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A novel octamer structure of bacterial cellulose synthesis component AxCesD

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Cellulose, a linear homopolymer of D-glucopyranose rings (DGPRs) with β-1, 4 linkages is an affluent biopolymer in the nature, that is used in production of many commercial materials such as paper, films, textiles, explosive, and food additives. Although previous studies showed that cellulose can be produced by many organisms including plants, algae, some bacteria, even some animals, the aspects of cellulose biosynthesis are not yet fully understood. Cellulose produced by bacteria such as Gram-negative bacterium *Acetobacter xylinum* (*A. xylinum*) is called bacterial cellulose (BC), which has the different structural and physical characters from those of cellulose produced by plants. *A. xylinum* synthesizes cellulose from UDP-glucose and forms cellulose fibril, and has a BC synthase operon, AxCes that includes three (*axcesAB*, *axcesC*, and *axcesD*) or four (*axcesA*, *axcesB*, *axcesC*, and *axcesD*) genes encoding a transmembrane protein complex called terminal complex (TC). The AxCesA and AxCesB (or AxCesAB) are identified as the catalytic subunits, while the AxCesC is suggested to form the pore in the cell walls for export of cellulose fibril. The AxCesD protein is the smallest member in BC synthase components, and responsible for celluloses crystallization. Moreover, it has been reported that lack of the *axcesD* gene product resulted in a 40% reduction in the rates of bacterial cellulose synthesis. We have solved the structures of AxCesD in three forms, N-terminus His-tagged and C-terminus His-tagged proteins, and one complex with β-D-glucopyranose ring (β-DGPR). AxCesD exits as an octamer forming a molecular ring. Combining with biochemical experiment, this molecular ring may play the role of gate for forming cellulose fibril.

Keywords: crystal structures of new compounds, cellulose biosynthesis, macromolecular assemblies

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Structure of Narcissus pseudonarcissus lectin complex with mannobiose at 1.7 Å resolution, FORM II

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A second preparation of crystals of the daffodil lectin in complex with alpha-1,3 mannobiose revealed a new stacking pattern, compared with the previously determined structure, 1NPL. Tetramers observed in the old, Form I, preparation were packed in layers that repeated by unit cell repetition normal to the layers (the z direction). Form II also has the same layering pattern, except that the repeat in the direction normal to the layers was shifted by 2.7Å in the plane. The result was a doubling of the z cell dimension, and the loss of the binding ability in the fourth binding site, at the interface between layers. Another crystallographic consequence was the incidental, purely translational, non-crystallographic symmetry, which gave faint bands in the diffraction pattern. The reason for the stacking loss of register is unclear, although the sample preparation was the same as before. Interaction energies across the interfaces were also calculated, in an attempt to clarify the differences.

Keywords: lectin crystallography, protein-saccharide interactions, molecular recognition

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Crystal structure of a lectin from the octocoral

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Lectins are sugar-binding proteins or glycoproteins that recognize specific carbohydrate structures and agglutinate various types of animal cells. In marine animals, lectins are believed to contribute as non-self recognition factors to the defence mechanism. Interestingly, it has been theorized that some lectins from marine animals mediate the interaction between symbiont and host. SLL-2 is a D-galactose binding lectin isolated from an octocoral, *Sinularia lochmodes*. It was found that SLL-2 was distributed densely on the surface of symbiotic dinoflagellate *Symbiodinium* sp. cells. Recently, SLL-2 was indicated to transform free-swimming stage *Symbiodinium* cells into non-motile stage *Symbiodinium* cells and keep them in their non-motile stage (K. Koike, *et al*., 2004). These results show that SLL-2 is a chemical cue in the symbiosis between dinoflagellates and coral. The three-dimensional structure of SLL-2 will provide information about the symbiosis mechanism. The purified SLL-2 was crystallized and the best crystals were obtained from the solutions containing 2-methyl-2,4-pentanediol as a precipitant and calcium formate as an additive reagent without the presence of any sugars. The structure of SLL-2 was solved by the molecular replacement method using atomic coordinates of HPA lectin (PDB code: 2ccv) as a search model. Three SLL-2 monomers form a trimer around a non-crystallographic 3-fold axis, and two trimers formed a hexmeric assembly using hydrogen bonds of β-strand (β1N) from each monomer. The structure of SLL-2 monomer shows the β-sandwich lectin fold with six β-strands (β1-β6). The sites of N-glycosylation and sugar binding were identified in the crystal structure.

