STUDENTSKÁ PŘEHLÍDKA

S1

STRUCTURAL AND FUNCTIONAL STUDY OF BI-FUNCTIONAL ANHYDROLASE

Andrea Štěpánková[1, 2], Tereza Skálová[1], Jarmila Dušková[1], Tomáš Koval'[3], Jindřich Hašek[1], L. H. Østergaard[4], Jan Dohnálek[1]

[1]*Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovského nám. 2, 162 06, Prague 6, Czech Rep.*
[2]*Dept. of Solid State Physics, FNSPE, CTU, Trojanova 13, 120 00, Prague 2, Czech Republic*
[3]*Institute of Physic AS CR, Cukrovarnická 10, P162 00, Prague 6, Czech Republic*
[4] *Novozymes A/S, Brudelysvej 26, DK-2880 Bagsvaerd, Denmark*
stepanko@imc.cas.cz

The bacterial enzyme Organophosphorus acid anhydrolase (OPAA) is able to catalyze the hydrolysis of both proline dipeptides (Xaa-Pro) and several types of organophosphate (OP) compounds. The OP compounds are highly toxic they are commonly used as pesticides and unfortunately also as nerve agents. Enzymatic biodegradation is safer and more economical than detoxification via purely chemical means or incineration, so recombinant OPAA can be applied as an enzymatic tool for detoxification of widespread pesticide waste and for deactivation of chemical warfare agents.

Three X-ray structures of recombinant wild type enzyme OPAA from a marine bacterium *Alteromonas macleodii* are presented here. The data were collected at the beam line BL14.1 of the source of synchrotron radiation Bessy II (Helmholtz-Zentrum, Berlin). In two cases, the crystals belong to space group *C*2 with unit cell parameters a = 134.3 Å, b = 49.1 Å, c = 97.2 Å and = 125.0°. Data were collected up to resolutions 1.8 Å and 1.9 Å, respectively. In other case, the crystal belongs to space group $P2_12_12_1$ with unit cell parameters a = 75.6 Å, b = 111.2 Å, c = 138.1 Å and data were collected to the resolution 2.2 Å.

The data were processed using *HKL*2000 and the structures were refined by Refmac5. All the 442 amino acids of the recombinant protein were located in electron density. The protein fold is mainly -helical. The binuclear metal center is located within the pita bread domain in the active site. Manganese ions, which are required for protein activity, were observed in full occupancy in the active sites. The enzyme form dimers. Existence of dimers was confirmed both in crystal and also in solution by dynamic light-scattering. The enzyme shares the so-called "pita bread" fold of the C-terminal domain with other enzymes with prolidase activity.

1. Vyas, N. K. *et al.,* (2010). Structural insights into the dual activities of the nerve agent degrading organophosphate anhydrolase/prolidase. *Biochemistry*, **49**, 547-559.
2. Raushel, F. M. (2002). Bacterial detoxification of organophosphate nerve agents. *Current opinion in Microbiology*, **5**, 288-295.

This work was supported by the Czech Science Foundation project 305/07/1073. The authors wish to thank Dr. Uwe Müller of the Helmholtz-Zentrum Berlin, Albert-Einstein-Str. 15 for support at the beam line BL14.1 of Bessy II.

S2

CRYSTALLIZATION AND X-RAY STRUCTURAL ANALYSIS OF BIFUNCTIONAL NUCLEASE TBN1

T. Koval[1], P. Lipovova[3], T. Podzimek[3,4], J. Matousek[4], J. Duskova[2], T. Skalova[2], A. Stepankova[2], J. Hasek[2] and J. Dohnalek[1,2]

[1]*Inst.of Physics, Acad. Sci. of the Czech Republic,v.v.i., Na Slovance 2,182 21 Praha 8, Czech Rep.,*
[2]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic,v.v.i., Heyrovskeho nam. 2, 162 06 Praha 6, Czech Republic*
[3]*Institute of Chemical Technology, Technicka 5, 166 28 Praha 6, Czech Republic*
[4]*Institute of Plant Molecular Biology, Biology Centre, Academy of Sciences of the Czech Republic,v.v.i., Branisovska 31, 370 05 Ceske Budejovice, Czech Republic*
koval.tomas@gmail.com

Bifunctional nuclease TBN1 (UniProt sequence accession no. AM238701) from *Solanum lycopersicum* is a Zn^{2+}- dependent plant glycoprotein composed of 277 amino acids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated). TBN1 belongs to plant nuclease I group. Nuclease I proteins are Zn^{2+}, Mg^{2+} or Ca^{2+} dependent and capable of cleaving both RNA and DNA in single and double stranded forms with a preference for bonds adjacent to adenine. They produce 5'-mononucleotides as end products at pH range 5.0 - 6.5. TBN1 also plays a considerable

role in specific apoptotic functions, vascular system development, stress response and tissue differentiation in plants [1]. In addition, TBN1 exhibits anticancerogenic properties [2]. Therefore, a detailed structural study of this enzyme can contribute to development of new drugs for cancer, bacterial and viral disease treatment. Nuclease P1 from *Penicillium citrinum* with 24% sequence identity, the structure of which is known (PDB ID 1ak0) [3], is probably the closest structural homologue of TBN1.

