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Structure of the Herpes Simplex Virus Capsid: Effects of Extraction with Guanidine Hydrochloride and Partial Reconstitution of Extracted Capsids

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Viral B capsids were purified from cells infected with herpes simplex virus type 1 and extracted in vitro with 2.0 M guanidine hydrochloride (GuHCl). Sodium dodecyl sulfate-polyacrylamide gel analyses demonstrated that extraction resulted in the removal of greater than 95% of capsid proteins VP22a and VP26 while there was only minimal (less than 10%) loss of VP5 (the major capsid protein), VP19, and VP23. Electron microscopic analysis of extracted capsids revealed that the pentons and the material found inside the cavity of B capsids (primarily VP22a) were removed nearly quantitatively, but extracted capsids remained otherwise structurally intact. Few, if any, hexons were lost; the capsid diameter was not greatly affected; and its icosahedral symmetry was still clearly evident. The results demonstrate that neither VP19 nor VP23 could constitute the capsid pentons. Like the hexons, the pentons are most likely composed of VP5. When B capsids were treated with 2.0 M GuHCl and then dialyzed to remove GuHCl, two bands of viral material were separated by sucrose density gradient ultracentrifugation. The more rapidly migrating of the two consisted of capsids which lacked pentons and VP22a but had a full complement of VP26. Thus, VP26 must have reassociated with extracted capsids during dialysis. The more slowly migrating band consisted of torus-shaped structures approximately 60 nm in diameter which were composed entirely of VP22a. These latter structures closely resembled torus-shaped condensates often seen in the cavity of native B capsids. The results suggest a similarity between herpes simplex virus type 1 B capsids and procapsids of *Salmonella* bacteriophage P22. Both contain an internal protein (VP22a in the case of HSV-1 B capsids and gp8 or "scaffolding" protein in phage P22) that can be extracted in vitro with GuHCl and that is absent from mature virions.

Herpesvirus capsids are icosahedral in shape and highly uniform in overall dimensions. In herpes simplex virus type 1 (HSV-1) and equine herpesvirus 1 (EHV-1), for example, the capsid shell is 125 nm in diameter and approximately 15 nm thick. The shell is composed entirely of protein, and, as in all herpesviruses, it is organized into 162 capsomers, 150 hexavalent capsomers (hexons), and 12 pentavalent capsomers (pentons). The capsomers lie on a $T = 16$ icosahedral lattice, with pentons located at the vertices and hexons occupying the capsid faces and edges (3, 10, 27). Three-dimensional reconstructions computed from cryoelectron micrographs of HSV-1 and EHV-1 capsids demonstrate that all capsomers are roughly cylindrical (diameter, ~10 nm), with central channels extending all the way from outside to inside the capsid cavity. Hexons have sixfold and pentons have fivefold symmetry. All capsomers are connected in groups of three by trigonal nodules or "triplexes" on the capsid surface (2, 30). In the intact virus, the capsid contains the viral DNA and it is surrounded by a glycoprotein-containing membrane.

Three types of capsids, called A, B, and C, can be recovered from cells infected with herpesviruses. C capsids contain the entire DNA genome and are probably identical to capsids found in native virions. In contrast, A and B capsids lack DNA and are found in the infected-cell nucleus (9, 21, 23). Pulse-chase experiments with EHV-1 indicate that B capsids can package DNA and mature into infectious virions, while A capsids cannot (22). A capsids are considered to result from failed attempts to package DNA into B capsids. Studies with temperature-sensitive morphogenetic mutants of HSV-1 (24, 31) and primate cytomegalovirus (16) support the view that B capsids are competent to package viral DNA.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel analyses have clarified the protein composition of purified capsids from at least seven different herpesvirus species (reviewed by Dargan [3]). In all cases capsids are found to consist of four to nine distinct proteins. One of these, called the major capsid protein (VP5 in HSV-1 and HSV-2), has a molecular weight of about 150,000 and constitutes 60 to 70% of the total capsid protein. HSV-1 A capsids contain four additional polypeptides, VP19 (M_r 53,000), VP23 (M_r 33,000), VP24 (M_r 25,000), and VP26 (M_r 12,000), which together consititue the remainder of the A capsid protein. C capsids are closely similar to A capsids in protein composition (9; unpublished observations), while HSV-1 B capsids differ in that they contain an additional polypeptide, VP22a (M_r 40,000), which accounts for 15 to 20% of the B capsid protein (9, 19). Other herpesvirus species, including HSV-2, EHV-1, and cytomegalovirus, have proteins analogous to HSV-1 VP22a in that they are present in B but absent from A and C capsids. Copy numbers for all EHV-1 A and B capsid proteins have been determined from scanning transmission electron microscopic and SDS-polyacrylamide gel analyses (20). The values obtained are in good overall agreement with those of an earlier biochemical study of HSV-1 (11).

