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A Thermally Induced Phase Transition in a Viral Capsid Transforms the Hexamers, Leaving the Pentamers Unchanged

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Abstract

Scanning calorimetry combined with cryo-electron microscopy affords a powerful approach to investigating hierarchical interactions in multi-protein complexes. Calorimetry can detect the temperatures at which certain interactions are disrupted and cryo-EM can reveal the accompanying structural changes. The procapsid of bacteriophage HK97 (Prohead I) is a 450 Å-diameter shell composed of 60 hexamers and 12 pentamers of gp5, organized with icosahedral symmetry. Gp5 consists of the N-terminal Δ-domain (11 kDa) and gp5* (31 kDa): gp5* forms the contiguous shell from which clusters of Δ-domains extend inwards. At neutral pH, Prohead I exhibits an endothermic transition at 53°C with an enthalpy change of 14 kcal/mol (of gp5 monomer). We show that this transition is reversible. To capture its structural expression, we incubated Prohead I at 60°C followed by rapid freezing and, by cryo-EM, observed a capsid species 10% larger than Prohead I. At 11 Å resolution, visible changes are confined to the gp5 hexamers. Their Δ-domain clusters have disappeared and are presumably disordered, either by unfolding or dispersal. The gp5* hexamer rings are thinned and flattened as they assume the conformation observed in Expansion Intermediate I, a transition state of the normal, proteolysis-induced, maturation pathway. We infer that, at ambient temperatures, the hexamer Δ-domains restrain their gp5* rings from switching to a lower free energy, EI-I-like, state; above 53°, this constraint is overcome. Pentamers, on the other hand, are more stably anchored and resist this thermal perturbation.

Keywords

Differential scanning calorimetry; cryo-electron microscopy; virus capsid structure; bacteriophage HK97; conformational changes

*“All capsomers are quasi-equivalent but some capsomers are more quasi-equivalent than others.” — after G. Orwell, *Animal Farm*.*

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1. Introduction

Macromolecular assemblies and machines represent a key level in the biological hierarchy, both as the entities responsible for many fundamental activities and as biomaterials (Alberts, 1998). Viruses belong to this class of particles, and study of their assembly has disclosed many subtle regulatory mechanisms that are likely to be general in their applicability. Understanding assembly requires elucidating the interactions within and between the many protein subunits that make up a given complex. As one way to address this problem, we have started to develop a hybrid approach with three components (Ross et al., 2005; Ross et al., 2006): differential scanning calorimetry is used to detect the disruption of sets of interactions and quantitate the associated energy exchanges (Makhatadze and Privalov, 1995); cryo-electron microscopy and image reconstruction may be used to characterize the structural changes that accompany a given thermal transition; and modelling based on subunit crystal structures allows cryo-EM data to be interpreted in molecular detail.

Bacteriophage HK97 capsid affords an advantageous system (Hendrix and Duda, 1998) in which to study assembly mechanisms that include the employment of: transient molecular scaffolds to guide assembly; regulatory proteolysis to trigger programmed transitions; and conformational changes to transform the properties of the maturing capsid. The HK97 system also uses a less common but highly efficacious mechanism - covalent cross-linking - to stabilize the mature capsid (Duda, 1998; Gan et al., 2004). The precursor capsid protein, gp5, is a fusion of the scaffolding protein (its Δ -domain, residues 2 to 103) with the building-block of the mature capsid (gp5*, residues 104 to 385). Gp5 forms two kinds of capsomers - hexamers and pentamers - that assemble into a procapsid called Prohead I with $T=7I$ icosahedral symmetry. If the protease gp4 is also expressed, about 50 copies are incorporated into Prohead I (Fig. 1). Maturation commences with activation of the protease and proteolysis of the Δ -domains, producing Prohead II. This particle is primed to undergo a major structural transformation - "expansion" (Conway et al., 1995; Duda et al., 1995b). *In vivo*, expansion is triggered by DNA packaging, wildtype procapsids having a portal channel for the entry of DNA. However, expansion may be induced *in vitro* on portal-less Prohead II by adjustments of pH (Duda et al., 1995b; Lata et al., 2000; Lee et al., 2004) - Fig 1. This simplified system has facilitated investigation of the expansion transformation.

