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Abstract

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HEXAGONAL CRYSTAL STRUCTURE OF CATALASE AND THE STRUCTURE OF ITS MOLECULES

Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. The molecular weight of catalase is about 240-250 thousand ([1]). The molecule contains 4 hemes and can undergo dissociation into 2 and then 4 subunits ([2],[3]). In ([4]) the authors described a hexagonal modification of catalase from bovine liver. This electron-microscopic and X-ray study made it possible to determine the unit-cell dimensions of wet (I) and dry (III) crystals, given in Table 1. The space group of the structure is $P3_{121}$ (or its enantiomorph $P3_{221}$); the number of molecules in the cell is $n = 6$. The molecules are packed in an openwork structure, forming corrugated rows that extend along directions of the type $[10\bar{1}0]$, as if strung on 2_1 axes. These rows are located at heights $z = 0$, $1/3$, and $2/3$, and are transformed into one another by the operation 3_1. It was concluded that, in the transition from the "wet" to the "dry" structure, certain shifts of the molecules take place.

Table 1

	X-ray diffraction	Electron microscopy	Electron microscopy
	wet I ([4])	intermediate II	dry III ([4])
a, Å	173	165–155	130
c, Å	237	235	200–180
Ω, 10^6Å^3	6.14	5.5–4.7	2.9–2.6
x_0	0.33	0.36–0.42	0.67–0.75

Results close to the data of ([4]) were obtained by Longley ([5]), who used an electron-microscopic sectioning technique that gives somewhat lower resolution. In addition to the hexagonal modification of catalase, its rhombic forms are known ([6],[7]), as are packings of it into flat monomolecular layers ([4],[8],[9]) and tubes ([9],[10]).

Fig. 2

Figure 1: Fig. 2

In the present work, crystals I and II were studied; data on them, together with the previously investigated structure III ([4]), are given in Table 1. Here Ω is the volume of the unit cell. The volume of the catalase molecule, Ω_m, found from density measurements, is 300 000 Å^3. The protein volume-filling coefficient of the cell is $x_0 = n\Omega_m/\Omega$.

Isolation and crystallization of the enzyme. Catalase was isolated from bovine liver and crystallized by the procedure described earlier ([4]). For electron microscopy, suspensions of microcrystals obtained from a 12% protein solution in 0.4 M ammonium phosphate buffer, pH 7.0, were used. The crystals for X-ray photography, grown by the method of ([4]), were needles of hexagonal cross section with a diameter of 0.2-0.3 mm. The study was carried out at 4°.

Electron microscopy. The suspension of microcrystals was contrasted with 5% PTA, pH 6.0. The specimens were examined in a JEM-6C microscope at a direct magnification of 50,000.

Figure 1 shows an electron micrograph of structure II along $[10\bar{1}0]$ and an enlarged portion of it, representing an xz-projection of the structure. Images were also obtained along [0001].

With negative staining, the crystallization liquid of the protein crystal is replaced by a contrasting substance containing salts of heavy metals, and the protein molecules appear as weakly absor–

electrons "empty" in a strongly absorbing matrix. Taking into account the law of absorption of electrons in the contrasting substance and in the protein, as well as the law of blackening of the photographic plate for electrons, we proceeded to a quantitative description of the electron-microscopic photograph in Fig. 1– the xz-projection of the structure–in terms of the thickness $L(xz)$ of the protein illuminated at the point xz of the unit cell (see Fig. 2a).

Fig. 2. a–averaged projection of the structure, obtained after quantitative processing of the electron-microscopic photograph in Fig. 1; b–two-dimensional section of the catalase structure, calculated from Fig. 2a at $z = 0$

If the structure possesses a definite symmetry, then one can pass from the projection to the spatial structure. De Rosier and Klug [(11)] realized this possibility for the case of cylindrical symmetry.

We considered the path of transition from projections of the crystalline structure to the spatial distribution of the protein in the cell. In our case of $P3_121$ symmetry, any section of the cell $S(xyz)$, z = const, projected onto the one-dimensional section $L(xz)$, z = const, of the projection in Fig. 2a, owing to the presence of the 3_1 axis, is also projected–but already rotated by 120°–onto the

Fig. 1. Electron-microscopic image of the catalase structure along $[10\bar{1}0]$

Figure 2: Fig. 1. Electron-microscopic image of the catalase structure along $[10\bar{1}0]$

section $L(x, z + 1/3)$ of the projection, and once more—again rotated by 120°—onto the section $L(x, z + 2/3)$ of the projection. Thus, in fact, we have three different one-dimensional projections L of one and the same two-dimensional section $S(xyz)$, $z = \text{const}$. We developed an algorithm for the transition from L to S. In Fig. 2b such a section is given as an example. The entire set of sections over the symmetrically independent region of the cell describes the spatial contour of the protein molecules.