With the structure and polypeptide composition of herpesvirus capsids now reasonably well defined, the challenge to investigators is to clarify the location of individual proteins

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in the capsid structure. Substructural features need to be identified with particular protein components. Some information about this topic is already available. For example, immunoelectron microscopic studies have suggested that HSV-1 hexons are composed of VP5 (35), while VP22a has been shown to reside inside the cavity of HSV-1 and EHV-1 B capsids (2, 19). In order to define the locations of the remaining polypeptides, we have extracted HSV-1 B capsids with guanidine hydrochloride (GuHCl) and then examined extracted capsids by SDS-polyacrylamide gel electrophoresis and by electron microscopy. The goal was to associate specifically solubilized proteins with structural features lost during extraction.

MATERIALS AND METHODS

Virus growth and B capsid purification. All experiments were carried out with HSV-1 strain 17MP, which was grown at 37°C on monolayer cultures of BHK-21 cells as previously described (19). B capsids were isolated from infected cells essentially by the method of Perdue et al. (21). This procedure involved disruption of cells by sonication in the presence of 1% Triton X-100 and separation of A, B, and C capsids by centrifugation on linear 20 to 50% sucrose gradients prepared in TNE buffer (0.5 M NaCl, 1 mM EDTA, 20 mM Tris hydrochloride [pH 7.5]). After centrifugation, the band of B capsids was removed from the gradient with a Pasteur pipette, diluted in TNE, and pelleted by centrifugation for 1 h at 24,000 rpm in an SW28 rotor. Typical preparations beginning with five 850-cm^2 roller bottles of infected cells yielded 1 to 2 mg of purified B capsids, which were found, by electron microscopic analysis of negatively stained preparations (see below), to contain less than 10% A capsids and 5% or less C capsids.

Extraction with GuHCl and dialysis to remove GuHCl. Pelleted capsids to be extracted with GuHCl were resuspended in TNE at a concentration of 0.5 to 1.5 mg/ml at 4°C. Then 6.0 M GuHCl (ICN Biochemicals, Cleveland, Ohio; ultrapure grade) in TNE was added slowly with vigorous stirring to give the desired final GuHCl concentration. The mixture was incubated with occasional stirring for a total of 30 min at 4°C. Extracted capsids were then recovered by centrifugation through a 150-μl layer of 25% sucrose in a 0.375 in. by 1.625 in. (ca. 10 by 41 mm, 0.8 ml capacity) tube. Centrifugation was for 1 h at 23,000 rpm in a Beckman SW50.1 rotor. Capsids were resuspended in TNE for electron microscopy or lyophilized for SDS-polyacrylamide gel electrophoresis.

For reconstitution experiments, GuHCl was removed from extraction mixtures by dialysis overnight in 0.5- to 1.0-ml aliquots against TNE at 4°C (with Spectra/Por 2 [molecular weight cutoff, 12,000 to 14,000] tubing). When mixtures contained 2.0 M GuHCl at the outset, dialysis did not markedly change the light-scattering properties of the capsid suspension. After dialysis, mixtures were analyzed by centrifugation on linear 20 to 50% sucrose gradients (in TNE) prepared in 5-ml tubes; centrifugation was for 45 min at 23,000 rpm in an SW50.1 rotor. Thereafter, bands of viral material were identified by light scattering, removed from the gradient with a Pasteur pipette, and dialyzed to remove sucrose.

Electron microscopy. Electron microscopy of capsids was carried out with specimens adsorbed to standard carbon-Formvar-coated copper (400 mesh) electron microscope grids and fixed for 2 min in 1% glutaraldehyde. Capsids to be examined without shadowing were then stained for 5 min with 0.3% uranyl acetate in 40% ethanol. All samples were critical-point dried in a Tousimis samdri 780 critical-point dryer and rotary shadowed, if necessary, with Pt-C at a 13° angle in a Balzers BAE 080 vacuum evaporator. Capsids were photographed at 20,000 to 50,000× in a JEOL 100CX transmission electron microscope operated at 80 keV. Counts of pentons in control and extracted capsids were made with positive prints of electron microscope negatives enlarged to yield a total magnification of 150,000×. The data points reported represent counts of 40 to 75 pentons (or gaps) in a minimum of 30 capsids. Negative staining of reassembled toroids and other materials was performed with 2% aqueous uranyl acetate as described previously (33).

