Analysis of the Quaternary Structure of the Putative HCMV Portal Protein PUL104†

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ABSTRACT: In this report we analyze the UL104 open reading frame of human cytomegalovirus (HCMV) genome that encodes the putative portal protein. An affinity-purified monospecific antiserum directed against a GST-UL104 fusion protein identified proteins of approximate M_r 73000 and 145000 in HCMV-infected cells and purified virions. Furthermore, using an in vitro assay the ability of pUL104 to bind double-stranded DNA was shown. Analysis under native conditions of pUL104 revealed that the monomeric and dimeric forms of the protein also form high molecular weight complexes upon sucrose gradient centrifugation. The protein has been purified from recombinant baculovirus UL104 infected cells. The quaternary structure of rpUL104 was investigated by gel permeation chromatography and electron microscopy. The purified rpUL104 was found to assemble into high molecular weight complexes, a prerequisite of portal proteins which form channels for DNA import into capsids.

Herpesvirus DNA replication leads to concatenated DNA that is cleaved into unit-length genomes prior to packaging into preassembled procapsids (*1*−*7*). DNA replication involving a concatemer is a common feature for many double-stranded DNA bacteriophages and herpes viruses (*8*). In the case of phage P22, T7 and λ DNA is translocated into procapsids (proheads) through the portal protein, a dodecameric ring representing one vertice of the 12 vertices of the capsid structure (*9*−*12*). This process requires a terminase that generates unit-length genomes from concatemers and provides ATP hydrolysis for packaging (*13*). The terminase−DNA complex interacts with the portal protein, and viral DNA is translocated into the capsid. DNA packaging is terminated by cleavage of concatemers into genomes either by sequence-specific cleavage (e.g., phage λ) or by a headful mechanism (e.g., P22; *8*).

The HCMV[1] terminase consists of the viral proteins pUL56, the large terminase subunit (*2*, *14*−*15*), and pUL89, the small terminase subunit (*16*). Recently, we identified the three-dimensional structure of pUL56 (*17*). We could demonstrate that the protein is a homodimer with a *C*2 symmetry and that it consists of two rings on top of each other. This structure is a prerequisite for its DNA-binding activity. While pUL56 binds to DNA and mediates ATP hydrolysis (*18*, *19*), pUL89 is required for DNA cleavage into the unit-length genome. It is postulated that in addition to the terminase subunits the gene products pUL51, pUL52, pUL77, and pUL104 are essential for DNA packaging. Recently, Newcomb and Brown (*20*) identified the portal protein of herpes simplex virus type 1. Homologues of pUL6 are conserved among all herpes viruses that have been sequenced (*21*−*24*). This protein is attached to a unique site of the capsid forming a ring-like structure composed of 12 monomers, corresponding to the portal vertex described in dsDNA bacteriophages. The HCMV homologue of HSV-1 UL6 is encoded by ORF UL104. In this study analyses were performed in an attempt to determine whether pUL104 like all known portal proteins forms oligomers.

EXPERIMENTAL PROCEDURES

Cells and Viruses. Embryonic lung fibroblasts, MRC5 cells, were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, penicillin (5 units/mL), and streptomycin (50 μg/mL). MRC5 cells at passages 10−15 were used for infections, and experiments were carried out with confluent cell monolayers (1.5×10^7 cells). Preparation of HCMV AD169 was performed after infection of MRC5 cells at a MOI of 0.1. At 96 h postinfection (pi) the supernatant was sedimented (100000*g*, 2 h, 4 °C) prior to purification by gradient centrifugation according to Talbot and Almeida (*25*). Infection of MRC5 with HCMV at a MOI of 1 was carried out as described before (*15*).

Insect cells 5B1-4 (High five) were grown in TC-100 medium supplemented with glutamine and gentamicin (60 μg/mL) and 10% fetal calf serum. Recombinant baculovirus UL104 were generated by transposition into the bacmid bMON14272 as described by Luckow et al. (*26*). High five cells were infected at a MOI of 2 with recombinant baculovirus UL104. The cells were harvested at 48 h after infection, sonicated, and centrifuged at 2000*g* for 5 min. The supernatant was used for UL104 purification by chromatography.

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[1] Abbreviations: HCMV, human cytomegalovirus; HSV-1, herpes simplex virus type 1; GST, glutathione *S*-transferase; FCS, fetal calf serum; DMEM, Dulbecco's minimal essential medium; IPTG, isopropyl β-D-1-thioglactopyranoside; MOI, multiplicity of infection; DTT, 1,4-dithio-DL-threitol; EDTA, ethylenediaminetetraacetic acid; MES, 2-morpholinoethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; ORF, open reading frame.

