Acta Cryst. (1986). B42, 602–605

Bovine Phospholipase A_2 Crystals Soaked in 30% Methanol: the First Structure Determination with a FAST Diffractometer at High Resolution

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(*Received* 17 *February* 1986; *accepted* 27 *May* 1986)

Abstract

Data collection has always been a time-consuming operation in protein X-ray crystallography. This situation drastically changed with the introduction of two-dimensional area detectors. In this article the first successful refinement of a protein structure using data collected with an Enraf–Nonius Fast Area-Scanning TV-detector (FAST) diffractometer is reported. The test case was bovine pancreatic phospholipase A_2 in a crystal form grown from 2-methyl-2, 4-pentanediol and subsequently soaked in 30% v/v methanol containing buffer. The change in medium caused intensity changes without affecting the cell dimensions. The crystal structure of the 2-methyl-2, 4-pentanediol form is known to high resolution [Dijkstra, Kalk, Hol & Drenth (1981). *J. Mol. Biol.* **147**, 97–123]. To elucidate the structural basis for the observed X-ray differences a data set to 1·75 Å resolution was collected for the methanol-soaked crystals on the FAST diffractometer at a speed of 1700 reflections h^{-1}. Refinement of the structure resulted in an R factor of 0·183, which proves the high quality of the diffraction data.

Introduction

Protein crystallography requires efficient registration of large numbers of reflections at a high level of accuracy. For years data collection on film with an oscillation camera (Arndt & Wonacott, 1977) has been the method of choice for crystals with unit-cell dimensions larger than about 100 Å. However, the film method has some inherent drawbacks, such as variation of film quality and non-uniformity from the developing process. Also, the scanning and processing of film data are rather time consuming. Therefore, electronic area detectors have been developed which can collect data for some hundreds of reflections simultaneously at high speed and with the same accuracy as the classical diffractometer. Two main types of detector have evolved from this development: the multiwire proportional-counter area detector (Xuong, Sullivan, Nielsen & Hamlin, 1985) and the TV detector (Arndt, 1982). We used a fast area-scanning TV detector (FAST) for data collection. The instrument consists of a modified κ-bracket (CAD-4) goniostat and the actual TV detector. The instrument is manufactured by Enraf–Nonius in Delft, The Netherlands. It consists essentially of a phosphorescent screen which is excited by the X-ray photons. The resulting image on this screen is intensified by an image amplifier and then scanned by a TV tube, recording position and intensity information for the X-ray reflections at the same time. This information is channeled to a computer for further processing. The X-ray pattern can also be displayed on a TV monitor for real-time inspection (Arndt & Thomas, 1982).

In this study we present results showing that the FAST detector produces data useful for the refinement of a protein structure at high resolution. The test case was bovine pancreatic phospholipase A_2 in a crystal form grown from 50% v/v 2-methyl-2,4-pentanediol (MPD) and soaked in 30% v/v methanol. The purpose of this experiment was to decrease the hydrophobicity of the mother liquor and thereby facilitate binding of phospholipids to phospholipase A_2 in the crystalline state. Upon soaking in methanol, the cell dimensions were not noticeably affected, but distinct intensity changes occurred compared with the MPD crystals, the structure of which is known at high resolution (Dijkstra, Kalk, Hol & Drenth, 1981). The observed intensity changes were reversible upon transferring the methanol-soaked crystals to the original MPD mother liquor. In order to elucidate the structural basis for the observed changes we collected a three-dimensional data set on the FAST diffractometer using the early pre-release PDP version of *PHASE* III software written by Thomas (1982) and refined the BPLA$_2$†-methanol structure starting from the structure in MPD.

Materials and methods

(*a*) *Crystallization and soaking*

Bovine phospholipase A_2 was a generous gift of Professor G. H. de Haas and co-workers and was

* On leave from Dalhousie University, Halifax, Nova Scotia, Canada.

† Abbreviation used: BPLA$_2$ = bovine pancreatic phospholipase A_2.

