Identification of Multiple Forms of the Noncapsid Parvovirus Protein NCVP1 in H-1 Parvovirus-Infected Cells

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Analysis of extracts of H-1 parvovirus-infected cells with virus-specific antiserum led to the identification of two forms of the noncapsid protein NCVP1. These two proteins had apparent molecular weights of 84,000 (NCVP1) and 92,000 (NCVP1') and were structurally related, based on their immunological reactivity and on peptide map analysis. Both of these proteins appeared early in the virus infection, about the same time that capsid proteins appeared. NCVP1' was a highly phosphorylated protein which was apparently derived from NCVP1 via a post-translational event. Phosphoserine was the predominant phosphorylated amino acid in NCVP1' and appeared to be localized in one site or a few sites on the protein. The possible involvement of these noncapsid proteins in parvovirus DNA replication is discussed.

The H-1 parvovirus is a member of the family of autonomously replicating parvoviruses. These small, single-stranded DNA-containing viruses have been identified in a wide variety of species, including humans (10, 21). These viruses are of particular interest because of their nearly complete dependence upon the replication system of the host cell for their DNA synthesis.

The H-1 parvovirus genome (5,176 nucleotides) contains two large open reading frames which encode the noncapsid virus protein NCVP1 and the two capsid proteins VP1 and VP2' (20). The capsid protein genes are in the 5' half of the viral sequences and are largely homologous, differing in their RNA splicing patterns, the point of initiation, or perhaps both. NCVP1 has thus far only been identified by translation of H-1 mRNA. It is encoded in the 3' half of the genome and has an apparent molecular weight of 84,000 (84K) on polyacrylamide gels but 76K on the basis of the amino acid composition predicted by the DNA sequence.

The discovery of a noncapsid virus protein is consistent with results of studies of deletion mutants and defective-interfering particles of H-1 which identified a *trans*-acting replicative-form (RF) *rep* gene product (16-18). Because the parvovirus genome encodes only the two capsid proteins and NCVP1, it seems likely that NCVP1 is the RF *rep* gene product. Further support for the noncapsid nature of the RF *rep* protein has recently been supplied by Merchlinsky et al. (9), who reported that a deletion in the capsid protein gene of a related parvovirus, minute virus of mice, did not inhibit RF DNA replication. Although the function of the RF *rep* protein is not known, several possibilities have been suggested. The RF *rep* protein could function as the protein covalently bound to the 5' end of both strands of the RF DNA molecule (14). It could also be the predicted site-specific nickase(s) which appears to be required at various stages of the RF DNA replication cycle (20). It is also possible that both of these functions are provided by the same protein.

In the present study I sought to identify and study NCVP1 in cells infected with H-1. My findings indicate that there are two cellular forms of NCVP1 with apparent molecular weights of 84K and 92K. The 92K protein is a highly

phosphorylated, post-translationally modified form of NCVP1.

MATERIALS AND METHODS

Preparation and immunoprecipitation of cell lysates. Simian virus 40-transformed human newborn kidney (NB) cells were grown to confluency and infected with H-1 at a multiplicity of ca. 100 PFU per cell. Human diploid fibroblast (MRC-5) cells were infected similarly except that they were only 50 to 75% confluent at the time of infection. The temperature-sensitive mutant of H-1, *ts2*, was kindly supplied by Solon L. Rhode III. The cells were labeled with either [³⁵S]methionine at 20 μ Ci/ml in methionine-poor medium (Flow Laboratories, Inc.) or ³²P, at 50 to 200 μ Ci/ml in phosphate-free medium for 2 h at various times postinfection. For the inhibition experiments with cytosine arabinoside or aphidocolin, the drugs were added 20 min before the 2-h labeling period and maintained throughout the labeling period at 10 μ g/ml for cytosine arabinoside and 5 μ g/ml for aphidocolin. The lysates were prepared by removing the labeled media, washing the cells twice with phosphate-buffered saline, and then scraping the cells into 20 mM Tris-hydrochloride (pH 8.5)-1% Nonidet P-40-1% deoxycholate-0.2% sodium dodecyl sulfate (SDS)-0.15 M NaCl (0.15 ml per 60-mm petri dish) at room temperature. The lysates were frozen at -70°C, thawed, and centrifuged at 10,000 $\times$ g for 10 min to remove insoluble components.