Expansion proceeds via three intermediates, called Expansion Intermediates I, II and III (Fig. 1). Each step involves rotations and translations of the gp5* subunit cores that progressively transform the capsid (Conway et al., 2001; Wikoff et al., 2006). Expansion facilitates the formation of a network of covalent crosslinks between subunits in adjacent capsomers (Duda, 1998; Wikoff et al., 2000; Gan et al., 2004). The first crosslinks form in EI-II and the final ones when EI-III converts to Head II (Fig. 1). EI-I and EI-II are both about 10% larger than Prohead II: our current understanding is that EI-I differs from EI-II primarily in having no cross-links and, consequently, may revert to the Prohead II state (Fig. 1). EI-III is 10% larger still and almost perfectly spherical. Finally, the pentamers move 40 Å radially outwards as EI-III converts to icosahedral Head-II.

In previous studies, we used DSC in combination with cryo-EM to study the process of thermal denaturation for HK97 capsids at successive stages of maturation (Ross et al., 2005; Ross et al., 2006). Each thermogram exhibits a denaturation endotherm of characteristic temperature, enthalpy, width and shape. However, some thermograms were found to include smaller endothermic events at lower temperatures that presumably represent thermally induced phase transitions of the capsid. Here we report the structural expression of a reversible transition exhibited by Prohead I.

2. Materials & Methods

2.1 Preparation and characterization of Proheads

Prohead I was prepared by plasmid expression of HK97 gene 5 in *E. coli* and purified as described previously (Duda et al., 1995a; Duda et al., 2006; Li et al., 2005). Purity and homogeneity were assessed by SDS-PAGE, agarose gel electrophoresis, and electron microscopy. To check the effect of heating on the mobility of Prohead I in native agarose gels, samples were diluted to 2 mg/ml in our standard calorimetry buffer (SCB: 17.2 mM K_2HPO_4 , 2.8 mM KH_2PO_4 , 0.1 M KCl, pH 7.5) and 50 μ l samples in 0.2 ml thin-walled tubes were incubated at 60°C in an Applied Biosystems GeneAmp PCR System 2700 thermocycler for 10, 20, 30, 40, or 50 min. Samples were cooled on ice and 9 μ l samples of each and an unheated control were run in a 0.9% agarose gel in TAMg buffer and stained for protein using a Coomassie Brilliant Blue R250 stain. The gel was run at ambient temperature using a temperature-stabilized Hoefer Minnie the Gel-Cicle gel box with no pre-cooling.

2.2 Differential scanning calorimetry

Calorimetry was performed as described (Ross et al., 2005; Ross et al., 2006) using a MC-2 calorimeter (Micro-Cal, Springfield, MA) equipped with 0.6 ml cells. Prohead concentrations, determined as described previously (Ross et al., 2005), were ~0.7 mg/ml protein in SCB. Scans were performed at 1°C/min, half the rate used in our previous calorimetric study of Prohead I and related assemblies (Ross et al., 2006); accordingly, corresponding transition temperatures are ~1° lower in the present study, on account of differing kinetic effects.

2.3 Thermo-cryo-electron microscopy

To vitrify particles for cryo-EM observation as handily as possible under defined conditions of temperature and humidity, we used an environmental chamber (Fig. 2) that mounts over our Reichert KF80 cryo-fixation unit. This chamber is manually accessible for grid preparation operations and can maintain temperatures of up to 80°C and humidity up to 100%, as set via an electronic control unit. This device was designed and constructed by Mr. G. Melvin; plans and other specifications are available on request.

