JOURNAL OF VIROLOGY, Mar. 1977, p. 1079-1084
Vol. 21, No. 3

Printed in U.S.A.

Characterization of Components Released by Alkali Disruption of Simian Virus 40

GUNNA CHRISTIANSEN, TERRY LANDERS, JACK GRIFFITH,* AND PAUL BERG
Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Received for publication 21 September 1976

Treatment of simian virus 40 (SV40) particles at pH 9.8 in the presence of 1 mM dithiothreitol for 5 min at 37°C disrupted the virions into a 60S DNA-protein complex and DNA-free 7S protein particles. The DNA-protein complex contained approximately equal amounts of DNA and protein, and appeared by electron microscopy to be relaxed circular structures with an average of 21 beads joined by short, thin bridges. The major protein components in the complex were host cell histones, but SV40-specific proteins VP3 and VP2 were also present. The 7S protein particles were almost exclusively VP1 and, in negatively stained samples, resembled the capsomer structures of intact virions.

Aside from the icosahedral arrangement of capsomers in simian virus 40 (SV40) virions, little is known about how the individual capsid polypeptides, the DNA, and the internal proteins are organized in the virions. It is clear, however, that the 5,100-base pair double-stranded circular DNA is associated with four histones, H2A, H2B, H3, and H4 (8), and that this nucleoprotein is enclosed in a capsid consisting of 72 subunits constructed from the virus-coded polypeptides VP1, VP2, and VP3 (1, 3).

SV40 DNA occurs in the nucleus of infected cells as a complex that contains equal amounts of DNA and histones and closely resembles cellular chromatin in its beaded appearance (5, 9, 13). However, Huang et al. (6) found that disruption of SV40 virions by dialysis at pH 10.5 (24 h at 0°C) released a nucleoprotein complex that subsequently was found to have half as much histone as DNA (11). We have re-investigated the composition and structure of the intravirion and intranuclear SV40 nucleoproteins to determine whether the two are indeed different. When SV40 virions are treated at lower pH than used by Huang et al. (6) (0.1 M glycine [pH 9.8] plus 1 mM dithiothreitol [DTT] for 5 min at 37°C), the viral DNA is released as a circular chromatin-like complex that is indistinguishable by electron microscopy (EM) from the intranuclear forms described earlier (5).

MATERIALS AND METHODS

Virus and cells. SV40 (strain *wt*830 obtained from SVS of Takemoto [12]) was grown on CV1 cells in Dulbecco modified Eagle medium (MEM) with 2% fetal calf serum. Cells were infected at an input multiplicity of 1 to 5 PFU/cell and harvested 5 to 8 days after infection.

Virus purification: Method A. Method IV of Estes et al. (2) was modified as follows. Virions were concentrated from a crude freeze-thawed lysate by mixing with 7% (wt/vol) polyethylene glycol 6000 and stirring overnight. The precipitated virions were centrifuged, and the pellet was extracted three times for 12 h with 0.15 M NaCl-0.025 M Tris (pH 7.5). The combined supernatants were layered over two 3-ml steps of CsCl (1.30 and 1.33 g/ml) and centrifuged in an SW27 rotor for 3 h at 25,000 rpm (4°C).

Virus purification: Method B. An infected-cell pellet was frozen and the virions were extracted according to Ozer (10). The pellet was thawed in 0.01 M sodium phosphate (pH 7.2) (0.5 ml/plate) and sonicated by three 30-s bursts with a Heat Systems microtip. The debris was spun out, and Nonidet P-40 was added to the supernatant to 1%. This solution was layered over 1 ml of 15% sucrose in 0.01 M sodium phosphate (pH 7.2)-0.15 M NaCl, which had been layered over CsCl (1.33 g/ml) 0.01 M sodium phosphate (pH 7.2) in an SW50.1 rotor tube. The samples were centrifuged for 80 min at 40,000 rpm. The opalescent band of virions below a band of empty capsids was removed through the side of the tube with a syringe. Virions produced by either method were found to contain less than 5% nonvirion proteins upon acrylamide gel electrophoresis.

