MS23.P07

Acta Cryst. (2011) A**67**, C354

SR Beamline for macromolecular assemblies by the institute for protein research

Atsushi Nakagawa[a] Eiki Yamashita,[a] Yasushi Umena,[a] Masato Yoshimura,[b] Mamoru Suzuki,[a] Kazuya Hasegawa,[c] Yukito Furukawa,[c] Toru Ohata,[c] Takashi Kumasaka,[c] Go Ueno,[d] Masaki Yamamoto,[d] Shinya Yoshikawa,[e] Tomitake Tsukihara,[ae] [a]*Institute for Protein Research, Osaka University (Japan).* [b]*NSRRC (Taiwan).* [c]*JASRI/SPring-8 (Japan).* [d]*RIKEN/SPring-8 (Japan).* [e]*Graduate School of Life Science, University of Hyogo (Japan).* E-mail: atsushi@protein.osaka-u.ac.jp

Biological macromolecular assemblies play significant roles in many biological reaction systems. Detailed understanding of the functions of the macromolecular assemblies requires information derived from three-dimensional atomic structures. X-ray crystallography is one of the most powerful techniques to determine the three-dimensional structures of macromolecular assemblies at atomic level. It is usually be known that biological macromolecular assembles are difficult to be crystallized or grown to larger size crystals. In addition, the unit cells of these crystals are quite large. Because of these features of the crystals of biological macromolecular assemblies, it is usually very difficult to obtain good diffraction data. The difficulties of diffraction data collection of biological macromolecular assembles are as follows; extremely weak diffraction power, narrow space between diffraction sports, x-ray radiation sensitive etc. High brilliance and highly paralleled synchrotron radiation from the undulator is an extremely powerful tool for diffraction data collection from macromolecular assembly crystals with large unite cell.

The Institute for Protein Research (IPR) of Osaka University is operating a synchrotron radiation beamline for crystal structure analysis of biological macromolecular assemblies at SPring-8 (BL44XU). This beamline is designed to collect diffraction data from biological macromolecule assembly crystals with large unit cells. The light source is a SPring-8 standard type in-vacuum undulator. A liquid-nitrogen cooled double crystal monochromator and a horizontal focusing mirror are used as the optical components. A high precision diffractometer combined with a specially designed large image-plate detector, DIP6020, and a high performance CCD detector, MX-225HE, are installed. BSS (Beamline Scheduling Software), a SPring-8 protein crystallography beamline standard GUI, is installed to unify user operation throughout protein crystallography beamlines in the SPring-8.

Present status and future plan of the beamline will be discussed.

Keywords: diffration_data, synchrotron_radiation, macromolecular_assembly

MS23.P08

Acta Cryst. (2011) A**67**, C354

Structural studies of bacterial Lon ATP-dependent proteases

Ramona Duman, Jan Löwe, *Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, (UK).* E-mail: ramona@mrc-lmb.cam.ac.uk

Lon ATP-dependent proteases are key components of the protein quality control systems of bacterial cells and eukaryotic organelles. As well as being responsible for removing misfolded proteins from cells, they are involved in regulating cellular processes by degradation of short-lived proteins. They are also the last of AAA+ proteases for which structural, as well as functional, information is far from being complete. Lon proteases are subdivided into two classes: LonA and LonB. Eubacterial LonA proteases contain an N-terminal domain, an ATPase domain, as well as a protease domain, all on one polypeptide chain. The N-terminal domain is thought to be involved in substrate recognition, the ATPase domain in substrate unfolding and translocation into the protease chamber, while the protease domain hydrolyses polypeptides into small peptide fragments. LonB proteases lack the large N terminal domain and have, instead, a membrane-anchoring insertion into the ATPase domain. Like other AAA+ ATPases and self-compartmentalising proteases, Lon functions as an oligomeric complex. Despite the abundance of functional and biochemical knowledge accumulated about these proteases, structural information is still very fragmentary. I shall present two crystal structures of truncated versions of a LonA protease from *Bacillus subtilis* (BsLon), which reveal significant architectural features of Lon complexes [1].

The 3.5 Å structure of BsLon-AP is the first ADP-bound form of a hexameric complex consisting of a small part of the N-terminal domain, the ATPase and protease domains and reveals the approximate arrangement of the three functional domains of Lon. It also shows a resemblance between the architecture of Lon proteases and the bacterial proteasome-like protease HslUV. BsLon-AP was crystallized in five different spacegroups, all showing helical arrangements of the six subunits, which has often been encountered with many AAA+ ATPases.

