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Dijkstra, Bauke W.; Nes, Gerard J.H. van; Kalk, Kor H.; Brandenburg, Niko P.; Hol, Wim G.J.; Drenth, Jan

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The Structure of Bovine Pancreatic Prophospholipase A_2 at 3·0 Å Resolution

BY BAUKE W. DIJKSTRA, GERARD J. H. VAN NES, KOR H. KALK, NIKO P. BRANDENBURG, WIM G. J. HOL AND JAN DRENTH

Laboratory of Chemical Physics, University of Groningen, Nijenborgh 16, 9747 *AG Groningen, The Netherlands*

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Abstract

Bovine pancreatic prophospholipase A_2, the precursor of the lipolytic enzyme phospholipase A_2, crystallizes in space group $P3_121$ with cell dimensions $a = b = 46{\cdot}95$, $c = 102{\cdot}0$ Å. The structure was determined at 3·0 Å resolution using rotation and translation functions with transaminated phospholipase A_2 as the model structure. The rotation-function calculations appeared to be sensitive to the resolution range selected. The results of the translation function were very well defined. Positioning of the model molecule according to the rotation- and translation-function results yielded an R factor of 0·44 for 2697 reflections between 7·1 and 3·0 Å. This could be decreased to 0·40 by a rigid-body R-factor search. Subsequent restrained refinement gave $R = 0{\cdot}27$. The position of the calcium ion, which was excluded from the structure factor calculations, shows up as one of the highest features in difference Fourier syntheses. From the difference maps it also appears that the ten N-terminal residues of prophospholipase A_2 are disordered. Disorder is also observed in the loop consisting of residues 62 to 73. This is quite in contrast to the situation in phospholipase A_2 where this loop is well defined. The observed disorder may account for the large difference in activity of phospholipase and prophospholipase with respect to aggregated substrates like micelles.

Introduction

Phospholipase A_2 (EC 3.1.1.4) catalyses the hydrolysis of naturally occurring as well as synthetic phosphoglycerides. Calcium is an essential cofactor for this reaction (van Deenen & de Haas, 1964). In mammals the enzyme is synthesized in the pancreas as a precursor, or zymogen: prophospholipase A_2. After entering the gastrinal tract, the precursor is transformed by trypsin into the native enzyme, phospholipase A_2. Trypsin removes the first seven residues from the zymogen (de Haas, Postema, Nieuwenhuizen & van Deenen, 1968). The catalytic properties of precursor and native enzyme with respect to monomeric substrates are quite similar. However, a dramatic difference exists in their activity *versus* aggregated substrates such as micelles: the native enzyme is two to three orders of magnitude more active in the degradation of the phospholipids in these aggregates than the zymogen (Pieterson, Volwerk & de Haas, 1974).

To explain the remarkable difference in activity between these two closely related proteins, high-resolution X-ray diffraction studies of phospholipases are being undertaken in our laboratory. This has resulted so far in (i) a proposal for the structure of porcine prophospholipase at 3·0 Å resolution (Drenth, Enzing, Kalk & Vessies, 1976); (ii) a 2·4 Å resolution structure of bovine pancreatic phospholipase A_2 (Dijkstra, Drenth, Kalk & Vandermaelen, 1978) which has subsequently been refined and extended to 1·7 Å (Dijkstra, Kalk, Hol & Drenth, 1981); and (iii) the 3·0 Å structure of transaminated bovine phospholipase A_2 (Slotboom & Dijkstra, in preparation), in which a minor modification of the N-terminal alanine residue destroys the tremendous activity of native phospholipase towards micelles. In the present article we describe the structure determination of bovine prophospholipase A_2 at 3·0 Å resolution and, in view of the limited resolution of this structure determination, discuss the results in a preliminary way.

Experimental procedures

The enzyme was kindly supplied by Professor de Haas and coworkers at the Biochemical Laboratory, University of Utrecht, The Netherlands. The lyophilized material received was dissolved in 0·1 *M* tris–HCl buffer, 5 m*M* $CaCl_2$, pH 8·2, to a final protein concentration of 15 mg ml^{-1}. Except for a slightly higher protein concentration and a difference in pH, the crystallization procedure, from a 1:1 mixture of 2-methyl-2,4-pentanediol and buffer, was the same as reported previously for native phospholipase A_2 (Dijkstra *et al.*, 1978). Hexagonally shaped crystals of dimensions 0·8 × 0·3 × 0·3 mm were grown in about 2–8 weeks. They have space group $P3_121$ or $P3_221$, *i.e.*

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different from the $P2_12_12_1$ symmetry of the native enzyme crystals.

