CRYSTALLIZATION OF CATALASES FOR STRUCTURE INVESTIGATION

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Catalase (EC 1.11.1.6; H_2O_2:H_2O_2-oxidoreductase) is an enzyme that is present in cells of almost all living organisms. It decomposes hydrogen peroxide to molecular oxygen and water:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The reaction consistis of two steps: the first step in the catalytic process is the interaction of the first hydrogen peroxide molecule with enzyme. It forms compound I, that is an oxidized form of the protein.

$$E + HOOH \rightarrow HOH + EO \text{ (compound I)}$$

At the second step compound I reacts further with the second hydrogen peroxide molecule, which reduces it to enzyme:

$$HOOH + EO \rightarrow HOH + E + O_2$$

Most biochemical and physico-chemical studies of catalase were done with beef liver catalase [1]. Three-dimensional structure of beef liver catalase was investigated by r. Rossmann's group [2]. Less studied are catalases from other sources such as plants, fungi and bacteria.

We crystallized the catalases from the fungus *Penicillium vitale* [3] and two bacteria catalases *Thermus thermophilus* [4] and *Micrococcus lysodeikticus* [5].

Molecular weights of all catalases are in region 250 – 300 kDa. Table 1 shows molecular weights and subunit composition of these catalases.

All catalases are oligomeric enzymes and consist of several subunits. Molecules of *Penicillium vitale* catalase (PVC) and *Micrococcus lysodeikti-*

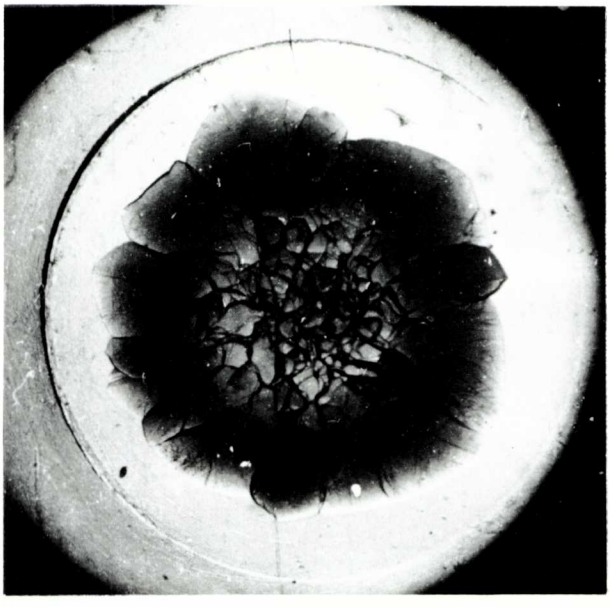

Fig. 1. Crystalline precipitate at the bottom of an ultracentrifuge tube.

Fig. 2. The single crystals from ultracentrifuge precipitate (PVC-I).

cus catalase (MLC) are tetramers and each subunit of the molecule contains one heme group with an iron atom in the active site.

Catalase from bacteria *Thermus thermophilus* (TTC) is non-heme catalase. It consists of six subunits and in the active site contains two manganese atoms.

Sources of isolation of these three catalases were the protein suspensions. After several stages of extraction, precipitation and purification by the biochemical methods, we have got the homogeneous solutions of catalases, which were suitable for crystallization.

Table 1. Molecular weights, subunit number of catalases.

Protein	Molecular weight	Number of subunits	Metal in active site
Catalase from *Penicillium vitale*	290,000	4	Fe
Catalase from *Thermus thermophilus*	210,000	6	2 Mn
Catalase from *Micrococcus lysodeikticus*	232,000	4	Fe

Table 2. Crystallization of *Penicillium vitale* Catalase (PVC), *Thermus thermophilus* Catalase (TTC) and *Micrococcus lysodeikticus* Catalase (MLC)

Protein	Concentration of protein mg/ml	pH	Buffer	Precipitant	Size of crystals	Growth time
PVC I (at 4°C)	0.15–0.20	5.5	0.1M Sodium-acetate	MPD 15-18%	1.0x1.5 mm	180 hours
PVC II	18.0–24.0	5.5	0.1M Sodium-acetate	$(NH_4)_2SO_4$ 1.4M	0.5-0.7 mm	4-7 days
TTC	25.0–30.0	6.0	0.05M Potassium phosphate	$(NH_4)_2SO_4$ 1.5M	1.0-1.5 mm	4-7 days
MLC	20.0–25.0	5.2	0.05M Sodium-acetate	$(NH_4)_2SO_4$ 1.2M	0.5x1.2 mm	4-7 days