The cleared lysates (up to 25 μ l) were incubated with 1 μ l of either hamster anti-H-1 serum from an animal surviving a neonatal H-1 infection or guinea pig anti-H-1 serum raised against the H-1 capsid proteins (20). After 1 h at 4°C, 10% Formalin-fixed *Staphylococcus aureus* cells (75 μ l; Pansorbin; Calbiochem-Behring) were added, pelleted, and prepared for gel electrophoresis as described previously (20).

Gel electrophoresis and autoradiography. Polyacrylamide gel electrophoresis was performed as described by Laemmli (8). Fluorography of ³⁵S-labeled gels was performed as described by Bonner and Laskey (2), and autoradiography of ³²P-labeled gels was performed with preflashed film and an intensifying screen at -70°C. The molecular weight markers used were cytochrome *c* (12.3K), β -lactalbumin (18.4K), α -chymotrypsinogen (25.7K), ovalbumin (43K), bovine serum albumin (68K), phosphorylase *b* (92.5K), and myosin (200K)

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(prestained standards from Bethesda Research Laboratories, Inc.).

Purification of NCVP1 and NCVP1'. ^{35}S - and ^{32}P -labeled proteins were purified from preparative gels. Either cleared lysates or immunoprecipitated proteins were run on a 7.5% SDS-containing gel, and the bands were localized by autoradiography of either wet or dried gels. The bands were cut out, and the gel was reswelled if necessary, homogenized in gel running buffer (8), and incubated at 37°C for 1 h. The acrylamide was removed by centrifugation at $10,000 \times g$ for 20 min. For enzyme digestion and hydrolysis, the proteins were collected by acetone precipitation with 5 volumes of acetone- NH_4OH (18:1 [vol/vol]).

Protease digestions. For digestions with *S. aureus* V8 protease (Sigma Chemical Co.), acetone precipitates were solubilized in 25 to 50 μl of 0.125 M Tris-hydrochloride (pH 6.8)–0.1% SDS–1 mM EDTA, followed by the addition of enzyme in the same buffer and incubation at 37°C for 30 min unless otherwise noted. Digestion with proteinase K (Boehringer-Mannheim) was done in 50 mM Tris-hydrochloride (pH 7.5)–0.15 M NaCl–10 mM EDTA–1% SDS–0.5 mg of proteinase K per ml at 37°C for 2 h.

Phosphoamino acid analysis. Phosphoamino acids were separated in two dimensions on thin-layer cellulose sheets. Acetone-precipitated protein was hydrolyzed in 6 N HCl in vacuo at 110°C for 90 min and spotted in the presence of 10 μg of unlabeled phosphoserine, phosphothreonine, and phosphotyrosine. The first dimension was electrophoresis at pH 1.9, followed by ascending chromatography in *n*-butanol-formic acid-isopropanol- H_2O (3:1:1:1 [vol/vol]) (6). The standards were identified with ninhydrin, and autoradiography was performed at –70°C with an intensifying screen.

RESULTS

Identification of 84K and 92K forms of NCVP1 in vivo. Lysates of ^{35}S -labeled, H-1-infected NB cells were prepared

FIG. 1. Specific immunoprecipitation of virus protein from H-1-infected NB cell lysates. NB cells were infected with H-1 and labeled with [^{35}S]methionine (20 $\mu\text{Ci}/\text{ml}$) for 2 h from 12 to 14 h postinfection. Lysates were prepared, immunoprecipitated, and run on a 7.5% gel as described in the text, except that the lysate in lane 4 was prepared in the absence of detergent. Precipitations were done with serum from a hamster surviving a neonatal H-1 infection (lane 1), guinea pig anti-capsid protein serum (lane 2), and serum from a normal hamster (lane 3). Total cell lysate was run in lanes 4 and 5, and ^{35}S -labeled H-1 empty capsid protein was run in lane 6.

FIG. 2. Comparative protease maps of NCVP1 and NCVP1'. Purified ^{35}S -NCVP1, ^{35}S -NCVP1', and ^{32}P -NCVP1' were cleaved with *S. aureus* V8 protease as described in the text, and the cleavage products were separated on a 12.5% SDS-polyacrylamide gel. Lanes 6, 2, and 5 contain ^{35}S -NCVP1' digested with 0, 0.0014, and 0.007 units of absorbance at 280 nm of protease per ml, respectively. Lanes 7, 3, and 4 contain ^{35}S -NCVP1 digested with 0, 0.0014, and 0.007 units of absorbance at 280 nm of protease per ml, respectively. Lane 1 contains ^{32}P -NCVP1' digested with 0.007 unit of absorbance at 280 nm of protease per ml. Arrows indicate two major cleavage products common to both NCVP1 (lane 4) and NCVP1' (lane 5).