The cell dimensions, determined on an Enraf–Nonius CAD-4F diffractometer, are $a = b = 46{\cdot}95$, $c = 102{\cdot}0$ Å. There is one molecule per asymmetric unit.

Three-dimensional X-ray diffraction data were measured on one single crystal at room temperature on the diffractometer, with Ni-filtered Cu $K\alpha$ radiation. The reflections were measured with a continuous scan of 0·8° for 2 min of which two times 20 s were spent on background measurements at each end of the scan. Friedel pairs were measured and averaged during data processing. After approximately every 50 intensity measurements five standard reflections were recorded which allowed correction for radiation damage. An empirical absorption correction was applied (North, Phillips & Mathews, 1968). 5857 intensities were recorded. After averaging of the multiply measured reflections, 2844 unique reflections remained. R_{sym}, defined as $\sum |I_h^+ - I_h^-| / \frac{1}{2}\sum (I_h^+ + I_h^-)$, was 5·1% for 2797 Friedel pairs.

Results

(a) *Rotation-function studies*

As the amino acid sequence of the precursor differs from the native enzyme only by the presence of seven extra N-terminal residues, and because of their similar catalytic properties with respect to monomeric substrates, it was reasonable to assume that the structures would not be too different. Further evidence for structural similarity comes from CD measurements (Jirgensons & de Haas, 1977). Consequently, the structure of the native enzyme would probably have been a good starting model for the molecular replacement method (Rossmann, 1972). However, as we had at our disposal the structure of the transaminated phospholipase A_2, which resembles prophospholipase in its lack of activity *versus* aggregated substrates, it was decided to use this as the starting model, although the differences between the transaminated and the native structures are small [r.m.s. difference for the C^α atoms, 0·2 Å (Slotboom & Dijkstra, in preparation)].

The atoms of a single transaminated phospholipase molecule were positioned in a rectangular unit cell with $a = 74$, $b = 63$ and $c = 78$ Å. These cell dimensions are such that no intermolecular vectors shorter than 20 Å occur. This was considered important for the rotation function where the use of a radius of integration of about 20 Å was envisaged and the absence of intermolecular vectors in the Patterson function of the model compound should decrease the noise due to superposition of inter- and intramolecular vectors. The model comprised 956 non-hydrogen atoms; the Ca^{2+} ion and water molecules were omitted. A structure factor calculation with an overall temperature factor of 15 Å^2 was carried out with a maximum resolution of 3·0 Å. These calculated structure factors for transaminated phospholipase were, together with the observed structure amplitudes of prophospholipase, used as input for Crowther's (1972) fast rotation-function program.

The results of the rotation-function program appeared to depend on the resolution limits selected. Studies on bovine liver rhodanese (te Riele, 1978) had given good results with data between 4·3 and 3·2 Å resolution. Excellent results with data between 4·5 and 3·7 Å had been obtained by Musick & Rossmann (1979) in the case of lactate dehydrogenase. Consequently, we employed initially reflections between 4·3 and 3·2 Å with a radius of integration of 19·2 Å. This radius is the maximum allowed at 3·2 Å resolution with the current version of the program. This rotation function yielded about five peaks significantly above background (Fig. 1). However, the rotation parameters of the highest peak gave no interpretable results in the translation-function calculations. The same negative result was obtained with the highest maximum in the rotation function obtained from data between 10 and 6 Å. The situation was quite different, however, when data between 6·0 and 3·2 Å were used. 1894 observed structure amplitudes and 6355 calculated terms were selected. The fast rotation function showed a distinct maximum of 89 (arbitrary units) at $\alpha = 24{\cdot}8$, $\beta = 56{\cdot}0$ and $\gamma = 87{\cdot}5°$; the second highest peak was 75 and occurred in the section $\beta = 60°$ (Fig. 1). The rotational parameters corresponding to the highest peak gave very clear-cut results in the translation-function calculations to be described in the following section.