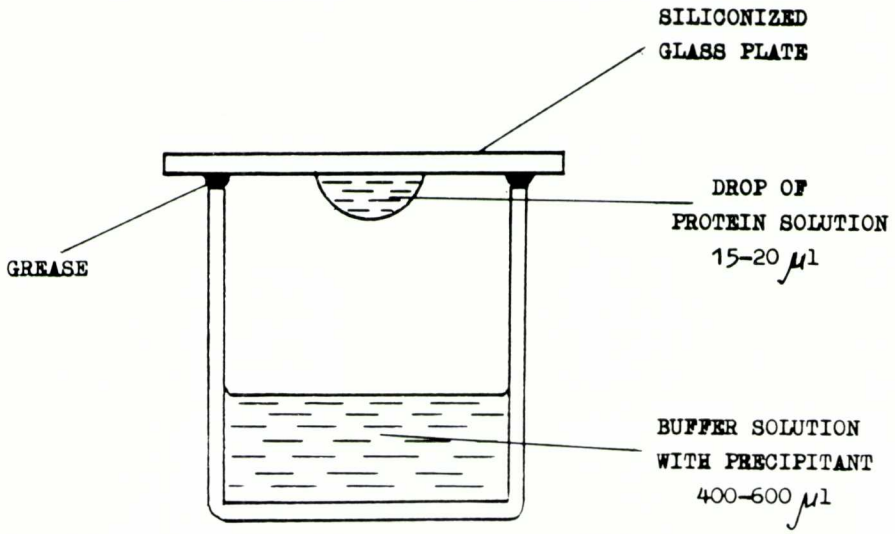

Fig. 3. Scheme of crystallization cell.

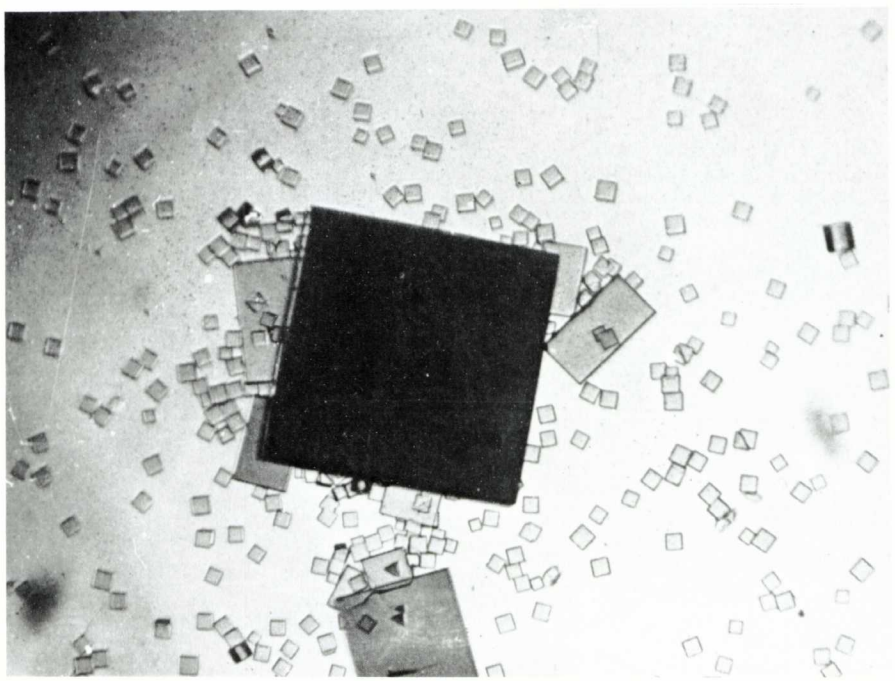

Fig. 4. Crystals of *Penicillium vitale* catalase (PVC-II).

