Crystallographic Studies on L-Asparaginase from Proteus vulgaris

II. SYMMETRY AND LOCATION OF THE TETRAMERIC MOLECULE*

(Received for publication, March 10, 1975)

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SUMMARY

Analyses of the x-ray diffraction intensity data by the Patterson synthesis and rotation function techniques show that the true space group of the monoclinic crystals of L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) from Proteus vulgaris is $P_2_1$, that the molecular centers lie at $x = 0.054$, $y = 0$, $z = 0.256$, and its symmetry related positions, and that the tetramer molecules possess three approximate, mutually perpendicular 2-fold rotational symmetries, the axes of which run along the directions of the crystallographic $a^*$-, $b$-, and $c$-axes. In addition, an investigation of the molecular packing arrangement in the crystal indicates that the tetramer molecules possess an approximately regular tetrahedral subunit structure.

L-Asparaginases (L-asparagine amidohydrolase, EC 3.5.1.1) are enzymes that catalyze the hydrolysis of L-asparagine to L-aspartic acid and ammonia (1). The x-ray crystallographic studies on the tetrameric enzymes from Escherichia coli (2) and from Erwinia carotovora (3, 4) revealed that the molecules have varying degrees of molecular symmetry.

L-Asparaginase from Proteus vulgaris is also a tetrameric enzyme, of molecular weight 120,000. The four subunits appear to be chemically identical as indicated by the sedimentation and polyacrylamide gel electrophoretic studies in the presence of denaturants and from the NH$_2$- and COOH-terminal end group analyses (5). Large crystals of this enzyme can be grown (6) that are stable over long periods of time and give diffraction patterns extending to a resolution of at least 3.4 Å. Preliminary studies of these photographs showed (7) that the crystals belong to the monoclinic space group $P_2_1$ or $P_2$, with the cell dimensions, $a = 69.3$ Å, $b = 125.6$ Å, $c = 101.4$ Å, $\beta = 97^\circ 20$ min. While the symmetry of the crystals and the cell dimension and density measurements indicate that the molecules are not required to have crystallographic symmetries, visual examination of the x-ray photographs led us to suspect that the tetramer molecules might possess a 222 molecular symmetry (7). We have since collected a set of x-ray diffraction data and analyzed them using Patterson synthesis and rotation function (8) techniques. These analyses lead us to conclude that the enzyme molecules indeed possess a high degree of 222 symmetry and that therefore all four subunits of the molecule have a similar three-dimensional structure. Also an investigation of the optimum packing of the subunits in the crystal indicates that the most probable structure for the tetramer molecule has a nearly regular tetrahedral arrangement of the subunits. An account of these studies is presented herein.

DATA COLLECTION

The x-ray diffraction data were collected using a Syntex P1 autodiffractometer and graphite-monochromated CuK$_\alpha$ radiation. A stepping $\omega$-scan technique (9) and an empirical absorption correction scheme (10) were used. A fuller account of the data collection and handling procedures will be presented elsewhere. A total of 3250 strong independent reflections within 6.3 Å resolution were chosen from this data set and used for the analyses described.

PATTERSON MAP

Previous photographic examinations (7) established that the crystals were monoclinic and belonged to the space group $P_2_1$ or $P_2$. It was also observed that they contained an $A$-centering translational pseudo-symmetry and that within about 10 Å resolution the space group was $A_2$. This latter observation was fully confirmed by the three-dimensional Patterson map. This map showed only one large, non-origin, symmetry-independent peak. This peak, located at $x = -0.108$, $y = \frac{1}{2}$, and $z = 0.488$,
is sharp and well defined and reaches a maximum at a little less than half the height of the origin peak (Fig. 1).

