Herpes Simplex Virus DNA Cleavage and Packaging: Association of Multiple Forms of U_L15-Encoded Proteins with B Capsids Requires at Least the U_L6, U_L17, and U_L28 Genes

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The U_L15 gene of herpes simplex virus (HSV) is one of several genes required for the packaging of viral DNA into intranuclear B capsids to produce C capsids that become enveloped at the inner nuclear membrane. A rabbit antiserum directed against U_L15-encoded protein recognized three proteins with apparent M_rs of 79,000, 80,000, and 83,000 in highly purified B capsids. The 83,000-M_r protein was detected in type C capsids and comigrated with the product of a U_L15 cDNA transcribed and translated in vitro. The 83,000- and 80,000-M_r proteins were readily detected in purified virions. Inasmuch as (i) none of these proteins were detectable in capsids purified from cells infected with HSV-1(ΔU_L15), a virus lacking an intact U_L15 gene, and (ii) corresponding proteins in capsids purified from cells infected with a recombinant virus [HSV-1(R7244), containing a 20-codon tag at the 3′ end of U_L15] were decreased in electrophoretic mobility relative to the wild-type proteins, we conclude that the proteins with apparent M_rs of 83,000, 80,000, and 79,000 are products of U_L15 with identical C termini. The 79,000-, 80,000-, and 83,000-M_r proteins remained associated with B capsids in the presence of 0.5 M guanidine HCl and remained detectable in capsids treated with 2.0 M guanidine HCl and lacking proteins associated with the capsid core. These data, therefore, indicate that U_L15-encoded proteins are integral components of B capsids. Only the 83,000-M_r protein was detected in B capsids purified from cells infected with viruses lacking the U_L6, U_L17, or U_L28 genes, which are required for DNA cleavage and packaging, suggesting that capsid association of the 80,000- and 79,000-M_r proteins requires intact cleavage and packaging machinery. These data, therefore, indicate that capsid association of the 80,000- and 79,000-M_r U_L15-encoded proteins reflects a previously unrecognized step in the DNA cleavage and packaging reaction.

At least three types of capsids, designated A, B, and C, that differ in density and electron microscopic appearance accumulate in the nuclei of cells infected with wild-type herpes simplex virus type 1 (HSV-1). All three capsid types have an outer protein shell approximately 120 nm in diameter made from hexons and pentons of VP5, the major capsid protein. The hexons and pentons are linked by triplexes composed of VP19c and VP23 (22). The culmination of HSV capsid assembly is the insertion of viral DNA into B capsids to produce C capsids that become enveloped at the inner nuclear membrane. During DNA packaging, internal proteins of B capsids (including a scaffold composed of VP22a [or ICP35] and a viral protease, VP24) are lost as DNA is inserted (12, 14). A capsids are believed to be the products of an aborted packaging reaction in which the internal proteins are lost but DNA is not inserted.

Two types of B capsids, distinguished by the appearance of the internal scaffold, which can be of large diameter (large-cored B capsids, or procapsids [21]) or small diameter (small-cored B capsids), can be seen in electron micrographs of infected cell nuclei. Cleavage of ICP35 in procapsids by the viral protease likely releases the scaffold from the inner side of the outer capsid shell, allowing the scaffold to collapse inward to produce small-cored B capsids (33, 34, 36). Such capsids are readily purified from HSV-infected cells (14). Whether it is the large-cored or small-cored B capsid that receives viral DNA is currently controversial (15, 25, 34).

Mutations in at least seven genes (U_L6, U_L15, U_L17, U_L25, U_L28, U_L32, and U_L33) prevent production of C capsids but are dispensable for assembly of B-like capsids (1, 2, 18, 24, 27, 29, 30, 35, 37). Although the functions of the proteins encoded by these genes are not known, at least the U_L6 and U_L25 proteins can associate with capsids (18, 23). Such capsid-associated proteins could play roles analogous to those of bacteriophages that comprise portal vertices into which DNA is inserted or could act as terminases which link the portal vertices to DNA and help mediate DNA packaging (9). Given the observation that U_L15 and its homologs in other herpesviruses display homology to the T4 bacteriophage terminase gp17 (11), the primary goal of the present study was to determine if the U_L15-encoded protein is a capsid component.

