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Herpesvirus Capsid Assembly: Insights from Structural Analysis

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Abstract

In all herpesviruses, the capsid is icosahedral in shape, composed of 162 capsomers, and assembled in the infected cell nucleus. Once a closed capsid has formed, it is packaged with the virus DNA and transported to the cytoplasm where further morphogenetic events take place. Herpesvirus capsid populations are highly uniform in shape, and this property has made them attractive for structural analysis particularly by cryo electron microscopy followed by three-dimensional image reconstruction. Here we describe what is known about herpesvirus capsid structure and assembly with emphasis on herpes simplex virus and on the contribution of structural studies. The overall analysis has demonstrated that herpesvirus capsids are formed by a pathway resembling that established for dsDNA bacteriophage such as P22 and HK97. For example herpes capsid assembly is found to: (1) involve a scaffolding protein not present in the mature virus; (2) proceed through a fragile, spherical procapsid intermediate; and (3) result in incorporation of a portal complex at a unique capsid vertex.

Introduction

More than 130 characterized viruses are now included in the Herpesvirus family. Most infect vertebrate host animals with a high degree of species specificity and an ability to cause both lytic and latent infections. Humans are the primary host for eight herpesviruses that cause diseases ranging in severity from self-limiting skin infections in the case of herpes simplex virus to mononucleosis, birth defects and cancer in infections by Epstein-Barr virus, human cytomegalovirus and Kaposi's sarcoma-associated herpesvirus, respectively. Herpesviruses all have the same basic structure consisting of four concentric layers, (a) a core composed of the virus dsDNA, (b) a sturdy, icosahedral capsid composed of 162 capsomers that surrounds and protects the DNA, (c) a thick layer of virus-encoded protein called the tegument that lies between the capsid and the envelope membrane, and (d) a membrane derived from the host cell that contains virus-encoded glycoproteins involved in entry and other functions (Fig. 1a).

Herpesvirus capsids are highly uniform, robust and symmetric in structure making them attractive for analysis by the methods of structural biology. Cryo electron microscopy followed by three-dimensional image reconstruction (cryoEM) has proved to be well-suited to capsid studies, and structures have now been determined for the capsids of human and several animal herpesviruses. The nature of the capsid structure has suggested testable hypotheses about how it is assembled, and revealing studies have now been pursued with a

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variety of in vivo and in vitro systems. Here we give an account of herpes capsid structure followed by a description of what has been learned about the mechanism of its assembly. Emphasis is on herpes simplex virus and on the results of structural analysis, an approach that continues to yield important advances.

Capsid structure

Initial studies of herpes simplex virus (HSV-1) capsid structure were carried out with capsids isolated from the infected cell nucleus. Three types of capsids, A, B and C capsids, were isolated and all were found to have the same shell structure. The three differ in the content of the capsid cavity with only C capsids containing the virus DNA and able to mature into infectious virus. A and B capsids are considered to be developmental dead ends [1,2].

CryoEM analysis has been carried out with all three capsid types [3–5]. The resolution has continued to improve in successive reconstructions with 8.5 Å the current standard [6**]. In the future, enhanced resolution is expected as advances are made in cryoEM and in methods of sample preparation. Reconstructions have demonstrated that the three capsid types are icosahedral in shape with a diameter of 125 nm. They resemble the capsids of all herpesviruses in that their major structural components are 162 capsomers (150 hexons and 12 pentons) that lie on a T=16 icosahedral lattice (Fig. 1b). Capsomers are connected by way of the capsid floor layer and by 320 triplexes (blue in Fig. 1b; [7]). The triplexes are smaller than capsomers, compact in shape, quite uniform in size and located on the surface of the capsid floor (Fig. 1b). Each triplex lies at the local three-fold position created by a group of three capsomers.

One of the twelve pentons, the portal, differs in structure from the other eleven. It is about the same size as a penton and cylindrical in shape with twelve-fold rotational symmetry rather than the five-fold symmetry found in other pentons (Fig. 2a; [8–10]). The portal has an axial channel through which the virus DNA enters and exits the capsid.

C capsids and capsids in the intact virion have an additional structural feature called the C capsid specific component (CCSC) not found in HSV-1 A or B capsids [11–12*]. CCSCs are rod-shaped with five rods located near each capsid vertex (Fig. 2b; green color). Each rod extends radially outward from the penton connecting it with two adjoining triplexes. The CCSC is considered to support its neighboring penton against the pressure created by the packaged DNA mass. It also provides an interface with the tegument as a prominent tegument protein, UL36, is found to bind the CCSC [13*].

