Structural Study of the Serratia entomophila Antifeeding Prophage: Three-Dimensional Structure of the Helical Sheath

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The sheath of the Serratia entomophila antifeeding prophage, which is pathogenic to the New Zealand grass grub Costelytra zealandica, is a 3-fold helical tube formed by a 4-fold symmetric repeating motif disposed around a helical inner tube. This structure, determined by electron microscopy and image processing, is distinct from that of the other known morphologically similar bacteriophage sheaths.

The antifeeding prophage (Afp) of Serratia entomophila and Serratia proteamuculans is a naturally occurring virus tail-like structure which delivers a putative toxin molecule that leads to starvation of the New Zealand grass grub Costelytra zealandica (5). Afp is composed of 18 different gene products (molecular masses of 6.5 to 263 kDa). The first 16 open reading frames have orthologues in the insecticidal bacterium Photobacterium phosphoreum and the virulence cassette (PVC) of the marine bacterium Photorhabdus luminescens TTO1 genome (5). Afp and PVCs morphologically resemble a typical R-type bacteriophage (6, 12, 16) However, Afp is the only known phage tail-like protein complex that is not a bacteriocin-protein complex of considerable medical relevance that targets the same or closely related bacterial strains (1, 3, 8, 12). The major component of Afp is a contractile cylindrical outer sheath encasing an inner tube speculated to house the toxin molecule (6). A dome-shaped “head” defines one extremity of the tube, while the other end is attached to a “bell-shaped” structure with a base morphologically similar to the base plate of the T4 bacteriophage tail (9).

Transmission electron micrographs of two-dimensional (2D) projections of negatively stained (Fig. 1A) or frozen-hydrated and vitrified (Fig. 1B) recombinant Afp particles (see Fig. S1 in the supplemental material) were used for computational image analysis. A globally averaged image of the Afp particle in the major configuration (called E here) (Fig. 1C), generated using negatively stained specimens, clearly distinguished the morphology of the various constituent structural parts. Thus, the cylindrical sheath appears to be formed by a periodic structure harboring a distinctive, inverted-V-shaped feature. A minor population of Afp particles displays an alternate configuration (called C here) where, concomitant with contraction of the sheath (averaged axial compression of ~52% [see Table S1 in the supplemental material]), the inner tube, shorn off the bell-shaped structure, is revealed (Fig. 1A) (6). Several other bacteriocins undergo such a high degree of compression, which has been characterized in detail for the tail sheath of bacteriophage T4 (9). We also generated individual global averages for the periodic sheath structure, for the bell-shaped structure, and for the inner tube (Fig. 1C) which provide more accurate dimensions of these different sections (see Table S1 in the supplemental material) than those reported earlier (6).

