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Crystallization and preliminary crystallographic analysis of manganese superoxide dismutase from *Bacillus halodenitrificans*

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Abstract

Manganese superoxide dismutase (GP-MnSOD), a component of the so-called 'green protein' (green protein complex) from the facultative anaerobic halodenitrifier *Bacillus halodenitrificans*, has been crystallized using the hanging-drop vapor diffusion method. Crystals have unit-cell parameters $a = b = 93.4\,\text{Å}$, $c = 65.0\,\text{Å}$, and belong to the space group $P4_32_12$. Preliminary analysis indicates there is one monomer in each asymmetric unit. The structural information from this enzyme will enrich our knowledge on its high catalytic activity and its possible role in green protein complex.

Keywords: Superoxide dismutase; Green protein complex; *Bacillus halodenitrificans*; Anaerobic; Halophilic; Denitrification; Crystallization

Superoxide dismutase (SOD) (EC 1. 15. 1. 1) is an important metal enzyme of aerobic organisms for protection against toxic superoxide radical, a normal by-product of the aerobic metabolism, and also many key biological processes [1–5]. Superoxide dismutase can convert superoxide radical to oxygen and peroxide by disproportionation on a metal ion. At present, four forms of SOD with different catalytic metal ions have been identified: Cu, Zn-SOD, FeSOD, MnSOD, and NiSOD. The functional and structural aspects of Cu, Zn-SOD, FeSOD, and MnSOD have been extensively reviewed, while the structure of NiSOD is not yet available. FeSOD and MnSOD are closely related in sequence and three-dimensional structure, but are unrelated to Cu, Zn-SOD.

Iron- or manganese-dependent SODs exist as dimers or tetramers in solution with dimers as functional units. Their active sites display conserved metal-binding residues and active-site structures, including similar shells of aromatic residues that surround the active-site metal and its ligands. The iron or manganese ion is cyclically reduced and oxidized during successive encounters with the superoxide substrate. The kinetics of the process is dominated by diffusion of the negatively charged superoxide radical anion into the substrate channel and the active site. The diffusion rate is controlled by electrostatic potential attributed to basic residues lining the substrate channel at the dimer interface [6–8]. There is currently no research evidence showing that iron- or manganese-dependent SODs can exist stably and function properly as monomers.

Manganese superoxide dismutase (GP-MnSOD) purified from *Bacillus halodenitrificans* (ATCC 49067), a facultative anaerobic halodenitrifier that can tolerate high concentration of nitrite [9], shows some unusual features in this bacterium. Under anaerobic conditions, GP-MnSOD exists as a monomeric form in a so-called 'green protein' (referred to as green protein complex (GPC) in this paper), which has been considered to play an important role in nitrate/nitrite denitrification of the bacterium [10,11]. After exposure to air, monomers of GP-MnSOD dissociated from the GPC assemble into

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functional dimers that play important roles in protecting the cell against the toxicity of both superoxide radicals and peroxynitrites. Besides these characteristics, GP-MnSOD exhibits high catalytic activity in comparison with other MnSODs. Its specific activity, expressed as the amount of enzyme inhibiting by 50% the reduction of cytochrome *c*, is 5150 units/mg, in contrast to about 3500 units/mg for other MnSODs [9]. The structural study of GP-MnSOD will further our understanding of its properties. This paper reports the crystallization and preliminary crystallographic analysis of GP-MnSOD.

Materials and methods

Bacillus halodenitrificans was grown as previously described [10]. GP-MnSOD and GPC were each purified from *B. halodenitrificans* under aerobic and anaerobic conditions [9,10]. No SOD activity can be detected in the fresh GPC. However, after exposure to air, SOD activity can be assayed in its dissociated polypeptides.

Before crystallization, GP-MnSOD was transferred to 20 mM sodium cacodylate (pH 6.5) by ultrafiltration and diluted to a concentration of 6 mg/ml with the same buffer. Crystallization trials were performed using the hanging-drop vapor-diffusion method. Optimal crystal growth was obtained by combing 2 μl of protein solution and 2 μl of reservoir solution at 281 K. The reservoir solution contained 1 ml of 10% (w/v) polyethylene glycol 4000, 10 mM zinc acetate buffered with 0.1 M sodium cacodylate, pH 6.5. Tetragonal crystals were obtained after two weeks (Fig. 1).

A preliminary X-ray diffraction analysis of GP-MnSOD crystal was performed at room temperature on a Mar345 image plate at the National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences. Graphite-monochromatic CuKα radiation ($\lambda = 1.5418$ Å) was provided by a sealed tube generator operated at 40 kV and 50 mA. The intensity data were collected using one crystal of GP-MnSOD with dimensions of approximately $0.2 \times 0.2 \times 0.2\,mm^3$. The detector was placed at a distance of 120 mm from the crystal, and $200 \times 1°$ oscillation images were recorded with an exposure time of 4 min per image (Fig. 2). The data were processed using the programs *DENZO* and *SCALEPACK* [12].

