Short Communication

Three-dimensional structure of the Epstein–Barr virus capsid

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Epstein–Barr virus (EBV), a gammaherpesvirus, infects >90% of the world’s population. Primary infection by EBV can lead to infectious mononucleosis, and EBV persistence is associated with several malignancies. Despite its importance for human health, little structural information is available on EBV. Here we report the purification of the EBV capsid by CsCl- or sucrose density-gradient centrifugation. Cryo-electron microscopy and image analysis resulted in two slightly different three-dimensional structures at about 20 Å resolution. These structures were compared with that of human herpesvirus 8, another gammaherpesvirus. CsCl-gradient purification leads to the removal of part of the triplex complex around the fivefold axes, whereas the complexes between hexons remained in place. This may be due to local differences in stability resulting from variation in quasi-equivalent interactions between pentons and hexons compared with those between hexons only.

Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV4), is a gammaherpesvirus that infects >90% of the world’s population. Primary infection is mostly asymptomatic, but can lead to infectious mononucleosis (Thorley-Lawson & Gross, 2004). The viral persistence that is established after primary infection remains mostly silent, but can be associated with several malignancies (Niedobitek et al., 2001). Information on the structure of EBV is mainly based on its similarity to other herpesviruses. All virions of this family have a capsid with icosahedral symmetry that contains the viral dsDNA genome, surrounded by a membrane carrying various surface glycoproteins. Tegument proteins fill up the space between the membrane and the inner icosahedral capsid (Grünewald et al., 2003). The three-dimensional (3D) structures of human herpesvirus capsids (1250 Å in diameter) are known from cryo-electron microscopy (cryo-EM) for herpes simplex virus type 1 (alpha herpesvirus) (Zhou et al., 2000), cytomegalovirus (beta herpesvirus) (Butcher et al., 1998) and human herpesvirus 8 (HHV8, gammaherpesvirus) (Trus et al., 2001) at 8.5, 22 and 24 Å resolution, respectively. Their common properties are a triangulation number of T=16 imposed by the 162 capsomers (150 hexamers and 12 pentamers) of the major capsid protein (MCP). An extra protein, the small capsid protein (SCP), is located at the outer surface of each MCP hexon, but is absent from the pentons. A heterotrimeric αβ2 complex (triplex complex) is located at each junction between three capsomers (three hexons or one penton and two hexons; 320 triplex complexes per capsid).

