduced to $-0.09e$ and $0.10e$, respectively.

Energy minimization was carried out mainly to relax the constraints induced by the modeling of the 52–74 peptide, and also to remove the bad contacts that remain in the crystal structure. The App–K49 was energy minimized with a conjugate gradient algorithm, with decreasing harmonic constraints on all atoms during the first few hundred cycles to smoothly remove the bad contacts. Calculations stopped after about 2000 minimization cycles, upon reaching the convergence criterion of 0.01 kcal/mol per Å.

MD calculations were accomplished on the PLA_2 using the following procedure:

(1) A 6-ps thermalization, from 0 K to 300 K in 5-K steps (the temperature is defined by assigning a Maxwellian distribution of velocities to the atoms with a variance equal to this temperature)
(2) A 20-ps equilibration with reassignment of the velocities every 0.25 ps
(3) A 30-ps equilibration with rescaling of the velocities if the temperature exits a 10-K window around the room temperature
(4) A 100-ps "productive" MD simulation (i.e., MD which is not thermalization or equilibration).

The integration of the Newtonian equations of motion was performed using the Verlet algorithm[15] with a 0.25-fs integration step and without constraint on the internal coordinates; particularly, the SHAKE algorithm[16,17] was not used. The nonbonded interactions lists were updated every 0.01 ps. Finally, "mean" (time-averaged over a dynamics simulation and energy-minimized) structures were calculated using the following process: the coordinates were averaged over the "productive" dynamics, then the energy was minimized during approximately 10 steepest descent cycles to quickly remove bad contacts and during 200 conjugate gradient cycles to improve the model.

The observed water molecule, near the active site in the crystal structure of App–K49, was not considered in the calculations to avoid constraints on the coordinates which would have rigidified the active site and hindered its reorganization.

Finally, it was necessary to check whether the charge densities modifications protocol could bias the final results. The same calculations (energy-minimization and molecular dynamics) were therefore performed on structures with several different charge densities on the hydroxyl atoms of TYR 52 and on the amino atoms of HIS 68, everything else being kept unchanged. The modifications given to these charge densities were up to 50% higher or lower than the values given above.

RESULTS AND DISCUSSION

During the energy minimization, HIS 68 already moved several Å away from its initial position in order to get closer to ASP 99, while TYR 52 was pushed away to the surface of the molecule. The movement initiated during the energy minimization was amplified during the dynamics simulation and a hydrogen bond was effectively formed between HIS 68 and ASP 99 (Figure 2c). This movement was facilitated by the relaxation of the constraints induced on the main chain by the replacement of PRO 68 with an histidine and, to lesser extent, by electrostatic interactions between HIS 68 and ASP 99 that occur when the distance between the two residues is sufficiently short. The movement of HIS 68 results in a conformational change of loop 52–74, which becomes close to the crystal conformation (Figure 3). The most significant displacements are listed in Table 1. Table 2 shows the hydrogen bond lengths in the catalytic network for the crystal structure, as a reference, for the modeled

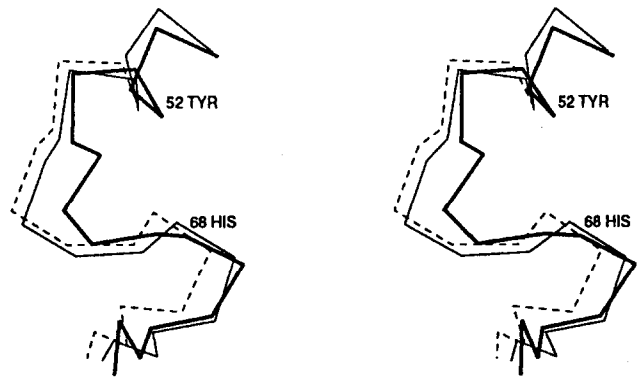


Figure 3. Stereo plot of the α-carbon backbone of loop 49–74; the loop of the crystal structure is represented in thin lines, the loop of the modeled structure, prior to the calculations, is represented in dashed lines and the loop of the time-averaged and energy-minimized structure is represented in bold lines. The conformation of the loop after the dynamics simulation is by far closer to the conformation observed in the crystal structure than to the one in the modeled structure prior to the calculations. The dynamics simulation has thus corrected modeling errors.

