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Expression of the Human Papillomavirus Type 11 L1 Capsid Protein in *Escherichia coli*: Characterization of Protein Domains Involved in DNA Binding and Capsid Assembly

MAOLIN LI,[1] TIMOTHY P. CRIPE,[1]† PATRICIA A. ESTES,[1] MARY K. LYON,[2] ROBERT C. ROSE,[3] AND ROBERT L. GARCEA[1]*

Section of Pediatric Hematology/Oncology, Department of Pediatrics, University of Colorado School of Medicine, Denver, Colorado 80262[1]; Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309[2]; and Infectious Disease Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642[3]

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The L1 major capsid protein of human papillomavirus type 11 (HPV-11) was expressed in *Escherichia coli*, and the soluble recombinant protein was purified to near homogeneity. The recombinant L1 protein bound DNA as determined by the Southwestern assay method, and recombinant mutant L1 proteins localized the DNA-binding domain to the carboxy-terminal 11 amino acids of L1. Trypsin digestion of the full-length L1 protein yielded a discrete 42-kDa product (trpL1), determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, resulting from cleavage at R415, 86 amino acids from the L1 carboxy terminus. Sucrose gradient sedimentation analysis demonstrated that trpL1 sedimented at 11S, while L1 proteins with amino-terminal deletions of 29 and 61 residues sedimented at 4S. Electron microscopy showed that the full-length L1 protein appeared as pentameric capsomeres which self-assembled into capsid-like particles. The trpL1 protein also had a pentameric morphology but was unable to assemble further. In an enzyme-linked immunosorbent assay, the trpL1 and L1 capsids reacted indistinguishably from virus-like particles purified after expression of HPV-11 L1 in insect cells. The carboxy terminus of L1 therefore constitutes the interpentamer linker arm responsible for HPV-11 capsid formation, much like the carboxy-terminal domain of the polyomavirus VP1 protein. The trypsin susceptibility of HPV-11 L1 capsids suggests a possible mechanism for virion disassembly.

Papillomaviruses are a family of nonenveloped, double-stranded DNA viruses which infect many species, and at this time more than 70 different types of human papillomaviruses (HPVs) have been identified. Image analysis of cryoelectron micrographs of bovine papillomavirus type 1 (BPV-1) and HPV-1 has demonstrated a common structure comprised of 72 pentameric capsomeres arranged on a T=7 icosahedral capsid lattice (2). This overall structure is similar to that previously described for simian virus 40 (SV40) and murine polyomavirus (15, 27), although possible differences in capsomere morphology and intercapsomere contacts are suggested by the image reconstruction. The papillomavirus genome encodes two structural proteins, L1 and L2, synthesized late in infection. In the virion, the ratio of L1 (55 kDa) to L2 (74 kDa) has been estimated over a range from 5:1 to 30:1 (26a). By analogy to polyomavirus and SV40, the capsomeres are likely formed by pentamers of L1. L2 may associate with the L1 pentamer, although given the uncertain ratio of L1 to L2, all or a subset of the pentamers may be occupied.

Papillomaviruses are difficult to propagate in vitro. Therefore, recombinant expression systems have been used to express the L1 and L2 proteins in order to characterize their immunologic characteristics as well as to analyze their structural properties. Capsid proteins for HPV-16, BPV-1, HPV-11, and HPV-33 have been expressed in insect cells with baculovirus vectors (12, 23, 28), and those for HPV-1, HPV-16, and BPV-1 have been expressed in mammalian cells with vaccinia virus (10, 30). These expression systems all generate virus-like particles (VLPs) similar in appearance to empty capsids, and these VLPs have immunologic characteristics suggesting a native conformation. When cells expressing L1 are examined by electron microscopy, the VLPs are seen exclusively in the nuclei of the cells (12, 23, 30). These results are also analogous to observations with polyomavirus (17), suggesting not only common structural features between these virus families but also similar control of the intracellular site of capsid assembly.

The polyomavirus major capsid protein VP1, purified after expression in *Escherichia coli*, self-assembles in vitro into capsid-like structures (25). This self-assembly reaction has provided a useful method for assaying conditions favoring capsid assembly as well as for examining mutant VP1 proteins for their ability to assemble (8, 26). In order to analyze the assembly properties of L1, we have purified the HPV-11 L1 protein after expression in *E. coli*. This recombinant protein also self-assembled in vitro into capsid-like particles; however, truncations of the L1 protein revealed a domain structure that differs from that of the polyomavirus VP1 protein.

MATERIALS AND METHODS

Plasmid constructions. The full-length HPV-11 L1 DNA sequence was obtained by PCR amplification from the plasmid pVL11L1 (23), with a forward primer, 5′-GCCGCGAAGCTTCATATGTGGCGGCCTAGCGACAG, containing an *Nde*I restriction enzyme site at the initiator methionine codon, and a reverse primer, 5′-GGGCCTGGATCCAGATCTCACAACACACTGACAC AC, containing a *Bgl*II restriction enzyme site at the 3′ terminal sequence. The PCR-amplified fragment was purified with a Geneclean II Kit (Bio 101) and then subcloned into a PCR II vector (Invitrogen). To generate the pET-3a-HPV-11 L1 plasmid, the plasmid pET-3a-SV40Agno (4a) was digested with *Nde*I and *Bam*HI, and the *Nde*I-*Bgl*II fragment of HPV-11 L1 subcloned from the PCR was then ligated.

* Corresponding author. Mailing address: Section of Pediatric Hematology/Oncology, Department of Pediatrics, University of Colorado School of Medicine, 4200 E. Ninth Ave., Box C229, Denver, CO 80262.

† Present address: University of Wisconsin Comprehensive Cancer Center, Madison, WI 53792.

