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Structural Polypeptides of Simian Virus 40

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To determine the number and molecular weights of the structural polypeptides of simian virus 40, we have analyzed purified virus by electrophoresis on 14% polyacrylamide gels containing sodium dodecyl sulfate. Full virus purified by several different methods showed six distinct bands with molecular weights of approximately 43,000 (VP1, containing 70% of virion protein), 32,000 (VP2, 9%), 23,000 (VP3, 10%), 14,000 (VP4, 6%), 12,500 (VP5, 4%), and 11,000 (VP6, 3%) both by analysis of radioactively labeled virions and by visualization of the polypeptide bands after staining. "Empty" virions contain decreased amounts of VP4, 5, and 6. The approximate molecular ratios of the polypeptides were 6.0, 1.0, 1.5, 1.5, 1.1, and 1.0. When virus degraded in an alkaline buffer was analyzed by velocity centrifugation in sucrose gradients, the two larger polypeptides (VP1 and VP2) remained at the top of the gradient, whereas the three smallest polypeptides (VP4, 5, and 6) sedimented as a complex with the viral deoxyribonucleic acid. VP3 was found in association with either VP1 and 2 or VP4, 5, and 6, depending on the conditions of degradation. Presumably, VP1 and VP2, comprising about 80% of the protein, form the capsid of the virus. VP4, 5, and 6 may form a nucleoprotein in the virion, and VP3 may serve as an intermediate structural component.

Simian virus 40 (SV40) with deoxyribonucleic acid (DNA) of molecular weight 3.2×10^6 daltons (4) contains enough genetic information to code for only 1,500 amino acids or for three large (molecular weight 50,000) or six to eight small (molecular weight 20,000) proteins. Their functions are not yet defined, but they must at least include the structural proteins of the virus. We report here analyses of the polypeptide components of SV40 made by electrophoresis of purified virions on polyacrylamide gels containing sodium dodecyl sulfate. In contrast to the results of an earlier analysis made by a different technique (2) and in agreement with Girard's findings (9), we find six structural polypeptides ranging from 43,000 to 11,000. These values leave only about 25% of the viral genome available for coding of other than structural components. An analysis of the products of virus degraded in alkaline medium suggests that three of these polypeptides may be localized internally in the virion.

MATERIALS AND METHODS

Cell cultures. MA-134 (Microbiological Associates), Vero cells, and CV-1 cells, continuous lines of African green monkey kidney cells, were cultivated in roller bottles (surface area 10^3 cm) containing about 10^8 cells per monolayer for the production of virus. The cells were cultivated in Eagle's minimal essential medium (Grand Island Biological Co.) containing 10% fetal calf serum and 50 μg of neomycin per ml. All cells were free of mycoplasma.

Virus production. A large-plaque strain of SV40 (17) was propagated with an exposure multiplicity of 30 to 50 plaque-forming units (PFU)/cell. The crude virus seed, used at a dilution of 10^{-1} came from a single pool of virus (titer $10^{9.6}$ PFU/ml). After 90 min of adsorption at 37 C, medium containing 2% inactivated fetal calf serum was added. With advanced cytolysis in 5 to 7 days, the cell-culture fluids were harvested by freezing and thawing.

Preparation of radioactively labeled virus. Twenty-four hours after infection, the cells in plastic flasks (75 cm^2) were washed with phosphate-buffered saline (0.14 M NaCl in 0.01 M phosphate, *p*H 7.4), and 1 μCi of ^3H- or 0.5 μCi of ^{14}C-labeled reconstituted protein hydrolysate per ml was added in medium containing 0.1 the usual concentration of amino acids. After 48 hr, 5 ml of complete medium was added; the virus was harvested when cytolysis was evident in about 3 days. Amino acid mixtures with different compositions were used to label virus to insure that the amounts of radioactivity in the polypeptide peaks reflected the amounts of proteins in the virus rather than variations in the amino acid composition of the virions.

