1P017
Crystal structure and molecular characterization of NEAT domain from Staphylococcus aureus

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Iron transport into the cell is critical for the growth of an organism. Pathogenic bacteria, which survive within an animal, are able to sequester iron from the iron-containing proteins of the host. It was reported that pathogenic gram-positive bacteria acquire nutrient iron from its host using iron-regulated surface determinant (Isd) system. In S. aureus, four proteins, IsdA, IsdB, IsdC, and IsdH, compose Isd system, and each protein contains commonly one or a few NEAT (NEAr Transporter) domains. IsdB and IsdH are especially to be addressed due to their potential as a vaccine against S. aureus infection. Extensive studies focused on these proteins have been done so far, and recently the solution structure of one of the NEAT domain, NEAT1, in IsdH has been reported. Although IsdH contains three NEAT domains, NEAT1, NEAT2, and NEAT3, NEAT3 shares little sequence identity with other two domains, suggesting that the function of NEAT3 is distinct from others. To investigate its function from structural viewpoint, we determined the crystal structure of NEAT3 in IsdH and characterized its heme binding property. In this conference, we will discuss its function based on the results from structural and biological analyses.

1P018
Structures of binary complex of human BACE1 (β-secretase)

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The BACE1 (β-secretase) cleaves the amyloid precursor protein (APP) into the amyloid β-peptide, which is the primary constituent of amyloid plaques found in the brain of Alzheimer’s disease victims. Thus, BACE1 is a major target for the screening of inhibitors since it occupies an initial step in the pathological cascade of Alzheimer’s disease. Recently, we were able to crystallize and determine the crystal structure of BACE1 in complex with ligands and inhibitors. These structures show significant conformational change in the cleft of active site. Different/new binding modes in complex structures are also observed. Here we will present our most recent data and discuss the analysis of the complex structures of BACE1, highlighting insights into the structural changes and mechanisms of this enzyme’s specificity.

1P019
Crystal structure of histidine kinase and response regulator complex

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Prokaryotes, fungi and plants exploited a signal transduction mechanism termed two-component system to sense and respond sudden changes in environments. The system consists of a sensor histidine kinase (HK) and a response regulator (RR). HK is composed of a sensor domain, a dimerization domain (DHp), and a catalytic domain (CA). In response to sensing signals in the sensor domain, CA phosphorylates a special His residue in the DHp, and the phosphoryl group is subsequently transferred to a special Asp residue of the RR. The final output from the two-component system is the interaction of the phosphorylated RR with specific proteins or with specific DNA motifs. Since the signal transduction of HK/RR complex is accomplished through inter-domain and inter-molecular interactions, we need to know the structure of the full-length HK or its complex with RR. We previously determined the structure of HK/RR (ThkA/TrkA) complex from hyperthermophilic at low resolution. To elucidate the molecular mechanism of signaling pathway in detail, we determined crystal structures of PAS-sensor (ScMet), CA (Hg complex) of ThkA and TrkA (ScMet) at 1.70, 1.63, and 1.65 Å resolution, respectively, and assigned these structures and DHp (built model from other HK) into HK/RR complex structure at 3.7 Å resolution based on Se and Hg atom position. FG-loop region of PAS-sensor, which is proposed as a switching region, interacted with CA. Moreover, α-helix of TrkA, which occurs the conformational change upon phosphorylation, interacted with PAS-sensor.

1P020
Crystal Structure of Wild type Putidaredoxin from Pseudomonas Putida

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The [2Fe-2S]-containing ferredoxin, putidaredoxin (Pdx), carries two electrons sequentially from the putidaredoxin reductase to the acceptor cytochrome P450cam in the catalytic cycle of camphor hydroxylation of Pseudomonas putida. Oxidized wild-type Pdx had been believed to be unstable protein and thus many efforts to crystallize the wild-type Pdx had failed. Recently, it was reported that Cys73 mutants of Pdx improved its stability and crystallized. Our highly purified wild-type Pdx (Rz > 0.6) kept on being more stable than the previously reported and was successfully crystallized at 25°C. Rod-like crystal clusters appeared in a few days and belonged to the tetragonal space group P4,2,2. The crystal structures of the oxidized and the dithionite-reduced forms of wild-type Pdx were solved to 1.75 Å and 1.70 Å resolution using synchrotron radiations at -180°C. The crystal structure of the oxidized wild-type Pdx was quite similar in overall fold to that of the reduced wild-type Pdx, which was performed by reduction of the oxidized Pdx crystal using sodium dithionite. The carbonyl oxygen atom of Cys45 in the Cys45-Ala46 peptide bond was directed toward the [2Fe-2S] cluster in the oxidized Pdx, whereas the carbonyl oxygen atom of Cys45 was found to point away from the [2Fe-2S] cluster in the reduced form. This indicated that photooxidation of the oxidized wild-type Pdxs did not occur during exposure to powerful synchrotron radiation, contrary to the recently reported crystal structures of Cys73Ser/Cys85Ser and Cys73Ser Pdx mutants.