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Three-Dimensional Structure of Vaccinia Virus-Produced Human Papillomavirus Type 1 Capsids

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The capsid proteins of papillomavirus self-assemble to form empty capsids or virus-like particles that appear quite similar to naturally occurring virions by conventional electron microscopy. To characterize such virus-like particles more fully, cryoelectron microscopy and image analysis techniques were used to generate three-dimensional reconstructions of capsids produced by vaccinia virus recombinants (V capsids) that expressed human papillomavirus type 1 L1 protein only or both L1 and L2 proteins. All V capsids had 72 pentameric capsomers arranged on a T=7 icosahedral lattice. Each particle (~60 nm in diameter) consisted of an ~2-nm-thick shell of protein with a radius of 22 nm with capsomers that extend ~6 nm from the shell. At a resolution of 3.5 nm, both V capsid structures appear identical to the capsid structure of native human papillomavirus type 1 (T. S. Baker, W. W. Newcomb, N. H. Olson, L. M. Cowsert, C. Olson, and J. C. Brown, Biophys. J. 60:1445–1456, 1991), thus implying that expressed and native capsids are structurally equivalent.

Investigation of the capsid structure of the human papillomavirus has been limited because of the inability to propagate HPV in vitro. The three-dimensional structures of virions of bovine papillomavirus type 1 and human papillomavirus type 1 (HPV-1), purified from clinical isolates, have been determined by means of cryoelectron microscopy and image analysis (4). Both papillomaviruses were shown to be ~60 nm in diameter, with capsid structures formed exclusively of 72 pentameric capsomers arranged on a T=7 icosahedral lattice. This all-pentamer structure was similar to the related viruses simian virus 40 (SV40) (1, 2, 14) and polyomavirus (11, 16) with the exceptions that the papillomavirus capsomers were larger and had a pronounced, star-shaped morphology. Interactions between adjacent capsomers were also distinctly different (4) and, therefore, may be unique to the papillomaviruses.

Early studies, in which purified papillomaviruses were isolated from clinical specimens, revealed the presence of a major protein of 55 kDa (L1), a minor protein of 74 kDa (L2), and numerous low-molecular-weight bands that were identified as histones (9, 10, 15). Virions contain 5 to 10 times more L1 than L2 (15). The proposed papillomavirus structure consisted of the capsid, composed of the L1 and L2 proteins, and a core which contained the double-stranded DNA genome complexed with histones. Recent investigations with polyomavirus and SV40 have added complementary structural information that may be applicable to the papillomaviruses. X-ray crystallographic studies of SV40 (14) and polyomavirus (16) have shown that the majority of the capsid is composed of the ~40-kDa major capsid protein, VP1. Each capsomer contains five subunits of VP1, and intercapsomer contacts are confined to the shell layer. An in vitro assembly system was used to demonstrate that polyomavirus VP1 expressed and purified from *Escherichia coli* could assemble into capsid-like particles (18). Similar experiments on papillomavirus structure, utilizing X-ray crystallography or mutagenesis, have been hampered or have not been possible because of the lack of a culture or in vitro assembly system.

Several groups have recently produced virus-like particles from various papillomaviruses by using either the vaccinia virus (12, 20) or baculovirus (13, 17) expression system. We have described the production of HPV-1 capsids with vaccinia virus vectors that express the L1 protein alone or both the L1 and L2 proteins (12). Conventional electron microscopy and immunogold techniques demonstrated that the vaccinia virus-produced capsids (V capsids) were indistinguishable from authentic HPV-1 virions. The V capsids have also been used to develop serologic assays for HPV-1 (6, 7) and have led to the detection of human antibodies that recognize conformational epitopes on the L1 protein. The antigenic targets possessed by the V capsids were assumed to be identical to those from authentic warts. In this study, the three-dimensional structures of V capsids are reported and compared to the structure that was previously determined for HPV-1 virions obtained from plantar warts (4).

MATERIALS AND METHODS

The V capsids were produced and purified as previously described (12). Cryoelectron microscopy was performed as described previously (3, 19) on vitrified aqueous samples of V capsids that contained 50 mM phosphate buffer (Fig. 1). Images were recorded under minimal exposure conditions (~10 to 20 $e^-/Å^2$) (1 Å = 0.1 nm) at 80 kV and at a nominal magnification of ×36,000 on an EM420 microscope (Philips Electron Optics Inc., Mahwah, N.J.). Selected micrographs were then digitized at 25-μm step size. Individual capsids with a uniform background and well separated from other capsid particles were selected for the image reconstruction analyses. The particle translational (x,y) and rotational (θ,ϕ,ω) parameters were determined by use of an iterative procedure that correlated two-dimensional projections of native HPV-1 reconstruction against each of the particle images (8). In order to reduce the noise level in the reconstructions, the number of particle images was increased by including images from two micrographs in each of the L1 and L1L2 reconstructions. The two micrographs selected for each sample had a similar

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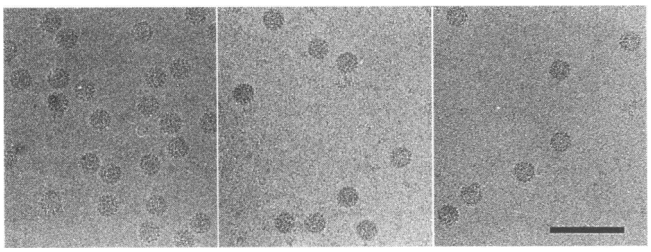

FIG. 1. Frozen-hydrated samples of HPV-1 (left), L1 V capsids (middle), and L1L2 V capsids (right). Bar = 200 nm.

objective lens defocus level (~1.2-μm underfocus), and each image was radically scaled to the reconstruction in real space. 47 L1 V capsid and 40 L1L2 V capsid images were used to compute three-dimensional reconstructions at ~2.9- and ~3.5-nm resolution, respectively.