The second structure, BsLon-N, at 2.6 Å resolution, represents the first 209 amino acids of the N-terminal domain of BsLon and consists of a globular domain and an additional four-helix bundle, which is part of a predicted coiled-coil region, for which no role has been assigned yet. The overall architecture of BsLon-N is reminiscent of the N terminal domain of proteasome-activating nucleotidases (PAN) from archaea. An unexpected dimeric interaction between BsLon-N monomers reveals the possibility that Lon complexes may be stabilised by coiled-coil interactions between neighbouring N-terminal domains, which is also seen in PAN ATPases. Together, BsLon-N and BsLon-AP are 36 amino acids short of offering a complete picture of a full-length Lon protease.

The architecture of membrane-bound LonB proteases has now been elucidated, with the recently reported structure of an archaeal Lon [2], however the overall structure of LonA proteases remains elusive. Since their discovery, Lon proteases have remained very difficult crystallization targets and this is mainly due to their large size (550 KDa) and flexible, multi-domain nature. The large N termini of LonA proteases are themselves multi-domained and contribute substantially to the flexibility and instability of the ensemble. It is most probable that these flexible structures can only be stabilised in the presence of a substrate.

Current work is aimed at obtaining a crystal structure of a full-length LonA and identifying a cellular substrate with the view of investigating the process of substrate translocation through the inner protease chamber.

[1] R.E. Duman, J. Lowe, *J Mol Biol*, **2010,** *401***,** 653-670. [2] S.S. Cha, et al., *EMBO J*, **2010,** *29***,** 3520-3530.

Keywords: lon, protease, AAA+

MS23.P09

Acta Cryst. (2011) A**67**, C354-C355

Structure determination complicated by tetragonal pseudosymmetry

Stephan Barden, Hartmut Niemann, *Department of Chemistry, Bielefeld University, Bielefeld (Germany).* E-mail: stephan.barden@uni-bielefeld.de

Here, we report on an α-helical protein which crystallizes

reproducible as tetragonal bipyramides. Diffraction images of different crystals show split or smeared spots but symmetry is clearly visible. Indexing works properly using different programs suggesting a primitive tetragonal lattice with a = b = 66.8 Å and c = 113.2 Å and one molecule in the asymmetric unit. Merging statistics and automatic space group assignment in various program packages suggests point group 422 with a $4_{1/3}$ screw axis along the fourfold and also a twofold screw axis (space group $P4_12_12$ or $P4_32_12$). But some strong violations of systematic absences at low resolution along the fourfold and one twofold axis indicate that space group $P4_{1/3}2_12$ may not actually be correct. Despite this, a three-wavelength SeMet-MAD dataset was measured with a resolution of 2.8 Å and useable anomalous signal to about 3.5 Å. SHELXD [1] consistently found a solution for the Se-substructure in space group $P4_12_12$ (4 out of 5 Se atoms with CC all of 52.9 and CC weak of 33.6). After solvent flattening with SHELXE [1], the experimental electron density revealed the expected three helical bundle. No improvement was achieved in further attempts of density modification using different programs from the CCP4 program suite [2].

Using the Se positions as anchors for sequence assignment, around 80 amino acids (out of a total of 221) could be built, another 40 could be placed with some uncertainty. The free R-factor stalled at ~46 % with a FOM of ~50 %, and little difference density that would have allowed to extend the model. This model was taken for molecular replacement with PHASER [3] into the single datasets of the MAD experiment and other datasets from the native protein. Single solutions were found but with rather low Z-scores of ~3.6 (rotation function) and ~6 (translation function). We also tried molecular replacement with lower symmetry down to $P4_1$, $P2_12_12$ and $C222_1$ as well as experimental phasing in these space groups. But this did not result in better phases that allowed extending the structure or refining it to lower R-factors.

Apparently, this is a case of severe pseudosymmetry as refinement of an initial model in the suggested high symmetry spacegroup $P4_12_12$ did not work. Pseudo-merohedral twinning in a lower symmetry space group seems possible but analysis with PHENIX Xtriage [4] did not detect any indication for this. Experimental phasing in space groups $P2_1$ or P1 did not work due to lower multiplicity of the data. After all, other crystallographic or biochemical approaches may be required to determine the structure of this protein.

[1] G.M. Sheldrick, *Acta Cryst.* **2008**, *A64*, 112-122. [2] Collaborative Computational Project, Number 4, *Acta Cryst.* **1994**, *D50*, 760-763. [3] A.J. McCoy, R.W. Grosse-Kunstleve, P.D. Adams, M.D. Winn, L.C. Storoni, R.J. Read, *J. Appl. Cryst.* **2007**, *40*, 658-674. [4] P.D. Adams, P.V. Afonine, G. Bunkóczi, V.B. Chen, I.W. Davis, N. Echols, J.J. Headd, L.W. Hung, G.J. Kapral, R.W. Grosse-Kunstleve, A.J. McCoy, N.W. Moriarty, R. Oeffner, R.J. Read, D.C. Richardson, J.S. Richardson, T.C. Terwilliger and P.H. Zwart. *Acta Cryst.* **2010**, *D66*, 213-221.

