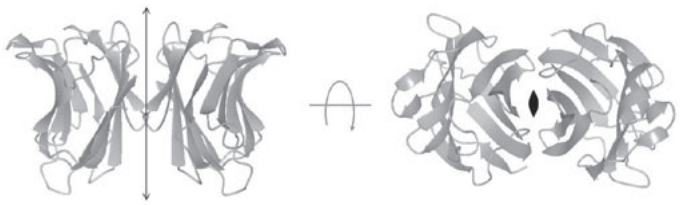
Yahaba, Iwate, 028-3694, Japan, [2]Faculty of Pharmaceutical Sciences, Josai University, [3]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology, [4]School of Pharmaceutical Sciences, Teikyo University, [5]Department of BioEngineering, Nagaoka University of Technology, E-mail：titagaki@iwate-med.ac.jp

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

Hiroshi Kida[1], Yuri Sugano[2], Ryo Iizuka[2,3], Masahiro Fujihashi[1], Masafumi Yohda[2], Kunio Miki[1]

[1]Kyoto University, Department of Chemistry, Graduate School of Science, Sakyo-ku, Kyoto, Kyoto, 606-8502, Japan, [2]Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan, [3]University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, E-mail：hirokida@kuchem.kyoto-u.ac.jp

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

Shuji Kanamaru, Tomoko Nakao, Tatsuya Nagao, Fumio Arisaka

Tokyo Institute of Technology, Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, B-39 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa-ken, 2268501, Japan, E-mail：skanamar@bio.titech.ac.jp

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