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 GTPase, 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 interactions between switch 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 regions (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 GTPases, 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- κ B [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, Horng, 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 Ca^{2+}/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 (Ca^{2+}/CaM) to a Calmodulin Binding/Autoinhibitory Domain (CBD/AID), adjacent to the catalytic core, has been proposed as the mechanism to release the autoinhibion 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 Ca^{2+}/CaM, solved at 2.1 Å resolution using X-ray crystallography. The interaction occurs mainly through a hydrophobic interface generated by the collapse of Ca^{2+}/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 Ca^{2+}/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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Phytochromes of plants, fungi, algae and bacteria are the photoreceptors that bind linear tetrapyrroles and show reversible photoconversion between red-absorbing form (Pr) and far-red-absorbing form. Cyanobacteriochromes with various spectral properties are the recently emerged photoreceptors that are distinctive relative of the phytochromes. Among them, AnPixJ-GAF2 from cyanobacterium *Anabaena* sp. PCC 7120 is a novel photoreceptor that covalently binds phycocyanobilin and shows green/red reversible photoconversion. It is suggested that the Pr form of AnPixJ-GAF2, which corresponds to that of phytochrome, is photoconverted to unusual blue-shifted green-absorbing form (Pg) via phytochrome-like intermediate states. To get structural insights into this unique photoconversion mechanism, we tried to crystallize AnPixJ-GAF2 in both forms. As a result, we obtained blue crystals of the Pr form by hanging-drop vapor diffusion method. The crystals belong to space group $P4_32_12$ and contain one monomer in an asymmetric unit. The crystal structure was solved at 1.8 Å resolution by iodide-SAD method. The overall structure and the chromophore structure of the Pr form are very similar to those of the Pr forms of bacterial phytochromes, although relative position of the chromophore to the apoprotein is significantly deviated. In correspondence with the deviation, amino acid residues surrounding the chromophore are quite diverged. Nevertheless, we can point out a common structural feature conserved between the two Pr forms. Moreover, some residues unique to AnPixJ-GAF2 are suggested to be crucial for the formation of the unusual Pg form. These results shed light on the universal and unique aspects of photosensory mechanism of phytochromes and cyanobacteriochromes.

Keywords: cyanobacteria, photoreceptor, phytochrome

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Fes kinase structure reveals cooperative interactions between SH2-kinase domains and substrate

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The Fps/Fes cytoplasmic tyrosine kinase was originally identified as a transforming protein encoded by avian (Fps) and mammalian (Fes) retroviral oncogenes, in which retroviral Gag protein sequences are fused to the N-termini of cell-derived Fps/Fes gene products. Peptide insertions to the viral oncoprotein led originally to the identification of the SH2 domain which exerted positive effects on the activity and substrate specificity of the adjacent tyrosine kinase domain, through a proposed intramolecular interaction. However, structures of Src family members revealed an inhibitory function of the SH2 domain, which binds to the C-terminal tail of these kinases, locking them in an inactive state by docking of the SH2-SH3 domain to the catalytic domain. Fes does not contain a SH3 domain and a C-terminal phosphorylation site and the mechanism of its activation by the SH2 domain remained enigmatic. In order to understand the molecular basis for Fps/Fes regulation, we solved the structure of a polypeptide containing the SH2 and kinase domains of human Fes. In its active conformation, the N-terminal region of the SH2 domain interacts with the N-lobe of the kinase domain and positions the αC helix in an active configuration. SH2 mutations that perturb this interface inhibit kinase activity, and the absence of an SH2 ligand destabilizes the active SH2-kinase conformation. The activation segment of the kinase domain can be ordered both by autophosphorylation and by binding to a substrate peptide, and substrate phosphorylation is enhanced by the presence of an appropriately spaced SH2-binding site on the same peptide. We present a model in which the active state of the Fps/Fes kinase results from a series of cooperative interactions between the SH2-kinase domains, and substrate.

Keywords: oncogenes, enzyme structure mechanism, biological crystallography

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Development of superagonist ligands for the vitamin D nuclear receptor, AMCR277A, -B and 2MeAMCR

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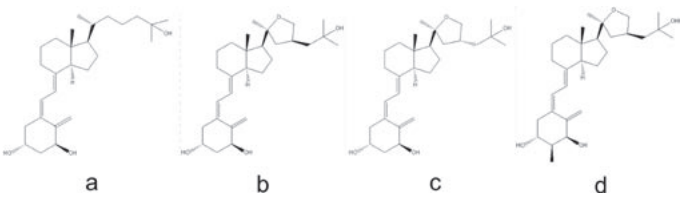
Vitamin D Receptor (VDR), a ligand-dependent transcriptional regulator, is an important target for multiple clinical applications like osteoporosis and cancer. However, high level of the natural ligand, 1alpha,25-(OH)2D3 (Fig. a), induces hypercalcemia. In order to minimize this side effect, chemical modifications have been made on the natural ligand. Based on the crystal structures of human VDR (hVDR) bound to 1alpha,25-(OH)2D3, superagonist KH1060, 2alpha-methyl vitamin D, we designed three new vitamin D analogues, AMCR277A, AMCR277B and 2MeAMCR (Fig. b, c and d, respectively). The crystal structures of hVDR bound to AMCR277A, -B and 2MeAMCR were solved at 2.0, 1.8 and 1.9 angstrom, respectively. Compared to the natural ligand, the three compounds make additional van der Waals (VDW) contacts with Val300 of hVDR. These contacts have been also found in the other superagonist-hVDR structures. The modified methyl group of 2MeAMCR at position C-2alpha of the A-ring makes additional VDW contacts. Therefore, the 2MeAMCR inherits structural features of both AMCR277A and 2alpha-methyl vitamin D. In addition, in vitro assays showed that AMCR277A and 2MeAMCR exhibit superagonist activity.



Keywords: nuclear receptors, vitamin D, drug discovery and