The Lability of Quaternary Structure: Cryo-EM of Archaeal Filaments Built from Homologs of Bacterial Type IV Pilin
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While the overall fold of a protein is quite resistant to multiple amino acid changes, we have suggested that quaternary structures are much more labile, and rapid changes in such quaternary arrangements may be an underappreciated mechanism for evolutionary divergence (Galkin et al., 2008). Cryo-EM remains the pre-eminent tool for studying such quaternary arrangements, and increasingly high resolutions are now being achieved for studying helical polymers. We previously used the IHRSR method (Egelman, 2000) to reconstruct Type IV pili (T4P) from Neisseria gonorrhoeae at 12.5 Å resolution (Craig et al., 2006) and suggested that this structure would serve as a good model for all T4P. We can now show that two very different archaeal surface filaments, both formed from homologs of bacterial Type IV pilin, have very different quaternary interactions and helical symmetries both from each other and from the N. gonorrhoeae T4P. We have reconstructed the adhesion filaments from I. hospitalis at better than 8 Å resolution, and the pili from S. acidocaldarius at better than 9 Å resolution. While the I. hospitalis filaments appear to have a rather fixed and constant symmetry, the S. acidocaldarius filaments are quite variable and show a large variability in the axial rise per subunit. Single particle approaches to sorting have allowed us to overcome this heterogeneity. We have sufficient resolution to dock models of the N-terminal T4P α-helices into the cores of these structures. The adhesion filaments appear to be very similar to archaeal flagellar filaments, but have a completely different structure from bacterial flagellar filaments. Since the archaeal flagellar filaments also supercoil, our results suggest that the mechanism for supercoiling a homopolymer must have arisen at least twice by convergent evolution.

Reference List


A side (a) and top (b) view of the three-dimensional reconstruction of the *S. acidocaldarius* pili. When the density threshold is raised (c), it can be seen that the central density is weaker than on the outside. A single α-helix fits very nicely into the tubular densities seen in the inner core of the reconstructed volume (d), consistent with the notion that the hydrophobic N-terminal α-helices are forming this core, with the globular heads on the outside of the filament (Craig et al., 2006).

Surface of reconstruction of *I. hospitalis* lho670 filament from cryo-EM is at ~ 8 Å resolution (a). The core of the *I. hospitalis* filament reconstruction (diameter ~ 43 Å) is fit nicely with a homology model for the first 31 residues that we have generated, based on the bacterial Type IV pilin crystal structure (b). The two aromatic residues (Tyr29,Trp31) are shown in red, and they fit very well into a large bulge at this position. The smallest residues, Ala, are in green, and this is where the density is constricted. We thus have some very strong reality checks. The symmetry of this filament is 106.6° rotation, 5.0 Å axial rise.