Polyoma Virus Major Capsid Protein, VP₁

PURIFICATION AFTER HIGH LEVEL EXPRESSION IN ESCHERICHIA COLI

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We have expression-cloned in Escherichia coli the major polyoma virus capsid protein, VP₁. Under the inducible control of the hybrid tac promoter, VP₁ constituted between 2 and 3% of the total host cell protein. The expressed VP₁ was purified to near homogeneity with initial yields to 10%. Optimal expression was temperature-dependent, and significant intracellular degradation could be demonstrated. The final product was obtained as one predominant isoelectric focusing species, without the pattern of post-translational modification seen in virus-infected eukaryotic cells. The purified VP₁ from E. coli will be useful as a substrate for the purification of VP₁ modification enzymes and in the study of inter-VP₁ oligomerization.

Polyoma is an endogenous mouse virus whose icosahedral capsid symmetry has been an important model for the mechanism of protein subunit packaging. X-ray crystallographic studies of polyoma originally suggested that the capsid consists of 12 five-coordinated and 60 six-coordinated morphological subunits (capsomeres) arranged on a T = 7d icosahedral surface (Klug, 1965). Recent x-ray diffraction data derived from polyoma capsid crystals, however, have shown that the 72 capsomeres are all pentamers, suggesting that the contacts between protein subunits are not all quasi-equivalent (Rayment et al., 1982). The packing of the pentamers in the hexavalent and pentavalent environments has been attributed by Baker et al. (1983) to a "variable bonding potential" of the pentameric capsomeres. Klug (1983) has pointed out that this variability may be a property of a simple multifunctional protein (VP₁) which is chemically modified in the different environments or a differential association of identical capsomeres with the minor structural proteins (VP₂ and VP₃).

The major capsid protein, VP₁, is extensively modified by both acetylation and phosphorylation, and these modifications appear to occur prior to virion assembly (Beien et al., 1981; Ponder et al., 1977; Garcea et al., 1985). In addition, there appears to be a relationship between the modification of VP₁, the assembly of intact virus, and the function of the viral early proteins, middle, and small tumor antigens (Garcea and Benjamin, 1983). Mutations in these early proteins lead to a defect in effective virion assembly which is associated with a marked under-modification of VP₁ (Garcea et al., 1985).

The elucidation of the structural roles of modified VP₁ molecules and the enzymes modifying VP₁ therefore connects the divergent areas of virion assembly and the mechanism of action of polyoma's transforming genes.

In order to study the relationship between VP₁ modification and virion structure and to generate a substrate for the purification of VP₁ modification enzymes, we have expression-cloned VP₁ in Escherichia coli. We chose a prokaryotic expression system in an attempt to avoid the post-translational modifications which occur in eukaryotes. The ability to isolate unrestricted quantities of VP₁, not only makes possible the study of the nature of inter-VP₁ bonding but also its association with the viral minichromosome in the condensation reaction to form the final virion.

MATERIALS AND METHODS

Expression of Polyoma Capsid Protein VP₁ in E. coli—The general methodology of Guarente et al. (1980) was used to express VP₁ in E. coli. The first step involved the construction of a plasmid carrying the 5' terminus of VP₁ fused to lacZ of E. coli and is shown in Fig. 1. A 5' fragment of VP₁ was obtained from a HindIII digestion of pPY322. The single-stranded ends of all HindIII digest fragments were made blunt with T₄ DNA polymerase. Phosphorylated SacI linkers were ligated to the HindIII fragments, and the entire mixture was digested with SacI and HindIII. A 242-bp fragment contained the initial (5') 156 bp of VP₁ terminating in a HindIII sticky end and 80 bp of 5' flanking polyoma DNA terminating in a SacI sticky end. Subsequent ligation of this fragment into pLG200 yielded a plasmid pAL100 in which the amino terminus of the VP₁ gene is fused in frame to a large carboxyl fragment of the lacZ gene.

