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# Chemical Cleavage of Polyomavirus Major Structural Protein VP1: Identification of Cleavage Products and Evidence That the Receptor Moiety Resides in the Carboxy-Terminal Region†

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As a first step toward identifying the various functional regions of the polyomavirus major capsid protein VP1, we used recently developed methods for the chemical cleavage of proteins and the available polyomavirus sequence data to devise a scheme to produce large, identifiable peptides and generate a cleavage map of VP1. Formic acid (75%) was found to cleave VP1 at only two sites, producing three peptides of apparent molecular weights of 29,000, 16,000, and 2,000. The order of peptides in intact VP1 was determined by recleavage of partial products and was found to be 29,000, 16,000, and 2,000. Two-dimensional peptide mapping studies of $^{125}$I-labeled VP1 formic acid peptides established that the limit products of formic acid digestion contained mutually exclusive sets of labeled peptides when either trypsin or chymotrypsin was used and that together the formic acid peptides contained all of the $^{125}$I-labeled tryptic and chymotryptic peptides found in VP1. Iodosobenzoic acid (IBA) digestion produced four peptides separable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with apparent molecular weights of 12,000, 8,000, 7,000, and 5,000. The approximate positions of the IBA peptides in the VP1 sequence were determined by cleavage of formic acid fragments with IBA. The number of peptides produced, their respective sizes, and their order in the intact VP1 molecule agree with predictions made from available sequence data, both for formic acid cleavage and IBA cleavage. In addition, the numbers of $^{125}$I-labeled tryptic peptides produced from digestion of VP1 formic acid peptides also agree with predictions made from the sequence information. These data establish with reasonable certainty that the peptides produced by formic acid cleavage and IBA cleavage of VP1 are indeed those predicted. Antibodies raised against spontaneously produced, previously undefined polypeptides resulting from degradation of VP1 reacted exclusively with the formic acid peptides derived from the C-terminal portion of VP1. These antibodies inhibited hemagglutination and neutralized polyomavirus virions. We interpret this to mean that at least some of the antigenic determinants of the receptor moiety reside in this portion of the VP1 sequence.

The major structural protein of polyomavirus, VP1 (molecular weight, 42,500), forms the highly cross-linked capsid shell protecting the nucleoprotein core of the virion (9, 10, 15, 18, 30). In addition to forming the capsid, VP1 appears to perform other functions during the course of the viral life cycle. Brady et al. (10) have shown that VP1 is stably associated with nucleoprotein complexes released by in vitro dissociation of polyomavirus virions, and it has been suggested that VP1 may by involved in the regulation of transcriptional activity in simian virus 40 (6–8). The most clearly defined nonstructural role for VP1, however, is its responsibility for cell binding. VP1 possesses the related activities of hemagglutinin and a specific receptor (3, 30).

Isoelectric focusing of disrupted polyomavirus virions resolves at least six isospecies, which have been designated A through F (3, 19, 20). Earlier work performed in our laboratory suggested that the phosphorylated species D, E, and F, which together comprise only about 12 to 15% of the total VP1 present in polyomavirus virions, perform the functions of viral hemagglutinin and receptor (3). Two lines of evidence support this contention. First, highly purified empty capsids fail to bind specifically to primary mouse kidney cells; these empty capsids also do not compete with intact virions for specific binding sites on mouse kidney cells (4). The purified, empty capsids were found to lack a

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major portion of the phosphorylated species. Second, when empty capsids were used to affinity fractionate hemagglutination-inhibiting and neutralizing antibodies, antibodies eluting from the column without binding to empty capsids were found to bind to virions, to neutralize infectivity but not to inhibit hemagglutination, and to preferentially immunoprecipitate phosphorylated species of VP1 (5). Interestingly, antibodies which were specifically bound to capsids in these studies were found to have hemagglutination-inhibiting activity but to be incapable of neutralizing viral infectivity. Both populations of antibodies were specific for VP1. These data suggested that the antibodies were capable of distinguishing different conformational states of VP1 and that posttranslational modification of VP1 by phosphorylation correlated with these apparent conformational alterations.

We wish to further investigate the apparent relationship between the phosphorylation and cell-binding functions of polyomavirus VP1. One approach in studying this question is to physically map the functional sites within the VP1 sequence. We have taken advantage of the facts that the genome of polyomavirus has been sequenced and that the amino acid sequences of the proteins have been predicted (12, 28) to devise a scheme of chemical cleavage which produces large fragments of VP1 which are separable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and are easily ordered. In this report, we describe the identification of peptides produced by the cleavage agents formic acid and iodosobenzoic acid (IBA), which we found to be those predicted from the sequence data, and present evidence that at least some of the determinants responsible for generating neutralizing antibodies lie within the carboxy-terminal portion of the VP1 sequence.