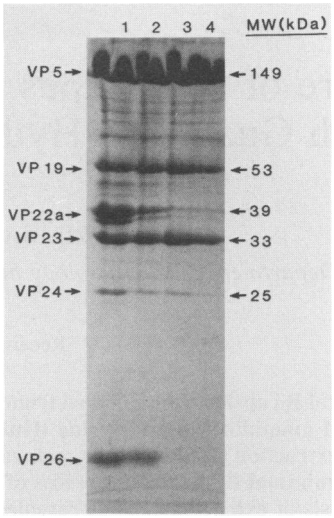

FIG. 1. SDS-polyacrylamide gel analysis of control HSV-1 B capsids (lane 1) and of B capsids after extraction with 1.0 M (lane 2), 1.5 M (lane 3), and 2.0 M (lane 4) GuHCl. B capsids were prepared and extracted with GuHCl in TNE as described in Materials and Methods.

SDS-polyacrylamide gel electrophoresis and quantitation of stained bands. SDS-polyacrylamide gel analyses were performed with capsid samples that were dissolved in 1% SDS–1% dithiothreitol and boiled for 2 min. Previously described procedures (18) were used for electrophoresis on 12% polyacrylamide slab gels and for staining of gels with 0.4% Coomassie blue. The amount of protein present in individual bands was determined quantitatively by scanning stained gels in an LKB UltroScan XL laser densitometer. The LKB 2400 Gelscan program (version 1.2) was used to integrate the data. Background staining was determined from the areas just above and just below each peak.

RESULTS

Effects of GuHCl extraction on B capsid structure. Figure 1 shows the results obtained when SDS-polyacrylamide gel analyses were performed with control B capsids (lane 1) and with B capsids after extraction with 1.0 M (lane 2), 1.5 M (lane 3), and 2.0 M GuHCl (lane 4). Proteins VP22a and VP26 were found to be removed nearly quantitatively by extraction with 1.5 or 2.0 M GuHCl, and there was a substantial loss of VP24. All three proteins (VP22a, VP24, and VP26) were partially extracted at 1.0 M GuHCl. In contrast, there was little apparent loss of VP5, VP19, or VP23 at any of the three GuHCl concentrations tested.

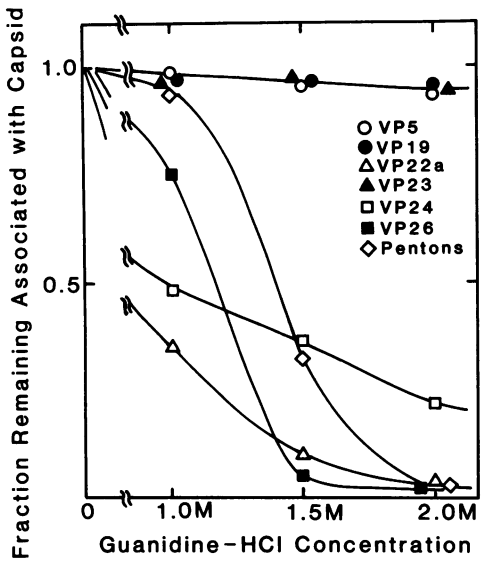

FIG. 2. Loss of specific proteins and pentons from GuHCl-extracted B capsids. The protein composition of control and GuHCl-extracted capsids was determined by densitometric scanning of stained SDS-polyacrylamide gels as described in Materials and Methods. The proportion of pentons present was determined by scoring capsid penton positions as either containing or not containing a capsomer.

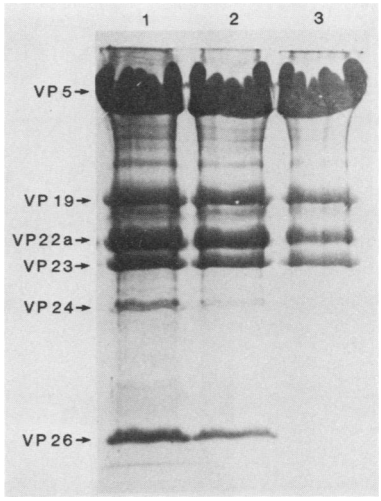

FIG. 3. SDS-polyacrylamide gel electrophoresis of control HSV-1 B capsids (lane 1) and of B capsids after extraction with 1.0 M GuHCl (lane 2) and 2.0 M GuHCl (lane 3) in low-ionic-strength buffer (20 mM Tris hydrochloride [pH 7.4], 1 mM EDTA). B capsids were prepared and extracted as described in Materials and Methods.

Quantitative analyses of the stained gels (Fig. 2 and Table 1) revealed that greater than 95% of VP22a and VP26 was removed by 2.0 M GuHCl extraction, while loss of VP24 was approximately 70%. Losses of VP5, VP19, and VP23 were in the range of 5 to 10%. None of the six capsid proteins was removed appreciably when extractions were performed at GuHCl concentrations of 0.1 M or lower. Capsids were completely solubilized (yielded a clear solution), however, at or above 3.0 M GuHCl (data not shown).