0108-7681/86/060602-04$01.50 © 1986 International Union of Crystallography

crystallized as described previously (Dijkstra, Drenth, Kalk & Vandermaelen, 1978): freeze-dried phospholipase A_2 was dissolved in a buffer containing 50 m*M* tris-maleate,* pH 7·2, and 5 m*M* $CaCl_2$, to a concentration of 15 mg ml^{-1}. 25 µl portions of this solution were frozen at 255 K and 25 µl MPD was layered on top of the frozen solution. Crystals grew within a week or two at room temperature. They were transferred to a solution of 30% *v/v* methanol in 50 m*M* tris-maleate, 5 m*M* $CaCl_2$, pH 7·2, and soaked for one week.

(*b*) *Equipment*

Data were collected on an Enraf-Nonius FAST diffractometer which was controlled by a PDP 11/24 under RSX-11M with 256 kbyte memory and 450 Mbyte disk storage capacity. The X-ray source was a sealed-off microfocus (0·15 × 0·2 mm) copper tube (AEG fff) operated at 30 kV and 26 mA (800 W). The radiation was Ni filtered and aligned by a collimator with 0·3 mm aperture. The capillary containing the crystal was mounted on a eucentric goniometer head on a standard four-circle diffractometer cradle with κ geometry, as is general for a CAD-4 diffractometer.

(*c*) *Software*

All steps involved in data collection and in the reduction of the raw data to *hkl* and F^2 were carried out with the *PHASE* III program system (Thomas, 1982). This system uses the detector in a diffractometer set up. The basic idea behind this mode of operation is to determine an initial orientation matrix for the crystal from two or three small scans at different orientation angles, which allows the prediction of the expected reflection spot positions on the detector surface as a function of the orientation angles. The advantage of this mode of operation is that no special mounting or alignment is required.

(*d*) *Data collection*

The crystal was mounted in an arbitrary orientation on the diffractometer cradle. The focus-to-crystal distance was 215 mm and the crystal-to-detector distance 50 mm. The detector area was 48 × 64 mm, corresponding to 512 × 512 pixels. The φ axis about which the crystal was rotated was set vertically. The crystal orientation was determined by the program from small scans at three different φ angles. Cell dimensions could be determined from the orientation matrix: $a = 47{\cdot}0$, $b = 64{\cdot}45$ and $c = 38{\cdot}1$ Å, space group $P2_12_12_1$. The crystal was block shaped with edges 0·4 × 0·4 × 0·4 mm. It was slightly larger than the X-ray beam. Data from one crystal were collected in separate φ scans (Table 1) at two different swing-out positions (θ_s) of the detector: $\theta_s = 0$ and 23°. The total φ range in both cases was 90°. The scan speed was 0·25° min^{-1}. Since the television tube registers 25 frames s^{-1} the data were collected in φ slices of 0·00017°. Dark current is integrated in the total background correction. Non-uniformity correction was done pixel by pixel. The crystals are extremely stable in the X-ray beam and showed little radiation damage. Data reduction was done by the *PHASE* III program with evaluation of the diffraction spots in boxes of 11 × 9 pixels.

(*e*) *Refinement*

Refinement was carried out on a VAX 11/750 with the $P2_12_12_1$-specific version of the fast-Fourier-transform refinement (*FFTREF*) program developed by Agarwal (1978) with intermittent regularization by the procedure of Dodson, Isaacs & Rollett (1976). Fourier maps on an arbitrary scale were calculated with the *XRAY* system (1976) on a CDC Cyber 170/760, and inspected on minimaps and on an Evans & Sutherland Picture System 2 using the program *GUIDE* (Brandenburg, Dempsey, Dijkstra, Lijk & Hol, 1981).

Results

(*a*) *Data collection and averaging*

Data collection and averaging is summarized in Table 1. 27 151 reflections were measured in 16 h. Data reduction on the PDP 11/24 took 120 h. The data were converted to structure-factor amplitudes and scaled on each other by an extended version of the procedure given by Hamilton, Rollett & Sparks (1965) using isotropic relative temperature factors. A uniform weighting scheme was used because the data collection program did not supply standard deviations. No absorption correction was applied at any stage but it was expected that any absorption effects could be taken care of, at least partly, by the scaling procedure used. The data in Table 1 seem to support this assumption.