If the true space group were $A_2$, one would expect a peak of the same height as the origin peak at 0 $\frac{1}{2}$ $\frac{1}{2}$ corresponding to the $A$-centering translational symmetry. However, if the translation vector deviates from the exact $A$-centering operation, the corresponding Patterson peak will split into two symmetry-related peaks, each of about half the origin height, around the ideal position. This is exactly what one sees in Fig. 1, thus confirming the existence of an $A$-centering translational pseudo-symmetry. As was noted earlier (7) the existence of this pseudo-symmetry, combined with the information that one unit cell of this crystal contains only 2 molecules, leads to the conclusion that the molecule possesses a 2-fold rotational symmetry parallel to the $b$-axis.1

The knowledge of the exact nature of the translational pseudo-symmetry enables us to go one step further and to establish unambiguously the true space group of the crystal and the position of the molecular centers with respect to the symmetry elements. As was pointed out earlier (7), if the true space group were $P_2$, molecules must lie on crystallographic 2-fold axes1 (Fig. 2A) and the pseudo-$A$-centering translation vector would be represented by 0 $t_y$ $\frac{1}{2}$, where $t_y$ has a value close to $\frac{1}{2}$. The Patterson map would then show two peaks at 0 $\pm t_y$ $\frac{1}{2}$. This is not what one observes in Fig. 1, as the peaks have very definite nonzero $x$ components (as well as a small but definite non-half $z$ component). The true space group is therefore $P_2$. The molecules lie on pseudo-2-fold axes at $x_0$, $y_0$, $z_0$, and its symmetry-related position, where $x_0$ is close to zero, $y_0$ is arbitrary and may be set to zero, and $z_0$ is close to $\frac{1}{2}$ (Fig. 2B). Patterson peaks will then be expected at $-2x_0$, $\frac{1}{2}$, $1-2z_0$ and $2x_0$, $\frac{1}{2}$, $2z_0$. Identifying these with the observed peaks, one finds $x_0 = 0.054$, $y_0 = 0$, and $z_0 = 0.256$. This pseudo-2-fold rotational symmetry appears to be very closely parallel to the $b$-axis and seems to involve no appreciable translation along the $x$ axis such as that observed in hexokinase B (11). Otherwise Patterson peaks should be elongated or even separated into two peaks along the $y$ direction. This is not the case; the peaks are sharp along the $y$ direction and accurately centered on the $y = \frac{1}{2}$ plane.

**FIG. 1.** Patterson map section at $y = \frac{1}{2}$. The lowest contour level and the interval between successive contour levels are both at $\frac{1}{4}$ of the origin peak height.

**Fig. 2.** A, molecular arrangement when the true space group is $P_2$. Filled symbols represent crystallographic symmetries, open symbols the noncrystallographic pseudo-symmetries. The 2 nonequivalent molecules in a unit cell need not be separated by exactly half the unit cell translation along the $y$ direction. Relative orientation of the molecules need not be identical either. Unless the tetramer molecule contains a large hollow volume at its center, this arrangement will produce a Patterson peak at 0 $t_y$ 0, where $t_y$ is close, but not necessarily equal, to $\frac{1}{2}$. The peak will be broad on the $x-z$ plane. B, molecular arrangement when the true space group is $P_2$. Filled symbols represent crystallographic symmetries, open symbols the noncrystallographic pseudo-symmetries. The 2 molecules in a unit cell are separated by an exact half of the unit cell translation along the $y$ direction. Also, to the extent that the pseudo 2-fold symmetry is locally true, the relative orientation of the 2 molecules is identical. This arrangement will, therefore, produce a sharp Patterson peak at $x$, $\frac{1}{2}$, $z$, where $x$ and $z$ are close but not necessarily equal to 0 and $\frac{1}{2}$, respectively.

**ROTATION FUNCTION CALCULATION**

The crystals have additional pseudosymmetries. These could be seen in the $h0l$ layer photograph (Fig. 1 of Ref. 7) as approximate 2-fold rotation symmetries along the $a$-* and $c$-axes. In order to confirm this, rotation function calculations were made. The computer program for these calculations was kindly supplied to us by Dr. W. Eventoff in Professor M. G. Rossmann's laboratory at Purdue University, Lafayette, Indiana. The program calculated the rotation function in the form of $R = \Sigma_{a} |F_{h}|^2 |\Sigma_{p} |F_{p}|^2 G_{h,p}|$, where $h$ and $p$ are two sets of Miller indices, $F_{h}$ and $F_{p}$ are the structure factors, and $G_{h,p}$ is an interference function whose value depends on the two indices $h$ and $p$, the angles of rotation, and the radius $r$ of a sphere in the Patterson space within which the degree of symmetry is being investigated. The first summation in the formula included 850 strongest reflections and the second 2776 strongest ones. The radius $r$ was chosen to be 20 Å. The function $R$ attains large values when the Patterson map within this sphere overlaps with itself after the specified rotation.

