Determining the mechanism of bacterial fibre assembly using non-covalent electrospray ionisation mass spectrometry

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Bacterial fibre assembly

The highly pathogenic *Salmonella enterica* subspecies I produce extra-cellular Saf fibres, composed of β-sheet subunits. The Saf operon codes for four proteins: SafA, the fibre subunit; SafB, the chaperone; SafC, the usher; and SafD, a fibre capping adhesin. In the periplasm of the bacterium, a chaperone-subunit complex exists, whereby completion of a seven-strand immunoglobulin-like fold in a subunit protein is achieved by donation of a β-strand from the chaperone. Fibre assembly is known to occur by a β-strand exchange mechanism, in which an N-terminal extension 'Nte' strand from one subunit is donated to an adjacent subunit molecule, causing subunit polymerisation. This donor strand exchange mechanism causes dissociation of the chaperone-subunit complex and formation of the subunit-subunit complex (and thus fibre) at the site of the usher in the bacterial outer membrane. The details of this mechanism were largely unknown, but it was hypothesised to proceed *via* a ternary intermediate in a concerted manner, whereby the Nte strand 'zippers' into the chaperone-subunit complex, gradually displacing the chaperone β-strand.

Probing the mechanism of DSE using mass spectrometry

Mass spectrometry has been used to monitor the reaction between the chaperone-subunit complex (SafA-SafB) and the Nte peptide of an incoming subunit. Using nano-electrospray ionisation, the reaction has been followed over time (Figure 1), allowing direct observation of the decrease in concentration of the SafA-SafB non-covalent complex, and the subsequent appearance and increase in concentration of the SafA-Nte peptide non-covalent product. The presence of a SafA-SafB-Nte peptide ternary complex has been detected (Figure 1), confirming directly, and for the first time, that the reaction proceeds by a concerted mechanism involving a stable ternary intermediate.

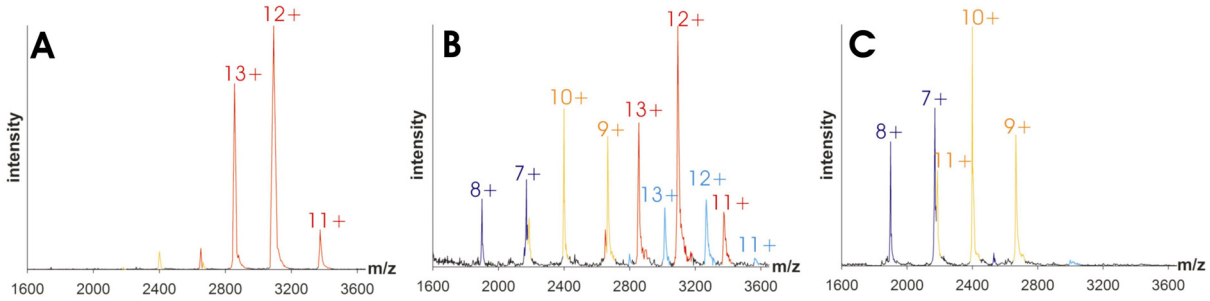

Figure 1. Mass spectra of samples taken during a donor strand exchange reaction between SafA-SafB (red peaks) and wild-type Nte peptide (not shown). (A) Sample prior to peptide addition, (B) 4 minutes after reaction initiation and (C) 24 hours after reaction initiation. These data provide the first evidence of the existence of a ternary intermediate (cyan peaks) during donor strand exchange (B). The products of the reaction, free SafB and SafA-Nte, are shown by the yellow and dark blue peaks, respectively.

Peptides of the Nte strand with single amino acid substitutions, plus variants of SafB with single-point mutations, have also been used to probe the roles of individual residues in the subunit exchange mechanism. The results showed that the reaction rates change significantly depending on nature of the residues at the interface between subunit and chaperone or within the Nte of the incoming subunit (Figure 2). This allowed residues involved in the initial docking site of the Nte to SafA-SafB to be identified, in addition to a capping interaction which stabilises the donor strand exchange product. The data point to a mechanism whereby hydrophobic residues on the N-terminal extension sequentially replace residues of the

chaperone, causing dissociation of the chaperone-subunit complex in a zip-in zip-out mechanism.

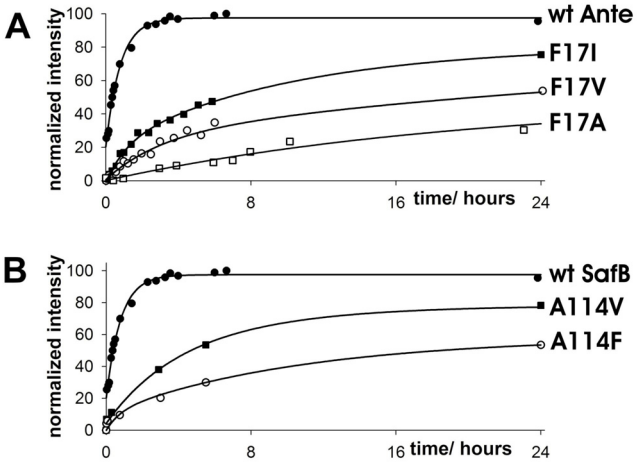

Figure 2. Kinetic traces of donor strand exchange reactions between SafB-SafA and the Nte peptide. (A) The rate of DSE decreases as residue F17 in the Nte peptide is substituted with smaller sidechains; (B) The rate of DSE also decreases as residue A114 in SafB is substituted with larger sidechains, stabilising the SafA-SafB complex. Since F17 binds to SafA at the same location as A114 in SafB, the data suggest that F17 is the initial site of Nte binding.

Current work is focussing on more complex pilus systems, including the P-pilus from *Escherichia coli*, in which six different subunits are assembled in a precise order and copy number. Using non-covalent mass spectrometry techniques, the specificity of the donor strand exchange reactions of each subunit is being monitored, providing detailed mechanistic insights into the manner by which the order of assembly is dictated and controlled.

Collaborators

This work has been in collaboration with Prof. Gabriel Waksman and Dr. Han Remaut from the School of Crystallography, UCL/ Birkbeck College, London. On-going work is in collaboration with this group, including additionally Dr. Denis Verger and Dr. Tina Daviter.

Publications

Remaut, H., Rose, R.J., Hannan, T.J., Hultgren, S.J., Radford, S.E., Ashcroft, A.E. & Waksman, G. (2006) Donor-strand exchange in chaperone-assisted pilus assembly proceeds through a concerted β-strand displacement mechanism. *Mol Cell* **22**, 831-842.

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