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Analysis of the Protein-Protein Interactions in the Parvovirus H-1 Capsid

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The structure of the icosahedral capsid of the H-1 parvovirus was probed by chemical cross-linking methods. Treatment of empty capsids with high-molecular-weight polyethylene glycols resulted in irreversible aggregation of the minor capsid protein VP1. Multimers of VP1 containing at least five and perhaps six molecules were obtained, but only with empty capsids and not with the full, DNA-containing virus. Cross-linking of the empty capsids with dimethylsuberimidate confirmed the assignments of the products formed after treatment with polyethylene glycol. With dimethylsuberimidate the most abundant product was a heterologous dimer containing VP1 and the major capsid protein VP2′. A small amount of homologous VP2′ dimer was also obtained, but the majority of VP2′ remained unreacted even at high concentrations of dimethylsuberimidate. The capsid proteins of the full virus, on the other hand, were completely unreactive to dimethylsuberimidate. The data suggest that the minor protein VP1 may be clustered in the capsid and perhaps composes one or two of the morphological units of the icosahedral shell.

The H-1 parvovirus is a small, single-stranded-DNA-containing virus which infects eucaryotic cells (20). The structure of the H-1 virus, which is representative of the autonomous, nondefective parvoviruses, is characterized by an icosahedral protein shell which surrounds the single-stranded DNA genome (11, 24). The capsid is made up of two proteins, VP1 and VP2′, in the empty particles, and three proteins, VP1, VP2′, and VP2, in the full, DNA-containing virus (13). VP1 (88,000 daltons [88K]) and VP2′ (68K) are viral gene products with overlapping sequences such that most of the sequences of VP2′ are contained within VP1 (19, 23). VP2 (65K), on the other hand, is a proteolytic cleavage product of VP2′; this cleavage occurs only after assembly of the DNA-containing particles, but not at all in the empty capsids (5, 22). Whereas the viral capsid might contain various amounts of VP2′ and VP2, the sum of the amounts of VP2′ and VP2, as well as the amount of VP1, remains constant (21).

At present, details of the protein interactions in the parvovirus capsid are not known. Peterson et al. (17) have shown that for four parvoviruses, H-3, H-1, Kilham's rat virus, and minute virus of mice, VP1 comprised around 15% of the total particle protein, with the other 85% being either VP2′ (empty capsids) or the sum of VP2′ and VP2 (full capsids). Similarly Kongsvik et al. (12) have calculated that the empty capsids of H-1 contain 20% VP1 and 80% VP2′. Electron microscopic studies of H-1 by Karasaki (11) and of Kilham's rat virus by Vasquez and Brailovsky (25) led to the determination that the capsids had icosahedral symmetry. Furthermore, both studies, following the rules of Caspar and Klug (3), concluded that a pentagonal dodecahedron containing 32 capsomeres was the most likely structure. It should also be noted that an earlier electron microscopic study of a defective parvovirus by Mayor et al. (15) suggested that the capsid was a polyhedron composed of 12 subunits, each located at a vertex of the icosahedron.

In this study I examined the protein-protein interactions in the capsid of the H-1 parvovirus via chemical cross-linking. Cross-linked products were observed only in the empty capsid, and the data suggest that the minor protein VP1 may be clustered in the virus.

MATERIALS AND METHODS

Growth and purification of H-1 virus. The H-1 virus was purified by one of two methods and will be referred to as cell-purified H-1 and polyethylene glycol (PEG)-precipitated H-1. In both cases, the virus was grown in NB (simian virus 40-transformed newborn human kidney) cells which had been grown to confluency in roller bottles and infected at a multiplicity of infection of 0.1. The virus was harvested after cytopathic effect was complete (around 5 days postinfection).

Cell-purified virus was obtained by a modification of the procedure of Rhode (18) as follows. The cells were

scraped off the bottles directly into the medium, the pH was adjusted to 6.7 with 1 N HCl, and 1 ml of guinea pig erythrocytes was added to bind the free virus. The cells were collected by centrifugation, suspended in 25 mM Tris-hydrochloride (pH 8.5)–5 mM EDTA–5 mM NaCl, frozen to −70°C, and then thawed to room temperature. This suspension was then adjusted to 1% in sodium dodecyl sulfate (SDS), layered onto a 5-ml cushion of 20% sucrose in 0.05 M Tris-hydrochloride (pH 8.0)–0.1 M NaCl–1 mM EDTA–0.2% SDS, and spun at 16,000 rpm for 16 to 20 h in an SW25.1 rotor. The pellet was suspended in 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.2% Sarkosyl, and the solution was sonicated until the pellet was completely dispersed. This suspension was then adjusted to a density of 1.38 g/cm^3 in CsCl, and the virus was banded in an SW60.1 rotor at 35,000 rpm for 20 to 24 h. The empty and full virus were visualized by vertical light, recovered by side puncture, and rebanded in 1.33 and 1.43 g/cm^3 CsCl, respectively. The empty capsids were recovered as described above, but the full virus was eluted from the bottom of the tube and the heavy and light full virus fractions were identified by determinations of absorbance at 260 nm and density. The pooled virus was dialyzed into 20 mM Tris-hydrochloride (pH 8.5)–1 mM EDTA. Virus concentration was estimated by using absorbance of a 1% solution at 280 nm = 17 for the empty capsids and absorbance at 260 nm = 94 for the full virus.