Virus purification. Four methods of virus purification were used. The final products contained between 1 and 2 mg of protein per ml as determined by the Lowry procedure (14) and had titers ranging from 10^{11} to 10^{12} PFU/ml. Two of the methods, which

follow standard procedures, are summarized as follows: I, concentrations with Carbowax 20 M, pelleting the virus at 90,000 × *g*, and isopycnic centrifugation in CsCl; II (A), pelleting is replaced by sedimentation through a sucrose gradient onto a cushion of CsCl; II (B), same as (A) but isopycnic banding is done both before and after the sedimentation step. Method III is that of Anderer et al. (12).

Method IV, which we prefer because it is rapid, efficient, and gives minimal losses of virus (78 to 85% recovery), is as follows. (i) Polyethylene glycol precipitation. To crude cell lysates, dry polyethylene glycol 6,000 (PEG) was added with stirring to 6% (w/v). After 2 hr at 4 C, the precipitate was collected by centrifugation for 30 min at 16,000 × *g*. The pellets were resuspended in 0.15 M NaCl buffered with 0.025 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.4 (TBS), at 0.2 to 0.1 the starting volume. The virus was eluted by stirring at 4 C overnight. After centrifugation for 10 min at 16,000 × *g*, the pellets were extracted twice more at 4 C for 2 hr. The supernatant fluids from the three extractions were pooled and dialyzed against TBS. After solid CsCl was added to give a density of 1.33 gm/cm^3, the virus-containing mixture was spun to isopycnic equilibrium. Full virion bands had a density of 1.34 gm/cm^3 and "empty" virion bands had a density of 1.30 gm/cm^3. The viral bands were collected, dialyzed against TBS, and sedimented through a 5 to 30% sucrose gradient on a CsCl cushion in 30% sucrose (ρ = 1.55).

(ii) Ion-exchange chromatography. The purified virus (1 mg) in 0.05 M Tris, *p*H 7.4, was placed on a diethylaminoethyl (DEAE) cellulose (Whatman microgranular DE-52, Cl^- form) anion-exchange column (0.6 by 15 cm) that had been equilibrated with 0.05 M Tris, *p*H 7.4. The column was developed by elution with 100 ml of a gradient of salt ranging from 0.01 to 0.5 M NaCl. The fractions (1 ml each) containing virus were concentrated by negative dialysis. The virus eluted at a concetration of approximately 0.15 M NaCl. The $A_{260/280}$ was 1.40 for full virions and 1.02 for empty virions.

Polyacrylamide gel electrophoresis. Purified virus was dialyzed against 0.03 M $NaHPO_4$, *p*H 7.4, and degraded at 40 C in 8 M urea, 1% SDS, and 1% β-mercaptoethanol for a minimum of 2 hr. Electrophoresis (5 ma per gel) was carried out as described by Maizel (15) in 14% neutral SDS-polyacrylamide gels (0.5 by 7 cm) with an acrylamide-bisacrylamide ratio of 10:1, polymerized with 0.14% $(NH_4)_2S_2O_8$. The gels and electrode buffer contained 0.1% SDS; Bromphenol blue (0.05%) was the tracking dye. The duration of electrophoresis was 5 to 7 hr. Approximately 50 to 200 μg of virus in 50-μliter volumes was applied to each gel. The gels were stained with Coomassie brilliant blue, destained 3 to 5 hr at 150 volts, and scanned with a Guilford spectrophotometer at 580 nm at a slit width of 0.2 by 2.36 mm.

Ovalbumin (molecular weight 45,000), chymotrypsinogen (molecular weight 25,000), myoglobin (molecular weight 17,800), and cytochrome *c* (molecular weight 12,400) were the standards for the molecular weights.

Gels of radioactive virus were fractionated into 0.8-mm slices and dissolved in 0.5 ml of 20% H_2O_2 at 37 C; the radioactivity was counted in Bray's solution in a liquid scintillation spectrometer. Counting data have been corrected for background radioactivity. Over 90% of the counts were recovered from the gels.