Radioactively labeled virions. Virions containing [^{3}H]thymidine-labeled DNA were prepared by adding [^{3}H]thymidine to the cultures 3 days after infection (50 μCi/plate) and isolating virus particles by method A. Virions with [^{35}S]methionine-labeled proteins were isolated from infected cells exposed to 2 mCi of [^{35}S]methionine per plate (in 6 ml of MEM lacking methionine, with 2% dialyzed calf serum) for 18 to 24 h before the cells were harvested. Particles were purified by method B. Approximately 75% of the ^{35}S label in the virions was in VP1.

Disruption of SV40 virions in alkaline pH. SV40 virions were disrupted by three methods. The first was dialysis against 0.15 M sodium carbonate (pH 10.5) for 24 h at 4°C, as described by Huang et

al. (6). In the second, equal volumes of virions in 0.01 M Tris (pH 7.5)-0.15 M NaCl were mixed with 0.2 M glycine (pH 9.8)-1 mM DTT. After 5 min at 37°C, the suspension was chilled and centrifuged immediately as described below. The third method was identical to the second but with glycine at pH 9.2.

Sucrose gradient centrifugation. Alkali-treated virions were layered over 5 to 20% sucrose gradients containing 0.1 M NaCl, 0.01 M Tris (pH 7.5), 1 mM EDTA, and 0.25% Triton X-100 and were centrifuged for 60 min at 50,000 rpm (4°C) in an SW56 rotor. Sedimentation coefficients were determined by comparison to SV40 form 1 DNA (21*S*).

EM. DNA protein samples were fixed in buffers containing 0.02 M sodium phosphate (pH 7.5) and 0.01 M NaCl by the addition of 10% formaldehyde to 1% for 15 min on ice, followed by the addition of 6% glutaraldehyde to 0.6% for 15 min on ice. Fixed samples were mounted onto glow-charged, carbon-supporting films, were dehydrated, and were shadowed with tungsten (4). Alternatively, samples were stained with freshly prepared 1% uranyl acetate and blotted dry. A Philips EM 300 was used at 20 and 40 kV.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoretic separation of viral proteins was carried out on flat-bed acrylamide gels (14 by 14 by 0.16 cm) containing linear gradients of 7.5 to 15% acrylamide in 0.42 M Tris (pH 8.8 at 1.5 M) at 120 to 150 V for 3 to 3.5 h (7).

RESULTS

Sedimentation analysis of alkali-disrupted virus. After incubation of SV40 virus at pH 9.2 for 5 min at 37°C, the labeled capsid proteins cosedimented with the viral DNA at about 90*S* (Fig. 1A). Intact virions sediment at 250*S*. When virus particles were exposed to pH 9.8 for 5 min at 37°C, the viral DNA sedimented at approximately 60*S* and was associated with only a small fraction of the viral protein; the majority of the labeled protein sedimented at 7*S* (Fig. 1B). Treatment of the virus at pH 10.5 by the method of Huang et al. (6) produced a broad peak of viral DNA at about 30*S*; most of the labeled proteins were at the top of the gradient (Fig. 1C).

The DNA-protein complex isolated from infected-cell nuclei (4) sediments in a broad peak at about 75*S*, whereas the complex we obtained by disruption of virus particles at pH 9.8 sedimented at 60*S*. This difference may be due to the association of some histone H1 in the complex obtained directly from infected cells. Histone H1 has not been detected by polyacrylamide gel electrophoresis of whole virions. In 0.6 M NaCl, which removes histone H1 but not other histones from DNA, the SV40 DNA-protein complex obtained from cells also sedimented at 60*S* (Fig. 2).