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Plasmids. The UL104 gene was amplified by PCR from the cosmid pCM1028 (*27*) with the following pair of oligonucleotides (restriction sites for *Eco*RI and *Not*I are underlined): 5′-GCGAATTCATGGAGCGAAACCACTG-GAACGAA-3′ and 5′-GCGCGGCCGCCTAGTGAAATC-CGTATGGACCTCCAGC-3′. The PCR product as well as the vector pcDNA3.1HisC, pGEX-6P-1, and pFastBac HTa (Life Technologies) were separately digested with *Eco*RI and *Not*I, and the PCR product was ligated in-frame into the vectors yielding plasmids pcDNA-UL104, pGEX-UL104, and pBac-UL104.

Purification of GST Fusion Proteins. A fresh overnight culture of *Escherichia coli* BL21 carrying the plasmid pGEX-UL104 was grown in 250 mL of LB media. After the cells reached an A_{600} of 0.6, the GST fusion protein expression was induced by addition of 0.1 mM IPTG and incubation for 2 h at 32 °C. Sedimented cells were lysed in PBS containing lysozyme (10 mg/mL) by incubation on ice for 30 min. After cycled freezing/thawing 0.5% Triton X-100 was added prior to incubation for 30 min at 4 °C. After separation of undissolved material the proteins were loaded on a GSTrap column (1 mL bed volume). The purification was performed at 4 °C using an ÄKTA Prime (Amersham Bioscience) according to the instruction of the manufacturer. The isolated GST-104 (1.00 mg/mL) was stored in elution buffer (50 mM Tris, pH 8.0, 10 mM glutathione) at −80 °C.

PreScission Protease Treatment. To cleave the GST tag from GST-104, the protein was after binding to the GSTrap column incubated with 80 units of PreScission protease (Amersham Bioscience) for 12 h at 4 °C under rotation. After removal of the GST tag from GST-UL104 during incubation with the protease the resulting proteins were eluted in 10 fractions (1 mL) of PreScission buffer (50 mM Tris, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT). The fractions containing the protein (0.97 mg/mL) were stored at −80 °C.

Antibody. HCMV pUL104 specific human polyclonal antibody pAbUL104 was purified from high titer human serum (IgG-positive patient serum selected by CMV diagnostic) by column affinity chromatography (Affi-Gel 10/15-pUL104). Affinity purification of the anti-UL104 antibody was done as follows. The fusion protein GST-UL104 was coupled to activated immunoaffinity supports Affi-Gel 10 and Affi-Gel 15 (1:2 Affi-Gel 10/Affi-Gel 15; Bio-Rad Laboratories) as described by Bio-Rad, yielding Affi-Gel 10/15-pUL104. Unspecific binding sites were blocked by incubation with buffer b (1 M ethanolamine hydrochloride, pH 8.0, 0.02% NaN_3) for 1 h. The resin was equilibrated with start buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.02% NaN_3). High titer human convalescent serum was incubated overnight with the prepared affinity matrix. After the matrix was washed twice with 10 bed volumes of ice-cold start buffer, the bound antibody was eluted in 200 mM glycine hydrochloride (pH 2.5). The purified anti-pUL104 antibody, pAbUL104, was neutralized with 100 mM Tris-HCl (pH 8.0). The specificity of the purified pAbUL104 was determined using Western blots with pUL104 or with stripes containing immunodominant epitopes (*28*): the carboxy-terminal half of the tegument protein ppUL83 (pp65; 31 kDa) and the carboxy terminus of ppUL32 (pp150; 26 kDa) and of the glycoproteins the internal AD-1 domain of gpUL55 (gB; 24 kDa) and the amino terminus of gpUL75 (gH; 20 kDa).

DNA Binding Assay. The TNTT7 quick Master Mix (Promega) was used to synthesize [^{35}S]methionine-labeled (Amersham Bioscience) pUL104 or MCP in a coupled transcription/translation reaction by using 1 μg of plasmid pcDNA-UL104 or pcDNA-MCP as template according to the instructions of the supplier (Amersham Bioscience). For in vitro DNA-binding analysis 200 mg of dsDNA cellulose (Sigma) packed on a 1 mL column was equilibrated with 2 mL of DNA-binding buffer (DBB; 50 mM Tris-HCl, pH 8.0, 10% glycerol, 1 mM DTT, 1 mM EDTA). The column was then loaded with pUL104, or MCP diluted 1:10 in DBB, and washed with 2 mL of DBB. Bound protein was eluted in 0.5 mL fractions of DBB with increasing concentrations of NaCl (200, 400, 800, and 1500 mM NaCl), which were analyzed by SDS−PAGE and autoradiography.