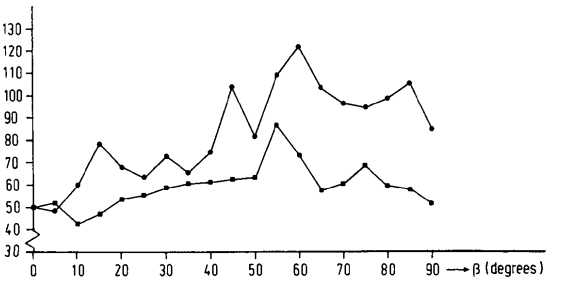

Fig. 1. Fast-rotation-function results (Crowther, 1972). The highest maxima in each section with constant β are plotted in the vertical direction on an arbitrary scale. Squares indicate the results obtained with data in the resolution range 6–3·2 Å. Circles represent the results with data between 4·3–3·2 Å resolution. The radius of integration was in both cases 19·2 Å. The highest maximum in the 4·3–3·2 Å range is a peak at position $\alpha = 10$, $\beta = 60$, $\gamma = 85°$ (without interpolation) which is not the correct rotation. The highest maximum in the 6·0–3·2 Å range is a peak at position $\alpha = 25$, $\beta = 55$, $\gamma = 87{\cdot}5°$ (without interpolation) which appeared to be the correct solution of the rotation problem.

(b) Translation-function, R-factor search and initial difference Fourier synthesis

The coordinates of one single transaminated phospholipase A_2 molecule were rotated according to the rotation-function result and centred on the origin of a cell which had precisely the dimensions of the unit cell of prophospholipase A_2: $a = b = 46{\cdot}95$, $c = 102{\cdot}0$ Å, $\gamma = 120°$. Structure factors for this triclinic structure were calculated out to a resolution of 3 Å with an overall temperature factor of 15 Å^2. Together with the observed prophospholipase structure amplitudes the calculated values served as input for a translation-function program (Crowther & Blow, 1967).

In all calculations data between 3·2 and 5·0 Å were included, resulting in 9341 terms for the translation function (as the translation function worked perfectly no other resolution limits were tested). The self-vectors of the six orientations of the model molecule were subtracted from the observed Patterson synthesis. In a translation-function search for the vectors between the model molecule in its starting orientation and the molecule rotated about the 3_1 axis, the sections $z_{tr} = \frac{1}{3}$ and $z_{tr} = \frac{2}{3}$ were calculated. The section at $z_{tr} = \frac{1}{3}$ contained a peak of 115 (arbitrary units), about ten times the average background in this section. The second highest peak in this section had a height of 38 (see also Fig. 2). The highest peak in the section at $z = \frac{2}{3}$ was 48. This shows that the space group is $P3_121$, and not its enantiomorph, $P3_221$.

The peak in the section $z_{tr} = \frac{1}{3}$ occurs at position $x_{tr} = 0{\cdot}260$, $y_{tr} = 0{\cdot}414$ and this allows calculation of the position of the molecular centre with respect to a 3_1 axis. The peak occurs at a position [A]**s** − **s** where [A] is the 3_1-fold rotation matrix and **s** the shift vector with a threefold screw axis as origin, analogous to the case described by Crowther & Blow (1967). Solving [A]**s** − **s** = (0·260, 0·414) shows that the shift in the x coordinate is −0·036 and in the y coordinate −0·224. As the unit cell contains three different 3_1 axes, the following possible (x,y) combinations for the molecular centre satisfy this result: (i) $x = -0{\cdot}036$, $y = -0{\cdot}224$, (ii) $x = 0{\cdot}333 - 0{\cdot}036 = 0{\cdot}297$, $y = 0{\cdot}667 - 0{\cdot}224 = 0{\cdot}443$, and (iii) $x = 0{\cdot}667 - 0{\cdot}036 = 0{\cdot}631$, $y = 0{\cdot}333 - 0{\cdot}224 = 0{\cdot}109$. This translation-function calculation does not provide information about the z coordinate of the molecular centre.