The presence of 0.5 M NaCl was found to be necessary for the complete or near-complete removal of VP22a by 2.0 M GuHCl. When NaCl was omitted from the extraction solution, much more VP22a remained associated with capsids, as shown in Fig. 3. The presence of NaCl had little effect on removal of VP24 or VP26 by GuHCl.

Electron microscopic analysis revealed two further effects of GuHCl extraction on B capsid structure. First, in shadowed preparations (Fig. 4), it was seen that extraction resulted in a highly selective loss of the capsid pentons. Whereas pentons were clearly observable in images of unextracted specimens (Fig. 4A, arrows), in comparable images of 2.0 M GuHCl-extracted capsids, virtually all (greater than 95%) of the identifiable penton positions were found to lack capsomers (Fig. 4B). Penton loss was negligible when extraction was performed at 1.0 M GuHCl and intermediate (approximately 70%) at 1.5 M GuHCl (Fig. 2). In all cases examined, pentons were either entirely present or entirely absent. Fragmented pentons or partial penton structures were never observed. In contrast to the situation with pentons, 2.0 M GuHCl extraction produced no significant (less than 5%) loss of hexons. This can be appreciated by examining the sites from which capsomers have been removed (i.e., gaps) in the extracted capsids shown in Fig. 4B. In all cases where gaps can be clearly discerned, they are found to be surrounded by five rather than six neighboring capsomers. No systematic damage to hexons could be detected by electron microscopy of shadowed specimens.

Second, examination of unshadowed capsids revealed that the material inside the B capsid cavity was removed by GuHCl extraction. Removal was complete or nearly so when extraction was performed with 2.0 M GuHCl, as shown in Fig. 5, and partial with 1.0 M GuHCl (not shown).

Except for the very specific effects on the pentons and the internal material, as noted above, extraction with 2.0 M GuHCl had relatively little effect on the basic structure of the capsid. The shells of extracted capsids were whole (i.e., not fragmented); neither the overall diameter nor the shell thickness was greatly affected by extraction; and the capsid icosahedral symmetry was still clearly evident, although its angularity was decreased to some extent (Fig. 4 and 5).

Partial reconstitution of extracted capsids. Since 2.0 M GuHCl was found to solubilize proteins VP22a and VP26 from B capsids, it was natural to ask whether the process could be reversed. Would the solubilized proteins reattach to extracted capsids if GuHCl were removed? To test this idea, B capsids were treated with 2.0 M GuHCl to solubilize VP22a and VP26 and then dialyzed against TNE to remove

TABLE 1. Protein composition of control, 2 M GuHCl-extracted, and partially reconstituted HSV-1 B capsids[a]

Protein	% of total protein		
	Control B capsids	GuHCl-extracted B capsids	Partially reconstituted B capsids
VP5	50.7	75.1	62.8
VP19	8.3	10.0	15.3
VP22a	22.4	<0.3	<0.3
VP23	9.2	14.0	11.1
VP24	1.7	0.5	<0.3
VP26	6.7	<0.3	10.3

[a] The procedures for gel electrophoresis and protein quantitation are described in Materials and Methods. Data are averages of values obtained in three, two, and two determinations for control, 2 M GuHCl-extracted, and partially reconstituted capsids, respectively.