Purification of Recombinant pUL104. High five cells (4×10^8) expressing the recombinant pUL104 (rpUL104) were harvested 48 h pi. Sedimented cells were lysed in 50 mL of cation-exchange buffer (20 mM MES, pH 6.5, 150 mM NaCl, and protease inhibitors) and sonicated on ice. Cell lysates were sedimented and passed through a 0.2 μm filter prior to being loaded onto an equilibrated cation-exchange column (HiTrap SP HP, 1 mL bed volume; Amersham Bioscience). The purification was performed at 4 °C by using a ÄKTA Prime (Amersham Bioscience). Elution was achieved using a linear salt gradient from 50 mM to 2 M NaCl. A total of 40 fractions were collected prior to separation by gel electrophoresis. Fractions containing the protein were subjected to the second purification step with gel filtration carried out with a HiLoad 16/60 Superdex 200 prep grade gel permeation column using a ÄKTA FPLC (Amersham Bioscience) as described before (*16*). A total of 70 fractions were collected and analyzed by a native gel electrophoresis. Fractions containing the protein were aliquoted and stored at −80 °C.

Immunoprecipitation. Infected or mock-infected cells (T-75 cm^2 flasks; 2×10^6 cells) were 60 h pi radiolabeled with 50 μCi/mL [^{35}S]methionine for 12 h. For immunoprecipitation total cell extracts were prepared from labeled cultures by solubilization in immunoprecipitation buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1% NP-40, 5 mM EDTA, 25 mM iodacetamide, 0.4% sodium deoxycholate, 1 mM PMSF, 100 units/mL Trasylol) and ultrasonic treatment. Insoluble material was sedimented for 30 min at 100000*g*. Comparable amounts of extracts (6×10^5) and affinity-purified pUL104-specific antibody (pAbUL104; 1:20) were used for precipitation as described previously (*15*). Immunoprecipitates were analyzed by 8% SDS−PAGE and autoradiography.

PAGE and Western Blot Analysis. Cell extracts or virions after purification using a sodium tartrate gradient (*25*) were solubilized in 4 × sample buffer [4% (v/v) β-mercaptoethanol, 0.01% (w/v) bromphenol blue, 4% (w/v) glycerol, 4% (w/v) SDS, 0.2 M Tris-HCl (pH 6.8)] prior to separation by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) on 8% polyacrylamide gel. Proteins were electrotransferred to nitrocellulose sheets (Protran, Schleicher & Schuell; 0.8 V/cm^2 for 1.5 h), and membranes were blocked with PBS containing 0.1% Tween 20 and 3% powdered milk. Antibodies were diluted in PBS containing 0.1% Tween 20. The pAbUL104 antibody (1:10) specific

for pUL104 was used as the primary antibody. With the Western blot stripes of immunodominant epitopes, mAb27−156 (1:10) specific for glycoprotein B (gB), mAb58−15 (1:10) specific for the major tegument protein pp65, and affinity-purified pAbgH (1:10; *29*) specific for glycoprotein H (gH), respectively, served as primary antibodies. For detection of primary antibody binding, horseradish peroxidase-conjugated anti-human or anti-mouse $F(ab')_2$ fragments (1:5000) and the enhanced chemiluminiscence detection system (Amersham Bioscience) were used according to the manufacturer's specification.

Urea gel electrophoresis was performed by solubilization of the proteins in 2 × urea sample buffer [2% (v/v) β-mercaptoethanol, 0.01% (w/v) bromphenol blue, 10% (w/v) glycerol, 4% (w/v) SDS, 15 mM Tris-HCl (pH 6.8), 8 M urea] prior to SDS−urea gel electrophoresis (8% SDS gel with 3.2 M urea). Proteins were transferred to nitrocellulose prior to Western blot analysis with pAbUL104.

Sucrose Gradient Analysis. Extracts from purified pUL104 or marker proteins (catalase, 247 kDa; ferritin, 440 kDa; thyroglobulin, 672 kDa; Sigma) were layered on 13 mL gradients of 5−30% sucrose (sucrose solvated in 150 mM Tris-HCl, pH 7.5) with a 500 μL 40% sucrose cushion at the bottom. The gradients were sedimented for 16 h [100000*g*, 4 °C in a TLS55 rotor (Beckman)]. Twelve fractions were collected by bottom puncture of the tube. The fractions were analyzed on a native 7.5% polyacrylamide gel and followed by immunoblotting.

Indirect Carbon Support Films. Carbon was evaporated from 1.0 mm carbon threat at a high of approximately 17 cm onto a glass slide. The carbon is reflected from the glass slide at an angle of 45° to a freshly cleaved mica in a coating unit with an operating vacuum of $<10^{-4}$ bar. Direct coating is prevented by a metal shield between the evaporation source and the mica (*30*).