In the experiments reported here, 30 μ l samples of Prohead I were sealed with parafilm in 0.2 ml thin-walled Eppendorf tubes and incubated at 60°C for 10 min in a microprocessor-controlled water bath (Precision, model 208). The Eppendorf was then transferred to a beaker containing water at 60°C and installed in the environmental chamber pre-equilibrated at 60°C and 80% humidity. The chamber also contained tweezers holding an EM grid with a holey carbon film substrate, pipet tips, and blotting paper, all at 60°C. The humidity was increased above 80% just before grid preparation, otherwise condensation interferes with visual observation. 3 μ l drops were applied to grids and vitrified thin film specimens were prepared in an otherwise usual manner. Finally, the grid was transferred into the electron microscope and low-dose micrographs recorded using conventional procedures at 50,000x magnification on a CM200-FEG electron microscope (FEI, Mahwah, NJ) equipped with a Gatan 626 cryo-holder (Cheng et al., 1999). Micrographs chosen for image reconstruction were checked for optical quality by diffraction.

2.4 Image Reconstruction

Micrographs included particles of two sizes. Five focal pairs, selected on the basis of their optical diffraction patterns, were digitized on a SCAI scanner (Z/I Imaging, Huntsville, AL) at a step size of 7 μ m, corresponding to 1.4 Å pixels. Image reconstruction, including contrast transfer function (CTF) correction, was performed as described (Conway and Steven, 1999), and orientations were significantly improved with the POR refinement program (Ji et al.,

2006). The micrographs yielded 2439 smaller and 614 larger particles (focal pairs) of which 1634 and 307 were included in their respective final maps. Resolutions, assessed in terms of the Fourier Shell Correlation (FSC) coefficient (Saxton and Baumeister, 1982) with a threshold of 0.3, were 11.0 Å for the smaller particles, and 12.3 Å for the larger ones. For illustration (Fig. 5), these density maps were calculated to 11 Å and 13 Å, respectively. The Prohead I map, reproduced from Ross et al. (2006), included 3988 focal pairs from a total of 5950, and was band-limited to 12 Å to facilitate comparisons, although the data extend considerably further (J.F.C. – unpublished results). Data for the EI-I/II reconstruction (Lata et al., 2000; Ross et al., 2005) were re-refined with the POR software, as above, from which 1065 particles of 1590 yielded a resolution (FSC 0.3 limit) of 13.2 Å. This map was calculated to 13 Å. Analyses were performed on Macintosh G5 computers (Apple Computer, Cupertino CA), and panels in Figure 5 were prepared on Linux workstations (Dell, Austin TX) with Amira 3.1 software (Mercury Computer Systems/3D Viz group, San Diego CA and Merignac, France).

3. Results

3.1 Reversibility of the 53° endotherm of Prohead I

On thermal scanning, Prohead I exhibits an endothermic transition between 50°C and 55°C, peaking at a T_p of 53°C (Fig. 3a; Ross et al., 2006). Thereafter, no significant transition is detected until 74°C, which marks the onset of the denaturation endotherm (T_p , 81°C; Fig. 3b). Although the enthalpy associated with the 53° event is only about 7% of that incurred in thermal denaturation, it is - at 14 kcal/mole of gp5, i.e. 5880 kcal/mole of capsid - large enough to potentially disrupt interactions within Prohead I, and thus effect structural changes in the surface lattice. To determine whether this event is reversible, we scanned a sample up to 60°C and then allowed it to cool. In a second scan, this time to 95°, we observed an event at the same temperature (53°) and with the same enthalpy (Fig. 3b), followed by the denaturation endotherm at 81°. We conclude from this observation that the 53° transition is fully reversible. However, after Prohead I is denatured by heating to 95°, neither event is observed upon reheating (Fig. 3c).

To confirm the reversibility of the 53° transition, we measured the electrophoretic mobility of Prohead I in non-denaturing agarose gels after incubating samples at 60°C for 10, 20, 30, 40 and 50 min and then cooling them on ice before running the gel at room temperature. The mobility of all samples was the same as that of an unheated control.