and selectively immunoprecipitated with serum from a neonatally H-1-infected hamster or with guinea pig antiserum against the H-1 capsid proteins (20). The results (Fig. 1) show that the hamster serum reacts with the capsid proteins as well as with an 84K protein identical in size to NCVP1, previously characterized by *in vitro* translation of H-1 mRNA (20) but not previously identified in infected cells. The guinea pig serum (Fig. 1, lane 2) reacted only with the capsid proteins, as expected. Of particular interest in Fig. 1, lane 1, however, was the appearance of a previously unidentified 92K virus protein. This protein appeared to behave immunologically in the same manner as NCVP1, suggesting that it was a noncapsid virus protein. Because there is not room on the H-1 genome for an additional protein of this size, it seemed likely that the 84K and 92K proteins were structurally related. This was in fact confirmed by peptide mapping of purified ^{35}S -labeled protein (Fig. 2), which demonstrated primary sequence homology between these two proteins. The arrows in Fig. 2 show that two of the major peptides resulting from protease cleavage are common to both NCVP1 and NCVP1'. Because my studies with H-1 mRNA translation revealed only an 84K gene product, I assumed at this point that the larger, 92K protein represented a post-translationally modified form of NCVP1; therefore, the 92K protein will herein be referred to as NCVP1' to indicate this relationship.

Analysis of the appearance of the 84K and 92K proteins with respect to time postinfection (Fig. 3) revealed that both proteins were produced early in the infectious process. To more clearly see the NCVP1 and NCVP1' bands, I immunoprecipitated the cellular lysates with the guinea pig anti-capsid protein serum, which removed much of the VP1 and VP2' (Fig. 3A). The capsid proteins first become apparent at 9 h postinfection and are obvious by 12 h postinfection. Similarly, both NCVP1 and NCVP1' can be discerned at 9 h

FIG. 3. Time course of virus protein production in H-1-infected NB cells. H-1-infected NB cells were labeled with [^{35}S]methionine for 2 h before being harvested. The lysates were then immunoprecipitated with guinea pig anti-capsid protein serum (A) and then with hamster anti-H-1 serum (B), and the proteins were separated on a 7.5% gel. Lanes 1 through 6 correspond to lysates prepared at 6, 9, 12, 14, 18, and 24 h postinfection.

postinfection (Fig. 3B, lane 2). The production of the non-capsid proteins peaked at around 18 h postinfection, whereas the capsid proteins were being produced in ever greater amounts at least up to 24 h postinfection. The decrease in NCVP1 and NCVP1' at these later times may reflect the condition of the cells as they succumb to the viral infection and release proteolytic enzymes to which the capsid proteins are remarkably resistant (3, 11).

Phosphorylation of NCVP1 in vivo. In an attempt to probe the structural differences between NCVP1 and NCVP1', I labeled infected cells with ^{32}P , and immunoprecipitated the cellular lysates with anti-H-1 serum. As seen in Fig. 4A, NCVP1' is very strongly phosphorylated and, in fact, at 15 h postinfection is one of the most strongly phosphorylated proteins in the lysate. NCVP1 is also labeled under these conditions but to a considerably lesser degree than NCVP1'. These ^{32}P -labeled proteins had the same immunospecificity described above for the ^{35}S -labeled noncapsid virus proteins (Fig. 4B). That is, both protein forms reacted only with serum from an infected animal and not with the anti-capsid serum. The capsid proteins VP2' and VP1 were also consistently labeled with ^{32}P but to a lesser degree than NCVP1 (data not shown). The low level of phosphorylation of capsid proteins has previously been shown for the related parvovirus minute virus of mice (13) and was therefore not unexpected for H-1.

The high level of phosphorylation and the 8K increase in size from 84K (NCVP1) to 92K (NCVP1') led me to investigate the possibility that NCVP1' was an NCVP1-oligonucleotide complex perhaps formed during viral replication. Attempts at digesting NCVP1' with various endonucleases and exonucleases were greatly hindered by the insolubility of the protein in the absence of detergents. So, although I was never able to demonstrate nuclease sensitivity, there was always the concern that the substrate was not present in a suitable form for digestion. The best evidence for the absence of an oligonucleotide associated with NCVP1' resulted from complete digestion of the purified protein with protein-

ase K in the presence of SDS (Fig. 5A). When this digest was run on an 18% SDS-containing gel, no distinct band representing a residual oligonucleotide could be discerned; only a smear of ^{32}P label probably in the form of small peptides could be seen. An oligonucleotide as small as 1K would have been detected on this gel.