PEG-precipitated virus was obtained as described previously (16). Briefly, the infected cells were lysed directly in the roller bottles by three cycles of freezing and thawing at pH 8.5, followed by centrifugation to remove debris. The supernatant was adjusted to 0.5 M in NaCl and 3.4% in PEG (either from Union Carbide Corp. [Carbowax 20M], with an average molecular weight of 15,000 [PEG-15,000], or from Sigma Chemical Co., with an average molecular weight of 20,000 [PEG-20,000] or 6,000 [PEG-6,000]) and left overnight at 4°C. The virus was collected by centrifugation at 8,000 × *g* for 30 min and then resuspended in 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.2% Sarkosyl at a density of 1.38 g/cm^3 in CsCl. The remainder of the purification was as described above for the cell-purified virus.

Labeling of H-1 virus. ^{35}S-labeled H-1 virus was obtained by infecting NB cells at a multiplicity of infection of 20 in methionine-poor medium (Flow Laboratories, Inc.) made to 20 μCi/ml in [^{35}S]methionine (New England Nuclear Corp.; 1,100 Ci/mmol). The cells were harvested at 24 h postinfection, and the labeled H-1 virus was purified as described above for cell-purified virus.

Radioiodinations were done with chloramine-T as described previously (16).

Chemical cross-linking with DMS. Cross-linking of the capsid proteins with dimethylsuberimidate (DMS) (Eastman Organic Chemicals) was done at room temperature in 0.2 M triethanolamine at pH 8.5. The reactions were done in a 30- to 50-μl volume with 20 to 30 μg of capsid protein and were terminated by the addition of SDS gel disruption buffer (14) followed by boiling for 3 min. These samples were then loaded directly onto a slab gel for electrophoresis. Reversal of the cross-linkages for subsequent analysis on two-dimensional gels is described below.

Gel electrophoresis. SDS- polyacrylamide slab gels were made according to the method of Laemmli (14). Fluorography was performed as described previously (2). Two-dimensional gels, with reversal of the DMS cross-linkage products after the first dimension, were made basically as described by Horden et al. (10). The products were separated in the first dimension on a 5% polyacrylamide gel, and the appropriate lane was sliced out and soaked for 24 h at room temperature in a solution containing 15 parts NH_4OH, 1 part acetic acid, and 1% 2-mercaptoethanol. The gel was then soaked for 30 min in running buffer, polymerized onto the top of a 7.5% polyacrylamide gel, and electrophoresed in the second dimension until the ion front was near the bottom of the gel.

The molecular weights of the multimers formed after PEG treatment were determined on a 5% polyacrylamide gel (14) by using the following marker proteins: phosphorylase *a* (92K), myosin (200K monomer and 400K dimer), reduced chicken embryo fibronectin (230K monomer), and nonreduced chicken embryo fibronectin (460K dimer).

Purification and amino acid analysis of viral proteins. VP2′, VP1, and the 170K protein formed by PEG precipitation were purified out of a 7.5% preparative slab gel with a single 10-cm sample lane after staining with Coomassie brilliant blue. Proteins were eluted from stained gel bands by soaking in 70% formic acid as described by Gibson and Garcy (7) except that the stain was removed by passing the concentrated eluate (100 μl) over a 1-ml Bio-Gel P-6 column in 70% formic acid and collecting everything that eluted ahead of the dye. Samples were then dried down and hydrolyzed in 6 N HCl–0.2% phenol for 24 h at 110°C, with subsequent amino acid analysis performed on a Beckman 121M analyzer equipped with an Autolab computing integrator.

RESULTS

The traditional method for purifying H-1 virus involves the extraction of virus from an H-1-infected cell pellet in the presence of SDS, followed by various combinations of high-speed centrifugation, lipid extraction, and equilibrium and velocity sedimentation (18). (The product is referred to here as cell-purified H-1.) In the interest of simplifying the purification procedure for large-scale preparations, I attempted to precipitate the virus with PEG as had been described previously for other viral systems (26). I found that lysis of the cells by freeze-thawing them directly in the roller bottles, followed by the addition of NaCl and either PEG-15,000 or PEG-20,000 to the cleared lysate, resulted in the precipitation of at least 80% of the viral hemagglutinin and infectivity. The PEG pellet, which was largely virus, can be taken up directly into the appropriate CsCl solution, and purified empty and full virus can be recovered from one gradient run. I routinely pooled the full virus and recentrifuged it to equilibrium in CsCl to separate the heavy and light full species.

