was carried out at 291 K in the presence of 20% (*w*/*v*) PEG 3350 by the hanging-drop method (McPherson, 1982). Crystals can be grown to sizes up to 0·1 × 0·3 × 0·7 mm in one week.

For the X-ray analysis, a crystal was mounted in a thin-walled glass capillary containing a small amount of mother liquor to prevent dehydration and sealed with wax. A data set to 3·2 Å resolution was collected on a Siemens X100 area detector using double mirror focused 5 kW Cu $K\alpha$ X-rays generated from a Rigaku RU200 rotating anode. The data collection was carried out using the Harvard *COLLECT* routines (Blum, Metcalf, Harrison & Wiley, 1987). Crystal orientation, integration and scaling were performed using the *XENGEN* (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987) program suite.

Results and discussion

Analysis of the three-dimensional data set indicates a monoclinic space group with cell parameters $a = 119{\cdot}7$, $b = 96{\cdot}2$, $c = 136{\cdot}7$ Å and $\beta = 103{\cdot}3°$. Systematic absences in $0k0$ for $k = 2n + 1$ suggest that the space group is $P2_1$. The unit-cell volume (1 558 237 Å^3) implies that there may be five or six dimers of 52 000 daltons each per asymmetric unit with volume per mass ratio, V_m (Matthews, 1968) about 3·0 and 2·5 respectively. Both are within the normal range for protein crystals. These crystals represent the first crystallization of GST in the absence of inhibitor.

When the mother liquor contained methylmercury chloride or ethylmercury chloride with the presence of β-octylglucopyranoside (10 m*M*), crystals of a different morphology were obtained. These crystals diffract to at least 2·5 Å resolution. Methylmercury chloride (MeHgCl) has been reported as an inhibitor to GST (Reddy, Scholz & Massaro, 1981). Following the activity assay method of Habig & Jakoby (1981) we found that complete inhibition is observed at 1 m*M* MeHgCl and 90% inhibition is observed at 0·6 m*M* MeHgCl. Ethylmercury chloride (EtHgCl), however, is less effective as an inhibitor, giving only 24·4 and 14·6% inhibition at 0·6 and 0·1 m*M* EtHgCl respectively. Diffraction data sets were collected on a Siemens area-detector system for crystals co-crystallized with both the MeHgCl and EtHgCl using procedures similar to those mentioned above for the $P2_1$ crystals. Analysis of the three-dimensional data indicates that crystals of the MeHgCl and EtHgCl complexes are isomorphous and belong to space group $C2$ with cell parameters, $a = 88{\cdot}3$, $b = 69{\cdot}7$, $c = 81{\cdot}4$ Å and $\beta = 105{\cdot}3°$. Assuming one dimer per asymmetric unit, the V_m is calculated to be 2·32 and the solvent content is estimated to be 47%.

These $C2$ crystals represent the weakly or partially inhibited GST's. They appear to be isomorphous with those of rat liver GST 3-3 grown (Sesay *et al.*, 1987) in the presence of a glutathione-based inhibitor. It would be interesting to compare these structures as well as that of the $P2_1$ crystals which contain no inhibitors. Crystallographic studies of both crystal forms of GST are underway.

References

Blum, M., Metcalf, P., Harrison, S. C. & Wiley, D. C. (1987). *J. Appl. Cryst.* **20**, 235–242.

Cowan, S. W., Bergfors, T., Jones, T. A., Tibbelin, G., Olin, B., Board, P. G., & Mannervik, B. (1989). *J. Mol. Biol.* **208**, 369–370.

Habig, W. H. & Jakoby, W. B. (1981). *Methods Enzymol.* **77**, 398–405.

Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulos, T. L., Ohlendorf, D. H. & Salemme, F. R. (1987). *J. Appl. Cryst.* **20**, 383–378.

Hsieh, J.-C., Liu, L.-F., Chen, W.-L. & Tam, M. F. (1989). *Biochem. Biophys. Res. Commun.* **162**, 1147–1154.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*, pp. 96–97. New York: John Wiley.

Mannervik, B. & Danielson, U. H. (1988). *CRC Crit. Rev. Biochem.* **23**, 283–337.

Mannervik, B. & Guthenberg, C. (1981). *Methods Enzymol.* **77**, 231–235.

Matthews, B. M. (1968). *J. Mol. Biol.* **33**, 491–497.

