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Crystal Structure of a Cyanobacterial BLUF Protein, Tll0078

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The sensor proteins for blue-light using the FAD (BLUF) domain belong to the third family of the photoreceptor proteins using a flavin chromophore, where the other two families are phototropins and cryptochromes. We have determined the crystal structure of the Tll0078 protein from *Thermosynechococcus elongatus* BP-1, which contains a BLUF domain bound to FAD, at 2 Å resolution.

The crystals belonged to space group $P2_12_12_1$ with cell dimensions of a=89.5 Å, b=109.9 Å, and c=169.9 Å. The asymmetric unit contains 10 monomers of Tll0078 (one decamer). Five Tll0078 monomers are located around the non-crystallographic five-fold axis to form a pentamer, and two pentamers related by two-fold noncrystallographic symmetry form a decameric assembly. The monomer consists of two domains, the BLUF domain at the N-terminal region and the C-terminal domain. The overall structure of the BLUF domain consists of a five-stranded mixed β-sheet with two αhelices running parallel to it. The isoalloxazine ring of FAD is accommodated in a pocket formed by several highly-conserved amino acid residues in the BLUF domain.

Keywords: flavoprotein structure, photosensors, protein crystallography

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SR Beamline for Macromolecular Assembly Crystallography Operated by IPR at SPring-8

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Biological macromolecular assemblies play significant roles in many biological reactions systems. To reveal the function of the macromolecular assemblies at atomic level, three-dimensional structure of the complex molecules is essential. A synchrotron radiation beamline for biological macromolecular assemblies at SPring-8 is operated by the Institute for Protein Research, Osaka University. Since crystals of biological macromolecular assemblies are often x-ray radiation sensitive and extremely weak diffraction power, it is essential to use high-brilliance and highly paralleled synchrotron radiation for diffraction data collection. This beamline is designed to collect high resolution and high quality diffraction data from macromolecular assembly crystals with large unit cells. A newly designed detector, DIP6040, which is a hybrid-type of image plates and a CCD, is installed.

Diffraction data from crystals with large unit cell (a=b=630, c=350Å) has been collected above 3.6 Å resolution. The present status and recent results of the beamline will be presented.

Keywords: biological macromolecular crystallography, synchrotron radiation crystallography, biological macromolecular assemblies

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Calculation of Biological Units from Protein Crystallography Data

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Solution of protein structures by means of X-ray diffraction delivers crystal structure and protein coordinates that form an asymmetric unit of the crystal. These coordinates do not necessarily identify the biological unit, or protein assembly that performs a certain physiological function. However, it is reasonable to expect that biological units last out to the crystallization and therefore it may be possible to find their structure and composition from the crystal data.

We propose a method for the calculation of protein assemblies from crystal structures, which is different from previously published [1,2] in that it is based on general principles of chemical thermodynamics. Our method employs graph techniques for the identification of all potential assemblies in a crystal, represented as a periodic graph, and each assembly is then analyzed for chemical stability on the basis of protein affinity and entropy change upon dissociation. As found for structures with experimental evidence of their oligomeric states, our method achieves 89% of correct predictions, which is higher then previously reported [1,2].

The developed software has been made available to public by setting up a web service that can take uploaded PDB and mmCIF coordinate files for analysis. The service also provides protein interfaces and protein assemblies precalculated for all PDB entries of structures solved by X-ray diffraction.

[1] Henrick K., Thornton J., *Thrends Biochem. Sci.*, 1998, **23**, 358. [2] Ponstingl H., Kabir T., Thornton J., *J. Appl. Cryst.*, 2003, **36**, 1116.

Keywords: protein assembly, protein interactions, protein crystals

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X-ray Structure of Recombinant Core 8D of the Nuclear Chaperone Nucleoplasmin

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The efficient assembly of histone complexes and nucleosomes involves the participation of molecular chaperones. The histone chaperone nucleoplasmin represents the most abundant protein in the *Xenopus* oocyte nucleus. It mediates nucleosome assembly by removing basic proteins from sperm chromatin and exchanging them with histones. This function is modulated by phosphorylation of nucleoplasmin at multiple sites.