Structural studies of EBV particles have been limited because it has been difficult to obtain a high virus yield in cell culture. Henson et al. (2009) have studied the self-assembly of the EBV capsid using baculovirus expression vectors for BcLF1 (MCP), BORF1 (triplex protein α), BDLF1 (triplex protein β), BFRF3 (SCP), BdRF1 (scaffold protein) and BVRF2 (pro tease) in insect cells. They observed capsid formation and produced negative-stain EM images of these recombinant capsids. More recently, Wang et al. (2011) studied the interaction of BDLF1 and BORF1 recombinant proteins produced in 293T cells. Here, we report the production of EBV capsids in the B95-8 lymphoblastoid cell line. We obtained two 3D structures of the EBV capsid, reconstructed from cryo-EM images with about 20 Å resolution, that showed slight differences depending on the purification method used.
B95-8 cells were grown in 1 l RPMI 1640 medium (Invitrogen) supplemented with 10 % heat-inactivated FBS, 100 U penicillin, 100 μg streptomycin ml⁻¹ and 1 % L-glutamine in a 5 % CO₂ humidified incubator. When the cells were growing exponentially with a density of 10⁶ cells ml⁻¹, they were exposed to adverse culture conditions (medium supplemented with only 5 % heat-inactivated FBS and incubation at 37 °C without CO₂) for 11 days in order to increase the production of extracellular EBV in the cell supernatant (Hinuma et al., 1967). At the end of this culture, we obtained 3.6 × 10⁹ viral DNA copies l⁻¹, as determined by quantitative PCR (Brengel-Pesce et al., 2002). Polyethylene glycol 6000 (6 %, w/v; Sigma) was added to the cell-free supernatant and incubated at 4 °C overnight under stirring. Virus was sedimented by centrifugation at 4000 g for 30 min and capsids were obtained by resuspending the pellet in 10 ml Tris-buffered saline (0.15 M NaCl in 0.05 M Tris, pH 7.4) containing 0.25 % Nonidet P-40. The capsid suspension was purified either by discontinuous CsCl density-gradient centrifugation (2 ml of 4.0 M and 4 ml of 2.2 M CsCl in 0.016 M Tris, 0.05 M NaCl, pH 7.4; centrifugation at 110 000 g for 2 h at 4 °C in a Beckman SW40Ti rotor) or by discontinuous sucrose density-gradient centrifugation (4.5 ml of 20 %, 2 ml of 40 % and 2 ml of 60 % sucrose in 0.05 M Tris, 0.05 M NaCl, pH 7.4; centrifugation at 110 000 g for 2 h at 4 °C in a Beckman SW40Ti rotor). For each purification method, two visible bands were collected, and observation by negative-stain EM indicated that the lower band contained capsids plus cellular debris, while the higher band contained only debris. The 100 μl capsid-containing fractions were further concentrated down to 15 μl using a Microcon YM-100 centrifugal filter device (Millipore) and subsequently used for cryo-EM preparation. Sample (4 μl) was deposited on Quantifoil R2/1 grids (Quantifoil Micro Tools GmbH) and prepared for cryo-EM using a vitrobot (FEI Netherlands). In the case of the CsCl-purified virus and prior to freezing, an extra layer of thin carbon was added on the top of the grid in order to increase the apparent virus concentration. The EBV particles were imaged using either a LaB6 Philipps CM200 electron microscope equipped with a Gatan 626 cryoholder operated at 200 kV and ×20 000 nominal magnification (CsCl purification) or an FEI F30 Polara electron microscope operating at 300 kV and ×31 000 magnification (sucrose purification). Cryo-electron micrographs of fields containing capsids are shown in Fig. 1(a). Using CsCl-gradient purification, the amount of capsid was just about enough to make four EM grids, but was not enough to allow purification of the A, B and C forms of the capsid (Yu et al., 2003) or to allow a proteomics analysis. Using sucrose-gradient purification, the amount of capsids obtained was even lower; 700 (CsCl) and 429 (sucrose) particles that, by eye, appeared to be A and B capsids, were carefully selected from respectively 19 and 36 Kodak SO163 films digitized at a 7 μm sampling step on a z/i Imaging PhotoScan.

After model-based image processing using a method described previously (Schoenh et al., 2008) and starting with the 3D structure of an adenovirus (scaled to the right size) as an initial reference, the 500 (CsCl) and 370 (sucrose) best particles were used to calculate the two reconstructions of the EBV capsid shown in (b). A and B capsids are indicated by arrows. Bar, 100 nm. (b) 3D structure of the EBV capsid (sucrose-purified) determined at 20 Å resolution, viewed in an isosurface representation down the twofold axis. (c) Fourier shell correlation (FSC) curves for the EBV reconstructions.
number that characterizes the *Herpesviridae*. Independently of the purification protocol used, the EBV capsid structures were very similar to that of the sucrose gradient-purified HHV8 capsid, in both size (1250 Å in diameter) and capsomer organization (Fig. 2b). The hexons and pentons are similar in shape even though the top of the capsomers appears to be a little more uneven and spiky in the EBV capsid, although this may be due to the slightly higher resolution. For the sucrose-purified capsid (middle row, Fig. 2b–d), the structure is very similar to that of HHV8. The triplex complexes are present everywhere at their expected positions and their shapes are similar to those of HHV8. However, with the CsCl-purified capsid structure, there was a significant difference between the EBV and all the other herpesvirus capsids: around the fivefold axis of the EBV capsid, at the expected position of the triplex proteins, only low densities were present (Fig. 2d, left and Fig. 3a, b). The reproducibility of this observation was confirmed by calculating a second reconstruction from another CsCl-purified sample that also showed the same reduced triplex density around the fivefold axis (data not shown). It is unlikely that the peripentonal triplex complex is smaller due to partial occupancy, because this low density is as strong as that of the rest of the capsid (Fig. 3b). By decreasing the isosurface contour level, the peripentonal triplex appearance did not change (not shown). The difference in density of the peripentonal triplex is therefore probably due to the absence of some of the proteins, possibly the dimer of BDLF1, in the triplex complex. As this difference was not present in capsids purified by sucrose-density gradient, the lack of density is the result of protein coming off the capsid during the purification process. It

