Inhibition of Phospholipase A_2; a Molecular Recognition Study

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A model of the enzyme PLA_2 with a phospholipid substrate bound in the active site was derived using molecular graphics and molecular mechanics modelling techniques, and its ability to account for competitive inhibition tested by modelling the analogous complex of the enzyme and a known inhibitor; the model was then applied to the study of the binding of a new inhibitor, 3-arachidonyl-4(*O*-phosphoethanolamino)-methyltetrahydrofuran-2-one and the absolute stereochemistry for active site binding of this inhibitor predicted to be (3*S*,4*R*).

Phospholipases A_2 (PLA_2) hydrolyse the *sn2* acyl esters of phosphoglycerides (**1**). Membrane bound and intracellular enzymes liberate arachidonic acid, this being the precursor of the family of inflammatory mediators. There is much current interest in designing inhibitors of this enzyme as its control could be chemotherapeutically beneficial.

Our objective was to develop a model for the binding of ligands and known inhibitors and to analyse the optimal conformational and electrostatic interactions in terms of the reaction mechanism. Although the active site domain has been identified by experiment,[1] there is no *X*-ray structure of PLA_2 complexed to a ligand.[2] Thus we have based our modelling on the *X*-ray structure of an apoenzyme.[3] The time averaged *X*-ray structure including 288 waters was relaxed by energy minimization with respect to all degrees of freedom. Docking a phospholipid according to the Drenth/de Haas mechanism[1,4] requires co-ordination to the active site calcium ion and nucleophilic attack by a water molecule hydrogen bonded to His[48] to give the tetrahedral intermediate of ester hydrolysis. Thus, a phospholipid (**2**) of known *X*-ray structure[5] was successfully docked, with retention of conformation which was also in accord with commonly observed features in solution and membranes.[6,7] Adjustments to the torsion angles of the conformationally mobile phosphatidylcholine group were necessary, with some local modifications to some side chains at the active site which are currently being refined. The complex was energy minimized[8] and in the resulting structure the phospholipid is snugly bound into the active site with the

(1)

(2)

(3)

(±)-(4)

Table 1.

Stereochemistry	RMS deviations/Å
3*S*,4*R*	0.313
E,4*R*	0.528
3*R*,4*R*	0.637
3*S*,4*S*	1.275
Z,4*R*	1.291
E,4*S*	1.473
3*R*,4*S*	1.903
Z,4*S*	2.399

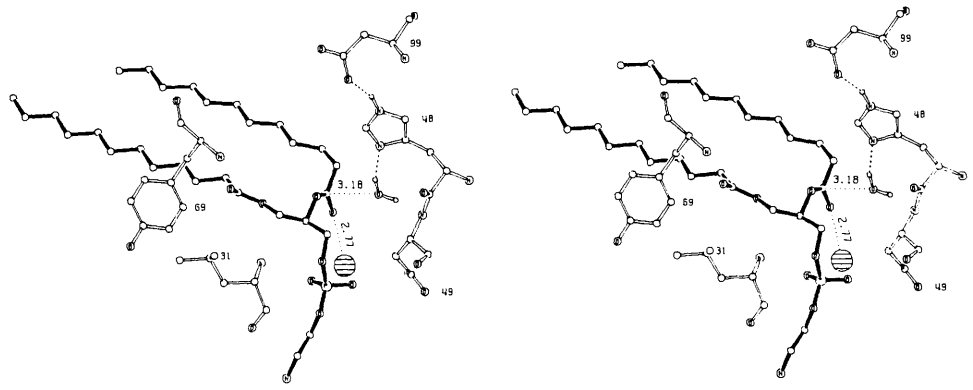

Figure 1. Active site of energy minimized PLA_2 (open bonds, calcium hatched) – model substrate (filled bonds) complex.