## MATERIALS AND METHODS

**Cells and virus propagation.** Polyomavirus used for these studies was grown in primary cultures of baby mouse kidney cells on 100-mm dishes. The preparation of baby mouse kidney cells has been described previously (27). Viral stocks were maintained by passage at low multiplicity in primary mouse embryo cells. Infected cultures were maintained in serum-free Dulbecco modified Eagle medium.

**Virus purification.** Virions were purified as described previously (23). CsCl gradients used in the purification were prepared as described by Brunck and Leick (11).

**Radioactive labeling procedures.** Purified polyomavirus virions were labeled in vitro with $^{125}I$ (carrier-free Amersham Corp.) by a modification of the method described by Frost and Bourgaux (17). Before labeling the virions, we incubated them in 0.2% SDS–0.01 M Tris, pH 7.5. This treatment disrupts the virion structure sufficiently to remove the noncovalently cross-linked proteins and the nucleoprotein core from the capsid structure (15) and yields a relatively even incorporation of iodine into all VP1 tyrosine residues.

Virions were labeled in vivo with [$^{35}S$]methionine (1,200 Ci/mmol; New England Nuclear Corp.) by removing the serum-free Dulbecco modified Eagle medium 18 h postinfection, washing the cells with phosphate-buffered saline, and replacing the medium with serum-free Eagle medium containing 10% of the standard amino acid concentration and 20 μCi of [$^{35}S$]methionine per ml. Virions were similarly labeled in vivo with 3 μCi of [$^{14}C$]histidine per ml (300 mCi/mmol, Schwarz/Mann). $^{14}C$-mixed amino acids (Schwarz/Mann) were used to label virions by adding 2 μCi per dish to infected monolayers in serum-free Eagle medium containing 10% of the standard amino acid concentration. In all cases, before the virus was harvested, the infection was allowed to proceed until maximum cytopathic effect was observed.

Purified *Staphylococcus aureus* protein A was $^{125}I$ labeled by the Bolton-Hunter procedure (technical bulletin; New England Nuclear Corp.).

**Electrophoresis.** SDS-PAGE was performed as described previously, with 15% acrylamide and 0.2% bisacrylamide (10).

**Chemical cleavage.** Formic acid, which efficiently and specifically cleaves aspartic acid-proline linkages (21), and IBA, which hydrolyzes typtophanyl peptide bonds (22), were used in these studies. Normally, VP1 was isolated from purified virions by SDS-PAGE before treatment. The position of VP1 in the gel was located either by staining or by autoradiography, and the band was excised.

For formic acid treatment, gel slices containing VP1 were swollen with 0.1 ml of 75% formic acid, which was completely absorbed, and were then incubated in tightly closed containers (12- by 75-mm polypropylene tubes) at 37°C for 18 to 24 h. Digestion was stopped by removing the formic acid in vacuo. The gel slices were then equilibrated with several changes of SDS-PAGE gel sample buffer until the bromphenol blue pH indicator remained blue. The cleavage products were analyzed by placing the equilibrated gel slices in the sample wells of a second SDS-PAGE gel and electrophoresing.

IBA cleavage was performed on SDS-PAGE-purified VP1 as described for formic acid cleavage, except that SDS was removed from the gel slices before treatment by washing in 10% (vol/vol) acetic acid–25% (vol/vol) isopropanol or by Coomassie blue staining. This was necessary because solubilization of IBA requires 4 M guanidine, and guanidine and SDS sometimes form an insoluble gel. After removal of SDS, gel slices containing VP1 were dried, and 0.1 ml of 10 mg of IBA per ml dissolved in 80% acetic acid–4 M guanidine was added. The gel slices were then incubated in tightly sealed tubes at room temperature for 24 h. Guanidine was removed by washing with acetic acid; the gel slices were dried in vacuo and equilibrated as described above, and the products were analyzed as described for formic acid.

**Two-dimensional peptide mapping.** Two-dimensional peptide mapping was performed on $^{125}I$-labeled VP1 and VP1 digestion products essentially as described by Elder et al. (14), except that proteins and peptides were always iodinated before SDS-PAGE. Chymotrypsin and L-(tosylamido, 2-phenyl) ethyl chloro-

methyl ketone–trypsin were purchased from Worthington Diagnostics, Freehold, N.J., and used at concentrations of 50 μg/ml. After lyophilization, the resulting peptides were dissolved in 20 μl of acetic acid-formic acid-water, 15:5:80 (buffer I), spotted on high-efficiency thin-layer chromatography silica gel thin-layer plates (10 by 10 cm; Analtech Inc.), and electrophoresed at 500 V on a cooling plate until the lead component of the tracking dye (2% orange G [wt/vol]–1% acid fuchsin [wt/vol]) traversed the plate. Chromatography in the second dimension was performed in n-butanol-pyridine-acetic acid-water, 32.5:25:5:20 (buffer II). The plates were dried and analyzed by autoradiography with Kodak XAR or Cronex 4 X-ray film and Cronex Lightning-Plus screens.