The final data set showed an overall $R_{\rm sym}$ of 3·4% in *F* for 4562 different reflections and contained 8206 unique reflections between 38 and 1·75 Å resolution, corresponding to an overall completeness of 67%. Table 2 gives a more detailed picture of the completeness of the data with respect to resolution ranges. It has to be noted here that completeness of the data was limited by the experimental set up: owing to the coincidence of the φ and θ axes the maximal resolution obtainable in the direction parallel to the φ axis (about 3 Å) was not affected by the θ setting, *i.e.* reflections at higher resolution along this direction could not be observed.

* Tris = 2-amino-2-hydroxymethyl-1,3-propanediol.

Table 1. *Summary of data collection and averaging*

Set no.	θ_s(°)	φ(°) start	φ(°) end	Total number of observations	Usable observations	R^*_{sym}	k	B	$R(i)$†
1	0	−45	−29	1584	1541	0·040	1·0145	0·96	0·033
2	0	−30	−14	1572	1546	0·034	1·0229	1·75	0·031
3	0	−15	+1	1577	1551	0·033	1·0145	1·25	0·030
4	0	0	16	1569	1549	0·035	1·0234	2·42	0·032
5	0	15	31	1568	1550	0·037	1·0041	1·65	0·033
6	0	30	46	1583	1553	0·061	0·9804	1·56	0·039
7	23	35	45	1794	1719	0·052	0·9482	−0·23	0·037
8	23	26	36	1804	1744	0·051	0·9624	−0·46	0·042
9	23	17	27	1779	1703	0·059	0·9821	−0·77	0·045
10	23	8	18	1795	1693	0·056	1·0015	−0·80	0·048
11	23	−1	9	1771	1701	0·068	1·0199	−0·35	0·052
12‡	23	−10	0	1803	1517	0·081	—	—	—
13	23	−19	−9	1775	1693	0·060	1·0173	−1·01	0·054
14‡	23	−28	−18	1783	1453	0·093	—	—	—
15	23	−36·5	−27·5	1625	1530	0·048	1·0151	−1·67	0·057
16	23	−46	−36	1769	1671	0·049	1·0145	−1·83	0·057
	0	−45	+46	9453					
	23	−46	+45	17698					
Total				27151					

* $R_{sym} = \sum_{hkl}\sum_i |I_i - \bar{I}| / \sum_{hkl}\sum_i \bar{I}$; this is R_{sym} within a data set.

† $R(i) = \sum_{hkl} \| F_{hkl,i}| - |\bar{F}_{hkl}\| / \sum_{hkl} |\bar{F}_{hkl}|$; here the structure-factor amplitudes of each data set are compared with the final average F.

‡ Because of crystal slippage during the exposure data sets 12 and 14 could not be used.

The R factor between the observed amplitudes for $BPLA_2$-MPD crystals measured with a CAD-4 diffractometer and the methanol-soaked crystals measured with the FAST diffractometer, after fully anisotropic scaling, was 0·146 including 7411 reflections between 1·8 and 8·0 Å. A comparison of data sets from different $BPLA_2$ crystals, both in MPD, gave for the amplitudes an R factor of 0·064 between CAD-4 and FAST data.

Table 2. *Completeness of data as a function of resolution*

Resolution range (Å)	Possible reflections	Observed reflections	Completeness of shell (%)	Overall completeness (%)
10–3·0	2102	2086	99·2	99·2
3·0–2·50	2132	1822	85·5	92·3
2·50–2·18	2099	1487	70·8	85·2
2·18–2·00	1854	1190	64·2	80·4
2·00–1·86	2010	1135	56·5	75·7
1·86–1·75	1948	416	21·4	67·0

(*b*) *Refinement*

A structure-factor calculation using the coordinates of refined $BPLA_2$-MPD without solvent molecules and without the Ca ion gave an initial R factor of 27·3% for 8076 reflections between 8 and 1·75 Å resolution. The highest peak in the corresponding difference Fourier map coincided with the position of the Ca ion in the $BPLA_2$-MPD structure. Refinement was terminated after 26 cycles of *FFTREF.* After cycles 6, 10 and 18 solvent molecules were included up to a total of 119. The R factor after the final cycle was 18·3%.