When purified ^{32}P -labeled NCVP1 and NCVP1' were digested with *S. aureus* V8 protease as described for the ^{35}S -labeled proteins (Fig. 2), the patterns were quite different. Digestion of ^{32}P -NCVP1 resulted in a fairly uniform distribu-

FIG. 4. ^{32}P labeling of NCVP1'. H-1-infected NB cells were labeled with 100 μCi of ^{32}P per ml from 12 to 14 h postinfection and then harvested as described in the text. (A) Total lysate was run in lane 2, and lysate immunoprecipitated with hamster anti-H-1 serum was run in lane 1. (B) ^{32}P -labeled cell lysate proteins were precipitated with normal hamster serum (lane 1), guinea pig preimmune serum (lane 2), hamster anti-H-1 serum (lane 3), and guinea pig anti-capsid protein serum (lane 4). The proteins were separated on 7.5% gels.

FIG. 5. Proteolytic cleavage of ^{32}P -NCVP1 and ^{32}P -NCVP1'. (A) Purified ^{32}P -NCVP1' was digested with either 0.5 mg of proteinase K per ml (lane 1) or 0.1 unit of absorbance at 280 nm of *S. aureus* V8 protease per ml (lane 2) at 37°C for 2 h and run on an 18% gel. I.F., Ion front which did not run off the bottom of the gel. (B) Purified ^{32}P -NCVP1' was digested with 0, 0.0014, 0.007, and 0.028 units of absorbance at 280 nm of V8 protease per ml and run in lanes 1, 3, 5, and 7, respectively. Similar digestions of ^{32}P -NCVP1 were run in lanes 2, 4, 6, and 8, respectively. Molecular weights at the right and left point out the size distribution of the ^{32}P -NCVP1' digestion products and were derived from molecular weight markers run in an adjacent lane.

tion of label into the numerous peptide products which, as expected, decreased in size as the enzyme concentration was increased (Fig. 5B, lanes 4, 6, and 8). Digestion of ^{32}P -NCVP1', on the other hand, yielded distinct subsets of products which were grouped at 70K to 90K, 45K to 55K, and 20K to 30K, depending on the concentration of enzyme used (Fig. 5B, lanes 3, 5, and 7); this trend continued (see Fig. 5A, lane 2) at higher concentrations of protease. This pattern was very reproducible and suggests a unique, localized concentration of ^{32}P on the protein rather than the more random distribution seen on NCVP1. Figure 2, lane 1, shows the digestion of ^{32}P -labeled NCVP1' under conditions similar to those used for the digestion of ^{35}S -labeled proteins and permits direct comparison of rather distinct labeling patterns.

Phosphoamino acid analysis of NCVP1'. The ^{32}P label in both NCVP1 and NCVP1' was found to be very alkali resistant. Incubation of gel-purified NCVP1' in 0.1 M

NH_4OH , NaOH , or KOH at either 55°C for 90 min or 70°C for 30 min had no effect on the protein label after the mixture was neutralized and run on an SDS-polyacrylamide gel. Higher concentrations of base resulted in hydrolysis of the protein. However, when dried gels were reswelled and alkali treated with 1.0 M KOH at 55°C for 1 h as described previously (4), the label in both NCVP1 and NCVP1' was essentially unaffected. Similarly, the label in the proteolytic cleavage products of NCVP1' (Fig. 5B) was also unaffected.

Phosphoamino acid analysis of NCVP1' revealed that phosphoserine was the most prominent amino acid (Fig. 6). A small amount of phosphothreonine was also seen, but phosphotyrosine was never detected. In all of the hydrolysates, there was substantial free ^{32}P , about equal to the phosphoserine content. The label which migrated just to the cathode side of the origin probably represents incomplete hydrolysis because it could be eliminated with longer hydrolysis times. The predominance of phosphoserine was somewhat surprising because of the demonstrated alkali resistance of the labeled protein. Although this phenomenon is not unprecedented (7), the cause is unknown.

