MS01.P29

Acta Cryst. (2011) A**67,** C225

Crystallographic analysis at atomic resolution of the N-terminal lectin module of the LBL protein from *Laccaria bicolor*

Iván Acebrón,[a] Blanca de las Rivas,[b] Rosario Muñoz,[b] José Miguel Mancheño,[a] [a]*Department of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid (Spain).* [b]*Group of Bacterial Biotechnology, Instituto de Ciencia y Tecnología de la Alimentación y Nutrición, CSIC, Madrid (Spain).* E-mail: xivan@iqfr.csic.es

Currently, production of recombinant soluble protein material suitable for structural analyses represents a bottleneck in Structural Biology. Statistics from structural genomics initiatives reveal that more than 80% of the cloned targets cannot be produced in a soluble form (http://targetdb.sbkb.org). With the aim to develop generic protocols aimed at producing soluble recombinant proteins in E. coli with high yield, we have demonstrated that a beta-trefoil lectin from *Laetiporus sulphurous* exhibits properties typical of solubility enhancers and can be used as fusion tag for the efficient production and purification of recombinant proteins. Now, we have found and characterized at atomic resolution a novel beta-trefoil from *Laccaria bicolor* which behaves as an excellent affinity tag.

Recently, we identified the gene 318163 (gene lacc) from the reported sequence of the *L. bicolor* genome [1], that encodes for a protein whose N-terminal region (152 amino acids) presents high sequence identity with the lectin module of the protein LSLa from the fungus *L. sulphureus* that we have already used as a fusion tag for recombinant protein production (Patent: WO 2009/121994 (CSIC) 8.10.2009). We have cloned the complete gene and the region coding for the first 152 amino acids from a cDNA library kindly provided by Dr. F. Martin. Both proteins have been over-expressed and purified in a just a single affinity chromatography step on Sepharose® 4B what reveals that this gene encodes a functional lectin that we have called LBL. Even though it was not possible to produce soluble LBL in *E. coli*, the lectin module (LBL_{152}) has been produced with a high yield (100 mg per liter of culture).

Crystals of LBL_{152} has been prepared in presence of 0.2 M lactose. The crystals grew quickly with a high quality in several different conditions. Best crystals were obtained in 20% (w/v) PEG 6000, 0.2 M ammonium chloride, 0.1 M sodium acetate pH 5.0. Complete diffraction data at 1 Å resolution were collected at 100 K at beamline ID23-2 of the ESRF (Grenoble, France). The subsequent analysis shows that crystals belong to the space group P212121, with a = 52.53 Å, b = 61.27 Å and c = 44.79 Å which indicates that there is only one molecule in the asymmetric unit. The structure has been solved with the molecular replacement method, using the structure of LSL_{150} (N-terminal lectin module of the protein LSLa) [2] as model. As expected, LBL_{152} is a lectin with a beta-trefoil fold.

[1] F. Martin, *et al. Nature* **2008**, *452*, 88-93. [2] J.M. Mancheño, *et al. J Biol Chem* **2005**, *280(17),* 17251-17259.

Keywords: lectin, purification, biocrystallography

MS01.P30

Acta Cryst. (2011) A**67,** C225

Time-resolved analysis of catalytic reaction of copper amine oxidase from *Arthrobacter globiformis.*

Hiroshi Yamaguchi,[a] Misumi Kataoka,[a] Hiroko Oya,[a] Ayuko Tominaga,[a] Masayuki Ohtsu,[a] Toshihide Okajima,[b] Katsuyuki Tanizawa,[b] [a]*School of Science and Technology, Kwansei Gakuin University,* [b]*Institute of Scientific and Industrial Research, Osaka University, (Japan).* E-mail: hiroshi@kwansei.ac.jp

Copper amine oxidases catalyze oxidative deamination of various primary amines to the corresponding aldehydes with concomitant production of ammonia and hydrogen peroxide. The enzyme contains a quinone cofactor, 2,4,5-trihydroxyphenylalanine quinone (topaquinone or TPQ), produced by post-translational modification of the conserved precursor tyrosine residue in the presence of Cu^{2+} ion and O_2. In a previous study, three intermediary structures formed during the TPQ formation have been determined and the detailed reaction mechanism of TPQ biogenesis has been proposed on the basis of their structures [1]. On the other hand, the catalytic reaction of amine oxidase proceeds by a Ping-Pong bi-ter mechanism, consisting of reductive and oxidative half-reactions [2]. In the former reductive half-reaction, a substrate Schiff-base is formed between an amine substrate and the cofactor in the initial oxidized form (TPQ_{ox}), which is finally converted to a semiquinone radical (TPQ_{sq}), yielding an aldehyde product.

To elucidate the structure-based reaction mechanism of copper amine oxidase, the reductive half-reaction catalyzed by the recombinant enzyme from *Arthrobacter globiformis* (AGAO) was analyzed by time-resolved X-ray crystallography. The AGAO crystals were grown anaerobically in a nitrogen-filled globe box. The crystals were soaked in a solution containing its substrate, phenylethylamine, also anaerobically. The crystals were immersed into liquid nitrogen at appropriate time intervals to freeze-trap the reaction intermediates transiently formed in the crystals. Before X-ray data collection, the crystals were subjected to single-crystal microspectrometry for monitoring the absorption spectrum of TPQ that reflects its chemical structure. Diffraction data were collected at 100 K in the BL38B1 and BL44B2 at SPring-8, Japan. Diffraction images were processed by using *HKL2000* and structure refinements were performed by using the program *Refmac5*. Crystal structures of four distinct intermediates formed during the reductive half-reaction have been determined at atomic resolution. Concerted conformational changes of TPQ, several active-site residues located in the substrate-binding pocket and gate residues at the entrance of the substrate channel are observed with the progress of reductive half-reaction.

[1] M. Kim *et al., Nature Struct. Biol.* **2002,** *9,* 591-596. [2] Y.-Ch.Chiu *et al., Biochemistry*. **2006,** *45,* 4105-4120.

Keywords: enzyme, catalytic_reaction, structure

MS01.P31

Acta Cryst. (2011) A**67,** C225-C226

The influence of nickel ions on the photoswitching of the GFP-like protein PDM1-4

Luc Van Meervelt, Ngan Nguyen Bich, Kristof Van Hecke, *Chemistry Department, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Leuven (Belgium).* E-mail: luc.vanmeervelt@chem.kuleuven.be

PDM1-4, a reversible photoswitchable fluorescent protein, has been generated by random mutation on Dronpa in order to be more effective in far-field fluorescent nanoscopy techniques. Compared to Dronpa, PDM1-4 exhibits slower switching properties, increasing the resolution in the photoactivated localization microscopy (PALM) technique [1]. We now have determined the crystal structure of both the *on*- and *off*-state of PDM1-4.

Plate-like crystals of PDM1-4 were obtained by the hanging- drop vapor-diffusion method. The *on*-state crystals strongly exhibit green fluorescent emission under ultraviolet light. The *off*-state crystals were