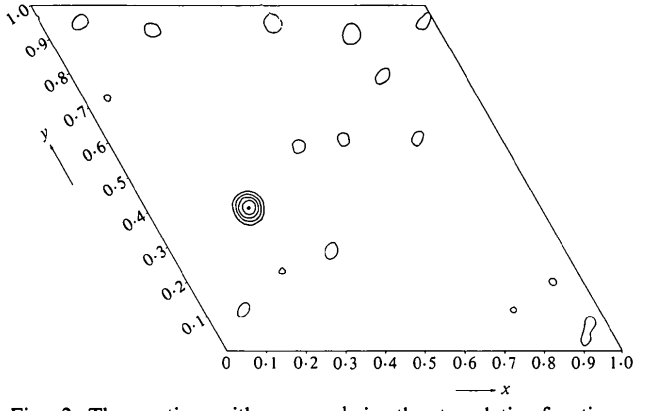

Fig. 2. The section with $z_{tr} = \frac{1}{3}$ in the translation-function (Crowther & Blow, 1967) calculation. The model molecule was oriented in the unit cell according to the rotation-function maximum obtained with data between 6·0 and 3·2 Å (Fig. 1). The calculated cross-vectors in the translation function were those between the model molecule and the molecule rotated about the 3_1 axis. The contour levels are drawn at intervals of 20 arbitrary units, starting at level 20.

The z coordinate can be determined by a translation-function search for the vectors between the model molecule and the model rotated about one of the twofold axes perpendicular to the 3_1 axis. Using the twofold axis parallel to the [110] direction, which lies in the plane $z = 0$, a peak of height 106 was observed at (0·473, 0·527, 0·651). As expected, this peak lies in the plane $x_{tr} + y_{tr} = 1$, *i.e.* in a plane through the origin perpendicular to the twofold axis. The peak was more than twice the height of the second highest peak. Solving the appropriate equations yields −0·326 as the z coordinate for the molecular centre. The molecular centre has to be on the plane $x - y = 0{\cdot}527$ parallel to the twofold axis, which follows from considering the x and y components of [A]**s** − **s** = (0·473, 0·527) for the twofold rotation operation about the [110] direction. This allows the resolution of the ambiguity left by the first translation function: from the three possible (x,y) combinations only the last agrees with the result of the second translation function. This gives the approximate position of the molecular centre: $x = 0{\cdot}631$, $y = 0{\cdot}109$, $z = 0{\cdot}674$.

The positioning of the molecule in its appropriate orientation in the $P3_121$ unit cell of prophospholipase A_2 yielded $R = 0{\cdot}44$ for 2697 unique reflections between 7·1 and 3·0 Å. An overall temperature factor of 15 Å^2 was assumed.

To improve the overall placement of the molecule in the unit cell, rigid-body movements with concomitant R-factor calculations were carried out. Initial orientational steps were 1° in the three Eulerian angles with translational steps of 0·01 in fractional coordinates. After an initial search of 12 positions in the six-dimensional search space, one more step was made for those parameters which showed a reduction in R. The steps were then reduced by a factor of three and the procedure was repeated. The final result was a reliability factor of 0·40. The corresponding rotation and translation parameters are: $\alpha = 26{\cdot}4$, $\beta = 56{\cdot}8$, $\gamma = 89{\cdot}15°$, $x = 0{\cdot}630$, $y = 0{\cdot}111$, $z = 0{\cdot}674$.

An $(|F_o| - |F_c|)$ difference synthesis calculated at this stage showed a distinct peak at the position corresponding to the Ca^{2+} ion in the phospholipase A_2 structure. Although this was not the highest feature of the difference map it did provide further evidence for the reasonable quality of the phases at this point of the

structure determination. In the $|F_o|$ electron density map calculated with the phases available at this stage, most residues were lying reasonably well in density. However, low density was detected at the N terminus, especially for Ala1, Leu2, Trp3 and Gln4, *i.e.* the first four residues of the model structure. The electron density for the extra seven residues at the N terminus* was hardly discernible and did not allow a straightforward interpretation. In addition, the density for the loop comprising residues 62–73 was weak. To allow more definite conclusions to be drawn a restrained refinement of the structure was commenced.

(c) *Restrained refinement*

The restrained-refinement program for proteins written by Konnert & Hendrickson (Konnert, 1976; Hendrickson & Konnert, 1980) was adapted for the Cyber 170/760 of the University of Groningen.