Figure 1A shows a polyacrylamide gel of various forms of the PEG-precipitated H-1 virus. Lanes 2 and 3, containing the heavy full and

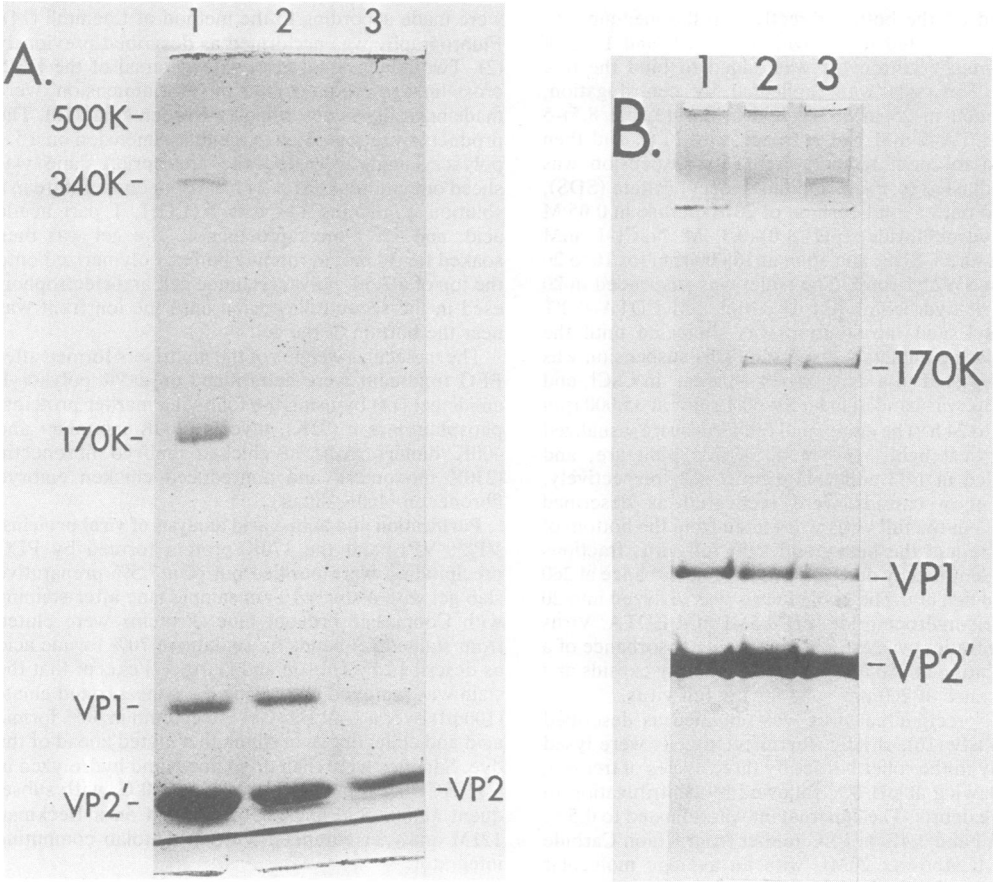

FIG. 1. Protein analysis of PEG-precipitated H-1 virus by polyacrylamide gel electrophoresis. (A) H-1 virus was isolated by PEG precipitation, and the empty capsid, heavy full virus, and light full virus were purified as described in the text. Samples were run on a 5% polyacrylamide gel and stained with Coomassie brilliant blue. Lanes: 1, empty capsid; 2, heavy full virus; 3, light full virus. (B) Cell-purified empty capsids (lane 1) were diluted to 10 μg/ml, precipitated with PEG, and rebanded in CsCl. Samples of the rebanded capsid (lane 2) and empty capsid initially purified by PEG precipitation (lane 3) were electrophoresed on a 7.5% polyacrylamide gel and stained with Coomassie brilliant blue.

light full virus, respectively, show the three H-1 capsid proteins VP1, VP2′ and VP2. Lane 1 contains empty capsid and has the two expected viral proteins, VP1 and VP2′. Of particular interest in lane 1 is the presence of three minor protein species migrating with apparent molecular weights of 170K, 340K, and 500K. The 170K protein is the predominant extra band, with progressively lesser amounts of the higher-molecular-weight species. When the lower-molecular-weight PEG-6,000 was used, the virus was efficiently precipitated but these higher-molecular-weight species were not seen. The extra associated proteins have been detected in all of the empty capsid preparations in which 15K to 20K PEG precipitation is included, but they have never been seen in either form of the full virus. That these bands are the result of treatment of the capsids with PEG is demonstrated in Fig. 1B. Cell-purified empty capsids (lane 1) were diluted to 10 μg/ml, precipitated with PEG-20,000, and banded in CsCl. This precipitated virus (lane 2) then contained the new proteins, as did the capsids which were initially purified by PEG precipitation (lane 3). These proteins were also resistant to disruption in 8 M urea at 100°C, 6 M guanidine hydrochloride, and 3 M potassium thiocyanate.

