Proteins in Intracellular Simian Virus 40 Nucleoprotein Complexes: Comparison with Simian Virus 40 Core Proteins

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Intracellular nucleoprotein complexes containing SV40 supercoiled DNA were purified from cell lysates by chromatography on hydroxyapatite columns followed by velocity sedimentation through sucrose gradients. The major protein components from purified complexes were identified as histone-like proteins. When analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, complex proteins comigrated with viral core polypeptides VP4, VP5, VP6, and VP7. [³H]tryptophan was not detected in polypeptides from intracellular complexes or in the histone components from purified SV40 virus. However, a large amount of [³H]tryptophan was found in the viral polypeptide VP3 relative to that incorporated into the capsid polypeptides VP1 and VP2. Intracellular complexes contain 30 to 40% more protein than viral cores prepared by alkali dissociation of intact virus, but when complexes were exposed to the same alkaline conditions, protein also was removed from complexes and they subsequently co-sedimented with and had the same buoyant density as viral cores. The composition and physical similarities of nucleoprotein complex and viral cores indicate that complexes may have a role in the assembly of virions.

Protein becomes associated with SV40 and polyoma DNA during replication of the viral DNA (11, 14, 23). Upon completion of progeny DNA molecules, nucleoprotein complexes containing supercoiled viral DNA are released from replication sites and can be extracted in the cytoplasmic fraction prepared by lysis of cells with nonionic detergents (11, 12, 13, 14, 25, 29).

SV40 nucleoproteins containing supercoiled viral DNA have an average sedimentation rate of 61S and consist of about equal parts of DNA and protein (14). The complexes are highly compact structures which are converted to more extended forms upon removal of protein by exposure to high salt concentrations (14, 29). Chemical and enzymatic studies revealed that the polyoma and SV40 complexes contain at least two or more different protein components, but these proteins were not identified (11, 14, 29).

We succeeded in devising a method for purification of complexes which enabled us to characterize the protein components of the SV40 complexes. The major proteins associated with intracellular complexes have properties similar to the core proteins extracted from virus in association with viral DNA.

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MATERIALS AND METHODS

Virus and cells. SV40 strain RH 911 was propagated in the TC7 subline of CV-1 cells (22). Monolayers were grown to confluence in Eagle medium supplemented with 10% calf serum in 9-cm plastic petri dishes (A.S. Nunc) at 36 C. Cell cultures were infected 1 or 2 days after confluence with 0.2 ml of virus (2 × 10⁸ PFU/ml) and the virus was allowed to absorb for 90 min. After absorption, 8 ml of medium containing 2.5% of dialyzed horse serum was added to each culture.

Purification of radioactive virus. Immediately after infection, cells were overlayed with Eagle medium containing 2.5% dialyzed horse serum and either 1 µCi of [¹⁴C]lysine per ml, 1 µCi of [³H]lysine per ml, or 10 µCi of ³H-labeled L-tryptophan per ml, and 2 µCi of [¹⁴C]lysine per ml. After 6 to 7 days, lysates were harvested and then frozen and thawed three times. Lysates were made 0.001 M with MgCl₂ and incubated for 3 h at 37 C with 10 µg of DNase per ml and 5 µg of RNase per ml. Lysates were clarified by centrifugation for 20 min at 10,000 rpm in a Beckman J-21 centrifuge (JA20 rotor), and both pellets and supernatant fluids were retained for further purification. Pellets were suspended in about 5 ml of Eagle medium, an equal volume of 1,1,2-trichlorotrifluoroethane was added, and solutions were homogenized in a Virtis S23 at maximum rates for 3 to 4 min. Phases were separated by low speed centrifugation and the top aqueous phase, containing SV40 particles, combined with the original supernatant fluids (10). Virus was pelleted by centrifugation in a 30 rotor at 25,000

rpm for 3 h at 5 C. Pellets were suspended in CsCl (ρ = 1.33 g/ml in 0.02 M Tris-hydrochloride, pH 7.5, and 0.001 M EDTA) and centrifuged to equilibrium for 48 h at 35,000 rpm at 25 C in the SW50 rotor (30). The peak fractions of the lower virus band were pooled and dialyzed against 0.02 M Tris-hydrochloride, pH 7.5, and 0.001 M EDTA. Virus was then sedimented for 35 min through linear, 10 to 40% (wt/wt) sucrose gradients (in 0.3 M NaCl, 0.05 M Tris-hydrochloride, pH 8.0) in the SW50.1 rotor at 32,000 rpm at 20 C. The virus band was collected by puncturing a hole in the bottom of the centrifuge tube and collecting 10-drop fractions with a 19-gauge needle.

