03-Crystallography of Biological Macromolecules

MS-03.06.08 STRUCTURE OF GLYCOSYLATED LIGNIN PEROXIDASE. By Klaus Piontek*, Tuomo Glumoff§ and Kaspar Winterhalter, Department of Biochemistry I, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland.
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Lignin peroxidase (ligninase, LiP) is a 40 kD, heme-containing glycoprotein produced by wood-rotting fungi like *Phanerochaete chrysosporium*. It takes part in the complex reaction pathway, which leads from lignin (structural component of woody plants) to carbon dioxide and water. Ligninase is produced only in rather low amounts and expression system to produce larger quantities remain awaited. However, the enzyme is well characterized biochemically and despite of the problems in getting crystallizable protein we recently solved the crystal structure of LiP415 (Klaus Piontek, FEBS-Lett., 1993, 315, 119-124), the isozyme with an isoelectric point of 4.15. LIP415 is a single polypeptide chain of 343 amino acids, one heme molecule, two N-acetylglucosamines with 16 mannose residues bound to it and possibly at least one O-glycosylation site. Single crystals ($P2_12_12_1$, a=61.3 Å, b=74.9 Å, c=106.4 Å, 1 molecule/a.u.) were obtained with $(NH4)2SO4$ as precipitant and diffract to at least 1.8 Å resolution. Data sets of the native and three derivative crystals have been collected with a Xentronics X100A area detector to a resolution of 2.0 Å for the native crystals. The quality of a MIR-map (FOM=0.71) calculated with data up to 3.0 Å resolution could be improved by solvent flattening (FOM=0.87). That maps allowed us to assign about 86 % of the amino acids to the electron density. Refinement with simulated annealing through molecular dynamics (XPLOR) and further model building resulted in an R=20.2 % for data of 2.5 to 6.0 Å resolution. Refinement including data up to 2.0 Å is in progress and data collection to higher resolution is planned. The current model comprises all amino acids of the polypeptide chain, one heme molecule, and 127 water molecules. The chain fold is in general similar to those of cytochrome c peroxidase (CcP). The C_α-atoms of residues 15 to 275 of CcP can be superimposed on the LIP415 structure with an r.m.s of 1.8 Å. Despite binding of the heme to the same region and a similar arrangement of the proximal and distal histidine as in CcP a significantly larger distance of the iron ion to the proximal histidine is observed. The weaker ligation of the heme iron with the proximal histidine in LIP415 might cause the heme being more electron deficient than in CcP. Discrete electron density extending from ND2 of asparagine 257 and from the hydroxyl group of serine 334 indicate ordered sugar residues at this N- respectively O-glycosylation site. In addition ordered electron density in the active site channel is observed which has been interpreted as carbohydrate molecules. Details of the structure determination, the refinement, the newest structural model, and the possible role of the carbohydrate chain will be presented.

PS-03.06.09 CRYSTALLIZATION AND CRYSTAL DATA OF A NEW D-MANNOSE-BINDING LECTIN (KM+) FROM *Artocarpus integrifolia*. By P.S. L. Oliveira, L.M. Beltramini, R.A. Silva-Lucca, R.C. Garratt, G. Oliva,M. C. Roque-Barreira+, and Y.P. Mascarenhas*, Dept. de Fisica,Universidade de São Paulo, CP 369, 13560-970 São Carlos, S. P. Brazil.+ Depto. de Microbiologia, Imunologia e Parasitologia , Fac. de Medicina,Universidade de São Paulo, 14049-900, Ribeirão Preto, S.P. Brazil.

In the present work we report the crystallization and crystal data determination of KM+. This lectin induces migration of neutrophils, proliferation of Balb/c mouse spleen cells and hemeagglutination of AB or O blood red cells. KM+ is a tetrameric protein, MW= 54000 daltons with four identical non-covalently bonded subunits of 13,500 daltons each. Preliminary solubility studies revealed that this protein precipitates reversibly from ammonium sulphate and phosphate solutions. The crystallization experiments were carried out at 18°C using the vapour diffusion technique (hanging drop). Crystals,grown as clusters of triangular plates up to a final size of 0.3x0.2x0.1mm, were obtained by equilibrating solutions containing 5.0 mg/ml of protein in 25% ammonium sulphate in 0.1 M TRIS pH=8.0 against 50% ammonium sulphate in 0.1 M TRIS at the same pH. Crystal data are: monoclinic system, $P2_1$, a=54.31, b=99.79, c=69.34 Å, ß=112.9° measured using a R-AXIS II (Rigaku Automated X-ray Imaging System) and the data processing software provided by Rigaku Corporation.

Acknowledgements are due to FAPESP,CAPES,FINEP AND CNPq.

PS-03.06.10
BINDING STUDIES OF SELECTED SUGARS AND OTHER ACTIVE SITE ANALOGS IN CRYSTALLINE D-XYLOSE ISOMERASE. H.L. Carrell*, J.P. Glusker*, H. Hoier†, C.A. Batt§, A. Karplus§, R. Whitaker§, Yunje Cho§, and Jaeho Cha§. *The Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia, PA 19111, USA; †Institut fur Organische Chemie, University of Stuttgart, Federal Republic of Germany; §Cornell University, Ithaca, NY 14853 USA.

D-xylose isomerase (glucose isomerase) catalyzes the conversion of D-xylose to D-xylulose (D-glucose to D-fructose) by the transfer of a single proton from C_2 to C_1. It is known that the divalent metal ions (Mg^{++}, Mn^{++}, Co^{++}) are required for activity. The mechanism of action was first proposed to be the base transfer of a proton via ene-diol intermediate. More recently, a hydride transfer mechanism has become the popular explanation for the enzymic activity.

In an effort to more fully understand the binding properties and activity shown by this enzyme, a variety of sugar molecules and active site analogs, including inhibitors, have been studied in both wild type enzyme and in mutant enzyme. The crystal structures investigated have been determined at resolutions between 1.9 to 1.6 Å. Studies have been carried out as a function of pH and metal content. The current results help to clarify the requirements at the active site of D-xylose isomerase.

Work supported by grant CA-10925 from the National Institutes of Health.