The second step in constructing the VP₁ expression vector was to place the necessary transcriptional and translational regulatory elements 5' to the VP₁-lacZ fusion. Plasmid pGL101, when digested with PvuII and PstI, yields a 851-bp "portable promoter" fragment which contains the reasonably strong lacUV5 promoter and, just 5 bp 5' to the PvuII site, a ribosome-binding site (Shine-Delgarno sequence). In order to place the lacUV5 promoter and Shine-Delgarno sequence at

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Cloning and Purification of Polymya Virus VP<sub>1</sub>

**FIG. 1. Construction of the VP<sub>1</sub> expression plasmid under the control of the lacUV5 promoter.** Plasmid pPY322 contains the late region of polynoma from the EcoRI to BamHI restriction sites cloned into pBR322. A 242-bp fragment from pPY322 was cloned in the optimal distance 5' to the VP1-lacZ fusion gene of pAL100, with variable amounts of Delgarno sequence provided from pGL101. Plasmid pAL164 was constructed consecutively with nuclease Bsi31, S1 nuclease, and PstI. The linearized PAL100 plasmid was then digested with E. coli tryptophan promoter (Amann et al., 1983), and Fig. 2 outlines this construction. Plasmid pALVP1 was digested with EcoRI and HindIII, and the small, approximately 380-bp fragment was isolated. Plasmid pPY322 was digested with HindIII and PvuII, and the fragment containing the distal 992 bp of the VP1 gene as well as the 894 bp of 3' flanking polynoma DNA was isolated. These three fragments were ligated, transformants were screened by restriction endonuclease digest mapping, and one plasmid, pALVP1, was used for further studies.

To assay for the reconstructed VP<sub>1</sub> protein, total cell lysates of E. coli, with or without pALVP1, were electrophoresed on 10% SDS-PAGE gels. Several attempts using strains HB101 and P90C failed to show Coomassie Blue-stainable VP<sub>1</sub> protein. However, these gels exhibited intensely staining cellular proteins at the expected molecular weight of VP1, which would make detection of a foreign protein difficult in this region of the gel. To test for low level production of VP<sub>1</sub> protein from pALVP1, immunoprecipitation using anti-VP<sub>1</sub> antibody was performed after labeling with <sup>35</sup>S. The results of this experiment (data not shown) demonstrated that a protein from pALVP1, which migrated at the expected molecular weight of VP1 (45,000), co-migrated with <sup>35</sup>S-labeled VP1 from polyoma virions, and was not present in an isogenic strain lacking pALVP1.

**Addition of the tac Promoter—** VP<sub>1</sub> protein expression was improved by changing the lacUV5 promoter to a hybrid promoter composed of fragments of the lacUV5 promoter and the E. coli tryptophan promoter (Amann et al., 1983), and Fig. 2 outlines this construction. Plasmid pALVP1 was digested with EcoRI and HindIII. The large fragment containing the beta-lactamase gene was saved. The small fragment, which contains the lacUV5 promoter and the 5' end of the VP1 gene, was then digested with HpaII, and the larger fragment containing the -10 segment and the 5' end of VP1 was isolated.

**FIG. 2. Changing the lacUV5 promoter to a tac promoter 5' to VP1.** Details are described under "Results."
transcriptional control of the tac promoter, RB791 was grown with and without pALVP,TAC. As shown in Fig. 3, a protein of approximately 45 kDa accumulated in strain RB791 containing pALVP,TAC but not in the isogenic strain lacking this plasmid. Furthermore, the 45-kDa protein was present only after the addition of the inducer IPTG. An additional band of approximately 70 kDa also appears to be induced and only after the addition of the inducer IPTG. An additional protein was present in RB791 without IPTG; RB791 without IPTG; lane c, pALVP,TAC/RB791 with IPTG; lane d, pALVP,TAC/RB791 without IPTG. The VP, protein in lane c is indicated by the arrowhead at left. Molecular weight markers are indicated in kilodaltons.

