pumps are proposed, which visualizes how MFPs link TolC and inner membrane transporters bridging the inner and outer membranes.

Keywords: MFP, efflux pump, MacA

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Structural basis of regulatory inactivation of DnaA

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Regulatory inactivation of DnaA (RIDA) is an important mechanism to prevent over initiation during bacterial chromosomal replication. RIDA is dependent on the protein Hda and the sliding clamp of DNA polymerase III. Hda, a member of the AAA+ ATPase superfamily and a homolog of DnaA, can trigger the transformation of DnaA from the active DnaA-ATP form into the inactive DnaA-ADP, when bound to the sliding clamp loaded on the duplex DNA. The crystal structure of the dimeric Hda from Shewanella Amazonensis SB2B was determined to 1.75 Å resolution by X-ray crystallography. The arrangement of the two domains in Hda differs dramatically from that of DnaA, despite structural similarities within each domain. A CDP molecule, bound to Hda, anchors the two domains in a conformation which promotes dimer formation. The Hda dimer displays a novel mode of oligomeric assembly for AAA+ proteins in which the arginine finger (Arg 161), that is crucial for ATP hydrolysis, is fully exposed. The structure suggests that Hda has not retained the ability to bind and hydrolyze nucleotide triphosphates. However, the dimer arrangement is compatible with an Hda-DnaA interaction, similar to the DnaA self-assembly at the origin of replication, which allows Hda to hydrolyze DnaA-ATP through a typical AAA+ type mechanism. Two sliding clamp binding motifs at the N-terminus (13-18) of Hda form an antiparallel β-sheet at the dimer interface. This surface could interact with the sliding clamp through hydrophobic interactions. A model for the Hda-DnaA, sliding clamp and duplex DNA complex is proposed. The JCSG is funded by NIGMS/PSI, U54 GM074898. SSRL operations are funded by DOE BES, and the SSRL SMB program by DOE BER, NIH NCRR BTP and NIH NIGMS.

Keywords: regulatory inactivation of DnaA (RIDA), Hda, DNA replication initiation

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Crystallographic analysis of the Phycobilisome antenna complex: Assembly and disassembly of a giant

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In cyanobacteria and red algae, the major light harvesting pigment-protein complex is the phycobilisome (PBS), an enormous complex with a molecular weight of 3-7MDa. Three dimensional structures of isolated components have been determined by X-ray crystallography for a variety of species; however our understanding of the overall structure of the PBS is still dependent on low resolution studies. We describe here high resolution structural information obtained on isolated components of the PBS from the thermophilic cyanobacterium T. vulcanus: trimeric phycocyanin (1.4Å), rod phycocyanin (MW of ~500kDa) in the presence of linker proteins (1.8 Å) and trimeric allophycocyanin (2.9 Å). These structures shed light on the process of PBS energy transfer mechanisms, on its ability to self-assembly and on its resistance to thermal or chemically induced denaturation. We have isolated, purified, functionally characterized and crystallized intact PBS of a molecular of greater than 3MDa. The crystals diffract to at least 3Å and we will describe our progress in determining its structure. Under certain conditions of nutrient starvation, the PBS is disassembled in an ordered manner, and its components are degraded as a secondary source of metabolites. We report here the determination of the three-dimensional structures of the NblA protein (2.5Å), an essential component in the disassembly process, from two cyanobacterial species (T. vulcanus and S. elongatus). Random mutagenesis in the S. elongatus protein shows that critical residues affecting the disassembly process in vivo can be found at internal positions as well as at the polypeptide termini leading to a model for the NblA proteins mode of action which is different than previously suggested.

Keywords: macromolecular assemblies, photosynthesis, biological structure-activity relationships

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A topological model of the baseplate of lactococcal phage Tuc2009

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The knowledge on phages infecting gram (+) bacteria and belonging to the Siphoviridae family lags behind that accumulated for members of Myoviridae. The receptor binding proteins (RBPs) are 20-30 kDa proteins that are attached to the baseplate, a large phage organelle, located at the tip of the non contractile tail. These proteins allow the recognition of a host cell wall receptor by the phage. The 3D structures of the RBPs of three phages infecting Lactococcus lactis have been determined recently (1-3). Tuc2009 phage baseplate is formed by the products of 6 ORFs, including the RBP (4). Since phage binding to its receptor induces DNA release, it has been postulated that the baseplate might be the trigger for DNA injection. Structural features of the Tuc2009 phage baseplate were established using blue native PAGE and also size exclusion chromatography coupled to on-line UV/VIS absorbance, light-scattering and refractive index detection (MALS/UV/RI). Using this latter system, we determined the self-association and the inter-association of the baseplate components, the stoichiometry of the interacting components and we also measured their hydrodynamic radii. We will present how the results obtained with this approach and combined with literature data led us to propose a “low resolution” model of Tuc2009 baseplate (5). We will also show how this strategy could be helpful to submit relevant complexes to crystallization trials for Tuc2009 in particular and for phages in general.
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