Electron Microscopy. Purified protein (fraction 23, elution volume 39.5−41 mL) was applied to the carbon on the mica and negative stained with 4% aqueous uranyl acetate (w/v) for 20 s according to Valentine et al. (*31*). Micrographs were obtained using a Zeiss EM T109 electron microscope operated at a calibrated magnification (25200×, by using a cross-ruled diffraction grating replica with 2160 lines/mm; *32*). Selected micrographs were digitized using a Microtex Scan Maker E6.

RESULTS

Identification of pUL104 in Infected Cells. To identify the protein encoded by ORF UL104, a specific antibody against pUL104 (pAbUL104) was purified from high titer human serum by column affinity chromatography against a GST-UL104 fusion protein according to the purification of pAbUL56 (Figure 1A). As a control for the specificity of the antibody immunoblots with different purified viral proteins were performed. While stripes containing recombinant polypeptides encoding immunodominant epitopes of the viral tegument proteins pp65 (31 kDa) and pp150 (26 kDa) and the glycoproteins gB (24 kDa) and gH (20 kDa) only reacted with the specific antibodies and reconvalescent serum (Figure 1C), the purified antibody pAbUL104 only detected the UL104 antigen on stripes with rpUL104 (Figure 1B). These observations demonstrate that pAbUL104 is a monospecific antibody against pUL104.

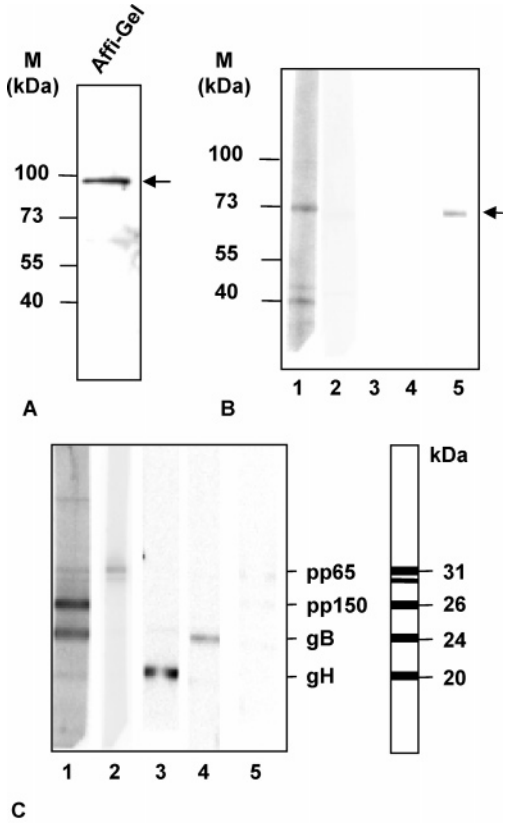

FIGURE 1: Characterization of the purified antibody pAbUL104. (A) An aliquot of the GST-UL104-resin was subjected to 10% SDS−PAGE prior to transfer onto nitrocellulose and immunoblot analysis using convalescent serum. (B) Purified GST-UL104 was separated by 10% SDS−PAGE and transferred to nitrocellulose. Immunoblot stripes were reacted with convalescent serum (lane 1), anti pp65 (lane 2), anti gH (lane 3), anti gB (lane 4) and pAbUL104 (lane 5). (C) Recombinant proteins encoding immunodominant epitopes of pp65 (31 kDa), pp150 (26 kDa), gB (24 kDa), and gH (20 kDa) were subjected to 11% SDS−PAGE prior to transfer onto nitrocellulose. Immunoblot analysis was performed with convalescent serum (lane 1) and antibodies against pp65 (lane 2), gB (lane 3), gH (lane 4), and pAbUL104 (lane 5). Molecular mass markers are indicated on the left, and the position of the specific protein is indicated on the right.

