pH Reduction as a Trigger for Dissociation of Herpes Simplex Virus Type 1 Scaffolds

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Assembly of the infectious herpes simplex virus type 1 virion is a complex, multistage process that begins with the production of a procapsid, which is formed by the condensation of capsid shell proteins around an internal scaffold fashioned from multiple copies of the scaffolding protein, pre-VP22a. The ability of pre-VP22a to interact with itself is an essential feature of this process. However, this self-interaction must subsequently be reversed to allow the scaffolding proteins to exit from the capsid to make room for the viral genome to be packaged. The nature of the process by which dissociation of the scaffold is accomplished is unknown.

In mature herpes simplex virus type 1 (HSV-1) virions, the viral genome is enclosed within a T=16 icosahedral capsid made up of 12 pentons, 150 hexons, and 320 connecting densities called triplexes (52). The capsid shell is composed of multiple copies of four sequence-unrelated proteins, VP5 (149 kDa), VP19C (50 kDa), VP23 (34 kDa), and VP26 (12 kDa), which makes up the pentons and the bulk of the hexons (60, 68), represents 70% by mass of the capsid shell. The hexons also contain VP26, six copies of which are arranged in a ring on the top of each hexon (59, 67). The two remaining proteins, VP23 and VP19C, associate in a 2:1 ratio as a heterotrimer, called the triplex, that forms connections between neighboring hexons and between hexons and pentons (36, 47, 66). The initial step in capsid assembly involves a cocondensation of shell and scaffolding proteins to form a spherical particle designated the procapsid. In the procapsid, the shell surrounds the internal scaffold, which is formed predominantly by a single protein, pre-VP22a (32, 34). Pre-VP22a provides the framework around which a shell of the correct size and symmetry is constructed (7, 53, 55). In order to fulfill this role, pre-VP22a must interact both with the capsid shell proteins and with itself. The association with the shell has been extensively studied, and it is known that sequences at the C terminus of pre-VP22a interact directly with VP5 (20). These sequences are essential for capsid assembly (21, 30, 53). The self-interaction of pre-VP22a is less well characterized (38, 42), but it is known that it can occur in the absence of the capsid shell proteins (31, 39, 53), when it results in the formation of particles resembling free scaffolds (referred to in this paper as scaffold particles).

The scaffolding proteins are encoded by the gene UL26 locus, which comprises two 3′-coterminal overlapping genes (UL26 and UL26.5) that encode proteins from a single open reading frame (26, 27, 43). UL26.5 maps to the 3′ portion of UL26 and encodes pre-VP22a. UL26 encodes a protease, which cleaves itself at two positions known as the R (release) and M (maturation) sites. Cleavage at the R site generates two products. One of these, pre-VP21, shares amino acid sequences with pre-VP22a and is a minor scaffolding component, while the other, VP24, contains the protease activity. The M site is present 25 amino acids upstream from the C termini of both pre-VP21 and pre-VP22a (8, 26, 43). These C-terminal 25 amino acids include the residues that interact with VP5 (20), and cleavage of pre-VP22a at the M site, to produce the shortened form VP22a, destroys this interaction. This cleavage also triggers the reconfiguration of the spherical procapsid shell into the polyhedral form found in mature capsids (2, 14, 46). Three types of polyhedral capsid are generated, designated A (empty), B (intermediate), and C (full) (16). In B capsids the scaffold is retained, while A capsids contain no internal material. In C capsids the internal space is occupied by the viral genome. To accommodate the genome, the scaffold must exit from the capsids. However, since the available channels through the procapsid and capsid shells are too small to allow the passage of large structures, the effective size of the scaffold must be reduced. Therefore, mechanisms presumably exist to reverse the self-association of the scaffolding proteins. Studies of viral mutants suggest that DNA packaging and scaffold release are coordinated events that take place before or during the conversion from procapsids to polyhedral capsids (2, 40, 46) and that loss of scaffold takes place only in circumstances...
where DNA packaging is actively occurring (4). Under conditions where DNA packaging is blocked, only B capsids are usually formed (49). Since loss of scaffold appears to be closely linked to the actual process of DNA packaging, it seems likely that entry of the DNA itself into the capsid could be an important factor in the process. We speculate that the introduction of a large amount of negatively charged DNA into the interior of the capsid, by altering the ionic environment, affects the interaction of the scaffolding proteins. To investigate this process, we examined the structural integrity of purified scaffold particles under mildly acidic conditions and in the presence of low concentrations of urea, which appears to bring about analogous changes in the protein.