The nucleoplasmin core domain structure forms a pentameric arrangement where each monomer consists of two domains: a core, that forms a stable ring-like pentamer, and a tail, which holds a polyglutamic tract and the nuclear localization signal. The lacking of the poly-Glu region, a putative binding site for basic proteins, does not affect its capacity for the binding of the sperm basic proteins and the chromatin decondensation. This activity has been reproduced artificially in a recombinant core domain through mutation of putative phosphorylation sites to aspartate, thus mimicking the charge effect of phosphorylation. The crystallographic studies of this recombinant domain (called CORE8D) at 2.5 **Å** resolution show the presence of these mutations which do not affect the folding of the monomer and so the formation of the pentameric structure, even though they are located in exposed flexible regions. The crystal packing has revealed the formation of a nucleoplasmin-core decamer that could represent its normal biological oligomerisation state. This decamer has localized negative charges near the interface (interactions between the Asp58 and Lys82 from all the opposite monomers), with a network of hydrogen bond waters which serve to maintain together the opposite pentamers.

Keywords: nucleoplasmin, protein, crystallography

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Hinge Peptide and Intersubunit Interface in Domain Swapping

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The domain swapping is a phenomenon, which is observed in an increasing number of proteins. Among the swapped proteins, bovine seminal ribonuclease (BS-RNase) represents a unique example, as the

process occurs in the native mixture between two dimeric forms, MxM (swapped) and M=M (unswapped), held together by the additional constraints of two inter-chain disulphide bridges. This peculiarity offers the opportunity to study the effects of selected mutations at the O-interface on the M=M / MxM equilibrium, within a substantially invariant quaternary assembly. Two variants, having Pro 19 (P19A) or the whole sequence of the hinge peptide 16-22 replaced by the corresponding residues of RNase A (BS-hinge-A), show equilibrium and kinetic parameters of the swapping similar to those of the parent protein. On the contrary, mutation of L28 (L28Q, P19A/L28Q) significantly affects the swapping processes. The X-ray structures of the MxM forms of P19A and BS-hinge-A, and the MxM and M=M forms of the double mutant P19A/L28Q have been determined. The structural effects of the mutations are discussed on the basis of the MxM/M=M equilibrium data measured in solution. The relative insensitivity of the swapping tendency to the substitutions in the hinge region, and in particular to the replacement P19A, contrasts with the results obtained for other swapped proteins and can be rationalized in terms of the unique features of the seminal enzyme. On the other hand, the substitution in position 28 points to a crucial role of the interface residues in the swapping of BS-RNase.

Keywords: swapping , ribonuclease mutations, structural analysis

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Molecular Mechanisms of Thrombin-Mediated Fibrin Assembly

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Conversion of fibrinogen into fibrin (fibrin assembly) is mediated by thrombin, which cleaves two pairs of fibrinopeptides, fpA and fpB, from fibrinogen Aα and Bβ chains, respectively. Fibrin assembly occurs in two stages starting with the removal of fpA, which triggers formation of two-stranded protofibrils, followed by the removal of fpB, which enhances lateral association of protofibrils into thicker fibers. Non-substrate interaction of thrombin with the central part of fibrin(ogen) seems to play an important role in these processes. To establish the structural basis for the sequential cleavage of fpA and fpB, we crystallized thrombin in complex with a fragment corresponding to the central part of fibrin(ogen), and solved the structure of this complex at 3.65 Å resolution [1]. Next, we modeled possible arrangements of fibrinopeptides-containing NH_2-terminal portions of the Aα and Bβ chains, which were not identified in the electron density map. The resulting structure reveals that thrombin binds fibrinogen in a way that promotes positioning of the Aα chains in its active site cleft. This explains the preferential removal of fpA at the first stage of fibrin assembly. Subsequent modeling of a protofibril by docking the structure of the dimeric fibrin-derived D-D fragment into the complex suggests that the fpB-containing portions in such structure should interact with D-D to restrict their positions to the vicinity of the active site cleft. In agreement, surface plasmon resonance experiments reveal that these portions indeed interact with D-D. These provide the rationale for the accelerated removal of fpB upon the formation of protofibrils.