Infected or mock-infected cells were radiolabeled with 50 μCi/mL [^{35}S]methionine at 60 h pi for 12 h and subjected to immunoprecipitation. In AD169 infected cell extracts the antibody precipitated two proteins while no protein was detected in the control (Figure 2A, lanes 1 and 2). The molecular mass of 73 kDa corresponds to the calculated mass of pUL104 (78 kDa) whereas the high molecular mass form could be due to dimerization of pUL104. The reactivity of the antiserum to these proteins was inhibited by preadsorption of the antiserum with the GST-UL104 fusion protein (Figure 2A, lane 3), thus confirming that both proteins are of the same origin. As a loading control an aliquot of extracts used for the immunoprecipitation was separated by gel electrophoresis followed by autoradiography (Figure 2B). Identical precipitates were subjected to native gel electrophoresis prior to autoradiography. Under these conditions only one polypeptide was detected, thus indicating that the 75 and 150 kDa represent different forms of one protein (Figure 2C). Interestingly, both polypeptides were detected in purified virions, whereas the high molecular mass form of the protein was predominant (Figure 2D, lane 2). These results show that

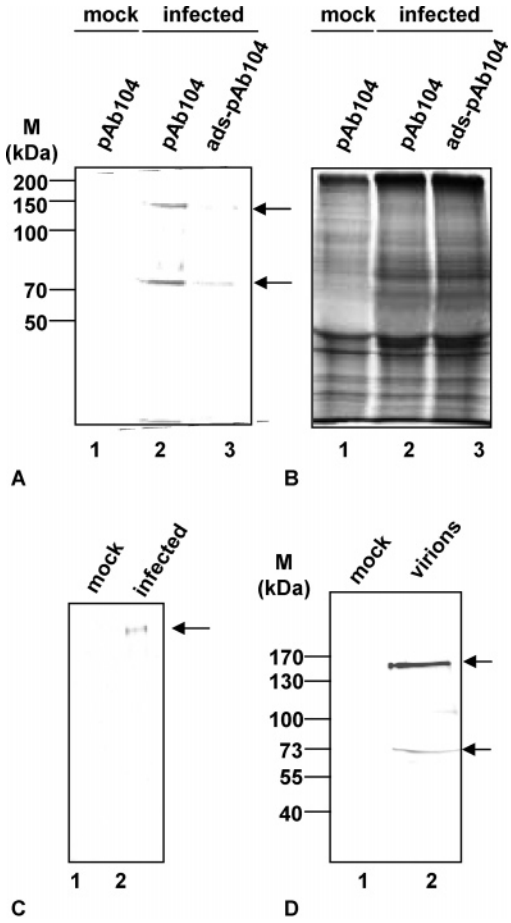

FIGURE 2: Identification of HCMV pUL104 and recognition of related proteins. (A) Mock-infected (lane 1) or HCMV-infected (lanes 2 and 3) MRC-5 cells were 60 h pi radiolabeled with 50 μCi/mL [^{35}S]methionine for 12 h. At 72 h pi the cells were immunoprecipitated with pAbUL104 (1:10; lanes 1 and 2) or preadpAbUL104 (1:10; lane 3) and subjected to SDS−PAGE prior to transfer onto nitrocellulose and autoradiography. (B) As a loading control extracts used for the immunoprecipitation in (C) were subjected to 10% SDS−PAGE prior to autoradiography. (C) An aliquot of the precipitated extracts from (A) was subjected to 7.5% native gel electrophoresis followed by autoradiography. (D) Mock-infected cells (lane 1) or purified AD169 virions (lane 2) were subjected to 10% SDS−PAGE prior to immunoblot analysis with pAbUL104 (1:10). The position of pUL104 is indicated by arrows, and molecular mass standards are indicated on the left side.

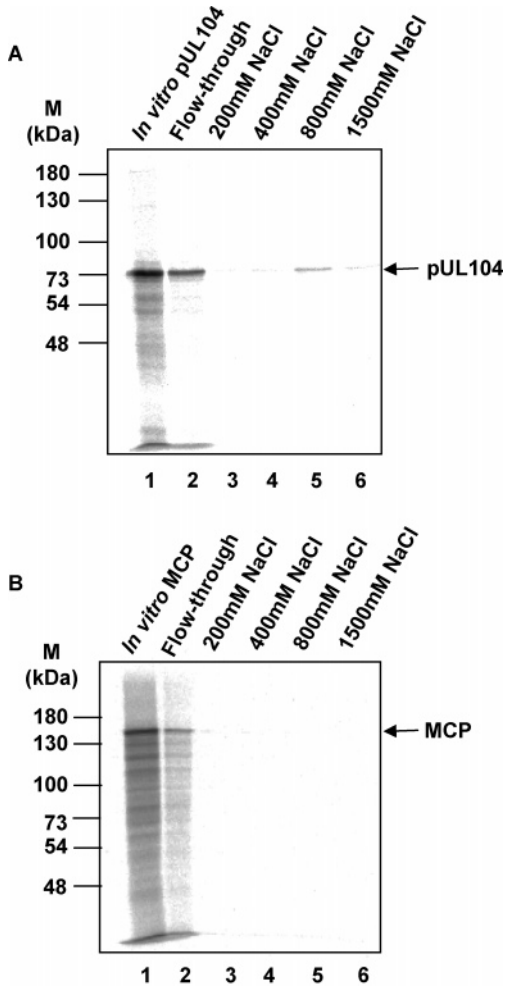

FIGURE 3: Interaction of the portal protein with DNA. (A) In vitro translated pUL104 (lane 1) was applied to a dsDNA cellulose column. After the column was washed, DNA-binding proteins were eluted with increasing NaCl concentrations (lanes 3−6). (B) In vitro translated MCP (lane 1) was loaded onto a dsDNA cellulose column. The column was washed prior to elution of DNA-binding proteins (lanes 3−6). Molecular mass markers (M) are indicated on the left; the positions of the proteins are indicated on the right.