MATERIALS AND METHODS

Viruses and cells. G5 transformed cells were derived from Vero cells and contain HSV-1 DNA from U_L16 to U_L21 (13). Clone 17 cells were derived from rabbit skin cells and contain a cDNA copy of the U_L15 gene (3). The C1 cell line was derived from Vero cells and contains the entire U_L28 gene and the U_L27 gene minus a 969-bp *Bst*EII fragment at the 5′ end (32). The G33 cell line was derived from Vero cells and contains HSV-1 DNA from U_L6 to U_L8 (24). Vero, rabbit skin, HEp-2, G5, C1, G33, and clone 17 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum, penicillin, and streptomycin as previously described (3, 4, 7, 13, 24, 32).

The titers of the wild-type viruses HSV-1(F) and HSV-1(17) and the mutant virus R7244 were determined on Vero cell monolayers. Virus R7244 contains a 20-amino-acid epitope of the human cytomegalovirus glycoprotein B gene incorporated into the C terminus of U_L15 protein encoded by a cDNA inserted into the native position of U_L15 exon I (4). Stocks of R7244 were grown in clone 17 cells. HSV-1(ΔU_L17) contains a *lacZ* expression cassette from a *Not*I site 105 bp from the 5′ end of U_L17 to a *Xho*I site 516 bp from the 3′ end of U_L17; it was grown and its titers were determined on G5 cells (29). HSV-1(ΔU_L15) contains a *lacZ* expression cassette in place of 226 codons of exon II of U_L15; it was grown and its titers were determined on clone 17 cells (3). The mutant gCB contains a 1,881-bp deletion in the U_L28 gene; it was grown and its titers were determined on the C1 cell line (32). Cos-U_L6^- was derived from the HSV-1(17) strain and

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contains a 4-bp insertion at a site corresponding to amino acid residue 381; it was grown and its titers were determined on G33 cells (24).

In vitro expression of U_L15 protein. PRB4503 contains a cDNA of the U_L15 gene inserted into the pGEM3Z vector (Promega) and delimited at the 5′ end by a *Pst*I site at position 28840 and a *Bsu*36I site at the 3′ end at position 35093 of the published HSV-1(17) sequence (4, 17). PRB4503 was transcribed and translated for 1 h at 30°C with the TNT^R T7/SP6 coupled reticulocyte system (Promega) according to the manufacturer's protocol.

Capsid purification and analysis. In a typical purification, Vero cell monolayers from three or four 850-cm^2 roller bottles were infected at a multiplicity of infection of 5.0 PFU per cell and incubated at 34°C for 18 h. Nuclear lysates were prepared as described previously (26) and were separated on a 20 to 50% continuous sucrose gradient at 23,000 rpm for 1 h in a Beckman SW41 rotor. Light-scattering bands near the middle of the tube were collected with either a Pasteur pipette or a fractionating device (Haake Buchler) starting at the top of the tube. The collected material was diluted in TNE (0.5 M NaCl, 20 mM Tris-HCl [pH 7.4], and 1 mM EDTA) and pelleted at 20,000 rpm in a SW41 rotor for 2 h. The capsid-containing pellets were resuspended in TNE by sonication and were separated by centrifugation on a second continuous sucrose gradient. For fractionation experiments, 0.5-ml fractions were collected and pelleted by centrifugation. Fractionated material was either (i) dialyzed against TNE, negatively stained, and viewed by electron microscopy or (ii) resuspended in a buffer containing sodium dodecyl sulfate followed by separation on a denaturing 10% polyacrylamide gel. Electrophoretically separated proteins were either stained with Coomassie brilliant blue or transferred to nitrocellulose and probed with specific antibodies. The intensities of protein bands on Coomassie blue-stained gels or immunoblots were determined from digitized video images produced on a Stratagene Eagle Eye II digital camera with Eagle Sight software (version 3.1). Capsid purity was verified by (i) examination by electron microscopy, (ii) Coomassie blue and silver staining of electrophoretically separated capsid proteins, and (iii) immunoblotting with antibodies against HSV tegument proteins encoded by U_L17 and U_L16 that do not associate with highly purified capsids (19, 29).

Electron microscopy. Purified capsids were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and adsorbed to standard carbon-Formvar-coated copper (300-mesh) electron microscopic grids. Samples were stained for 30 s in a 1:1 solution of potassium phosphotungstic acid and bacitracin and viewed through a Zeiss 902 transmission electron microscope at an accelerated voltage of 80 kV.