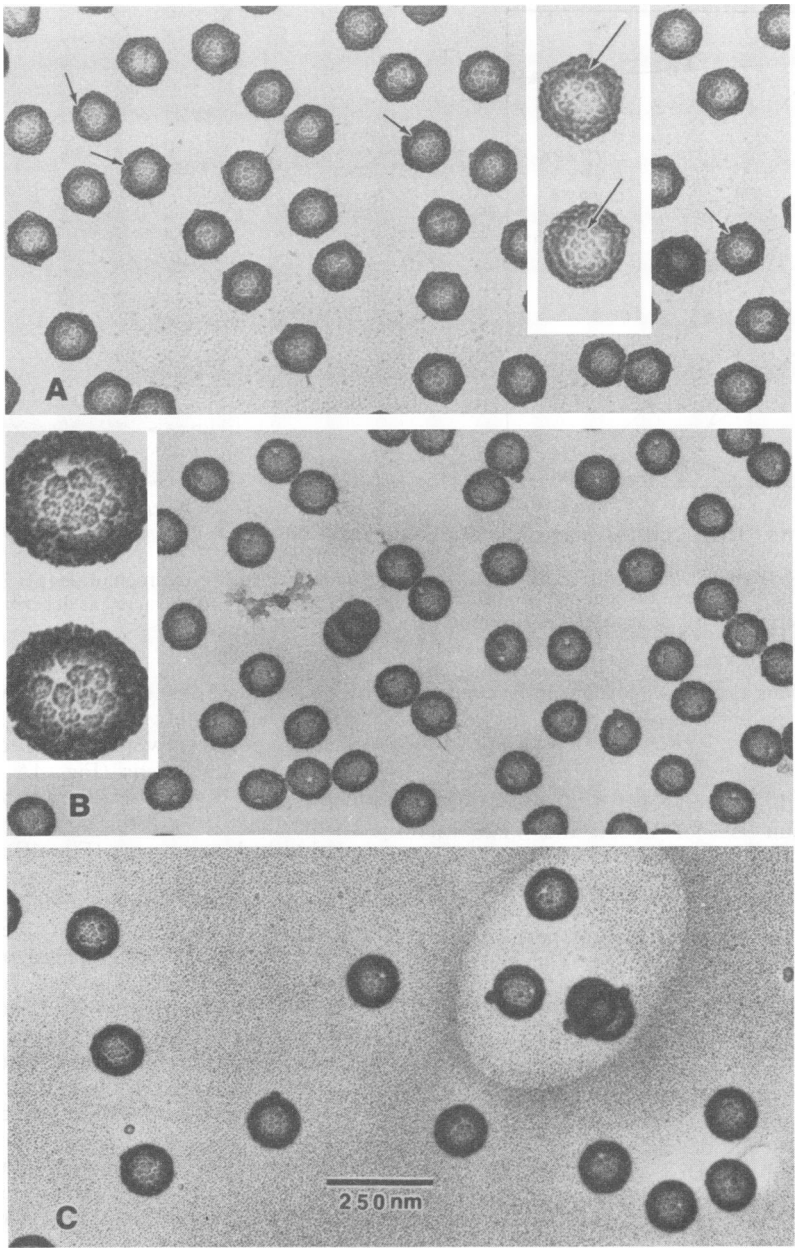

FIG. 4. Electron microscopic analysis of control HSV-1 B capsids (A), B capsids after extraction with 2.0 M GuHCl (B), and partially reconstituted B capsids (C). Arrows in panel A indicate the positions of capsid pentons which are absent (gaps) in 2.0 M GuHCl-extracted (B) and partially reconstituted (C) capsids. The inset in panel A shows a higher (1.8× higher) magnification of control B capsids. Pentons here can be identified by the fact that they are surrounded by five rather than six capsomers. In panel B the inset shows a 3× enlargement of 2.0 M GuHCl-extracted B capsids. Here it can be seen that gaps are surrounded by five rather than six capsomers. All specimens were prepared for electron microscopy by critical-point drying and shadowing with Pt-C as described in Materials and Methods.

the denaturant. When the resulting mixtures were centrifuged on 20 to 50% sucrose gradients, two bands of viral material were observed by scattered light. One had a sedimentation rate approximately the same as that of 2.0 M GuHCl-extracted capsids, while the second sedimented more slowly. The two bands were removed separately from gradients and analyzed by electron microscopy and by SDS-polyacrylamide gel electrophoresis. Figure 4C shows the results obtained when material from the more rapidly migrating (capsidlike) band was shadowed and examined in the electron microscope. Images showed capsids (hereinafter called partially reconstituted capsids) that were quite similar to 2.0 M GuHCl-extracted B capsids. Pentons were absent, but virtually all hexons appeared to be present. The overall diameter was not greatly different from that of control B capsids or of 2.0 M GuHCl-extracted B capsids. Gaps at the penton positions, however, were somewhat smaller in partially reconstituted than in 2.0 M GuHCl-