pUL104 is expressed as a 73 kDa monomer that might have the tendency to form a dimer.

DNA Binding of pUL104. To determine the ability of pUL104 to bind DNA, in vitro binding studies were performed. ^{35}S-Labeled pUL104 or MCP as a negative control was loaded on a dsDNA cellulose column. After the washing steps bound proteins were eluted with a NaCl gradient. pUL104 could be detected in the eluted fractions (Figure 3A), especially in the one containing 800 mM NaCl, thus implying an interaction with dsDNA. In contrast to this observation there was no significant signal for MCP (Figure 3B). These results demonstrated that pUL104 could bind dsDNA.

Complex Formation by Sedimentation. To determine whether pUL104 has the ability to form a high molecular mass complex under native conditions, purified protein was applied to a sucrose gradient (5−30% with a 500 μL 40% cushion density) centrifugation. Each fraction was analyzed for pUL104 by native gel electrophoresis followed by Western immunoblotting. The results demonstrated that pUL104 was only found in fraction 6 (Figure 4B, lane 6). This fraction corresponded to a sedimentation mass near 669 kDa based on the markers catalase (232 kDa) and thyroglobulin (669 kDa) sedimented on a parallel gradient (Figure 4C, lane 6). This observation underlines our observations that the protein has the ability to form high molecular mass complexes.

Analysis of the Quaternary Structure of Purified rpUL104. To study the multimeric structure of pUL104, the protein was purified. Purification of High five cells expressing the recombinant pUL104 (rpUL104) was carried out by fast liquid chromatography using a cation-exchange column. The purified protein and molecular mass markers (Figure 5A as indicated: thyroglobulin, 669 kDa; ferritin, 440 kDa; catalase, 232 kDa; aldolase, 158 kDa) were then analyzed by gel permeation chromatography without detergent. The rpUL104 eluted from the gel permeation column had an apparent M_r of 669000 (Figure 5A,B, fraction 23). When the peak fractions were analyzed by urea gel electrophoresis

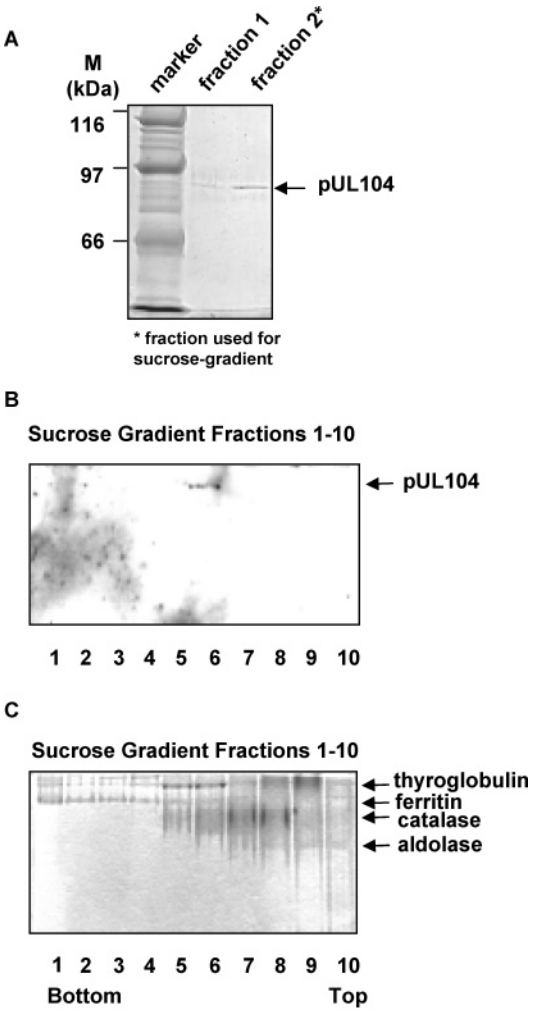

FIGURE 4: Determination of the oligomerization of pUL104 after sedimentation on sucrose density gradients. (A) GST fusion protein GST-UL104 was purified by using affinity chromatography of *E. coli* expressed extracts including cleavage of the GST tag. The first two fractions were subjected to SDS−PAGE and analyzed by silver staining according to the manufacturer (Amersham Bioscience). (B) Fraction 2 was loaded onto a 5−30% sucrose gradient, and gradient fractions were separated by native PAGE. Proteins were detected by immunoblot with anti-UL104 pAbUL104 antiserum. (C) Sedimentation standards were run in a parallel gradient: thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and aldolase (158 kDa).