3.2 Capturing the thermally transformed state of Prohead I

We wished to observe what structural changes might accompany the 53° transition. However, we did not know how rapidly the procapsid reverts to its original state (or, possibly, to an energetically and electrophoretically equivalent but structurally altered state) on cooling. Accordingly, we sought to capture the altered state by cryo-fixation by incubating Prohead I at 60°C and then freezing as rapidly as possible for cryo-EM. In our usual vitrification protocol (Cheng et al., 1999), a small drop is applied to a holey carbon film, blotted to a thin film, and after several seconds, plunged into the cryogen. However, under normal laboratory conditions, thin aqueous films evaporate rapidly when maintained at 60°C and such drying may dehydrate the specimen or, at the least, significantly alter the ionic strength, temperature and pH of its environment (Frederik and Hubert, 2005), and hence, potentially alter its structure. Accordingly, it was important to maintain samples at high humidity during the interval between blotting the sample to a thin film and freezing it. For this purpose, we constructed an environmental specimen preparation chamber that was mounted over a commercial cryo-fixation unit (see Methods). Using it, the elapsed time between applying a 3-µl drop of sample at 60°C to an EM grid preheated to the same temperature and having a vitrified specimen at ~-190°C, ready for observation, was less than 4 seconds. The time that the specimen spends

below 53°C before it is cryofixed is not known precisely but is expected to be very much less (see Discussion).

A typical micrograph is shown in Fig. 4a. In addition to normal-appearing Prohead I, the field contains particles that are about 10% larger – some examples are marked with arrows. They constitute about 16% of the population observed in this experiment. A somewhat smaller fraction of enlarged capsids, 10%, was recorded in a second experiment performed with an independent preparation of Prohead I (Table 1). No such particles were observed in control experiments with unheated aliquots of the same Prohead I preparations, nor in a sample that was cooled to room temperature after the 60° incubation and prepared for cryo-EM 10 min later (Fig. 4b; Table 1). Nor were any particles of intermediate size present, according to visual assessment of the micrographs. The conclusion that the capsids observed when Prohead I is vitrified as rapidly as possible after a 60° incubation are two-component populations was confirmed by image analysis and classification.

3.3 Structure of thermally perturbed Prohead I

Three-dimensional reconstructions of the normal-sized particles and the enlarged ones are compared in Figs. 5b and 5c, and Figs. 6b and 6c. The former capsids very closely resemble unheated Prohead I (cf. with Figs. 5a and 6a, respectively). Their outer surface shows the same skewed hexamers and symmetric pentamers, and E-loops are visible as small nodules grouped in triplets around the axes of local threefold symmetry. The most prominent features on their inner surfaces are the Δ -domain-associated gondolas of density. In grayscale slices through the density map (Figs. 6a,b), the gondola density is fuzzy compared to the sharply defined features in the contiguous shell, indicative of some disorder. Nevertheless, it is apparent that the gondolas under the pentamers are single 5-fold clusters, while those under the hexamers are doublets, both components presumably having contributions from the Δ -domains of three subunits in the hexamer (Fig. 5a). The only respect in which Prohead I in the 60° sample appears to differ from the unheated control is in that the doublets of Δ -domain trimers are less evident by surface rendering (Figs. 5a, b), presumably because this density is slightly more diffuse.

In the thermally enlarged particles, the pentamers are quite unchanged (cf. Figs. 5c and 6c with Figs. 5a, b and 6a,b); however, the gondolas have disappeared from under the hexamers. We assume that their Δ -domains have unfolded or dissociated in response to thermal stress. The hexamer-associated portion of the contiguous shell is also markedly altered, having become thinner, smoother, and wider in the plane of the shell. This region does not match the corresponding region of Prohead II (which is essentially the same size as Prohead I, not 10% larger; Conway et al., 1995). However, it is essentially superimposable on the corresponding region of Expansion Intermediate I, the transition state produced by inducing Prohead II to expand at acidic pH (Lata et al., 2000). To make a more precise comparison, we re-analyzed our earlier data on EI-I, extending the resolution to ~13 Å, similar to that for the thermally expanded capsid (cf. Fig. 5c with 5d and Fig. 6c with 6d). The resulting map confirms the close structural similarity of the respective hexamers.