[1] Pechik I., Madrazo J., Mosesson M.W., Henandez I., Gilliland G.L., Medved L., *Proc. Nat. Acad. Sci. USA*, 2004, **101**, 2718.

Keywords: protein complex structure, fibrinogen, blood coagulation

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The Complex of the Polycomb Group Proteins Ring1B (RNF2) and Bmi1 –A Structure-function-analysis

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The Ring finger proteins Ring1B and Bmi1 belong to the Polycomb group proteins (PcG), which form large multimeric protein complexes. These complexes covalently modify histones leading to the transcriptional repression of specific genes. Mammalian homologs of Ring1B and Bmi1 are part of the PRC1L complex (Polycomb repressive complex 1-like), which acts as an E3 ligase in the ubiquitination of histone H2A [1].

Here we present the crystal structure of the Ring1B-Bmi1 Ring domain dimer at a resolution of 2.0 Å. The structure reveals that the interaction is mainly determined by structural elements that flank the Ring motifs. Details of the structure will be presented.

An *in vitro* ubiquitination assay was used to study E3 ligase activity of the Ring1B-Bmi1 complex. Adding either the full length Ring-Ring complex or the truncated form significantly activated ubiquitination of histone H2A.

[1] Wang H., Wang L., Erdjument-Bromage H., Vidal M., Tempst P., Jones R.S., Zhang Y., *Nature*, 2004, **431**, 873.

Keywords: ubiquitin system, Ring1B (RNF2), Bmi1

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Structure of a Giant Hemoglobin of the Gutless Beard Worm *Oligobrachia mashikoi*

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Pogonophorans and vestimentiferans obtain their nutrition solely from their symbiotic chemoautotrophic sulfur-oxidizing bacteria because they have no mouth or gut. These animals have sulfide-binding 400 kDa and/or 3500 kDa hemoglobin (Hb) which transports oxygen and sulfide simultaneously, and the symbiotic bacteria are supplied with sulfide by these giant Hbs. We have determined for the first time the crystal structure of a 400 kDa vascular Hb from pogonophoran *Oligobrachia mashikoi* at 2.85 Å resolution as an intact oligomeric form. The structure is hollow-spherical, with outer and inner diameters of about 120 Å and 50 Å, respectively, and composed of a total of 24 globins as a dimer of dodecamer. This dodecameric assemblage would be a common unit to both 400 kDa and 3500 kDa Hb. The structure of the mercury derivative used for phasing provides insights into the sulfide-binding mechanism. The mercury compounds bound to all free Cys residues that have been expected as sulfide binding sites. Some of the free Cys residues are surrounded by Phe aromatic rings, and mercury atoms come into contact with these residues in the derivative structure; it is strongly suggested that sulfur atoms bound to these sites could be stabilized by aromatic-electrostatic interactions by the surrounding Phe residues.

Keywords: proteins, hemoglobins, supramolecular assemblies

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Structural Biology of Type IV Secretion System

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Type IV secretion systems (T4SSs) are machineries used for the transport of macromolecules across the bacterial cell envelopes of Gram-negative bacteria. The archetypal T4SS, the VirB/D system was first defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefasciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4) whereas T4SS in other bacteria can display homologues for only a subset of those proteins. T4SSs are highly versatile and have been found in many bacterial pathogens such as *Helicobacter pylori* (Cag PAI), *Brucella suis* (VirB/D), and *Legionella pneumophila* (Dot, Icm). The secretion machinery spans both bacterial