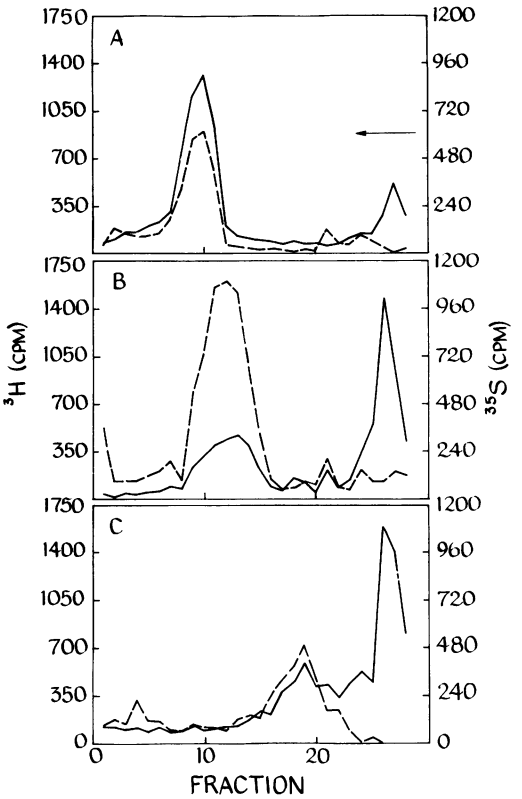

FIG. 1. *Sedimentation of alkali-disrupted virions in neutral sucrose gradients. SV40 virus particles with the viral DNA (^{3}H) and protein (^{35}S) labeled (75% of the ^{35}S was in VP1) were exposed to (A) 0.1 M glycine (pH 9.2)-1 mM DTT for 5 min at 37°C or (B) 0.1 M glycine (pH 9.8)-1 mM DTT for 5 min at 37°C; or (C) were dialyzed against 0.15 M sodium carbonate (pH 10.5) for 24 h at 4°C (8). Samples were layered immediately over 5 to 20% sucrose gradients with 0.1 M NaCl and centrifuged for 60 min at 50,000 rpm (4°C) in an SW56 rotor.* (- - -) ^{35}*S*; (——) ^{3}*H*.

Visualization of SV40 DNA-protein complexes by EM. Fractions containing DNA from the sedimentations shown in Fig. 1A, B, and C were examined by EM, using a two-step fixation procedure that does not alter the sedimentation behavior of SV40 chromatin but stabilizes it for various manipulations and treatments used in microscopy (G. Christiansen and J. Griffith, unpublished data). The DNA-protein complexes obtained at pH 9.2 (90*S* peak in Fig. 1A) were dense, rod-shaped structures (Fig. 3A), whereas complexes obtained at pH 10.5 (Fig. 1C) appeared as loops of a DNA-like fiber containing occasional beaded structures (Fig. 3B). Material obtained after treatment of virions at pH 9.8 (Fig. 1B) appeared as a con-

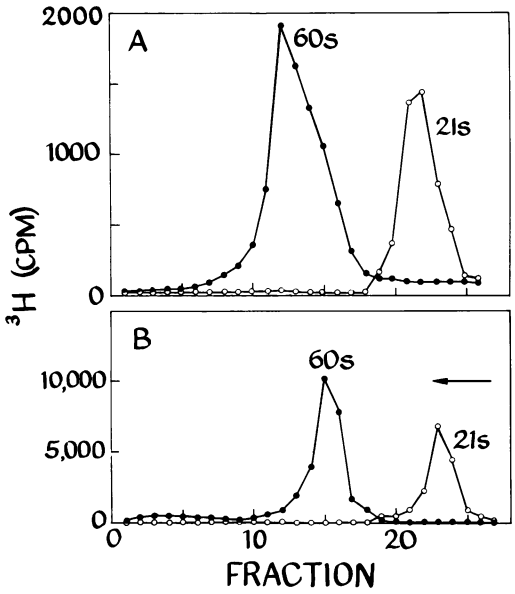

FIG. 2. *Sucrose sedimentation of complexes of virus and cell origin. (A) [^{3}H]thymidine-labeled SV40 virions were treated at pH 9.8, as described in the legend of Fig. 1-(B), and centrifuged. (B) SV40 chromatin isolated from nuclei of productively infected cells (labeled with [^{3}H]thymidine) (5) were sedimented as in (A) but with 0.6 M NaCl present to remove traces of histone H1. Open circles correspond to SV40 form I DNA centrifuged in parallel gradients.*