**Antisera.** Purified polyomavirus virions electrophoresed on SDS-polyacrylamide gels were used as the antigen source. Protein bands were excised and injected into New Zealand white rabbits for production of antisera. In this manner, antisera were made against VP1, VP2, VP3, and the virion histone region of SDS-PAGE. Details of the antigens, the antisera produced, and the characterization of their specificities have been described previously (24). Immunoglobulin G (IgG) was purified as previously described (24).

**Western blot analysis.** Proteins and peptides fractionated by SDS-PAGE were electrophoretically transferred to nitrocellulose sheets by the method described by Bittner et al. (2). The residual binding potential of the nitrocellulose was blocked by incubation with 4% (wt/vol) bovine serum albumin, fraction V (Sigma Chemical Co.), in 0.05 M NaCl–0.002 M EDTA–0.01 M Tris, pH 7.5 (BTDN buffer), and the peptides were probed with the respective antibodies in BTDN buffer. After incubation for 12 to 18 h, the antibody was removed, the nitrocellulose paper was washed with five changes of 0.05 M NaCl–0.002 M EDTA–0.01 M Tris, pH 7.5 (TDN buffer), for 10 min each change, and the mixture was incubated for 1 h with Bolton-Hunter reagent $^{125}$I-labeled *S. aureus* protein A, as described by Renart et al. (26). The unbound protein A was removed by washing with five changes of TDN buffer for 10 min each change. Bound IgG was then detected indirectly by autoradiography.

## RESULTS

**Chemical cleavage of VP1.** By using the previously described, predicted sequences for polyomavirus VP1 as a guide (12, 28), we selected methods of chemical cleavage with the criterion that they yield the fewest (and thus most easily identified and ordered) peptides possible. The cleavage agents we chose were IBA and formic acid, which hydrolyze at tryptophan residues and aspartic acid-proline linkages, respectively. Table 1 shows the products which should result from treatment of polyomavirus VP1 with these reagents. The sequence of Deininger et al. (12) was used to construct Table 1, but the sequence of Soeda et al. (28) predicts very similar results, differing primarily in the presence of an additional tryptophan residue. Three peptides are predicted to result from formic acid cleavage: an amino-terminal fragment of 231 amino acids, the largest peptide, with an approximate molecular weight of 26,000 (26K); a central peptide of 133 amino acids, with a molecular weight of 15K; and a small carboxy-terminal peptide of 20 amino acids, with a molecular weight of approximately 2K. The Deininger et al. (12) VP1 sequence contains six tryptophan residues, but since the conditions used for IBA cleavage (80% acetic acid) also result in the cleavage of aspartyl-prolyl sites, nine fragments are predicted to be produced by this treatment, ranging in size from 107 amino acids to 3 amino acids. However, nearly 80% of the VP1 sequence resides in peptides with molecular weights of 5K or greater, which are separable by SDS-PAGE.

To facilitate studies of microgram quantities of VP1, we developed convenient methods of performing the cleavages on SDS-PAGE-purified VP1 in gel slices without elution and of analyzing the cleavage products with a second SDS-PAGE. Proteins used for these studies

TABLE 1. Peptides predicted to be produced by limit cleavage with formic acid treatment and IBA treatment[a]

| Formic acid | | | IBA | | |
|---|---|---|---|---|---|
| Cleavage site | Position[b] | Size[c] | Cleavage site | Position[b] | Size[c] |
| | | | $Trp_{76}$-$Ser_{77}$ | 1 to 76 | 76 |
| | | | $Trp_{99}$-$Ser_{100}$ | 77 to 99 | 23 |
| | | | $Trp_{121}$-$Glu_{122}$ | 100 to 121 | 22 |
| | | | $Trp_{228}$-$His_{229}$ | 122 to 228 | 107 |
| $Asp_{231}$-$Pro_{232}$ | 1 to 231 | 231 | $Asp_{231}$-$Pro_{232}$ | 229 to 231 | 3 |
| | | | $Trp_{300}$-$Arg_{301}$ | 232 to 300 | 69 |
| | | | $Trp_{315}$-$Val_{316}$ | 301 to 315 | 15 |
| $Asp_{364}$-$Pro_{365}$ | 232 to 364 | 133 | $Asp_{364}$-$Pro_{365}$ | 316 to 364 | 49 |
| $Pro_{365}$-$C_{ter}$ | 365 to 384 | 20 | $Pro_{365}$-$C_{ter}$ | 365 to 384 | 20 |

[a] Predictions are made from the sequence data of Deininger et al. (12).
[b] Positions of the first and last amino acids of the resulting peptide within the VP1 sequence.
[c] Number of amino acids at a cleavage site.