The rotation was specified initially by means of the three Eulerian angles. Each of these angles was varied in 30° steps over the entire range of its possible values. The rotation map...
thus produced showed only one symmetry-independent non-origin peak at a position corresponding to 2-fold rotations around the crystallographic $a^*$- and $c$-axes. (Because the Patterson space has a crystallographic 2-fold rotation symmetry along the $b$-axis, presence of a 2-fold rotation symmetry along the $a^*$-axis generates the same symmetry along the $c$-axis and vice versa.)

The calculation was then repeated using polar angles. A given rotation is described as a rotation of angle $\kappa$ around an axis, the direction of which is specified by the two polar angles $\psi$ and $\phi$. The former is the angle that the rotation axis makes with the crystallographic $b$-axis. The latter is the angle that the projection of the rotation axis onto the $a^*$-$c$ plane makes with the $a^*$-axis. The angle $\kappa$ was fixed at 180$^\circ$. Other angles were varied in 15$^\circ$ steps in a coarse search and 5$^\circ$ steps in a fine search. The rotation map thus produced is given in Fig. 3. In full confirmation of the expectation from the photographic examination, the map contains two non-origin peaks at positions corresponding to 2-fold rotations along the crystallographic $a^*$- and $c$-axes. In order to check the possibility that this pseudorotation symmetry might involve an angle that is close to but not quite 180$^\circ$, a separate 5$^\circ$-step fine search was made by varying all three angles, including $\kappa$, within a range of 20$^\circ$ each around the peak position of the $\kappa = 180^\circ$ search. This search confirmed that the rotation angle is indeed 180$^\circ$ within the limit of the resolution of the search. We conclude, therefore, that the tetramer molecule possesses a high degree of 222 molecular symmetry with its axes running along the crystallographic $a^*$-, $b$-, and $c$-axes.

FIG. 3. Polar graph of the rotation function calculated for $\kappa = 180^\circ$. The radial distance from the center measures angle $\psi$, the outer rim corresponding to $\psi = 90^\circ$. Angle $\phi$ is measured clockwise around the circle. Numbers are contour levels in arbitrary units. The graph has a 2-fold symmetry. The lower half shows coarse search, the upper half fine search.

If the four subunits of the tetramer were in a regular tetrahedral arrangement, the molecule could possess a degree of 3-fold rotation symmetry. This symmetry cannot be exact since, if it exists, its axis must pass through a subunit and thus requires that subunit to have a 3-fold rotation symmetry by itself. However, if the remaining three subunits conformed to a 3-fold rotation symmetry, one might expect to see a corresponding small peak in the rotation map. A 3-fold search was, therefore, made with $\kappa = 120^\circ$. This map, however, did not show any non-origin peak significantly above the background.

Molecular Packing

The molecular packing arrangement in the crystal was investigated by systematically moving a subunit in the unit cell and calculating the volume of overlap among it and all other subunits generated by the crystallographic and noncrystallographic symmetries. Each subunit was assumed to be a sphere of 41.2 Å diameter. This latter value was estimated from the specific volume and the molecular weight of the protein.

