

Three Dimensional Structure of native Di-Manganese Catalase from *Thermus thermophilus* at 1.05Å Resolution.

S.V. Antonyuk^{1,4}, A.N. Popov^{1,2}, V.S. Lamzin² and V.V. Barynin^{1,3}

¹ Institute of Crystallography, Russian Academy of Sciences, Leninskii pr. 59, Moscow, 117333 Russia

² EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, Hamburg, 22603 Germany

³ Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology,
University of Sheffield, Sheffield, S10 2TN UK

⁴ CRLC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England, UK

Di-manganese catalase is a metalloenzyme which protects living cells from the toxic metabolite hydrogen peroxide by catalysing its disproportion to dioxygen and water: $2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$. Structures of Mn containing catalases isolated from *Thermus thermophilus* and *Lactobacillus plantarum* have been solved [1,2]. Though both proteins cycle between (Mn^{2+} , Mn^{2+}) and (Mn^{3+} , Mn^{3+}) states in the catalytic mechanism [3], their structures show important differences in the active sites, leading to different ways of peroxide decomposition.

Preliminary X-ray [4] and EPR [5] studies of di-Mn catalase from *T. thermophilus* indicate that the enzyme contains 6 identical subunits of 33.3 kDa and each subunit contains two Mn ions in the active site, situated in a 4-helix bundle. Spectroscopic studies indicate that Mn ions exist in different binuclear redox states: (Mn^{2+} , Mn^{2+}), (Mn^{2+} , Mn^{3+}), (Mn^{3+} , Mn^{3+}) and (Mn^{3+} , Mn^{4+}). It was shown that the oxidation state (Mn^{2+} , Mn^{2+}) is reversibly inhibited by one electron anions such as Cl^- , N_3^- , NO_2^- , NO_3^- , F^- [5].

Diffraction data were collected at 100K at the EMBL Hamburg outstation.

Table 1. Data collection and refinement statistics

Diffraction limit (Å)	1.05	R factor (free) (%)	9.8 (11.0)
Number of reflections	354906	Number of atoms (protein)	5864(4954)
Completeness (%)	99.9	Number of water molecules	880
Completeness outer shell (%)	98.9	Number of duple side chain conformations	27
R-merge (%)	7.0	RMS bonds length (Å)	0.014
I / σ (outer shell)	2.5	B factor for main chain (Å ²)	9.7

The initial model was that of the native protein (1.6 Å resolution, data collected at room temperature) [6]. Subsequently, the data was improved to 1.05 Å resolution. The structure was refined by the programs REFMAC and SHELX [2].

Refinement at atomic resolution gives precise geometry at the active site which is necessary for understanding the details of the reaction mechanism of the *T. thermophilus* catalase (fig.1, table 2). Three water molecules are ligated to one of the Mn ions, two of them are coordinated to the second Mn. Analysis of the electron density distributions in the active centre of the native form of the enzyme revealed that the most flexible side chains of the amino acid residues Lys162 and Glu36 exist in two interrelated conformations each. This allowed us to obtain structural evidence which is necessary for an understanding of the mechanism of enzymatic activity of di-Mn catalase.

Table 2. Ligand distances at the active site from atomic resolution structure (Å).

Conformation	I	II	Conformation	I	II
Mn1Mn2	3.13				
Mn1 coordination	6 (5)	6	Mn2 coordination	5	
Mn1Glu36 OE1	2.10(2.13)	2.05(2.03)	Mn2Glu70 OE2	2.12(2.13)	
Mn1Glu36 OE2	2.35(2.89)	3.39(3.36)	Mn2His188 ND1	2.26(2.25)	
Mn1Glu70 OE2	2.12		Mn2Glu155 OE1	2.17(2.17)	
Mn1His73 ND1	2.22(2.20)		Mn2Glu155 OE2	2.64(2.63)	
Mn1W1	2.03(2.06)		Mn2W1	2.12(2.13)	
Mn1W2	2.07(2.07)		Mn2W2	2.18(2.21)	
Mn1W3	2.27(2.29)				

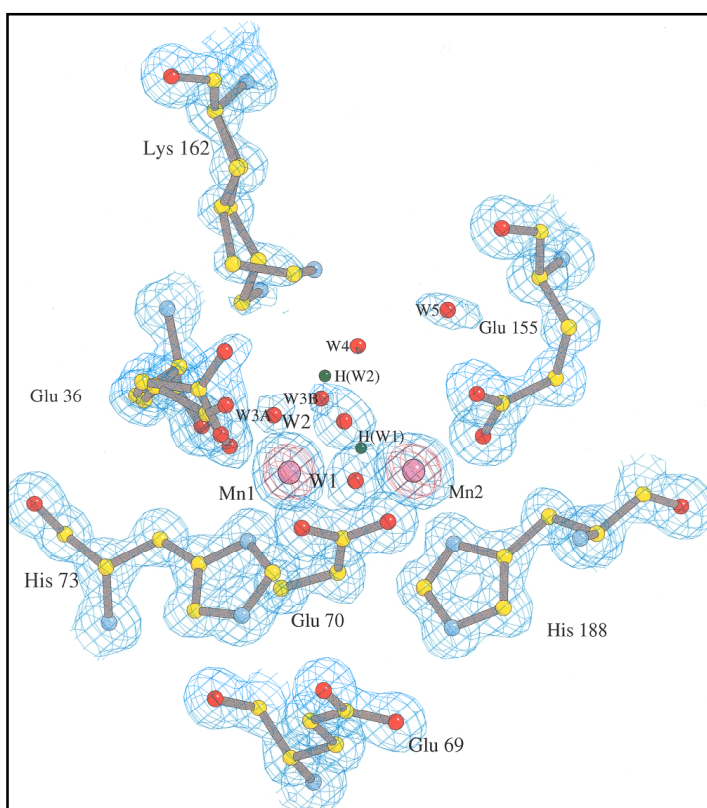


Figure 1: The electron density distributions in the active centers of the native enzyme is shown.

References

- [1] V.V.Barynin, M.M.Wittaker, S.V.Antonyuk, V.S.Lamzin, P.M.Harrison, P.J.Artymiuk and J.W.Wittaker, *Structure* 9(8), 725-738 (2001)
- [2] S.V. Antonyuk, W.R. Melik-Adamyan, A.N. Popov, V.S. Lamzin, P.D. Hepstead, P.M. Harrison, P.J. Artymiuk and V.V. Barynin, *Crystallografia reports*, **45**(1), 111-122 (2000)
- [3] S.V. Khangulov, V.V. Barynin, S.V. Antonyuk-Barynina, *Biochim. Biophys. Acta*, **1020**, 25-33 (1990).
- [4] V.V. Barynin, A.A. Vagin, W.R. Melik-Adamyan, A.I. Grebenko, S.V. Khangulov, A.N. Popov, M.E. Andrianova & B.K. Vainshtein *Dokl. Acad. Nauk. SSSR*. **288**, 877-880 (1986).
- [5] S.V. Khangulov, V.V. Barynin, N.V. Voevodskaya and A.I. Grebenko, *Biochim. Biophys. Acta*, **1020**, 305-310 (1990).
- [6] V.V. Barynin, P.D. Hempstead, A.A. Vagin, S.V. Antonyuk, W.R. Melik-Adamyan, V.S. Lamzin, P.M. Harrison & P.J. Artymiuk, *J. Inorg. Biochem.* **67**, 196 (1997).