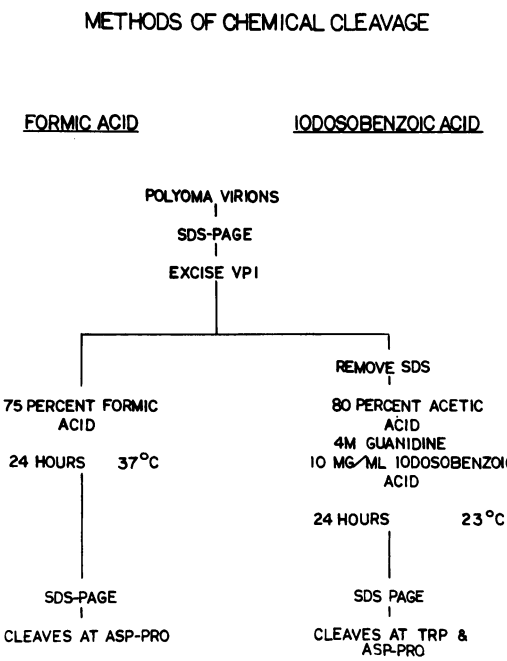


FIG. 1. Methods of chemical cleavage. The cleavage conditions shown are those used for all experiments described in the text.

were obtained from purified polyomavirus virions; VP1 was separated from other virion proteins by SDS-PAGE. Preliminary studies were performed to optimize the cleavage conditions, and the conditions shown in Fig. 1, which outlines the cleavage protocols, are those which we used in subsequent experiments. Incubation of VP1 with 75% formic acid for 24 h at 37°C resulted in a 60 to 80% cleavage and yielded all possible combinations of complete and partial products. Longer incubations resulted in progressively more complete digestion, approaching 100% at 48 h (data not shown). Treatment with IBA yielded nearly complete cleavage, except at the $Trp_{121}$-$Glu_{122}$ site, which was relatively refractory, but with the conditions used, it was nevertheless at least 70% cleaved. For both formic acid and IBA, the cleavage rate appeared to be independent of the protein concentration within the range used (20 μg per gel slice or less).

Figure 2A shows the results obtained when VP1 was cleaved with formic acid under the conditions shown in Fig. 1. Four major products resulted, with apparent molecular weights of 40K, 29K, 18K, and 16K. Figure 2B shows the fragments produced by IBA cleavage of purified polyomavirus VP1. The major peptides migrated with estimated molecular weights of approximately 12K, 8K, 7K, and 5K. The molecular weights of fragments generated by both methods of cleavage were in agreement with those predicted by the sequence data, either as limit products in the case of the 29K and 16K formic acid fragments and the 12K, 8K, and 7K IBA fragments or as the result of partial cleavage, as in the case of the 40K and 18K formic acid fragments.

**Identification of peptides produced by formic acid cleavage and IBA cleavage.** To confirm which of the peptides represented nonoverlapping portions of the VP1 sequence, we peptide mapped $^{125}$I-labeled formic acid fragments (Fig. 3). The Deininger et al. (12) sequence predicts 13 tyrosine residues present in 11 separate tryptic peptides. We were able to identify 11 $^{125}$I-labeled peptides resulting from tryptic digestion of $^{125}$I-labeled VP1 (Fig. 3A). Digestion of the 29K and 18K fragments, which together should represent the entire VP1 sequence, produced four and seven peptides, respectively, which is also in agreement with the sequence information (Fig. 3A and 3B). Mixing the $^{125}$I-labeled tryptic peptides of the 29K and 18K formic acid fragments and running them together produced a peptide map indistinguishable from that of VP1 (data not shown). When we compared their chymotryptic peptide maps, similar results were obtained, except that more labeled peptides were generated from the sequence than we predicted (Fig. 4). This may be due simply to lapses in specificity or, alternatively, to a failure to