Keywords: pseudosymmetry, phasing, helical_protein

MS23.P10

Acta Cryst. (2011) A**67,** C355

Low energy SAD experiments performed at the photon factory BL-1A

Naohiro Matsugaki, Yusuke Yamada, Leonard M.G. Chavas, Masahiko Hiraki, Masato Kawasaki, Ryuichi Kato, Noriyuki Igarashi, Soichi Wakatsuki. *Structural Biology Research Center, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK).* E-mail: naohiro.matsugaki@kek.jp

Recent developments in SAD (single anomalous dispersion) phasing techniques facilitate to solve macromolecular crystal structures using light atoms such as sulfurs or phosphors. Longer wavelength beam ranging from 1.7 Å to Cr K-edge (~2.3 Å) has been mainly used in data collection to enhance the weak anomalous signals. The method would be attractive for de-novo structure solutions without any derivatives, including membrane proteins or macromolecular complexes, for which heavy atom or selenomethionine derivative crystals are difficult to prepare.

We have developed a synchrotron beamline dedicated to long wavelength SAD experiments. The beamline is designed to take full advantage of a long wavelengh X-ray beam at around 3 Å to further enhance anomalous signals. The light source is an in-vacuum short gap undulator optimized at around the wavelength with the fundamental harmonics to obtain maximum brilliance. The vacuum section of the beamline has only one terminal beryllium window, followed by a diffractometer equipped with a helium cryostream and a specially designed helium chamber to minimize the attenuation of the lower energy beam and background noises. A cryo-cooled channel-cut monochromator and bimorph KB focusing mirrors compose a simple optics to deliver a focused beam with a good stability. The focused beam size (FWHM) at the sample position is 70 µm (H) x 10 µm (V), and the measured beam intensity is in the order of 10^{10} photons/sec on the area of 10 µm square.

Diffraction experiments using the wavelength of 2.7 Å was performed against various protein or nucleic acid crystals. X-ray detector was the commonly used CCD area detector (ADSC Quantum 270). Some of the crystals are mounted in the 'mother-liquor free' condition following the method developed by Hokkaido University [2][3] to decrease the background noise and anisotropic absorptions. Fully automated structural solutions were obtained for some crystals. In the presentation, the effectiveness of the mounting method, the helium cryostream and variable beam sizes are discussed. The exploration of the parameters used for phasing (resolution cut-off, number of frames, etc.) will be presented in the relationship with the radiation damage.

The development is supported by the national project 'Targeted Proteins Research Program', MEXT Japan

[1] K. Djinović Carugo, J.R. Helliwell, H. Stuhrmann, M.S.Weiss, *J. Synchr. Rad.* **2005**, *12,* 410-419. [2] Y. Kitago, N. Watanabe, I. Tanaka, *Acta Cryst.* **2005**, *D61,* 1013-1021. [3] Y. Kitago, N. Watanabe, I. Tanaka, *J. Appl. Cryst.* **2010**, *43*, 341-346.

Keywords: synchrotron_beam_line, long_wavelength, single_anomalous_dispersion

MS24.P01

Acta Cryst. (2011) A**67,** C355-C356

Coordinated ligand effects in the substitution kinetics of $[Re(CO)_3]^+$ complexes

Marietjie Schutte, Hendrik G. Visser, Andreas Roodt. *Departament of Chemistry, PO Box 339, University of the Free State, Bloemfontein, 9301, (South Africa).* E-mail: schuttem@ufs.ac.za

The coordination chemistry of rhenium gained a lot of interest in the last few years, since Alberto's *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ complex were remarkably synthesized from $[^{99m}TcO_4]^-$ in water and under mild conditions [1].

Synthetically, a lot of work has been published on $[M(CO)_3]^+$ (M = Re, Tc) systems with a huge variation in ligand systems [2 – 7]. The aqua ligands on the starting synthon, $[Re(CO)_3(H_2O)_3]^+$, can readily be substituted by a variety of combinations of ligands to produce potential radiopharmaceuticals with many different characteristics.

Our interest lies with the *fac*-$[Re(CO)_3]^+$ moiety and adopting the [2+1] approach [8]. The solid state behaviour and different effects like charge of the complexes and the influence of coordinated bidentate ligands on the rate of substitution can be explored.