[MS6-03] Elucidation of the ScaC cohesin to ScaB dockerin Type I protein:protein interactions in *Acetivibrio cellulolyticus*

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The cellulosome, a highly elaborate extracellular multi-enzyme complex of cellulases and hemicellulases, is responsible for the efficient degradation of plant cell wall carbohydrates in anaerobic micro-organisms. Cohesin and dockerin recognition pairs are integral to the architecture of the cellulosome. Type I dockerins present in the modular cellulosomal catalytic components bind to cohesins located in primary scaffoldins and this constitutes the basic mechanism for cellulosome assembly. In contrast, type II dockerins located in primary scaffoldins bind to anchoring scaffoldins, contributing to the cell surface attachment of the entire complex. The mesophilic, gram-positive bacterium Acetivibrio cellulolyticus possesses an extremely complex cellulosome which is organized by 16 different scaffoldin that together comprise 41 cohesins [1]. The main components in the organization of A. cellulolyticus cellulosome are the primary enzyme-binding scaffoldin (ScaA), two anchoring-scaffoldins (ScaC, ScaD) and an unusual adaptor-scaffoldin (ScaB). It is now well established that type I dockerins usually display an internal two fold symmetry that supports the evolution of two similar cohesin binding interfaces. Residues that dominate cohesin recognition are usually located at positions 11 and 12 of either calcium binding loops. In ScaB dockerin these residues are conserved in the two duplicated segments (IN) suggesting that this type I dockerin should indeed express a dual binding mode. To understand the mechanisms that modulate the different specificities expressed by A. cellulolyticus cohesin-dockerin complexes, we have solved both the ScaC cohesin 3 complexed with divergent dockerin of ScaB mutated at the first helix and again, but mutated at the third helix. Structural characterization shows that ten key hydrogen bonds between the cohesin and dockerin are conserved in both complexes. The first mutant binds through the third helix at residues 50-61 and the second mutant binds through the first helix at 15-26. Interactions in dockerin mutant one are between the Ile 51 and Asn 52 and in mutant two, Ile 15 and Asn 16, making tight van der Waals interactions with the Leu 34 and Tyr 122 on the cohesin, respectively. A slightly larger surface is buried between the cohesin and dockerin in mutant two (~860 Å2) than in mutant one (~800 Å2). In mutant one the crystal structure is a dimer with tight interactions between the two cohesin modules, burying a surface of 960 Å2 and having 18 H-bonds between some 20 amino acid residues on each cohesin. This dimerisation indicates that cohesins might be tightly packed on the anchoring scaffoldin. However, in mutant two this is not the case. The crystal structure is a dimer with tight interactions between the two cohesion modules, burying a surface of 960 Å2 and having 18 H-bonds between some 20 amino acid residues on each cohesin. This dimerisation indicates that cohesins might be tightly packed on the anchoring scaffoldin. As expected, ScaB dockerin binds the ScaC cohesin through the 3rd helix (residues 50-61), with the Ile 51 and Asn 52 on the dockerin making tight van der Waals interactions with the Leu 34 and Tyr 122 on the cohesin, respectively. About 800 Å2 of surface is buried and there are 10 H-bonds between the two modules.

References:
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