MS08-P02

Structural insight into protein-aided bacterial biofilm formation

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Microorganisms form surface-attached communities, termed biofilms, which can serve as protection against host immune reactions or antibiotics. *Bacillus subtilis* biofilms contain TasA as major proteinaceous component in addition to exopolysaccharides. In stark contrast to the initially unfolded biofilm proteins of other bacteria, TasA is a soluble, stably folded monomer, whose structure we have determined by X-ray crystallography.

In this work, we present a high-resolution crystal structure of soluble, monomeric TasA in its mature secreted form. Despite its apparent homology to camelysins, biochemical experiments suggest that TasA is not an active protease. As a basis for understanding the structural changes occurring during fiber and biofilm formation, the monomer and multimeric forms were investigated in vitro and in vivo by NMR, analytical ultracentrifugation (AUC), and other biophysical techniques. In particular, we analyzed in vitro the transformation of soluble monomeric TasA into two different states, a gel-like form and fibrils. Magic-angle spinning (MAS) NMR applied to biofilms that were generated by adding soluble, monomeric and isotope-labeled TasA to the medium of *B. subtilis* ΔtasA cultures allowed probing of the in vivo situation, revealing the formation of homogeneous TasA fibers as the major proteinaceous extracellular matrix component. Thereby, we characterized the transition of folded TasA into fibrils, both in vitro and in its natural biofilm environment on a molecular level.

Understanding the formation and structure of protective bacterial biofilms will help to design and identify antimicrobial strategies. Our experiments with the secreted major biofilm protein TasA characterize on a molecular level in vivo the transition of a folded protein into protease-resistant biofilm-stabilizing fibrils. Such conformational changes from a globular state into fibrillar structures are so far not seen for other biofilm-forming proteins. In this context, TasA can serve as a model system to study functional fibril formation from a globular state.

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X-ray solution scattering and crystallization experiments towards time-resolved structural studies of the membrane protein transporter, Mhp1

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Secondary-active trans-membrane transporters facilitate the movement of essential substrates, such as neurotransmitters or metabolites, across the cell membrane. Disruption of these vital processes is implicated in severe diseases in humans, e.g. Parkinson's disease. Our aim is to investigate the structural basis of the transport mechanism of secondary-active transporters, using the bacterial sodium-hydantoin transporter Mhp1 [1] as a model system examined by serial time-resolved crystallographic methods. Mhp1 has been previously crystallized in two crystal forms in its outward ($P2_12_12_1$) and inward ($P6_1$) facing conformations, and soaking experiments have so far only shown substrate bound in the outward facing cavity, suggesting that crystal packing may limit the ability of the transporter to complete its full structural cycle in the crystalline state. Interestingly, SEC-SAXS experiments suggest that different buffer conditions result in the formation of differently arranged small oligomers (< 6 monomers) that may result in distinct crystal forms. We are therefore exploring the use of SEC-SAXS as a tool to guide the choice of crystallization conditions in order to obtain crystal packing arrangements that will enable the structural transitions associated with substrate transport.

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