CRYSTAL STRUCTURE OF DI-MANGANESE CATALASE FROM *LACTOBACILLUS PLANTARUM.*

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Catalases carry out disproportionation of hydrogen peroxide, forming oxygen and water. Two distinct families of catalases are known: they are the haem-containing catalases and the manganese containing catalases. The manganese containing catalases do not contain haem and their sequences and structures are quite different. Structures of di-manganese catalase from the extreme thermophile *Thermus thermophilus* (Fig.1) have been previosly refined at 1.6 Å [1,2] and 1Å [3] resolution.

The three-dimensional structure of the mesophile *L. plantarum* catalase is currently being investigated. Monoclinic crystals of space group $P2_1$ with a=75.5 b=97.6 c=108.0 Å, α=90.0 β=107.2 γ=90.0 were obtained using PEG 8000 as precipitant. Tests indicated that the crystals are capable of diffraction to 1.2 Å resolution at room temperature. The first data set at 293K to 1.84 Å resolution was collected on a MAR Research imaging plate at beamline X11 at the EMBL-Hamburg outstation (DESY). A self-rotation function revealed the strong 32 NCS symmetry of molecule. The *L. plantarum* catalase structure was solved by molecular replacement. The initial model used was that of a core of the *T.thermophilus* manganese catalase hexamer structure (32% identity) [1]. The structure of the *L. plantarum* catalase has been refined to an R-factor of 14.8% (free R of 20.3%) . The structure has confirmed the homohexameric nature of this enzyme with a novel arrangement of di-Mn catalase domains in the monomer (Fig.2). The first coordination sphere of the di-Mn cluster in the active site is composed of three Glu and two His ligands. Only the His ligands (His-69, His-181) and a (Mn,Mn) bridging Glu-66 have the same geometry as the equivalent *T.thermophilus* manganese catalase residues His-73, His-188 and Glu-70, respectively. The positions of the Mn-monodentate ligands Glu-35 and Glu-148 of the active site are different. There are also three solvent ligands to the Mn's. Both the Mn ions of the *L. plantarum* catalase active site are six-coordinate. Two new residues Arg-147 and Glu-178 were found in the active site. Comparison of the *L. plantarum* and *T.thermophilus* catalase structures has revealed that there are two distinct but related classes of catalase in the manganese catalase family with different designs of the hexamers and the active sites.

Two atomic resolution data sets of *L. plantarum* catalase from two different oxidation states of Mn were also collected at 100K in May 2000 (table 1) at the EMBL beamline BW7B (EMBL/DESY, Hamburg).

Table 1. Data processing statistics

Crystal	Resolution (Å)	Unique reflections	Rmerge	Completeness (%)	I/σ	Multiplicity
1	17 - 1.84	113270	5.5	90.4	16.1	1.4
2	35 - 1.14	500409	4.6	99.1	16.5	3.5
3.	35 - 1.10	505014	7.7	92.2	9.0	2.2

Refinement of the L.plantarum atomic resolution structure is in progress.