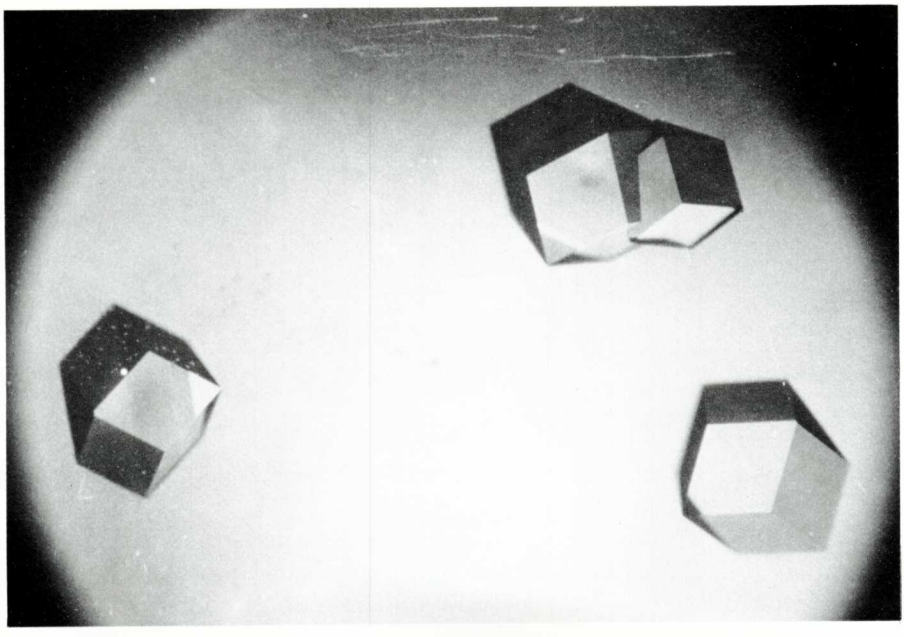

Fig. 5. Crystals of *Thermus thermophilus* catalase.

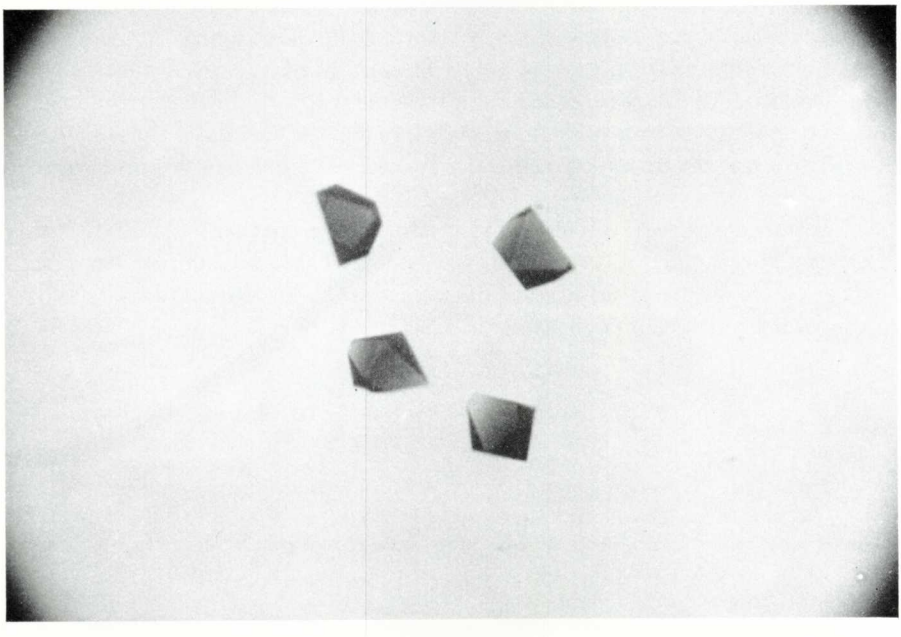

Fig. 6. Crystals of *Micrococcus lysodeikticus* catalase.

Table 3. Parameters of catalase crystals.

Catalase of	Mol. mass in kDa	Space group	Unit cell in Å	Mol. in asymm. unit	X-ray resol. in Å
Penicillium vitale	290 (4 x 72)	$P3_121$	a = 144.4 c = 133.8	1/2	2.0
Thermus thermophilus	210 (6 x 35)	$P2_13$	a = 133.4	1/3	3.0
Micrococcus lysodeikticus	232 (4 x 58)	$P4_22_12$	a = 106.7 c = 106.3	1/4	2.5

We used different methods for crystallization of the proteins. For crystallizatioin of PVC-I we used a rather unusual method, method of crystallization in an ultracentrifuge [6].

Test tubes with the protein solution were rotated in a Beckman ultracentrifuge at 15 000 rev./min for 180 hours, at 4°C. Crystals of PVC-I as can be seen from Table 2 were grown from protein solution, containing 15-18% 2-methyl-2,4-pentandiol in Sodium acetate buffer.

Crystalline precipitate of irregular shape crystals was at the bottom of the centrifuge tube (figure 1). Crystals reached a size of 1.5 mm and several single crystals were separated easily from precipitate (figure 2).

For crystallization of PVC-II, TTC and MLC we used a more common method of crystallization of proteins: vapour diffusion method at room temperature in a special cell (See figure 3). Crystals of these proteins were grown at conditions specified in Table 2. Crystals are shown in figures 4-6.

All catalase crystals had a good quality and were investigated by X-ray analysis. Parameters of the crystals are listed in Table 3. Quantity of crystals was good for X-ray investigation of three-dimensional structure of catalase molecules of different organisms [3, 5, 7].

ABSTRACT

Catalases from three microorganizms, the fungus *Penicillium vitale* and two bacteria *Thermus thermophilus* and *Micrococcus lysodeikticus* were crystallized for X-ray structure investigation. The suitable crystals were grown by ultracentrifuge technique and vapour diffusion hanging drop technique using 2-methyl-2,4-pentandiol and ammonium sulphate as the precipitants.

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