For a better insight, we determined the 3D structure of the central periodic section of the Afp particle in the E state. A global power spectrum derived from the cryoimages established the structure to be helical with a clear first meridional reflection at 1/78.5 Å and the strongest nonmeridional reflection at 1/118 Å (Fig. 1D). These reflections correspond to the axial rise (Δr) and the pitch of the helix, respectively, and reflect a turn angle (Δφ) of about ±240° (32 helix) for the repeating motif. The correct sign, i.e., the hand, of the helix remains to be determined. Computationally excited overlapping segments of this helical section from images of vitrified and negatively stained Afp particles were subjected to 3D reconstruction using the iterative helical real-space reconstruction (IHRSR) algorithm (2) using the determined helical parameters (see Fig. S2 in the supplemental material). After a few iterations, the presence of an in-plane 4-fold symmetry (C4) was apparent in the density map (see Fig. S2 in the supplemental material), which was then imposed in the subsequent reconstruction exercises. However, no stable solution was forthcoming, even after many (e.g., 30) iterative cycles. This is generally indicative of the presence of heterogeneity in the form of variations in helix translation and/or twist angle (15) in the structure. As a first step, we focused our attention on the pitch value, and following classification (see Fig. S3 in the supplemental material), we found that the majority of the image segments correspond to a pitch of 120 Å. These segments were then selected out of the full data set and led to a stable and refined 3D reconstruction. We also obtained very comparable results for the helical section when images of negatively stained Afp sheath sections were used, thus supporting our computational approach (see Fig. S4 in the supplemental material) and general conclusions about the E state described below.
Figure 2A is an isosurface representation of the density map of the helical Afp sheath in the E state calculated at \( \sim 21.5 \text{ Å} \) resolution (see Fig. S5 in the supplemental material). To the best of our knowledge, a 4-fold rotational symmetry has not been seen for any other contractile T4 bacteriophage tail-like structure, which points to the unique architecture of the Afp sheath. A power spectrum generated using the 2D projection from the final density map, compared to the experimental global power spectrum (Fig. 2D), showed strong agreement, confirming the fidelity of the computational image analysis. The density map displays protein layers, \( \sim 80 \text{ Å} \) thick, that are stacked on each other in a periodic fashion. The uneven outer surface of the sheath is perforated and decorated with \( \sim 35 \text{ Å} \) protrusions. When rendered with a raised threshold, a characteristic feature of the map is a contiguous, high-electron-density region having an inverted-Y-shaped structure (Fig. 2C and E; see Fig. S4 in the supplemental material). At the modest \( \sim 21.5 \text{ Å} \) resolution, the boundary of the repeating subunit cannot be defined. A 25 \( \pm 3 \text{ Å} \)-wide central lumen is seen clearly when viewed along the helix axis (Fig. 2B) and likely represents the pore of the inner tube (see also below). Using scanning transmission electron microscopy (STEM) (see Fig. S6 in the supplemental material), we estimated the averaged molecular mass of the central helical section of an Afp particle to be 9.8 \( \pm 0.4 \text{ kDa/Å} \) (Fig. 3) (14) and that of only the inner tube to be \( \sim 2.5 \text{ kDa/Å} \), based on a relatively small pool of such images. These values translate to a mass contribution of approximately 145 kDa of the subunit whose periodic arrangement forms the outer component of the sheath (i.e., excluding the inner tube) (see Fig. S6 in the supplemental material). This value is not very different from the cumulative mass of the different proteins, i.e., homologous afp2, afp3, and afp4, thought to be involved in Afp sheath formation (5) (see Fig. S7 in the supplemental material).

A paucity of images of the C state (\( \sim 5\% \) of the complete data set) precluded a full, refined 3D reconstruction, but based on the available 2,774 overlapping image segments of the isolated inner tube, a global average was calculated. A plot of contrast variation (Fig. 2F) indicates that the surface is characterized by \( \sim 40 \text{ Å} \) spaced elevated crests and invaginated...
grooves, in agreement with the calculated axial rise of \( \approx 39 \) Å for the subunit (see Fig. S8 in the supplemental material) comprising the tube. Based on these preliminary results, it appears that the helical symmetry of the inner tube is markedly different from that of the sheath.

Our observation that the pitch of the helix in the E state can vary by as much as \( \approx 50 \) Å attests to the flexible nature of the sheath, which is required for compressibility and may be facilitated by the somewhat porous nature of the sheath (Fig. 2). Preliminary deductions (data not shown) based on a small pool

**FIG. 2.** Orthogonal isosurface rendering, at 1 \( \sigma \) (standard deviation) of the computed \( \approx 21.5 \) Å density map of the helical sheath of the vitrified Afp particle viewed normal (A) and parallel (B) to the helix. The images were generated using the software package CHIMERA (13). The arrow indicates a surface perforation. (C) The Afp density map rendered at 3.5 \( \sigma \) to highlight the largest contiguous high-electron-density regions; one circumscribed by a black ellipse is computationally extracted and shown in panel E. (D) Comparison of the experimental, averaged power spectrum of the helical sheath of Afp (left) with that computed (right) from the 2D projection of the calculated density map. (F) Global average of the inner tube of the Afp particle and a plot of the surface density variation (scaled from 0 to 1) along the helical \( y \) axis. The dimension along the tube is plotted on the \( x \) axis.

**FIG. 3.** (A) Dark-field micrograph of a freeze-dried, unstained preparation of Afp particles used in STEM measurements. An Afp particle in the E state, an Afp inner tube with the attached bell-shaped structure, and a tobacco mosaic virus particle, used as a calibration standard, are marked by the arrowhead and the gray and white arrows, respectively. (B) Histogram plot of the measured distributions of mass per unit length corresponding to the uniform periodic section of the Afp particles overlaid with a fitted Gaussian curve produced by using the ORIGIN6 software package.
of images of the C state suggest profound rearrangement of the elements of the sheath. How that translates to extrusion of the toxin remains to be revealed.

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REFERENCES

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