Extraction of nucleoproteins from SV40-infected cells. Nucleoprotein complexes containing SV40 DNA were extracted by procedures described previously (13, 14). Between 38 and 46 h after infection, monolayers were washed twice with ice-cold, Tris-buffered saline containing magnesium and calcium (30). Monolayers were treated with 0.9 ml of a solution containing 0.25% Triton X-100, 0.01 M EDTA, and 0.01 M Tris-hydrochloride, pH 7.9 (TTE) (13). After 10 min at room temperature, 0.1 ml of 2.0 M NaCl was added to each petri dish and they were maintained at room temperature for an additional 10 min. This treatment lysed cells leaving nuclei morphologically intact. Lysates were poured into centrifuge tubes, and nuclei and large cellular debris were pelleted by centrifugation at 2,000 rpm for 5 min at 4 C in a Sorvall GLC centrifuge (HL-4 rotor). The supernatant fluid (Triton cytoplasmic fraction) containing SV40 nucleoprotein complex was decanted and stored at 4 C for no more than 36 h.

Purification of SV40 nucleoprotein complex on columns of hydroxyapatite. The Triton cytoplasmic fractions from 10 petri plate cultures were pooled and applied to a column of hydroxyapatite (1.0 by 2.5 cm bed dimensions) equilibrated with 0.14 M sodium phosphate (pH 6.8) buffer at room temperature. The column was then washed with 50 to 70 3-ml portions of 0.22 M sodium phosphate (pH 6.8) buffer containing 0.1% Triton X-100 to remove RNA and most cellular proteins. SV40 nucleoprotein complex, as well as some other cellular proteins, were eluted from the column with several 2.0-ml washes of 0.35 M sodium phosphate (pH 6.8) buffer containing 0.1% Triton X-100. Since both polyoma and SV40 nucleoproteins are unstable in high ionic environments (11, 13, 29), complexes were eluted directly into equal volumes of ice-cold TTE. The column was then washed with 0.48 M sodium phosphate (pH 6.8) buffer containing 0.1% Triton X-100. At this salt concentration, no additional nucleoprotein complex was eluted from the column. However, a small amount of supercoiled SV40 DNA and some protein were removed. Peak fractions containing nucleoprotein complex were pooled, dialyzed, and concentrated against TTE by vacuum dialysis using a Sartorius cellulose nitrate collodion bag.

Velocity sedimentation. Linear, 5 to 20% (wt/wt) sucrose gradients were prepared in TTE containing 0.2 M NaCl. Samples (0.2 ml) were layered onto 4.8-ml gradients and centrifuged at 36,000 rpm for either 120, 135, or 180 min at 4 C in a Spinco SW50.1

rotor (14). Fractions were collected through a hole punctured in the bottom of the tube. Portions from each fraction were spotted on filter papers (Schleicher and Schuell 740-E), and the filter papers were treated for trichloroacetic acid precipitable radioactivity using a batch technique (18).

Buoyant density centrifugation of glutaraldehyde-fixed nucleoprotein complex. Peak fractions from sucrose gradients containing SV40 nucleoprotein complex were pooled and fixed for 12 h at 4 C in 5% neutralized glutaraldehyde by the procedures of Baltimore and Huang (3). Samples (0.2 ml) were layered onto 4.8-ml preformed linear CsCl gradients (1.3 to 1.6 g/cm³) containing 0.01 M Tris-hydrochloride (pH 8.0), 0.01 M EDTA, 0.1% Triton X-100. Gradients were centrifuged at 35,000 rpm for 7 h at 4 C in a Spinco SW50 rotor. For density determination, fractions were collected into mineral oil in microtiter plates, and the index of refraction was measured in a Zeiss refractometer. Fractions were then assayed for radioactivity as described above.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis of SV40 nucleoprotein complexes and SV40 virions was performed in either 10% sodium dodecyl sulfate (SDS) gels using a phosphate buffer system (17) or in 13% SDS gels employing an acetate buffer system (16). For the 10% gel system, acrylamide was polymerized (acrylamide: bis-acrylamide, 30:0.8) in plastic tubes (0.6 by 10 cm) in 0.03 M sodium phosphate (pH 7.4) buffer (gel buffer) containing 0.1% SDS; the same buffer was used as a running buffer. Purified virus or nucleoprotein complexes were dialyzed into gel buffer, made 1% with SDS and 1% with β-mercaptoethanol, incubated 1 h at 50 C, and then heated to 100 C for 4 min. Following a 2-h gel "pre-run" at 5 mA/tube, digested samples (0.1 ml) containing bromophenol blue (0.01%) and sucrose (10%) were applied to the top of gels, and electrophoresis was carried out at 5 mA/tube for 4 to 5 h. Runs were terminated about 45 min after tracking dye had eluted from the bottom of gels. Gels were removed from tubes, frozen, and sliced into 1-mm pieces with a gel slicer (Misco Specialties Co., Berkeley, Calif.). Gel slices were digested with 0.2 ml of 30% H_2O_2 for 16 h at 37 C. Radioactivity was determined in a Nuclear Chicago scintillation counter using a toluene-Triton X-100 (2:1, vol/vol), Omnifluor scintillation counting fluid (10 ml).