GuHCl treatment of capsids. Purified capsids to be extracted with guanidine hydrochloride (GuHCl) were resuspended in TNE at 4°C at 0.5 to 1.5 mg of protein per ml, essentially as described previously (20) except that five 4.5-ml GuHCl solutions were prepared such that addition of 50 μl of purified capsids produced final concentrations of 0.05, 0.1, 0.5, 1.0, and 2.0 M GuHCl, respectively. The extracted capsids were pelleted through a 300-μl cushion of 25% (wt/wt) sucrose by centrifugation in a Beckman SW50.1 rotor for 1 h at 23,000 rpm and were analyzed on denaturing 10% polyacrylamide gels.

Virion purification. Virions were purified essentially as described previously (31). Briefly, Vero cell monolayers in roller bottles were infected at a multiplicity of infection of 3.0 PFU per cell and were held at 34°C. Forty hours later, the cells were resuspended in 1.0 mM NaPO$_4$ (pH 7.4) and homogenized in a Dounce homogenizer; the nuclei were immediately stabilized with 1.25 M sucrose in 1.0 mM NaPO$_4$, and the virions were pelleted. The supernatant was then loaded onto a linear dextran T10 gradient and centrifuged in a Beckman SW28 rotor at 20,000 rpm for 1 h. The virion band, visible as a hazy region just above the middle of the tube, was collected and pelleted at 25,000 rpm for 2 h. For the second purification, virions were resuspended by sonication and trituration, solid sucrose was added to produce a solution of 50% sucrose (wt/wt), and the material was placed in a SW28 tube and overlaid with a discontinuous gradient formed by successive layers of 40, 30, and 20% sucrose (wt/wt). The gradients were then centrifuged at 25,000 rpm for 18 h in a SW28 rotor. The bands containing virions were collected with a Pasteur pipette. The virions were pelleted and suspended in a small volume of 0.01 M Tris buffer, and associated proteins were denatured in buffer-containing sodium dodecyl sulfate and separated on a denaturing polyacrylamide gel.

Immunoblotting. Electrophoretically separated capsid or virion proteins were electrically transferred to nitrocellulose and probed with either a previously characterized polyclonal antiserum directed against U_L15-encoded proteins at a dilution of 1:750 or with ICP5-specific polyclonal antiserum (NC1) at a dilution of 1:5,000 (10). The U_L15-specific rabbit polyclonal antibody was generated by immunization with an affinity-purified bacterial protein (U_L15-MBP) containing the *malE* gene product fused to the protein encoded by the majority of U_L15 exon II (4). Bound antibody was visualized by the addition of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin followed by the addition of chromogenic substrate (Bio-Rad) as previously described (6). The apparent M_rs of proteins were calculated based upon the migration of prestained standards (Bio-Rad). The migration of each standard in a given batch was verified by comparison to electrophoretically separated, unlabeled proteins.
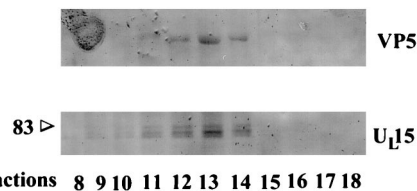

FIG. 1. Scanned digital images of immunoblots probed with anti-U_L15-MBP antibody. Fractions (0.5 ml) of a 14-ml continuous sucrose gradient containing purified B capsids were collected starting at the top of the tube, and pelleted material was electrophoretically separated, transferred to nitrocellulose, and reacted with the U_L15-MBP antiserum. Bound immunoglobulin was visualized by addition of alkaline phosphatase-conjugated anti-rabbit antibody followed by fixation of colored substrate. The two panels show regions of the immunoblot containing VP5 (upper panel), and U_L15-encoded proteins (lower panel). The positions of bands corresponding to the 83,000-M_r (83) U_L15 proteins are indicated. Only fractions 8 to 18 are shown; fractions 1 to 7 and 19 to 25 did not contain detectable levels of U_L15 or VP5.

RESULTS

Multiple U_L15 proteins are components of B capsids. To test the possibility that the U_L15 protein is capsid associated, type B capsids were purified from HSV-1(F)-infected Vero cells on two successive continuous sucrose gradients (see Materials and Methods). The capsids were then separated on a third continuous sucrose gradient, fractions were collected, and the capsids were pelleted by centrifugation. Capsid-associated proteins were electrophoretically separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and reacted with a previously described antiserum directed against U_L15 exon II-encoded protein sequences and maltose-binding protein (U_L15-MBP) (4). Bound antibody was visualized by the addition of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin followed by the addition of chromogenic substrate. The results are shown in Fig. 1.