We conclude, therefore, that the 53° thermal transition leaves the gp5 pentamers unperturbed, while dispersing the Δ -domains of the hexamers and causing their gp5* moieties in the contiguous shell to switch into their EI-I conformation.

4. Discussion

4.1 Thermally enlarged Prohead I: Kinetics of reversion

The fractions of enlarged capsids observed after incubating Prohead I at 60° were 16% and 10%, in two experiments (Table 1). No enlarged particles were seen in the starting preparations,

which were homogeneous by all criteria applied – native gel electrophoresis, SDS-PAGE, and cryo-EM. Moreover, the 53° event is fully reversible in the capsid population, i.e. it is not confined to a subset of particles, to judge by the fact that the same enthalpy was recorded on re-scanning, and there was a 100% reversion rate to particles of the original size after 10 min (Fig. 4b; Table 1). Accordingly, we interpret these data as follows: in the 53° transition, the procapsid switches to the enlarged state in which it has the pentamers of Prohead I and the hexamers of Expansion Intermediate I but with disordered Δ -domains still attached (Fig. 7). Upon cooling, reversion initiates stochastically on any given particle, and then proceeds rapidly, to account for the fact that no partially enlarged particles were observed.

4.2 Thermo-cryo-electron microscopy

With our apparatus for preparing vitrified specimens for cryo-EM from defined conditions of elevated temperature and humidity, the time required to complete preparation of a cryo-EM grid is a few seconds. (Vitrification itself is accomplished in milliseconds). This is slow on the time-scale of many macromolecular processes. In retrospect, our procedure appears to have been just fast enough to capture significant fractions of particles in the thermally enlarged state of Prohead I. We cannot give a precise figure for the half-life for reversion once Prohead I is cooled below 53°C, but estimate that it is of the order of a tenth or a few tenths of a second. If it were to be shorter by a factor of, say, three or four, then the fraction of capsids cryofixed in the thermally enlarged state would have been too small to be noticed. At the outset of this project, we were aware of other cryo-EM set-ups that have been used to investigate short-lived states in the millisecond range (Berriman and Unwin, 1994; Subramaniam et al., 1997; White et al., 2003), but we were dubious that, with them, we would be able to capture the large fields of capsids embedded in uniformly thin ice films that we needed for image reconstruction. However, to investigate states that are shorter-lived than the 53° Prohead I transition, they must necessarily be tried.

With regard to the potential applicability of this hybrid approach, we note that although the number of multi-protein complexes that have been analyzed, to date, by calorimetry is small, pre-denaturation transitions appear to be quite a common phenomenon. Specifically, there are thermally inducible events in other conformational states of the HK97 capsid that occur at other temperatures (than 53°) and may therefore represent different structural effects. For instance, Head II has three minor events, all reversible; and Head I, a mutant capsid with a Lys to Tyr substitution at position 169, that is defective for crosslinking but otherwise structurally mature, has a prominent event at 72° (Ross et al., 2005). Moreover, such transitions are not exclusive to the HK97 system. T4 procapsid-like polyheads – tubular, uncapped, particles composed of hexamers – have an event at 45°, well separated from their denaturation endotherm at 65° (Ross et al., 1985); and pre-denaturation transitions have also been detected in the P22 system (Galisteo and King, 1993). We suspect that comparable events may also occur in other, non-viral, assemblies.

4.3 Quasi-equivalence and Non-equivalence

The theory of quasi-equivalence (Caspar and Klug, 1962) whereby small non-uniformities in the conformations and mutual interactions of protein subunits were invoked to allow the assembly of icosahedral capsids with more than 60 subunits has enjoyed considerable success and longevity. However, it is not universally applicable. There are now a number of well documented cases of non-equivalent packings in which the subunits maintain largely similar folds but engage in substantially different interactions. Examples include the 120-subunit capsids of double-stranded RNA viruses (e.g. (Butcher et al., 1997; Cheng et al., 1994; Grimes et al., 1998); the 360-subunit, all-pentamer, capsids of papillomaviruses and polyomaviruses (Belnap et al., 1996); and the icosahedrally ordered envelopes of flaviviruses such as dengue (Kuhn et al., 2002).