Parker, M. W., Bello, M. L. & Federici, G. (1990). *J. Mol. Biol.* **213**, 221–222.

Reddy, C. C., Scholz, R. W. & Massaro, E. J. (1981). *Toxicol. Appl. Pharmacol.* **61**, 460–468.

Schaffer, J., Gallay, O. & Ladenstein, R. (1988). *J. Biol. Chem.* **263**, 17405–17411.

Sesay, M., Ammon, H. L. & Armstrong, R. N. (1987). *J. Mol. Biol.* **197**, 377–378.

Acta Cryst. (1991). B**47**, 814–816

Crystallographic refinement of bovine pro-phospholipase A_2 at 1·6 Å resolution.

By B. C. Finzel,* P. C. Weber, D. H. Ohlendorf and F. R. Salemme,† *The DuPont Merck Pharmaceutical Company, DuPont Experimental Station, PO Box* 80228, *Wilmington, DE* 19880-0228, *USA*

(*Received* 14 *December* 1990; *accepted* 8 *April* 1991)

Abstract

Bovine pro-phospholipase A_2 ($M_r = 14\,520$), trigonal, $P3_121$, $a = b = 46{\cdot}5$, $c = 102{\cdot}0$ Å, one molecule per asymmetric unit, λ(Cu $K\alpha$) = 1·54 Å. The model incorporating 895 protein atoms, two molecules of 2-methyl-2,4-pentanediol, and 60 solvent water molecules, was refined by restrained least squares to a residual $R = 0{\cdot}194$ for 14 667 reflections from 5 to 1·6 Å resolution.

Introduction

Phospholipase A_2 (PLA2; EC 3.1.1.4) is a small enzyme catalyzing fatty acid hydrolysis from the *sn*-2-position of

* Current address: Physical and Analytical Chemistry, The Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001, USA.

† Current address: Chemical Research and Development, Sterling Research Group, 9 Great Valley Parkway, Malvern, PA 19355, USA.

0108-7681/91/050814-03$03.00

glycerophospholipids. A distinctive property of PLA2 is its enhanced catalysis of substrates aggregated in membranes or micelles. In mammalian pancreatic enzymes, this ability is associated with activation from a proform involving cleavage of a seven-residue polypeptide from the proenzyme amino terminus (Dennis, 1983). Previous structural comparisons of the mature enzyme (Dijkstra, Kalk, Hol & Drenth, 1981; Dijkstra, Renetseder, Kalk, Hol & Drenth, 1983) with either the proenzyme structure (Dijkstra, van Nes, Kalk, Brandenburg, Hol & Drenth, 1982) or a modified form of the mature enzyme altered at its amino terminus (Dijkstra, Kalk, Drenth, de Haas, Egmond & Slotboom, 1984), have suggested that activation is associated with a disorder–order transition mediated in part through hydrogen-bond interactions formed by the amino terminus of the mature enzyme and the remainder of the protein (Wierenga, 1986; Kuipers *et al.*, 1989). The present work extends the structure determination of the bovine proenzyme, previously examined at 3·0 Å resolution (Dijkstra *et al.*, 1982), to 1·6 Å resolution. The work was carried out as part of a program to design inhibitors of PLA2 that catalytically liberate the fatty-acid precursors to leukotrienes, prostaglandins and thromboxanes which act as mediators of the inflammatory response.

Experimental

Lyophilyzed bovine pro-phospholipase A_2 (pro-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 50 mg ml^{-1}. Crystals with average dimensions of 0·3 mm were grown by free interface diffusion between equal volumes of a concentrated protein solution and a 75% (*v*/*v*) 2-methyl-2,4-pentanediol/water solution as precipitant (Drenth, Enzing, Kalk & Vessies, 1976). Amino-terminal analysis and partial amino-acid sequencing indicated that the material supplied was a mixture of proform and mature enzymes. However, composition analysis of dissolved crystals showed them to be predominantly (> 90%) composed of the proenzyme.

A complete three-dimensional data set was collected from a crystal mounted in a general orientation on a Siemens X-ray area detector with a graphite-monochromated Cu X-ray beam produced by an Enraf-Nonius GX-21 X-ray generator operated at 2·5 kW on a 0·3 × 3·0 mm focal spot. Two crystal settings were used to collect a total of 1566 oscillation data frames (focal length = 10 cm, time = 180 s per frame) at 0·25° increments on ω and a detector offset angle of 35°.