The mutant L1 coding sequences with amino-terminal and carboxy-terminal deletions were generated with the cloning methods described above. Two proteins with amino-terminal deletions, NΔ29L1 and NΔ61L1, were PCR amplified with two forward primers, 5′-GCGAAGCTTCATATGACCAACATATTTTAT CATGCC and 5′-GCGAAGCTTCATATGGTGTCTGGATATCAATATAGA, and a reverse primer as described above. Two mutant L1 proteins with carboxy-terminal deletions, CΔ11L1 and CΔ86L1, were PCR amplified with a forward primer as described above and two reverse primers, 5′-CCTGGATCCAGATC TCATGTAGAGGGCTTAGACACAGC and 5′-CCTGGATCCAGATCTCAT CTATAAGTATCCTCCAGTGT.

Recombinant L1 expression and purification. L1 proteins were expressed from the T7 promoter of pET-3a-HPV-11 L1 in the host bacteria BL21(DE3). Bacteria were grown at 37°C in M9 medium with ampicillin (100 μg/ml) overnight. Fresh medium (50 ml) was then inoculated with 1 ml of this overnight culture, and cells were grown for 6 to 8 h at 37°C. This culture was used to inoculate 500 ml of fresh medium containing 100 μg of ampicillin per ml. Bacteria were induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and grown for 6 h. The cells were then pelleted and stored at −20°C.

Bacteria from 2 liters of culture were thawed in 200 ml of buffer A with 0.25 M NaCl (50 mM Tris-HCl [pH 7.9], 5% glycerol, 2 mM EDTA, 15 mM 2-mercaptoethanol, 250 mM NaCl), and lysozyme was added to 200 μg/ml. After 20 min at 4°C, sodium deoxycholate and phenylmethylsulfonyl fluoride were added to final concentrations of 0.05% (wt/vol) and 1 mM, respectively. All subsequent steps were carried out at 4°C. The cell suspension was sonicated with a Heat System Ultrasonics W-225 for three 40-s bursts, at 1-min intervals. The sonicated suspension was subjected to Dounce homogenization 20 times with a B pestle. The homogenate was centrifuged at 12,000 × *g* for 20 min, and Polymin P was added slowly to the supernatant to a final concentration of 0.5% (wt/vol) and incubated for 20 min. After centrifugation at 10,000 × *g* for 15 min, the pellet was resuspended in 50 ml of buffer B with 1 M NaCl (10 mM Tris-HCl [pH 7.9], 5% glycerol, 2 mM EDTA, 15 mM 2-mercaptoethanol, 1 M NaCl) with a Dounce homogenizer. The suspension was centrifuged at 10,000 × *g* for 15 min. The pellet was reextracted with 50 ml of the same buffer, and the supernatants were combined and precipitated by the addition of ammonium sulfate to 35% saturation. The pellet from the first homogenate was extracted with 100 ml of buffer B with 1 M NaCl with a Dounce homogenizer. The suspension was centrifuged at 10,000 × *g* for 15 min, and the supernatant was precipitated with ammonium sulfate as described above. This precipitate was resuspended in 20 ml of buffer C with 0.1 M NaCl (10 mM Tris-HCl [pH 7.2], 5% glycerol, 2 mM EDTA, 15 mM 2-mercaptoethanol, 100 mM NaCl) and dialyzed against the same buffer. The insoluble material was removed by centrifugation at 10,000 × *g* for 20 min. The supernatant was subjected to chromatography on a DE-52 cellulose column in buffer C with 0.1 M NaCl. The flowthrough was applied to a P11 phosphocellulose (Whatman) column. The column was washed once with buffer C with 0.25 M NaCl and once with buffer C with 0.5 M NaCl. L1 was eluted with buffer C with 1 M NaCl. Further purification was carried out by pelleting the protein from sucrose gradients (see below) or directly from the 1 M NaCl eluant by centrifugation at 120,000 × *g* for 20 min. Purification steps were monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining and immunoblotting (23).

Trypsin digestion and protein sequence determination. Purified L1 protein was dialyzed against trypsin digestion buffer (10 mM Tris-HCl, 2 mM EDTA, 15 mM 2-mercaptoethanol, 100 mM NaCl, 100 mM $NaHCO_3$, pH 7.8). Trypsin digestion was carried out for 2 h at 4, 25, or 37°C, with the ratio of protein to trypsin at 1:100 (wt/wt). Trypsin-digested L1 peptides were resolved by SDS–7.5% PAGE and electroblotted to an Immobilon-P membrane in CAPS buffer (10 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 10% methanol, pH 11.0) (1). Proteins were stained with 0.1% Coomassie blue R-250 in 50% methanol for 5 min and destained with 50% methanol–10% acetic acid with several changes. The L1 proteolytic fragments were excised and subjected to protein sequencing. The amino-terminal 8 amino acids of the trypsin products were determined with an Applied Biosystems gas phase sequencer.

Southwestern assays. L1 was separated by SDS–9% PAGE, transferred to nitrocellulose membranes, and then incubated for 20 min with 2% bovine serum albumin. Proteins were renatured in buffer D (50 mM Tris-HCl [pH 7.4], 1 mM EDTA, 200 mM NaCl, 10% glycerol, 0.1% Nonidet P-40) at 4°C overnight. The DNA probe was prepared from the plasmid SP65 digested with *Eco*RI and labeled with [^{32}P]dCTP, using the Prime-a-gene System (Promega). The DNA binding assay was carried out in buffer E (30 mM HEPES [pH 7.4], 5 mM $MgCl_2$, 50 mM NaCl) with ^{32}P-labeled plasmid DNA for 30 min at room temperature (19). The nitrocellulose filters were washed four times with binding buffer and then subjected to autoradiography.

Sucrose gradient sedimentation of L1 proteins. L1 fractions eluting from the phosphocellulose column in buffer C with 1 M NaCl were dialyzed against buffer A with 0.25 M NaCl. Four hundred microliters was layered onto a 5 to 20% sucrose gradient in buffer A with 0.25 M NaCl and centrifuged at 39,000 rpm for 16 h in an SW41 rotor. Fractions (0.5 ml) were collected from below. The fractions were assayed for L1 by immunoblotting or Southwestern assay following SDS-PAGE. The L1 protein pelleted in the sucrose gradients was near homogeneous.