Alkaline degradation of virus. Purified virus was degraded in an alkaline buffer by the method of Anderer (1); virus solutions were dialyzed against Tris-ethanolamine buffer (*p*H 10.5) at 4 C for 20 hr. Virus was also degraded by dialysis against 0.15 M $NaHCO_3$ buffer (*p*H 10.5). The dissociated virus (2.5 ml) was layered on a 5 to 20% sucrose gradient in *p*H 9.0 buffer and centrifuged at 25,000 rev/min at 4 C for 8 hr in an SW27 rotor. Fractions were collected by removal from the top of the tube. Fractions were also dialyzed against deionized water, concentrated by lyopholization, and then reconstituted in buffer for gel electrophoresis.

RESULTS

Influence of method of purification on number of polypeptides. Figure 1 illustrates the results of electrophoresis of degraded virions purified by the procedures indicated earlier. In all cases, six similar bands are distinct. Pelleted virions show additional large polypeptides, probably cellular or serum contaminants. The faint second and fifth bands are visible only if at least 80 μg of protein is applied to the gels. The same six bands were also detected in an analysis of purified virions that had been propagated in Vero and CV-1 cells. The results were not affected by sonic treatment of the crude virus or by extraction with such lipid solvents as chloroform.

Figure 2 illustrates the results of electrophoresis of radioactively labeled PEG-purified virions. Again, six peaks are distinct. A slight shoulder off VP3 is also regularly present in labeled preparations but is rarely visible in the stained gels; its significance is unknown. Peaks containing larger polypeptides are sometimes detected in labeled preparations, but these are believed to be contaminants.

Molecular weights of SV40 virion polypeptides. Estimates of the molecular weights of the viral proteins were made by comparison with proteins of known molecular weights, electrophoresed under identical conditions (5, 18) with the standard curve shown in Fig. 3.

In Table 1 are given representative molecular weights determined from virions purified by the different methods. Six polypeptides were detected regularly regardless of the method of purification. Full and empty virions contain the same six polypeptides; however, VP4, 5, and 6 are decreased in amount in empty virions. Pelleting of virions either by method I or III leaves larger polypep-

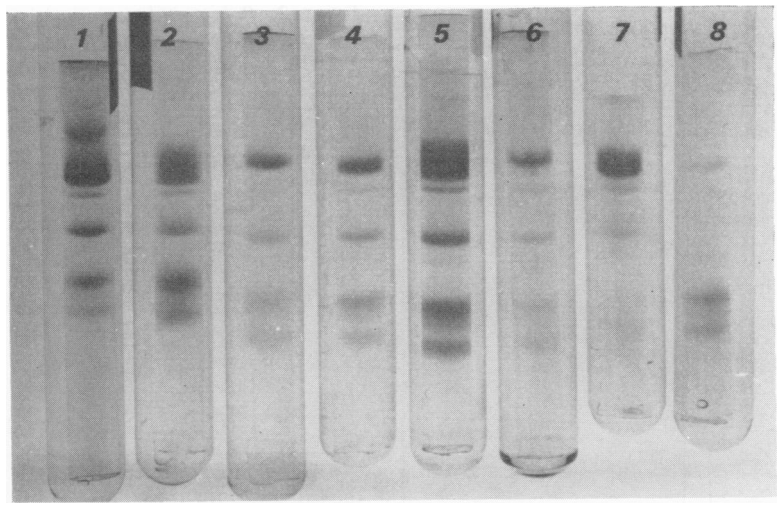

FIG. 1. *Electrophoresis of SV40 on SDS-polyacrylamide gels. Examples of the results of analyses of virus purified by different methods; our preferred method is (5). (1) Pelleted virus. (2) Anderer's procedure. (3) Nonpelleted virus. (4) PEG method. (5) PEG and DEAE cellulose method. (6) Empty virions. Analyses of polypeptides from the experiment described in Figure 4 and the text: protein (7) and protein-DNA (8) containing fractions from virus degraded in alkaline buffer and fractionated by centrifugation on a sucrose gradient. The three smallest polypeptides are associated with the DNA.*

TABLE 1. *Sodium dodecyl sulfate polyacrylamide gel electrophoresis of SV40 virion polypeptides*