To further compare the empty-capsid preparations from the two purification procedures, as well as to demonstrate that the extra protein bands were part of the capsid structure, the following experiment was done. ^{125}I-labeled cell-purified virus and unlabeled PEG-purified virus were sedimented in a 5 to 20% sucrose gradient. Figure 2 shows the elution profile of

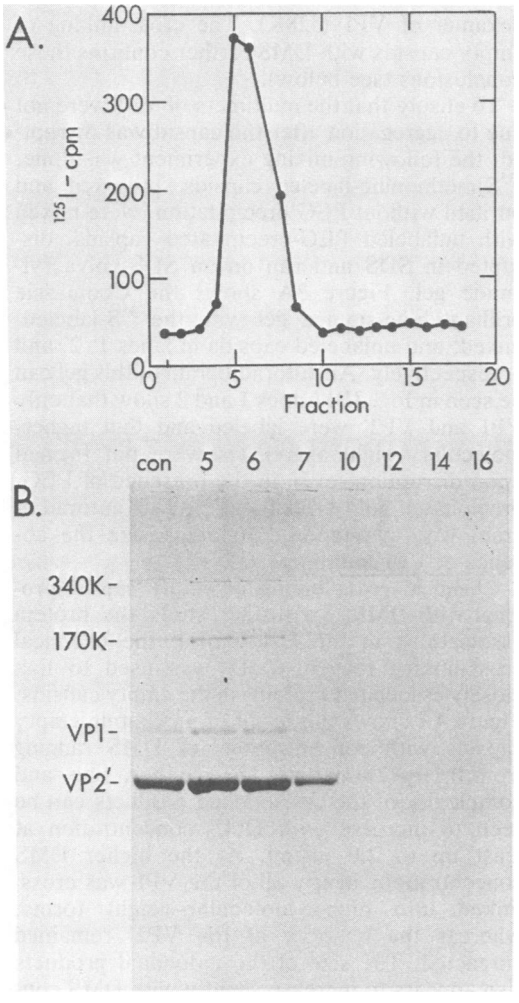

FIG. 2. Sucrose gradient and polyacrylamide gel analysis of PEG-precipitated empty capsids. PEG-precipitated empty capsid (150 μg) was made to 1% in SDS and run on a linear 5 to 20% sucrose gradient (4 ml) containing 10 mM Tris-hydrochloride (pH 8), 1 mM EDTA, 0.1 M NaCl, and 0.2% SDS in an SW60.1 rotor at 20°C and 45,000 rpm for 85 min. In an adjacent tube, ^{125}I-labeled cell-purified capsid (2,000 cpm) was spun under the same conditions. Both gradients were eluted from the bottom, and 0.25-ml fractions were collected. (A) Aliquots (150 μl) of the ^{125}I-labeled gradient fractions were counted and plotted. (B) Samples containing the PEG-precipitated capsid were run on a 7.5% polyacrylamide gel which was stained with Coomassie brilliant blue. A sample of the original material (con) was also run.

the ^{125}I-labeled material from this gradient (A) and the protein content of the various indicated fractions (B). The PEG-precipitated virus cosedimented with the ^{125}I-labeled cell-purified virus as the monomer 70S capsid. Also, the extra protein bands in the PEG-precipitated virus cosedimented with the capsid, indicating that they are part of this structure. The resolving power of this gel separated only the 170K protein, but the other two species can be seen at the top of the separating gel. It should also be emphasized that this sucrose gradient was run in the presence of 1% SDS, conditions under which most protein-protein interactions are destroyed but the H-1 capsid is stable.

Origin of the high-molecular-weight capsid proteins. The ability to form these high-molecular-weight species by treatment of purified empty capsids with PEG suggested that these proteins were derived from VP1 or VP2′ (or both) after the capsid was formed. Peptide mapping of the 170K protein by the method of Cleveland et al. (4) (data not shown) indicated that it was of viral origin, but due to the sequence homology between VP2′ and VP1, a definitive assignment of this protein as a multimer of either VP2′ or VP1 could not be made. Also, VP2′, VP1, and the 170K protein were purified from a preparative SDS gel and subjected to amino acid analysis. The compositions of these three proteins are very similar (Table 1), and it is clear that the 170K protein is derived from the viral capsid proteins. The major difference in composition between VP2′ and VP1 is the higher lysine content of the latter, which is consistent with the findings that VP1 is more basis (as determined by isoelectric focusing) than VP2′ (17; P. R.

TABLE 1. Amino acid composition of VP2′, VP1, and the 170K protein[a]

Amino acid	Amt (mol %) in:		
	VP2′	VP1	170K protein
Asx	14.02	12.93	13.00
Thr	9.83	8.61	7.79
Ser	6.46	6.64	6.32
Glx	10.28	10.12	9.68
Pro	6.38	6.92	6.91
Gly	9.12	9.46	10.22
Ala	8.84	10.06	9.71
Val	4.12	4.26	4.10
Met	1.67	1.54	1.36
Ile	4.10	3.68	3.53
Leu	6.19	6.24	6.23
Tyr	4.01	3.74	4.35
Phe	4.87	4.54	4.91
His	2.91	2.87	2.81
Lys	3.45	5.37	5.51
Arg	3.77	3.01	3.57

[a] VP2′, VP1, and the 170K protein were purified from a preparative SDS-polyacrylamide gel and subjected to amino acid analysis as described in the text. The values for cysteine and tryptophan were not determined and were not included in the mole precent calculations. The compositions of VP2′ and VP1 have been presented previously (19).

