Microsymposia

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Activation of the complement cascade involves multiple proteolytic reactions mediated by large macromolecular complexes. Upon initiation, the three complement pathways converge into the critical step of the conversion of C3 to its activated form C3b by means of short-lived enzymatic complexes called C3 convertases. These convertases amplify C3b production near target surfaces, resulting in opsonization of target cells, activation of the complement terminal pathway and stimulation of the adaptive immune response. Generation and activation of complement convertases are multi-step processes that require localization of serine protease-containing proenzymes on large protein subunits. The interplay between formation of large multi-domain enzymes, extensive conformational changes and specific proteolytic cleavages highlights the complexity of the complement system.

We investigated the process of convertase formation in the alternative pathway of complement. In this pathway, the C3 convertase complex is generated when the proenzyme factor B (FB) interacts with surface-bound C3b to form the pro-convertase C3bB, which is then specifically cleaved by the soluble, self-inactivated serine protease factor D (FD), yielding the active yet unstable C3 convertase C3bBb. We present here the crystal structures of the pro-convertase complex C3bBb, formed by C3b (12 domains, 160 kDa) with factor B (5 domains, 90 kDa) at 4-Å resolution and the transient complex formed by C3bB with an inactive mutant of factor D (1 domain, 27 kDa) at 3.5-Å resolution. In agreement with previously published electron microscopy data, our structures highlight the equilibrium between an initial (closed/loading) state and a subsequent (open/activation) state of factor B. The structures reveal unexpected conformational changes that create the “open” state which exposes the scissile loop of FB for proteolytic cleavage. In the C3bBb complex, we show how the open state of FB provides a docking platform for factor D distant from its catalytic site, which has been caught in an activated conformation.

Additional biochemical and biophysical analyses confirm the observed structural features and reveal a highly concerted and specific activation mechanism based on cofactor-dependent and substrate-induced proteolysis, which provides an important “double-safety” catch to restrict complement amplification to C3b-tagged target cells. By adding new frames to the “structural movie” of complement activation, our data also provide new valuable information for potential structure-based drug design of complement inhibitors.

Keywords: complement, immunology, proteolysis

MS.01.5

Crystal structure of the Usher:Chaperone:Adhesin subunit complex - insights into pilus assembly mechanism

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Gram-negative bacteria express different classes of adhesive surface organelles that allow them to specifically bind and infect human cells. Among them, the chaperone-usher (CU) fibers constitute the most abundant group of bacterial cell surface appendages and have been the most extensively studied [3]. Type 1 pili are the archetypal representative of this class of adhesive multisubunit fibres. During pilus assembly, subunits dock as chaperone-bound complexes to an usher platform which catalyses their polymerization and mediates pilus translocation across the outer membrane. We report the 2.8 Å crystal structure of the usher (FimD) in its active conformation, bound to the chaperone (FimC) and adhesin subunit (FimH) complex [1]. The structure shows the adhesin subunit inserted inside the usher 24-stranded-barrel translocation channel, held in place through interactions with the two C-terminal periplasmic domains of the usher (CTD1 and CTD2). To accommodate the adhesin subunit, the usher plug domain is displaced from the barrel lumen to the periplasm, concomitant with a dramatic conformational change in the β-barrel [2]. The displaced plug positions the N-terminal domain of the usher (NTD) in an ideal conformation to catalyse incorporation of the next recruited chaperone: subunit complex. The usher:chaperone:adhesin subunit (FimD:FimC:FimH) structure captures the first view of a membrane protein transporter in the act of secreting its cognate substrate, giving insight into the molecular mechanism of the pilus assembly and certainly will serve as a new basis for drug-design.


Keywords: microbial, transporter, pilus

MS.02.1

Cryo–EM of the ribosome–SecYEG complex in nanodiscs

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The ubiquitous SecY/Sec61–complex translocates nascent secretory proteins across cellular membranes and integrates membrane proteins into lipid bilayers. Several structures of mostly detergent solubilized Sec–complexes have been reported. Here, we solved a single–particle cryo–electron microscopy structure of the SecYEG complex in a membrane environment at sub–nanometer resolution, bound to a translating ribosome. Using the SecYEG complex reconstituted in a so–called Nanodisc, we could trace the nascent polypeptide chain from the peptidyl transferase center into the membrane. In order to build a near complete molecular model we used existing X-ray structures, homology models and Molecular Dynamics-based models of the nanodisc, and docked them into our...