Electrophoresis through 13% SDS-polyacrylamide gels (acrylamide: bisacrylamide, 40:1) was by the procedure of Lake et al. (16). The running buffer and gel buffer was 0.04 M Tris, 0.02 M sodium acetate, 0.002 M EDTA, pH 7.4 (A buffer) containing 1% SDS. Samples were precipitated with 25% cold trichloroacetic acid, washed once in ice-cold acetone containing 0.5% HCl (vol/vol) and twice in acetone. Precipitates were air-dried and then solubilized in A buffer containing 1% SDS, 8 M urea, and 2% β-mercaptoethanol (vol/vol). After incubation at 50 C for 2 h and heating to 100 C for 3 min, 1 μg of pyronin Y marker dye was added, and samples were made 10% sucrose and then layered on top of 13% SDS-gels. Electrophoresis was for 5 to 6 h (5 mA/tube) as described above for the 10% gels. Gels were frozen and sliced as described above,

but 13% gels had to be digested with 0.5 ml of 30% H_2O_2 for 60 h at 60 C.

Alkaline degradation of virus and nucleoprotein complex. Purified virus and nucleoprotein complex were degraded in an alkaline buffer by the method of Friedmann (7). Virus or nucleoprotein were dialyzed against 0.2 M Na_2CO_3-$NaHCO_3$, pH 10.6, 0.01 M dithiothreitol for 3 h at 4 C. After dialysis, preparations were sedimented through neutral sucrose gradients as described above.

Chemicals. Chemicals were obtained from the following sources: [methyl-^3H]thymidine (55.3 Ci/ mmol), [2-^{14}C]thymidine (54 mCi/mmol), L-[4,5-^3H]lysine (N) (55 Ci/mmol), L-[^{14}C]lysine (U) (306 mCi/mmol), L-[^3H]tryptophan (G) (2.1 Ci/mmol), ^3H-labeled L-amino acid mixture (G), L-[4,5-^3H]leucine (N) (5 Ci/mmol), and L-[3-^3H]arginine (N) (30 Ci/ mmol), New England Nuclear Corp. (Boston, Mass.); DNase and RNase, Sigma Chemical Co. (St. Louis, Mo.); Eagle minimal essential medium, Grand Island Biological Co. (Grand Island, N.Y.); hydroxyapatite (Bio-Gel HTP), Bio Rad Laboratories (Richmond, Calif.); glutaraldehyde, Aldrich Chemical Co. (Milwaukee, Wisc.); CsCl (optical grade), Harshaw Chemical Co. (Solon, Ohio); sucrose (RNase-free), Schwartz-Mann (N.Y.); and Triton X-100, Atlas Chemical and Manufacturing Co. (San Diego, Calif.).

RESULTS

Purification of SV40 nucleoprotein complexes. Figure 1 shows an elution profile from a hydroxyapatite column of Triton cytoplasmic fractions prepared from cells labeled for 45 h after infection with ^3H-labeled mixed L-amino acids, and additional L-[^3H]leucine, L-[^3H]arginine, and [^{14}C]thymidine. About 90% of ^3H-labeled proteins eluted from the column with a 0.22 M sodium phosphate buffer, whereas ^{14}C-labeled DNA, presumably as a nucleoprotein complex, remained on the column at this salt concentration. When the phosphate concentration was increased to 0.35 M, 96% of the ^{14}C-labeled complexes was eluted together with about 6% of the ^3H-labeled protein. However, only about 3% of the ^3H label was coincident with the peak of ^{14}C-labeled DNA (Fig. 1).

To establish that ^{14}C-labeled DNA was in SV40 nucleoprotein complexes and not as free or degraded DNA, peak fractions from 0.35 M phosphate washes containing ^{14}C-labeled DNA were pooled (fractions 51 to 53, Fig. 1), concentrated against TTE buffer, and sedimented through a 5 to 20% sucrose gradient (Fig. 2). As markers, ^{14}C-labeled SV40 DNA and ^{14}C-labeled SV40 complex from crude Triton lysates were run in two additional tubes. About 25% of the ^3H-labeled protein co-sedimented with ^{14}C-labeled DNA to the lower half of the gradient at a position characteristic of SV40 nucleoprotein complexes, i.e., 61S (14). The finding that no
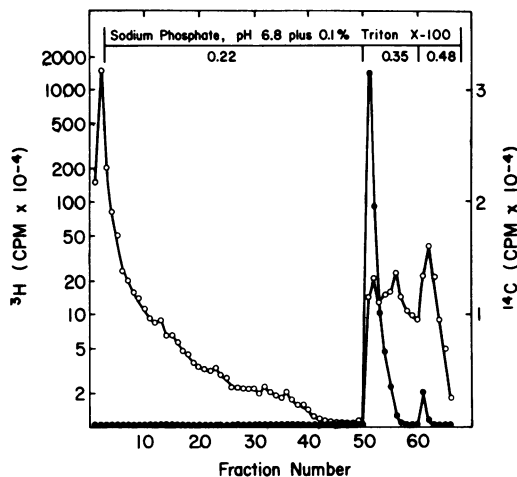