Three proteins with M_rs of approximately 83,000, 80,000, and 79,000 were readily detected by the anti-U_L15-MBP serum in fractions 11 to 14 and were faintly detectable in fractions 9, 10, and 15 of the sucrose gradient, whereas other fractions did not contain these proteins at detectable levels. Electron microscopic analysis revealed that fractions 11 to 14 contained capsids without other electron-dense material greater than 2.0 nm in diameter (data not shown). In some immunoblots of capsid proteins probed with the anti-U_L15-MBP serum (such as that shown in Fig. 1), it was noted that the anti-U_L15-MBP antibody reacted weakly with large amounts of VP5. Thus, lanes of the immunoblot that contained fractions 11 to 14 also contained a band corresponding to the major capsid protein, VP5. Both the VP5 and U_L15 proteins were most readily detected in fraction 13. Gradient fractions 1 to 7 and 20 to 25 did not contain proteins that reacted with the anti-U_L15-MBP antibody, nor was VP5 detectable (data not shown). Taken together, these data indicate that U_L15 proteins with apparent M_rs of 83,000, 80,000, and 79,000 are readily detectable in purified capsids.

To insure that the proteins with apparent M_rs of 83,000, 80,000, and 79,000 recognized by the anti-U_L15-MBP antiserum were encoded by U_L15, B capsids were purified from Vero cells infected with HSV-1(F) or a virus lacking most of the second exon of U_L15, designated HSV-1(ΔU_L15) (3). Proteins associated with the purified capsids were electrophoretically separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and reacted with the anti-U_L15 antiserum. As shown in Fig. 2A, the proteins with apparent M_rs of 83,000, 80,000, and 79,000 present in HSV-1(F) capsids were not detectable in capsids purified from HSV-1(ΔU_L15)-infected cells.
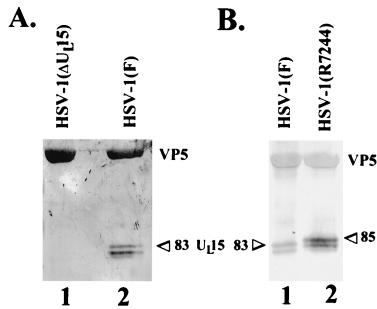
FIG. 2. Scanned digital images of immunoblots probed with anti-U$_L$15-MBP antibody. Electrophoretically separated proteins were transferred to nitrocellulose and probed with the antiserum directed against the U$_L$15-MBP fusion protein. (A) Type B capsids were purified from cells infected with wild-type HSV-1(F) or with HSV-1(ΔU$_L$15), lacking most of the U$_L$15 second exon. (B) B capsids were purified from cells infected with HSV-1(F) or with HSV-1(R7244), containing a 20-codon epitopic tag at the 3′ end of the U$_L$15 gene. The positions of the bands corresponding to the 83,000- and the 85,000-M_r U$_L$15 proteins (83 and 85, respectively) are indicated.

Cross-reactivity with VP5 in the same lanes of the immunoblot showed that similar amounts of capsid proteins were loaded in each lane of the polyacrylamide gel. We conclude that, inasmuch as they are not detectable in capsids purified from cells infected with the U$_L$15 deletion mutant, the 83,000-, 80,000-, and 79,000-M_r proteins recognized by the U$_L$15-MBP antiserum are products of the U$_L$15 gene.

To further demonstrate that the 83,000-, 80,000-, and 79,000-M_r proteins were derived from translation of the U$_L$15 gene, we took advantage of an available recombinant virus, designated R7244, which contained DNA encoding a 20-amino-acid epitopic tag inserted within the 3′ end of a U$_L$15 cDNA. Lysates of cells infected with R7244 contain U$_L$15 protein with decreased electrophoretic mobility relative to the U$_L$15 protein in HSV-1(F)-infected cell lysates (4). B capsids were purified from Vero cells infected with HSV-1(F) or HSV-1(R7244). Capsid-associated proteins were then electrophoretically separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and probed with the anti-U$_L$15-MBP serum. As shown in Fig. 2B, HSV-1(F) B capsids contained proteins with M_rs of 83,000, 80,000, and 79,000 that were recognized by the U$_L$15-MBP-specific antiserum. In contrast, the anti-U$_L$15-MBP serum recognized three proteins with apparent M_rs of 85,000, 82,000, and 81,000 in lysates of B capsids purified from R7244-infected cells. Thus, capsids purified from R7244-infected cells contain capsid-associated U$_L$15-encoded proteins with decreased electrophoretic mobilities relative to those of the corresponding wild-type proteins. These data demonstrate that the 83,000-, 80,000-, and 79,000-M_r proteins are products of U$_L$15, since they contain protein encoded by the 3′ end of the U$_L$15 gene.