Electron microscopy. L1 protein obtained after the phosphocellulose purification step was dialyzed into buffer containing 0.1 M NaCl, 20 mM Tris-HCl (pH 7.2), 1 mM EDTA, and 15 mM 2-mercaptoethanol. Samples were applied to glow-discharged carbon-coated 400-mesh grids and stained with 2% uranyl acetate. Images were photographed with a Philips CM 10 electron microscope at nominal magnifications of ×39,000 or ×73,000.

Enzyme-linked immunosorbent assay (ELISA). VLPs were purified as described previously (22, 23). Methods used for the production of rabbit polyclonal HPV-11 virion and HPV-11, HPV-16, and HPV-18 VLP antisera have been described previously (4, 22, 24). Purified antigens were diluted in phosphate-buffered saline to a concentration of 0.01 mg/ml, and 100-μl aliquots were dispensed into 96-well microtiter plates. The plates were incubated overnight at 4°C and then blocked for 2 h at room temperature, as previously described (24).

Threefold serial dilutions of primary antisera were made in a diluent solution containing 1% bovine serum albumin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) and 10% (vol/vol) wild-type *Autographa californica* nuclear polyhedrosis virus-infected cell culture supernatant, added to reduce background seroreactivities (22). Diluted sera were preabsorbed overnight at 4°C, centrifuged for 1 min at 14,000 × *g*, and then added in 100-μl aliquots to replicate wells containing antigen (test wells) or phosphate-buffered saline alone (control wells). Secondary antibody conjugate (anti-rabbit immunoglobulin G-alkaline phosphatase at a 1:2,000 dilution; Kirkegaard & Perry Laboratories) and enzyme substrate were then added as previously described (24). Specific absorbance values were calculated by subtracting control well values from test well values, and replicate differences were averaged.

RESULTS

Purification of the HPV-11 L1 protein after expression in *E. coli*. The full-length HPV-11 L1 coding sequence was cloned into the vector pET-3a to yield pET3aL1, and protein expression was induced from the T7 promoter of the vector by addition of IPTG. L1 was expressed more efficiently in the *E. coli* strain BL21(DE3) than in HMS174(DE3) (data not shown). The L1 protein was purified according to a protocol based on that used for the purification of the polyomavirus VP1 protein (14). The steps in the purification were assayed by SDS-PAGE and immunoblot analysis with an anti-L1 rabbit antiserum (Fig. 1) (23). After cell lysis, approximately 50% of the L1 was soluble. L1 remaining in the bacterial cell pellet at this stage could be partially recovered by further extraction in a buffer containing 1 M NaCl. Under the loading buffer conditions, L1 was found in the flowthrough fraction from the DE-52 cellulose column (Fig. 1, lanes 3) and was retained on the P11 phosphocellulose column (Fig. 1, lanes 4 and 5). L1 eluted in 1 M NaCl buffer from the phosphocellulose column (Fig. 1, lanes 6). This chromatographic behavior is similar to that of the polyomavirus VP1 protein (14). The eluant from the phosphocellulose column contained various amounts of minor contaminating proteins, and final purification was achieved with sedimentation through a sucrose gradient (Fig. 1, lanes 6 and 7).

L1 binds DNA. Analysis of L1 DNA binding was performed with the Southwestern blot assay (19). In order to characterize the DNA-binding domain identified by this assay, L1 proteins with amino- and carboxy-terminal deletions were generated by site-directed mutagenesis of the L1 expression vector. Mutant proteins were partially purified after expression in bacteria. The L1 proteins were separated by SDS-PAGE, electrotransferred to nitrocellulose, and hybridized with ^{32}P-labeled plasmid DNA. As seen in Fig. 2, DNA binding similar to that of the full-length protein was seen for two mutant proteins with amino-terminal deletions, NΔ29L1 and NΔ61L1. However, two mutant proteins with carboxy-terminal deletions, CΔ86L1 and CΔ11L1, failed to bind DNA. DNA binding to the full-length L1 protein was also tested with increasing concentrations of NaCl in the Southwestern blot assay, and DNA binding was lost when greater than 200 mM NaCl was present in the wash buffer (data not shown).

The mutant CΔ11L1 also behaved differently from the full-length L1 protein during its purification, in that CΔ11L1 eluted from the phosphocellulose column in a buffer containing 0.5 M

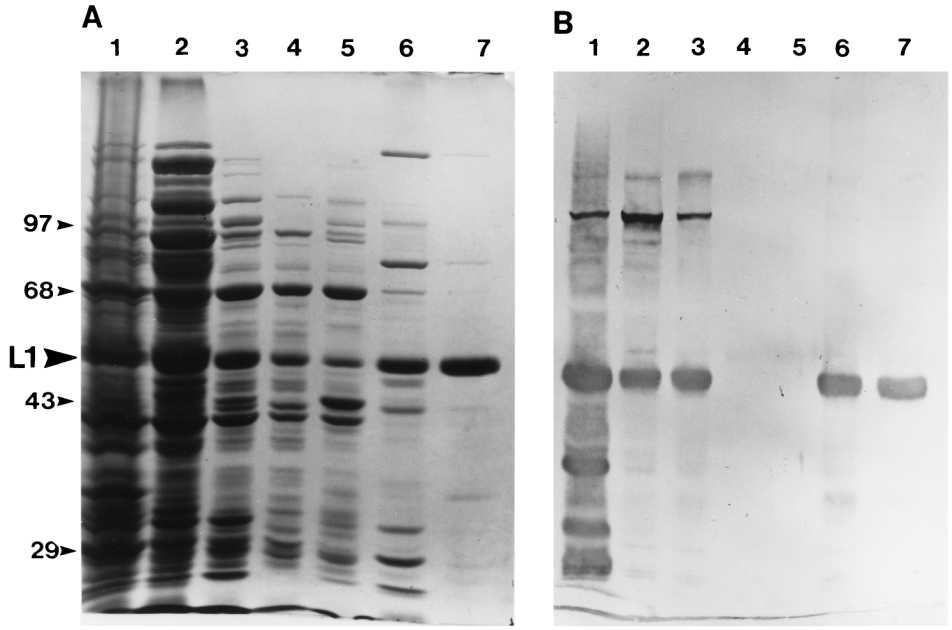

FIG. 1. Purification of HPV-11 L1 after expression in *E. coli*. SDS-PAGE analysis of L1 fractions either stained with Coomassie blue (A) or immunoblotted with an anti-L1 antiserum (B). Lanes: 1, whole-cell lysate; 2, ammonium sulfate precipitate; 3, flowthrough from DE-52 column; 4, flowthrough from phosphocellulose column; 5, 250 mM NaCl eluant from phosphocellulose; 6, 1 M NaCl eluant from phosphocellulose; 7, L1 pelleted after sucrose gradient sedimentation. Molecular mass standards (in kilodaltons) appear on the left.

