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An Unexpected Twist in Viral Capsid Maturation

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Abstract

Lambda-like dsDNA bacteriophage undergo massive conformational changes in their capsid shell during the packaging of their viral genomes. Capsid shells are complex organizations of hundreds of protein subunits that assemble into intricate quaternary complexes that ultimately are able to withstand over 50 atm. of pressure during genome packaging¹. The extensive integration between subunits in capsids is unlikely to form in a single assembly step, therefore requiring formation of an intermediate complex, termed a procapsid, from which individual subunits can undergo the necessary refolding and structural rearrangements needed to transition to the more stable capsid. Though various mature capsids have been characterized at atomic resolution, no such procapsid structure is available for a dsDNA virus or bacteriophage that undergoes large scale conformational changes. We present a procapsid x-ray structure at 3.65 Å resolution, termed Prohead II, of the lambda like bacteriophage HK97, whose mature capsid structure was previously solved to 3.44 Å². A comparison of the two largely different capsid forms has unveiled an unprecedented expansion mechanism that describes the transition. Crystallographic and Hydrogen/Deuterium exchange data presented here demonstrates that the subunit tertiary structures are significantly different between the two states, with twisting and bending motions occurring in both helical and β -sheet regions. We have also discovered conserved subunit interactions at each 3-fold of the virus capsid, from which capsid subunits maintain their integrity during refolding, facilitating the rotational and translational motions of maturation. Calorimetric data of a closely related bacteriophage, P22, showed that capsid maturation was an exothermic process that resulted in a release of 90KJ/mol of energy³. We propose the major tertiary changes presented in this study reveal a structural basis for an exothermic maturation process likely present in many dsDNA Bacteriophage and possibly viruses such as Herpes which share the HK97 subunit fold⁴.

Main Text

HK97 is a favorable system for studying capsid maturation because capsid particles can be assembled in *E. coli* from the expression of just two viral gene products, gp4 (protease) and gp5 (capsid subunit), and maturation can be triggered and analyzed *in vitro*^{2,5-8}. The 384-residue gp5 subunits assemble into hexameric and pentameric oligomers, termed capsomers, that first assemble to form the Prohead I capsid (P-I). The 17.7-Megadalton, T=7 *laevo* icosahedral particle contains 12 pentamers and 60 hexamers and encapsidates approximately 60 copies of the gp4 protease⁹⁻¹¹. Prohead I particles can be made with either a defective

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protease or without protease and can be disassembled *in vitro* into free capsomers and then re-assembled when exposed to specific chemical treatments¹². When active gp4 is present, the particles spontaneously mature to the Prohead II (P-II) form following digestion of residues 2-103 from all subunits. The proteolytic fragments and auto-digested protease diffuse out of the Prohead II capsid leaving a 13-Megadalton particle with an external morphology closely similar to Prohead I. *In vivo*, the packaging of the DNA genome induces capsid maturation, but *in vitro*, a construct lacking a portal protein necessary for DNA packaging may be induced to expand using chemical or low pH treatments. Constructs lacking the portal were used both for current and previous structural studies. During *in vitro* maturation, the particle expands through discrete intermediates, EI, and Balloon, on the pathway to the mature Head II form (Fig. 1a). The resulting Head II particle has a morphology indistinguishable from the authentic virion. Concomitant with capsid expansion, inter-subunit crosslinks form, topologically interlocking the capsid into protein rings characterized as molecular chainmail^{2,13}. During the maturation, subunit reorganization facilitates a particle expansion from 540 Å (Prohead II) to 660 Å (Head II) in diameter (Fig. 1c). The kinetics of maturation were previously studied using time-resolved solution X-ray scattering¹⁴ and the structures of the intermediates were determined with Cryo-EM^{6,7,15} and X-ray crystallography^{2,5}. Near atomic resolution structures have characterized the late maturation states (Balloon, Head II), but only lower resolution Cryo-EM models were previously available for the Procapsid and EI forms, which used the 3.44 Å Head II structure as a basis for pseudo atomic models. The previous 12 Å resolution Cryo-EM study of Prohead II suggested that the majority of the capsid structural changes in expansion were the result of rigid-body rotations and translations of the central domains of the subunit, while the E-loop and N-arm regions moved independently⁶.