FIG. 1. *Hydroxyapatite column chromatography of SV40 nucleoprotein complexes. SV40-infected monolayers from 10 petri plates were labeled with ^3H-labeled mixed L-amino acids (12 μCi/ml), additional L-[4,5-^3H]leucine (N) (16 μCi/ml), and L-[3-^3H]arginine (N) (16 μCi/ml) in Eagle medium containing 2.5% dialyzed horse serum for 32 h after infection. Labeled media was replaced with fresh media containing 5% horse serum and 0.5 μCi of [^{14}C]thymidine per ml, and cultures were incubated an additional 2 h. Cells were lysed with Triton X-100 (0.25%) and Triton cytoplasmic fractions prepared as described. The Triton cytoplasmic fraction was loaded onto a hydroxyapatite column and the column was washed under pressure with 50 3.0-ml portions of 0.22 M sodium phosphate (pH 6.8) containing 0.1% Triton X-100 (average flow rate was 1.5 ml/min). To elute complex, the column was washed with 10 2.0-ml portions of 0.35 M sodium phosphate (pH 6.8) containing 0.1% Triton X-100 followed by six 2-ml washes of 0.48 M sodium phosphate (pH 6.8) and 0.1% Triton X-100. Beginning with fraction 51, washes were collected into an equal volume of ice-cold TTE. Samples were removed from each fraction and assayed for trichloroacetic acid radioactivity by procedures described previously (18). The total radioactivity in each fraction is presented. Symbols: O, ^3H; ●, ^{14}C.*

^{14}C-labeled DNA sedimented as free DNA (fractions 35 to 36) indicated that all DNA which elutes from the column with 0.35 M sodium phosphate is in the form of a nucleoprotein complex. About 75% of the ^3H-labeled protein in the 0.35 M phosphate fraction is found at the top of the gradient and presumably consists of cellular proteins.

Buoyant density of glutaraldehyde-fixed SV40 nucleoprotein complexes in CsCl. To obtain an estimate of the degree of purity of SV40 nucleoprotein complex after hydroxyapatite chromatography and sedimentation through sucrose gradients, fractions 13 through
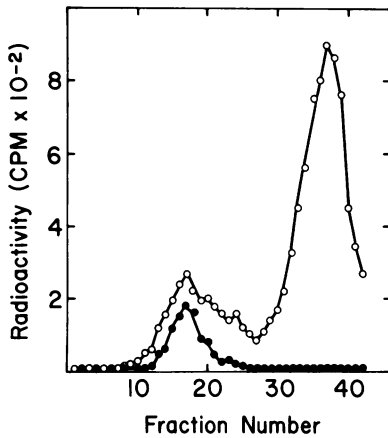
FIG. 2. *Velocity sedimentation in neutral sucrose gradients of SV40 nucleoprotein complexes from hydroxyapatite columns. Fractions 51 to 53 from Fig. 1 were pooled and concentrated, and a sample was sedimented in 5 to 20% sucrose gradients for 120 min. Fractions were collected and trichloroacetic acid-precipitable radioactivity was determined as described. Sedimentation in this and all other gradients is from right to left. Symbols:* O, ^3H; ●, ^{14}C.

18 from the gradient (Fig. 2) were pooled, fixed in glutaraldehyde, and centrifuged to equilibrium in CsCl density gradients (11). As shown in Fig. 3, 80% of the ^3H-labeled and all of the ^{14}C-labeled DNA banded at a position in the gradient which corresponds to an average buoyant density of 1.447 g/cm^3. This value is in very good agreement with the buoyant density reported for glutaraldehyde-fixed SV40 nucleoprotein complexes (14). The finding that some ^3H-labeled material bands at the top of these gradients as free protein indicates that not more than about 20% of the ^3H-labeled protein was unrelated to nucleoprotein complexes. In later experiments, the amount of contaminating material was reduced to less than 6% by increasing the number of washes of 0.22 M sodium phosphate applied to the hydroxyapatite column (Fig. 4) and by sedimenting nucleoprotein complexes further into sucrose gradients.

Proteins in SV40 nucleoprotein complex. A series of experiments was performed to identify the polypeptide components of isolated nucleoprotein complexes and to compare these to proteins of purified virus. SV40 nucleoprotein complexes were labeled with 20μCi of [^3H]lysine per ml for 45 h immediately after infection. After a 1-h chase with medium minus label, cells were lysed with Triton X-100, nucleoprotein complexes were extracted, and the Triton cytoplasmic fraction was applied to a hydroxyapatite column. After washing the column

with 210 ml of 0.22 M phosphate, SV40 nucleoprotein complexes were eluted with 0.35 M phosphate buffer, concentrated against TTE buffer, and then sedimented through sucrose gradients (Fig. 4). Fractions from the nucleoprotein peak (Fig. 4, brackets) were pooled and mixed with ^{14}C-labeled SV40 virus, and after digestion they were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 5).