Capsid proteins; VP5

The protein content of HSV-1 C capsids is shown in Table 1. All the hexons and eleven of the twelve pentons are found to be composed of the major capsid protein (MCP; VP5); hexons and pentons contain six and five molecules, respectively. At their distal ends, the hexons also contain six molecules of VP26 arranged in a ring and attached one to each VP5 molecule [14–16]. The outline of VP5 molecules in the hexons and pentons is well described in cryoEM reconstructions (Fig. 1c). In both, VP5 molecules are found to be arranged to create a roughly cylindrical structure (12 nm in diameter × 15 nm long) with an axial channel running through the center. The channel is thought to function as an exit site for the scaffolding protein as DNA enters the capsid (see below). A constriction is found near the center of the axial channel suggesting the channel may be closed at some point in the virus life cycle. High resolution cryoEM reconstructions of the capsid show the location of alpha helices in the VP5 structure [6**].

Further information about VP5 structure was obtained by X-ray crystallography. When purified VP5 (1374 aa) was digested with trypsin in solution, a specific fragment corresponding to amino acids 451–1054 was found to be protected from proteolysis [17**]. After purification, this fragment was found to crystallize, and its structure was determined by X-ray crystallography at 2.9 Å resolution [17**]. Examination of the structure demonstrated that it fits uniquely into the upper domain of the VP5 volume as defined by cryoEM reconstruction of HSV-1 capsids (Fig. 1c). The fragment, now called the VP5 upper domain (VP5ud), is observed to consist mostly of short regions of α -helix connected by short coils. Two regions of β -structure are found near the fragment base.

The VP5ud fold is unique in that it is not found in the coat protein of any other virus or indeed in any other protein [17**]. The fold is expected to be found, however, in the major capsid proteins of other herpesviruses. Resistance of the VP5ud to trypsin digestion suggests it is a structurally robust region perhaps serving as a nucleation site for folding of the overall molecule [17**]. Determination of the VP5ud structure was a most significant advance in herpes virology as it was the first high resolution structure of any herpes major capsid protein.

Information about the VP5 floor layer came from a most un-expected source. Investigators using X-ray crystallography to study the capsids of dsDNA bacteriophage such as HK97 and T4 noticed that the capsid protein fit reasonably well into the volume of the VP5 floor layer as defined by cryoEM (Fig. 1c; [18*]). It is reasonable to speculate that this structural conservation may underlie the similar mechanisms of capsid formation observed in herpesviruses and dsDNA bacteriophage (see below). The structural conservation also supports the idea that there may be an evolutionary relationship linking herpesviruses and dsDNA bacteriophage [19].

Capsid proteins; triplex proteins

All the triplexes are composed of one VP19C and two VP23 molecules yielding a particle molecular weight of ~119 kDa [7, 20]. Six distinct triplex types, Ta-Tf, can be distinguished in cryoEM reconstructions. The six differ slightly in structure and in the identity of their neighboring capsomers (Fig. 1b; [21**]). The triplexes are required for capsid assembly where they function to hold capsomers together as the structure is formed. High resolution structure analysis of the triplexes has frustrated the best efforts of crystallographers. When insect cells are infected with recombinant baculoviruses (rBV) encoding VP19C and VP23, triplexes form readily and can be purified. Crystals suitable for analysis have not been obtained, however, despite many attempts. It is speculated that the conformational heterogeneity of the triplexes may affect crystal formation.

Capsid proteins: the portal

The portal is composed of twelve copies of the portal protein, pUL6 [22**]. The UL6 protein is required for HSV-1 replication, and homologs are encoded in the genomes of all herpesviruses. Structural analysis of the HSV-1 portal has been carried out with purified portals [8] and with portals in situ in the capsid [9–10]. The two structures are in agreement. They show that the portal is cylindrical in shape with an outside diameter of 16.5 nm, a length of 9 nm and an axial channel ~3 nm in diameter (Fig. 2a). The channel is slightly wider in diameter at the end facing outside the capsid. The structure of the HSV-1 portal is found to be recognizably similar to the portals of dsDNA bacteriophage (Fig. 2a). Like the HSV-1 portal, the dsDNA phage structures are also located at a single capsid vertex, 12-fold symmetric in shape and involved in transport of DNA into and out of the capsid.