The extent to which the HK97 capsid complies with the provisions of quasi-equivalence depends on its conformational state. Prohead I self-assembles from pentamers and hexamers of gp5, in keeping with quasi-equivalence; however, the asymmetry of the gp5* hexamers in the contiguous shell is so pronounced as to call into question whether the conformations and interactions of the six subunits are close enough to be considered quasi-equivalent. More extreme is the clustering of the Δ -domains in two interacting groups of three (Fig. 5a) instead of one ring of six – definitely a non-equivalent arrangement. These delinquencies are resolved in subsequent steps of maturation: in Prohead II, the Δ -domains are removed; and as Prohead II switches to EI-I, the hexon asymmetry disappears (Lata et al., 2000). The thermally enlarged conformation of Prohead I, although non-physiological, represents the thermodynamically favored state of a protein array under certain defined conditions. It exhibits non-equivalence of a different kind, in which pentamers of Prohead I are combined with hexamers in essentially the same state as EI-I as far as their gp5* moieties are concerned, but with the Δ -domains disordered (unlike those of pentamers).

There are precedents for quasi-autonomous behavior of the pentamers and hexamers in capsid surface lattices. In the herpes simplex virus system, which has much in common with tailed bacteriophages (Steven et al., 2005), the maturing hexamers undergo extensive remodeling whereas pentamers make only small shifts (Heymann et al., 2003). When capsids of bacteriophage P22 are heated at 70°C, their pentamers are dislodged and their hexamers remain in place (Teschke et al., 2003).

4.4 Why do the hexamers switch to their EI-I state, not their Prohead II state?

The structural changes observed in the thermal transition of Prohead I point to a greater susceptibility of its hexamers, as compared to its pentamers, to thermal perturbation. We tentatively attribute this difference in susceptibility to differential stability of their Δ -domain clusters that, in the case of pentamers, keep the capsomer anchored in its initial conformation. In this context, it is noteworthy that the hexamers switch, not to the next state of the pathway – Prohead II, in which the conformation of their gp5* moieties is only slightly different from that of Prohead I (J.F.C. et al., unpublished observations) – but to their substantially altered EI-I state. We propose the following explanation. Prohead II is a metastable particle that may be induced to embark on expansion by any of a number of different stimuli (Duda et al., 1995b). As such, it may be considered as occupying a shallow potential well in which it cannot remain at temperatures above 53°C. In this scenario, the 53° transition of Prohead I involves two effects: disordering the hexamer Δ -domains to relax the constraint that they impose on the rest of this capsomer; and inducing gp5* hexamers in the contiguous shell to mature past the Prohead II conformation to the EI-I state.

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Figure 1. HK97 capsid assembly with and without the maturational protease

The pathways shown, involving only the capsid protein (gp5) and protease (gp4), are absent of the portal/connector protein and DNA packaging. If the protease is present, the resulting particles may be used to study the maturation events *in vitro*. The proteolytically processed capsid, Prohead II may be matured by adjustments of pH as shown. Without gp4, the assembly pathway does not progress beyond the initial Prohead I stage.

Figure 2. Environmental chamber for preparation of vitrified specimens for cryo-EM
In this chamber, specimens are maintained under defined conditions of (elevated) temperature and humidity until they are quenched in the underlying cryogen.

Figure 3. The 53°C thermal transition of HK97 Prohead I is reversible

After an initial thermal scan to 65°C (a) detected an endothermic transition at a peak temperature of 53°C, the sample was cooled and then scanned to 95°C (b). The second scan again showed the 53° event followed by thermal denaturation, peaking at 81°C. A third scan of the same specimen (c) exhibited neither event.

Figure 4. Cryo-electron micrographs of Prohead I

(a) Sample frozen immediately after incubation at 60°C. Some of the capsids - about 16% overall - are appreciably enlarged (by about 10%). Some examples are marked with arrows.
(b) Sample frozen 10 min after cooling to room temperature from a 10 min incubation at 60°C. None of these capsids remained in the enlarged state. Bars = 500 Å.