RESULTS AND DISCUSSION

Capsids of HPV-1 virions and the V capsids were ~60 nm in diameter and were composed of 72 pentameric capsomers arranged on a T=7 icosahedral lattice (5) (Fig. 2, top row). Sixty capsomers occupy hexavalent (six neighboring capsomers) locations, and the remaining capsomers occupy the twelve pentavalent (five neighboring capsomers) positions. No differences in the numbers or relative orientations of the hexavalent and pentavalent capsomers in the three reconstructions were seen. Capsids containing only L1 appeared identical to capsids containing both L1 and L2 and also identical to the capsid portion of naturally occurring virions, demonstrating that most of the capsid density must be attributed to L1.

The external and internal features of the capsid structures in the three density maps also appear to be quite similar in cross section (Fig. 2, middle row). All particles had a maximum radius of ~30 nm with a fairly contiguous rim of density (shell) that had a radius of 22 nm. The shell, ~2-nm thick, appeared much smoother on the internal surface than the external surface. Capsomers extended radially 6 nm from the shell, and interactions between capsomers were confined to the shell layer. The close similarities of the cross-sectional views of the three-dimensional maps confirm that L1 protein constitutes most of the capsid density. The five-pointed star capsomer morphology is similar in all three reconstructions (Fig. 2, bottom row). This morphology is consistent with a capsomer structure consisting of five L1 molecules and is, therefore, similar to the five-VP1 subunit structure of polyomavirus (11, 16) and SV40 (1, 2, 14) capsids. Each papillomavirus capsomer has a maximum diameter of 11 nm and an axial dimple of ~3 nm in diameter. Minor differences in the papillomavirus capsomer morphologies (e.g., at the tips of the pentamers) are more likely a reflection of the noise level of the data than genuine differences in structure. Both intra- and intercapsomeric interactions in the HPV-1 virions and V capsids appear identical at 3.5-nm resolution. Considering the similarities in capsomer interactions between the polyomaviruses (14) and papillomaviruses (4), it is indeed possible that they exhibit the same kind of bonding specificity. It is not possible at this resolution, however, to show that the C-terminal arms of the papillomavirus subunits interact in the same way as those of SV40 do (14). The answer to this question awaits high-resolution analysis of the papillomaviruses.

In this report, the structures of V capsids that contain the L1 and L2 proteins or only the major capsid protein, L1, were compared with the structure of HPV-1 virions. The outer surfaces of all three particles appear identical, with the majority of the capsid protein attributed to L1. The techniques of scanning-transmission electron microscopy and cryomicroscopy are now concurrently being used on samples of L1L2 V capsids to unequivocally locate the position of L2 in the capsid.

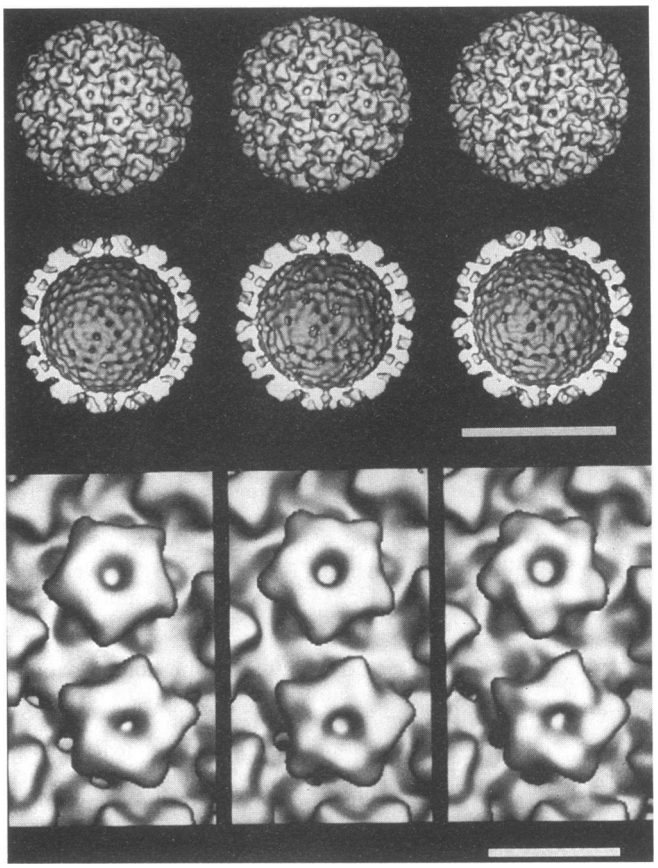

FIG. 2. Surface-shaded representations of reconstructions of HPV-1 from warts (left column), L1 V capsids (middle column), and L1L2 V capsids (right column). Outside view of capsids (top row), inside view (middle row), and a close-up view of pentavalent (top) and hexavalent (bottom) capsomers (bottom row). Internal density out to a radius of 20 nm was computationally removed to better observe the internal features of the capsid. Bars = 50 nm (top and middle rows) and 10 nm (bottom row).

We have used vaccinia virus-derived HPV-1 capsids to develop a radioimmunoprecipitation assay (6) or enzyme-linked immunosorbent assay (ELISA) (7) that identifies human antibodies reactive to one or more conformational epitopes on L1. Good correlation between human sera with a positive ELISA or radioimmunoprecipitation assay and a history of foot warts was seen. Serologic data coupled with the reconstruction data presented here confirms that V capsids possess antigenic targets similar to authentic HPV-1 virions. This validates the use of vaccinia virus-derived HPV capsids in the development of serological assays as well as other potential uses in vaccine development and identification of a cellular receptor.

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