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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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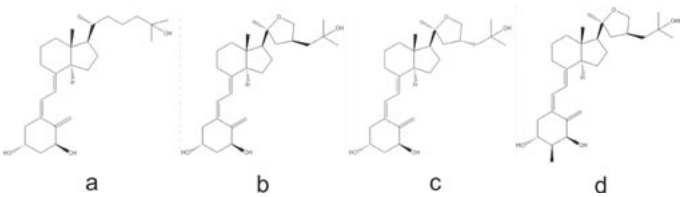
Vitamin D Receptor (VDR), a ligand-dependent transcriptional regulator, is an important target for multiple clinical applications like osteoporosis and cancer. However, high level of the natural ligand, 1alpha,25-(OH)2D3 (Fig. a), induces hypercalcemia. In order to minimize this side effect, chemical 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

design

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Crystal structure of p62 ubiquitin associated (UBA) domain

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Posttranslational modification by ubiquitin regulates a broad range of cellular activities such as protein degradation, transcriptional regulation, endocytosis, and DNA repair. p62 is one of the proteins which is known to recognize poly-linked ubiquitin chains through the ubiquitin associated (UBA) domain. Physiological function of p62 is implicated in the formation of protein inclusions which can be observed in neurodegenerative diseases such as Huntington's disease. p62 is known to accumulate in ubiquitin-positive inclusions of polyubiquitinated proteins, and p62 protein lacking the UBA domain failed to form inclusions in HEK cells, suggesting the important role of the p62 UBA domain in the formation of those inclusions. We solved the crystal structure of the p62 UBA domain at 1.4 angstrom resolution. Phases were obtained by MAD technique using the selenomethionine derivative of the p62 UBA domain. The structure of the p62 UBA domain was a homodimer in a crystallographic asymmetric unit. Two molecules form a dimer with a large hydrophobic interface. Results of analytical ultracentrifugation and NMR spectroscopy strongly supported that the p62 UBA domain also adopts a dimer configuration under an aqueous condition. We further examining how this dimeric structure changes upon ubiquitin binding and whether the dimerization affects the binding to the specific poly-ubiquitin chains.

Keywords: ubiquitin associated domain, polyubiquitin, protein inclusion

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Crystal structure of human DAAM1 formin homology 2 domain

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Reorganization of actin filaments is an essential process for cell motility, cell-cell attachment, and intracellular transport. Formin protein family promotes nucleation and elongation of the actin filament, which is catalyzed by the conserved Formin-homology 2 (FH2) domain. FH2 forms a dimer and directly binds to the barbed end of the actin filament. The active dimeric FH2 structure has been reported in yeast formin, Bni1p, but not in any mammalian formin. Dishevelled-associated activator of morphogenesis (DAAM) is a Rho-regulated formin implicated in neuronal development. To elucidate the mechanism of the actin filament assembly by mammalian FH2, we crystallized human DAAM1 FH2. The native crystal belongs to the triclinic space group $P1$, with unit-cell parameters a = 69.2 Å, b = 91.9 Å, c = 97.7 Å, α = 98.1°, β = 90.3°, γ = 104.8°, and diffracts to 2.8 Å resolution. The structure was solved by multiple-wavelength anomalous dispersion method using the SeMet-labeled crystal, and refined to an Rfree value of 28.9% at 2.8 Å resolution. The present DAAM1 FH2 structure consists of five subdomains (termed as "lasso", "linker", "knob", "coiled-coil", and "post"), and forms a dimeric ring in a head-to-tail manner similar to that of Bni1p. In contrast, the orientation of the FH2 dimeric ring was remarkably different between DAAM1 and Bni1p. Further docking analysis of the DAAM1 FH2-actin filament complex suggests that the dimeric ring should be expanded by elongation of the linker subdomain. We showed importance of the linker length by pyrene-labeled actin assembly assay using mutants with various linker lengths. To understand the Rho-regulated actin filament assembly by DAAM1, crystallization of the full-length DAAM1 is now under way.

Keywords: actin, GTP-binding proteins, cytoskeleton

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Crystallographic study of the ubiquitin-binding zinc finger domain of human polymerase eta

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Y-family DNA polymerases play central roles in replication across a wide variety of distorted DNA lesions in a process known as translesion synthesis (TLS). TLS polymersases, however, show relatively low fidelity compared with replicative DNA polymerases and their activity thus needs to be tightly regulated in order to avoid error prone replication due to their low fidelities. Switching from normal DNA replication to TLS is mediated by monoubiquitination of proliferating cell nuclear antigen (PCNA) on K164. Although polymerase eta can bind directly to PCNA, recent studies revealed that monoubiquitination of PCNA enhances their interaction through novel ubiquitin-binding Zn finger (UBZ) domains or ubiquitin-binding motifs (UBM). The crystal structure of UBZ of human polymerase eta at 1.65 Å resolution shows that UBZ domain is composed of beta-beta-alpha fold forming a classic CCHH-type zinc finger and the outer surface of the C-terminal helix provides a possible site for ubiquitin interaction.

Keywords: DNA repair, zinc fingers, ubiquitin system