To confirm that the 45-kDa protein encoded by pALVP,TAC is VP, strains RB791 and pALVP,TAC/RB791 were labeled with 35S and cell extracts were immunoprecipitated using anti-VP, antibody. When the 35S-labeled immunoprecipitates were electrophoresed on 10% SDS-PAGE gels, a 45-kDa IPTG-inducible protein was present in strain pALVP,TAC/RB791 but not in strain RB791 (Fig. 4). In addition, multiple bands of less than 45 kDa were present in pALVP,TAC/RB791 (lane b). These bands were not seen in the absence of IPTG induction (not shown) nor were they present in RB791 alone. Furthermore, the identical banding pattern was reproducible in multiple immunoprecipitation experiments. Thus, since these bands are cross-reactive with anti-VP, antibody, they are not nonspecifically bound E. coli proteins and may represent either degradation products or polypeptides synthesized from internal translation initiation sites.

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FIG. 3. SDS-PAGE of total cell lysates from E. coli producing unfused VP1. Cells were grown in TYE media to early logarithmic phase and split into two equal volume samples. IPTG was added to one sample to a final concentration of 5 mM, and all samples were then incubated for an additional 3 h. Aliquots were then removed from each sample and prepared as described in the legends to Fig. 4. Total cell lysates from equal numbers of cells were then electrophoresed on a 10% SDS-PAGE gel. Lanes a, RB791 with IPTG; lane b, RB791 without IPTG; lane c, pALVP,TAC/RB791 with IPTG; lane d, pALVP,TAC/RB791 without IPTG. The VP, protein in lane c is indicated by the arrowhead at left. Molecular weight markers are indicated in kilodaltons.

FIG. 4. Immunoprecipitation of E. coli cell lysates with anti-VP1 antibody. Cells were grown in supplemented minimal media, induced with 5 mM IPTG, and labeled with 35S (20 μCi/ml) for 3 h. Total cell lysates and immunoprecipitations were then performed as described under “Materials and Methods.” The 10% SDS-PAGE gel shows immunoprecipitates of RB791 with anti-VP, (lane a), pALVP,TAC/RB791 with anti-VP, (lane b), RB791 with preimmune sera (lane c), and pALVP,TAC/RB791 with preimmune sera (lane d). The arrowhead indicates the position of a VP, virion standard. Reducing these bands were not seen in pALVP,TAC/RB791 without IPTG. The VP, protein in lane c is indicated by the arrowhead at left. Molecular weight markers are indicated in kilodaltons.

The molecular weight determined by SDS-PAGE is identical to virion derived VP1; (b) it is immunoprecipitated by anti-VP1 antibody; and (c) on two-dimensional gel analysis, it has the same isoelectric point as virus-derived VP1. Furthermore, VP1 protein is synthesized in E. coli at levels detectable by Coomassie Blue staining when total cell lysates are electrophoresed on SDS-PAGE gels (Fig. 3). Densitometric analysis of such a gel shows that the 45-kDa band, less the host bacterial contri-
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FIG. 5. Two-dimensional gel electrophoretic analysis of proteins synthesized by pALVP1/TAC/RB791. Cells were grown in supplemented minimal media into mid-logarithmic phase in the presence of $^{35}$SO$_4$ (20 µCi/ml) and 5 mM IPTG. Total cell lysates and immunoprecipitations were as described under “Materials and Methods.” A, RB791 total cell lysate; B, pALVP1/TAC/RB791 total cell lysate; C, ALVP1/TAC/RB791 immunoprecipitated with anti-VP1 antibody. The large arrowheads indicate VP1 protein. The small arrowheads represent presumed VP1 degradation products, as discussed in the text. The degradation products are seen both in total cell lysate and in the immunoprecipitation, indicating that the degradation spots in panel C are not simply a result of the technique. The isoelectric focusing dimension is oriented with the left basic and the right acidic.

bution at that molecular weight, constitutes between 2 and 3% of the total host cell Coomassie Blue-stainable proteins.

