12th Heart of Europe bio-Crystallography Meeting

Třeš , Czech Republic, 24. 9. - 26. 9., 2009

Thursday Evening Session I - September 24

L1

INFLUENCE OF RNA BINDING ON THE STRUCTURE OF BORNA DISEASE VIRUS MATRIX PROTEIN

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Borna disease virus (BDV) is the causative agent for Borna disease, a non-cytolytic, persistent infection of the central nervous system originally detected among horses in Borna, Germany. BDV has been used as a model system to investigate and understand persistent viral infections of the brain. The known hosts of BDV range from rodents to non-human primates and perhaps humans. Within the order *Mononegavirales*, which includes among others the viruses Marburg, Ebola and Rabies, BDV is the representative and only member of the family of *Bornaviridae*.

BDV has the smallest genome among all known negative stranded non-segmented RNA viruses, with a size of 8.9 kb, encoding for six proteins. The matrix protein of BDV (BDVM), a 16.2 kDa protein that forms a stable homotetramer, is associated with virus assembly and budding and may also be associated with the regulation of the viral ribonucleoprotein activity. We have recently shown that BDVM binds single stranded RNA, as does the matrix protein VP40 of the Ebola virus.

To further investigate the structural and functional influence of RNA binding on BDVM, we mutated the specific RNA binding site, creating the variant BDVM H112W. Here, we present two different crystal structures of BDVM H112W and their biophysical characterization, providing new insights into the influence of RNA binding on the structure and suggesting alternative functionalities of BDVM.

1. Staeheli P, Sauder C, Hausmann J, Ehrensperger F, Schwemmle M. Epidemiology of Borna disease virus. *J. Gen. Virol.* 2000; 81(Pt 9):2123-35.
2. Cubitt B, Oldstone C, de la Torre JC. Sequence and genome organization of Borna disease virus. *J. Virol.* 1994; 68(3):1382-96.
3. Kraus I, Bogner E, Lilie H, Eickmann M, Garten W. Oligomerization and assembly of the matrix protein of Borna disease virus. *FEBS Lett.* 2005; 579(12):2686-92.
4. Chase G, Mayer D, Hildebrand A, u. a. Borna disease virus matrix protein is an integral component of the viral ribonucleoprotein complex that does not interfere with polymerase activity. *J. Virol.* 2007;81(2):743-9.
5. Neumann P, Lieber D, Meyer S, u. a. Crystal structure of the Borna disease virus matrix protein (BDV-M) reveals ssRNA binding properties. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106(10):3710-3715.

L2

TWO-DOMAIN LACCASE FROM *Streptomyces coelicolor*: A LINK BETWEEN LACCASES AND NITRITE REDUCTASES

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Laccases (EC 1.10.3.2) are multicopper oxidases catalyzing the reduction of molecular oxygen to water accompanied by oxidation of a substrate, with broad substrate specificity (polyphenols, methoxy-substituted phenols, aromatic diamines). Common laccases consists of three domains. The laccase from *Streptomyces coelicolor* reported here (PDB code 3CG8) was the first two-domain laccase structure of which was solved (Skálová et al., J. Mol. Biol. 2009, 385, 1165-1178).

Multicopper blue proteins form an interesting protein family with variability of positions of copper ions in proteins and variability of protein oligomerization states. An evolutionary theory of multicopper blue proteins was proposed by Nakamura and Go (Cell. Mol. Life. Sci., 62 (18), 2050-2066), with evolution from monomeric proteins over two-domain trimeric proteins of three types (A, B and C) towards three-domain laccases and ascorbate oxidases, two-domain nitrite reductases and six-domain ceruloplasmin.

The structure of laccase from *Streptomyces coelicolor* confirms expectations of hypothesis of Nakamura and Go concerning protein arrangement (two-domain trimeric protein type B) and brings detailed structural information.