One noteworthy observation is that the band with an apparent M_r of 79,000 was not detected in all capsid preparations, possibly due to the inability to resolve the 80,000- and 79,000-M_r proteins in some experiments. We also cannot exclude the possibility that the protein with an apparent M_r of 79,000 may arise from degradation of either the 83,000- or the 80,000-M_r protein. However, at least the 83,000- and 80,000-M_r U$_L$15-encoded proteins were always detected in wild-type B capsids.

Effects of GuHCl extraction on the association of the U$_L$15 protein with B-type capsids. Treatment of purified capsids with various concentrations of GuHCl has been shown to result in removal of selected proteins. Specifically, the majority of the capsid pentons, comprised mostly of VP5, VP26 (which binds
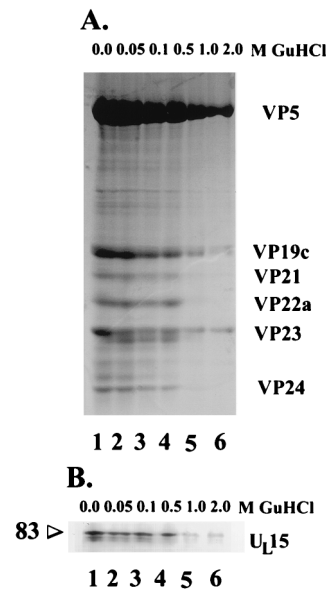

FIG. 3. (A) Scanned image of Coomassie blue-stained denaturing polyacrylamide gel. Equal amounts of purified B capsids were extracted with the indicated concentrations of GuHCl, and proteins which remained capsid associated were electrophoretically separated and stained with Coomassie blue. The identities of capsid proteins are indicated to the right. (B) Immunoblot of GuHCl-treated capsids. Samples of the preparations shown in panel A were electrophoretically separated, transferred to nitrocellulose, and reacted with the anti-U$_L$15-MBP serum. The position of a band corresponding to the 83,000-M_r U$_L$15 protein (83) is indicated.

the tips of the hexons), and components of the capsid core (VP22a), are removed upon treatment with 2.0 M GuHCl. In contrast, VP5, comprising the capsid hexons, and VP19c and VP23, which comprise the capsid triplexes, are more resistant to extraction under this treatment (20, 22, 23).

To determine whether U$_L$15 proteins were tightly associated with capsids, B-type capsids were purified and equal amounts were extracted with 0.05, 0.1, 0.5, 1.0, or 2.0 M GuHCl, pelleted through a sucrose cushion to remove solubilized material, separated on a denaturing polyacrylamide gel, stained with Coomassie blue, and analyzed by densitometry (see Materials and Methods). Compared to the amounts in untreated capsids (Fig. 3A, lane 1), approximately 40% of VP5, 21% of VP19c, and 30% of VP23 were retained in pelletable material upon

TABLE 1. Percentages of capsid proteins remaining after extraction with GuHCl[a]

Protein	% Protein after extraction with indicated concn (M) of GuHCl				
	0.05	0.1	0.5	1.0	2.0
VP5	100.0	100.0	93.0	79.0	40.0
VP19c	95.0	81.0	67.0	40.0	21.0
VP21	97.0	68.0	49.0	16.0	ND[b]
VP22a	96.0	68.0	74.0	12.0	ND
VP23	89.0	60.0	54.0	35.0	30.0
VP24	92.0	58.0	37.0	3.0	ND
U$_L$15 protein	81.0	83.0	75.0	16.0	15.0

[a] Percentages were calculated by dividing densitometric values of Coomassie blue-stained proteins in capsids extracted with GuHCl by values of corresponding proteins in untreated capsids. For the U$_L$15 protein, densitometric values of the immunoblot reacted with the U$_L$15-MBP antibody were used.
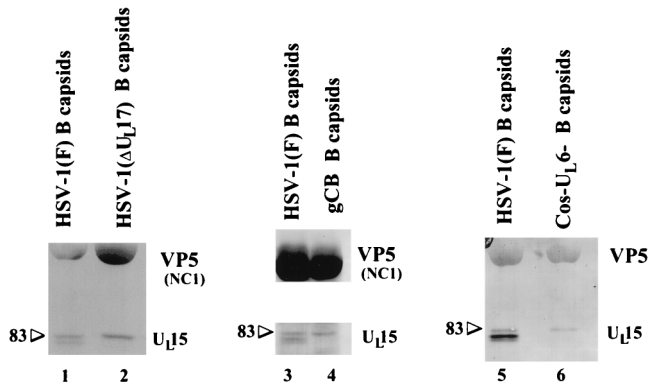[b] Nondetectable.