VP1 Protein Stability in E. coli—Because the VP1-β-galactosidase fusion protein transcribed from the lacUV5 promoter constitutes 3–4% of the total cell protein, whereas the complete VP1 protein was not detected on SDS-PAGE gels stained with Coomassie Blue, we tested by pulse-chase experiments the possibility that rapid degradation of the unfused VP1 protein contributed to the inability to detect VP1 by Coomassie Blue staining. Control experiments demonstrated that the conditions for the pulse-chase experiments were such that: (a) the rate of VP1 synthesis was linear, and (b) antibody was present in excess during all immunoprecipitations. Fig. 6B shows the results of pALVP1/RB791 grown at 30 and 37 °C, with one-half of the $^{35}$S-labeled VP1 protein from a 2-min pulse disappearing in approximately 16 min at 30 °C and approximately 7.5 min at 37 °C. Thus, VP1 protein has a

FIG. 6. The rate of VP1 protein degradation determined by pulse-chase experiments. The pulse-chase experiments and anti-VP1 immunoprecipitations were performed as described under "Materials and Methods." Samples were electrophoresed on 10% SDS-PAGE gels. The amount of VP1 was determined by densitometry of the gel autoradiogram. The relative amount of VP1 present at each time point was normalized to a value of 10 for the 30 °C pulse sample at t = 0, and data were plotted using linear regression analysis. A, pALVP1/TAC/RB791 grown at 30 °C (●) and 37 °C (△). The correlation coefficient is −0.19 for the 30 °C experiment and −0.77 for the 37 °C experiment. B, pALVP1/RB791 grown at 30 °C (○) and 37 °C (●). The correlation coefficient is −0.96 for the 30 °C experiment and 0.96 for the 37 °C experiment. The normalized value at t = 0 for pALVP1/TAC (A) is approximately 3-fold greater in absolute amount as that for pALVP1 (B).

TABLE I

<table>
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<tr>
<th>Stage of purification</th>
<th>Vol ml</th>
<th>Total protein mg</th>
<th>VP1 mg</th>
<th>Yield %</th>
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<td>100</td>
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<td>14.5</td>
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<td>32</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>8.1</td>
<td>2.6</td>
<td>18</td>
</tr>
<tr>
<td>Post-DE52 precipitate</td>
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<td>1.8</td>
<td>0.7</td>
<td>5</td>
</tr>
<tr>
<td>P-11 eluant pooled</td>
<td>2</td>
<td>1.6</td>
<td>1.4</td>
<td>9</td>
</tr>
</tbody>
</table>


Summary of purification (from 5.5 g of cells)
temperature-dependent half-life when synthesized in E. coli. Unexpectedly, the results in Fig. 6A also show slower degradation of VP₁, both at 30 and 37 °C, when the tac promoter is present versus the lacUV5 promoter. Densitometric comparison between pALVP₁ and pALVP,TAC of the pulse-labeled VP₁ (t = 0) reveals a 3-fold increase in the relative synthesis of VP₁ between the tac and lac constructions.

Purification of VP₁ from Cell Extracts—We reasoned that VP₁ is a structural virion protein tightly associated with the DNA genome and would thus adsorb to anionic supports. Because of the lack of an enzymatic assay, we followed the purification by SDS-gel electrophoresis.

The cell extraction procedure (see "Materials and Methods") efficiently solubilized VP₁ until the dialysis step following ammonium sulfate precipitation of the Polymin P extract. At this stage, a precipitate formed which contained up to 60% of the total VP₁ extracted. The VP₁ could be recovered from this precipitate by solubilization in 1 M NaCl, dialysis to 250 mM NaCl, and then proceeding to the phosphocellulose (P-11) column step described below. The soluble extract after dialysis to 100 mM NaCl was first applied to a DE52 cellulose column equilibrated with buffer A, pH 7.2, 100 mM NaCl, and the flow-through plus 4 column volumes collected. These fractions contained all the VP₁ applied. The eluate was precipitated with ammonium sulfate to 35% saturation (see "Materials and Methods"), and the precipitate was dissolved in 1 M NaCl. The soluble fraction was then applied to a phosphocellulose (P-11) column equilibrated with buffer A, pH 7.4, 100 mM NaCl and washed with buffer A, pH 7.4, 250 mM NaCl, and VP₁ was subsequently eluted with 1 M NaCl.