MATERIALS AND METHODS

Cells and virus. Spodoptera frugiperda (SF21) cells were grown in TC100 medium (Invitrogen) supplemented with 5% fetal calf serum. The construction of the recombinant baculoviruses AcUL26.5 (53) and AcVP22a (21), expressing pre-VP22a and VP22a, respectively, have been described previously.

Purification of scaffold particles. Three-hundred-milliliter cultures of SF21 cells at 10^7 cells/mL were infected at 5 PFU/cell with either AcUL26.5 or AcVP22a. After 48 h of incubation at 28°C, the cell culture was harvested by centrifugation and the pellet was resuspended in 1.60 volume of phosphate-buffered saline (PBS) (170 mM NaCl, 3.4 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2). Aliquots containing approximately 10^8 cells were pelleted and stored at −20°C until required. The method of scaffold purification was similar to that described by Newcomb et al. (33). Frozen aliquots were resuspended in 500 μL of PBS and subjected to five cycles of freeze-thawing in dry ice-methanol before the cellular debris was removed by centrifugation at 13,000 rpm in an MSE Microcentrifug centrifuge. The supernatant was transferred to a fresh tube, and 90% saturated ammonium sulfate was added to a final concentration of 10%. The solution was mixed at 4°C for 30 min, and the precipitated proteins were pelleted by centrifugation at 13,000 rpm. The supernatant was discarded, and the pellet was resuspended in 0.5 mL of PBS. The suspension was layered onto a 13-ml gradient centrifugation and in the presence of low concentrations of urea, which appears to bring about analogous changes in the protein.

RESULTS

Scaffold particle stability. The preparation of scaffold particles purified from AcUL26.5-infected cells (see Materials and Methods) consisted almost entirely of pre-VP22a as determined by Coomassie brilliant blue staining (Fig. 1a, lane 6). When examined by electron microscopy, the particles appeared to be roughly spherical and approximately 60 nm diameter (Fig. 1b). Analysis by light scattering indicated a diameter of ~70 nm with a molecular mass of 14,700 to 18,400 kDa (Brian Bowman, personal communication), corresponding to approximately 500 copies of pre-VP22a. This is considerably larger than the size of the scaffolds purified by Newcomb et al. (33), presumably reflecting the different properties of their human cytomegalovirus-HSV-1 fusion construct and the wild-type HSV-1 scaffolding protein used here.

To investigate the stability of the scaffold particles, they were treated with a range of urea concentrations. Since the scaffolding protein undergoes extensive disulfide cross-linking when stored under nonreducing conditions (69), all experiments were carried out in the presence of 1 mM DTT. The presence of DTT did not alter the appearance of the scaffold particles when examined by negative staining (Fig. 2a). However, when exposed to urea at a concentration of 1 M or higher, no scaffold particles were visible (Fig. 2b), although on close inspection the grids were seen to be coated with a layer of protein that presumably arose from dissociated scaffold particles. This suggests that the presence of low concentrations of urea can cause the dissociation of the scaffold particles into much smaller units that do not form distinctive structures visible by electron microscopy.

The stability of scaffold particles under different pH conditions was tested by dialyzing them against PBS at a range of pHs between pH 5.0 and 6.5 at intervals of 0.5. At pH 6.5 (not shown) there was no obvious alteration in the numbers or appearance of the scaffold particles compared to the untreated control at pH 7.2 (Fig. 2a). At pH 6.0 (Fig. 2c) scaffold particles were still present in similar numbers, although they appeared to be smaller and less uniform, with a tendency to aggregate. However, at pH 5.0 (not shown) and pH 5.5 (Fig. 2d), no particles were visible and the grids were covered with a protein layer similar to that seen following treatment with 1 M urea.