Here we report a 3.65 Å resolution X-ray crystal structure of W336F, E-loop truncated Prohead II (PDB ID: 3E8K) that changes the previous conceptions of capsid maturation (crystallographic statistics listed in Supplementary Table 1). The structure reveals that 3-fold contacts between subunits, mediated by “P-loops” as well as their surrounding β -strands on each subunit, are preserved during maturation from Prohead II to Head II. As a result, the previously proposed rigid subunit motions at lower resolution could now be resolved as domain motions corresponding to a twist of the subunit about three β -strands (β D, β J, and β I) (Fig. 1b), and a simultaneous bending and unwinding of the long (spine) helix with respect to the fixed 3-fold interaction sites. The extent of the subunit twist and the helix bend vary among subunits and depend on their quasi-equivalent position.

The overall morphologies between the Prohead II and Head II states are very distinct. The subunits in Prohead II are oriented radially relative to the capsid surface, but are roughly tangential in Head II (Fig. 1c and 1e). A striking feature of Prohead II, which was seen in the previous Cryo-EM study, is that the skewed hexamers comprised of trimers of subunits with a trapezoidal arrangement give the hexamers a pseudo 2-fold appearance.

The refined P-loop contacts in Prohead II bear a striking similarity to the same contacts in Head II. The P-loop of each subunit is tightly associated with the P-loop of two other subunits from separate capsomers at all 3-fold and quasi-3-fold axes (Fig. 2). In the previous cryo-EM-based model, the P-loop of Prohead II was kept fixed relative to the subunit core, changing the trimer associations when compared to those in Head II. It is now clear that the position and quaternary interactions of the P-loops and surrounding β -strands (region colored blue in figure 2c) are unchanged during expansion, demonstrating that it functions as a fixed point of subunit interaction in an otherwise highly plastic quaternary structure. Figure 2b and c shows salt bridge interactions between Glu 344 and 363 from 2 of the β -strands surrounding the P-loop on one subunit with Arg 194 (located on the turn following the spine helix) and Arg 347 (located on the P-loop) of a neighboring 3-fold related subunit. The salt bridges as well as a putative metal binding site coordinating three glutamate (E348) residues directly underneath each 3-fold axis

(Fig. 2b) remain unchanged during capsid maturation. Three of these residues (R194, E344, and E363) are proximal to the borders of the region that remains fixed during maturation, defining the boundaries of the pivot points of tertiary rearrangement (Fig. 2c). In accord with this newly recognized structural constraint, the tertiary structure of the subunit is now seen to have a significant twist about the P-domain β -sheet (Supplementary Movie 1).

To corroborate the conclusions from the crystallographic data, we characterized the dynamics of the 3-fold P-loop interactions with H^2H exchange coupled to MALDI mass spectrometry on Prohead II, Head I, and free capsomers. The technique measures the solvent accessibility of amide protons (in native proteins in solution) whose rate of exchange with deuterium is influenced by secondary, tertiary and quaternary structure interactions^{17,18}. Following incubation in deuterium, the capsid protein is digested with pepsin protease and the masses of previously determined peptide fragments are quantified. Regions with greater solvent accessibilities will have larger shifts in their mass envelopes, which are quantified as described in the supplementary methods. A K169Y mutant was used instead of WT-Head II for the study since covalent crosslinks inhibited efficient pepsin digestion and subsequent analysis by mass spectrometry. The mutant is able to expand through similar intermediate forms as wild-type Prohead II (Figure 1a), though maturation stops at the penultimate, Head I state, which was shown by crystallography to have very similar subunit tertiary structures as wild-type Head II⁵. The crosslink-defective mutant therefore permitted comparisons of H^2H exchange profiles between subunits in Prohead II and subunits in a virtually mature particle form. H^2H exchange was also performed on capsomers that were disassociated from the Prohead I state and were no longer able to form 3-fold P-loop associations. One of the peptide fragments spanned residues 345-353 of the P-loop (colored lime-green in Fig. 2A), which lies at the junction of the trimer interface. As seen in Figure 2E, this P-loop fragment is highly solvent protected in both the Prohead II and Head I states, while in free capsomers it is nearly five times more solvent accessible. Quaternary interactions are therefore limiting the rate of amide proton exchange in these intact particle forms, while P-loops in the unassociated capsomers are more free to exchange. Data generated for EI (Supplementary Fig. 1) yielded nearly identical exchange profiles as seen for Prohead II and Head I, verifying the presence of P-loop interactions during intermediate stages of expansion as well. Consistent with the Prohead II crystal structure, the H^2H data confirmed that strong interactions remained fixed at the P-loop 3-fold sites, despite the large subunit rotational motions.

