

Diverse ligand-binding domain combinations at the distal end of bacterial RTX adhesins are postal codes for biofilm formation.

Tyler D. R. Vance, Shayan Assaie-Ardakany, Adam P. Sage, Brigid Conroy, Phil Guo, Corey A. Stevens, Laurie A. Graham and Peter L. Davies

Many Gram-negative bacteria produce Repeats-In-Toxin adhesion proteins (RTX adhesins) to facilitate microbial adhesion to a variety of biotic and abiotic substrates. These large, multi-domain proteins anchored on the outer membrane share a common architecture comprised of three regions: 1) an N-terminal cell-membrane-anchor region; 2) a large extension region consisting of tens to hundreds of tandem Bacterial Immunoglobulin-like (BIg) domains; and 3) a C-terminal region containing domains responsible for adhesion and cohesion. Bioinformatic analysis of putative RTX adhesins in various bacterial species show that the first two regions are largely conserved, while the C-terminal regions contain a variety of currently unidentified ligand-binding domains, or domain homologues whose ligands are not yet known. Here we illustrate the variability in C-terminal regions having solved the structure of RTX adhesin ligand-binding domains from two divergent bacteria: the oil-eating bacterium *Marinobacter hydrocarbonoclasticus* and the opportunistic pathogen *Aeromonas hydrophila*. The structure of a sugar-binding domain from the *M. hydrocarbonoclasticus* adhesin was solved by molecular replacement as a PA14-like β -sandwich (1.0-Å resolution). The protein uses Ca^{2+} to coordinate a glucose molecule via the C1 and C2 hydroxyl groups of the sugar. The importance of similar domains in previously studied multispecies biofilms implicates this sugar-binding domain in cohesion between microorganisms, either through binding to surface glycans or exopolysaccharides. The adhesin from *A. hydrophila* was found to contain a von Willebrand Factor A-like (vWFA) domain within its C-terminal region. The structure was solved using SAD phasing, with Ca^{2+} as the heavy atom (1.4-Å resolution). A Rossman fold made up the majority of the structure, but an unexpected β -sandwich domain was found inserted between secondary structure elements. The predicted metal-ion-dependent adhesive site (MIDAS) contained a calcium ion that was bound to the C-terminus of another vWFA domain within the crystal. The similarity of this domain to known extracellular-matrix-binding proteins, like integrin, leads us to hypothesize that this protein may be involved in adhesion of the pathogen to host tissue. Using the structures of these recombinantly-expressed proteins in concert with a series of biochemical assays, we have begun to identify the ligands for each domain, and understand how differences in domain complement reflect the organism-specific substrate-binding needs.

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