Table 1. Displacement (Å), between the modeled and the time-averaged (over the "productive" dynamics) structures, of the alpha carbons and of the side chain polar atoms of residues SER 1, HIS 48, TYR 52, HIS 68, TYR 73 and ASP 99; the name of the side chain polar atoms is indicated between brackets

	Alpha carbons	Polar atoms
Ser–1	2.00	1.80 (N)
His–48	1.62	1.86 (ND1)
		1.54 (NE2)
Tyr–52	2.37	3.53 (OH)
His–68	2.70	5.53 (ND1)
		5.56 (NE2)
Tyr–73	1.81	3.90 (OH)
Asp–99	0.92	1.74 (OD1)
		1.76 (OD2)

Table 2. Distance (Å) between the polar atoms of the side chains of the residues of the catalytic network, in the crystal, the initial model and the time-averaged (over the "productive" dynamics) structure

	Distance with 99–OD1				
	48–NE2	52–OH	68–ND1	68–NE2	73–OH
Crystal	2.84	7.88	4.90	2.79	2.61
Initial model	2.84	2.56	8.45	10.15	2.89
Time averaged	2.97	6.83	2.87	4.65	2.93
	Distance with 99–OD2				
	48–NE2	52–OH	68–ND1	68–NE2	73–OH
Crystal	5.03	9.37	5.60	3.81	4.56
Initial model	5.03	3.49	9.14	10.86	4.86
Time averaged	3.01	6.45	4.25	6.22	3.35

structure before the calculations and for the structure after being time averaged over the dynamics and energy minimized. The electrostatic interactions between ASP 99 and TYR 52 or HIS 68 do not appear to influence greatly the reorganization of the loop, as the catalytic network was modified in the same way in the whole range of structures with various charge densities on the hydroxyl atoms of TYR 52 and on the imino atoms of HIS 68.

It should be noticed that the structure that has been time averaged over the dynamics simulation does not correspond exactly to the crystal structure. Nevertheless, it is reasonable to think that a larger simulation in a solvent box would have led to a better correlation.

These results are very significant because they show that molecular dynamics may correct a mistake in molecular modeling. This technique must thus be regarded as a required tool in graphics-based molecular modeling. While these simulations require large amounts of main processor time, the recent progress in computer technology will render the required power commonly available in the near future. The results also validate the protocol devised for the PLA_2, and show the importance of a prior tuning on reference molecules. Finally, if we follow the hypothesis that the inactivity of the molecule is related to the absence of TYR 52 in the catalytic network, our calculations confirm the stability of the protein in this region. Particularly, the hydrogen bond network is reorganized.

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REFERENCES

1 Renetseder, R., Brunie, S., Dijkstra, B.W., Drenth, J. and Sigler, P.B. *J. Biol. Chem.* 1984, **260**, 11627
2 Demaret, J.Ph. and Brunie, S. *Prot. Engng.* 1990, **4**, 163
3 Maraganore, J.M., Merutka, G., Cho, W., Welches, W., Kezdy, F.J. and Heinrikson, R.L., *J. Biol. Chem.* 1984, **259**, 13839
4 Maraganore, J.M. and Heinrikson, R.L. *J. Biol. Chem.* 1986, **261**, 4797
5 Maraganore, J.M. and Heinrikson, R.L. *Biochem. Biophys. Res. Com.* 1985, **131**, 129
6 Scott, D.L., Achari, A., Brunie, S. and Sigler, P.B., in preparation
7 van den Bergh, C.J., Slotboom, A.J., Verheij, H.M. and de Haas, G.H. *J. Cell. Biochem.* 1989, **39**, 379
8 Jones, T.A. *J. Appl. Cryst.* 1987, **11**, 268
9 Brooks, B., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan S. and Karplus, M. *J. Comp. Chem.* 1983, **4**, 187
10 Dijkstra, B.W., Drenth, J. and Kalk, K.H. *Nature* 1981, **289**, 604
11 Dijkstra, B.W., Kalk, K.H., Hol, W.G.J. and Drenth, J. *J. Mol. Biol.* 1981, **147**, 97
12 Dijkstra, B.W., Renetseder, R., Kalk, K.H., Hol, W.G.J. and Drenth, J. *J. Mol. Biol.* 1983, **168**, 163
13 Brunie, S., Bolin, J., Gewirth, D. and Sigler, P.B. *J. Biol. Chem.* 1985, **260**, 9742
14 Mouawad, L., Desmadril, M., Perahia, D., Yon, J.M. and Brochon, J.C. *Biopolymers* 1990, **30**, 1151–1160
15 Verlet, L. *Phys. Rev.* 1967, **159**, 98
16 Ryckaert, J.P., Ciccoti, G. and Berendsen, H.J.C. *J. Comput. Phys.* 1977, **23**, 327
17 van Gunsteren, W.F. and Berendsen, H.J.C. *Mol. Phys.* 1977, **34**, 1311