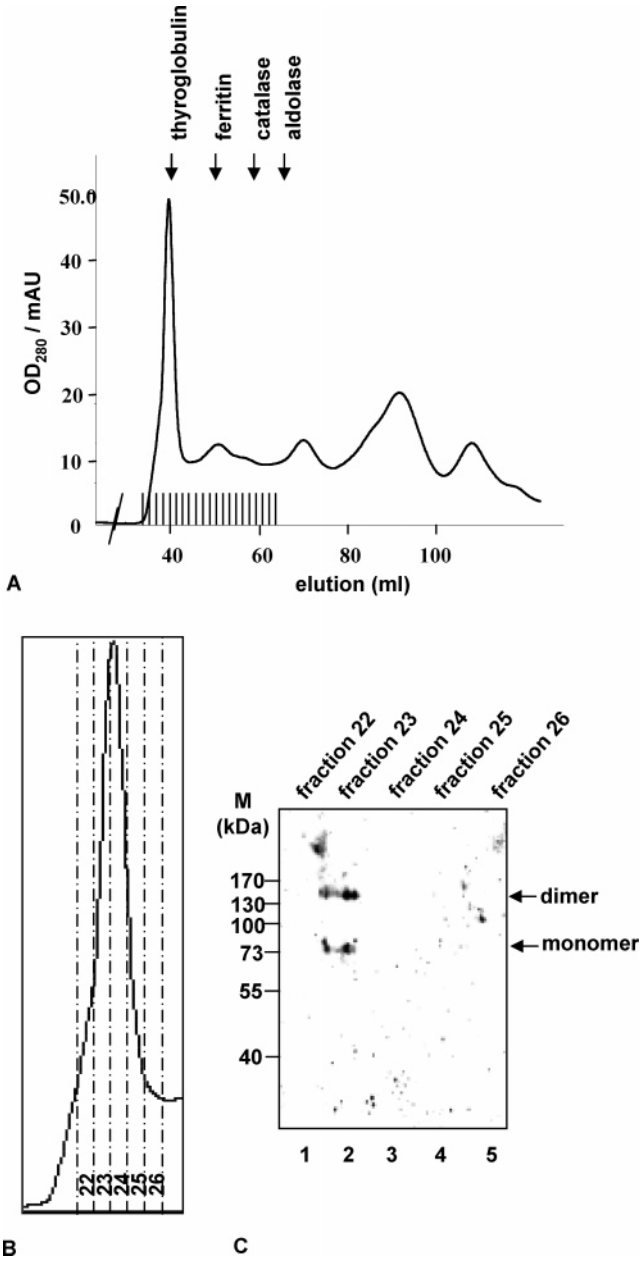

FIGURE 5: Analysis of purified recombinant rpUL104. (A) Gel permeation chromatography of rpUL104. Single step purified rpUL104 was subjected to chromatography through a HiLoad 16/60 Superdex 200 prep column using an ÄKTA Explorer recorded by OD_{280}. The column was calibrated with the following molecular mass standards, aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa), and thyroglobulin (669 kDa), as indicated by arrows. (B) An enlarged section of (A) is shown to indicate peak fractions 22−26. (C) Analysis of the peak fractions (22−26) in an urea gel. The fractions were separated on a urea gel prior to immunoblot analysis with pAbUL104. Molecular mass markers are indicated on the left side, and the position of the monomer and dimer is indicated on the right side.

followed by immunoblotting, rpUL104 no longer migrated as an oligomer but as a protein with an apparent M_r of 73000 and 145000, thus indicating the molecular mass of a monomer and a dimer (Figure 5C). These observations indicated that rpUL104 migrated as an oligomer under native conditions.

Electron Microscopy Study of rpUL104. Purified rpUL104 was analyzed by electron microscopy. A representative section of a micrograph is shown in Figure 6A. Galleries of rpUL104 particles are presented (Figure 6B). The proteins have a diameter of approximately 17 nm. On the basis of the findings of Zipper et al. (*33*) that a volume of 1.37 Å^3 is equivalent to 1 Da, a monomer of rpUL104 with a calculated mass of 78 kDa is approximate 5 nm in diameter. Since the diameter of rpUL104 in the electron microscope was measured to be 17.7 nm high and 17.5 nm wide, respectively, the molecule must form oligomers.