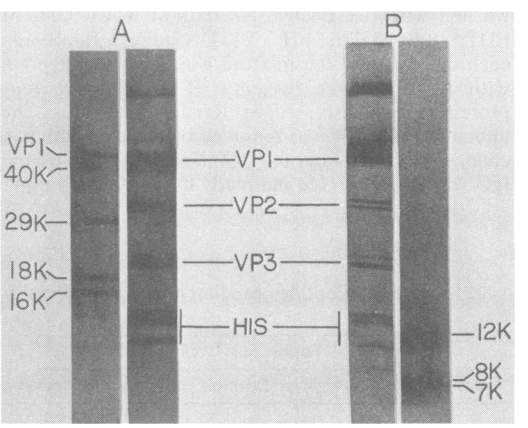


FIG. 2. Chemical cleavage of polyomavirus VP1. VP1 was prepared by SDS-PAGE of purifed polyomavirus virions, located by Coomassie blue staining, excised, and treated with either 75% (vol/vol) formic acid (A) or 10 mg of IBA per ml in 80% (vol/vol) acetic acid–4 M guanidine (B), as described in the text. Resulting peptides were detected by autoradiography; [$^{35}$S]methionine-labeled polyomavirus virion proteins were used as molecular-weight markers. Estimated peptide molecular weights are indicated. HIS, Histone.

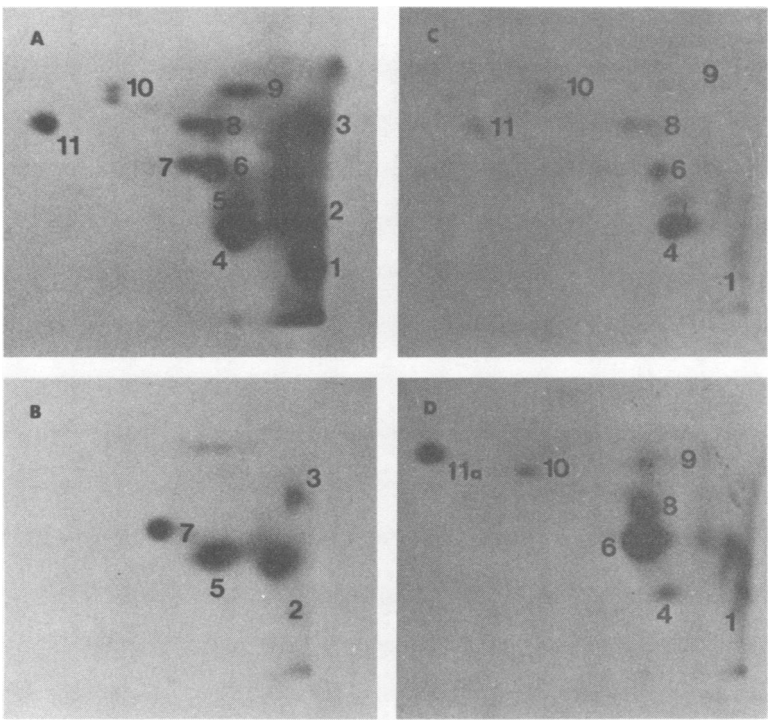


FIG. 3. Autoradiogram of two-dimensional tryptic fingerprint analysis of chloramine T $^{125}$I-labeled VP1 formic acid peptides. Peptides were prepared for analysis by labeling purified polyomavirus virions in the presence of 0.2% SDS before electrophoresis, excision of VP1, and treatment with 75% formic acid. After cleavage, peptides were separated by a second SDS-PAGE, located by autoradiography, and trypsinized as described in the text. Recovered tryptic peptides were dissolved in buffer I and subjected to peptide mapping on thin-layer plates. Origin is in the lower right-hand corner for each plate. Electrophoresis was performed in buffer I from right to left (500 V; 20 min), and ascending chromatography was performed in buffer II from bottom to top until the solvent reached the top of the plate. Spots were located by autoradiography. Peptides present in VP1 are identified with a number, and peptides held in common by the respective VP1 formic acid fragments are identified by the same numbering system. (A) VP1; (B) 29K; (C) 18K; (D) 16K.

cleave at some iodotyrosine residues. We conclude from these experiments that the 29K and 18K formic acid fragments do indeed represent nonoverlapping regions of the VP1 amino acid sequence, and furthermore, that together they probably represent the entire VP1 sequence, since all of the $^{125}$I-labeled VP1 tryptic peptides were found in either the 29K or the 18K fragment.

We studied the peptides produced by formic acid cleavage by excising them, recleaving them with formic acid, and analyzing the recleavage products by SDS-PAGE (Fig. 5). Recleavage of VP1 not cleaved in the first round of formic acid treatment produced the expected pattern, with all four major peptides being produced (lane 1). Recleavage of the 40K peptide produced only the 29K and 16K peptides (lane 2). Recleavage of the 29K peptide did not generate either of the smaller fragments, whereas retreatment of the 18K peptide generated the 16K peptide (lanes 3 and 4). The origin of the higher-molecular-weight peptides observed in lanes 4 and 5 is not resolved, although they probably represent dimerization of the recleaved 18K and 16K peptides. These results allow us to order the fragments. We conclude that the 29K peptide represents one terminal fragment, and a small fragment of about 2K is the other terminal peptide. The 2K peptide was not seen in our gels, probably because of its loss during equilibration, but its sequence was present in the 18K peptide formed by the incomplete cleavage of VP1. The 16K peptide resides between the two terminal peptides; together, the three peptides make up the entire VP1 sequence.