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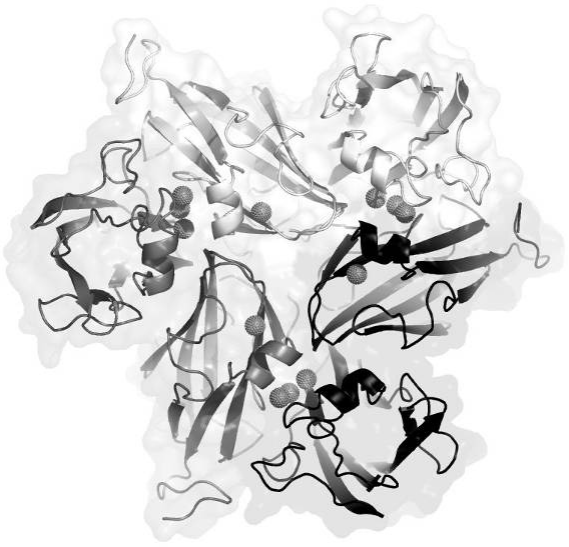

Figure 1. Trimer of two-domain laccase from *Streptomyces coelicolor* with denoted positions of twelve copper ions.

L3

CRYSTALLISATION AND STRUCTURAL ANALYSIS OF A NOVEL HALOALKANE DEHALOGENASE DBEA FROM *Bradyrhizobium elkani* USDA94

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A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkani* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. To understand the altered activity and specificity of DbeA enzyme, its mutant variant DbeA1, carrying the unique fragment of DbjA, was also constructed. Both the wild type DbeA and the DbeA1 were crystallised using the sitting-drop vapour-diffusion method. The crystals of DbeA belong to the primitive orthorhombic space group $P2_12_12_1$, while the crystals of the DbeA1 mutant belong to the monoclinic space group $C2$. Crystal structure of a DbeA and DbeA1 have been solved and refined to 2.2 Å resolution. The enzymatic molecular structure of DbeA was compared with those of known haloalkane dehalogenases already deposited in Brookhaven Protein Data Bank.

L4

CRYSTAL STRUCTURE OF A NOVEL INTRAMOLECULARCHAPERONE MEDIATING TRIPLE- -HELIX FOLDING

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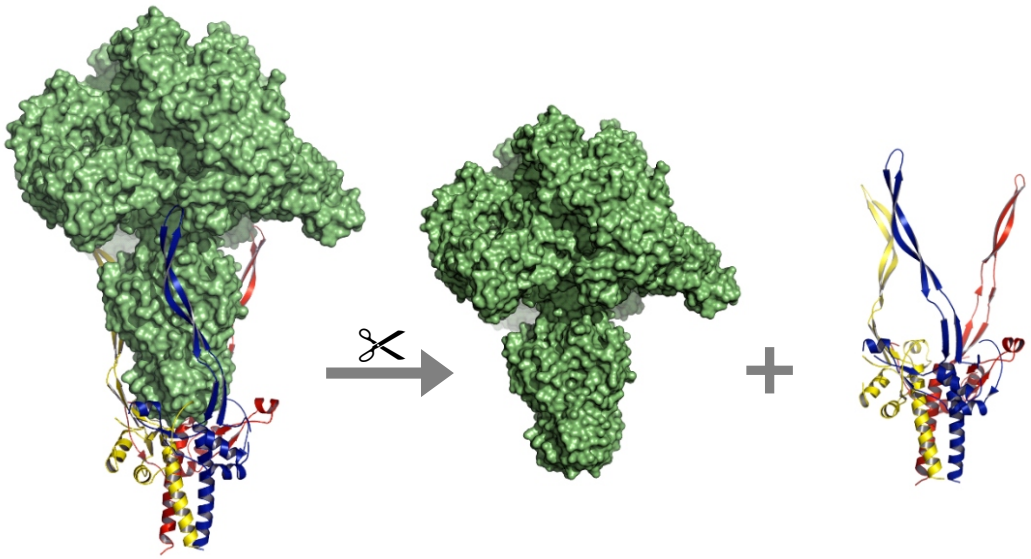
In order to prevent misfoldingprotein folding is often mediated by molecular chaperones. Recently, a novel class of intramolecular chaperones (IMC) has been identified in tailspike proteins of evolutionary distant viruses, which require a C-terminal chaperone for correct folding. The chaperone domains, which share a high sequence homology are interchangeable between pre-proteins and release themselves after proper protein folding.