Paradiso, unpublished data). The 170K protein is similar to VP1 in this respect, which suggests that the 170K protein is a dimer of VP1.

Several other lines of evidence suggest that the high-molecular-weight proteins formed by PEG treatment represent multimers of VP1. The first is that the quantity of VP1 is markedly reduced after PEG treatment. By scanning negatives of SDS gels stained with Coomassie brilliant blue, I have determined that in the empty capsid VP1 comprises 16.3 ± 2.3% of the total protein (VP1 plus VP2'). However, in the PEG-precipitated capsid the VP1 is reduced to less than 10% of the sum of VP1 and VP2'. The second line of evidence is that the apparent molecular weights of the two smaller species (170K and 340K) are close to those expected for a dimer (176K) and tetramer (352K) of VP1. The size of the largest protein species is difficult to determine since there are few available marker proteins in this region. By using myosin (200K) and chicken embryo fibronectin in the reduced (230K) and unreduced (460K) forms as markers, the largest protein species appears to be around 500K. It is possible that this species represents a hexamer of VP1 (528K). The cross-linking of empty capsids with DMS further confirms these conclusions (see below).

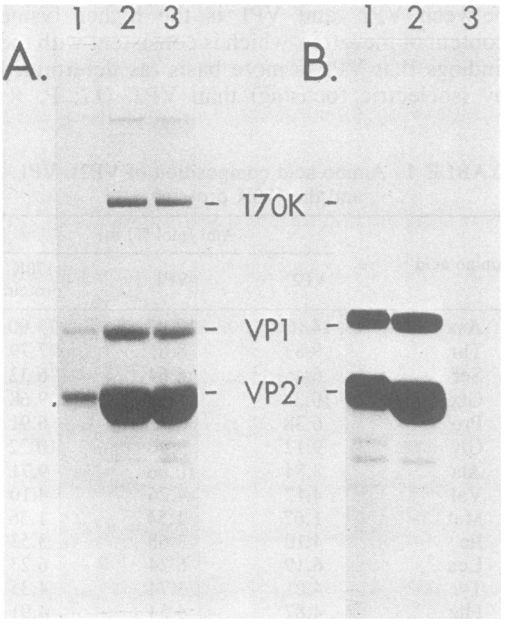

FIG. 3. Mixing of cell-purified and PEG-precipitated empty capsids. The following samples were run on a 7.5% polyacrylamide gel: ^{35}S-labeled cell-purified empty capsids (50,000 cpm) (lane 1); ^{35}S-labeled cell-purified empty capsid plus unlabeled PEG-precipitated empty capsids (15 μg), which were mixed before disruption (lane 2); and unlabeled PEG-precipitated empty capsids (15 μg) (lane 3). The gel was stained with Coomassie brilliant blue (A) and analyzed by fluorography (B).

To ensure that the multimers of VP1 were not due to aggregation after the capsid was disrupted, the following mixing experiment was done. [^{35}S]methionine-labeled capsids, prepared and purified without PEG precipitation, were mixed with unlabeled PEG-precipitated capsids, disrupted in SDS and run on an SDS-polyacrylamide gel. Figure 3A shows the Coomassie brilliant blue-stained gel with the ^{35}S-labeled, mixed, and unlabeled capsids in lanes 1, 2, and 3, respectively. An autoradiogram of this gel can be seen in Fig. 3B. Lanes 1 and 2 show that only VP1 and VP2' were labeled and that higher-molecular-weight aggregates were not formed upon disruption, even in the presence of PEG-precipitated cold virus (lane 2). This autoradiogram was overexposed to emphasize the absence of VP1 multimers.

Chemical cross-linking of empty capsid proteins with DMS. To further study the protein associations in the H-1 capsid, the chemical cross-linking reagent DMS was used to link closely associated proteins in the empty capsids. Figure 4A shows the result of incubating empty capsids with concentrations of DMS ranging from 0.1 to 2.0 mg/ml. The number, size, and complexity of the cross-linked products can be seen to increase with DMS concentration at least up to 2.0 mg/ml. At the higher DMS concentrations nearly all of the VP1 was cross-linked into higher-molecular-weight forms, whereas the majority of the VP2' remained unreacted. The size of the individual products also appears to increase slightly with DMS concentration, apparently due either to increasing amounts of bound DMS or perhaps to internal cross-linking.

The first set of cross-linked products was a triplet of bands of 135K, 155K, and 170K, which are around the size expected for dimeric forms of the capsid proteins. Figure 4B shows the cross-linking of PEG-treated capsids; this gave a very similar pattern except that the 170K protein was enriched at the lower DMS concentrations. The 170K protein formed after PEG treatment corresponds in size to the highest-molecular-weight dimer in Fig. 4A. This is particularly evident in Fig. 4B, where one would expect a doublet of around 170K if the DMS-induced protein were significantly different in size from the PEG-induced dimer.