The positions of the 12K, 8K, and 7K IBA peptides in the VP1 sequence were confirmed to be as predicted by recleavage of formic acid fragments with IBA (data not shown). The 12K and 8K peptides were generated by cleavage of the 29K formic acid fragment, and the 7K pep-

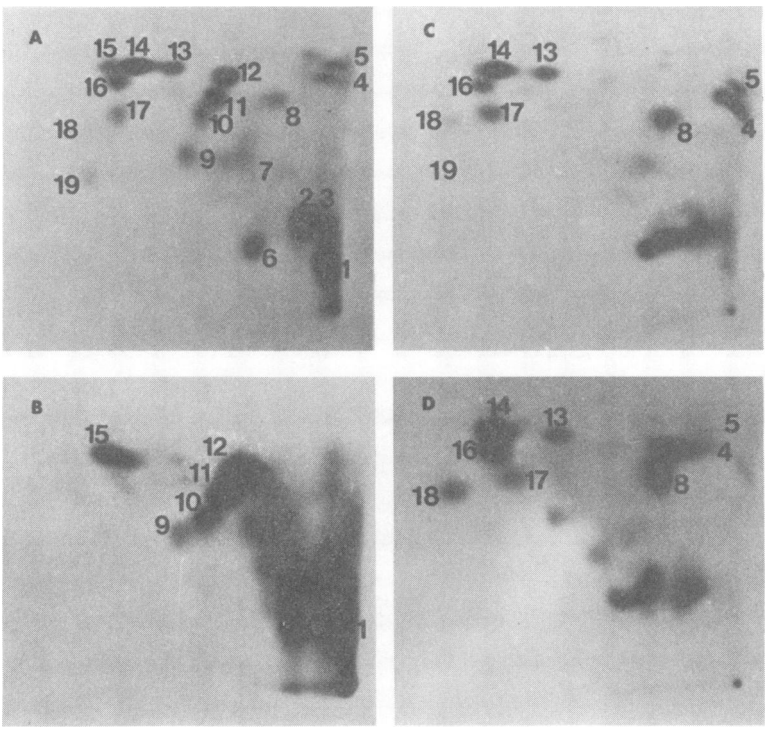


FIG. 4. Autoradiogram of two-dimensional chymotryptic fingerprint analysis of chloramine T $^{125}$I-labeled VP1 formic acid peptides. Peptides were prepared for analysis as described in the legend to Fig. 3. Individual formic acid peptides were treated with chymotrypsin. The resulting chymotryptic peptides were fingerprinted as described in the legend to Fig. 3. Origin is in the lower right-hand corner of each panel. (A) VP1; (B) 29K; (C) 18K; (D) 16K.

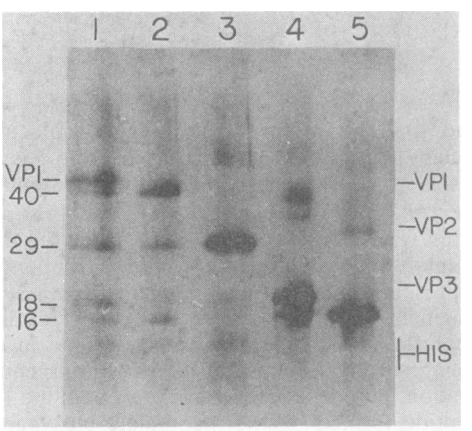


FIG. 5. Fluorogram of recleavage of formic acid peptides. $^{14}$C-amino acid-labeled VP1-containing gel slices were treated with 75% formic acid under the standard conditions described in the text. The resulting peptides were separated by SDS-PAGE and identified by Coomassie blue staining. The peptide bands were then excised and retreated with 75% formic acid, and the products were analyzed by a second SDS-PAGE. The bands were identified by fluorography. Lanes show products resulting from recleavage of the following: 1, intact VP1; 2, 40K; 3, 29K; 4, 18K; and 5, 16K. Positions of marker polyomavirus proteins are indicated on the right, and VP1 formic acid peptides are identified on the left. HIS, Histone.

tide was found to be produced by cleavage of the 18K formic acid fragment.