To confirm that lowering the pH was causing dissociation of the scaffold particles, their sizes were analyzed by sucrose gradient centrifugation. For these experiments both pre-VP22a and VP22a (the truncated form of pre-VP22a missing the C-
terminal 25 amino acids [41]) scaffold particles were prepared. Purified scaffold particles at a concentration of 1 mg/ml in PBS (pH 7.2) were treated with 1 mM DTT for 1 h to ensure the removal of intermolecular disulfide cross-links before being layered on top of 10 to 40% sucrose gradients prepared at either pH 7.2 or 5.5 (see Materials and Methods). After centrifugation at 40,000 rpm (Sorvall AH650 rotor) for 105 min, the gradients were examined for the presence of visible refracting bands. For both pre-VP22a and VP22a, a single band was visible near the bottom of the pH 7.2 gradients, but in neither case could a band be seen on the pH 5.5 gradients (data not shown). To ascertain the distribution of the scaffolding proteins, each gradient was fractionated and analyzed by Western blotting. Figure 3 shows the profiles for both the full-length and truncated forms. At pH 7.2, the abundance of both pre-VP22a (Fig. 3a) and VP22a (Fig. 3c) was maximal in fraction 3, in agreement with the position of the visible scaffold particle band. In contrast, at pH 5.5 both pre-VP22a (Fig. 3b) and VP22a (Fig. 3d) were shifted towards the top of the gradient, suggesting that the scaffold particles were dissociating into smaller components.

To determine the extent of dissociation, the sucrose gradient analysis at pH 5.5 was repeated as before, but the centrifugation was carried out at 40,000 rpm (Sorvall AH650 rotor) for 20 h. Bovine serum albumin (BSA) (250 μg/gradient; Sigma Aldrich) was added to each sample as a size marker. Following gel electrophoresis of the gradient fractions, the distribution of the BSA marker was determined by Coomassie brilliant blue staining (Fig. 4a and c), while the scaffolding proteins were detected by Western blotting (Fig. 4b and d). The gel profiles revealed that for both pre-VP22a (Fig. 4b) and VP22a (Fig. 4d) the peak fractions coincided with the BSA peak (Fig. 4a and c, respectively). Since BSA is a monomer with an Mr of 66,000, it is clear that under these conditions both pre-VP22a and VP22a, which have molecular masses of around 34 and 31 kDa, respectively, are sedimenting as small entities which are likely to be dimers or monomers.

Effects of denaturants on the secondary and tertiary structures of the scaffolding protein. The studies described above demonstrate that the association of scaffolding proteins into distinct scaffold particles is sensitive to experimental conditions and can be reversed by low concentrations of denaturants or by relatively small changes in pH. To determine whether these conditions were directly affecting the structure of the scaffolding proteins themselves, fluorescence spectroscopy and CD were used to assess the structural integrity of the scaffolding protein.

Figure 5 shows the CD spectra obtained from purified scaffold particles and the effects of pH changes and presence of urea on these spectra. The SELCON procedure (51) was used to estimate the secondary structure content of the sample from the far-UV CD spectrum (Fig. 5a). At pH 7.2, pre-VP22a was found to contain 19% α-helix, 24% antiparallel β-sheet, 2% parallel β-sheet, and 24% turn. The secondary structure prediction program PSIPRED gives a value of up to 21% α-helix but predicts less than 5% β-sheet. Lowering the pH from 7.2 to 5.5 or addition of 1 M urea led to small losses of secondary structure, with the changes in ellipticity at 225 nm of the order of 10% or less. Addition of 8 M urea led to a greater than 95% loss of ellipticity at 225 nm, indicating that the protein was completely unfolded under these conditions.