The magnitudes of rotation that bring the Prohead II subunit into the Head II conformation were measured (Fig. 2e) by superimposing the residues behind the fixed region colored blue in Fig 2c. The measurements therefore directly relate to the degree of tertiary twisting, which at lower resolution was quantified as whole subunit rotations in previous studies⁶. Subunits closest to the pseudo 2-fold axis (A and D) undergo the least rotation, while those furthest (B and E) undergo the most.

One of the fixed anchor points, Arginine 194, resides several residues N-terminal to the spine helix. The majority of the subunit beyond the fixed P-domain region (colored blue in Fig. 2c) twists as a rigid unit, causing significant bending of the helix, which is fixed at its N-terminal end. The degree of helix bending is therefore proportional to the extent of β -strand twisting (Supplementary Movie 1). The helix deformation in Prohead II can be seen in figure 3a and Supplementary Movie 3. Subunits B,C,E,F and G show dramatic helix bending while subunits A and D exhibit straighter helices as well as smaller twisting motions in the P-domain β -sheet.

To examine the dynamics of the spine helix in solution, H^2H exchange was measured for a peptide spanning residues 206-216, which covers the bent region. The average amount of deuterium exchanged in this region of Prohead II is nearly 5 times greater than in Head I, showing a more canonical helical structure with stronger hydrogen bonding in the mature Head

I form (Fig. 3b). H^2H measurements of the first expansion intermediate, EI, were also performed. The helix peptide shows nearly identical solvent exchange for EI as Head I, indicating that the increased hydrogen bonding in the helix occurs in the initial stage of expansion. The helix in the free capsomer state shows a similar level of solvent accessibility as Prohead II, indicating that the helix distortion is not just a result of the quaternary arrangement enforced in the intact capsid, but is likely occurring at the level of capsomer assembly and facilitated by interactions of the Δ -domain (residues 2-103 that function as a scaffold and are cleaved off of Prohead I to form Prohead II).

Quaternary associations are likely inducing different degrees of strain in the local tertiary structures of the 7 quasi-equivalent subunits. The subunits are not only in a skewed arrangement in the Prohead II hexamer, but they also exhibit different orientations depending on their positions in the hexamer. While the long axes of subunits B, C, E, and F lie more radial to the capsid surface, subunits A and D lie more parallel to the capsid surface and therefore do not need to rotate as much to assume their orientations in the mature hexamer (Supplementary Movie 2). Because P-loop contacts are preserved during maturation, there is a strong correlation between the orientation of the subunit relative to the capsid surface and the change in tertiary structure between Prohead II and Head II, with the more tangential subunits showing less tertiary structure change and the more radial displaying the larger tertiary structure change.