FIG. 4. Scanned image of immunoblot of B capsids purified from cells infected with a wild-type strain, HSV-1(F), and mutants which do not cleave and package DNA. Capsids were purified from cells infected with HSV-1(F) or the DNA cleavage and packaging mutants Cos-U_L6^-, HSV-1(ΔU_L17), or gCB, lacking functional U_L6, U_L17, and U_L28, respectively. Proteins associated with purified capsids were electrophoretically separated, transferred to nitrocellulose, and reacted with the U_L15-MBP-specific antibody (lanes 5 and 6) or anti-U_L15-MBP serum and NC1, a polyclonal antibody directed against VP5 (lanes 1 to 4), to clearly demonstrate the relative amounts of capsid proteins loaded in the lanes. The positions of the bands corresponding to the 83,000-M_r U_L15 protein (83) are indicated.

treatment with 2.0 M GuHCl (Fig. 3A, lane 6, and Table 1). The amounts of VP21, VP22a, and VP24 were diminished to undetectable levels upon extraction with 2.0 M GuHCl (Table 1 and Fig. 3A). These data suggest that in this experiment, treatment with 2.0 M GuHCl denatured some capsids and removed proteins associated with the cores of the remaining capsids.

Electrophoretically separated capsid proteins from the above preparations were transferred to nitrocellulose and were reacted with the anti-U_L15-MBP antibody. As shown in Fig. 3B, at least 75% of the reactivity with the proteins with apparent M_rs of 83,000, 80,000, and 79,000 was retained in capsids treated with 0.05, 0.1, and 0.5 M GuHCl whereas 15 and 16% of the reactivity was retained in capsids treated with 1.0 and 2.0 M GuHCl, respectively. The retention of U_L15 protein immunoreactivity at 1.0 and 2.0 M GuHCl was less than that of VP23 (35 and 30% retained, respectively) and VP19c (40 and 21% retained, respectively) but more than that of core proteins such as VP24, which were reduced to undetectable levels. This suggested that U_L15 proteins were more resistant to extraction than were core proteins such as VP24. A caveat of this interpretation is that a linear correlation between U_L15 protein concentration and reactivity on immunoblots has not been formally established. We can conclude, however, that U_L15 proteins remain associated with capsids extracted with 0.5 M GuHCl. This supports the conclusion that U_L15 proteins are integral components of capsids rather than contaminants of the capsid preparations.

Capsids from cells infected with cleavage and packaging mutants contain only the U_L15-encoded protein with an apparent M_r of 83,000. Because genes in addition to U_L15 are required for DNA cleavage and packaging, it was of interest to determine if capsid association of U_L15-encoded proteins was dependent on other gene products. To address this question, capsids were purified from Vero cell monolayers infected with the wild-type strain HSV-1(F) or with cleavage and packaging mutants lacking either the U_L17, U_L6, or U_L28 gene [HSV-1(ΔU_L17), Cos-U_L6^-, and gCB, respectively] (24, 29, 32). Proteins associated with the purified capsids were separated on a denaturing polyacrylamide gel, transferred to nitrocellulose,

and probed with the anti-U_L15-MBP serum. Surprisingly, while at least the 83,000- and 80,000-M_r U_L15 proteins were present in capsids purified from cells infected with HSV-1(F), only the 83,000-M_r protein was detectable in capsids purified from cells infected with HSV-1(ΔU_L17), Cos-U_L6^-, or gCB (Fig. 4). These data indicate that at least the U_L6, U_L17, and U_L28 genes are individually required for association of the U_L15 80,000-M_r protein with B capsids and suggest that the U_L15 80,000-M_r protein associates with capsids only in the presence of intact cleavage and packaging machinery.

The 83,000-M_r U_L15 protein is the predominant form in C capsids. To determine what forms of U_L15 proteins are associated with C capsids, Vero cell monolayers were infected with HSV-1(F) for 18 h and capsids were purified on a continuous 20 to 50% sucrose gradient. B and C capsids, distinguished by their sedimentations in the gradient, were individually collected, pelleted, and purified on separate sucrose gradients. Capsid-associated proteins were separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and reacted with the anti-U_L15-MBP serum. As shown in Fig. 5, whereas B capsids contained the 83,000-, 80,000-, and 79,000-M_r U_L15-encoded proteins (lane 1), the 83,000-M_r U_L15 protein was the predominant form of U_L15-encoded protein detected in C capsids (lane 3).