The near-UV CD spectrum of pre-VP22a at pH 7.2 (Fig. 5b) shows a small positive peak at 295 nm, characteristic of Trp side chains, and a larger negative peak at 275 nm, characteristic of Tyr side chains. Addition of 1 M urea led to a modest (less than 30%) decline in the amplitude of the near-UV CD spectrum, indicating that the tertiary structure of the protein had not been seriously affected. Despite several attempts, it proved impossible to obtain a satisfactory near-UV CD spectrum of the protein at pH 5.5. This was because at the concentrations (1 mg/ml or greater) required to record spectra, the sample...
had a tendency to precipitate while undergoing dialysis to lower its pH.

It should be noted that the amplitudes of the peaks in the near-UV CD spectrum of pre-VP22a are relatively small by

comparison with those found in several well-characterized proteins such as insulin, lysozyme, and carbonic anhydrase (63). While there are several factors that influence the intensities of near-UV CD spectra, one explanation for the low intensities of

FIG. 2. Dissociation of scaffold particles by urea and pH reduction. Scaffold particles prepared as for Fig. 1 (a) were treated with 1 M urea (b) or dialyzed against PBS that had been adjusted to pH 6.0 (c) or pH 5.5 (d) as described in the text. The samples were absorbed onto Parlodion-coated microscope grids, stained with 1% phosphotungstic acid, and examined by electron microscopy. Bar, 500 nm.

FIG. 3. Sucrose gradient analysis of scaffolds. Scaffold particles purified from AcUL26.5 (a and b)- or AcVP22a (c and d)-infected Sf21 cells were analyzed on 5-ml, 10 to 40% (wt/vol) sucrose gradients at pH 7.2 (a and c) or pH 5.5 (b and d). Centrifugation was at 40,000 rpm for 105 min in a Sorvall AH650 rotor. Nine fractions were collected from the bottom of the gradient, and 20-μl samples of each fraction were analyzed on SDS–10% polyacrylamide gels and transferred to nitrocellulose membranes. To reveal the distribution of the scaffolding proteins, the blots were probed with MCA406 antibody as described in Materials and Methods.
the peaks in pre-VP22a is that the interactions stabilizing the tertiary structure of the protein may be relatively weak.

From the CD studies it can be concluded that the lowering of the pH from 7.2 to 5.5 or the addition of 1 M urea leads to the retention of most of the secondary and tertiary structural features of native pre-VP22a.

Fluorescence spectroscopy reports on the environment of the tryptophan side chains and therefore provides an additional measure of the integrity of the folded state of a protein. A completely unfolded protein would have a $\lambda_{\text{max}}$ (wavelength of maximum emission) of 356 nm, indicating that the Trp residues are fully exposed to the solvent. A shift to shorter wavelengths indicates that the Trp residues are at least partially shielded from the solvent (11). Changes in fluorescence intensity during unfolding can often show a more complex pattern because a variety of factors, such as efficiency of quenching by solvent and by energy transfer, are involved.

Pre-VP22a scaffold particles were incubated for 15 min with a range of urea concentrations of from 1 to 8 M, and their fluorescence spectra were recorded (Fig. 6a). The untreated control sample had a $\lambda_{\text{max}}$ of 344.5 nm. This is significantly shifted from the value of 356 nm observed for the model compound N-acetyltryptophanamide and is consistent with the scaffolding protein having a tertiary structure in which the Trp side chains are buried to a moderate extent. In the presence of 8 M urea, the protein had a $\lambda_{\text{max}}$ of 355 nm, indicating that the protein is essentially completely unfolded under these conditions. However, in the sample treated with 1 M urea (where the dissociation of scaffold particles still occurs), there was only a minor shift in $\lambda_{\text{max}}$ (to 345.5 nm), representing ~10% of the total change seen between 0 and 8 M (Fig. 6b). When scaffold particles dialyzed against PBS at pH 5.5 were examined, they also gave a $\lambda_{\text{max}}$ of 355 nm. This suggests that reducing the pH has little effect on the average exposure of the Trp side chains and thus presumably the overall tertiary structural features of the protein.