Method of purification	Molecular weight (× 10^3 daltons)									
Pelleting										
Full virions	90	80	72	59	45	31	24	15	—[a]	11.5
'Empty' virions				60	46	33	24	14	—[a]	11
Nonpelleting										
Sedimentation step first										
Full virions					41	30	23	14	—[a]	11.5
Full virions					42	31	24	14	12	11
Isopycnic step first										
Full virions					45	34	26	16	13	12
Empty virions					42	32	22	14	—[a]	11
Anderer's method										
Full virions					42	32	24	15.5	—[a]	11
Full virions					40	30	23	14	—[a]	10
Full virions				60	45	34	25	15.5	13	11
Polyethylene glycol precipitation										
Full virions					42	32	24	15	12.5	11
Empty virions					43	32	23	14	12	11

[a] In early experiments with small amounts of viral protein, VP5 was not seen.

tides that are almost certainly host-cell contaminants associated with the virions.

Effect of different methods of virus degradation. The gel patterns were not altered by heating the virus at 100 C for 10 min, by higher concentrations of SDS up to 2.5%, or by the addition of 2 M urea to the gel or the electrode buffer. However, without β-mercaptoethanol for degradation, additional bands of different molecular weights including 80,000, 60,000, and 25,000 appeared; these bands presumably were aggregates of the polypeptides.

Molecular ratios of polypeptides in virus. The percentage of each of the six polypeptides in the virion was determined by analysis of ^{14}C-labeled virus (five determinations) as well as by optical scanning (Table 2) VP1 and VP2 are closely adjacent making separation difficult, which probably accounts for the greater variation in these two polypeptides than in those of the other four. The

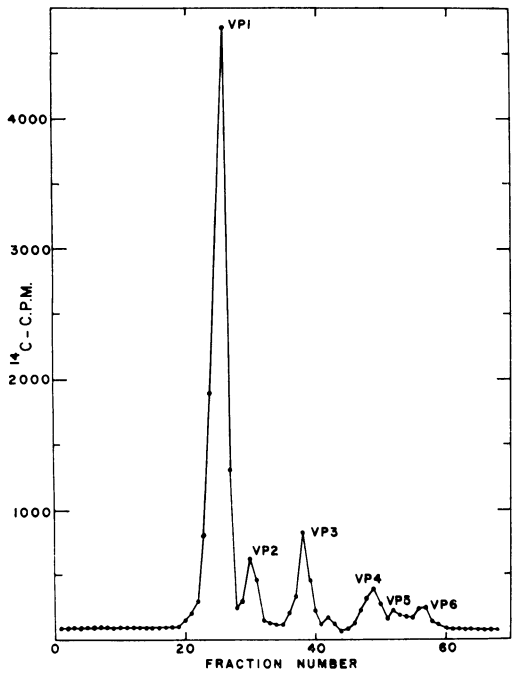

FIG. 2. *Polyacrylamide gel electrophoresis of ^{14}C-labeled protein from purified SV40. The virus, purified by method III, was degraded in 8 M urea, 1% β-mercaptoethanol, and 1% SDS and electrophoresed in 14% polyacrylamide gel, pH 7.4, containing 0.1% SDS. Gels were frozen and sliced, and the fractions were dissolved in 30% H_2O_2 at 60 C and counted in Bray's solution. Migration is from left to right.*

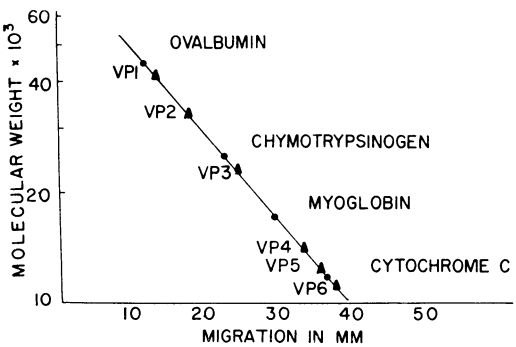

FIG. 3. *Migration of purified SV40 and standard proteins in SDS polyacrylamide gels as a logarithmic function of molecular weight. Standard proteins (●), ovalbumin (45,000), chymotrypsinogen (25,000), myoglobin (17,800), and cytochrome c (12,400) were electrophoresed under identical conditions as purified SV40 virion protein (▲) in 14% polyacrylamide-0.1% SDS gels which were then stained with Coomassie blue. The positions of the proteins were determined by scanning the stained gels at a wavelength of 580 nm in a Gilford model 240 spectrophotometer.*