The distribution of viral polypeptides shown in Fig. 5 is typical of that reported by others (4, 6, 15, 16, 19, 28). The major polypeptide component VP1 and VP2 are thought to be viral coat proteins (2, 4, 9, 15). Huang and co-workers (15) propose that VP3 might be an intermediate structural element with affinity both for the viral coat proteins and the internal core polypeptides, whereas Walter and co-workers (28) suggest VP3 is a constituent of the capsid penton. The low-molecular-weight components, designated as VP4, VP5, VP6, and VP7, comprise the internal proteins and appear to be host-derived histones (16). These components are isolated as subvirion nucleoprotein complexes after treatment of virions with mild alkali (16) and with DNA make up the viral core. (The nomenclature used in this study is that of Lake et al. (16). In the former nomenclature of Estes et al. (6) and Huang et al. (15) only three histone peaks were identified, VP4, VP6, and VP7, respectively, of Lake and co-workers.)
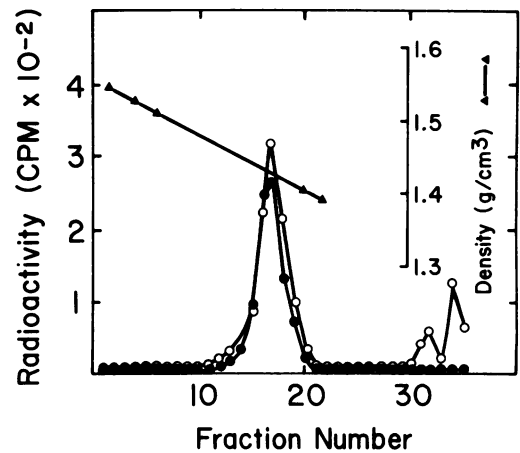

FIG. 3. *Equilibrium centrifugation in CsCl density gradients of glutaraldehyde-fixed nucleoprotein complexes. Peak fractions (13 to 18) from sucrose gradients (Fig. 2) were pooled and treated with 5% neutralized glutaraldehyde for 12 h at 4 C. Fixed samples were layered onto preformed CsCl gradients and centrifuged to equilibrium. Radioactivity and buoyant densities were measured as described. Symbols:* O, ^3H; ●, ^{14}C.
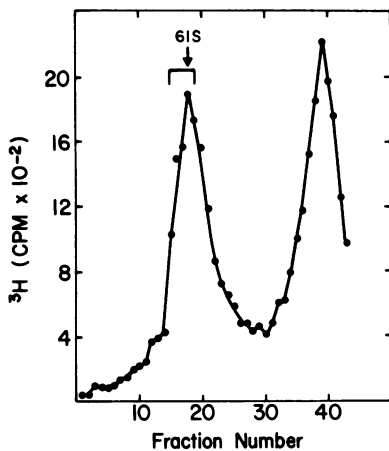
FIG. 4. *Velocity sedimentation in neutral sucrose gradients of [³H]lysine-labeled SV40 nucleoprotein complexes after chromatography on hydroxyapatite columns. Ten SV40-infected petri plate cultures were labeled with 20 μCi of [³H]lysine per ml for 45 h after infection. After a 1-h chase, Triton cytoplasmic fractions were prepared and loaded onto a hydroxyapatite column, the column was washed with 0.22 M phosphate buffer, and SV40 nucleoprotein complex was eluted as described in the text. After concentration, samples (0.3 ml) were sedimented as described in Fig. 2. The brackets indicate the fractions used for SDS-polyacrylamide gel electrophoresis (Fig. 5).*
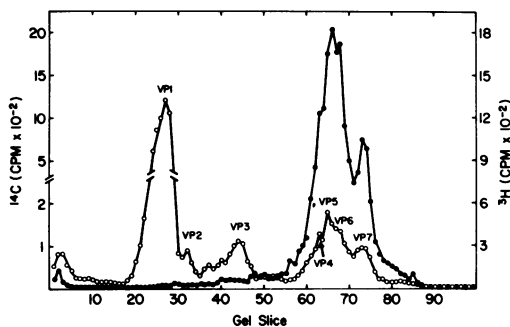

FIG. 5. *Polyacrylamide gel electrophoresis of polypeptides of purified nucleoprotein complexes. After hydroxyapatite column chromatography and sedimentation in sucrose gradients, [³H]lysine-labeled nucleoprotein complexes (Fig. 4, brackets) were precipitated with ice-cold trichloroacetic acid and resuspended in A buffer, and [¹⁴C]lysine-labeled SV40 virus was added as marker. After treatment with SDS, urea, β-mercaptoethanol, and heat (see Materials and Methods), polypeptides were separated in 13% Tris-acetate-SDS gels. Migration in this and all other gels is from left to right. Symbols: ●, ³H; ○, ¹⁴C.*

SV40 nucleoprotein complexes purified by hydroxyapatite column chromatography and sedimentation in sucrose gradients have polypeptides with electrophoretic mobilities similar

to SV40 core polypeptides and contain no polypeptides which correspond to viral components VP1, VP2, or VP3 (Fig. 5).

Complexes labeled with ³H-labeled mixed amino acids showed a similar polypeptide distribution in gels.