The combined crystallographic and H^2H exchange data demonstrate that the large subunit rotations concomitant with expansion from Prohead II to Head II are facilitated by a tertiary structural transition – the twist of the subunit core about a fixed hinge. H^2H exchange data of the helix, which appears to be bent in concert with the overall hinging motions, indicates that most, if not all of the change in tertiary structure occurs during the initial and irreversible expansion from PII to EI^{8,19} (Fig. 3c). This is reasonable considering nearly 60% of the expansion in size occurs in the first transition, as well as the symmetrization of the hexamers⁸ (Supplemental Movie 2). We propose that the bent helix and twisted β -strand in Prohead II place the subunits in a strained conformation of elevated free energy and that this accounts for both the meta-stability of Prohead II and the driving force for the initial expansion to EI. The 3-fold interactions at the P-loops stabilize inter-capsomer interactions during the expansion. Capsid integrity is augmented after transition to EI, which is competent for covalent crosslinking in the 3-fold region. The energy sources for the distorted tertiary structure in Prohead II likely stems from the initial assembly, in which the Δ -domains (residues 2-103) of each subunit putatively act as molecular scaffolds that promote capsomers assembly. The favorable association of Δ -domains in this early assembly product may induce the strained conformation (Fig. 4). The high level of deuterium exchange observed in the spine helix of free capsomers supports our hypothesis that the bent subunit conformation exists at the stage of capsomers, not just fully assembled capsid. Δ -domains interact in a trimer arrangement in the hexamers of Prohead I²⁰, which assume a skewed symmetry similar to Prohead II. Though Prohead I, prepared without the viral protease, is resistant to expansion when exposed to conditions that expand Prohead II, Cryo-EM of Prohead I particles heated to 55 degrees C showed a reversible transition to an EI like state²⁰. That study showed that heating the Prohead I particles causes a disruption of Δ -domain interactions, enabling the particle to expand beyond the Prohead II state to a state in which the hexamers were symmetric. Based on these data we argue that upon disruption of Δ -domain interactions and the formation of symmetric hexamers, the tertiary strain in the subunits is relieved. When the Δ -domains are present, as they are in Prohead I, cooling the particles causes the Δ -domains to re-associate, which induces the skewed hexamers and strained tertiary structure. When the Δ -domain is absent as in Prohead II, the particle is trapped in an elevated local energy minimum until it is perturbed to expand either by DNA packaging *in vivo*, or by chemical perturbation *in vitro*.

Tuma et al.²¹ previously proposed a mechanism for P22 expansion where the procapsid subunit existed as a late-folding intermediate that underwent further tertiary changes *en route* to the lower energy, mature conformation. Such a mechanism is now evident in HK97, and may in fact be the driving force for expansion. Systems in which tertiary structure folding events, comparable to those presented here for HK97, have been characterized include the CA domain of the HIV capsid protein, which has been shown to require a kinking of a helix in order to induce dimer activation²². Although such tertiary structural changes have not been characterized at high resolution in other dsDNA phage and viruses, they may in fact be present in P22, T4, T7, phi 29 and possibly animal viruses such as Herpes viruses, which all share an HK97-like fold^{23-26,4}.

Methods Summary

Mutagenesis and Crystallography

The W336F mutation suppresses the spontaneous expansion observed in wild-type Proheads and therefore increased the homogeneity of Prohead II preparations. The E-loop was truncated between residues 159-171 to improve crystallization, as previous studies showed that the tip of the full-length E-loop was partially disordered and protruded from the capsid surface⁶. Crystals were grown using the hanging drop vapor diffusion method with a mother liquor consisting of 0.1M CHES buffer, pH 9.0, 200mM Manganese chloride and 2.3-3.0% Peg 4000. A 200mM final concentration of NDSB-211 (Hampton Research) was added to the drop. Room-temperature diffraction data from 29 crystals was processed as described in the supplementary methods section. An atomic model for the Prohead II structure was initially derived by rigid-body fitting of the refined 3.44 Å structure of the mature Head II coordinates (PDB ID: 1OHG) into the Prohead II electron density. Model refinement is described in the supplementary methods.

H/²H exchange and sample preparation

H/²H exchanged samples were analyzed on a DE-STR MALDI-TOF mass spectrometer. H/²H exchange was performed at pH 7.5 for all protein samples. A detailed description of the protocol can also be found in the supplementary methods. Capsomers used in the experiment were obtained by diluting Prohead I in a final buffer concentration of 0.5M NaSCN, 20mM Tris pH 7.5 and incubating at room temperature for 12-24 hours. Capsomer preparation can be monitored as previously described¹². Gel analysis showed that following incubation, the dissociated capsomers consisted of a predominant population of hexons and a small quantity of penton subunits (gel not shown). The late expansion intermediate Head I was generated as in previous studies⁵. The EI-I particle form was obtained by treatment of Prohead II with 10% isobutanol followed by a 15 minute incubation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. HK97 assembly and morphology