TABLE 2. *Polypeptide composition of SV40: percentages of virion protein as determined from virus labeled with ^{14}C-mixed amino acids or by optical scanning*

Virion polypeptide	Per cent ^{14}C-labeled		Optical scans (%)[a]	
	Full virions	Empty virions	Full	Empty
VP1	71 ± 4			
VP2	9 ± 3			
VP1 + 2	78 ± 3	88 ± 2	61 ± 4	75 ± 5
VP3	10 ± 2	9 ± 2	12 ± 2	16 ± 3
VP4	6 ± 1			
VP5	4 ± 1			
VP6	3 ± 1			
VP4 + 5 + 6	12 ± 1	3 ± 2	25 ± 3	9 ± 2

[a] These values are the averages of 10 scans for the full and 5 for the empty. Scans are of gels with protein concentrations of VP1 that did not exceed the optical density range of the spectrophotometer. Such gels exhibited very faint VP2 and VP5 bands so the values for the individual polypeptides were not calculated.

total of VP1 plus VP2 is nearly constant. Ratios determined after analysis of alkaline-degraded virus agree with these results (Fig. 4). Less than 3% of the total counts may remain at the top of the gel. These counts represent viral proteins bound to the DNA. They can be removed by treating degraded virions with deoxyribonuclease. Deoxyribonuclease digestion, however, does not significantly change the polypeptide percentages.

The ratios determined from optical scanning of the stained gels are less accurate because of residual dye after decolorization, because protein concentrations high enough to distinguish clearly the six polypeptides exceed the optical density range of the spectrophotometer, and because the amount of absorbance of protein stained with Coomassie blue obeys Beer's law up to 20 μg of protein in a band (7). In stained preparations where all six polypeptides were distinguishable, the concentration of VP1 exceeded 20 μg. This would explain the lower percentages of VP1 plus VP2 calculated from scans compared with the values from labeled preparations. Also, the avidity of the polypeptides for the dye probably differs.

The approximate molecular ratios of the polypeptides were calculated from the averages of five determinations from ^{14}C-labeled virus (Table 3). We assumed that the radioactivity in each peak reflects directly the amount of polypeptide. We calculated the approximate number of polypeptide chains by assuming that the molecular weight of the total protein of the virion was 20×10^6.