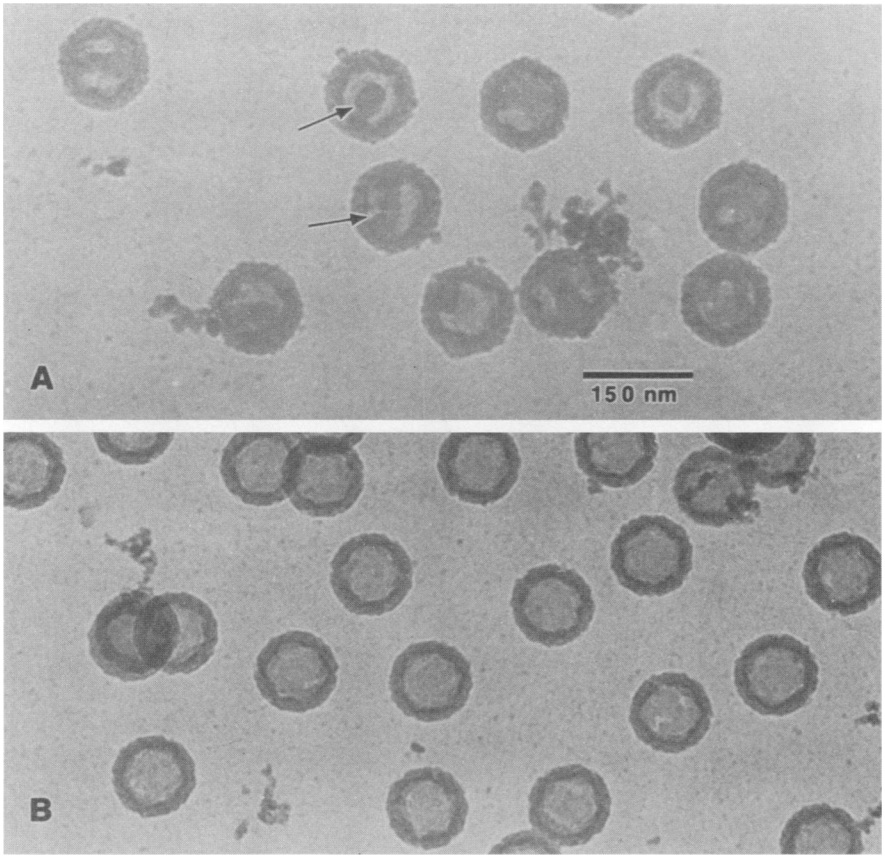

FIG. 5. Electron microscopic analysis of control HSV-1 B capsids (A) and B capsids after extraction with 2.0 M GuHCl (B). Arrows in panel A indicate material in the cavity of control B capsids that is lost after extraction. Specimens were prepared for electron microscopy by staining with 0.3% uranyl acetate followed by critical-point drying as described in Materials and Methods.

extracted capsids. In unshadowed preparations, partially reconstituted capsids were found to lack detectable material in the central cavity (not shown).

SDS-polyacrylamide gel analysis of partially reconstituted capsids showed that they contained all the proteins present in B capsids except for VP22a (Table 1 and Fig. 6, lane 2). Since both VP22a and VP26 were solubilized by GuHCl treatment, VP26 must have reassociated with extracted capsids during dialysis to remove GuHCl.

The more slowly migrating band was found by electron microscopic analysis to consist of a reasonably uniform population of toroidal or donut-shaped objects (Fig. 7A). No other regular structures were observed. Measurements of the micrographs showed that the overall torus diameter was 59.7 ± 3.4 nm (n = 33). Most toroids had a central depression or channel that was accessible to the negative stain (uranyl acetate). SDS-polyacrylamide gel electrophoresis demonstrated that the toroids consisted almost entirely of VP22a (Fig. 6, lane 1). Both subspecies of VP22a (26) observed in native B capsids (Fig. 1, lane 1) were also found in toroids assembled in vitro. Similar torus-shaped structures (Fig. 7B) were formed following dialysis (against TNE) of the clear supernatant obtained when B capsids were exposed to 2.0 M GuHCl and then centrifuged (at 24,000 rpm for 1 h in an SW50.1 rotor) to pellet the extracted capsids. Toroids formed in vitro by this latter procedure were also found by SDS-polyacrylamide gel analysis to consist primarily of VP22a (data not shown).

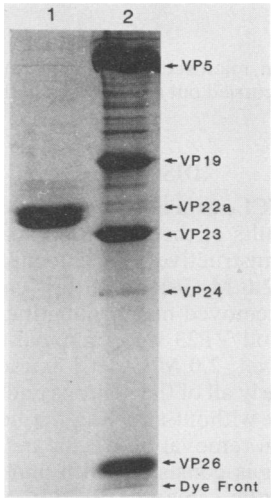

FIG. 6. SDS-polyacrylamide gel analysis of toroids (lane 1) and partially reconstituted B capsids (lane 2). Specimens were prepared by treating B capsids with 2.0 M GuHCl, dialyzing to remove GuHCl, and centrifuging the dialyzed mixture on a 20 to 50% sucrose gradient as described in Materials and Methods. Toroids constituted the upper (more slowly migrating) and partially reconstituted capsids constituted the lower band recovered after centrifugation of such gradients. Electrophoresis and staining of gels with Coomassie blue were carried out as described previously (18).