Unit-cell parameters and space group were determined by analysis of electronic area-detector data (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987) which are consistent with a primitive trigonal cell with refined parameters of $a = b = 46{\cdot}5$, $c = 101{\cdot}9$ Å, as reported previously (Dijkstra *et al.*, 1982). The space group was verified by inspection of integrated intensity patterns computed for principle zones of reciprocal space (Ohlendorf, 1991). The final merged data set contained 88 408 observations of 17 470 (of 18 736 possible) unique reflections to 1·5 Å resolution. The R_{merge}* for intensities of symmetry-related reflections was 0·054. Data in the shell beyond 1·6 Å where the average F was $< 0{\cdot}9\sigma(F)$, were discarded in the final analysis.

In order to obtain an initial phase set upon which to base high-resolution X-ray refinement, the molecular-replacement solution of pro-PLA2 was reproduced with the amino-terminal modified PLA2 (trans-PLA2) structure as the probe molecule (Dijkstra *et al.*, 1982). Coordinates for the trans-PLA2 structure (Brookhaven Protein Data Bank; Bernstein *et al.*, 1977) were stripped of solvent molecules and reoriented in the trigonal pro-PLA2 cell according to the published angular transformations (Dijkstra *et al.*, 1982). The orientation was then translationally refined with the grid-search program *BRUTE* (Fujinaga & Read, 1987) based on 595 reflections in a shell between 4 and 5 Å resolution. Successive searches on finer grids, *i.e.*, $(1{\cdot}0\ \text{Å})^3$, $(0{\cdot}5\ \text{Å})^3$, $(0{\cdot}2\ \text{Å})^3$, gave a maximum correlation factor of 0·65. The *R* factor* for the trans-PLA2 structure oriented in the trigonal pro-PLA2 cell was 0·43, computed for all reflections between 10 and 2·5 Å resolution.

The reoriented trans-PLA2 model was subjected to 40 cycles of restrained least-squares refinement (Hendrickson & Konnert, 1980; Finzel, 1987). Initial cycles incorporated data from 5 to 2·5 Å resolution, with gradual extension to include data to 1·6 Å. Automated refinement was interrupted after cycles 8, 14, 20, 26, 29, 33, 35 and 38 to examine $2F_o - F_c$ and $F_o - F_c$ difference electron density maps and to adjust the model manually. Bound water molecules were included when an isolated sphere of difference density (of at least 3σ intensity) was present in $F_o - F_c$ difference maps, and if the site allowed a water molecule to hydrogen bond with at least one well resolved protein atom or previously placed solvent molecule. Individual isotropic temperature factors (*B* values) were introduced into the model after cycle 14, when the resolution was extended to 2·0 Å.

Sixty water molecules were located during the course of the refinement, of which 39 were found in similar positions in both the mature and trans-PLA2 structures. In this comparison, similar water positions are defined as permitting at least one hydrogen bond to corresponding protein heteroatoms (or previously classified water molecules) and as being less than 1·5 Å apart following least-squares superposition of the protein molecules. In addition to water molecules, two binding sites for the solvent of crystallization (2-methyl-2,4-pentanediol; MPD) were located during refinement. The first, found also in the mature enzyme structure (Dijkstra *et al.*, 1983), is located in a hydrophobic cavity involving Leu19 (Cδ2), Leu2 (Cδ), Phe5 (Cδ2) and Phe106 (Cζ14). A second MPD is located on the opposite side of the molecule from the first in a large hydrophobic cavity between molecules related by crystal symmetry, and interacts with Leu20 (Cδ1) and Leu19 (Cδ1) of one molecule and Cys105 (Sγ) of another. This MPD is not observed in the mature PLA2 (Dijkstra *et al.*, 1983). Three water molecules occupy the second MPD binding site found in the mature enzyme structure (Dijkstra *et al.*, 1983). The final crystallographic *R* factor, computed over all data between 5 and 1·6 Å resolution with $I > 0{\cdot}1\sigma(I)$, is 0·194. The distribution of errors with

* $R_{merge} = \sum_h \sum_i |I_i^h - I^h| / \sum_h \sum_i I_i^h$ where I_i^h is the intensity of ith observation of the reflection h and I^h is the mean of these intensities.

* $R = \sum_h |F_c^h - F_o^h| / \sum_h F_o^h$ where F^h is the calculated (c) or observed (o) structure-factor magnitude for reflection h.