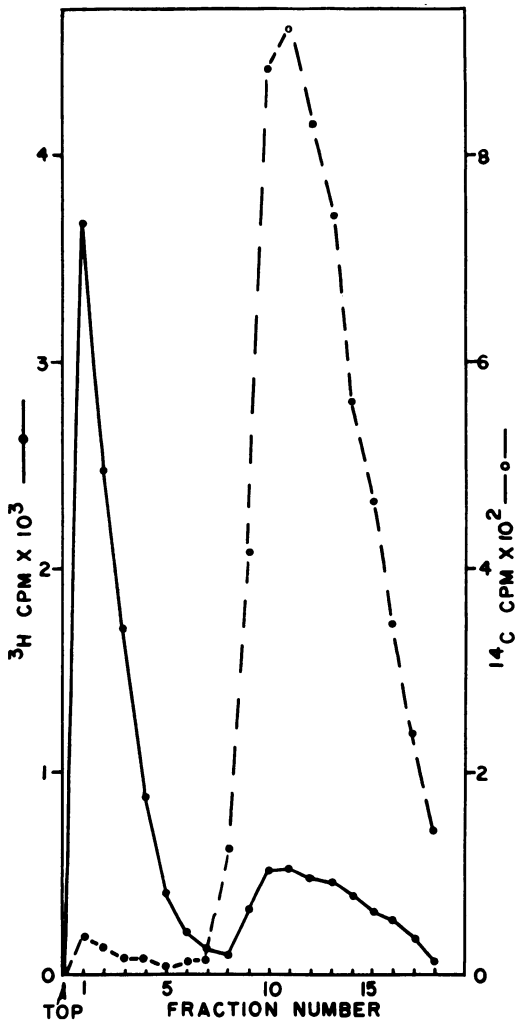

FIG. 4. *Velocity sedimentation of alkaline degraded SV40. Purified SV40 doubly labeled with ^{3}H-amino acids (1 μCi/ml) and ^{14}C-thymidine (0.05 μCi/ml) was degraded in pH 10.5 Tris-ethanolamine buffer for 20 hr at 4 C. The samples were layered on a 5 to 20% sucrose gradient, pH 9.0, and then centrifuged for 6.5 hr at 25,000 rev/min in an SW27 rotor at 4 C. The peak at the top of the gradient contains 70% of the viral protein and virtually no DNA, whereas the faster sedimenting peak contains about 30% of the viral protein and all of the viral DNA. Peak 1 contains VP1, 2, and some 3; peak 2 contains VP4, 5, 6, and some 3. See Fig. 1 (7 and 8) for photographs of gels.*

Polypeptides of full and "empty" virions. Analyses of full and empty virions show that there is a decreased amount of VP4, VP5, and VP6 in empty virions (Fig. 1). Although we do not propagate virus to enrich for defective particles, the analyses of empty virions is complicated by the fact that partly empty virions may be trapped in the band of empty virions despite their lesser density. Light pseudovirions also band with shells, but our preparations of virus made in MA-134 cells are devoid of pseudovirions as indicated by the absense of 14*S* DNA in phenol extracts of the DNA analyzed by analytical ultracentrifugation.

Because of the problems discussed above with scanning, the results of labeled virions cannot be compared directly with the scan results, but the full and empty scan results can be compared.

TABLE 3. *Relative proportions of the polypeptides of SV40*

Virion polypeptide	Molecular weight	Percentage of virion protein[a]	Approximate molecular ratio[b]	Approximate no. of chains per virion[c]
VP1	43,000 ± 2,000	71 ± 4	6.0	320
VP2	32,000 ± 2,000	9 ± 3	1.0	54
VP3	23,000 ± 1,100	10 ± 2	1.5	80
VP4	14,000 ± 800	6 ± 1	1.5	80
VP5	12,500 ± 500	4 ± 1	1.1	60
VP6	11,000 ± 500	3 ± 1	1.0	54

[a] Counts in each peak are expressed as a percentage of the total counts recovered from the gels.
[b] Calculated by dividing the average percentage of the virion protein by the average molecular weight of the corresponding polypeptide. The value obtained for VP2 was taken as 1.
[c] Calculated by assuming that the molecular weight of the total virion protein was 20 × 10^6.

Polypeptides associated with viral DNA. When virus labeled with ^{14}C-thymidine and ^{3}H-amino acids was degraded in an alkaline buffer and sedimented in a 5 to 20% sucrose gradient, two peaks were detected (Fig. 4). After degradation, 79% of the ^{14}C and 70% of the ^{3}H label in the virus were recovered in the gradient fractions. The peak sedimenting more slowly contained most (71%) of the ^{3}H counts, whereas the faster sedimenting material contained almost all of the ^{14}C label and only 28% of the ^{3}H counts after corrections for crossover. The second peak is broad, but its position estimated from 20*S* DNA used as a marker corresponds to the 46*S* value assigned it by Anderer (1). Degradation of the virus in either the Tris or the bicarbonate buffers gave essentially the same results.

Electrophoretic analysis of the slow peak revealed VP1 and VP2, whereas the DNA-containing peak contained VP4, VP5, and VP6 (Fig. 1, gels 7 and 8). VP3 was found in association with either VP1 and 2 or VP4, 5, and 6, depending on the conditions of degradation. Higher salt concentration appears to effect more complete degra-

dation, VP3 being associated with VP1 and VP2. VP5 is especially distinct in these analyses since 70 μg of only the DNA-associated polypeptides was applied to the gels. There was slight contamination of the DNA-associated protein with VP1 and VP2. One interpretation of these findings is that VP1 and VP2 are capsid polypeptides; they comprise about 80% of the virion protein. The smaller three polypeptides, making up about 13% of the virion protein, may be internal polypeptides as suggested by their persistent association with the viral DNA, and VP3 may be an intermediate structural component. However, after different degradation experiments, the so-called 46*S* component does not always appear as a homogeneous peak; it is sometimes rather broad. It may be that the DNA-polypeptide complex does not exist as a nucleoprotein structure in the virion but forms upon its disruption.