Using "patterns"of the type shown in Fig. 2b, plates of thickness $c/24$ were made, and their superposition yielded a three-dimensional model of the crystalline structure of catalase. Fig. 3a shows the general view of this model; Fig. 3b, c show two views of an individual molecule "removed" from the structure.

In Fig. 4a, b structure II is shown in two projections; Fig. 4c, d show the corresponding projections of the electron density of structure I.

X-ray diffraction data. The intensities of reflections with d down to 20 Å were recorded by the photographic method on precession X-ray photographs. Three-dimensional Patterson syntheses with a resolution of 50 Å and projections with a resolution of 20 Å were constructed, from which, to a first approximation, the coordinates of the centers of the molecules in structure I were obtained.

Refinement was carried out by minimizing the R-factor and by constructing Fourier projections of the electron density. In calculating the theoretical F_{hkl}, the electron density of the molecule was at first approximated by a spherically symmetric function with a radius of about 40 Å. Subsequently the molecule was described as a dumbbell of two spherical subunits, in accordance with the electron-microscopy data. Fourier projections of the electron density are shown in Fig. 4c, d.

Form, symmetry, and quaternary structure of the molecules. Their packing. In a first approximation, the molecule may be

Fig. 1. Electron-microscopic image of the catalase structure along $[10\bar{1}0]$

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has been described as an elongated triaxial ellipsoid or ovaloid, but in reality it is built more complexly. From sections (Fig. 2) of the model (Fig. 3) and projections (Fig. 4) it is clearly seen that the molecule is divided into two subunits with $M = 120\,000$, between which there is a gap, a narrow slit inside the molecule that widens outward. The subunit is approximately a flattened ellipsoid. The boundary contour of the subunits is shown in the projection in Fig. 4a. Consideration of the arrangement of the subunits and of the shape of

Fig. 3

Figure 3: Fig. 3

the molecule as a whole shows that they

Fig. 3. a—general view of the model of the catalase structure; **,** —two views of the model of an individual catalase molecule

can be symmetrically transformed into one another by two axes of the second order (Fig. 3). The third axis 2, perpendicular to the first two, is located along the long axis of the molecule and is an axis of symmetry of each of the subunits. Thus, the molecule has tetradich symmetry 222. According to biochemical data [(1–3)], the subunit with $M = 120\,000$ itself splits into two with $M = 60\,000$, which agrees with the symmetry 2 observed by us. However, at this stage we did not detect a distinct boundary between the subunits of 60,000.

The dimensions of the molecule along its three axes are about 70, 80, and 95 Å. The first two dimensions correspond to the averaged—smoothed—contour of the surface, since in reality (see Fig. 3 ,) precisely along the short axes the contour recedes inward, following the shape of the subunits.

Thus, independently of any initial assumptions, the intrinsic symmetry 222 of the catalase molecules appeared in the structure studied here. It is interesting that the second-order axes of the molecule that emerged proved to be arranged in such a way that their orientation and directions have no relation to the orientation and arrangement of the symmetry axes of the structure, i.e., of the group $P3_121$. The obtained shape and quaternary structure of the molecule are in good agreement with images of molecules observed in monomolecular layers [(4,8,9)].

Thus, quantitative processing of electron-microscopic images of protein crystals and the developed method of transition from

projections to the spatial structure made it possible to obtain data on the crystalline structure of hexagonal catalase, on the shape and quaternary structure of the molecules of this enzyme. On the other hand, the results obtained were independently confirmed by X-ray diffraction. The combination of the data from both methods made it possible to avoid, at least at the descriptive stage of the X-ray study, the use of the exceptionally laborious method of heavy isomorphous derivatives and to obtain syntheses of the electron density.

Fig. 4. *a, b*—projection of the structure shown in Fig. 3a along [0001] (*a*) and [10$\bar{1}$0] (*b*); *c, d*—projections of the electron density of the structure along [0001] (*c*) and [10$\bar{1}$0] (*d*)

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Note: Figure translations are in progress. See original paper for figures.