The molecular weights of the dimers correspond well to those predicted for a VP2' dimer, a VP2'-VP1 mixed dimer, and a VP1 dimer. To confirm these assignments, the cross-linked products from DMS at 0.2 and 2.0 mg/ml were analyzed by two-dimensional gel electrophoresis

with reversal of the cross-linkage after the first dimension. The two different DMS concentrations were used because there was generally a greater amount of the 170K dimer at the lower concentration and a greater amount of the 135K dimer at high concentrations (Fig. 4A). Both VP1 and VP2′ were released from the 155K protein band, and only VP1 was released from the 170K protein band (Fig. 5A). Figure 5B demonstrates further that the 135K protein is composed solely of VP2′. Thus, all of the assignments match those predicted by the molecular weights. The efficiency of reversal after the first dimension was never 100%. When similar experiments were done using dimethyl 3,3′-dithiobis-propionimidate as the cross-linker and 2-mercaptoethanol as the reversing agent, the patterns were the same and the reversal was no more efficient. The reason for this is not known, but it may reflect a resistance similar to the aggregation effect seen with PEG treatment. However, since only two proteins are available for cross-linkage, the assignments are straightforward and match those predicted by the molecular weights. These results also provide further evidence that the 170K protein formed with PEG is a dimer of VP1.

The VP1 dimer formed early and was cross-linked into higher forms at the higher DMS concentrations. The VP1-VP2′ mixed dimer was the most abundant dimeric species formed at all of the DMS concentrations used. This species also appears to be further cross-linked, but a large amount remains even at the higher DMS concentrations. The amount of VP2′ dimer increased with DMS concentration but never

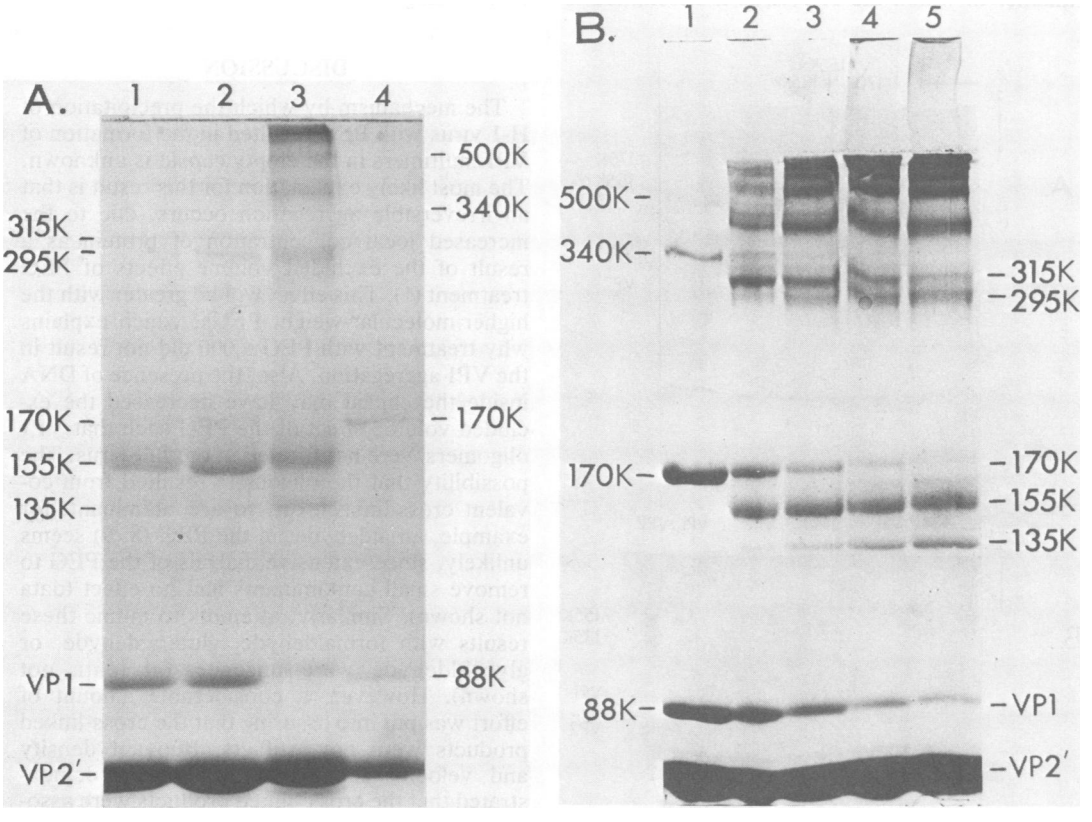

FIG. 4. Cross-linking of empty capsid protein with DMS. (A) Empty capsids (30 μg) were cross-linked with various amounts of DMS at room temperature for 2 h, and the products were analyzed by polyacrylamide gel electrophoresis (5%). Lanes 1–3 correspond to DMS concentrations of 0.1, 0.2, and 2.0 mg/ml, respectively. (B) PEG-precipitated empty capsids were cross-linked as in panel A, with lanes 2–5 corresponding to DMS concentrations of 0.2, 0.5, 1.0, and 2.0 mg/ml, respectively. The molecular weights in the right margin of panel A and the left margin of panel B correspond to the positions of the proteins in the PEG-precipitated virus, which were run in lane 4 of panel A and lane 1 of panel B for comparison. The numbers in the opposite margins correspond to the calculated molecular weights of the products of the DMS cross-linking. The gels were stained with Coomassie brilliant blue.