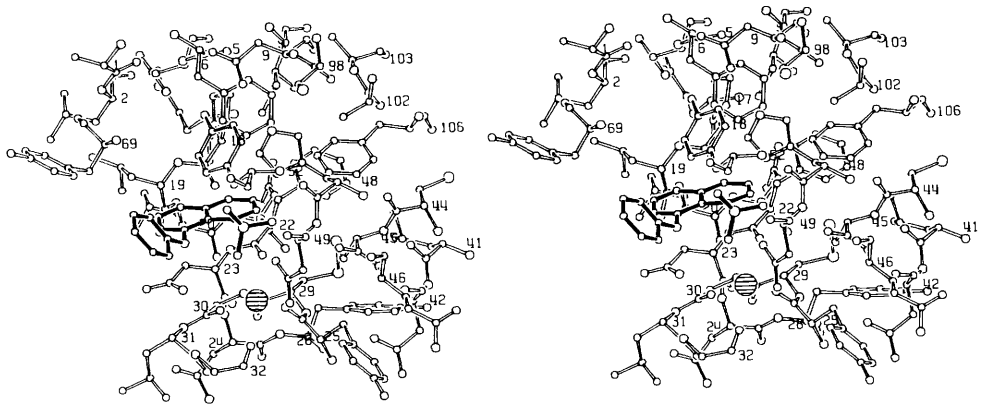

Figure 2. (**3**) (filled bonds) docked into active site of PLA_2 (open bonds, calcium hatched).

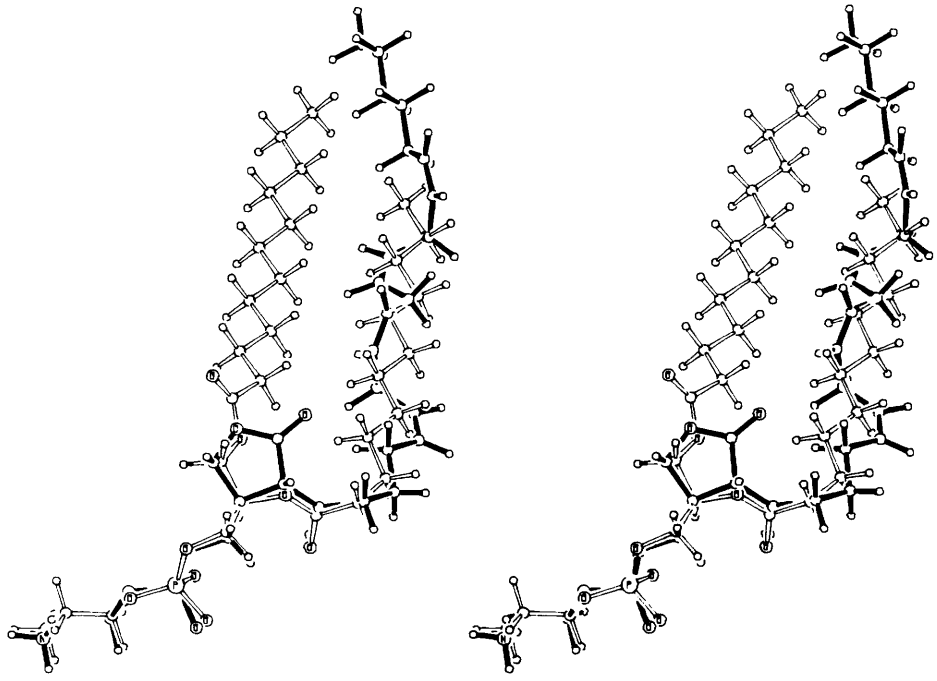

Figure 3. Furanone (3*S*,4*R*)-(**4**) (filled bonds) after constrained minimisation, superimposed on to minimized receptor site conformation of model substrate (open bonds).

catalytic water correctly poised on the beginning of the reaction co-ordinate (Figure 1), and the calcium ion correctly juxtaposed with respect to the phosphate and the ester.