(*c*) *The molecular structure*

The refined structure shows no major conformational changes with respect to the starting model, except for some side-chain rearrangements at the surface of the molecule (Fig. 1). These side chains are of residues Arg 43, Val 63, Glu 92 and Lys 121. They also show high temperature factors in the MPD structure and can easily take on a somewhat different conformation. The overall r.m.s. difference in atomic position for the 492 main-chain atoms is 0·17 Å and for all side-chain atoms 0·93 Å, but if we exclude those of residues 43, 63, 92 and 121 it is only 0·28 Å.

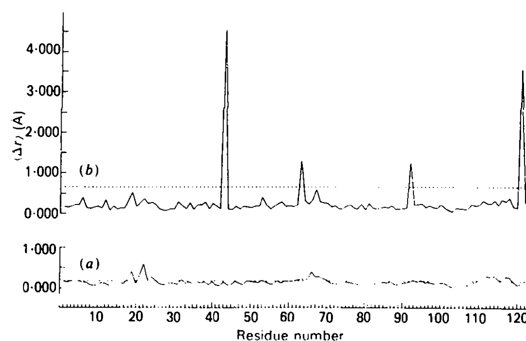

Fig. 1. R.m.s. displacement between $BPLA_2$ in MPD and in methanol for (*a*) main-chain atoms only, (*b*) all atoms of a residue. The broken line gives the average value.

For all 958 non-hydrogen protein atoms, including the calcium ion, the r.m.s. difference is 0·66 Å.*

Concluding remarks

The final proof in testing an X-ray detector is the collection and processing of crystal diffraction data, the determination of the structure and the refinement of that structure. The successful refinement of the $BPLA_2$ structure in methanol using data collected with a FAST diffractometer shows several things. Firstly, the TV area detector has now reached an operational state producing high-resolution data of good quality. The data were collected with the instrument in the diffractometer mode, at a speed of 1700 reflections h^{-1} which is quite good. Furthermore, the very complex software (Thomas, 1982) works satisfactorily. Data reduction on the PDP 11/24 took 120 h. This is rather long. It is determined by the efficiency of the reduction program and the memory and speed of the computer. The latter has now been replaced by a VAX 11/730. The hardware has also been further improved. Subsequent to the work described in this article we have used the updated instrument in the film mode with the Munich area detector NE software package *MADNES* (Pflugrath & Messerschmidt, 1985) for data collection on crystals of several proteins. In this system data were processed simultaneously with data collection. These results were also quite satisfactory.

We thank Professor G. H. de Haas and his coworkers for their generous supply of protein material. The assistance by Dr Niek van der Putten and Dr Conrad Kiers is gratefully acknowledged. We thank Professor W. G. J. Hol for helpful discussions. This work was supported by the Netherlands Foundation for Chemical Research with financial aid from the Netherlands Organization for the Advancement of Pure Research.

* Lists of structure factors and atom coordinates have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 42985 (72 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Acta Cryst. (1986). B42, 605-609

Thermal Motion Analysis in Tetraphenylmethane: a Lattice-Dynamical Approach

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(*Received* 11 *April* 1986; *accepted* 16 *July* 1986)

Abstract

A 'non-rigid' harmonic lattice-dynamical model has been used for analyzing thermal motion in crystals of tetraphenylmethane. Empirical internal and external force fields which were derived for a series of other aromatic hydrocarbons have been employed. The agreement between the calculated anisotropic temperature factors and the corresponding experimental values is excellent. Bond-length corrections for the general case of non-rigid thermal motion are obtained: the average C–C bond length in the phenyl

0108-7681/86/060605-05$01.50 © 1986 International Union of Crystallography