A three-dimensional overlap map was generated when the volume of the overlap calculated in this manner was plotted as a function of the position of a subunit. The maximum in this map occurs when all four subunits are placed at the same position. The overlap volume at this point is 6 times the volume of one subunit according to the algorithm we used for this computation. Aside from this trivial feature, the map contained one continuous “valley” where the overlap volume was markedly less than in other regions. The two highest points in this valley corresponded to rectangular subunit arrangements with overlap volumes of about 41.2 of the over-all maximum. The center-to-center distances between the neighboring subunits were approximately $\frac{1}{2} a^* = 34.4$ Å and $b/2 = 62.5$ Å for one (Structure $A$ of Fig. 4) and $\frac{1}{2} a^* = 34.4$ Å and $c/2 = 50.6$ Å for the other (Structure $D$ of Fig. 4). These structures are, therefore, compressed too much along the crystallographic $a^*$ direction and too loosely packed along the crystallographic $b$ or $c$ direction.

FIG. 4. Representative subunit structures in the low volume region of the overlap map. Structures $A$ and $D$ are represented by points of maxima in this region. Structures $B$ and $C$ are equivalent and represented by the points of over-all minimum.
Fig. 5. Molecular packing, as viewed down the b-axis, with the Structure B of Fig. 5. Each circle represents a subunit and has a 20.6 Å radius. The y-coordinate is indicated for each subunit at its center. Crystallographic symmetries are indicated by the filled symbols, noncrystallographic by the open symbols.

Other points in this valley of low overlap represented a series of tetrahedral structures that can be obtained by deforming either of the two rectangular structures in the direction of the other. The over-all minimum occurs when one of the subunits is at $x \approx -0.17$, $y \approx -0.1$ (with the origin of the y-coordinate at the center of the tetramer molecule), and $z \approx 0.10$. The overlap volume at this point was less than $0.1\%$ of the over-all maximum. This position is midway between those for the two planar structures and represents a nearly regular tetrahedral arrangement of the subunits (Structures B and C of Fig. 4). The three independent center-to-center distances between the subunits in this structure are 40 Å, 43 Å, and 47 Å. The low volume of overlap and the nice subunit packing within a molecule may be taken as indications that this structure is close to the true structure. A view down the y-axis of the packing arrangement with this structure is shown in Fig. 5.

Comparison with E. coli Enzyme

Epp et al. (2) studied L-asparaginase from E. coli using similar crystallographic techniques. This enzyme crystallizes in the monoclinic space group C2 with 1 tetramer molecule/asymmetric unit. The tetramer molecules were also found to have the 222 pseudosymmetry. At low resolution the tetramer then has the 1222 space group symmetry with the cell dimensions $a'$ = 136.7 Å, $b'$ = 62.9 Å, $c'$ = 71.5 Å. If one considers the tetramer molecule as an approximately cubic box of dimension $71 = (c') \times 68 = (a' / 2) \times 63 = (b')$ Å, this crystal is seen to have a very simple and compact packing scheme (Fig. 6A). The crystal consists of layers of boxes parallel to the $b'$-$c'$ plane. Neighboring layers are displaced with respect to one another such that the corner of the box in one layer is at the center of a box in the next layer. There is no space either between boxes in one layer or between adjacent layers.

If the P. vulgaris enzyme molecule is also considered as an approximately cubic box of dimension $69 = (a \sin \beta) / \sin \alpha \times 68 = (c / 1.5) \times 63 = (b / 2)$, it is apparent that the crystals of this enzyme have a more complex and open packing scheme (Fig. 6B). The crystal consists of rows of boxes running parallel to the $a$-axis. In each row the boxes are displaced along the $c$ direction by $0.1$ Å ($-0.5 \sin \beta$ = $0.5$ of the box dimension along $c$). A given row has four neighboring rows. One row and its neighbor are related by the crystallographic $2$ screw or the approximately $A$-centering translation symmetry. Each of these rows is surrounded by four channels of solvent. These channels are all equivalent by symmetry and are about $34$ Å = $0.5$ of the box dimension) wide along the $c$ direction and one full box size (63 Å) wide along the $b$ direction. The volume of the solvent in this crystal must, therefore, be about $50\%$ larger than that in the E. coli crystal. In fact, if the specific volume of the protein is assumed to be 0.74 ml/g, the volume fraction of solvent in the crystal is calculated to be $46\%$ for the E. coli crystal and $65\%$ for the P. vulgaris crystal.

References

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