A test of this model was to attempt to dock a known active site inhibitor with dissimilar structure, 1.8-anilinonaphthalenesulphonic acid (**3**).[9] As shown in Figure 2, an excellent fit was obtained with only minor changes to active site residues. The sulphonate co-ordinates to the calcium while the rest of the molecule occupies part of the large hydrophobic cavity which otherwise accommodates the substrate's alkyl side chains. Credence was thus given to the model.

In parallel, a novel structural analogue (**4**)[10] which is a more potent inhibitor of PLA_2 was analysed. The tetrahydrofuranone (**4**), which was racemic, could, in principle, adopt the *cis*- or *trans*-conformations or exist as *E*- or *Z*-exocyclic enols. Eight chiral conformations are thus feasible. Modelling studies were performed for each in order to elucidate which might be compatible with the ligand binding model derived above. Conformations were adjusted to mimic the defined substrate requirements by constrained minimizations and compared with the model substrate in terms of spatial fit and favourable orientation of functional groups. The results of the template forcing minimizations, with a harmonic potential of 5 kcal $Å^{-1}$ (cal = 4.184 J), between 15 heavy atoms of the phospholipid head group and corresponding atoms of (**4**) are given in Table 1. The best fit to our substrate model was achieved with (3*S*,4*R*)-(**4**) as shown in Figure 3. The two next best fits, (*E*,4*R*)- and (3*R*,4*R*)-(**4**), both have the plane of the lactone ring perpendicular to that defined by the phospholipid side chains. The poorest fits are observed with isomers having (4*S*)-stereochemistry.

We therefore predict that for optimal activity in structural variants based upon tetrahydrofuranones such as (**4**), (3*S*,4*R*)-stereochemistry will be required. Other potentially chiral

inhibitors of PLA_2 may also have to satisfy appropriate requirements. Further synthesis will challenge this hypothesis and the model upon which it is based. The co-ordinates of the model are available on request.

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References

1 H. M. Verheij, J. J. Volwerk, E. H. J. M. Jansen, W. C. Puyk, B. W. Dijkstra, J. Drenth, and G. H. de Haas, *Biochemistry,* 1980, **19**, 743.

2 Recently, the *X*-ray structure of an inhibitor covalently bound to PLA_2 has been described; B. W. Dijkstra, K. Huizinga, K. H. Kalk, and J. Drenth, *J. Mol. Biol.,* 1988, **200**, 181.

3 B. W. Dijkstra, K. H. Kalk, and J. Drenth, *J. Mol. Biol.,* 1981, **147**, 97.

4 B. W. Dijkstra, J. Drenth, and K. H. Kalk, *Nature,* 1981, **289**, 604.

5 M. Elder, P. Hitchcock, R. Mason, and G. G. Shipley, *Proc. R. Soc. London, A,* 1977, **354**, 157.

6 R. H. Pearson and I. Pascher, *Nature,* 1979, **281**, 499.

7 H. Hauser, W. Guyer, I. Pascher, P. Skrabal, and S. Sundell, *Biochemistry,* 1980, **19**, 366.

8 Energy minimization was performed using a valence force field program with fully flexible geometry using a force field parameterised for peptides and proteins, using both an ICL 39/80 (South Western Universities Computer) and a Cray XMP-4 (Rutherford Appleton Laboratories) to an average derivative of 0.02 kcal $Å^{-1}$. Docking used the interactive graphics INSIGHT running on a microVAXII/Evans & Sutherland PS330 system.

9 M. G. van Oort, R. Dijkman, J. D. R. Hille, and G. H. de Haas, *Biochemistry,* 1985, **24**, 7987.

10 Full synthetic and biological details will be reported separately. PLA_2 inhibition was demonstrated in rat neutrophil cell-free preparations (IC_{50} 64 μM) and likewise, the PLA_2 secreted from rat macrophage was inhibited (IC_{50} 44 μM).