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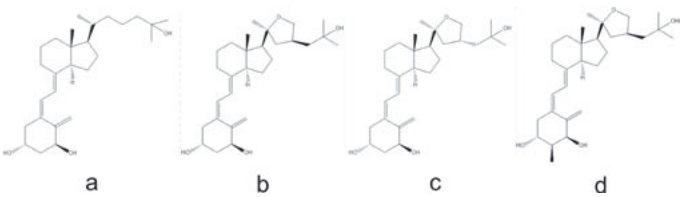
Vitamin D Receptor (VDR), a ligand-dependent transcriptional regulator, is an important target for multiple clinical applications like osteoporosis and cancer. However, high level of the natural ligand, 1alpha,25-(OH)2D3 (Fig. a), induces hypercalcemia. In order to minimize this side effect, chemical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