These findings suggest that dissociation of scaffold particles is due to subtle changes affecting the interacting surfaces and does not involve extensive changes in the structure of pre-VP22a monomers themselves.
Pre-VP22a appears to have a weakly defined tertiary structure but does not behave like a typical molten globule. The molten globule is a relatively compact state, in which a protein retains most of its native secondary structure but the interactions between side chains stabilizing the tertiary structure are weak (1, 44). The fluorescent dye ANS binds preferentially to nonpolar regions of proteins. A characteristic large increase in fluorescence at 470 nm (with excitation at 370 nm) is generally considered to be a sensitive test for the molten globule state of proteins, where ANS has access to the hydrophobic interior (1, 44). Although ANS has been shown to bind to the native state of some proteins via clusters of nonpolar residues, binding to typical molten globule states (Fig. 7b) is usually enhanced by at least an order of magnitude compared with either the native or fully unfolded states (45, 48). Replicate samples of scaffold particles were tested for ANS binding ability at physiological pH (pH 7.2) or after dialysis against PBS adjusted to pH 6.5, 6.0, or 5.5.

Scaffold particles were found to bind ANS weakly at pH 7.2, as shown by a 3.5-fold increase in fluorescence at 470 nm compared with free ANS (Fig. 7a). At pH 6.5 and 6.0 the increases in fluorescence were of a similar order but, the increase at pH 5.5 was about 50% greater (fivefold greater fluorescence than free ANS), suggesting that some additional nonpolar sites on pre-VP22a for binding ANS had become available as a result of dissociation of the scaffold particles. However, the increase in ANS fluorescence under any of the
conditions tested was only relatively modest, indicating that formation of a typical molten globule state was unlikely to have occurred in the case of pre-VP22a. This conclusion is supported by the data from the near-UV CD and fluorescence studies; namely, that although the interactions stabilizing the tertiary structure of pre-VP22a are weak, they persist under conditions where the scaffold is dissociated. In a typical molten globule state, the near-UV CD spectrum would be essentially abolished, reflecting the lack of stable tertiary interactions between side chains (1, 44).

Effect of reduced pH on capsids. Since dissociation of the scaffold seems to be a necessary precursor to its exit from the capsid, the effect of reduced pH was examined by dialyzing purified B capsids against PBS at pH 5.5 for 16 h at 4°C in the presence of 1 mM DTT. Figure 8 shows electron micrographs of negatively stained B capsids treated at pH 7.2 (Fig. 8a) and pH 5.5 (Fig. 8b). While the scaffold density could clearly be seen inside the capsids at pH 7.2, it was not observed at pH 5.5, suggesting that the nature of the scaffold had altered. When capsids treated in a similar way were examined after thin sectioning, the characteristic ring-shaped, central scaffold cores were clearly defined at pH 7.2 (Fig. 8c) but were not seen at pH 5.5 (Fig. 8d). Instead the internal material had either congregated into one or more clumps on the inner surface of the capsid or was more evenly distributed around the inside of the capsid shell. To confirm that the scaffolding proteins had remained inside the capsids, B capsids that had been treated at pH 7.2 (Fig. 9a and b) or pH 5.5 (Fig. 9c and d) were layered onto sucrose gradients of the same pH. Western blot analysis showed that at both pHs, VP22a (Fig. 9b and d) cosedimented with the major capsid protein, VP5 (Fig. 9a and c), in the position occupied by the visible capsid band. A similar result was obtained when capsids were treated with 1 M urea (data not shown). No dissociated VP22a was detected at the top of the gradients, confirming that the scaffolding proteins had remained associated with the capsids.