Incorporation of tryptophan into virions and complexes. Core polypeptides of virions are presumably host-derived histones (16) and therefore should be devoid of tryptophan (5). That this latter situation is indeed the case is shown by the distribution of radioactivity in polypeptides derived from virus grown in medium containing [³H]tryptophan and [¹⁴C]lysine (Fig. 6). Although [¹⁴C]lysine was incorporated into each major viral polypeptide, [³H]tryptophan was found only in peaks corresponding to VP1, VP2, and VP3. Interestingly, VP3 is relatively rich in tryptophan (Table 1).

To determine whether the proteins in SV40 complexes contain tryptophan, infected cells were grown in medium containing [³H]tryptophan and [¹⁴C]lysine and complex purified by hydroxyapatite column chromatography followed by velocity sedimentation in sucrose. Analysis by gel electrophoresis of purified com-
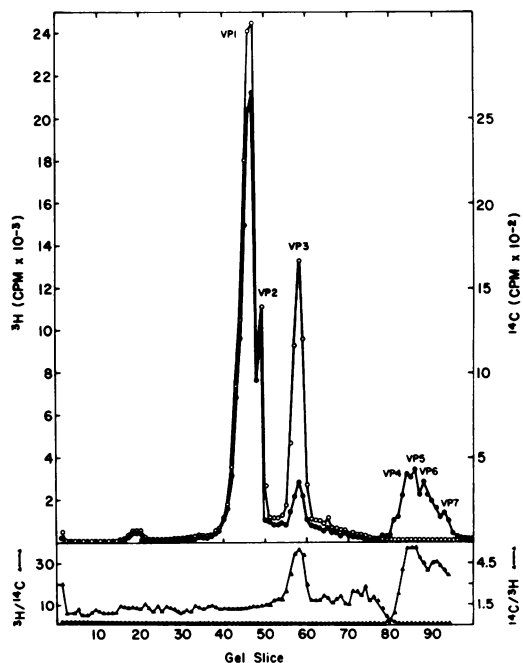

FIG. 6. *Polyacrylamide gel electrophoresis of [³H]tryptophan- and [¹⁴C]lysine-labeled polypeptides of SV40 virus. Virions were isolated from cells which were labeled with 10 μCi of [³H]tryptophan per ml and 2μCi of [¹⁴C]lysine per ml as described. Electrophoresis in 10% phosphate-SDS gels was for 5 h. Symbols: ○, ³H; ●, ¹⁴C.*

Table 1. *Incorporation of ^3H-labeled amino acids into SV40 polypeptides*

^3H-labeled amino acid	Viral polypeptide	% of total[a]
L-Tryptophan	VP1 + VP2	72.8
	VP3	26.6
	VP4 to VP7	0.6
L-Lysine	VP1 + VP2	71.7
	VP3	7.5
	VP4 to VP7	20.8
Mixed L-amino acids	VP1 + VP2	71.1
	VP3	11.5
	VP4 to VP7	17.4

[a] Calculated from the amount of radioactivity under each polypeptide component in polyacrylamide-SDS gels.

plexes is shown in Fig. 7. Although there was considerable incorporation of [^3H]tryptophan into cellular proteins (25×10^6 acid precipitable counts/min), no significant [^3H]tryptophan radioactivity is apparent in the region of the gel corresponding to polypeptides from complexes. From these results, it is concluded that the major protein components in the SV40 complex are core-like proteins.

Relationship of complexes to cores. The finding that the major protein components in the complex were core-like proteins led to a study in which SV40 complex was compared to SV40 cores. Subviral deoxyribonucleoprotein cores can be prepared by mild alkaline degradation of virions (1) (pH 10.6) and comprise about 12% of the protein mass (6). Cores contain at least four polypeptides and recent studies suggest that viral polypeptides VP4, VP5, VP6, and VP7 correspond to the four evolutionarily conserved histones F3, F2b, F2a2, and F2a1, respectively (16).

In neutral sucrose gradients, cores prepared by alkaline treatment of virions sediment at a rate of about 46S (6). In contrast, SV40 nucleoprotein complexes have an average sedimentation velocity of about 61S (14). Since both SV40 nucleoprotein complexes and core contain supercoiled DNA (14), the increased rate of sedimentation of complexes suggests that complexes (i) contain more protein than cores or (ii) have the same amount of protein but have a configuration that is more compact than cores.

Treatment of complexes with alkali under the same conditions used to make cores from virus removes about 30 to 40% of the proteins (Fig. 8, bottom). Modified complexes resulting from

this treatment sediment at a rate identical to that of cores derived from virions by treatment with alkali (Fig. 8, top).

In an attempt to determine which complex proteins were alkali sensitive, the following experiments were performed. [^3H]lysine-labeled alkali modified complex (Fig. 8, bottom, bracket a) and the protein portion released from complexes by alkali treatment (Fig. 8, bottom, bracket b) were mixed separately with [^{14}C]lysine-labeled virus and polypeptides separated by gel electrophoresis. Polypeptides which remain associated with alkali modified complex (Fig. 8, bottom, a) migrated with viral core polypeptides (Fig. 9a). However, none of the ^3H-labeled peaks corresponded exactly to the ^{14}C-labeled viral core polypeptides. Furthermore, comparison of the migration patterns of alkali modified complex (Fig. 9a) to the migration pattern of released protein (Fig. 9b) shows that alkali treatment of complexes removes primarily polypeptides that originally comigrated with viral polypeptides VP5 and VP6. Most polypeptides that comigrate with VP4 and VP7 do not appear to be removed during alkali treatment (Fig. 9a, see also Fig. 5).

