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An independent crystallographic refinement of porcine phospholipase A2 at 2·4 Å resolution.

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Abstract

Porcine phospholipase A2 (M_r = 13 980), trigonal, $P3_121$, $a = b = 69{\cdot}4$, $c = 70{\cdot}4$ Å, one molecule per asymmetric unit, λ(Cu $K\alpha$) = 1·54 Å. Model incorporating 975 protein atoms and eight solvent molecules refined by restrained least-squares fit to a residual $R = 0{\cdot}21$ for 6382 reflections from 5 to 2·4 Å resolution.

Introduction

Phospholipase A2 (PLA2) is a small enzyme catalyzing the hydrolytic removal of fatty acids from the sn-2 position of glycerophospholipids. A number of PLA2 structures have been reported including native and mutant enzymes from porcine pancreas (Dijkstra, Renetseder, Kalk, Hol & Drenth, 1983; Kuipers *et al.*, 1989), zymogen and mature PLA2 from bovine pancreas (Dijkstra, van Nes, Kalk, Brandenburg, Hol & Drenth, 1982; Dijkstra, Kalk, Hol & Drenth, 1981), and a snake venom enzyme (Brunie, Bolin, Gewirth & Sigler, 1985). All structures were refined to R factors under 0·19 except for porcine PLA2 and bovine proBPLA2, which have R factors of 0·241 and 0·219, respectively. As part of a program to design PLA2 inhibitors that might act as anti-inflammatory agents, independent refinements of these latter proteins were undertaken. This report describes the refinement of porcine PLA2.

Experimental

Lyophilyzed porcine PLA2 (Sigma Biochemical) was dissolved in 0·1 *M* tris(hydroxymethyl)aminomethane hydrochloride, pH 7·6, 5 m*M* $CaCl_2$, to a concentration of 25 mg ml^{-1}. The solution was then dialysed against the same buffer prior to twofold concentration with a Centricon-10 filter (Amicon). Crystals were grown by free interface diffusion using equal volumes of protein solution and either methanol or 2-methyl-2,4-pentanediol as precipitant (Drenth, Enzing, Kalk & Vessies, 1976). Crystals grew to approximately 0·2 mm in smallest dimension within 2–4 weeks.

A complete three-dimensional data set was collected from a randomly oriented crystal using a Nicolet X-ray area detector (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987). The X-ray source was a graphite-monochromated Cu X-ray beam produced by an Enraf–Nonius GX-21 rotating-anode X-ray generator, operating at 2·5 kW on a 0·3 × 3·0 mm focal spot. A total of 805 data frames were collected in 0·25° increments, for two goniometer crystal settings at a chamber offset of 15° and a camera length of 9 cm. Laue group and unit-cell parameters were determined by analysis of electronic area detector data (Ohlendorf, 1991). The refined parameters of the primitive trigonal cell were $a = b = 69{\cdot}4$, $c = 70{\cdot}4$ Å. The a, b cell parameters were similar to those reported previously (Dijkstra *et al.*, 1983), although the c axis was approximately 3 Å longer, an observation that initially suggested a new crystal form. In our hands, crystals of the porcine enzyme appear to diffract marginally better than those described previously (Dijkstra *et al.*, 1983). After integration and scaling, the final data set contained 42 862 observations of 8006 reflections (of a possible 8020) to 2·4 Å resolution. In the highest-resolution shell the mean intensity divided by σ of I was 0·97.

Table 1. *Final refinement statistics for porcine PLA2*

Values given are the r.m.s. deviation (Å) from ideal geometry. Thermal parameter values are the mean difference in isotropic temperature factor between pairs of atoms.

Space group	$P3_121$
Cell dimensions (Å)	$a = b = 69{\cdot}4$, $c = 70{\cdot}4$
Resolution limits (Å)	5–2·4
Reflections	6382 [$I > 0{\cdot}1\sigma(I)$]
R factor	0·210
Protein atoms	975
Water molecules	8
Geometric conformity	
Distances (Å)	
Bond	0·019
Angle	0·040
Planes (Å)	
Peptides	0·013
Other	0·016
Chiral volumes (Å^3)	0·249
Thermal parameters (Å^2)	
Main-chain bond	0·848
Main-chain angle	1·538
Side-chain bond	1·373
Side-chain angle	2·365
Hydrogen bond	3·510