Keywords: protein crystallization, protein structure analysis, lectins

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X-ray crystallographic analysis of galectin LEC-8 from *Caenorhabditis elegans*

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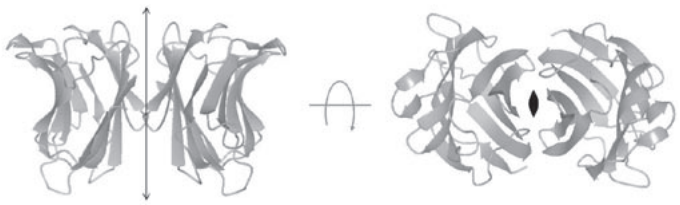
Galectin LEC-8 from nematode *Caenorhabditis elegans*, classified as a prototype galectin, consists of 180 amino acid residues and specifically binds to β-galactoside. We succeeded in crystallizing the carbohydrate recognition domain of LEC-8 (LEC-8CRD), ranging from the Met1 residue to the Gly139 residue, under a condition including ammonium sulfate as a precipitant and 1, 4-diethylene dioxide as an additive using the hanging-drop vapor-diffusion method. The structure was solved by molecular replacement using X-ray diffraction data collected to 1.9Å resolution at BL-17A of the Photon Factory. The crystals belong to the monoclinic space group *C*2, with unit-cell parameters a = 113.2, b = 40.1, c = 66.7Å, and β = 116.0°. In the asymmetric unit, there are two LEC-8CRD molecules, related by a noncrystallographic twofold axis as shown in the figure. Seven sulfate anions, seven glycerols, and 178 waters have been assigned in the current refined structure. LEC-8CRD, like other galectins, has the β-sandwich motif, and its dimerization pattern formed in the crystal is very similar to that of human galectin-7, which is known as a prototype galectin.



Keywords: lectin proteins, macromolecular X-ray crystallography, X-ray crystal structure analysis

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Structural and molecular characterization of the prefoldin beta subunit from *Thermococcus* strain

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Prefoldin (PFD) is a heterohexameric molecular chaperone that is found in eukaryotic cytosol and archaea. PFD is composed of α and β subunits and forms a "jellyfish-like" structure. PFD binds and stabilizes nascent polypeptide chains and transfers them to group II chaperonins for completion of their folding. Recently, a whole genome from *Thermococcus kodakaraensis* KOD1 was reported and shown to contain the genes of two α and two β subunits of PFD. The genome of *Thermococcus* strain KS-1 also possesses the two sets of α (α1 and α2) and β (β1 and β2) subunits of PFD (TsPFD). However, the functions and roles of each of these PFD subunit have not been well investigated. We crystallized the TsPFD α2-β1 complex. The obtained crystals belong to the space group *I*422 with unit cell dimensions of a=b=71Å, c=114Å and diffracted to 1.9Å resolution. The SIRAS phased electron density map showed clear peaks corresponding to the β1 subunit, whereas the α2 subunit was not observed. The refinement of the structure composed only of the β1 subunit reduced the crystallographic *R*work and *R*free factors to 17.7% and 19.7%, respectively. TsPFD β1 subunits form a tetramer with four coiled-coil tentacles resembling the "jellyfish-like" structure of heterohexameric PFD. β hairpin linkers of β1 subunits assemble to a β barrel "body" around a central four-fold axis. Size exclusion chromatography and multi-angle light scattering analysis shows that the β1 subunits form a tetramer at pH 6.8. The tetrameric β1 subunits can protect against aggregation of a relatively small proteins, such as insulin and lysozyme. The structural and biochemical analyses imply that PFD β1 subunits act as a molecular chaperone in living cells of some archaea.

Keywords: molecular chaperone, chaperone proteins, structural characterization

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Domain interaction analyses of gp7, gp10 and gp11 of bacteriophage T4 for crystallization

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Bacteriophage T4 changes the shape of the baseplate during infection process. Recently, three-dimensional reconstructions from cryo-EM images of the baseplate before and after infection were carried out. Combination of the low resolution structure of the baseplate and the high resolution structures of some components has revealed that the gross structural change of the baseplate is accompanied by the rearrangement of the subunit of the baseplate. In order to fully understand the mechanism of the structural change, however, determination of the atomic structures of all the subunits by x-ray crystallography is essential. Crystallization of structural components of multi-subunit complex often encounters difficulty. One possibility is the exposure of hydrophobic surfaces which are otherwise covered by other subunits; it may cause undesirable non-specific associations. Some flexible regions which are important for complex formation may interfere with crystallization. To overcome these difficulties, we chose gp7, gp10 and gp11 of bacteriophage T4 as a model for "trimmed complex" which is suitable for crystallization of multi-subunit protein complex. To determine the domain interactions of these proteins, gp7, gp10, gp11 as well as gp7-gp10 complex and gp10-gp11 complex were treated with lysyl-endopeptidase for limited proteolysis. Significant difference in digestion pattern between gp10 alone and gp10-gp11 was observed. The N-terminal sequence analysis of digested fragments showed that gp10 alone was cleaved at Lys289, but when complexed with gp11, it was digested at Lys194. When complexed with gp7, gp10 became absolutely resistant to lysyl-endopeptidase. It was concluded that the C-terminal domain of gp7 binds to gp10 and makes gp10 resistant to the protease.

Keywords: phage, protein assembly, complex protein interactions