NaCl. For the polyomavirus VP1 protein, disulfide reducing agents eliminated DNA binding in the Southwestern assay (19). However, neither 2-mercaptoethanol nor dithiothreitol affected L1 DNA binding (data not shown). A DNA-binding domain therefore can be localized to the carboxy-terminal 11 amino acids of L1.

Trypsinization of recombinant L1 yields a discrete 42-kDa fragment. Protease sensitivity of viral capsid proteins has been informative in assessing their native conformation, and for example, trypsin cleaves the polyomavirus VP1 protein at a single lysine residue (K28) near the amino terminus (19). In order to characterize protease-accessible domains of L1, the purified recombinant L1 protein was subjected to trypsin digestion. After digestion with trypsin, two progressive cleavage products were identified by SDS-PAGE (Fig. 3A). The first (L1a) was an ≈48-kDa species obtained after digestion at either 4 or 25°C. The second was an ≈42-kDa species (trpL1) which was obtained after digestion at 37°C for 2 h (Fig. 3A, lane 4) and which remained unchanged upon longer incubations. These cleavage products were characterized by amino-terminal protein sequencing after electrotransfer to an Immobilon filter. Both products had an intact amino terminus, indicating cleavage sites at the carboxy terminus. When directly compared, CΔ86L1 (Fig. 2A, lane 5) (calculated molecular mass, 45.7 kDa) migrated at the same apparent molecular weight by SDS-PAGE as trpL1 (Fig. 3A, lane 4), suggesting that R415 is the cleavage site used to generate trpL1. Based upon its SDS-PAGE mobility in relation to the full-length and trpL1 proteins, the L1a cleavage site is likely to be at residue K450, K460, or R471. The resistance of the amino terminus to digestion suggests that this domain of L1 is relatively inaccessible to digestion in comparison to the carboxy terminus.

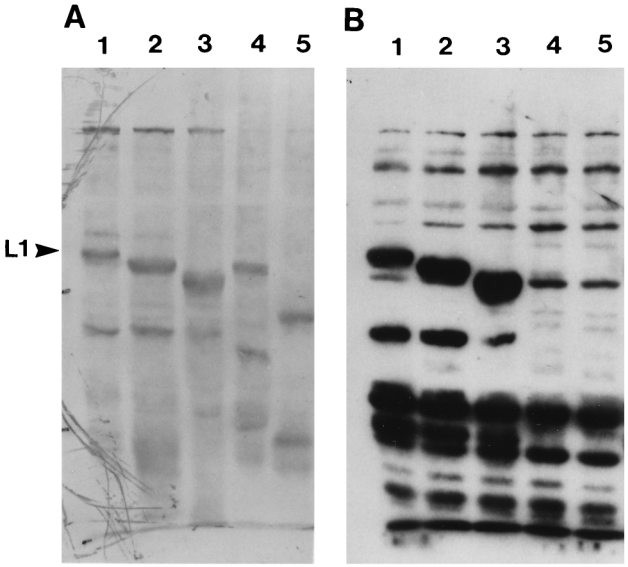

FIG. 2. Southwestern analysis of L1 mutant proteins. Recombinant L1 proteins were partially purified after expression in *E. coli*, subjected to SDS-PAGE, and either immunoblotted with an anti-L1 antiserum (A) or subjected to Southwestern analysis (B). Lanes: 1, wild type; 2, NΔ29L1; 3, NΔ61L1; 4, CΔ11L1; 5, CΔ86L1.

In order to confirm the results of the previous DNA binding experiments, the trypsin-digested L1 cleavage products were analyzed with the Southwestern assay (Fig. 3C). Consistent with the CΔ86L1 and CΔ11L1 results, both trypsin cleavage products did not bind DNA in this assay. In addition, both trpL1 and L1a eluted from phosphocellulose in buffers containing 100 mM NaCl (data not shown), again correlating decreased phosphocellulose binding with deletion of the putative L1 DNA-binding domain.

Sedimentation analysis identifies inter- and intrapentamer bonding domains. Sedimentation analysis has been used to separate polyomavirus subviral intermediates as well as to as-