Table I follows the yield of this purification scheme, not accounting for recovery of the VP₁ precipitated during the first dialysis. Fig. 7 follows the purity of VP₁ at each step. Densitometric determination of VP₁ yield in Table I reflects the amount of protein in the major VP₁ band, and all other bands are considered contaminants. The lower bands in Fig. 7, lanes E and F, correspond to anti-VP₁ antibody immunoreactive peptides (see Fig. 5C) and are most likely VP₁ degradation products. With recycling of the initially precipitated VP₁, up to 50% final yields have been obtained. Fig. 8A is a Coomassie Blue-stained two-dimensional gel of the P-11-eluted material shown in Fig. 7, lane F. The most intense spot, labeled VP₁, has the same isoelectric point as unmodified polyoma virion VP₁ (Fig. 8A). Two or more spots at the same molecular weight of the major spot in panel B correspond to those seen in Fig. 5C. The isoelectric focusing pattern of E. coli-produced VP₁ (panel B) is distinct from that seen in virus-infected cells (panel A) where greater than 50% of the subregions are modified (Hunter and Gibson, 1978; Hewick et al., 1977; Bolen et al., 1981).

**DISCUSSION**

The purpose of the present study was to generate large quantities of VP₁ so that the chemistry of inter-VP₁ bonding and VP₁ association with the viral minichromosome may be studied in further detail. We found that VP₁ produced in E. coli under control of the lacUV5 promoter was detected only by immunoprecipitation with anti-VP₁ antibody ([³⁵S]methionine labeling. By changing to the hybrid tac promoter, VP₁ could be detected by Coomassie Blue staining of total E. coli extracts and constituted 2-3% of the host cell protein. This result is consistent with previous reports concerning the tac promoter (Bikel et al., 1983) and may reflect not only enhanced transcription but also a saturation of the degradative pathways for VP₁. Alternatively, it is possible that increased levels of VP₁ result in a certain amount of self-aggregation which is protective against degradation. However, it should be noted that VP₁ does not form the type of insoluble complexes found for other eukaryotic proteins made in E. coli (Bikel et al., 1983). The 2-fold difference in protein half-life between induction at 32 °C versus 37 °C which we observed may reflect a partial activation of the heat shock proteolytic
system of E. coli (Goff et al., 1984), which may represent one degradative pathway for VP1.

We observed multiple anti-VP1 immunoreactive peptides smaller than VP1 in both whole cell lysates and during VP1 purification. Although the majority of these presumed proteolytic fragments may result from E. coli processing, VP1 may have an intrinsic autoproteolytic activity. Bowen et al. (1984) have detected a serine protease activity associated with VP1, identified by radiolabeling the protease with diisopropyl fluorophosphonate, which yields two well-defined proteolytic subtypes of 43.5 and 40 kDa. Autodigestion of VP1 may aid virion uncoating in specific intracellular compartments, and future studies with the E. coli-purified VP1 hopefully will clarify this activity.

The distinctive chemical properties of VP1, deduced from the amino acid sequence and its function as a probable DNA-binding protein, greatly facilitated its purification. The principal difficulty we anticipated was the tendency of VP1 to spontaneously aggregate. Indeed, although most VP1 was solubilized from the bacterial cell debris, dialysis to low ionic strengths did result in the precipitation of a fraction of the VP1. Further studies of the chemical properties of VP1 will require a soluble preparation. Brady and Consigli (1978) have previously shown that VP1 from virions, when completely denatured by guanidine HCl and 2-mercaptoethanol, can be gel-filtered through Sepharose CL-6B to obtain a monomer species of about 46 kDa. Subsequent step dialysis to non-denaturing conditions resulted in capsomere-like structures as defined by velocity sedimentation and electron microscopic appearance. This protocol provides a methodology for the initial studies of inter-VP1 interactions using the cloned protein.