Since proteins from intracellular complexes are removed and modified by alkali treatment, it was of interest to determine whether preparation of cores by exposure of virus to alkali also
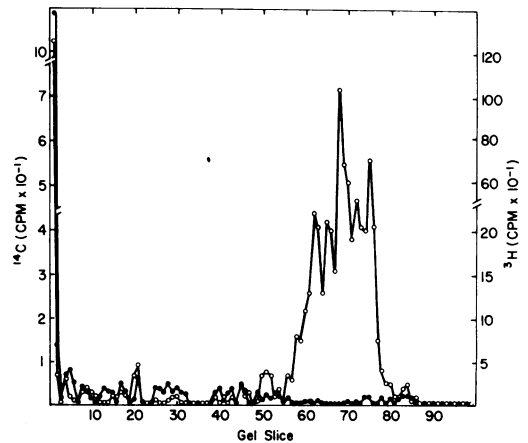

Fig. 7. *Polyacrylamide gel electrophoresis of polypeptides of [^3H]tryptophan- and [^{14}C]lysine-labeled SV40 nucleoprotein complexes. Cells were labeled with 20 μCi of [^3H]tryptophan per ml and 5 μCi of [^{14}C]lysine per ml for 41 h after infection. Nucleoprotein complexes were purified by hydroxyapatite chromatography and velocity sedimentation in neutral sucrose gradients (180 min at 36,000 rpm). Electrophoresis in 10% phosphate-SDS gels was for 4 h. Symbols: O, [^{14}C]lysine; ●, [^3H]tryptophan.*
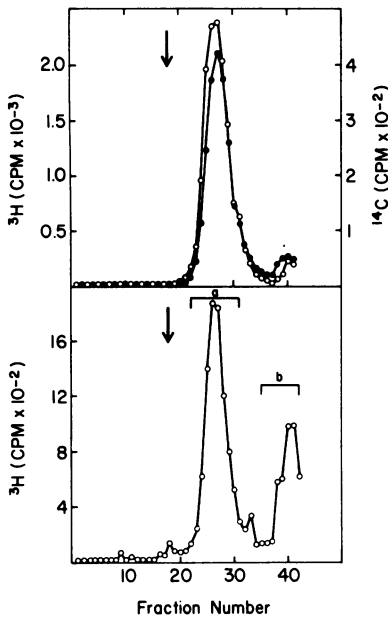
FIG. 8. *Velocity sedimentation in neutral sucrose gradients of alkaline-treated virions and nucleoprotein complexes. (Top) Purified [^{14}C]thymidine-labeled virus and purified [^{3}H]thymidine-labeled nucleoprotein complexes were mixed and dialyzed for 3 h against 0.2 M Na_2CO_3-$NaHCO_3$, pH 10.6, 0.01 M dithiothreitol at 4 C. Samples (0.2 ml) were sedimented for 135 min in 5 to 20% neutral sucrose gradients. (Bottom) Purified [^{3}H]lysine-labeled nucleoprotein complexes were alkaline treated as described above and subsequently centrifuged in sucrose gradients. The arrows indicate where the peak fraction of untreated nucleoprotein complexes sedimented in separate gradients. Symbols: O, ^{3}H; ●, ^{14}C.*

ban and Goor (4). Unfortunately, there was some degradation of viral capsid polypeptides into smaller components, and therefore, it is possible that a portion of the radioactivity in the viral histone region represents degradation products. This small amount of degradation is
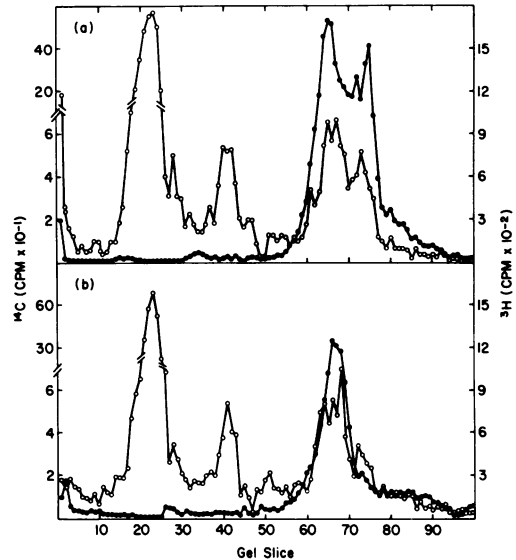