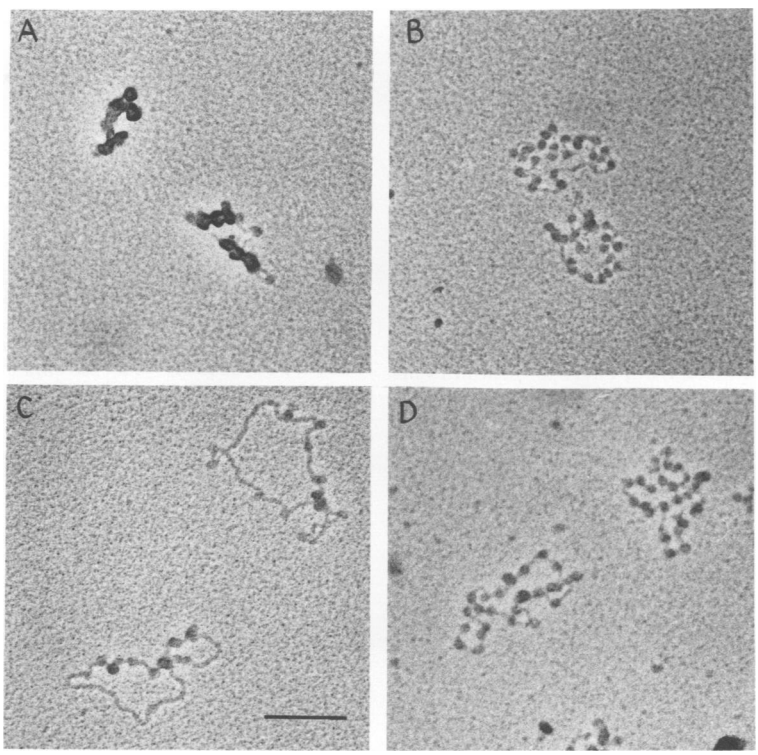

FIG. 3. *Visualization of SV40 complexes of virus and cell origin. Complexes were isolated by sucrose gradient centrifugation, fixed, and prepared for EM as described in the text. (A) Virus complexes derived from pH 9.2 treatment, (B) pH 10.5 treatment, and (C) pH 9.8 treatments. (D) Complexes isolated from nuclei of infected cells, depleted of histone H1. Bar represents 0.1 μm.*

tinuous, beaded structure with short, thin bridges (Fig. 3C). DNA-protein complexes produced at pH 9.8 were indistinguishable by EM from the SV40 chromatin obtained from nuclei of productively infected cells and treated with 0.6 M NaCl to remove traces of histone H1 (Fig. 3D).

Direct counts of the number of beads in complexes obtained from virions by treatment at pH 9.8 and from infected cells are summarized in Table 1. Both complexes had an average of 21 beads (range, 18 to 26) of 11- to 12-nm diameter; this is essentially the same value reported previously for the cell-derived complex (5). Complexes obtained from virions by alkali disruption at pH 10.5 have fewer and a more variable number of beads per DNA molecule.

Composition of the virus-derived DNA-protein complexes. The ratio of DNA to protein in each complex was determined from the buoyant density of material that had been fixed with formaldehyde and glutaraldehyde (Table 1). The complex liberated from virions at pH 9.2 had a protein-to-DNA ratio of 2, whereas the complexes obtained at pH 9.8, as well as the cell-derived complexes, had equal amounts of protein and DNA. The complex generated at pH 10.5 contained only half as much protein as DNA.

The proteins of the complex obtained from virions treated at pH 9.8 were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and compared with those of intact virions (Fig. 4). Gel A shows the band pattern of proteins from intact SV40 virions. The three upper bands are the virus-coded capsid proteins VP1, VP2, and VP3, and the lower bands are cellular histone proteins, H2A, H2B, H3, and H4. Gel B shows the pattern of the proteins in the complex obtained from sucrose gradients of the pH 9.8-treated virions. In addition to the four histones, there are clearly some VP3 and traces of VP1 and VP2.

Examination of the protein particles released by pH 9.8 treatment. The treatment of virus particles at pH 9.8 released, in addition to the DNA-protein complex, a slow-sedimenting, discrete peak of protein. Sedimentation for longer time (Fig. 5) showed that the DNA-free

TABLE 1. *Direct counts of beads in complexes*

Complex	Density[a] (g/cm³)	S value[b]	No. of "beads" (range, avg, no. counted)
Cell derived[c]	1.46	60 ± 5	18–26, 21, 40
Virus-derived			
pH 9.2	1.40	90 ± 5	Not beaded
pH 9.8	1.46	60 ± 5	18–26, 21, 26
pH 10.5	1.55	30 ± 5	3–11, 8, 30

[a] Measured by banding in CsCl after fixation with formaldehyde and glutaraldehyde.
[b] Measured by comparison with SV40 I DNA (21S).
[c] Purified from nuclei of productively infected cells and treated with 0.6 M NaCl to remove histone H1.