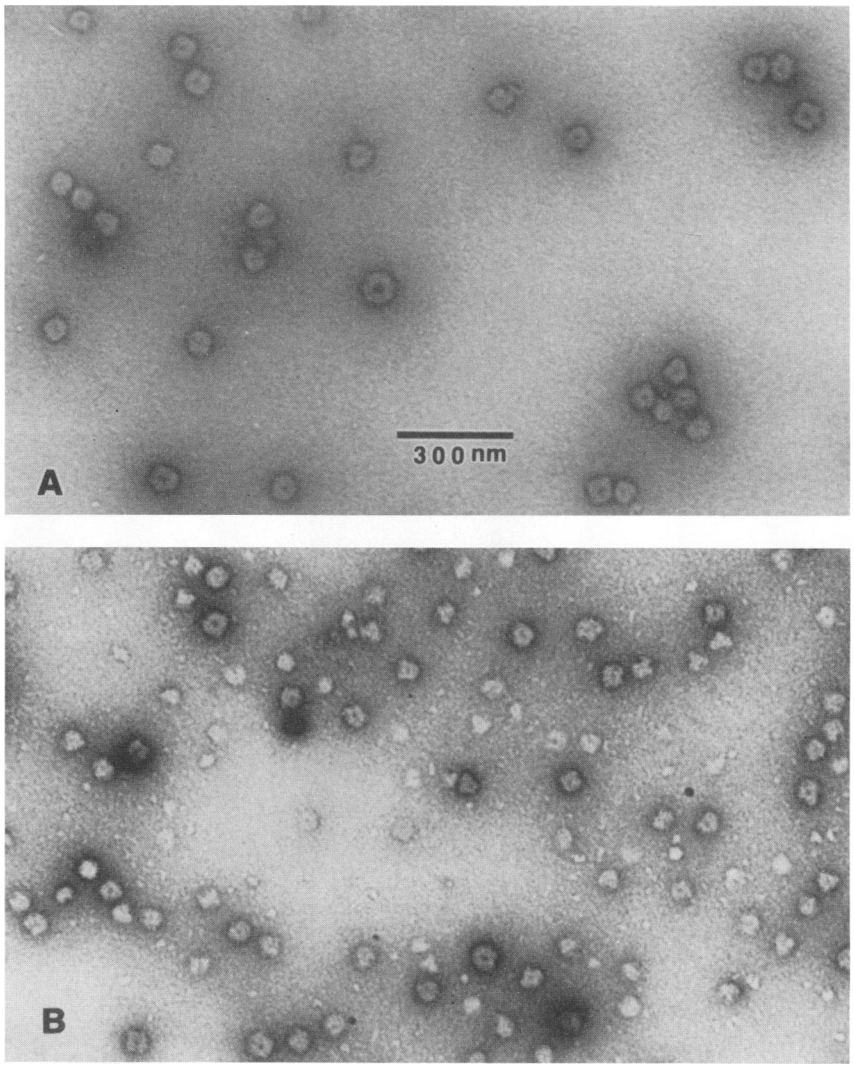

FIG. 7. Electron microscopy of toroid structures self-assembled in vitro in the presence (A) or absence (B) of capsids. In vitro self-assembly was carried out as described in the text. Specimens were prepared for microscopy by negative staining with 2% uranyl acetate.

DISCUSSION

Effects of GuHCl extraction and composition of the capsid pentons. The results of the extraction studies reported here are particularly instructive if one focuses on experiments performed with 2.0 M GuHCl. In this case proteins VP22a and VP26 were removed nearly quantitatively, while losses of VP5, VP19, and VP23 were minimal (5 to 10%). At the morphological level, 2.0 M GuHCl extraction was found to remove all or nearly all of the pentons and the material inside the capsid cavity without affecting the hexons appreciably. The simultaneous removal of VP22a and the material from the capsid center is consistent with and supports the view that the two are the same (2, 19)—i.e., that VP22a constitutes most of the material in the B capsid cavity. In this respect, VP22a resembles the scaffolding protein (gp8) of *Salmonella typhimurium* bacteriophage P22, which is present inside the phage procapsid and solubilized when procapsids are treated with GuHCl (6, 7). As in the case of the phage scaffolding protein, VP22a is removed while leaving the remaining capsid basically intact. Although NaCl was found to be required for quantitative removal of VP22a from B capsids (Fig. 3), its role in the process is not yet clear. NaCl could be required for GuHCl to gain access to the capsid cavity or possibly for freeing VP22a from an association with the capsid wall. NaCl is not likely to be directly involved in dissociation of VP22a aggregates, however, as it was present during B capsid purification and its presence was found to be permissive for in vitro assembly of VP22a into polymerized structures (i.e., toroids; see Fig. 7A).

The GuHCl extraction studies have also clarified the composition of the pentons to some extent. If it is assumed (on the basis of capsomer morphology) that each penton has 5/6 the mass of each hexon, then the 12 pentons are expected to have an aggregate mass of 9.0 MDa. This compares with observed extracted masses (as calculated from the data in Table 1, assuming there are 960 copies of VP5 present in each capsid) of 1.2 to 2.4 MDa for VP19, 1.3 to 2.6 MDa for VP23, 7.2 to 14.4 MDa for VP5, and 19.0 MDa for VP26. Thus, proteins VP19 and VP23 are eliminated as candidates for the penton subunit (assuming that pentons are composed of only one protein) because not enough of either is removed

to account for the extracted penton mass. This leaves VP5 and VP26 as the only viable candidates for the penton protein. VP5 suggests itself because of the morphological similarity of the pentons and hexons (2, 30). The dimensions of the two are similar; both have axial channels; and both have a "ribbed" external appearance. The major difference is simply that hexons have sixfold and pentons have fivefold symmetry. VP26 is suggested as the penton subunit because it is removed concomitantly with pentons during GuHCl extraction (Fig. 2). It also appears to bind at the penton positions when extracted capsids are partially reconstituted. For the present, therefore, both VP5 and VP26 must be considered possible penton components. Additional experimental work will be required to clarify the issue further.