NCVP1' as a function of RF DNA replication. The presence of NCVP1 and NCVP1' in cell lysates was analyzed in the presence of inhibitors of DNA synthesis. In the presence of either cytosine arabinoside or aphidocolin and under conditions which reduced RF DNA to around 10% of the control (S. Rhode, personal communication), the relative distribution of noncapsid protein shifted in the direction of NCVP1. Whereas the synthesis of capsid proteins was not affected by treatment with either inhibitor at 11 h postinfection and 20 min before the 2-h pulse, the amount of NCVP1 appeared to increase relative to the amount of NCVP1' (Fig. 7A). These experiments suggest that the inhibition of DNA synthesis might inhibit the conversion of NCVP1 to NCVP1'.

To look more specifically at the effects of inhibition of viral DNA synthesis, I took advantage of two other infectious processes involving the H-1 virus. The first system involved the use of the temperature-sensitive mutant of H-1, *ts2*, which is defective in progeny DNA synthesis at the restrictive temperature but which replicates RF DNA normally (15). Fig. 7B shows that in *ts2*-infected NB cells, phosphorylation of both NCVP1 and NCVP1' is the same as in wild-type infection; this was most clearly seen when the two lysates were mixed (Fig. 7B, lane 2). The second system involved the abortive infection of MRC-5 cells with wild-type H-1. In this infection, parental RF DNA synthesis and subsequent capsid virus protein synthesis occur, but RF DNA synthesis is at most 1% of normal, and no infectious

FIG. 6. Phosphoamino acid analysis of ^{32}P -NCVP1'. Purified ^{32}P -NCVP1' was hydrolyzed in 6 N HCl , and the phosphoamino acids were separated in two dimensions. The small circle refers to the origin. p-tyr, Phosphotyrosine; p-thr, phosphothreonine; p-ser, phosphoserine. P^- , Free ^{32}P .

FIG. 7. Effect of DNA synthesis on NCVP1 and NCVP1'. (A) H-1-infected NB cells were treated with 5 μ g/ml of aphidocolin per ml (lane 1), 10 μ g of cytosine arabinoside per ml (lane 3), or no drug (lane 2) for 20 min at 11 h postinfection and then for an additional 2 h in the presence of 50 μ Ci of 32 P per ml. The lysates were prepared and precipitated with hamster anti-H-1 serum. (B) NB cells were infected with either wild-type H-1 or *ts2* at the restrictive temperature (40°C) and labeled with 32 P (150 μ Ci/ml) from 13 to 15 h postinfection. Lysates were immunoprecipitated as described in (A). Lanes: 1, *ts2* lysate; 2, equal mixture of *ts2* and wild-type lysates; 3, wild-type lysate.

virus is formed (22). When infected MRC-5 cells were labeled with [35 S]methionine, both NCVP1 and NCVP1' were formed, and the time course of their appearance was similar to that for infection of NB cells. Similarly, both of these protein species were phosphorylated, as in NB cells (data not shown). Thus, specific inhibition of viral RF or progeny DNA does not affect the production or processing of NCVP1.

DISCUSSION

The autonomous parvoviruses are unique in their nearly complete dependence on the replication system of the host cell, having only two known functional genes. The capsid protein genes, located in the 5' half of the viral genome (20), encodes two highly homologous proteins, probably by independent transcriptional events but nonetheless by using essentially the same coding regions. The second gene,

located in the 3' half of the viral genome, encodes the 84K noncapsid protein NCVP1. Previous studies with temperature-sensitive (15) and deletion (16–18) mutants of H-1 led to the discovery of a *trans*-acting noncapsid viral gene product which was required for RF DNA replication (RF *rep* protein). The only other protein involved in RF DNA replication that might be of viral origin is the protein covalently bound to the 5' end of both strands of the RF DNA molecule (14). It is possible that the 5' covalently bound protein and the RF *rep* protein are the same. The identification of NCVP1 leads therefore to the speculation that this protein might function as the 5' covalently bound protein or the RF *rep* protein or both. Noncapsid proteins which are probably homologous to NCVP1 have also been detected in other parvovirus systems, including Aleutian disease virus (1), canine parvovirus (12), and minute virus of mice (5).