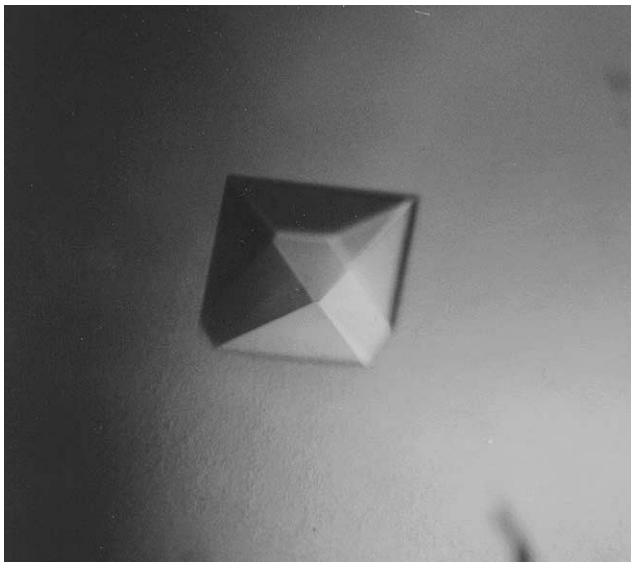

Fig. 1. A crystal of GP-MnSOD.

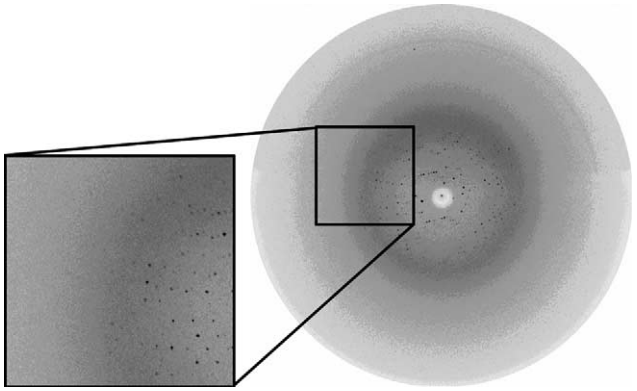

Fig. 2. A 1.0° oscillation frame of GP-MnSOD. Diffraction data reach a 3.2 Å resolution.

Results and discussion

Unlike known MnSODs [13–15], both crystallization and crystals of GP-MnSOD were very heat sensitive. No crystal was obtained when initial screening was performed using the sparse-matrix method [16] above 288 K. The appropriate crystallization temperature was finally found to be below 282 K. Crystals dissolved quickly in hanging drops at room temperature. The crystals were also easily damaged by X-ray radiation during data collection.

Data processing showed that the space group of GP-MnSOD belongs to either $P4_32_12$ or $P4_12_12$, with unit cell parameters $a = b = 93.4$ Å, $c = 65.0$ Å (Table 1). Given a Matthews coefficient [17] of 3.01 $Å^3\ Da^{-1}$ and a solvent content of 59%, the asymmetric unit contains one molecule, in contrast to multimeric forms of known MnSODs [13–15]. The rotation and translation searches verified the calculation with Matthews method and showed that the $P4_32_12$ space group was reasonable.

Table 1
Data-collection statistics

X-ray source	CuKα
Wavelength (Å)	1.5418
Resolution (Å)	10–3.2 (3.3–3.2)
Total observations	73544
Unique reflections	4892
Data completeness (%)	100 (100)
R_{merge}[a] (%)	15.3 (52.7)
Mean I/σ (I)	20.6 (5.8)
Space group	$P4_32_12$
Unit-cell parameters	$a = b = 93.4$ Å, $c = 65.0$ Å

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of *i*th measurement of the reflection h and $\langle I(h)\rangle$ is the mean value of the $I(h,i)$ for all i measurements.

The functional units of MnSODs are dimers [1,2]. There is also at least a functional dimer in an asymmetric unit of any known MnSOD crystal structure [13–15]. The preliminary structural analysis by molecular replacement method showed that monomers in a functional dimer of GP-MnSOD relate to each other by crystallographic 2-fold axes, so the crystal packing of GP-MnSOD is different from that of any known MnSODs and might be one of the elements which contribute to the heat sensitivity of GP-MnSOD crystals.

It is noteworthy that the 148th residue of GP-MnSOD is a hydrophobic isoleucine, while that of any other MnSOD is a hydrophilic asparagine according to their amino acid sequence alignment. In any known MnSOD structure, Asn148, which lies at the dimer interface and is exposed to the solvent region, contributes to the stabilization of the functional dimer [13–15]. However, owing to its hydrophobicity, Ile148 may have some disadvantageous thermodynamic influence on its local environment and destabilize the assembly of GP-MnSOD's functional dimer. This means that the functional dimer of GP-MnSOD may have a higher tendency to dissociate into monomers than that of any known MnSOD. Such an inclination towards dissociation into monomers may be of great importance to GP-MnSOD's monomeric existence in GPC [11]. This will be studied in future structural analysis.

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