came near the concentration of the mixed dimer. Longer incubations of the capsid at a higher DMS concentration never resulted in a significant increase in the VP2′ dimer, and the majority of VP2′ always remained un-cross-linked.

Significant levels of cross-linked products in the expected size range of trimers of VP2′ and VP1 (200K to 260K) were never found. Instead, the second set of cross-linked products was in the range of 290K to 340K, the size class that corresponds to tetrameric forms of the capsid proteins. Due to the loss of accuracy in determining the exact size of the larger cross-linked products and also because of the increase in the various possible protein combinations, I did not attempt to determine the exact composition of these larger species. However, it is apparent that large complexes are formed, including those which are unable to enter the 5% gel.

Treatment of full virus particles with DMS. The manner in which the H-1 virus is assembled is unknown, but it is clear that the protein structure of the capsid is altered in the full, DNA-containing virus as compared with the empty capsids. To probe the changes in the basic protein-protein interactions of the full virus, I treated it with DMS as was done with the empty capsids. Since the H-1 virus derived from NB cells contains predominantly VP2′ (Fig. 1, lanes 2 and 3) (12, 16), this virus was used because it most closely resembled the protein content of the empty capsids. However, incubation of full virus with various concentrations of DMS (0.1 to 4.0 mg/ml) did not result in any detectable levels of cross-linking (data not shown). To be sure that the cross-linking was not being inhibited by a component of the full virus preparation, mixing experiments were performed with empty and full virus. Cross-linking of empty-capsid protein was never inhibited by the presence of the full virus, indicating that the latter is resistant to cross-linkage.

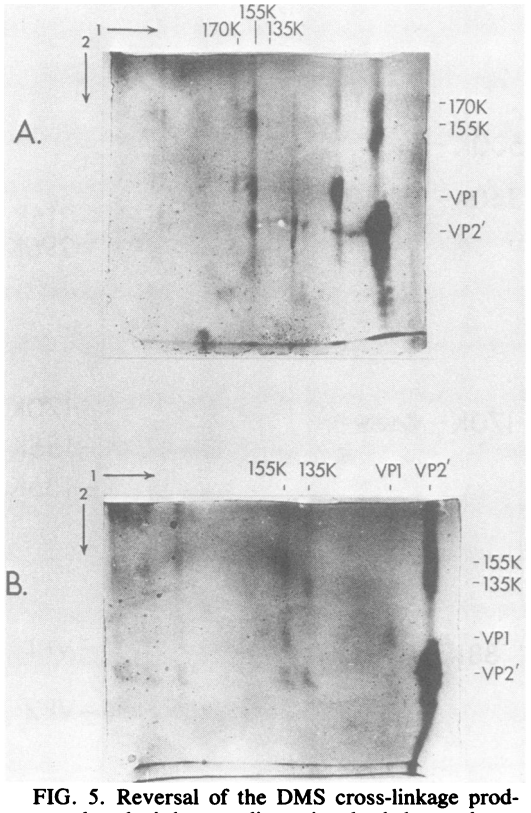

FIG. 5. Reversal of the DMS cross-linkage products and analysis by two-dimensional gel electrophoresis. Empty capsid protein (40 μg) was cross-linked with DMS at 0.2 mg/ml (A) or 2.0 mg/ml (B) and run on a 5% polyacrylamide slab gel in the first dimension. The appropriate lanes were cut out, the cross-linkages were reversed, and the second-dimension 7.5% gel was run as described in the text. The gels were stained with Coomassie brilliant blue.

DISCUSSION

The mechanism by which the precipitation of H-1 virus with PEG resulted in the formation of VP1 multimers in the empty capsid is unknown. The most likely explanation for this result is that an irreversible aggregation occurs, due to the increased local concentration of protein as a result of the excluded volume effects of PEG treatment (1). This effect will be greater with the higher-molecular-weight PEGs, which explains why treatment with PEG-6,000 did not result in the VP1 aggregation. Also, the presence of DNA inside the capsid may have decreased the excluded volume effect of the PEG such that VP1 oligomers were not formed in the full virus. The possibility that the oligomers resulted from covalent cross-linkage due to a contaminant, for example, an aldehyde, in the PEG (8, 9) seems unlikely, since extensive dialysis of the PEG to remove small contaminants had no effect (data not shown). Similarly, attempts to mimic these results with formaldehyde, glutaraldehyde, or glycoaldehyde were unsuccessful (data not shown). However, a considerable amount of effort was put into assuring that the cross-linked products were not artifacts. Buoyant density and velocity sedimentation analyses demonstrated that the cross-linked products were associated with the empty capsid structure and with monomer capsids rather than with aggregates. The mixing experiments with the ^{35}S-labeled capsids (Fig. 3) showed that the multimers of VP1 were not the result of a specific aggregation of the VP1 after the virus was disrupted. This was also indicated by the lack of cross-linkage in the full virus which was precipitated simultaneously. Thus, although the exact mechanism of

oligomer formation is not known, it is clear that the VP1 molecules in the capsid must be closely associated to permit such aggregation.