We chose expression in an E. coli vector in order to avoid the post-translational modifications of VP1, which occur in a eukaryotic system. Nonetheless, our two-dimensional gel profiles of purified VP1 show two to three distinct isoelectric forms at the same molecular weight as our presumed unmodified subspecies. Although these subspecies may also represent proteolytic heterogeneity, we have not definitely ruled out that E. coli enzymes are nonspecifically modifying VP1 to a small extent. Our interest in VP1 modification results from the observation that the host-range nontransforming mutants of polyoma underphosphorylate VP1, and that this defect is contributory to the failure of these mutants to produce wild type levels of virus in certain cell types (Garcea and Benjamin, 1983). Unmodified VP1 will be useful as a substrate for identifying and purifying the enzymes involved in modification. The definition of these enzymes may aid in establishing a link between the viral transforming genes and pathways of cellular metabolism.

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Cloning and Purification of Polymya Virus VP1

ANOREY POLYOMA VIRUS MAJOR CAPSID PROTEIN VP1: PURIFICATION AFTER HIGH LEVEL EXPRESSION IN ESCHERICHIA COLI

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MATERIALS

Trypsine, yeast extract, and MacConkey agar were from Difco. Polylin P was obtained from New England Nuclear. Ammonium acetate was from Fisher. Tris was from J. T. Baker Chemicals, and sodium dodecyl sulfate was from Sigma. Bovine serum albumin was from Miles Laboratories. All restriction enzymes, nuclease Bal-31, and T4-DNA ligase were from Bethesda Research Laboratories. XAR-5 X-Oxid X-ray film was from Eastman Kodak. [35S]Jodobenzoine (800 Ci/mmol) and Na235SO4 (10-100mCi/mol) were from New England Nuclear. Anti-VP, antibody was the gift of Bill Murakami, and was raised in rabbits against SOS-gel purified virion VP1. It is specific to all modified forms of VP1 (Garcea et al., 1983).

METHODS

DNA: Complex media (TVE) contained 10 g tryptone, 5 g yeast extract, and 8 g NaCl per liter. Minimal media was M9 as described by Miller (1972). L-arabinose acids were used at 20 g/ml. Biotin was used at 1 pglml, and glucose at 0.5% w/v. MacConkey agar was used at the appropriate range of digestion. DNA was otherwise specified: ampicillin at 35 g/ml, tetracycline at 12.5 g/ml.

Bacterial Strains and Plasmids: Plasmids used are as pBR322 (Bolivar et al., 1977) and pPV322 (Lascio et al., 1980). Protein extract for further purification was labelled by either of the following: 

1. [35S]Jodobenzoine (800 Ci/mmol) and Na235SO4 (10-100mCi/mol) were from New England Nuclear. Anti-VP, antibody was the gift of Bill Murakami, and was raised in rabbits against SOS-gel purified virion VP1. It is specific to all modified forms of VP1 (Garcea et al., 1983).

2. [35S]Methionine (18 pCi/ml) as described below.

Pulse-Chase Experiments: Cells were grown in M9 minimal media until early log phase. An aliquot equivalent to 0.05% x volume (ml) was removed, spun at 10,000g, and resuspended in 700 ml of M9 media. The culture was then placed at the desired temperature for 1 ml, 15 ml of 0.5M IPTG was then added, the cells incubated for an additional 10 min. [35S]Methionine was then added, and the cells were reincubated for 2 min. The cell mix was immediately diluted 1:1 with M9 minimal media containing methionine and 45 g/wl unlabeled methionine, spun at 10,000g, washed with 500 ml M9 containing methionine and 45 g/wl methionine, and resuspended in 700 ml of M9 minimal media containing methionine and 45 g/wl methionine (no IPTG). Seventy ml were removed as a pulse aliquot, the cells concentrated by centrifugation and frozen rapidly in a dry ice-ethanol bath. The remainder was aliquoted into 70 ml samples and incubated with agitator. At designated times after removal of the pulse sample, chase samples were spun, aspirated, and placed in a dry ice-ethanol bath. Subsequently, samples were immunoprecipitated as described below.