The structure was solved using molecular replacement (Crowther, 1972) from coordinates for porcine PLA2 (Dijkstra *et al.*, 1983) deposited in the Protein Data Bank (Bernstein *et al.*, 1977). The molecule was initially reoriented as a rigid body by searching a local grid in 0·5° angular, and 0·2 Å translational, increments for solutions that maximized the correlation coefficient between the observed and calculated structure factors. The search was carried out using the program *BRUTE* (Fujinaga & Read, 1987) implemented on a Star Technologies ST-100 array processor, and verified the space group as $P3_121$ (Dijkstra *et al.*, 1983). The reoriented PLA2 model (incorporating the protein atoms and two bound calcium ions) was refined by restrained methods (Hendrickson & Konnert, 1980; Finzel, 1987), during which the R factor was reduced from 0·322 to 0·210. Refinement was interupted once to rebuild and add four solvent molecules. Final statistics are summarized in Table 1.

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Discussion

Comparison of the present and previously reported PLA2 models (Dijkstra *et al.*, 1983) shows them to be extremely similar. After superposition the r.m.s. error between the models is 1·04 Å for all atoms (0·68 Å upon eliminating 57 atoms with r.m.s. errors $>2\sigma$ due mainly to flips of side chains such as leucine and asparagine) and 0·55 Å for Cα's (0·50 Å upon elimination of two atoms with r.m.s. errors $> 2\sigma$). The correlation coefficient between thermal parameters was 0·887. The mean thermal parameter for the model reported here is 42·0 $Å^{-2}$ compared with 21·46 $Å^{-2}$ for the model of Dijkstra *et al.* (1983). The increase in unit-cell *c* axis appears to be the result of a slight reorientation (a 1·65° rotation and 1·3 Å translation) of the PLA2 molecule in the crystal lattice. Complete atomic coordinate data and structure factors have been deposited in the Brookhaven Protein Data Bank.*

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 4P2P, R4P2PSF), and are available in machine-readable form from the Protein Data Bank at Brookhaven or one of the affiliated centres at Melbourne or Osaka. The data have also been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 37040 (as microfiche). Free copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

References

BERNSTEIN, F. C., KOETZLE, T. F., WILLIAMS, G. J., MEYER, E. E. JR, BRICE, M. D., RODGERS, J. R., KENNARD, O., SHIMANOUCHI, T. & TASUMI, M. (1977). *J. Mol. Biol.* **112**, 535–542.

BRUNIE, S., BOLIN, J., GEWIRTH, D. & SIGLER, P. B. (1985). *J. Biol. Chem.* **260**, 9742–9749.

CROWTHER, R. A. (1972). *The Molecular Replacement Method*, edited by M. G. ROSSMANN, pp. 173–178. New York: Gordon & Breach.

DIJKSTRA, B. W., KALK, K. H., HOL, W. G. J. & DRENTH, J. (1981). *J. Mol. Biol.* **147**, 97–123.

DIJKSTRA, B. W., RENETSEDER, R., KALK, K. H., HOL, W. G. J. & DRENTH, J. (1983). *J. Mol. Biol.* **168**, 163–179.

DIJKSTRA, B. W., VAN NES, G. J. H., KALK, K. H., BRANDENBURG, N. P., HOL, W. G. J. & DRENTH, J. (1982). *Acta Cryst.* **B38**, 793–799.

DRENTH, J., ENZING, C. M., KALK, K. H. & VESSIES, J. C. A. (1976). *Nature (London)*, **264**, 373–377.

FINZEL, B. C. (1987). *J. Appl. Cryst.* **20**, 53–55.

FUJINAGA, M. & READ, R. (1987). *J. Appl. Cryst.* **20**, 517–521.

HENDRICKSON, W. A. & KONNERT, J. H. (1980). *Computing in Crystallography*, edited by R. DIAMOND, S. RAMASESHAN & K. VENKATESAN, pp. 13.01–13.23. Bangalore: Indian Institute of Science.

HOWARD, A. J., GILLILAND, G. L., FINZEL, B. C., POULOS, T. L., OHLENDORF, D. H. & SALEMME, F. R. (1987). *J. Appl. Cryst.* **20**, 383–387.

KUIPERS, O. P., THUNNISSEN, M. M. G. M., DE GEUS, P., DIJKSTRA, B. W., DRENTH, J., VERHEIJ, H. M. & DE HAAS, G. H. (1989). *Science*, **244**, 82–85.

OHLENDORF, D. H. (1991). *J. Appl. Cryst.* **24**, 64–66.