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STRUCTURAL BASIS OF THE RADIXIN FERM DOMAIN COMPLEXED WITH ITS INTERACTIVE PROTEINS

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Radixin is a member of the ezrin-radixin-moesin (ERM) family of proteins, which play a key role in the formation of the membrane-associated cytoskeleton by linking actin filaments to plasma membranes. Members of this family possess the conserved N-terminal regions, which contain the FERM (4.1 and ERM) domain consisting of about 300 residues. The FERM domain interacts with membranes and membrane-integrated adhesion molecules such as CD44, CD43, and ICAMs. These adhesion molecules are key players in cell-extracellular matrix adhesion controlling cell growth, cell-cell repulsion and adhesion important for cell-cell communication, respectively. The FERM domain also binds cytoplasmic proteins regulating an ion channel activity. One such protein is the PDZ-containing adaptor protein, Na^+/H^+ exchanger regulatory factor (NHERF), which binds the ion channel NHE through the PDZ domain and modifies the activity. Here, we have identified the FERM-binding region of NHERF with optical biosensors based on surface plasmon resonance and determined the crystal structure of a complex between the radixin FERM domain and the FERM-binding region of NHERF at 2.5 Å resolution. The complex structure is compared with those of the FERM/IP3 (1) and FERM/ICAM-2 complexes.

(1) Hamada, K., Shimizu, T., Matsui, T., Tsukita, Sh., Tsukita, Sa., Hakoshima, T. (2000). Structural basis of the membrane-targeting and unmasking mechanisms of ERM proteins revealed by the crystal structures of the radixin FERM domain. EMBO J. 19, 4449-4462.

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CRYSTAL STRUCTURE OF THE EPSTEIN-BARR VIRUS PROTEASE

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Epstein-Barr virus (EBV) belongs to the family of γ-herpesviruses. The protease domain of the assemblin protein (ORF UL26) of EBV has been expressed in *E. coli*. We solved the structure of the protease by X-ray crystallography to 2.3 Å resolution after inhibition of the protease with diisopropyl-fluorophosphate (DFP). The structure was solved by molecular replacement and refined using merohedrally twinned data with a twinning fraction above 40 %. The overall structure confirms the conservation of the structure and the dimer formation throughout the family of herpesviruses. The substrate recognition could be modelled using information from the bound inhibitor, from a crystal contact suggesting that the substrate forms an antiparallel β-strand extending strand β2 and the comparison with the peptidomimetic inhibitor bound to a cytomegalovirus protease structure. 2 long, partially ordered loops are of particular interest: L2 which comprises strictly conserved residues shows the presence of the small helix AA as observed for CMV, VZV and HSV protease. Leu25 contributes to the P3 subsite of the substrate binding site. Loop L9 contributes to the substrate recognition and in contrast to previous structures, it got partially ordered probably due to the DFP inhibition. The protease shows a previously unrecognised recognition of the C-terminus of the protein in a specific binding pocket involving among others residues Phe210 of the second monomer interacting across the dimer interface. This suggests conformational changes of the protease domain after its release from the assemblin precursor followed by burial of the new C-terminus and a likely effect onto the monomer-dimer equilibrium.

Keywords: VIRUS PROTEASE TWINNING

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CRYSTALLOGRAPIC STUDIES OF THE N TERMINAL DOMAIN OF THE BLUETONGUE VIRUS NS2 PROTEIN

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The nonstructural protein, NS2, of Blutongue Virus (BTV) is a nonspecific single-stranded RNA-binding protein and the major constituent of viral inclusion bodies, which are the sites of BTV synthesis in virus, infected cells. The RNA-binding domain has been mapped to the N terminus of NS2. Due to its RNA-binding abilities, NS2 is believed to play an important role in the selection and condensation of the mRNA segments into precursor viral particles. We had been able to grow crystals from the controlled proteolysis of a construct with a high specificity TEV protease cleavage site inserted between the N and C terminal domains of the protein. A complete data set for the N terminal (RNA binding) domain was collected to a resolution of 2.4 Å on the X13 beam line at the EMBL Hamburg (λ = 0.80 Å, 193444 observations of 18244 unique reflections, Rmerge0.058 with 99.9% completeness). The space group is $P6_5$ (a = 102.3 Å, c = 77.92 Å) with a dimer in the asymmetric unit. Crystals of the selenomethionine containing protein have also been grown and these crystals diffract to 2.8 Å resolution. Data has been collected on the X11 beam line at the EMBL Hamburg to 2.8 Å (λ = 0.85Å, 140366 observations of 11475 unique reflections, Rmerge0.076 with 98.8% completeness) and to 4 Å at the selenium peak wavelength on BM14 at the ESRF (λ = 0.97Å, 27567 observations of 3940 unique reflections, Rmerge 0.100, with 99.8% completeness). Fourteen of the sixteen expected selenium atoms have been located and structure solution is in progress.

Keywords: NS2, CRYSTAL, STRUCTURE

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ASSEMBLY MECHANISM OF A DOUBLE-SHELLED VIRUS, RICE DWARF VIRUS

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Rice dwarf virus (RDV) is a member of the genus Phytoreovirus in the family Reoviridae possessing 12 segments of dsRNA genome within an icosahedral double-shelled particle. The outer capsid shell consists of 780 subunits of 46kDa protein P8. The inner capsid shell consists of 120 subunits of 114 kDa protein P3. This double-shelled capsid packages 12 segments of dsRNA, P1; a putative RNA-dependent RNA polymerase, P5; a putative guanylyltransferase and P7 proteins; an RNA-binding protein. A 26 Å resolution electron map reconstructed from cryoEM was used as a starting model, and extended to 3.5 Å resolution using the fifteen-fold non-crystallographic symmetry and solvent flattening. The resulting maps were unambiguous, and models for 2 subunits of P3 and 13 subunits of P8 in an icosahedral asymmetric unit were constructed using program O and refined at 3.5 Å resolution by CNS. The structure of P3 subunit forms a large, plate-like structure similar to the other reovirus inner capsid proteins. P3 subunits form dimers of two conformational states, and thus can fit into two distinct local environments. This atomic structures of P3 subunits revealed the mechanism of this conformational switching in the inner capsid shell. P8 subunits form trimers, which is organized on a T = 13l icosahedral lattice. P8 monomers are divided into the upper domain of the jellyroll motif and the lower domain constructed from mainly α-helices. Intra- and inter-P8 trimer, and inter-layer contacts revealed the assembly mechanism and the organization of viral capsid components to make a whole virus particle.

Keywords: VIRUS STRUCTURE MACROMOLECULAR ASSEMBLY RICE DWARF VIRUS