Capsid proteins; the CCSC

Each CCSC molecule is composed of one molecule of UL17 protein and one of UL25 (green rods in Fig. 2b; [11–12*, 23]). The two proteins are linearly arranged in the structure with the pUL17 molecule closest to the neighboring penton and pUL25 more distal [11]. CCSC molecules are attached to the capsid in the infected cell nucleus as DNA packaging is completed; pUL17 attachment is required for binding of pUL25 [24]. Binding of pUL25 is found to require amino acids 1–50 [25]. X-ray crystallographic analysis has been done with a fragment (aa 134–580) of the 580 aa pUL25 molecule [26]. The structure is found to consist of a compact core from which four loops project outward.

Capsid assembly

In HSV-1-infected cells, capsid formation takes place in the nucleus in large, spherical structures called inclusion bodies (~5–10/cell; [27]). Assembly-competent inclusion bodies form within 4 hours after initiation of an infection and persist throughout the infection increasing somewhat in number. In electron micrographs, inclusion bodies can be seen to have progeny DNA-containing capsids at their outer edges supporting the view that they are involved in capsid formation. Inclusion bodies stain darkly in electron micrographs, however, so images provide little information about the mechanism of capsid formation. Assembly-competent inclusion bodies have not been isolated from infected cells.

The capsid assembly pathway has been more productively studied with systems based on a panel of recombinant baculoviruses (rBV) encoding HSV-1 capsid proteins [28**–29**]. A uniform population of morphologically normal capsids is formed when insect cells are co-infected with rBV encoding the major capsid, triplex and scaffolding proteins. The panel of rBV is also central for in vitro systems of capsid formation involving: (a) extracts of rBV-infected insect cells [30]; and (b) capsid proteins purified from infected rBV extracts [31**]. Important information about capsid assembly has also been obtained with the use of HSV-1 mutants containing temperature-sensitive lesions in the viral maturational protease (ts1201 and tsProtA; [32–33]). At the non-permissive temperature, capsid formation with these mutants is arrested at the procapsid stage. When cells are transferred to the permissive temperature the ts lesion is reversed and capsid formation proceeds to completion.

Capsid assembly pathway

Fig. 3 shows the pathway of capsid assembly deduced from studies with the experimental systems described above. The basic assembly unit is a complex of the major capsid and scaffolding proteins. These associate with each other to form angular segments of the spherical procapsid with binding promoted by scaffold-scaffold interactions and by the triplexes which link VP5 molecules. The angular segments, called partial procapsids, enlarge progressively in size to create the closed, spherical procapsid [34*]. The procapsid has the same diameter as the mature capsid (125 nm), and the same T=16 icosahedral symmetry, but differs in that it is spherical rather than polyhedral in shape (Fig. 4a–4c). Like the major capsid protein, the portal is thought to be incorporated into the nascent procapsid by way of a complex with the scaffolding protein [35*–38]. Incorporation of the portal takes place during initiation or other early stage of procapsid formation [39**].

Once the procapsid is formed, it is packaged with the virus dsDNA genome. DNA transport into the capsid is mediated by a three-subunit complex of virus-encoded proteins called terminase [40]. As DNA enters, the scaffolding protein exits the procapsid and the overall structure angularizes to create the polyhedral shape of the mature capsid (Fig. 3). It is thought that the scaffolding protein exits the capsid by way of the capsomer channels or other openings in the procapsid structure. Detachment of the scaffolding protein from the

capsid shell is promoted by a proteolytic cut near the C-terminal end of the scaffolding protein molecule catalyzed by the virus-encoded maturational protease. The same proteolytic cut is also involved in initiation of procapsid angularization [41–43].

Structural analysis of assembly components

Structural analysis of the assembly pathway has focused on the procapsid and on the maturational protease. Procapsids have been isolated from cells infected with an HSV-1 mutant, *m100* [33], deficient in the maturational protease and the structure of the procapsids was determined by cryoEM [44]. Purified procapsids from the same source have been induced to mature in vitro, and cryoEM structures have been determined for intermediates in the maturation process [21**]. The resulting set of structures has been most revealing about the capsid maturation pathway. The structures have shown, for example, that the procapsid and mature capsid differ significantly in the floor layer; the floor is fully intact in the mature capsid but lacking almost entirely in the procapsid (compare Figs. 4f and 4g). Capsomers in the procapsid are connected only by the triplexes and the scaffolding protein leaving numerous openings in what will become the capsid floor. Many other differences are revealed including a “clam shell” shape to the hexons of the procapsid which mature to create the hexagonal shape found in the mature capsid (compare Figs. 4d and 4e).