In an initial refinement, all non-hydrogen atoms from the transaminated model structure were included, omitting the Ca^{2+} ion and water molecules. Some details of the refinement procedure are given in Table 1 (refinement *A*). For the reflections between 7·1 and 3·0 Å R decreased from 0·40 to 0·27. The overall temperature factor and scale factor were refined simultaneously. In this and all further refinements, the S–S bond distances were restrained to a value of 2·08 Å. The r.m.s. shift for the N, C^α and C main-chain atoms was 0·35 Å and for all atoms 0·55 Å (Fig. 3). In the subsequent difference map, the Ca peak was more pronounced than in the difference map prior to the refinement. The new map showed again disorder for the N-terminal region as well as for the loop 62–73 [henceforth also referred to as the 'asparagine loop' as it contains Asn71 which interacts directly with the α-NH_3^+ in the native enzyme (Dijkstra *et al.*, 1981)].

Further unrestrained refinement of the isotropic temperature factors, with fixed atom coordinates, reduced R to 0·22. From the subsequent $(F_o - F_c)$ map, no additional information about the N terminus and the asparagine loop could be obtained.

The temperature factors for these two regions of the molecule are considerably above average (Fig. 4). As this again pointed to conformational changes or disorder for these two segments, a new structure factor calculation using the refined coordinates was carried out, but omitting residues 1–8, 61–74 and also 100–110. This third region was a section of an α-helix close to the N terminus and was omitted mainly for comparative purposes. In the subsequent difference map, the helical segment 100–110 showed up very clearly, with all atoms in density; only weak and disconnected patches of density were observed for residues 62–73 and for the ten N-terminal residues.

* These extra residues are usually numbered −7 to −1 by workers in the field.

Table 1. *Summary of parameters and results obtained by the Konnert–Hendrickson (Konnert, 1976; Hendrickson & Konnert, 1980) restrained-refinement procedure*

Refinement *A* includes all model atoms; in refinement *B* residues 1–8 and 61–74 have been omitted.

	Refinement *A*	Refinement *B*
Number of atoms	956	776
Number of reflections (3·0–7·1 Å)	2697	2697
Number of cycles	12	12
$R = \sum\|\|F_o\| - \|F_c\|\|/\sum\|F_o\|$	0·27	0·28
$B_{overall}$ (Å^2)	10·2	7·6
Scale factor	2·05	2·14

	Input standard deviation	R.m.s. deviation from standard value after refinement *A*	R.m.s. deviation from standard value after refinement *B*
Bond lengths (1–2 distances)	0·03	0·018	0·021
Bond lengths (1–3 distances)	0·05	0·055	0·059
1–4 interactions	0·05	0·043	0·047
Plane distances (except peptide units)	0·05	0·037	0·039
Plane distances (peptide units)	0·01	0·001	0·001
Chiral volumes	0·20	0·212	0·207
Van der Waals distances	0·03	0·042	0·041
Structure factors	90	234	246
Number of distances		2623	2102
Number of planes		171	138
Number of chiral volumes		138	112

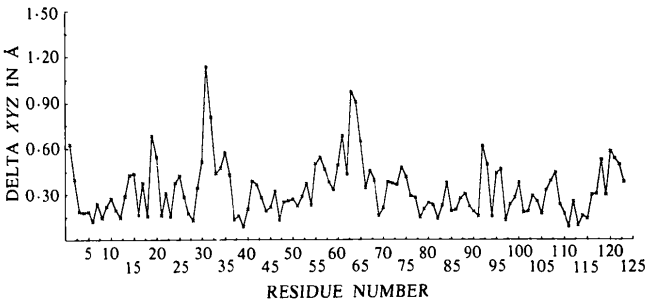

Fig. 3. Average deviations for the N, C^α and C atoms after optimal superposition of the atomic positions of phospholipase A_2 (Dijkstra *et al.*, 1981) and prophospholipase A_2 after refinement *A* (Table 1).