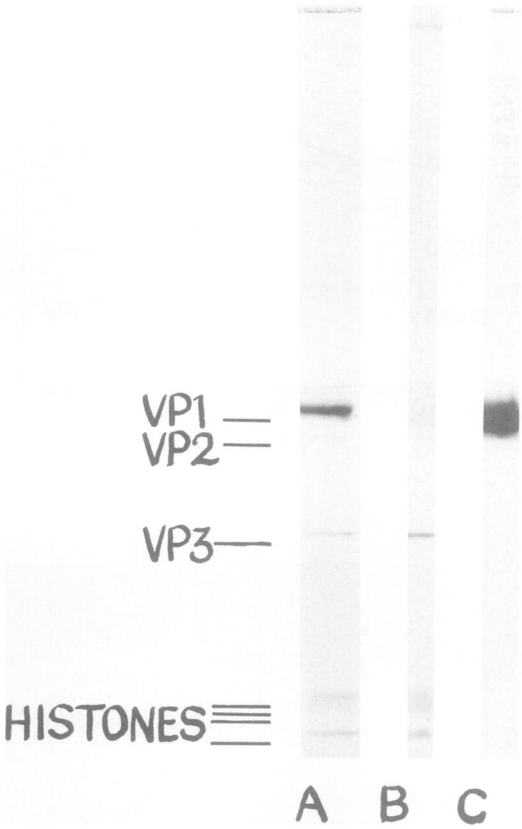

FIG. 4. *(A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins from purified SV40 virions. The bands (top to bottom) are VP1, VP2, VP3, and the four cell histones. (B) SV40 complex prepared from virions after pH 9.8 treatment. (C) Purified 7S protein particles obtained from virions after pH 9.8 treatment.*

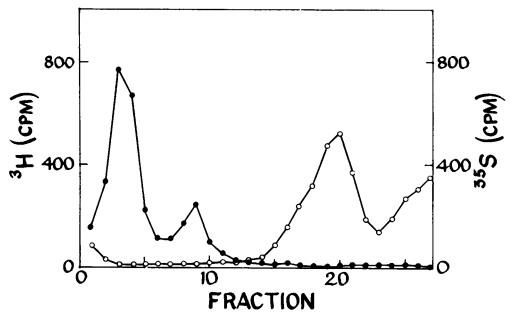

FIG. 5. *Sucrose gradient sedimentation of SV40 capsid proteins (^{35}S labeled) released after treatment at pH 9.8 (○). SV40 (I) and (II) DNA were run in a parallel gradient (●). Sedimentation was for 5 h at 55,000 rpm (4°C) in an SW56 rotor.*