In the present study, I have shown that NCVP1 is present in H-1-infected cells and that an apparently altered form, NCVP1', is also present. The time course of the appearance of these two proteins appears to parallel that of the capsid proteins, and as for the capsid proteins, it seems likely that NCVP1 mRNA is synthesized from parental RF DNA (19). This is also consistent with the finding that NCVP1 and NCVP1' are produced during the abortive infection of MRC-5 cells in the absence of RF DNA replication. In the wild-type infection, after the formation of the parental RF DNA molecule and the production of virus proteins, daughter RF DNA synthesis begins, and the monomer and dimer RF DNAs accumulate in the nucleus. The RF *rep* protein is required for this step, and if NCVP1 serves this *rep* function, modification to NCVP1' may also be required. The fact that NCVP1' is formed normally during the abortive infection of MRC-5 cells indicates that a blockage in the formation or modification of NCVP1 is not the cause of restriction in these cells. However, it is still possible that the formation of NCVP1' is linked to RF DNA synthesis. The blockage of RF DNA synthesis in MRC-5 cells may occur after initiation and NCVP1' formation. The experiments with inhibitors of DNA synthesis suggest a possible link between NCVP1 modification and DNA synthesis. However, this link may be nonspecific in nature, as in the loss of a cellular function required for NCVP1 phosphorylation. Clearly, more information on the function of NCVP1 is required.

The exact nature of the post-translational modification responsible for the 8K increase in the size of NCVP1 is not known, but it seems likely to be related to the high level of

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1 - MAGNAYSDV LGVINWLKDK SSQEVFSFVF KNEINVQLNGK DIGWNSYRKE LQDELKSLQ RGAETITWQDS EDMWESEAVD DMITKKQVFI
91 - DSLVKKCLFE VLSTKNIAP NVITWFVQHEW GKDQGWCHV LIGGKDFSQP QGKWWRRQLN VYWSRWLVTA CNVQLTPAER IKLREIAEDS
181 - EWVTLLITYKH KHTKKDYTKC VLFGNMIAAY FLSSKKICTS PPRDGGYFLS SDSGWKTDFN LKEGERHLVS KLYTFMKPET VETITVITAQE
271 - AKRGRIQTRE EVSIKTTILKE LVHKRVTSP EMMMQPDSY IEMMAQPGGE NLLKNTLEIC TLTARTKTA FDLILEKAET SKLANFSMAS
361 - TRICRIFAEH GWNYIKVCHA ICCVLNRQGG KRNIVLFHGP ASTGKSIIAQ ALAQAVGNVG CYNAANVNFP FNDCTNKNLI WVEEAGNFGQ
451 - QVNQFKAICS GQTRIDQKG KGSKQIEPTP VIMTINENIT WRIGCEERP EHTQPIRDM INIHLTRITLP GDFGLVDKHE WPLICAWLVK
541 - NGYQSTIMACY CAKWGKVPDW SEDWAEPKLD TPINSLGSMR SPSTLTPRSTP LSQNYALTPL ASDLADIALE PWSTPNTFVA GTAASTQNTGE
631 - AGSTACQGAQ RSPITWSEIEA DLRACFSQEQ LESDFNEELT LD

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FIG. 8. Primary sequence of NCVP1. The protein sequence of NCVP1 was derived from the nucleotide sequence starting at nucleotide 264 and terminating at nucleotide 2355 as previously predicted (20). The serine residues, which are the predominant site of phosphorylation, are highlighted with dots. The greatest concentration of serines is between residues 575 and 663 (14 serines), but other smaller areas of concentration can also be seen (e.g., between residues 220 and 233).

protein phosphorylation which is apparent in NCVp1'. The phosphorylation of NCVp1 has likely resulted in a considerable alteration in the conformation of the protein, evidenced by a retarded migration during SDS gel electrophoresis, which is not in proportion to the increase in protein mass. The apparent localization of the charged phosphate groups to one region or a few regions on the protein would tend to accentuate the charge effects resulting from this phosphorylation. The predicted primary sequence of NCVp1 is shown in Fig. 8 (20). On the basis of this sequence, the protein is 76K, somewhat smaller than the 84K estimate from gel electrophoresis. The protein contains 45 serines (6.7%), which are well distributed throughout the protein, except for a concentration at the carboxy terminus (14 of the last 100 residues). A concentration of phosphate groups in this region would be compatible with the data I have presented here. However, it should be pointed out that the possibility that an additional post-translational event is occurring and contributing to the conversion of NCVp1 to NCVp1' cannot be eliminated.

Replication of a single-stranded parvovirus requires some unique functions, most of which are supplied by the replication machinery of the cell. However, certain functions are virus specific and facilitate the integration of this process into the replication system of the cell. Further elucidation of the function of NCVp1 will hopefully help to define these viral parameters and the required cellular adaptations.

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