

Structural Analysis of the Saf Pilus by Electron Microscopy and Image Processing

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Abstract:

Bacterial pili are virulence factors important in pathogenesis. Pili are involved in initiating attachment to host cells, which is a key step required to establish successful infections. Here we studied *Salmonella* atypical fimbriae (or Saf pili). The organisation of the subunits in this atypical fibrillum is unknown. In our study, we have used negative stain electron microscopy (EM) and single-particle image analysis to determine the three-dimensional (3D) structure of the *Salmonella typhimurium* Saf pilus formed by SafA, the major pilus subunit. We have found that structures of the Saf atypical fibrillum present globular subunits (~49 Å long, ~25 Å deep and ~22 Å wide) arranged in a row. The subunits are linked to each other through thin, short stretches of density giving a 'beads on a string'-like appearance. Quantitative and objective fitting of the atomic resolution structure of SafA into the electron density maps, linker modelling and energy minimisation have enabled analysis of subunit arrangement and interactions in the Saf pilus. The combined results demonstrate the inherent flexibility of the Saf pilus about the linker regions, which act as molecular hinges allowing a large range of movement between consecutive subunits in the fibre. These results explain why atypical fibrillae are flexible and appear unstructured on bacterial surfaces.