MS23.P07

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

SR Beamline for macromolecular assemblies by the institute for protein research

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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

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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

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