The cross-linking experiments with DMS confirmed that the multimers formed by PEG treatment were oligomeric forms of VP1. However, with DMS more of the VP1 was cross-linked into a mixed dimer than into a homologous VP1 dimer. This indicates that whereas VP1 molecules may be closely associated in the capsid, the VP1 molecules are also in contact with VP2'. Given the abundance of VP2', this result is not surprising. More surprising is the fact that the VP2' did not cross-link more readily to itself, and, in fact, the majority of the VP2' remained as monomers even at the highest concentrations of DMS. The strength and stability of the capsid suggest that there are very strong protein-protein interactions which must include VP2'-VP2' interactions. However, these interactions are not very susceptible to cross-linking with DMS and perhaps involve highly hydrophobic regions of the molecules such that the required amino groups are not available for cross-linking. Neither the PEG-induced aggregation nor the DMS cross-linking resulted in the formation of trimeric products. This might suggest that there is some form of dimer clustering in the capsid such that only multimers of closely interacting dimers are obtained.

The capsid structure of the parvoviruses is different in the empty compared with the full virus particle. This is most clearly demonstrated by the different susceptibilities of the capsid proteins to proteolytic cleavage. The H-1 empty capsids are extremely resistant to a wide range of proteases, including trypsin, chymotrypsin (13), pepsin at pH 3, and proteinase K in the presence of 1% SDS (P. R. Paradiso, unpublished data). In the full virus, while the core capsid structure is still quite resistant to proteases, VP2' is readily cleaved to VP2. Thus, whereas the overall protein content of the full virus is similar, especially in the case of H-1 virus propagated in NB cells where there is very little VP2 (12, 16), the presence of DNA clearly alters the protein structure. Similarly, the full virus can exist in two forms with distinct densities with little change in the protein content (16). My inability to cross-link the proteins in the full virus under the same conditions used to link the empty capsid proteins again points up the considerable differences in the structure of the capsid in these two forms. It is possible that the presence of DNA has so completely changed the structure of the capsid that none of the specific protein-protein interactions which resulted in covalent linkage in the empty capsid exist in the full virus. However, if this were true, one might expect an alteration in the cross-linking pattern rather than a complete lack of cross-linked products. Since the majority of the cross-linkages in the empty capsid involved VP1, it is possible that the DNA in the full virus interacts strongly with the VP1 in such a way that the close contacts which result in the cross-linkages have been disrupted or blocked. In fact, Tattersall et al. (23) have previously suggested that the very basic region of VP1, which is absent from VP2', may be located internally and interact with the DNA in full particles. Such a basic region would also be most susceptible to cross-linking with reagents such as DMS or aldehydes.

As indicated above, details of the icosahedral structure of the parvoviruses are not known. Several reports analyzing electron microscopic data have suggested a pentagonal dodecahedron containing 32 capsomeres (11, 25), whereas another suggested a polyhedron composed of 12 subunits (15). Based on the ratio of VP1 to VP2', the molecular weights of the proteins, and the total protein content of the virus, Kongsvik et al. (12) have suggested an H-1 capsid structure containing 12 pentamers of VP2' (60 VP2' molecules) with a molecule of VP1 situated at the vertex of each pentamer (12 VP1 molecules). Tattersall et al. (23) have suggested that the total number of protein molecules in minute virus of mice is close to 60, which might be arranged as equivalent units on the surface of a sphere. This might imply that the structurally identical regions of VP1 and VP2' would interact equivalently in constructing the capsid (3). My data indicate that VP1 is clustered in the capsid, and it is tempting to speculate that one or two morphological units in the capsid are composed solely of VP1. For example, an arrangement of the capsid into 12 pentameric structural units (3), two of which are composed of VP1, could predict a structure containing 10 molecules of VP1 and 50 molecules of VP2'. Alternatively, a structure containing 72 monomers, such as that predicted by Kongsvik et al. (12), could have two pentamers of VP1 with a molecule of VP1 at the apex of each pentamer (12 molecules of VP1) and 10 pentamers of VP2' with a molecule of VP2' at the apex of each of these pentamers (60 molecules of VP2'). In either case, the basic structure would contain 12 morphological units produced by a clustering of pentamers as predicted by Caspar and Klug (3) for the simplest icosahedron. The clustering of VP1 into a pentameric unit permits close interaction between VP1 molecules as well as with VP2' molecules from adjacent pentamers (6) and thus conforms to the data I have presented.

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