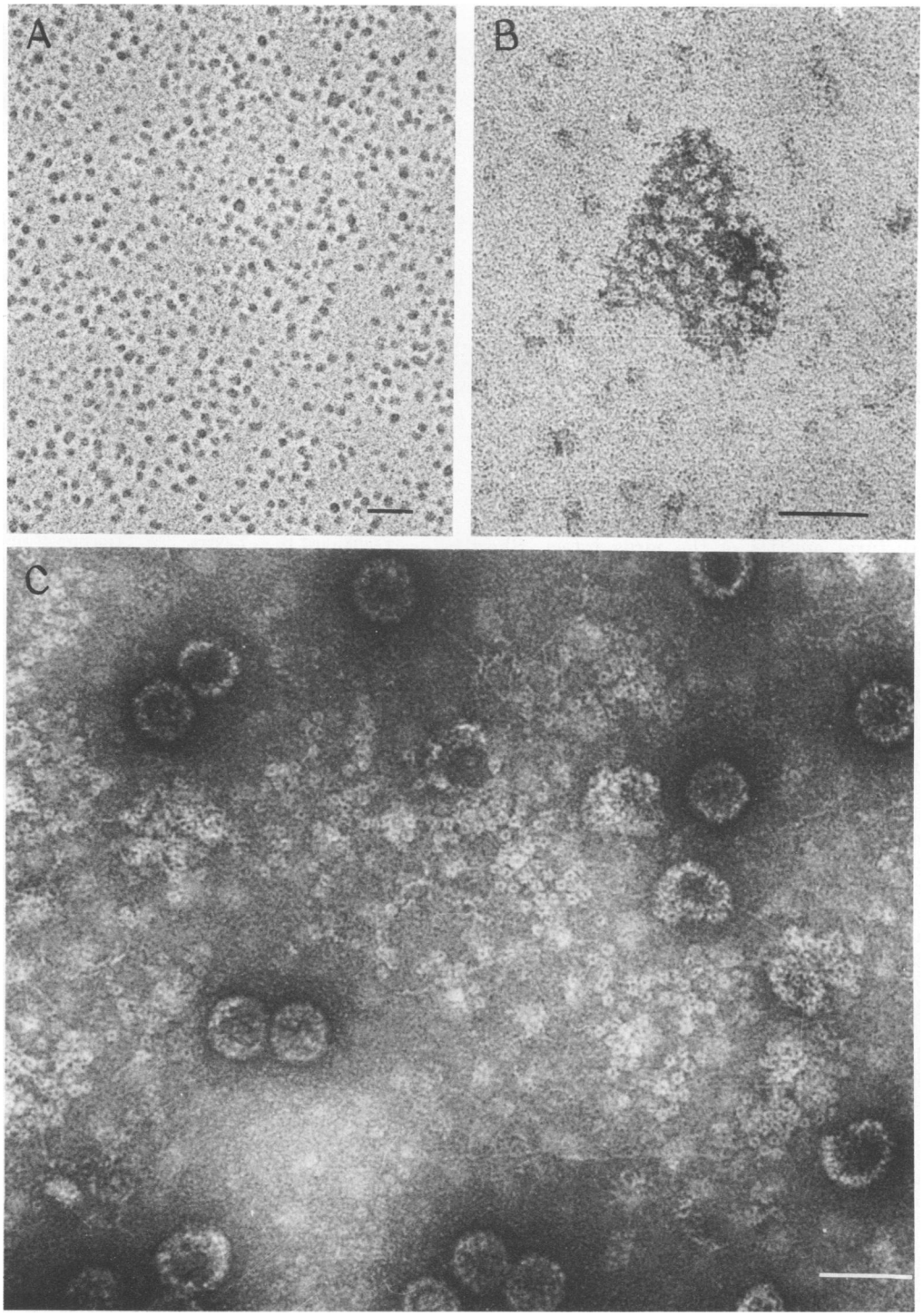

FIG. 6. *EM of SV40 virions and free capsomers, Purified 7S capsomer particles appear as a uniform field of dots after shadowing with tungsten (A) and as doughnut-shaped structures 6 nm in diameter after negative staining with 1% uranyl acetate (B). Similar 6-nm doughnut-shaped particles are found on the surface of intact SV40 virions and, in this virus preparation, were also free on the background (C). Bars represent 0.1 μm.*

protein sedimented at 7S. When examined by EM after tungsten shadowing, this material appeared as uniform-size particles (Fig. 6A); after negative staining with uranyl acetate, they appeared to be doughnut-like structures with a diameter of 6 nm (Fig. 6B). These doughnut-like 7S particles were similar in size and appearance to the capsomers of the SV40 virus shell previously observed by negative staining (Fig. 6C) in this and other laboratories (14).

Polyacrylamide gel electrophoresis of the 7S particles purified by sucrose gradient centrifugation showed that they consisted mostly of VP1, with only a minor amount of VP2; even after the gels were overloaded, histones or VP3 could not be detected (Fig. 4C).

DISCUSSION

Several conditions analogous to those described earlier (3, 8) were explored for disrupting SV40 virus particles to release the major structural entities of the virus intact. Short exposure (5 min) of SV40 virions to pH 9.8 at 37°C disrupts SV40 virus particles into a 60S DNA-protein complex fraction and a 7S protein particle fraction. The DNA-protein complex behaves and appears like the SV40 "minichromosomes" (depleted of histone H1) (5) isolated from nuclei of productively infected cells, and the 7S protein particles resemble the capsomer subunits of the virus shell (3, 14).