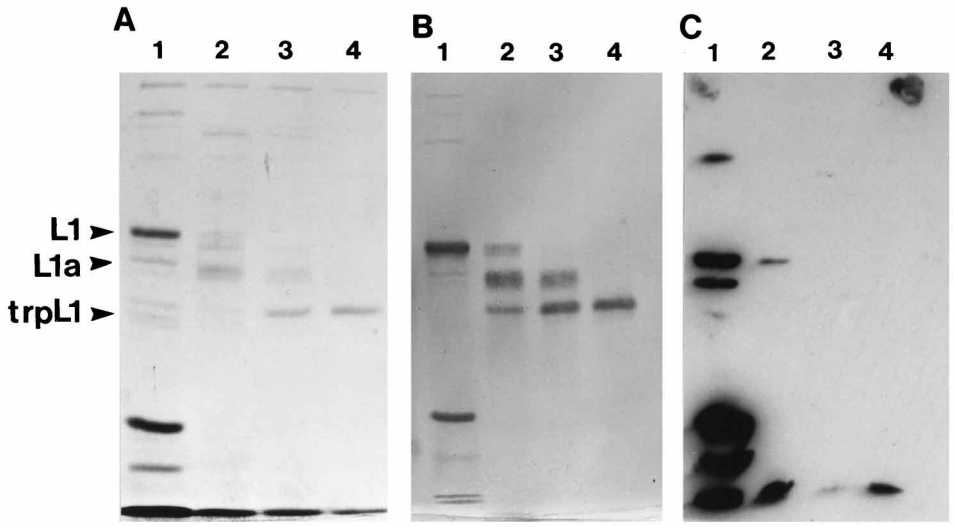

FIG. 3. Trypsin digestion of L1. L1 protein was partially purified after expression in *E. coli*; digested for 2 h with trypsin at the indicated temperatures; subjected to SDS-PAGE; and stained with Coomassie blue (A), immunoblotted with an anti-L1 antiserum (B), or subjected to Southwestern analysis (C). Lanes: 1, no trypsin at 37°C; 2 to 4, trpsin at 4, 20, and 37°C, respectively. L1a and trpL1 indicate major proteolytic products.

sess capsomere and capsid formation (9). For polyomavirus, the pentameric VP1 capsomere sediments at 7.5S, and the capsid sediments at 140S (25). To assay the effect of L1 mutations on capsomere and capsid formation, velocity sedimentation analysis of full-length, trypsinized, and mutant L1 proteins was performed. Given the molecular weight of L1, pentamers of ≈270 kDa might be expected to sediment at 10 to 14S. Under the conditions shown in Fig. 4, capsid-like particles and aggregates having a sedimentation value greater than 19S were pelleted to the bottom of the gradients. The associated nature of the full-length L1 protein was made evident by the small amount of protein sedimenting between 7 and 14S compared with the amount present in the pellet fraction. The majority of the CΔ11L1 protein also pelleted, suggesting capsid or aggregate formation in this preparation (data not shown). The trpL1 protein showed a major component with a sedimentation coefficient of 11 to 12S, a value consistent with that expected for unassociated pentamers.

The mutant proteins with amino-terminal deletions, NΔ29L1 and NΔ61L1, precipitated during the dialysis before the DE-52 column, and only a small amount of soluble mutant protein was obtained. Therefore, their presence in the sucrose gradients was monitored by SDS-PAGE and with the Southwestern assay. Both proteins sedimented with a predominant species at 4S (Fig. 4) (data for NΔ61L1 are not shown). Furthermore, a Centricon-100 spin column (Amicon) with a 100-

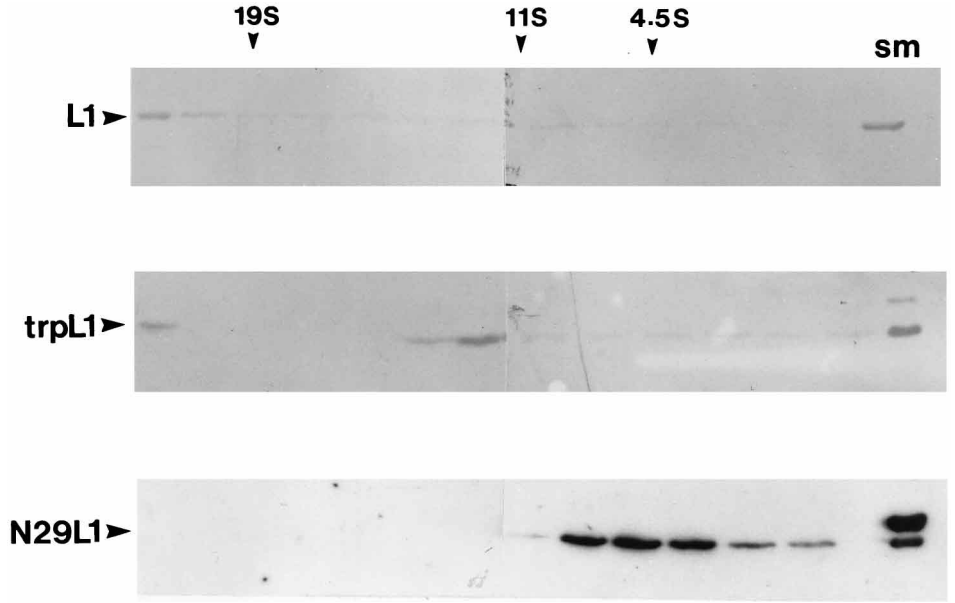

FIG. 4. Sucrose gradient sedimentation of L1 proteins. SDS-PAGE analysis of fractions after sedimentation of L1 proteins in 5 to 20% sucrose gradients. Proteins were detected by immunoblotting (L1 and trpL1) or Southwestern assay (NΔ29L1). Sedimentation markers in a parallel gradient were β-galactosidase (19S), catalase (11.3S), and hemoglobin (4.5S). sm, starting material.

kDa exclusion limit was used to concentrate the mutant NΔ29L1 and NΔ61L1 proteins. Under these conditions, pentameric capsomeres should be retained. Immunoblot analysis showed that the mutant proteins passed through the Centricon-100 (data not shown), providing additional evidence that these truncations at the amino terminus affected pentamer formation and resulted in an L1 protein species of approximately monomer-to-dimer size.

Recombinant L1 self-assembles in vitro into capsid-like particles. HPV-11 L1 protein purified after recombinant baculovirus expression in Sf9 insect cells self-assembles into VLPs (23). Based upon this result, L1 protein purified after expression in *E. coli* was analyzed for in vitro capsid assembly by electron microscopy. As shown in Fig. 5A, the L1 protein, which eluted from the phosphocellulose column in 1 M NaCl, self-assembled into capsid-like structures with a diameter similar to that of papillomavirus virions (55 nm). In addition, unassembled capsomeres with a central stain-filled hollow, which appeared similar to the pentameric capsomeres of polyomavirus VP1, could also be identified (25). Electron micrographs of the trypsin digestion products, L1a and trpL1, showed a uniform population of pentamer-like structures (Fig. 5B and C), although many of the pentamers appeared to lie on their sides, presenting an H or V shape. Capsid-like structures were no longer present after trypsin digestion of L1. Higher-magnification views (Fig. 5C) verified the similarity of these structures to truncated pentamers of the polyomavirus VP1 protein (8). The CΔ11L1 protein was further purified by sucrose gradient sedimentation in order to obtain a homogeneous preparation suitable for electron microscopy. Electron micrographs demonstrated that CΔ11L1 is also capable of in vitro self-assembly into capsid-like aggregates, although they are of less uniform appearance than those of the full-length L1 protein (Fig. 5D). Because of the difficulty in purifying the CΔ86L1 protein, this mutant protein could not be analyzed by electron microscopy.