Immunoprecipitation: The cell pellets from the pulse-chase experiments were thawed, 20 ml of 50% S0, 10 mM Tris pH 8.0, 100 g/ml EDTA was added, and the sample was placed at 100°C for 4 min. Six ml of the boiled sample were then added to 650 g/ml 5% Triton X-100, 50 ml 0.5M Tris pH 8.0, 100 g/ml EDTA pH 8.0 and vortexed well, and antibody (1-8) was added to the mix. The mix was then incubated at 0°C for 2 hr. 50 ml of Step-A-Sepharose beads were then added and the mix was again incubated at 0°C for 30 minutes with gentle agitation every 5 min. The mixture was then washed twice with 50 ml 15% Trition X-100, 50 mM Tris pH 7.5, and 1 M NaCl, and once with 50 ml 10 mM Tris pH 8.0. 70 ml of SDS-PAGE sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2.5% SDS, 0.0625 M Tris pH 6.8, 0.005% bromphenol blue) were then added to the mix, the samples placed at 100°C for 5 min, centrifuged at 10,000g for 8 min, and then applied to an SDS-PAGE gel.

Densitometry: Quantitation of protein bands from autoradiograms was performed by densitometry using a Quick-Scan scanner (Helena Laboratories). Scan results (graphic) were photocopied, and the weight of peaks were used to compare the relative amount of protein in various bands.

Two-dimensional gel electrophoresis: Two-dimensional gel electrophoresis was performed according to the methods of O'Farrell (1975). In the first dimension (isoelectric focusing), ampholites were pH 3.5-10/10 pH 7.0-9.2, 1:2 (vol/vol/vol). The second dimension gel contained 10% acrylamide.

Growth Curves: Growth curves were performed in the standard fashion (Miller, 1972), with absorbance readings taken at a wavelength of 650nm.

Preparation of Protein Extract for VP1 Purification: 2 liters of strain RB791 containing the plasmid pVP1/1ac were grown at 37°C and at early log phase, IPTG was added to a final concentration of 0.05% w/v and the suspension sonicated at 200 watt-seconds in a Branson sonication unit for 30 s. The sonicated suspension was then incubated at 0°C for 70 min, at which time an equal volume of buffer A (pH 7.2/250 mM Nacalil1150 mM Tris pH 7.2, 5% glycerol, 0.5% EDTA, 150 mM 2-mercaptoethanol, 250 mM NaCl), 150g/ml sucrose, and the suspension incubated at 4°C for 20 min. Sodium dodecyl sulfate (10% w/v) was then added to a final concentration of 0.05% w/v and the suspension sonicated at 200 watt-seconds in a Branson sonication unit for 30 s. The sonicated suspension was then incubated at 0°C for 70 min. To each sample was added the following buffer B (pH 7.2/2500 mM Nacalil111500 mM Tris pH 7.2, 5% glycerol, 0.5% EDTA, 150 mM 2-mercaptoethanol) using a dounce homogenizer. After centrifugation at 6,000g for 10 min the pellet was re-extracted with 2 volumes of the same buffer and the supernatants combined. Amonium sulfate was slowly added to 35% saturation, the solution stirred for an additional 3 h, and then centrifuged at 8,000g for 15 min. The pellet was resuspended in 1 ml of buffer B (pH 7.2/25000 mM Nacalil and dialyzed against buffer B (pH 7.2/210000 Nacalil.

The dialyzed extract was centrifuged at 8,000g for 15 min, and the supernatant used as the protein extract for further purification.