The infectivity of the DNA-polypeptide complex is a negligible fraction ($\sim 10^2$ PFU/ml) of the infectivity of the undegraded virus (10^{11} PFU/ml). The infectivity of the degraded material is increased 1,000- to 10,000-fold in the presence of DEAE dextran to about 10^6 PFU/ml (17). On electron microscopy, no virus particles are visible in this material.

DISCUSSION

These results indicate that the protein moiety of SV40 consists of one major polypeptide of molecular weight 43,000 and at least five other polypeptides of lower molecular weights. These results differ from those of Anderer et al. (2) who analyzed degraded virions by sedimentation velocity and by thin-layer electrophoresis and found three polypeptides, each of approximately 16,000 molecular weight. Our values for the molecular weights are slightly lower than Girard's (9), perhaps because of different standards. Our molecular weights for the three smaller polypeptides are probably more accurate since we used enough virus to permit analysis by staining gels as well as by counts. The resolution of these smaller components is also increased in 14% gels as is the determination of their molecular weights (5).

With polyoma virus, Kass (11) found a major polypeptide of molecular weight 45,000 and a possible minor polypeptide, but the methods were probably not sensitive enough to resolve other minor polypeptides. Hirt (Le Petit Colloquium, *in press*) has identified the major polyoma polypeptide by another method, guanidine hydrochloride-agarose chromatography. Its molecular weight was 43,000; there were several smaller unresolved polypeptides in the preparations. He has also identified five polypeptides with SDS polyacrylamide gels.

The method of purification of virions is important. We avoid pelleting of virions and prefer to precipitate virus with polyethylene glycol. With SV40, this procedure (method IV) takes 2 days for the concentration of 2 liters of crude virus to 4 ml of purified virus with only 10 to 20% loss, as measured by assays of infectivity (Pagano and Hutchinson, *in preparation*). A similar method has been published recently (8). We worked with a high concentration of pure viral protein, facilitated by the use of MA-134 cells which give a high yield of virus, with little of the adherence to membranes described with other cell lines (3). The high concentrations permitted the resolution of two proteins (VP2 and VP5) not seen at first. Resolution of the polyacrylamide gel system is limited by the requirement for approximately 70 μg to detect the minor bands. Perhaps additional minor SV40 polypeptides will be distinguished with better methods or with even greater concentrations of protein; recently we have detected a consistent trace polypeptide with a molecular weight of about 19,000 in labeled virions.

There are many questions as to the significance of these six isolated polypeptides. The large protein of molecular weight of 43,000 is the major viral component comprising about 70% of the virion protein. The alkaline degradation experiments suggest that VP1 and VP2 may be the capsid polypeptides. If one assumes that migration of proteins with the DNA implies a similar association in the virus, then VP4, 5, and 6 may be internal. VP3, which sediments either with VP1 and 2 or with VP4, 5, and 6, may serve as an intermediate structure. We are attempting to localize the polypeptides in the virions and to distinguish whether the nucleoprotein-DNA complex is merely a complex formed on disruption of the virus or a real structural element.

The significance of the nucleoprotein-DNA complex is unknown. The origin of VP4, 5, and 6 is of interest since the six polypeptides described leave only 25% of the SV40 genome to code for other than structural components of the virion. These DNA-associated proteins may be of host cell origin, in which case the number and quantity of them may vary. The incorporation of membranous proteins of host origin into viruses is, of course, commonplace, but histones or other basic proteins of cellular origin have not been identified in animal viruses. Reliable molecular weights of cell histones have been determined for F_2b (13,800; reference 10), F_1 (21,000; reference 19), and F_3 (20,000; reference 6). The possibility is relevant since pseudovirions containing host cell DNA comprise a variable proportion of purified polyoma and SV40 preparations (13, 16, 20). Our virus preparations do not contain detectable

pseudovirions and this fact helps to define the system for the investigation of the origin of the minor protein components of SV40.