DISCUSSION

It is well established that scaffolding proteins play a role in ensuring accurate and efficient assembly of capsid shells (9). Other functions have also been suggested, including the exclusion of material from the capsid interior during assembly (10) and the masking of DNA binding sites on the inner surface of
The procapsid (22). However, the link between the procapsid-capsid transition and the breaking of the interaction between the scaffolding protein and VP5 in HSV suggests that they may have an additional role in stabilizing the procapsid structure.

When they first assemble, the proteins forming the HSV procapsids are apparently not in their most stable state, since they can rearrange to form polyhedral capsids in a process that does not require energy input (58). Studies on temperature-sensitive mutants have shown that the procapsid state can be maintained for several hours (46) but that once the conversion to polyhedral capsids is initiated, it progresses quite rapidly (2). From this it can be concluded that the procapsid shell is maintained in an energetically suboptimal conformation. This has been demonstrated for P22, where head expansion has been shown to be an exothermic process, implying that the mature phage head is in a lower energy state than the procapsid (13). The maintenance of the procapsid shell in this relatively unfavorable state may be explained if the shell and scaffold are considered as a single entity rather than separately. Thus, the scaffold-shell complex would have a minimum-energy state that did not correspond to that of either the shell or the scaffold on its own. This minimum-energy state is the procapsid and represents the natural end point of assembly. When the link between the scaffold and the shell is broken, the constraints on the capsid shell are released. The proteins can then reconfigure into the minimum-energy state for the shell, which corresponds to the polyhedral capsid conformation. The fate of the scaffold would depend on the conditions applying when the scaffold-shell link was broken. If cleavage is accompanied by DNA entry and packaging, the scaffold will dissociate and exit from the capsid to be replaced by the viral DNA. However, if there is no packaging, the scaffolding proteins can refold into their own minimum-energy state, represented by the small core of the B capsid. The ability of the scaffold to maintain the shell in the procapsid configuration may have an additional purpose, since this would ensure that it remains in a suitable condition for DNA packaging for a longer time, thereby enlarging the pool of procapsids available for packaging.

Status of scaffolding proteins during exit from capsids. The form in which the scaffolding protein exits from the capsid is unknown, as is the route by which it leaves. In bacteriophage P22, the scaffolding protein is believed to be lost before the maturation from prohead to capsid is completed. The P22 prohead has a 30- to 45-A diameter channel at the center of each hexon (57). These channels are closed during the rearrangement of the shell proteins that accompanies DNA packaging, and the mature phage head forms a largely unbroken shell around the DNA (64). In HSV the mature capsid shell is not unbroken as in P22 but is perforated by channels through the pentons and hexons (60, 68), leaving open the question of when scaffold exit takes place. Indeed, it has been shown that moderate concentrations of chaotropic agents can remove VP22a from B capsids in the presence of 0.5 M NaCl, without apparently altering the structure of the capsid shell (36). However, by analogy with P22, it seems likely that the HSV scaffold is lost before or during the conversion from procapsid to capsid, as the HSV procapsid shell is more open than that of the mature capsid, with a poorly formed capsid floor and large channels through the pentons and hexons (37, 58).

Treatment of B capsids at low pH showed that although the characteristic scaffold cores had disappeared, the scaffolding proteins remained inside the capsids. This suggests that the internal scaffold can be disrupted by low pH but either that suitable channels, through which the scaffolding protein can pass, are not present in the B capsid shell or that the individual protein molecules are not in an appropriate form to leave the capsid.

Monomeric P22 scaffolding protein has been suggested to form a molten globule (61). The adoption of a molten globule form would be expected to provide the P22 scaffolding protein with greater flexibility, which might aid its passage through the narrow channels in the prohead. One of the HSV-1 capsid shell proteins (VP23) has already been shown to have the properties of a molten globule (23). However, fluorescence, far-UV CD, and near-UV CD analyses showed that the conditions used to dissociate HSV-1 scaffold particles (1 M urea or pH 5.5) do not induce large alterations in the structure of pre-VP22a. Although there was an increase in ANS fluorescence at pH 5.5 compared to pH 7.2 (Fig. 7), it was not large enough to indicate that the protein had adopted a molten globule state. Therefore, there is no definite evidence that pre-VP22a adopts a classical molten globule structure as a means of exiting from

![Image](http://jvi.asm.org/Downloadedfrom/September18,2019byguest)
the capsid. However, it should be pointed out that the relatively small magnitude of the near-UV CD spectrum indicates that the interactions involved in stabilizing the tertiary structure of the pre-VP22a protein may be relatively weak, and this degree of structural plasticity could be important for its role in forming a short-lived scaffold.