![Fig. 2. Comparison of the capsid structures of EBV and HHV8. (a) 3D structure of EBV capsids (left, CsCl-purified; right, sucrose-purified) determined at 20 Å resolution and with one icosahedral facet highlighted in colour. (b–d) Comparison of the 3D capsid structures of EBV (left, CsCl-purified; middle, sucrose-purified) determined at a resolution of 20 Å with the 3D structure of the HHV8 capsid (right) at 24 Å. (b) A single segmented facet, in which the hexons and pentons are coloured in blue and the heterotrimeric triplex complexes in red, is shown for each capsid. Bar, 20 nm. (c) Enlarged view of a hexon (blue) surrounded by six triplex complexes (red). The structures of the hexons, as well as the triplex complex, are similar between the two viruses. (d) Enlarged view of a penton (blue) surrounded by five triplex complexes (red). The structures of the pentons are similar for the two viruses. The size of the triplex complex in the CsCl-purified EBV structure is about one-third of the size of the sucrose-purified EBV and HHV8 triplex complex.](http://vir.sgmjournals.org)
capsid. Once the capsid is docked on the nuclear pore, this pentons and the portal could change the stability of the mimics what may happen during the release of DNA at the purification-induced disappearance of the BDLF1 dimer shock (Iwasaki et al., 2000). In T4, the absence of this trimeric complex (Soc) at around the fivefold axes (Duda et al., 2006; Iwasaki et al., 2000). The EBV capsid 3D maps were deposited in the EM database associated with the Macromolecular Structure Database under accession numbers 2092 (CsCl) and 2093 (sucrose). The Polara microscope is part of the IBS Structural Biology and Dynamics GIS-IBISA-labelled platform.

**Fig. 3.** Slices through the protein density of the EBV capsid 3D reconstructions obtained after CsCl- or sucrose-gradient purification. (a, b) View of a central section through the 3D reconstruction of the CsCl-purified (a) and sucrose-purified (b) EBV capsids seen in a 222 orientation (protein density is in dark grey). The direction of the fivefold symmetry axis is indicated. The right panels are enlarged views of the regions delineated by a black square on the left panels. Small and large circles highlight the triplex density position around pentons and hexons, respectively. Bars, 20 nm.

could be due to a location-dependent difference in stability of the triplex complex (the quasi-equivalent contacts for triplexes between hexons only are different from those between pentons and hexons). The fact that the triplex complex is located at the hexon–hexon and hexon–penton interfaces suggests that it plays a role in capsid stabilization. It has been shown that the capsids of members of the *Herpesviridae* and dsDNA bacteriophages are evolutionarily related. Interestingly, in T4 and SPO1 bacteriophage capsids, the corresponding proteins that occupy the same location as the triplex complex in EBV are also missing around the fivefold axes (Duda et al., 2006; Iwasaki et al., 2000). In T4, the absence of this trimeric complex (Soc) at the peripentonal position may weaken the capsid shell, rendering it susceptible to external stresses such as osmotic shock (Iwasaki et al., 2000). One could hypothesize that the purification-induced disappearance of the BDLF1 dimer mimics what may happen during the release of DNA at the nuclear-pore complex. Expulsion of the dimers around the pentons and the portal could change the stability of the capsid. Once the capsid is docked on the nuclear pore, this weakness might allow expulsion of the DNA from the capsid into the nucleus through the vertex closest to the nuclear pore. If our hypothesis is correct, then the harsh conditions of CsCl purification could mimic a first step of the decapsidation reaction.

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**References**