The maturational protease is required for HSV-1 replication and a homolog is encoded in the genomes of all herpesviruses. Its importance for replication has made the protease the focus of intense analysis including an X-ray crystallographic structure [45]. So far, however, the structure has not suggested the nature of effective anti-herpes therapeutics directed at the protease.

Future prospects

Despite the impressive progress so far, further clarification of herpesvirus capsid structure and assembly would be most welcome. For example, higher resolution in the structures of the capsid and procapsid could reveal why some VP5 molecules are incorporated into hexons and others pentons. There is much to be learned at higher resolution about the structure of the procapsid and how it is transformed into the mature form. The mechanism of DNA encapsidation and the structure of the tegument are areas where current understanding is at a more rudimentary level. In the case of DNA packaging, the players in the process are known, the capsid, portal and three-subunit terminase. Little is known, however, about the structure of the terminase and how it interfaces with the portal. The tegument attaches directly to the capsid [46], so it is an obvious way studies of the capsid could be extended. Its more fluid organization, however, will challenge even the most advanced methods of structural analysis.

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Highlights

- X-ray crystallographic structure determined for part of the HSV-1 major capsid protein.
- HSV-1 capsid assembly occurs by way of a procapsid intermediate as observed in dsDNA bacteriophage.
- use of cryoelectron microscopy to identify steps in the procapsid maturation pathway.
- HSV-1 portal identified at a unique capsid vertex.
- portal involved in initiation of HSV-1 capsid assembly.

Figure 1.

Structures of the HSV-1 virion and capsid. (a) Electron micrograph showing a single HSV-1 virion in cross-section. Note the four concentric layers that constitute the virion. The virion diameter is ~210 nm. (b) CryoEM reconstruction of an HSV-1 capsid shown in surface-shaded representation viewed along a face (3-fold axis). One of the capsid faces is illustrated in color with the hexons, pentons and triplexes shown in red, orange and blue, respectively. The capsid diameter is 125 nm. (c) View of the HSV-1 capsid with a single hexon and a single major capsid protein molecule extracted. Also shown are the VP5 upper domain whose structure was determined by X-ray crystallography (1NO7; [17**]), and an example of the bacteriophage capsid protein that docks into the major capsid protein floor domain (2FT1; [47]). Alpha helix, coil and beta structure are indicated in blue, gray and yellow, respectively.

(a) Portal structure

(b) CCSC structure

Figure 2.

Structures of the HSV-1 portal (a) and CCSC (b). In (a), the structure of the HSV-1 portal is shown beside the portals of phages T7 and P22 [8, 48–49]. Note that the three are similar in size and structure suggesting their function is the same. All are cylindrical in shape with 12-fold rotational symmetry and an axial channel through which virus DNA enters and exits the capsid. In (b) the CCSC is highlighted in green and shown in the full capsid (left) and at higher magnification in a single vertex (right). Note that five CCSC rods project radially outward from each capsid vertex. CCSC graphic courtesy of James Conway (University of Pittsburgh; [25]).

Figure 3.

Drawing illustrating the pathway of herpesvirus capsid formation. The basic assembly units are small complexes of the major capsid and scaffolding proteins. In the presence of the triplexes (not illustrated), complexes of the major capsid-scaffolding proteins assemble to form angular segments of the closed, spherical procapsid (partial procapsids). A complex of the portal and scaffolding protein is incorporated at an early stage of procapsid formation. Once procapsids are closed, they become the substrate for initiation of DNA encapsidation which occurs concurrently with loss of the scaffolding protein from the procapsid interior and angularization of the capsid shell.

Figure 4. Structure of the HSV-1 procapsid compared to the mature capsid. CryoEM reconstructions are shown in surface rendering as viewed along the 2-fold axis of icosahedral symmetry (a–c). Note that the procapsid is spherical in overall shape while the mature capsid is angular. At higher magnification it can be seen that the hexons in the procapsid are oval in exterior view while those of the mature capsid are hexagonal (compare d and e). The procapsid is found to have openings in the floor layer which are closed in the mature capsid (arrows in f and g).

Table 1

Protein composition of the HSV-1 C capsid

Gene	Protein	Amino Acids	Copies/capsid	Location in capsid
UL19	VP5	1374	955	Hexons; pentons
UL38	VP19C	465	320	Triplexes
UL18	VP23	318	640	Triplexes
UL35	VP26	112	900	Tip of hexon
UL6	pUL6	676	12	Unique vertex
UL17	pUL17	703	60	Near vertices
UL25	pUL25	546	60	Near vertices