DISCUSSION

DNA packaging comprises complex different reactions and is an essential step in herpes viral replication. Packaging proteins, especially the DNA−terminase complex, bind to the portal protein and translocate viral unit-length genome to the capsid's interior (*8*, *20*). Here we have characterized the oligomerization status of the gene product of the HCMV ORF UL104, the structural homologue of the herpes simplex

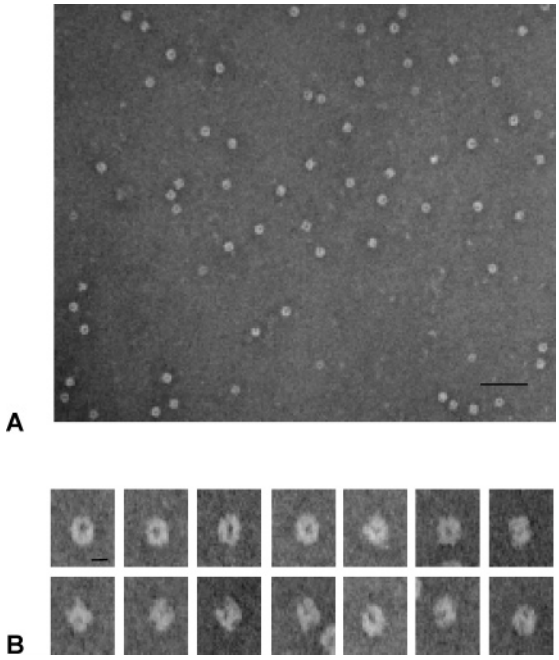

FIGURE 6: Electron microscopic analysis of purified rpUL104 negatively stained with 4% uranyl acetate. (A) Representative section of the micrograph. (B) A gallery shows molecules of rpUL104. The scale bars correspond to 100 (A) and 10 nm (B).

virus type 1 (HSV-1) portal protein pUL6 (*20*, *22*). For our analysis we used an affinity-purified antibody against pUL104. The specificity of the antibody was demonstrated by immunoblot analysis with purified pUL104 as well as with recombinant proteins expressing immunodominant epitopes of the tegument proteins pp65 and pp150 and the glycoproteins gB and gH. The antibody recognized only the specific antigen. This antibody detected in infected cells proteins with molecular masses of approximate 73000 and 145000. However, by native gel electrophoresis only one high molecular weight complex could be detected. In purified virions the high molecular weight form is predominant. Since the reactivity of the antibody against both protein forms is eliminated by preadsorption against pUL104, it is reasonable to speculate that (i) both proteins are of the same origin and (ii) the high molecular weight form represents a dimer of pUL104.

Another feature of portal proteins is the short-term binding of DNA during the insertion process. In this study, the sequence-independent binding of pUL104 to double-stranded DNA was shown by an in vitro assay. Therefore, this prerequisite for a portal protein is also fulfilled by pUL104. Sucrose gradient analyses demonstrated that the protein has also the ability to form oligomeric structures under native conditions. Comparable observations were found with the recombinant baculovirus expressed rpUL104. By using gel permeation chromatography of rpUL104 and molecular mass markers, it was found that also the recombinant protein forms multimeric structures. To directly demonstrate the quaternary structure of rpUL104, first structural analysis was performed by single particle analysis of negative stained specimens. On average the particles have a size of 17.7 nm high and 17.5 nm wide, thus indicating that rpUL104 forms multimers. This ability is consistent with all portal proteins that oligomerize in a cyclical form organized around a central channel through which DNA import occurs (*9*−*12*). Most bacteriophages analyzed today have 12-fold symmetric portal proteins. However, in the case of bacteriophage T7 or P2 populations of 12 and 13 monomers have been found (*34*−*35*). To determine the exact symmetry of rpUL104, we must await studies with the reconstruction of the three-dimensional structure. Recently, Newcomb et al. (*36*) provided evidence that the HSV-1 portal protein is incorporated into capsids as a complex with the scaffolding protein UL26.5. Their findings implicated that the oligomerization of pUL6 occurs before insertion into the capsids. Our results together with the report from Newcomb and Brown (*20*) of the portal protein in HSV-1, pUL6, demonstrated that oligomerization also occurs in herpes viruses and is a prerequisite for portal formation. Taken together, the data from the present study lead to the suggestions that pUL104 forms oligomers, thus representing one requirement for a portal protein. Further studies will need to be undertaken for obtaining higher resolution structural information of HCMV pUL104.

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