The sensitivity of HSV-1 pentons to GuHCl extraction contrasts markedly with that of the hexons. Whereas virtually all pentons were lost after extraction of capsids with 2.0 M GuHCl, nearly all the hexons remained in place. Pentons were also found to be lost more readily than hexons when intact B capsids were treated with pronase or chymotrypsin (unpublished observations). Similar observations have been reported for HSV-2 capsids (32). It must be, therefore, that pentons are differently and more loosely integrated into the capsid shell than hexons. The location of pentons at the capsid vertices may contribute to this effect, as these are the sites of greatest mechanical stress in an icosahedron. A similar situation is observed with adenovirus, in which pentons are lost more readily than hexons after exposure of intact virions to elevated (56°C) temperature (28) or low ionic strength (15).

Location of VP26. Although VP26 was specifically extracted from B capsids and later rebound in near-normal amounts, it was still impossible to define its location in the capsid by the techniques employed here. No structure observable in the electron microscope correlated uniquely with the presence of VP26. The most important clue to the location of VP26 is probably the fact that gaps at the penton positions were smaller in partially reconstituted than in 2.0 M GuHCl-extracted capsids. Since only VP26 rebound to extracted capsids, this suggests that it may lie in or around the capsid vertices.

On the other hand, it is attractive to speculate that VP26 may form the triplexes (2). VP26 shares several important properties with bacteriophage proteins, such as the T4 *soc* protein and gD of phage lambda, that form triplexlike structures on the capsid surface (1, 13, 14, 36, 37). For example, *soc* and gD have high copy numbers (1:1 ratio with the major capsid protein) and low molecular weights (in the range of 12,000) like VP26, and both are able to bind in vitro to capsids that lack them. A similar trigonal arrangement has been observed (8) for adenovirus protein IX (M_r 14,339). It is easy to imagine, therefore, that VP26 could have an analogous location (i.e., the triplexes) in the HSV-1 capsid. There, its role could be to provide mechanical stability for capsomer-capsomer interactions. The position of VP26 can be resolved definitively by three-dimensional reconstructions calculated from appropriate cryoelectron micrographs of extracted and partially reconstituted capsids. Such studies are in progress.

In vitro self-assembly of VP22a and implications for capsid morphogenesis. Few details are yet available about how herpesvirus capsids are assembled in vivo. As indicated above, DNA-free capsids containing an assembly or scaffolding protein (VP22a in HSV-1) are found to form in the nuclei of infected cells. These structures, analogous to the proheads of double-stranded DNA bacteriophages, are then thought to package viral DNA at or near the time that the scaffolding protein leaves the capsid cavity (16, 27, 31). The ability of VP22a to self-assemble into defined structures (toroids) in vitro, as described here, raises the possibility that this may be a part of its in vivo function as well. The toroid is large enough (~60 nm in diameter) that it could serve as a scaffold or jig around which other proteins (VP5, VP19, and VP23) could condense to form the capsid shell. Toroids of similar size are often seen in the center of B capsids in vivo (5, 16, 22, 24, 26) and after capsid purification in vitro (20, 21). The ability of VP22a to assemble into toroids in the absence of preformed capsids is consistent with its proposed role as a scaffold. It is also compatible with the view that VP22a may be involved in packaging of DNA into the B capsid (24, 26). The diameter of toroids formed in vitro is too great for them to correspond to the "ringlike component" (20 to 30 nm diameter) found in the nuclei of cells infected with HSV-1 or EHV-1 (22, 29).

The results described here emphasize the overall similarity of VP22a to the scaffolding proteins of double-stranded DNA bacteriophages (12). Like phage scaffolding proteins, VP22a is found in the prohead (B capsid) cavity but not in the mature virion (9). As mentioned above, VP22a resembles the phage P22 scaffolding protein in that both are extracted when proheads are treated with GuHCl. Although the structures formed are quite different, VP22a and the T4 phage scaffolding protein share the property that both self-assemble in vitro into polymerized structures (4, 34). The similarity of herpesvirus and phage capsids is further emphasized by recent studies (2a) showing that the DNA in HSV-1 C capsids is arranged in the same liquid-crystalline form found in double-stranded DNA bacteriophages (17, 25). In attempting to clarify the details of herpesvirus capsid morphogenesis, therefore, it may be particularly productive to continue to pay attention to the results of relevant studies with double-stranded DNA phages.

ACKNOWLEDGMENTS

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