In order to test whether L1 assembly occurred in vivo, bacteria expressing L1 were fixed, sectioned, and directly examined by electron microscopy. No capsid-like particles were seen in the bacteria, although inclusion bodies were present (data not shown). In addition, crude bacterial lysates were subjected to sucrose gradient sedimentation, and fractions were assayed both by immunoblotting for L1 and by electron microscopy. Only rare capsid-like particles could be detected in the presence of abundant L1 protein (data not shown). Thus, capsid assembly occurs after bacterial lysis and is likely facilitated by the exposure of the L1 protein to high ionic strength, e.g., the phosphocellulose column elution buffer, or to other in vitro conditions which may favor capsid formation.

Immunologic reactivity of bacterially expressed HPV-11 L1. Results from previous studies have demonstrated that VLPs have conformationally dependent capsid-neutralizing antigenic domains similar to those of native HPV-11 virions (24). In addition, these epitopes are antigenically distinct from similar epitopes of HPV-16 and HPV-18 (22). The antigenic properties of HPV-11 capsids and isolated capsomeres obtained after expression in *E. coli* were compared with VLPs in an ELISA (Fig. 6, top panel) (4, 24). Antisera raised against either native HPV-11 virions (4) or HPV-11 VLPs (24) reacted indistinguishably with all of the HPV-11 L1 antigens tested. Denatured VLPs reacted much less efficiently in this assay (data not shown), indicating that nonlinear epitopes were the predominant epitopes recognized by these antisera. Virus type specificity of the epitopes was verified by the failure of antibodies raised against either HPV-16 or HPV-18 capsids to react with the HPV-11 antigens (Fig. 6, bottom panel). These findings are consistent with previous results (22) and indicate that HPV-11 capsids and L1 capsomeres purified after expression in *E. coli* are antigenically similar to HPV-11 VLPs and, thus, to native HPV-11 virions.

DISCUSSION

We have expressed and purified the HPV-11 L1 protein in *E. coli*. The purified protein is soluble and has many of the biochemical properties found previously for the recombinant polyomavirus VP1 protein, such as DNA binding, pentameric morphology, and the ability to self-assemble into capsid-like particles. The introduction of site-directed mutations into the expression vector has allowed identification of three functional domains of the L1 protein: (i) a DNA-binding region near the carboxy terminus, (ii) a region within 86 amino acids of the carboxy terminus required for intercapsomere bonding, and (iii) a region near the amino terminus which affects pentamer formation. The positions of these domains distinguish HPV-11 L1 from polyomavirus VP1, in that the VP1 DNA-binding domain is located at the amino terminus and the intercapsomere bonding domain is at the carboxy terminus. This difference suggests an alternative bonding strategy for L1 in the papillomavirus capsid compared to that of VP1 in the polyomavirus capsid.

Digestion with trypsin was used to identify accessible residues and thus provide evidence for conformationally exposed regions of the L1 pentamer. Characterizations of papillomavirus structural proteins derived from purified virions by SDS-PAGE have previously noted minor capsid protein species which may be degradation products related to those generated by trypsin (6, 7). Interestingly, trypsin cleavage sites were found only at the carboxy terminus, extending inward approximately 86 residues. The inability of the resulting trpL1 pentamers to self-assemble while maintaining their pentameric morphology is similar to the behavior of the polyomavirus ΔNCO VP1 mutant protein (8). This result provides evidence that the intercapsomere linker arm of L1 is located at its carboxy terminus. The PPP motif around residue 404 (Fig. 6) may be similar to the PYP motif in polyomavirus (residue 320) and SV40 (residue 300) (15). These residues signal the exit of the polypeptide chain from the body of the pentamer and its redirection towards its capsomere neighbor, and therefore the PPP motif may represent the beginning of an interpentamer linker arm for L1. The ability of the CΔ11L1 mutant protein to assemble may therefore define the linker arm as the domain between L1 residues 407 and 490.

The sedimentation characteristics of the mutant and trypsinized L1 proteins were consistent with the morphology of the proteins determined by electron microscopy. The trpL1 protein is morphologically similar to the polyomavirus VP1 pentameric capsomere, and the 12S value is therefore representative of an L1 capsomere. The proteins with amino-terminal deletions, NΔ29L1 and NΔ61L1, sediment at less than capsomere size, suggesting that pentamer oligomerization is disrupted by these deletions. The motif PPP, residues 12 to 14 (Fig. 7A), may indicate a chain redirection at the amino terminus of L1, and it is possible that deletions before this signal may not affect pentamer formation.

With the Southwestern assay, a DNA-binding domain was localized to the carboxy-terminal 11 residues of HPV-11 L1 (Fig. 2). We assume that this DNA binding is sequence nonspecific, because a plasmid probe was used for its identification and because similar results for the polyomavirus VP1 protein have indicated no preference for the source of DNA (19). A previous reported study of the HPV-16 L1 protein in which our

