Cryo-EM structure of the HD6 defensin helical assembly

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Defensins are small antimicrobial proteins produced by animals, plants, and fungi. HD6 is a human defensin secreted into the small intestine. Unlike other defensins, HD6 is not directly bactericidal but rather self assembles into «nanonets» [1] thought to physically trap bacteria and prevent them from penetrating the intestinal epithelium. The existing model for the nanonet filament structure, a spiral of HD6 dimers proposed based on an HD6 crystal structure, does not fully explain the long persistence length (microns) of HD6 filaments in vitro [2]. To resolve this apparent discrepancy, we determined structures of HD6 nanonet filaments using SPA cryo-EM.

HD6 filaments were produced in phosphate buffer according to a previous protocol [2]. The filaments demonstrated polymorphous behavior, so data were collected at different protein concentrations to favor different polymorphs. Specifically, low concentrations yielded predominantly thin filaments ~40 Å in diameter. Higher concentrations yielded thicker filaments ~80 Å in diameter. Cryo-EM data were collected on a Talos Arctica (Thermo Fisher Scientific) operated at 200 kV. EER movies were collected using EPU software in AFIS mode on a Falcon 4i direct detector at a nominal magnification of 190kx, corresponding to a physical pixel size of 0.73 Å. Image processing was performed using cryoSPARC v4 [3].

HD6 thin filaments were found to consist of stacked pairs of dimers spiraling along the helix axis with apparent D2 symmetry. Strong densities were observed along the axis and were interpreted as phosphate ions, each coordinated by multiple copies of a histidine, H5, in HD6. Mutation of H5 abolished filamentation. Once maps of sufficient resolution were obtained, a symmetry break became apparent: the two spirals of dimers in the thin filaments were found to be related by two-fold symmetry perpendicular to the helix axis, but not by true two-fold symmetry along the axis as would have been expected for D2. The repeating unit of the thin filament consists of two stacked HD6 dimers, with one protomer out of the four displaying a loop in a slightly different conformation than in the other three molecules. The thick filaments, in turn, formed by wrapping two additional HD6 dimer spirals around the thin filament without coordination of additional phosphate. The outer spirals adopt D2 symmetry and show helical rise and twist that differ from the inner filament.

The robust multiple micron length HD6 filaments are explained by the fact that two or more HD6 dimers constitute each level of the supramolecular assembly. Moreover, phosphate plays a structural role in stabilizing the filaments. HD6 helical polymorphs arise from an optional wrapping of additional stacked-dimer spirals around the phosphate-coordinating inner filament, and a symmetry mismatch exists between inner and outer spirals.

Keywords:

Cryo-EM, SPA, Defensins, HD6

Reference:

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