Recombinant tomato nuclease R-TBN1 was produced by heterologous expression in *Nicotiana benthamiana* (tobacco) leafs and purified to homogeneity [2]. Crystals with sufficient quality for X-ray diffraction analysis was obtain after optimization from initial screening using vapor diffusion crystallization method and combination of salt and polymer in the crystallization conditions. The first diffraction experiments were performed using an in house Gemini Enhanced Ultra diffractometer with the Atlas CCD detector (Oxford Diffraction) and three different crystal morphologies were identified (orthorhombic, rhombohedral and trigonal). Datasets for structural analysis were collected at the synchrotron radiation source BESSY II (Helmholtz-Zentrum Berlin), beam line MX-14-1, with a MARmosaic CCD detector. Presence of zinc in the protein was confirmed by X-ray fluorescence and an absorption edge scan and two MAD datasets (for a rhombohedral and a trigonal crystal) were collected [4]. The phase problem was solved using the SHELXC, D and E program suite [5]. The TBN1 structure resembles some features of P1 nuclease with differences near the active site and in the glycosylation pattern.

We gratefully acknowledge support from Praemium Academiae of AS CR, Institution research plan AVOZ 10100521 of the Institute of Physics, Institution research plan AV0Z50510513 of the Institute of Plant Molecular Biology, Biology Centre. The authors wish to thank Dr. U. Müller of the Helmholtz-Zentrum Berlin, Albert-Einstein-Str. 15 for support at the beam line.

1. J. Matousek, P. Kozlova, L. Orctova, A. Schmitz, K. Pesina, O. Bannach, N. Diermann, G. Steger, D. Riesner, *Biol. CHem*., **388**, (2007), 1–13.
2. J. Matousek, T. Podzimek, P. Pouckova, J. Stehlik, J. Skvor, P. Lipovova, J. Matousek, *Neoplasma,* **57**, (2010), 339-348.
3. C. Romier, R. Dominguez, A. Lahm, O. Dahl, D. Suck, *Proteins,* **32**, (1998), 414–424.
4. T.Koval, P. Lipovova, T. Podzimek, J. Matousek, J. Duskova, T. Skalova, A. Stepankova, J. Hasek, J. Dohnalek, *Acta. Cryst.,* **F67,** (2011), 124-128.
5 G. M. Sheldrick, Acta Cryst., **A64**, (2008), 112-122.

S3

STRUCTURAL ANALYSIS OF DELTA-SUBUNIT OF RNA-POLYMERASE BY X-RAY

Demo G.[1], Papoušková V.[1], Šanderová H.[2,3], Wimmerová M.[1]

[1]National Center for Biomolecular Research, Masaryk University, Brno, Czech Republic
[2]Laboratory of Molecular Genetics of Bacteria, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[3] Department of Bacteriology, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic, guliver@mail.muni.cz

RNA polymerase (RNAP) is an essential enzyme that is responsible for transcription of DNA into RNA. It is a multisubunit enzyme and its composition is well conserved throughout all bacterial species. Gram-positive bacteria in comparison to gram-negative bacteria contain two additional subunits that associate with RNAP: [1] which is the subject of this work, and $_1$ [2]. The recombinant form of ä subunit from *Bacillus subtilis* is 173 aa long protein with an acidic pI of 3.6. It was shown to consist of two domains: the N-terminal domain displaying an ordered structure as determined by CD spectroscopy, and the C-terminal domain, which appeared flexible and unstructured. The N-terminal domain was shown to interact with RNAP [3].

The structure of N-terminal domain was determined by NMR. It consists mainly of three α-helices and one short β-sheet, yet the N-terminal part remains unstructured. The cause of this flexibility is probably the His_6-Tag attached at the N-terminus [4].

Therefore crystallization and structure determination by X-ray analysis were chosen. The N-terminal domain of subunit was conquested to high-throughput screening (sitting drop), where several crystallization conditions were found. Further optimization showed two conditions that are more favourable for the crystal growth and its quality. Hanging drop was the technique used for the crystallization in the optimizing step. Crystals were obtained after 7-9 days with an average size 500 m x 400 m. Diffraction data were collected at ESRF Grenoble, ID-23-1. The data were processed by MOSFLM [5] and the determination of the structure was done by molecular replacement with NMR structure used as a model.

1. S. Clark, Losick R. Losick, J. Pero, *Nature*, 252, (1974), 21–24.
2. J.D. Helmann, C.P. Moran, Washington, DC: ASM Press, 289–312, 2002.
3. F.J. Lopez de Saro, A.Y. Woody, J.D. Helmann, *J. Mol. Biol.*, **252**, (1995), 189–202
4. V. Motáčková , H. Šanderová, L. Žídek, J. Nováček, P. Padrta, A. Svenková, J. Korelusová, J. Jonák, L. Krásný, V. Sklenář, *Proteins*, **78**(7), (2010), 1807-10.
5. A.G.W. Leslie, *Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography*, **26**, (1992).