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STRUCTURAL STUDIES OF A DUAL LIGAND BINDING SH3 DOMAIN IN Pex13

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The Peroxisomal Targetting Signal 1 proteins (PTS1) comprising the three C-terminal residues SKL, are recognized by the import receptor Pex5p, which docks to the peroxisomal membrane proteins Pex13p and Pex14p. The C-terminus of Pex13p contains a Src Homology 3 (SH3) domain that is critical for the ternary Pex5p-Pex13p-Pex14p complex formation. Pex14p, containing a PxxP motif, is a classical SH3 domain ligand while Pex5p is void of such motif . To reveal the molecular basis of Pex13p(SH3) interaction to its two ligands, we have used a combination of X-Ray crystallography, NMR and CD experiments. The crystal structure of Pex13p SH3 domain was solved to 2.65 Å using MAD phases. It shows a unique hydrophobic network between the RT- and n-Src loops resulting from insertions in both loops. We have also mapped the Pex5p and Pex14p binding sites on the SH3 domain by NMR using Pex5p and Pex14p peptides. The two sites are not overlapping and are located at opposite surfaces. NMR titration experiments show an interaction in the micromolar range for both peptides. Furthermore, sequential titrations demonstrate that the two ligands bind independently. Additionally, several CD data indicate that the Pex14p peptide adopts a polyproline type II conformation, reminiscent of other PxxP-ligands. An α-helical conformation of the Pex5p peptide is induced upon binding to Pex13p(SH3) while being unfolded in its absence. Taken together, these results indicate two independent and non-competitive modes of interactions for Pex13p(SH3) to its ligands: Pex14p via the classical SH3-PxxP and Pex5p via a novel mode of interaction.

Keywords: SRC HOMOLOGY 3, PEROXISOME, IMPORT

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CRYSTAL STRUCTURE OF HAP3, A FLAGELLAR HOOK-FILAMENT JUNCTION PROTEIN AND A JUNCTION MODEL

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The bacteria flagellum consists of a long helical filament, a short curved segment called hook and a basal body. Between the filament and the hook, there are two junction proteins, called HAP1 (FlgK) and HAP3 (FlgL). The junction proteins are thought to be a kind of buffering structure connecting two structures that have mechanically distinct characteristics, where the hook is flexible with a small radius of curvature and the filament is rather rigid with a much larger radius of curvature. Here we report the crystal structure of a core fragment of HAP3. HAP3 tends to polymerize into filamentous aggregates. Therefore, its 26K dalton fragment (FL26) missing both terminal regions was overexpressed and purified for crystallization. The structure was solved using two heavy atom derivatives with anomalous data collected at SPring-8 BL41XU. FL26 consists of 4 helices and several β-hairpins. The overall feature is very similar to the D1 domain of flagellin. Two long helices and one relatively short helix, which are the major component of the D1 domain of flagellin, can be superimposed with the root mean square displacement of 1.3 Å. This allowed us to immediately construct the junction model in the filament structure by replacing the proximal flagellin subunits with FL26 in the R-type filament model. A β-hairpin corresponding to the possible mechanical switching unit in flagellin is also found in FL26, but its length and conformation are different from that of flagellin. Other β-hairpins are similar to those found in the D1 domain of hook protein.

Keywords: MACROMOLECULAR ASSEMBLY, FLAGELLUM, STRUCTURAL PROTEIN

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CRYSTAL STRUCTURE ANALYSIS OF PHOTOSYSTEM II FROM *SYNECHOCOCCUS VULCANUS* AT BL41XU OF SPRING-8

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Photosystem II (PSII), a membrane protein complex, possesses oxygen-evolving activity and plays important roles in photosynthesis. We have succeeded in crystallizing the PSII complex from *Synechococcus vulcanus* and analyzing the crystal structure at 3.7 Å resolution. The PSII monomer (310 kDa) is composed of at least thirteen trans-membrane subunits and three peripheral subunits, and many cofactors such as a Mn cluster, three iron atoms, one plastoquinone and 32-36 chlorophylls. X-ray diffraction experiments were carried out at BL41XU of SPring-8. Diffraction data were collected at 100 K and processed with DPS/MOSFLM and CCP4 program suit. Four heavy atom derivatives were prepared with Ta6Br14, K2Pt(CN)4, ethylmercuriphosphate, and KAu(CN)2. The Ta6Br14 derivative was a major contributor in phasing with SHARP up to 6 A resolution and much higher range (4.5 Å) was phased mainly by the K2Pt(CN)4 and ethylmercuriphosphate derivatives. Electron density maps were improved and extended up to 3.7 Å with a density modification program DM at 60% solvent ratio. The structure models were constructed for 36 trans-membrane helices (TMHs), several hydrophilic loops between TMHs, 3 peripheral subunits, and many cofactors. One of three peripheral subunits, 12kDa, was not identified for the PSII structure of S. elongatus (Zouni et al., Nature 409 (2000), 739). Whole arrangement of the components were very similar with the preceding report. Polypeptide chains surrounding the Mn cluster were newly assigned. One of them bound the cluster at its end, which could be C-terminus of D1 subunit.

Keywords: PHOTOSYSTEM II OXYGEN EVOLUTION PHOTOSYNTHESIS

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X-RAY STRUCTURE STUDIES OF H- AND L-PROTEINS OF GLYCINE CLEAVAGE SYSTEM FROM *THERMUS THERMOPHILUS*

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The glycine cleavage system (GCS) is a multi-enzyme complex composed of four different components (P-, H-, T- and L-proteins). In order to determine the structures of component enzymes and their complexes, we have recently over-expressed and purified all the component enzymes of GCS from *Thermus thermophilus* (Tth) HB8. In this session, we will report crystallization and structure determination of the recombinant Tth H- and L-proteins by molecular replacement method. Crystals of the H-protein were grown in ammonium phosphate as a precipitant. Synchrotron radiation from BL44B2 at SPring-8 was used to measure a native data set to 2.5 Å resolution. The crystals belong to the hexagonal space group $P6_5$, with unit-cell parameters a = b = 55.8, c = 191.2 Å. There are three monomeric molecules in the asymmetric unit, corresponding to a solvent content of 39%. Because of a large number of molecules in the unit cell, this structure could not be solved with AMoRe or X-PLOR, but was solved successfully with EPMR. Crystals of the L-protein were grown in polyethylene glycol as a precipitant. A native data set was collected to 1.6 Å resolution using the X-ray source mentioned above. The crystals belong to the triclinic space group P1, with unit-cell parameters a = 61.3, b = 62.7, c = 73.5 Å, α = 93.3, β = 107.4, γ = 108.7°. There is only one dimeric molecule in the asymmetric unit, corresponding to a solvent content of 52%. This structure was successfully solved with AMoRe.

Keywords: GLYCINE CLEAVAGE SYSTEM, LIPOAMIDE DEHYDROGENASE, GLYCINE DECARBOXYLASE