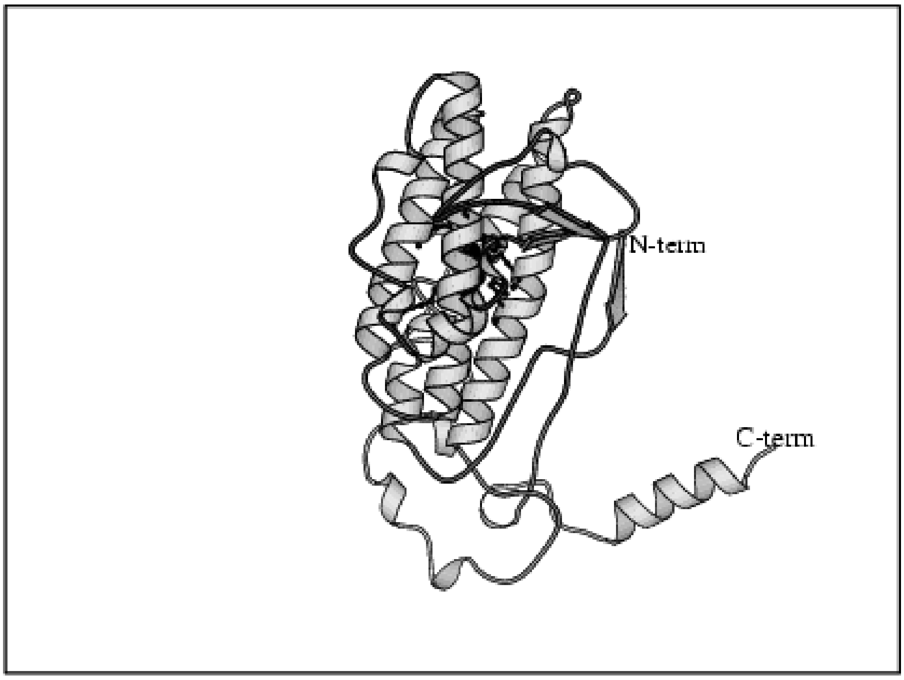

Figure1: Schematic drawing of the subunit of catalase from *T.thermophilus* [2]

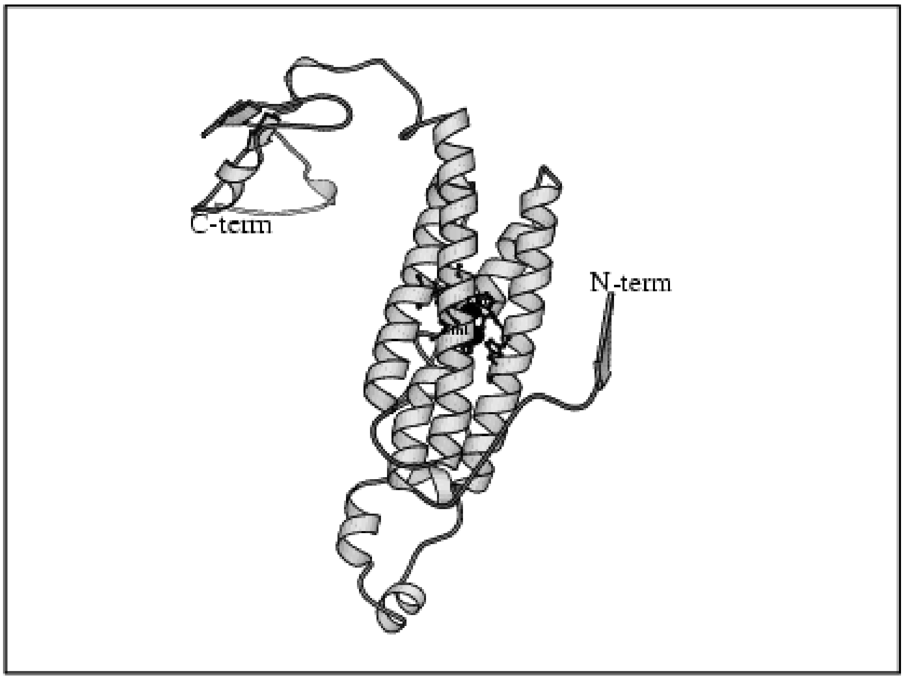

Figure2: Schematic drawing of the subunit of catalase from *L.plantarum*

References

[1] 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.Inorganic Biochem. 67, 196 (1997)
[2] V.V.Barynin, P.D.Hempstead, A.A.Vagin, S.V.Antonyuk, W.R.Melik-Adamyan, V.S.Lamzin, & P.M.HarrisonP.J.Artymiuk, EMBL Hamburg Outstation Annual Report 1998 p.283. (1998)
[3] S.V.Antonyuk, W.R.Melik-Adamyan, , A.N.Popov , V.S.Lamzin, P.D.Hempstead, P.M.Harrison, P.J.Artymiuk and V.V.Barynin, Crystallography Reports 45, 105 (2000)