**Evidence that the receptor moiety resides in the carboxy-terminal one-third of the VP1 sequence.** In earlier studies performed in this laboratory, various antisera were raised against polyomavirus structural proteins (24). The antigens used to raise these antisera were either intact polyomavirus virions, SDS-disrupted virions, or individual virion proteins denatured and separated by SDS-PAGE. Antisera were also raised against the polyomavirus virion histone region of SDS-PAGE. The anti-histone region antisera were very interesting because they were found to be more effective in hemagglutination inhibition and neutralization assays than were antisera directed against complete polyomavirus virions proteins VP1, VP2, and VP3 (24). Subsequently, the anti-histone region antisera were shown to be directed against two VP1-related peptides of

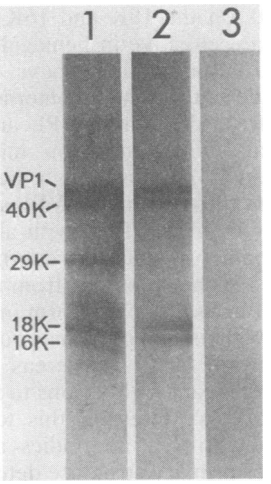


FIG. 6. Autoradiogram of Western blot analysis of antiserum specificity with respect to formic acid fragments of polyomavirus VP1. Unlabeled polyomavirus VP1 from polyomavirus virions was isolated by SDS-PAGE, identified by Coomassie blue staining, excised from the gel, and treated with 75% formic acid under the standard conditions described in the text. Resulting formic acid peptides were then separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose filter sheet. Residual protein-binding capacity of the filter was blocked by incubation in BTDN buffer, and the peptides were probed with IgG fractions of the respective antisera. After careful washing in TDN buffer, bound IgG was detected by incubation of the nitrocellulose sheet in BTDN buffer containing $5 \times 10^5$ cpm of Bolton-Hunter $^{125}$I-labeled *S. aureus* protein A ($5 \times 10^5$ cpm/μg), followed by washing in TDN buffer and autoradiography. Lanes represent formic acid peptides probed with the following: 1, anti-intact polyomavirus virion IgG; 2, anti-histone region IgG; 3, preimmune IgG. Positions of respective peptides are identified in the left margin.

unknown origin, whose molecular weights were estimated to be 16K and 14K (5), and these antisera did not react with either histones or other virion proteins. Evidence from our laboratory (5) and from others (16) indicated that the VP1-related peptides were degradation products of VP1, but the portion of the VP1 sequence they represented was not known.

To investigate the specificity of these antisera with respect to the formic acid fragments, we performed Western blot analysis on VP1 formic acid fragments (immunoblots) with IgGs purified from the respective antisera. The results are presented in Fig. 6. IgG prepared from anti-intact polyomavirus antiserum reacted with all of the fragments generated by formic acid cleavage, indicating that in intact virions, determinants from both the amino-terminal and carboxy-terminal portions of VP1 are exposed and recognized in intact virions (lane 1). When the anti-histone region antisera were tested, however, the 29K formic acid fragment, which constitutes the amino-terminal two-thirds of the VP1 sequence, was completely nonreactive; the 18K and 16K fragments, however, reacted strongly (lane 2). Thus, the anti-histone region antisera are directed against determinants present in the carboxy-terminal 153 amino acids of VP1. Since the anti-histone region antisera efficiently neutralize polyomavirus virions, we suggest that this region of VP1 contains the majority, if not all, of the linear determinants responsible for generating neutralizing antibodies and therefore probably constitutes or contributes to the structure of the viral receptor.

## DISCUSSION

Our goal in these studies was to cleave VP1 into a set of large identifiable peptides as a first step toward determination of the various functional domains. To accomplish this, we have taken advantage of recently developed methods for specific chemical cleavage of proteins and polyomavirus sequence data. Results of these cleavage studies are summarized in Fig. 7, which shows formic acid and IBA cleavage maps that we determined for VP1. Formic acid cleaves VP1 primarily at two sites, generating three peptides with estimated molecular weights of 29K, 16K, and 2K. The three peptides are linked in 29K, 16K, and 2K order in intact VP1. Because the molecular weights and order of these peptides concur with predictions made based on sequence data, as do tryptic and chymotryptic peptide analyses, we conclude that the formic acid peptides of VP1 are indeed those predicted from the sequence data in Table 1. As further support of these predictions, we found that IBA cleavage also produces peptides corresponding in size and order to those predicted in

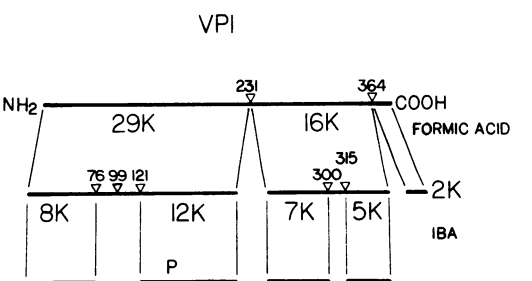


FIG. 7. Schematic representation of proposed VP1 cleavage map derived from data presented in this report. Cleavage sites are indicated by inverted triangles and identified by amino acid numbers. P, Phosphorylation domain (1). Sequence information is from Deininger et al. (12).