To check whether or not the inclusion of the atoms from the two flexible or disordered segments in the refinement procedure had affected the results in such a way that alternative positions for these segments had somehow been masked, a second refinement was

performed (Table 1, refinement *B*). An overall temperature factor and scale factor were refined with data between 7·1 and 3·0 Å. The starting coordinates were the same as for the first refinement, except that the first eight residues of the model molecule (*i.e.* the 15 N-terminal residues of prophospholipase) and residues 61–74 were omitted. *R* dropped from 0·42 to 0·28. In the subsequent difference synthesis, the Ca^{2+} ion, together with the S atom of Met8, showed up as the highest features in the map (Fig. 5). In the loop 61–74 reasonable density could only be observed for residues 61 and 74 (Figs. 6 and 7). The remainder of the loop could not be traced. In the N-terminal region, residues Phe5, Asn6 and Met8 appeared to be clearly present in the density. From Gln4 backwards the density fades away and gives virtually no indication for the positions of the first ten residues of the prophospholipase molecule (Fig. 8).*

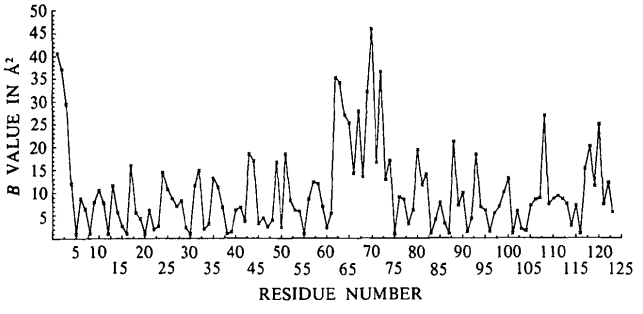

Fig. 4. Graphical representation of the mean temperature factor for the peptide atoms C, C^{α} and N against residue number. High values occur both for the first three residues of the N terminus and for the atoms in the asparagine loop (residues 62 to 73). During the refinement no restraints on the *B* values were used.

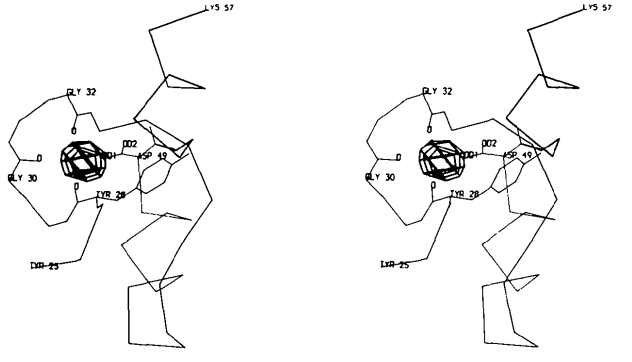

Fig. 5. Stereoscopic view of the electron density of Ca^{2+} in the difference synthesis after refinement *B* (Table 1), and the positions of a number of surrounding residues. All stereodiagrams in the present article, were drawn with an interactive graphics system and the program *GUIDE* (Brandenburg, Dempsey, Dijkstra, Lijk & Hol, 1981).

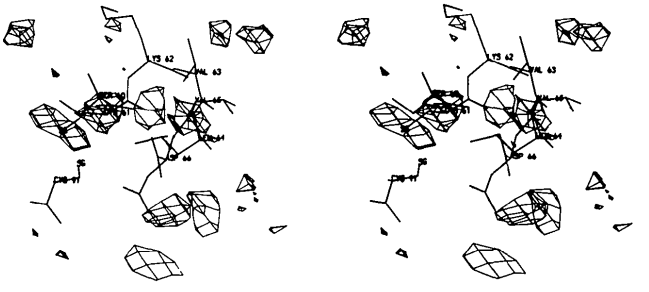

Fig. 6. Stereodiagram of the difference electron density at the beginning of the asparagine loop after refinement *B* (Table 1). In this refinement residues 61–74 and −7–8 were omitted. The positions of the residues are those after refinement *A* (Table 1).

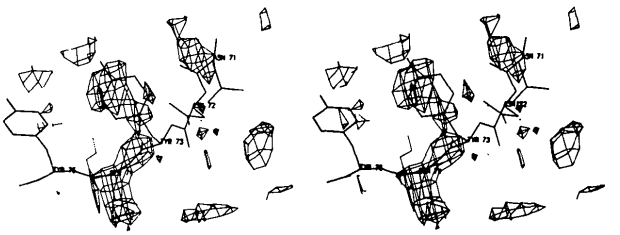

Fig. 7. Stereodiagram of the difference electron density at the end of the asparagine loop after refinement *B* (see legend to Fig. 6).

