Insight into pilus and non-pilus biogenesis from molecular dynamics studies

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Motivation

The analysis of protein-protein interactions involved in the formation of large macromolecular assemblies is one of the most investigated topics in structural biology. Frequently, protein-protein association is mediated by the exchange of well-defined secondary structural elements. In the assembly of multi-subunit fibres on the external cell surface of pathogens such as Escherichia coli and Haemophilus influenzae, key molecular events involve the addition of a -strand onto a -sheet or -strand of the receptor domain (Remaut H, Waksman G. Trends Biochem Sci. 2006. 31:436-44). The biogenesis of these fibres, which are essential to the bacterial pathogenicity, is driven by a complex protein secretion system denoted as chaperoneusher pathway. Several gene clusters encoding proteins involved in the fibre formation through the chaperone-usher pathway have been identified and described. Of these, the Pap gene cluster (PapA-PapK) of the uropathogenic E. coli involved in pilus biogenesis can be regarded as the prototype of this protein family.

A crucial role in this process is played by the chaperone PapD and by the PapE pilin subunit, which exhibits an IG-like fold with a missing strand. The missing complementary G strand is donated by the chaperone during pilin folding and by adjacent pilin subunits in the final fibre. Understanding the atomic details of the mechanism of fibre formation is also important for the development of new therapeutic agents (Pinkner et al. PNAS U S A. 2006. 103 :17897-902). In order to obtain a detailed picture at atomic level of the molecular events related to pilus biogenesis, we undertook molecular dynamics studies of the non-canonical imuno-globulin-like PapE in its unliganded state and in complex with both the G1 strand of the chaperone PapD and with the N-terminal end of the pilin PapK. These studies have been have extended to non-pilus systems, such as the F1 antigen of the plague pathogen Yersinia pestis.

The F1 antigen is a fibre built up of several copies of IG-like domains (named as Caf1). Since no structural information is available for this fibre, efforts have also been devoted to investigate the assembly of the Caf1 building blocks of the F1 antigen.

Methods

The coordinates of the starting models of PapE subunits were derived from the structure of the complex of PapE with the chaperone PapD (PDB code 1n0l) and from the crystal structure of the complex between PapE and the N-terminal strand (residues 1-11) of PapK (PDB code 1n12). Coordinates for Caf1 were extracted from the complex of the chaperone Caf1M with two Caf1 subunits (PDB code 1P5U). The coordinates of the starting model of the Caf1 dimer were derived from the same PDB entry by removing the coordinates of the entire chaperone Caf1M. Model building was used to reconstruct the missing regions, typically loops, of the models. MD simulations were performed using the GROMACS software package. For each model, MD simulations were carried out over a time scale of 20 ns. Electrostatic interactions were treated using the Particle Mesh Ewald (PME) method.

Results

Our results showed that the equilibrated structure of unliganded PapE, which is difficult to characterise experimentally, displayed unexpected features. Indeed, a significant rearrangement of the local structure of the groove, which hosts the complementary strand from an adjacent pilin, was observed. This reorganisation, characterised by the formation of several new hydrogen bonds, leads to a closure of the groove that likely makes pilin polymerisation more difficult. Altogether, our findings have important implications for the understanding of the strand-donor mechanism since they suggest that, besides helping the folding, the chaperone PapD is needed to avoid quick collapse of the pilin structure towards states which are not prone to polymerise (Vitagliano L, Ruggiero A, Pedone C, Berisio R .J Mol Biol. 2007. 367 :935-41). Simulations of the Caf1 monomer have shown that a similar mechanism of collapse holds for the F1 antigen in the absence of the chaperone Caf1M. Furthermore, simulations of the Caf1 dimer clearly indicate that this system undergoes large structural rearrangements in the early stages of the simulation. This initial evolution is followed by the formation of a complex, which is very stable throughout the rest of the trajectory. The analysis of Caf1 dimer structure emerged from the MD analysis clearly highlights the residues that are important for aggregation. Using this dimer as a building block, larger assemblies were generated. In line with available experimental data, these analyses show that the F1 antigen is formed by linear assemblies of Caf1 subunits.

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