The observation that the three minor polypeptides are present in reduced amounts in "empty" virions supports the notion that one or more of these three polypeptides form a viral nucleoprotein. These polypeptides may enter the virion with the DNA during its packaging in the capsid, or they may aid in the packaging itself. We have recently demonstrated that VP4, 5, and 6 have a pronounced effect on the transcription of the SV40 genome in vitro (E. S. Huang, et al., *manuscript in preparation*).

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LITERATURE CITED

1. Anderer, F. A., M. A. Koch, and H. D. Schlumberger. 1968. Structure of simian virus 40. III. Alkaline degradation of the virus particle. Virology **34**:452–458.
2. Anderer, F. A., H. D. Schlumberger, M. A. Koch, H. Frank, and H. J. Eggers. 1967. Structure of simian virus 40. II. Symmetry and components of the virus particle. Virology **32**:511–523.
3. Black, P. H., E. M. Crawford, and L. V. Crawford. 1964. The purification of simian virus 40. Virology **24**:381–387.
4. Crawford, L. V., and P. H. Black. 1964. The nucleic acid of simian virus 40. Virology **24**:388–392.
5. Dunker, A. K., and R. R. Rueckert. 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. **244**:5074–5080.
6. Fambrough, D. M., and J. Bonner. 1968. Sequence homology and role of cysteine in plant and animal arginine-rich histones. J. Biol. Chem. **243**:4434–4439.
7. Fazekas De St. Groth, S., R. G. Webster, and A. Datyner. 1963. Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. Biochim. Biophys. Acta **71**:377–391.
8. Friedmann, T., and M. Haas. 1970. Rapid concentration and purification of polyoma virus and SV40 with polyethylene glycol. Virology **42**:248–250.
9. Girard, M., L. Marty, and F. Suarez. 1970. Capsid proteins of simian virus 40. Biochem. Biophys. Res. Commun. **40**:97–102.
10. Iwai, K., K. Ishikawa, and H. Hayashi. 1970. Amino-acid sequence of slightly lysine-rich histone. Nature (London) **226**:1056–1058.
11. Kass, S. J. 1970. Chemical studies on polyoma and shope papilloma viruses. J. Virol. **5**:381–387.
12. Koch, M. A., H. J. Eggers, F. A. Anderer, H. D. Schlumberger, and H. Frank. 1967. Structure of simian virus 40. I. Purification and physical characterization of the virus particle. Virology **32**:503–510.
13. Levine, A. J., and A. K. Teresky. 1970. Deoxyribonucleic acid replication in simian virus 40-infected cells. II. Detection and characterization of simian virus 40 pseudovirions. J. Virol. **5**:451–457.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–280.
15. Maizel, J. V. 1969. Acrylamide gel electrophoresis of proteins and nucleic acids, p. 334–362. *In* K. Habel and N. P Salzman (ed.), Fundamental techniques in virology. Academic Press Inc., New York.
16. Michel, M. R., B. Hirt, and R. Weil. 1967. Mouse cellular DNA enclosed in polyoma viral capsids (pseudovirions). Proc. Nat. Acad. Sci. U.S.A. **58**:1381–1388.
17. Pagano, J. S. 1969. Assay of infectious DNA, p. 184–197. *In* K. Habel and N. P. Salzman (ed.), Fundamental techniques in virology. Academic Press Inc., New York.
18. Shapiro, A. L., E. Vinuela, and J. V. Maizel. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. **28**:815–820.
19. Teller, D. C., J. M. Kinkade, and R. D. Cole. 1965. The molecular weight of lysine-rich histone. Biochem. Biophys. Res. Commun. **20**:739–744.
20. Winocour, E. 1969. Some aspects of the interaction between polyoma virus and cell DNA. Advan. Virus Res. **14**:153–200.