(a) Particle assembly and maturation based on experimental data. P-I (Prohead I) and EI (Expansion intermediate) are derived from Cryo-EM data, while P-II (Prohead II), Balloon/Head I, and Head II are from crystal structures. Note, crosslinking occurs in the WT particle following formation of the EI state. Crosslinks (isopeptide bond) form between Lys 169 and Asn 356 located on different subunits. A crosslink defective mutant K169Y expands to Head I, a state nearly identical to Balloon minus the crosslinks. WT Balloon undergoes a final expansion step to Head II in which the pentons become more protruded and form one last class of crosslinks. (b) Crystal structure of subunit D of Prohead II at 3.65 Å (c) 3.65 Å electron density map (displayed as a solid surface) of the full Prohead II capsid. Electron density is contoured at $\sim 1\sigma$ in chimera. The Prohead II hexamers and pentamers are shown alongside the capsid with the 7 subunits of the viral asymmetric subunit labeled A-F for the hexamers and G for the pentamers. (d) A calculated electron density map of the Head II capsid shown at 3.65 Å, also rendered at $\sim 1\sigma$. (e) Prohead II and Head II hexamers shown tangential to capsid surface (rotated 90 degrees from view in 1c and 1d).

Fig. 2. P-loops located at 3-fold axes act as invariant pivot points

(a) Ribbon representation of P-II. The black triangle represents an icosahedral 3-fold, orange and magenta triangles represent two quasi-3-fold positions. A zoomed in view of subunits at a quasi-3 fold axis are shown viewed from outside of the capsid. Residues 345-353 of the P-loop are colored lime-green, and represent the peptide fragment analyzed by H²H exchange. (b) Side-chain interactions at 3-folds that remain invariant during expansion. The view is from the interior of the capsid directly underneath a quasi-3-fold axis, 180 degrees from the view in figure a. Electron density is contoured at 1 σ . The 3 outer circles highlight salt bridges while the center circle highlights three glutamates (E348) coordinated at a putative metal binding site. (c) Subunit G of P-II (yellow) and H-II (green) have been aligned by the region of the P-

domain which remains invariant (blue). (This motion is best captured in Supplementary Movie 1). (d) A P-II trimer (subunits A,F,G as shown in a) is aligned on the H-II trimer (green) by the regions that remain invariant (blue), illustrating the rotational motions in respect to the fixed trimeric interactions. The upper view is looking down a quasi 3-fold while the lower figure shows a perpendicular view, tangential to the capsid surface. (e) Table shows the angles of rotation (θ) of each subunit from P-II to H-II as illustrated in both c and d. (f) $H/{}^2H$ exchange curve of a peptide fragment spanning residues 345-353 of the P-loop (colored lime-green in a) shown for P-II, H-I, and free capsomer states. Time points are taken from 30 seconds to 10 minutes, with error bars representing standard deviations between successive measurements done in triplicate.

Fig. 3. Spine helix bends during maturation

(a) The spine helix (yellow) is shown for subunit F of Prohead II both in its corresponding electron density on the left (1σ), and aligned with Head II on the right (green). The subunits from the two states were aligned using the subunit core that acts mostly as a rigid body (residues 230-383) with an r.m.s.d of 1.3 Å or better for each alignment. The region colored blue represents the fragment spanning residues 206-216, analyzed by H²/H exchange. (b) H²/H exchange rate curves comparing deuterium exchange between P-II, EI-I, H-I, and free capsomer helix fragment.

Fig. 4. A working hypothesis for the formation, meta-stability, and subsequent maturation of HK97, represented with a single hexamer

Individual subunits are first assembled into hexamers and pentamers (far left figure is a hypothetical representation of the initial subunit organization). Based on H^2H exchange data subunit tertiary structures are distorted in free capsomers and we hypothesize that the hexamers are skewed (second figure). We believe that interactions of Δ -domains provide the driving force to skew the hexamers and distort the subunit tertiary structures. The energy required for these changes is exactly balanced by interactions between Δ -domains and other favorable quaternary contacts between subunits of the capsomer. Prohead I is formed by assembly of hexamers and pentamers into a T=7 particle with Δ -domains attached. Following proteolysis of the Δ -domains to form Prohead II, the skewed hexamers and distorted tertiary structures are preserved by quaternary structure interactions in the particle, raising the free energy of the particle to a meta-stable state maintained in a local minimum. Perturbation of these particles by dsDNA packaging (in vivo) or lowering the pH (in vitro) lowers the energy barrier, leading to an exothermic expansion of the particles producing symmetric hexamers and undistorted subunit tertiary structures (far right).