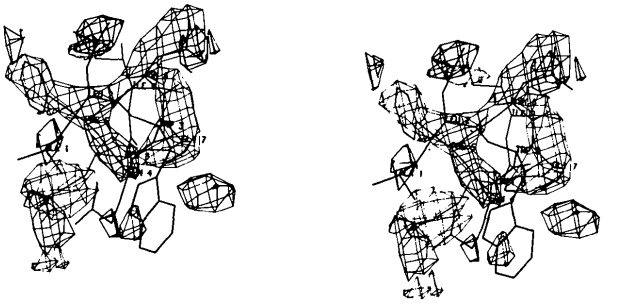

Fig. 8. Stereodiagram of the difference electron density at the N terminus of prophospholipase after refinement *B* (see legend to Fig. 6).

Discussion

The active-site residues in phospholipase A_2 (Dijkstra *et al.*, 1981) are positioned in a similar fashion as in prophospholipase A_2. Also, the Ca^{2+} ion appears to be bound in quite a similar way [Fig. 5 and Dijkstra *et al.* (1981)]. This is in agreement with the observed similarities of precursor and native enzyme in their

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 2BP2 and R2BP2SF), and are available in machine-readable form from the Protein Data Bank at Brookhaven or one of the affiliated centres at Cambridge, Melbourne or Osaka. The data have also been deposited with the British Library Lending Division as Supplementary Publication No. SUP 37003 (2 microfiche). Free copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

catalytic activities with respect to monomeric substrates.

As Fig. 9 shows, the structure of *bovine* prophospholipase A_2 obtained from the present investigation resembles that of the native enzyme to a considerable extent. The proposal for a *porcine* prophospholipase A_2 structure (Drenth *et al.*, 1976) was derived from a rather poor electron density map. In the interpretation of this map, the authors relied strongly on a connectivity for the seven disulphide bridges, two of which were later shown to be in error (Dijkstra *et al.*, 1978). The present bovine prophospholipase structure is derived from a much better quality map, although still of limited resolution. Considering the high homology in amino acid sequence between the bovine and porcine enzyme (Meyer, Verhoef, Hendriks, Slotboom & de Haas, 1979) the present study implies a correction of the proposed folding of porcine prophospholipase A_2.

Another important result obtained from this investigation is that bovine prophospholipase seems to exhibit a substantially greater flexibility than the native enzyme. This situation is analogous to the differences between trypsinogen and trypsin (Fehlhammer, Bode & Huber, 1977). The precursor, trypsinogen, exhibits a markedly greater flexibility in about 16% of the polypeptide chain than the active enzyme trypsin. These observations suggest that enhanced motility of considerable sections of an enzyme molecule may be a quite general feature employed by nature in manufacturing harmless enzyme precursors. However, the number of enzymes for which the structures of both precursor and native enzyme are known is quite limited. Moreover for chymotrypsinogen no greatly enhanced flexibility has been reported (Freer, Kraut, Robertus, Wright & Xuong, 1970) and for trypsinogen a second, much less flexible structure has been described (Kossiakoff, Chambers, Kay & Stroud, 1977).

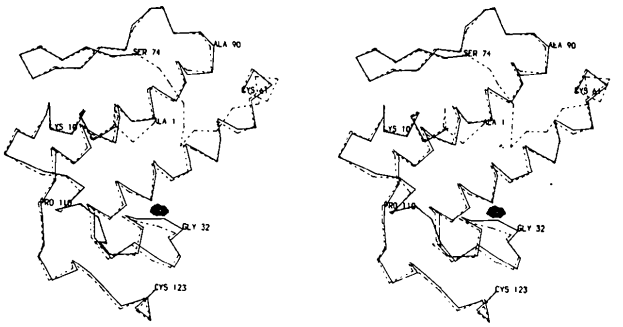

Fig. 9. Stereodiagram of the superposition of the C^{α} positions of phospholipase A_2 (Dijkstra *et al.*, 1981) and prophospholipase A_2 after refinement *B*. The native enzyme is indicated by dashed lines; the flexible or disordered regions in the precursor molecule are omitted. The electron density for the Ca^{2+} ion is added and indicates the active-site region.

Turning to the difference in structure between native and precursor phospholipase A_2, one has to refrain from detailed conclusions in view of the limited resolution of the present prophospholipase model. Nevertheless, it seems quite possible that the greater flexibility of the N-terminal loop and the asparagine loop is due to two factors. First, the disappearance of the hydrogen bond between the α-NH_3^+ of residue 1 with the main chain carbonyl oxygen of Asn71 in phospholipase (Dijkstra *et al.*, 1981), and, second, the extra length of the N terminus which would collide with the position of the asparagine loop in the native enzyme. The difference in activity *versus* micelles could be due to the changes in the N terminus alone, to the alterations in the asparagine loop, or to both. Only further investigations will settle this point.

