Poster Sessions

Keywords: starch binding domain, Rhizopus oryzae glucoamylase, cyclodextrin

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Crystal structure of the Sec4p:Sec2p complex in the nucleotide exchanging intermediate state

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Exocytosis is a basic membrane traffic event including transport, docking, and fusion of secretory vesicles. The yeast Rab GTase, Sec4p, regulates vesicular transport in exocytosis by oscillating between the active GTP-bound and inactive GDP-bound states. Sec2p is a guanine nucleotide exchange factor (GEF) for Sec4p, which catalyzes GDP release to promote GDP-GTP exchange for Sec4p activation. The N-terminal region (residues 1-160) of Sec2p is necessary and sufficient for its GEF activity while it shows no sequence homology to any other GEFs with known structures. The crystal structure of S. cerevisiae Sec2p revealed that the Sec2p GEF domain folds into a parallel dimeric coiled coil. To elucidate its GDP/GTP exchange mechanism, we have determined the crystal structure of the GEF domain of S. cerevisiae Sec2p in a complex with the nucleotide-free Sec4p. Upon complex formation, the Sec2p helices approach each other, and the switch I and switch II regions of Sec4p are largely deformed, to create a flat hydrophobic interface that snugly fits the surface of the Sec2p coiled-coil. These drastic conformational changes disrupt the interaction between the switches I and the bound guanine nucleotide, which facilitates the GDP release. In mammals, two GEFs, GRAB and Rabin3, are known as orthologs of Sec2p. The putative Rab binding domains (corresponding to residues 96 to 124 of Sec2p) share 93% similarity between Rabin3 and GRAB. In spite of this similarity, the specificity for Rab GTPases differs between Rabin8 and GRAB; Rabin3 exchanges GDP for GTP on Rab8, but not on Rab3A, while GRAB exchanges GDP for GTP on Rab3A. To elucidate their selectivity for Rab subfamily GTases, crystalization screening of the Rab8:Rabin3 and Rab3A:GRAB complexes is now under way.

Keywords: GEF, GTP-binding proteins, protein complex structure

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Crystal structure of hMyD88 at 1.8 Å resolution

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MyD88 (Myeloid differentiation primary response gene 88) is one of the signaling adaptor proteins with a Toll/interleukin receptor (TIR) domain. MyD88 has been recognized as a universal adaptor for all Toll-like receptors (TLRs), except for TLR-3, to activate transcript factor NF-κB [1]. We determined the crystal structure of hMyD88 at resolution 1.8 angstrom. The structure reveals that it may form an active signaling complex with other TIR domain containing adaptors. The conserved BB loop of the TIR domain was shown to play an important role in interaction with other TIRs [2]. The hMyD88 structure displays a different conformation of the BB loop in comparison with TIR1 and TIR2. We have observed distinct monomer and dimer species of hMyD88 in solution. hMyD88 dimer species can be reduced to monomer sizes upon addition of reducing agents, indicating that disulfides might also mediate its dimerization. We also observed a dimer formation in the crystal that utilizes the BB, DD and EE loops at the interface. Based on this structure, the models of hMyD88 in complexes with other TIRs and adaptors are proposed. [1] O’Neil & Bowie, Nature Reviews/Immunology, 2007, 7, 353-364. [2] Xu, Tao, Shen, Hornig, Medzhitov, Manley & Tong, Nature, 2000, 408, 111-115.

Keywords: signaling adaptor, Toll/interleukin receptor, Toll-like receptor

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First structure of a kinase domain in complex with Ca2+/CaM

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DAP kinase-1 is a CaM-regulated Ser/Thr kinase which acts as a cell death mediator and possesses tumor suppressor activity in humans. Like in other Calmodulin regulated kinases, direct binding of Calcium-activated Calmodulin (Ca2+/CaM) to a Calmodulin Binding/Autoinhibitory Domain (CBD/AID), adjacent to the catalytic core, has been proposed as the mechanism to release the autoinhibition through the removal of intramolecular interactions between this domain and key residues within the catalytic cleft. We present the structure of the kinase and CBD/AID domains of human DAPK-1 (residues 1-320) in complex with Ca2+/CaM, solved at 2.1 Å resolution using X-ray crystallography. The interaction occurs mainly through a hydrophobic interface generated by the collapse of Ca2+/CaM around the α-helical CBD. Several changes in the catalytic domain are imposed by this new conformation, when compared with the corresponding fragment of the autoinhibited DAPK-1 structure, that explain the catalytic activation and confirms the previously proposed ‘release-based’ mechanism. Electrostatic contacts, some of them involving extraregulatory domains, help to stabilize the complex and may participate in additional control mechanisms. Site-directed mutagenesis in combination with a biochemical approach was used to validate the structural data and determine the kinetics of the activation process. These findings allow to understand the mechanism of regulation of CaMKs by Ca2+/CaM, in the context of a biologically active, macromolecular assembly.

Keywords: apoptosis, calmodulin-mediated calcium signal transduction, protein kinases

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Novel crystal structure of red-absorbing form of cyanobacteriochrome AnPixJ-GAF2

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