Table 1. *Refinement statistics for bovine pro-phospholipase A2*

Space group	$P3_121$
Cell	$a = b = 46{\cdot}5$, $c = 102$ Å
Resolution limits	5 1·6 Å
Reflections	14667
Crystallographic *R* factor	0·194
Protein atoms	895
Water molecules	60
Geometric conformity*	
Distances (Å)	
1 2	0·024
1-3	0·037
1 4	0·047
Planes (Å)	0·017
Chiral volumes (Å^3)	0·258
Thermal parameters (Å^2)	
Main-chain bond	1·103
Main-chain angle	1·960
Side-chain bond	2·046
Side-chain angle	3·306
Hydrogen bond	4·845

*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. The notation 1–2, 1–3, and 1–4 refers to atom pairs related through a bond, a bond angle, or dihedral angle, respectively.

resolution can be used to estimate a mean error in atomic position of 0·17 Å (Luzzati, 1952). Geometric characteristics of the final model and other refinement statistics are summarized in Table 1.

Discussion

The phospholipase A_2 structure is organized around two antiparallel helices that are interconnected by three disulfide linkages. This helix pair forms a platform for two smaller subdomains incorporating an additional α-helix, some β-sheet and extended loop structures. This overall arrangement produces a large crevice between the subdomains where many of the catalytic residues, as well as the bound calcium cation required for activity, are situated.

Previous studies of the proenzyme structure carried out at 3·0 Å resolution (Dijkstra *et al.*, 1982) suggested a high degree of similarity with the mature enzyme. However, both the amino terminus (incorporating the seven-residue sequence prosequence Glu-Ala-Gly-Leu-Asn-Ser-Arg) and a loop forming one side of the substrate binding site (Thunnissen *et al.*, 1990) were disordered in the proenzyme. The structure of trans-PLA2, where the charge of the amino terminus is altered, was similarly disordered, suggesting that hydrogen bonds formed between the N-terminal ammonium ion and main-chain carbonyl oxygens of the 64–71 loop structurally ordered the enzyme and were important in proenzyme activation (Wierenga, 1986). Although the present structure determination is overall well resolved at 1·6 Å resolution, the previously observed proenzyme disordering effects persist.

Specifically, the refined pro-PLA2 map lacks defined electron density for the amino-terminal prosequence, active-site-loop residues 65–70, and the side chains of Trp3 and Leu64. Further, residues Ala1, Leu2, Val63 and Asn71, while included in the model, may be affected by partial disorder or multiple conformations. Otherwise the pro-PLA2 structure is similar to pancreatic enzyme structures determined at high resolution (Dijkstra *et al.*, 1981, 1984; Kuipers *et al.*, 1989). The r.m.s. difference in the positions of Cα atoms in pro-PLA2 and mature PLA2 is 0·54 Å while that between pro-PLA2 and trans-PLA2 is 0·48 Å. Differences including all atoms are 1·04 and 1·00 Å, respectively, which primarily reflect differences in lattice packing interactions of the surface-residue side chains of Leu31, Arg43, Glu81, Glu87, Asn89, Tyr111 and Lys121. Structure factors and atomic coordinates have been deposited with the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977).*

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 4BP2, R4BP2SF), and are available in machine-readable form from the Protein Data Bank at Brookhaven. The data have also been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 37045 (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. B., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.

Dennis, E. A. (1983). *The Enzymes*, Vol 16, edited by P. D. Boyer, 3rd ed., pp. 307–353. New York: Academic Press.

Dijkstra, B. W., Kalk, K. H., Drenth, J., de Haas, G. H., Egmond, M. R. & Slotboom, A. J. (1984). *Biochemistry*, **23**, 2759–2766.

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.* B**38**, 793–799.

Drenth, J., Enzing, C. M., Kalk, K. H. & Vessies, J. C. A. (1976). *Nature (London)*, **264**, 383–377.

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. Ramesshan & 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. D. (1989). *Science*, **244**, 82–85.

Luzzati, V. (1952). *Acta Cryst.* **5**, 802–810.

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

Thunnissen, M. M. G. M., Eiso, A. B., Kalk, K. H., Drenth, J., Dijkstra, B. W., Kuipers, R. D., de Haas, G. H. & Verheij, H. M. (1990). *Nature (London)*, **347**, 689–691.

Wierenga, R. K. (1986). *Trans. Am. Crystallogr. Assoc.* **22**, 49–62.