We thank Professor G. H. de Haas and coworkers (Utrecht) for the ample supply of protein material throughout the course of this investigation, IJsbrand van der Leeuw for his assistance in growing single crystals, Mrs C. Torfs-van Cothem for excellent technical support and Frits Hazelhoff for initial rotation-function studies. Dr Pullford (Department of Zoology, Oxford University) kindly provided us with a version of the Konnert–Hendrickson program. We are also grateful to Drs Crowther and Lattman for programs. The computations were performed on the Cyber 170/760 at the University of Groningen's Computer Centre.

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Molecular Structure of 21⟨O_7-(1,2)Benzeno.2_6coronand-7⟩4,18-dione–KSCN 1:1 Complex*

BY MÁTYÁS CZUGLER AND ALAJOS KÁLMÁN

Central Research Institute of Chemistry, Hungarian Academy of Sciences, Budapest 114, *POB* 17, *H*-1525, *Hungary*

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Abstract

$C_{18}H_{24}O_9.KSCN$, $M_r = 481{\cdot}57$, is monoclinic, $P2_1/n$, with $a = 8{\cdot}631$ (1), $b = 11{\cdot}338$ (1), $c = 24{\cdot}479$ (3) Å, $\beta = 109{\cdot}58$ (2)°, $V = 2257{\cdot}0$ (8) Å^3, $D_c = 1{\cdot}417$ Mg m^{-3}, $Z = 4$, $F(000) = 1008$. The structure, after inclusion of a conformational disorder for the C(9) and C(10) atoms, was refined to $R = 0{\cdot}050$ for 3122 diffractometer (CAD-4) data. The 21-crown-7 ligand is fairly puckered, wrapping round the guest cation; K^+ is located near the least-squares plane of the seven ligating ether O atoms (their mean deviation is 0·56 Å). All K···O distances (2·77–3·07 Å) are longer than the sum of the corresponding ionic and van der Waals radii (2·73 Å). The eighth atom coordinated to K^+ is N of SCN^- at a distance of 2·80 Å. The keto O atoms, pointing outward from the macro ring, do not coordinate to K^+. The established conformational disorder of atoms C(9) and C(10) suggests that these large rings may be subject to a low-energy-barrier pseudorotation.

Introduction

The alkali-metal salts are known to form various types of complexes with crown ethers. These polyether complexes may be classified into four groups according to the positioning of the cation relative to the ligand atoms (Dalley, 1978). Examination of the cation and ligand size parameters indicates that K^+ is just able to fit the cavity of an 18-crown-6 ligand (Seiler, Dobler & Dunitz, 1974). Accordingly, the title complex, KSCN with 21-crown-7, should fall into the third group, *i.e.* the cation is smaller than the cavity. To our knowledge, based on a search of the Cambridge Crystallographic Data File containing 27 988 entries to October 1980, no crystal structure of a K^+ (or any other metal) complex with a 21-crown-7 ligand has been reported hitherto. Only the structure of a substituted uncomplexed 21-crown-7 is known (Owen & Nowell, 1978). The structures of K^+ complexes with dibenzo-24-crown-8 and dibenzo-30-crown-10 ligands have been described by Mercer & Truter (1973*b*) and Bush & Truter (1972). In these cases the cavities are even larger than in the title complex. Owing to the actual host–guest relationship in the title compound, complexation of the keto O atoms was expected (Vögtle, 1980).

Experimental

Crystals of the compound were kindly supplied by Professor F. Vögtle (University of Bonn). Intensities were collected on a CAD-4 diffractometer with graphite-monochromated Cu $K\alpha$ ($\bar{\lambda} = 1{\cdot}5418$ Å) radiation. Cell constants were determined by least squares from the setting angles of 25 reflexions; 3122

* Nomenclature is as suggested by Weber & Vögtle (1980). Alternative name: 2,5,8,11,14,17,20-Heptaoxabicyclo[19.4.0]-pentacosa-1(25),21,23-triene-4,18-dione–KSCN complex.

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