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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