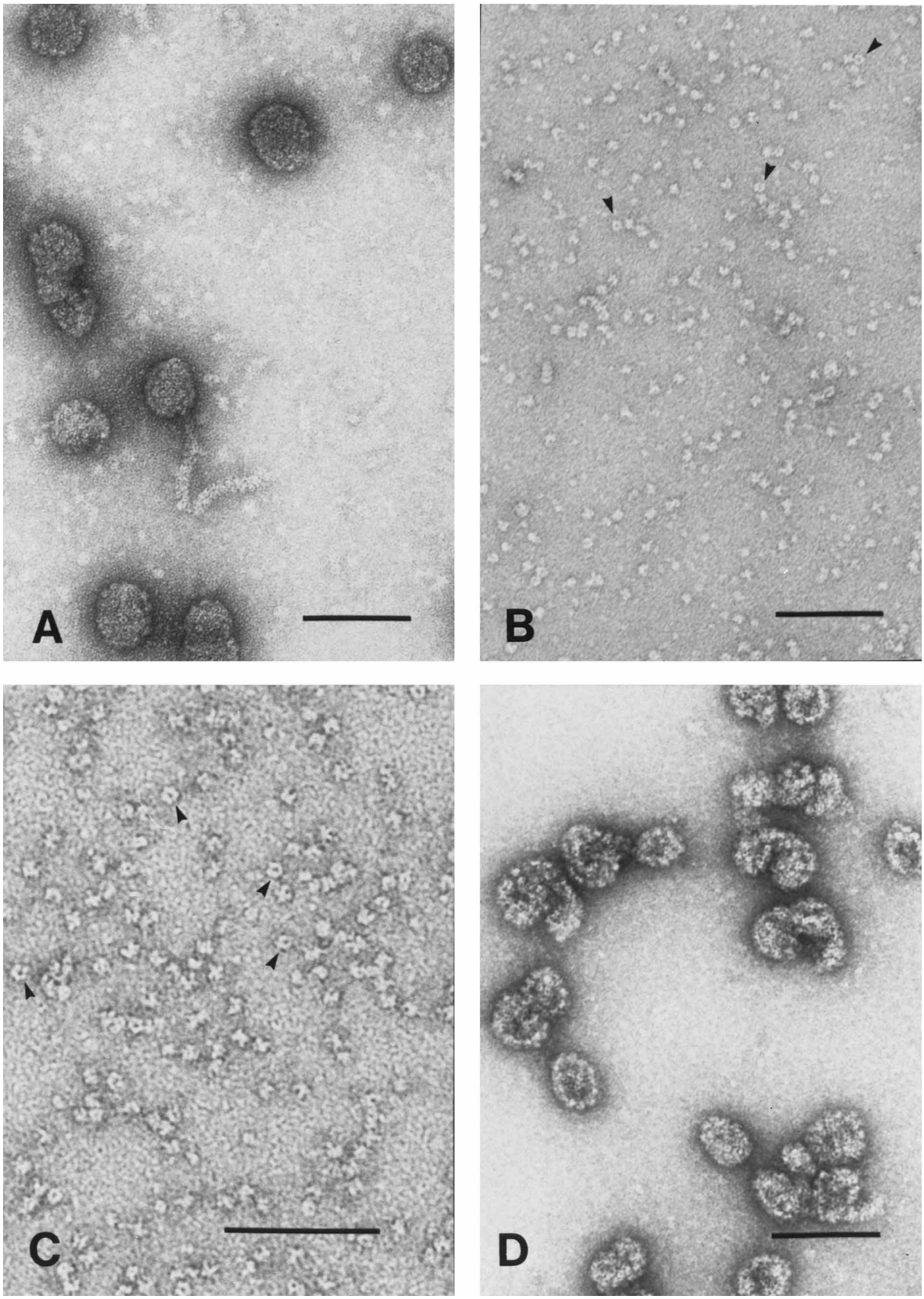

FIG. 5. Electron micrographs of L1 proteins after in vitro self-assembly. Shown are the full-length L1 protein (A), L1a (B), trpL1 (C), and CΔ11L1 (D). (B and C) Arrowheads indicate structures resembling the pentameric capsomeres of polyomavirus VP1 (8), with their stain-filled fivefold axis orthogonal to the grid. Bar, 100 nm.

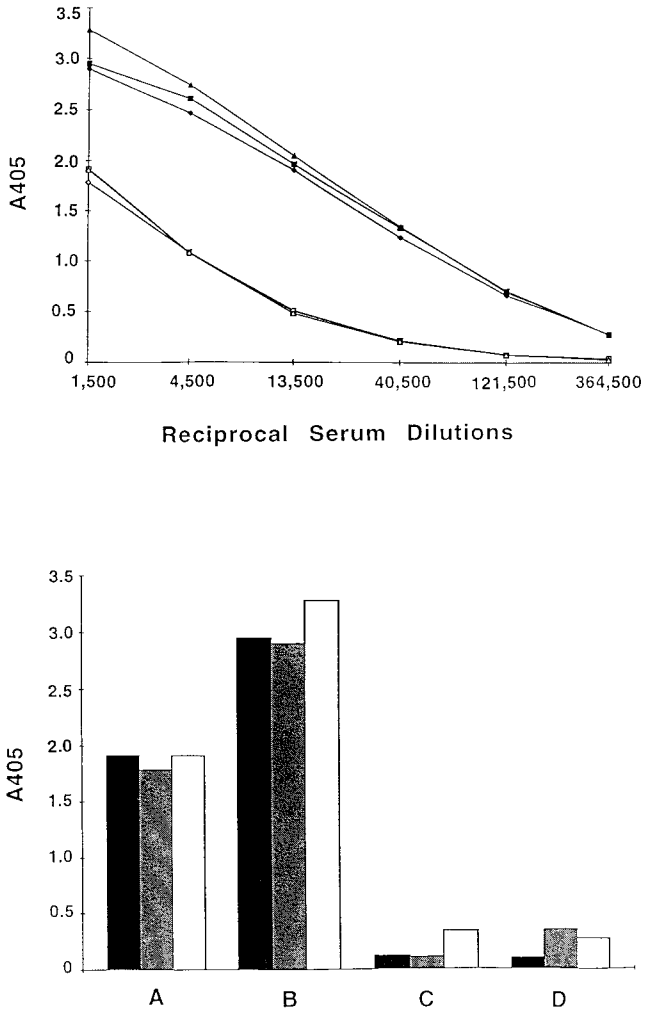

FIG. 6. Antigenic properties of the HPV-11 L1 protein purified after expression in *E. coli*. (Top panel) Polyclonal rabbit antisera against HPV-11 VLPs (filled symbols) or HPV-11 virions (open symbols) were compared in an ELISA for reactivity against VLPs (triangles), capsids prepared from L1 purified after expression in *E. coli* (squares), or trpL1 capsomeres (diamonds). (Bottom panel) Type-specific polyclonal HPV capsid antisera were tested for immunoreactivity in an ELISA against VLPs (white bars), trpL1 capsomeres (grey bars), or capsids assembled from L1 purified after expression in *E. coli* (black bars). Tested were HPV-11 virion antiserum (A), HPV-11 VLP antiserum (B), HPV-16 VLP antiserum (C), and HPV-18 VLP antiserum (D).