The disruption of virions into discrete 60S and 7S components was observed only within a narrow range of conditions. In general, treatments employing buffers of lower ionic strength or lower pH or using shorter incubations or lower temperatures than those described in method 2 (see Materials and Methods) yield structures resembling collapsed virus particles (Fig. 1A and 3A). Conversely, treatments employing higher pH or temperatures, or longer incubation times, produced DNA-protein complexes that were relatively depleted in histones and had no 7S protein components. Glycine buffers gave the most consistent results.

The 60S DNA-protein complex released from virions after treatment at pH 9.8 contained, in addition to the four histones, a significant amount of the virus-specified protein VP3. Conceivably, VP3 may play a role in the condensation and packing of the DNA-histone complex during virion maturation, but it may be due to adventitious association as well.

If, as the EMs suggest, the 7S particles are indeed virion capsomers, it should be possible to examine their detailed composition and structure. The method of dissociating SV40 virions presented here also may prove useful in attempts to reconstitute viable virions from the molecular components. Treatment of SV40 virions at pH 9.8 also provides a convenient way to obtain SV40 chromatin for a variety of studies on the structure and function of such complexes.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service International Research Fellowship 5 F05 TW02254-02 to one of us (G. C.), by a Jane Coffin Childs fellowship to T. Landers, and by grant GB 43576 from the National Science Foundation.

ADDENDUM

After completion of this work, a study reported by A. Varshavsky, V. Bakayev, P. Chumackov, and G. Georgiev (Nucleic Acids Res., 3:2101-2113, 1976) confirmed the presence of histone H1 in SV40 minichromosomes isolated in low-salt solutions.

LITERATURE CITED

1. **Anderer, F. A., H. D. Schlermberger, 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.
2. **Estes, M. K., E. S. Huang, and J. S. Pagano.** 1971. Structural polypeptides of simian virus 40. J. Virol. 7:635-641.
3. **Girard, M., L. Marty, and F. Suarez.** 1970. Capsid proteins of simian virus 40. Biochem. Biophys. Res. Commun. 40:97-102.
4. **Griffith, J. D.** 1973. Electron microscopic visualization of DNA in association with cellular components, p. 129-145. *In* D. M. Prescott (ed.), Methods in cell biology, vol. 7. Academic Press Inc., New York.
5. **Griffith, J. D.** 1975. Chromatin structure: deduced from a minichromosome. Science 187:1202-1203.
6. **Huang, E. S., M. K. Estes, and J. S. Pagano.** 1972. Structure and function of the polypeptides in simian virus 40. I. Existence of subviral deoxyribonucleoprotein complexes. J. Virol. 9:923-929.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
8. **Lake, R. S., S. Barban, and N. P. Salzman.** 1973. Resolution and identification of the core deoxynucleoproteins of the simian virus 40. Biochem. Biophys. Res. Commun. 54:640-647.
9. **Meinke, W., M. R. Hall, and D. A. Goldstein.** 1975. Proteins in intracellular simian virus 40 nucleoprotein complexes: comparison with simian virus 40 core proteins. J. Virol. 15:439-448.
10. **Ozer, H. L.** 1972. Synthesis and assembly of simian virus 40. J. Virol. 9:41-51.
11. **Polisky, B., and B. McCarthy.** 1975. Location of histones on SV40 DNA. Proc. Natl. Acad. Sci. U.S.A. 72:2895-2899.
12. **Takemoto, K. K., R. L. Kirschstein, and K. Habel.** 1966. Mutants of simian virus 40 differing in plaque size, oncogenicity, and heat sensitivity. J. Bacteriol. 92:990-994.
13. **White, M., and R. Eason.** 1971. Nucleoprotein complexes in simian virus 40-infected cells. J. Virol. 8:363-371.
14. **Williams, R. C., and H. W. Fisher.** 1974. An electron micrographic atlas of viruses. Charles C Thomas, Springfield, Ill.