Three Dimensional Structure of Chloride Inhibited Di-Manganese Catalase from *Thermus thermophilus* at 0.98Å Resolution.

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Di-manganese catalase is a metalloenzyme which protects living cells from the toxic metabolite hydrogen peroxide by catalysing its disproportion to dioxygen and water: $2H_2O_2 \bullet O_2 + 2H_2O$. Manganese containing catalases have been isolated from *Thermus thermophilus*, *Lactobacillus plantarum* and *Thermoleophilum album*. It is thought that di-Mn catalase cycles between (Mn^{2+}, Mn^{2+}) and (Mn^{3+}, Mn^{3+}) states in the catalytic mechanism [1].

Preliminary X-ray [2] and EPR [3] studies of di-Mn catalase from *Thermus 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. The six subunits assemble into a hexamer with 32 symmetry that can be described as a distorted hexagonal prism with dimensions 69Å in the direction of the molecular 3-fold axis and cross section diameter 95Å. 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^- [3].

The enzyme was crystallised in the cubic group P213 using 1.5 M ammonium sulphate from a precipitant at pH 5.5 by hanging drop evaporation. The chloride form was obtained by soaking the reduced (Mn^{2+}, Mn^{2+}) form of crystals in 0.1 M NaCl solution in 1.7 M ammonium sulphate at pH 5.5.

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

Table 1. Data collection statistics

Diffraction limit (Å)	0.98
Number of reflections	413309
Completeness (%)	98.5
Completeness outershell (%)	98.8
R-merge (%)	3.6
I /σ (outer shell)	3.5

The initial model used was that of native di-Mn catalase (1.05Å resolution, R-factor 9.8%). The structure was refined by the program REFMAC. For the final refinement the anisotropic version of the program was used.

The atomic model of the molecule was refined to an R factor of 10% (R_{free} of 11.2%). The structure of di-Mn catalase has been briefly described earlier [2,4]. The subunit structure consists of 14

helices, 2 two strand sheets and one strand which comprises an antiparallel sheet with the same strand of a neighbouring subunit. 42.7% of amino acids have α-helical conformation, 8.8% 3/10 helical conformation and only 4.3% sheet conformation [5].

Two chloride ions are situated between the two Mn ions. In a refinement test, different occupancies for the chloride atoms were applied to the model and subsequently Fo-Fc maps and the temperature B-factor of the chloride were monitored for residual density. From these tests we conclude that the occupancy for the first chloride ion (Cl1) is about 90% and for the second chloride ion (Cl2) 85% (Fig.1). Cl2 has a nearby secondary position occupied by water molecule W1 with an occupancy 15%. Analysis of the electron density distributions in the active centre of the inhibited 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.

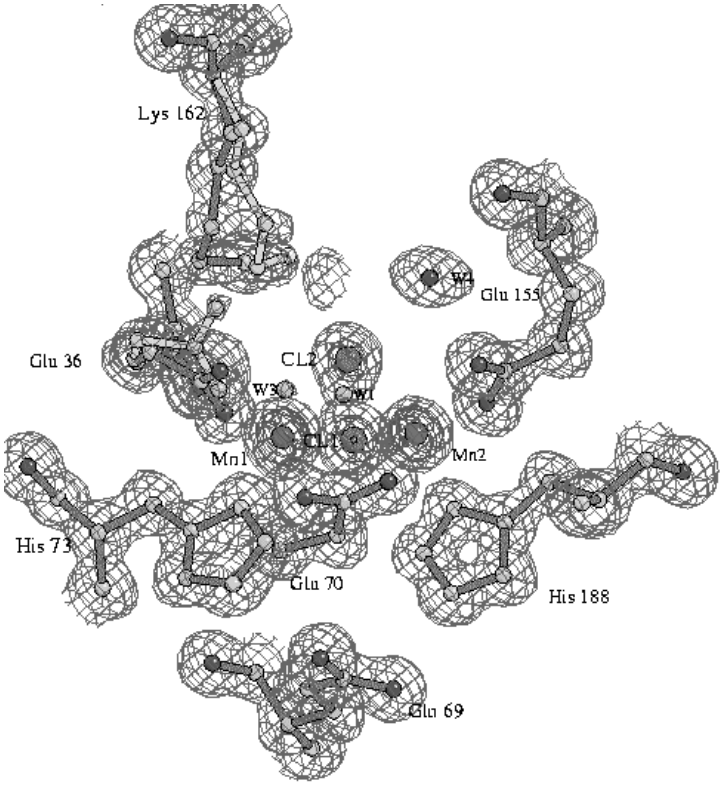

Figure 1: The electron density distributions in the active centers of the native enzyme and its complex with chloride ions are shown.

References

[1] S.V. Khangulov, V.V. Barynin, S.V. Antonyuk-Barynina, Biochim. Biophys. Acta, **1020**, 25-33 (1990).

[2] 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).

[3] S.V. Khangulov, V.V. Barynin, N.V. Voevodskaya and A.I. Grebenko, Biochim. Biophys. Acta, **1020**, 305-310 (1990).

[4] 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).

[5] S.V. Antonyuk, W.R. Melik-Adamyan, A.N. Popov, V.S. Lamzin, P.D. Hepstread, P.M. Harrison, P.J. Artymiuk and V.V. Barynin, Crystallografia. (Russian, in press) (2000)