Origin of the U_L15-encoded proteins. To identify which of the 83,000-, 80,000-, and 79,000-M_r U_L15 proteins constituted the full-length translation product of the U_L15 cDNA detectable in HSV-1-infected cells (5), a U_L15 cDNA was transcribed and translated in rabbit reticulocyte lysate. The proteins, and polypeptides associated with wild-type B and C capsids, were separated in separate lanes of a denaturing polyacrylamide gel, transferred to nitrocellulose, and reacted with the U_L15-MBP-specific antiserum. As shown in Fig. 5, lane 2, the electrophoretic migration of the in vitro transcription-translation product was indistinguishable from that of the 83,000-M_r protein present in B and C capsids (lanes 1 and 3). Also present in the in vitro transcription-translation reaction was a U_L15 product, possibly derived from initiation at a methionine codon (codon 35) in the U_L15 cDNA (17), that comigrated with the protein with an apparent M_r of 79,000 but not the protein with an apparent M_r of 80,000. A protein that comigrated with the U_L15-encoded protein with an apparent M_r of 80,000 in B capsids was not detected in the rabbit reticulocyte lysate programmed with the U_L15 cDNA. We therefore conclude that the U_L15-encoded protein with an apparent M_r of 83,000 comi-
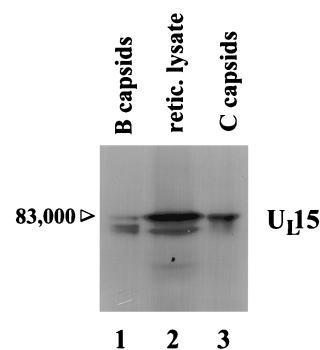

FIG. 5. Scanned image of immunoblot probed with U_L15-MBP-specific antiserum. B and C capsids were purified from HSV-1(F)-infected cells, and a cDNA of the known U_L15 mRNA was transcribed and translated in a rabbit reticulocyte (retic. lysate). Proteins were electrophoretically separated and reacted with the U_L15-MBP antiserum. The position of the band corresponding to the 83,000-M_r U_L15 protein is indicated.
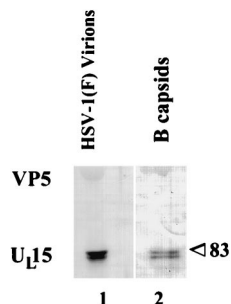
FIG. 6. Scanned image of immunoblot probed with the U$_L$15-MBP-specific antiserum. Virions and B capsids were purified from HSV-1(F)-infected cells, and associated proteins were electrophoretically separated and reacted with the U$_L$15-MBP-specific antiserum. The position of the band corresponding to the 83,000-M_r U$_L$15 protein (83) is indicated.

grates with the translational product of the full-length U$_L$15 cDNA whereas the 79,000-M_r protein comigrates with an additional protein produced in in vitro transcription-translation reactions. These data suggest that the 83,000-M_r protein is the product of the full-length U$_L$15 gene.

At least two forms of the U$_L$15 protein are associated with virions. To determine if U$_L$15-encoded proteins were virion components, Vero cell monolayers were infected at 3.0 PFU per cell and were purified as described in Materials and Methods (31). Virions were separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and reacted with the anti-U$_L$15-MBP antibody. As shown in Fig. 6, at least two different forms of U$_L$15, corresponding to M_rs of approximately 83,000 and 80,000, were detectable in lanes containing both virions and B capsid polypeptides. To confirm these results, virions purified through a dextran gradient were further purified through a sucrose flotation gradient as described previously (31). Proteins associated with the purified virions were analyzed on immunoblots probed with the anti-U$_L$15-MBP antibody as described above. The results reiterated those shown in Fig. 6 and indicated that both the 80,000- and 83,000-M_r proteins were detectable in virions (data not shown).

DISCUSSION

We have demonstrated that U$_L$15 proteins are components of B and C capsids and virions. Evidence supporting the observation that B capsids contain U$_L$15-encoded proteins includes the observations that U$_L$15 proteins (i) cofractionate with viral capsids in a sucrose gradient, (ii) remain associated with capsids extracted with 0.5 M GuHCl, and (iii) are detectable in capsids extracted with 1.0 and 2.0 M GuHCl. The observation that U$_L$15 proteins remain detectable in highly purified capsids treated with 2.0 M GuHCl argues that U$_L$15 proteins are integral components of capsids. Parenthetically, the 35,000-M_r U$_L$15 protein which has previously been reported (4) was not found to associate with capsids or virions (data not shown).