Table 1, as demonstrated by IBA cleavage of formic acid peptides.

These studies provide an interesting explanation for a puzzling observation made in our laboratory (5) and by others (16) concerning the degradation of VP1 from purified polyomavirus virions under conditions which disrupt virion structure. This degradation has generally been assumed to be proteolytic, although numerous attempts to inhibit cleavage by using standard protease inhibitors have been unsuccessful (16; unpublished data). The major observed products of this degradation are a polypeptide with a molecular weight of 29K and two smaller peptides migrating slightly above virion histones during SDS-PAGE (5). We suggest that these degradation products are generated not by proteolysis, but rather by spontaneous hydrolysis of the aspartyl-prolyl linkages attacked by formic acid. These linkages have previously been shown to be relatively unstable (21). Evidence for the spontaneous hydrolysis at formic acid sites is twofold. First, the spontaneously produced 29K peptide comigrates exactly with the 29K formic acid fragment identified in these studies (data not shown). Second, antisera raised against the polyomavirus virion histone region of SDS-PAGE react specifically with 18K and 16K VP1 formic acid fragments (Fig. 7). Earlier work presented by our laboratory has shown that these antisera also react specifically with two peptides whose molecular weights were estimated to be 16K and 14K and which were generated by incubation of virions under disruption conditions, without histones (5). These results suggest that the spontaneously produced peptides are the same as those produced by formic acid cleavage. We are currently making antisera against the formic acid fragments which will allow us to further define the relationship between formic acid peptides and spontaneously produced VP1 fragments.

It is not clear why such disparate agents as SDS, EGTA, and alkaline carbonate buffers should lead to enhanced hydrolysis at these sites. We note, however, that all of these agents disrupt the virion structure (9). It is possible that the aspartyl-prolyl linkages are involved in stabilizing secondary or higher-order interactions, which are lost subsequent to virion disruption. We have no evidence to suggest that hydrolysis at these sites is of functional significance, but it is noteworthy that the sites are conserved in the simian virus 40 sequence (29).

These studies also indicate that the polyomavirus hemagglutinin and receptor functions reside in, or at least have determinants contributed by, the carboxy-terminal 153 amino acids of the VP1 sequence. The anti-histone region antisera described above, which specifically recognize determinants on the 18K and 16K formic acid fragments, are effective in hemagglutination inhibition and neutralization assays. In contrast, antisera made in the same manner against SDS-PAGE-purified, denatured VP1 are relatively inefficient at hemagglutination inhibition and neutralization (24), even though they recognize determinants throughout the VP1 sequence, as indicated by their reactivity with all the formic acid fragments (unpublished data). A clue to why this should be so comes from the fact that the anti-VP1 antisera do not form immunoprecipitin lines with intact polyomavirus virions in immunodiffusion assays, whereas anti-histone region antisera react with virions to form precipitin lines (24). We interpret this to mean that most of the anti-VP1 antibodies are directed against either primary structure determinants or higher-order determinants which are not present or accessible in the native structure. In the case of the anti-histone region antisera, the VP1 peptides which served as the antigen either assumed a conformation which presented higher-order determinants similar to those exposed in the intact virion or which presented primary structure determinants present on the surface of intact virions.

These studies do not rule out the possibility that structural elements of VP1 other than those present in the carboxy-terminal portion contribute to cell-binding function. In similar studies performed by Dietzschold et al. (13) on the rabies virus glycoprotein, three cyanogen bromide peptides from widely separated regions of the molecule were found to generate neutralizing antibodies. The identification of VP1 peptides generated by formic acid and IBA reported here will allow us to examine in more detail the distribution of neutralizing epitopes in the VP1 sequence. We are currently making antisera against each of the fragments. In addition, we are attempting to use these VP1 peptides in affinity chromatography studies to fractionate antisera made against intact and disrupted polyomavirus virions.

This biochemical approach has general potential for the identification of structural and functional domains of polyomavirus VP1. In addition to the preliminary identification of the hemagglutinin and receptor regions of VP1 reported here, as well as ongoing studies to further define these functional sites, we have used this method to localize the phosphorylation sites (1). Furthermore, we are extending these studies to determine the organization of intramolecular and intermolecular disulfide cross-links of VP1 in the virion structure. These studies will complement the crystallographic studies of Rayment et al. (25), which have already produced startling information about polyomavirus virion struc-

ture, and will help to relate polyomavirus VP1 structure in the virion to the performance of its required functions.


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