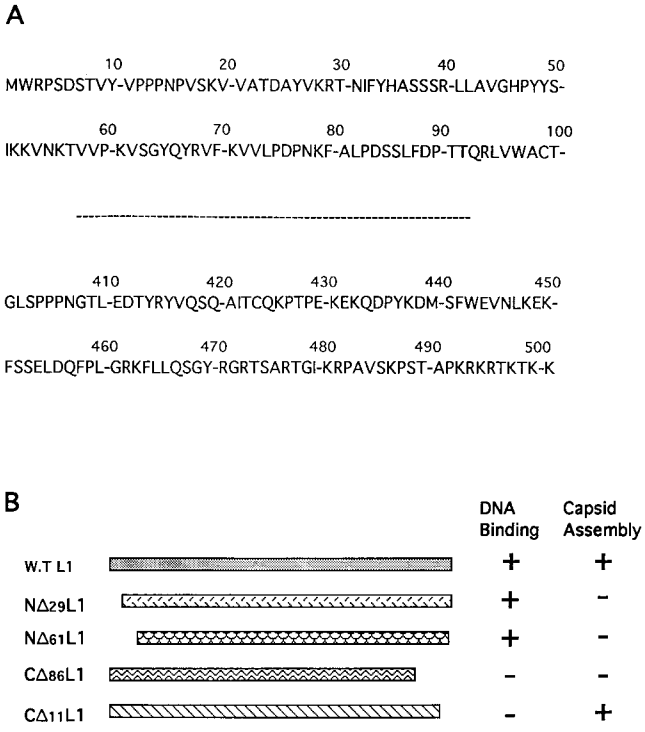

FIG. 7. Summary of L1 proteins analyzed. The L1 amino- and carboxy-terminal protein sequences (A) are shown along with the truncated L1 proteins (B) tested for DNA binding and capsid self-assembly. A consensus bipartite NLS is present at residues 480 to 481 through 492 to 495.

protocol was used found no DNA binding activity for this protein, although the HPV-16 L1 protein has a similar basic region at its carboxy terminus (31). However, fusion proteins for HPV-16 and 6b L1 proteins have demonstrated DNA binding (16); thus, with the current data we believe that L1 is a DNA-binding protein. However, this activity may be modulated if associated with L2 in a manner similar to VP1 association with VP2/VP3 (5). A nuclear localization signal (NLS) sequence was also identified in this region of the HPV-16 L1 protein (29). By inspection, it is likely that a similar NLS sequence may be present in the HPV-11 L1 protein as well (Fig. 7). By analogy to polyomavirus VP1, the DNA binding and NLS sequences may overlap, and further site-directed mutagenesis will be required to identify residues important for each function (18, 19). The biological significance of this DNA binding property also requires further investigation.

The assembly of capsid-like aggregates of HPV-11 L1 parallels the behavior of this protein when expressed in insect cells with baculovirus vectors (23). However, the VLPs purified from the nucleus of the insect cells were more homogeneous in appearance than those generated from in vitro assembly of the *E. coli* recombinant L1 protein. This behavior was previously noted for the polyomavirus VP1 protein and has been attributed to more ideal assembly conditions present intracellularly than in vitro (17). In contrast to polyomavirus VP1, the in vitro-assembled L1 is very difficult to disassemble under conditions of calcium chelation and disulfide bond reduction (14a), and by sucrose gradient analysis the majority of the protein was in an aggregated form. Only with concurrent trypsin digestion could capsid-like aggregates be fully disrupted, again suggesting differences in interpentamer bonding between L1 and polyomavirus VP1. The recent observation of "holes" in a subset of BPV capsids suggests an alternative mechanism of capsid dissociation (3). Calcium chelation and/or disulfide bond reduction may cause a conformational change in the capsid, resulting in the opening of holes between capsomeres. The carboxy termini of the L1 proteins may then become exposed to a trypsin-like protease, which cleaves L1, resulting in irreversible capsid disassembly. This type of conformational change with subsequent sensitivity to proteases has been well described for plant viruses (11, 13, 20) and may be utilized during virus uncoating after infection. Without induction of such a conformational change, however, BPV virions are resistant to trypsin and other proteases (21).

The ELISA analysis suggests that HPV-11 L1 purified after expression in *E. coli* is antigenically similar to VLPs and thus to native HPV-11 virions. The results obtained with the trpL1 protein also demonstrate that the unassembled capsomere subunit retains the overall antigenicity of VLPs and capsids, since the reactivities with polyclonal antisera were indistinguishable

between capsomeres (trpL1), capsids, and VLPs over the titration range of the ELISA. Previous attempts to generate native immunoreactivity with L1 proteins purified after expression in *E. coli* may have been unsuccessful because these proteins were denatured. Although the ability of individual capsomeres to induce HPV-11 virus-neutralizing antibodies remains to be tested, these results suggest that L1 capsomeres may possess a neutralizing conformational domain, characterized previously with the same antisera (4, 22, 24).

The ability to purify the HPV-11 L1 protein in a soluble, nondenatured form after expression in *E. coli* now permits a detailed analysis of the intercapsomere bonding domain and buffer conditions which favor papillomavirus capsid assembly. Further studies will be required to test whether the recombinant HPV-11 capsomeres can generate a neutralizing-antibody response which is biologically significant.

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