U$_L$15-encoded proteins with apparent M_rs of 83,000, 80,000, and 79,000 are detectable in B capsids. Previous results indicated that the major U$_L$15-encoded protein in electrophoretically separated lysates had an apparent M_r of 75,000 (4), but the use of more carefully calibrated standards in the present study indicated that the major protein in infected cell lysates, and the slowest-migrating U$_L$15 protein associated with capsids, migrated with an apparent M_r of 83,000 (data not shown). The fact that the 83,000-, 80,000-, and 79,000-M_r capsid proteins are derived from U$_L$15 was confirmed by the observations

that (i) these proteins were not detectable in capsids purified from cells infected with HSV-1(ΔU$_L$15), a virus lacking an intact U$_L$15 gene, and (ii) corresponding proteins in capsids purified from cells infected with a recombinant virus [HSV-1 (R7244)] containing a 20-codon tag at the 3′ end of U$_L$15 had decreased electrophoretic mobilities relative to those of the wild-type proteins. The latter observation also indicated that the 80,000- and 79,000-M_r species did not result from carboxy-terminal cleavage of the 83,000-M_r protein, since the tag was retained in all three proteins.

The observations that the 80,000- and the 79,000-M_r proteins were not detectable in capsids from HSV-1(ΔU$_L$17)-, Cos-U$_L$6$^-$-, or gCB-infected cells indicate that capsid association of these proteins is dependent on at least the U$_L$6, U$_L$17, and U$_L$28 genes and argue that capsid association of the proteins with apparent M_rs of 80,000 and 79,000 requires intact cleavage and packaging machinery. Additionally, the 83,000-, 80,000-, and 79,000-M_r proteins were detectable in capsids purified from complementing cell lines infected with the U$_L$17 and U$_L$6 null viruses (data not shown). One possibility is that the 83,000-M_r U$_L$15 protein associates with the capsid and is modified to produce the 80,000- and 79,000-M_r proteins, coincident with the conversion of large-cored B capsids to small-cored B capsids. Inasmuch as the 83,000-M_r protein comigrates with full-length U$_L$15 protein produced in rabbit reticulocyte lysates, such modifications would have to account for the increased electrophoretic mobility of the other U$_L$15-encoded proteins. We cannot rule out the possibility that the 80,000- and 79,000-M_r proteins are derived from (i) translation initiated at codon 35 (ATG) in the U$_L$15 mRNA (17) or (ii) translation of an alternatively spliced RNA. We do not favor the latter possibility because the cononical splice donor site is destroyed in R7244 U$_L$15 mRNA, suggesting that alternative splicing could not occur (4, 5). We also cannot rule out the possibility that the 79,000-M_r protein is a breakdown product of the 83,000- or 80,000-M_r proteins, which may account for its variability in different experiments.

We have noted that at least the 83,000- and 80,000-M_r U$_L$15 proteins are associated with virions, whereas the 83,000-M_r protein is the predominant form associated with C capsids, the progenitors of virions. We favor the hypothesis that the 80,000- and the 79,000-M_r U$_L$15 proteins are bound tightly to B capsids and become incorporated into the virion tegument by weak association with C capsids. Thus, the 79,000- and 80,000-M_r proteins are mostly lost during purification of C capsids. Alternatively, the 79,000- and 80,000-M_r proteins could associate with B capsids, disassociate from C capsids, and reassociate with C capsids as they mature into virions.

It is noteworthy that in the T4 bacteriophage system, a protein (gp23*) which has DNA-dependent ATPase and non-sequence-specific endonuclease activities remains enzymatically inactive until it is proteolytically cleaved during the cleavage and packaging reaction (8, 16, 28). This could provide a means to regulate enzymatic activity until all components required for DNA packaging are properly assembled. If such regulation were to occur during the HSV cleavage and packaging reaction, it could also serve to coordinate the onset of DNA cleavage with DNA packaging and scaffold cleavage and expulsion. In this scenario, the 83,000-M_r U$_L$15-encoded protein could represent an inactive form of U$_L$15-encoded protein, whereas the 80,000- and 79,000-M_r proteins might perform tightly regulated functions in the DNA-packaging reaction. Determining whether this is the case or whether the proteins perform entirely different functions will require additional studies.

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