Figure 5. Surface renderings of cryo-EM reconstructions of HK97 Prohead I and Expansion Intermediate I and related particles

(a) Unheated Prohead I (control); (b) the smaller capsid observed after incubating a Prohead I preparation at 60°C; (c) the enlarged capsid observed after incubating a Prohead I preparation at 60°C; (d) Expansion Intermediate I, observed at short times after shifting Prohead II to pH 4.1 (Lata et al., 2000). Left column: outer surfaces; middle: inner surfaces; right column: blow-ups of the rectangular areas of outer surface (top) and inner surface (bottom) delineated by dashes in (a), left and middle panels. The capsids are viewed along a 2-fold axis of symmetry. The same hexamer conformation is observed in (a) and (b), and an altered hexamer conformation in (c) and (d). The same pentamer conformation is observed in (a), (b) and (c).

Gondolas of Δ -domain-associated density are colored yellow in (a), (b) and (c). Gondolas are not seen under the hexamers in (c), even though the Δ -domains are still present (but disordered). The gondola under the Prohead I hexamer is not a 6-fold ring but a pairing of two components of equal size, presumably trimers, as shown particularly clearly in (a), center and right/bottom panels. In general, Δ -domain-density is more diffuse than in the contiguous shell, as illustrated in grayscale sections in Fig. 6, and more so in (b) than (a) of this Figure. In each case, nodules of density are seen on the outer surface that correspond to the β -hairpin motifs of the outward-protruding E-loops (Conway et al., 2001). These nodules are colored red in (d). As noted (Introduction), EI-I and EI-II differ primarily in that EI-II has some crosslinks, and the E-loops involved in these crosslinks lie flat in the plane of the contiguous shell and do not protrude outwards. As a result of these crosslinks, EI-II cannot revert. The heat-induced transition of Prohead I is eminently reversible (Results) and so its hexamer conformation relates to that of EI-I. The data used for this reconstruction (d) included both EI and EI-II particles (Lata et al., 2000), which at this resolution have essentially the same structure in their contiguous shells. Bar = 100 Å (for the left and middle panels).

Figure 6. Central sections through density maps of HK97 Prohead I and Expansion Intermediate I and related particles

(a) Unheated Prohead I (control); (b) the smaller capsids observed after incubating a Prohead I preparation at 60°C; (c) the enlarged capsids observed after incubating a Prohead I preparation at 60°C; (d) Expansion Intermediate I (see Figure 5 legend). These grayscale sections complement the surface renderings shown in Fig. 5(a)–(d). The capsids are viewed along a 2-fold axis of symmetry. In this view, the central section passes centrally through four pentamers, which are marked by overlying arcs in (a). Gondolas of Δ -domain-associated density underlie the pentamers in (a), (b) and (c), as well as the hexamers in (a) and (b); white arrows. This density is generally more diffuse than the features in the contiguous shell. Note the absence of

gondolas from under the hexamers in (c), indicating that the Δ -domains, although present, are disordered. Bar = 100 Å.

Figure 7. Schematic diagram with interpretation of the thermally induced transition of Prohead I Distinct conformations of the contiguous shell are denoted by different colors. Δ -domains, when present, are shown dangling beneath the gp5* subunits. Upon heating beyond 53°C, Prohead I undergoes a reversible phase transition in which the hexamer Δ -domains become disordered and the gp5* moieties switch to their EI-I/II conformation.

Table 1
Fractions of enlarged Prohead I particles observed in cryo-electron micrographs

Experiment		Fraction enlarged	Percentage
#1	Control (unheated)	0 / 385	0.0%
#1	Rapidly frozen from 60°C	206 / 1308	15.7%
#2	Control (unheated)	0 / 1496	0.0%
#2	Rapidly frozen from 60°C	195 / 1924	10.1%
#2	Frozen 10 min after cooling from 60°C	0 / 2483	0.0%