When purified scaffold particles, at relatively high concentrations (~1 mg/ml), were maintained at pH 5.5, they tended to precipitate. This was observed, for example, when they were dialyzed to lower their pH for near-UV CD analysis. The propensity to aggregate may reflect the exposure of additional nonpolar sites on the protein as suggested by the increase in ANS binding (Fig. 7). However, when scaffold particles at 1 mg/ml were sedimented through gradients at pH 5.5, the subunits dissociated and aggregation did not take place (Fig. 3 and 4). The most probable explanation is that the subunits were able to separate quickly from each other when they migrated into, and were diluted by, the gradient, as aggregation was not a problem at the lower protein concentrations (~0.2 mg/ml) used for far-UV CD and fluorescence. It seems likely that a similar process could occur during DNA packaging, as the exit of dissociated scaffolding proteins might be expected to be an efficient process that would remove the subunits before they had the opportunity to aggregate.

Role of DNA packaging in scaffold dissociation. In order to exit from capsids, scaffolding proteins have to reverse both their self-interaction and their interaction with the capsid shell. In HSV, reversing the interaction with the capsid shell is achieved by proteolysis of pre-VP22a. Studies of mutants with defects in cleavage (2, 14, 40, 46) suggested how pH-induced alterations in the protonation state of surface amino acids of the protein but would not necessarily have a dramatic effect on its overall structural properties. Studies on foot-and-mouth disease virus have demonstrated how pH-induced alterations in the protonation state of a small number of amino acids can overcome the ionic and hydrogen bonding forces holding the structural subunits together and lead to their dissociation (12). Self-interaction in pre-VP22a (5, 38, 42) and its human cytomegalovirus counterpart (62) has been shown to involve only limited portions of the protein, and it is easy to envisage how similar small-scale changes could cause the dissociation of scaffold particles without having any marked effect on the secondary or tertiary structures of the component proteins.

Based on the results described here, we propose that the disassembly of the scaffold core inside procapsids may result from a change in the local charge environment caused by the insertion of the viral genome. DNA is an acidic molecule with a substantial negative charge, which, within cells, is normally neutralized by being bound to basic proteins or to polyamines. Although spermine is found within the capsid in HSV-1 virions and is thought to be important for close packing of the genome, it is not known when it enters the capsid, and the amounts detected are sufficient to neutralize only about half the charges on the DNA molecule (15, 17). During packaging the viral DNA enters into a confined space by passing through a narrow channel, which may shield it from the cellular environment as has been suggested for the double-stranded RNA virus bluetongue virus (18). Recently it has been shown that
the 329 portal complex can exert a large force to counteract the combined resistance provided by the entropic, electrostatic, and bending energies of the packaged DNA (50). A similar portal complex is now known to be present in herpesvirus capsids (35). It is reasonable to assume, therefore, that the charges on the packaged DNA do not have to be fully neutralized. Indeed, it is likely that some residual repulsive force is important for initiating the release of the DNA from the capsid following infection (50). Nothing is known about the conditions within the capsid during DNA packaging, but again it seems reasonable to assume that localized reductions in pH would arise due to incomplete neutralization of the charges on the DNA. The experiments described here have clearly demonstrated that a relatively small reduction in pH induces the rapid dissociation of isolated scaffold particles into their constituent proteins. Equivalent local, and perhaps short-lived, alterations in pH within the capsid may provide a mechanism to drive the dissociation of the scaffold into a low-molecular-weight form capable of exiting through the available channels.

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