Here we report the crystal structures of two IMCs in either the released or the pre-cleaved form, revealing the role of the chaperone domain in formation of a triple- -helix motif. Tentacle-like protrusions enclose the pre-proteins during the folding process. After the assembly a sensory mechanism for correctly folded -helices triggers a Ser-Lys catalytic dyad to release the mature protein-autoproteolytic cleavage reaction. Sequence analysis shows a conservation of the IMCSsin functionally unrelated proteins presumably sharing -helices as a common structural motif.



L5

READY, SET, SCREEN: X8 PROSPECTOR

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The current bottle neck in protein crystallography is to get well diffracting crystals. The process of screening crystals is consequently vital, not only to monitor improvement in the crystallization trials but also to select the best crystals for the data collection at the synchrotron.

Thus, when screening a large number of potential candidates, it is important having a dedicated system, which is easy to use, has low maintenance costs, and has an excellent DCE [1] to enable the scientist to judge the scattering power of the crystal.

The only way to figure out if and how well your crystal diffracts is a direct check using a sufficiently strong X-ray beam. Why waste your valuable synchrotron time screening crystals there when you could easily screen in the lab? The X8 PROSPECTOR (Fig. 1) is a system designed to fulfil the demand for efficient screening, economical and reliable. With its compact design and small footprint, it can be placed in any laboratory. Moreover it gives you all the information needed prior to go to the beamline.

The source is completely air-cooled and therefore requires no external plumbing. Furthermore it uses regular, single-phase power without the need of special electrical wiring. A microfocus I S sealed tube coupled with the QUAZAR optics delivers a highly stable X-rays beam with intensity equivalent to the one of a traditional sources, such as rotating anode generator with a 300 micron focus combined with 6 cm mulilayer mirrors. The I S delivers the smallest beam – focussing the X-rays where you need them and minimising background. The rock-solid fourth generation APEX II CCD detector is the most sensitive available, and when coupled with this powerful I S source it enables you to measure diffraction from the most weakly diffracting crystals.

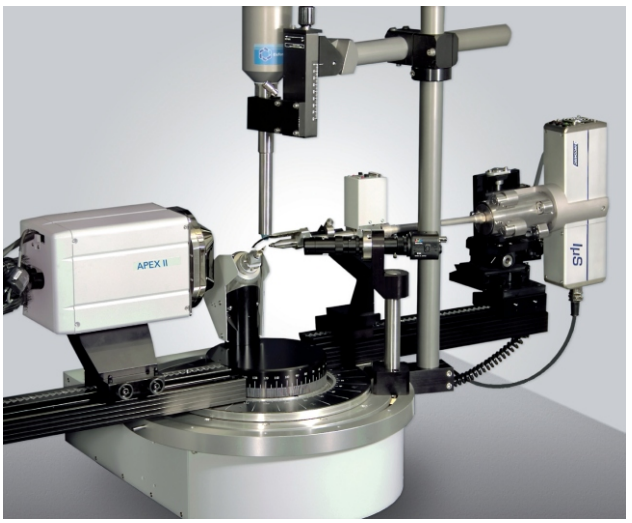

Figure 1. X8 PROSPECTOR with I S microfocus source, QUAZAR optics, three circles goniometer and APEXII CCD detector: the ideal system for screening crystals.

The X8 PROSPECTOR not only speeds up your screening work, it also leads the user through the whole process. The PROTEUM2 software controls the hardware and analyzes the results to give quick and reliable answers to your questions.

Examples will be presented showing how easily the X8 PROSPECTOR finds the jewels in your crystallization plates.

M. Stanton, *Nucl. Instrum. Methods*, **A325**, (1993), 550.