FIG. 9. *Polyacrylamide gel electrophoresis of polypeptides from alkaline-treated SV40 nucleoprotein complexes. (a) [^{3}H]lysine-labeled components corresponding to alkali modified complex (Fig. 8, bottom, bracket a) and (b) protein released from complexes by alkali, (Fig. 8, bottom, bracket b) were mixed separately with [^{14}C]lysine-labeled virions and polypeptides separated in 13% Tris-acetate-SDS gels. Electrophoresis was for 5 h. Symbols: O, ^{14}C; ●, ^{3}H.*

released some core histones. To test this possibility, [^{3}H]lysine-labeled virus was treated with alkali and then sedimented through sucrose gradients. The polypeptides of the fast sedimenting viral cores (Fig. 10, bracket a) and of the remaining slow sedimenting viral components (Fig. 10, bracket b) were then separated by gel electrophoresis. Viral cores yielded ^{3}H-labeled peaks corresponding to all viral core polypeptides, i.e., VP4, VP5, VP6, and VP7 (Fig. 11a).

Nevertheless, the migration pattern of the slow sedimenting material from the sucrose gradient (Fig. 10b) also revealed the presence of polypeptides which comigrated with all viral core polypeptides (Fig. 11b). Additionally, alkali treatment of virus splits the viral capsid polypeptide, VP1, into two components (fractions 21 to 33, Fig. 11b). Similar alkaline degradation of VP1 has been reported by Bar-
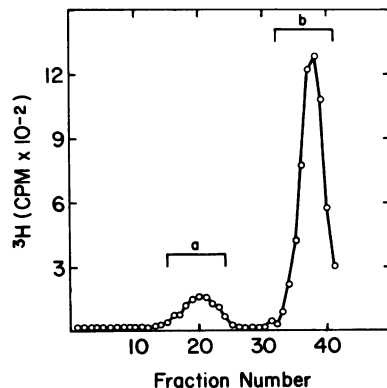

FIG. 10. *Velocity sedimentation in neutral sucrose gradients of alkaline-treated virions. Purified [^{3}H]lysine-labeled SV40 virus was dialyzed against carbonate buffer, pH 10.6 as described for Fig. 8. After dialysis, 0.2-ml samples were sedimented for 180 min in 5 to 20% neutral sucrose gradients.*
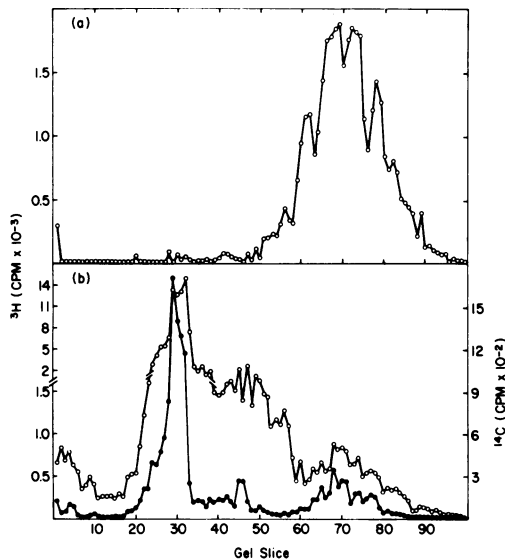
FIG. 11. *Polyacrylamide gel electrophoresis of [³H]lysine-labeled polypeptides from alkali dissociated SV40 virus. Fractions from Fig. 10 corresponding to viral cores (bracket a) and the slow sedimenting viral components (bracket b) were pooled separately. After addition of [¹⁴C]lysine-labeled virus to the slow sedimenting components, polypeptides of both viral cores (a) and the slow sedimenting viral components (b) were separated in 13% Tris-acetate gels. Electrophoresis was for 6 h. Symbols: ○, ³H; ●, ¹⁴C.*

emphasized by the large expansion of the ³H scale in Fig. 11b.

If it were assumed, however, that the portion of the ³H-labeled material which migrates like viral histones is indeed derived from viral core, this material would represent about 40% of the core histones. This value is based on the finding that generally about 20% of the lysine label is incorporated into VP4, VP5, VP6, and VP7 (Table 1), whereas after alkali degradation only about 8% of the label from the slow sedimenting peak migrated as histone (Fig. 11b).

Because of the degradation problem, it is not possible to make a definite conclusion as to the composition of the actual core. However, since alkali treatment does degrade viral components as well as intracellular nucleoprotein complexes, there is reason to believe that viral core made by alkali treatment of virus does not represent all of the intact internal viral histones. Thus, in our experiments, we were not able to establish that intracellular complexes are more representative of the viral core than nucleoprotein produced by treatment of virus with alkali. The fact that both intracellular

complex and viral core sediment at identical rates after alkali treatment and contain similar proteins, however, indicates that viral cores could conceivably be derived from an intracellular pool of nucleoprotein complexes.

DISCUSSION

Purification of intracellular SV40 DNA-protein complexes by chromatography on hydroxyapatite yields a product that is similar to the complexes from crude lysates. This is concluded from the fact that (i) purified complexes sediment with crude complexes in sucrose gradients, and (ii) purified and crude complexes have the similar buoyant densities in CsCl after fixation with glutaraldehyde. These methods, however, are not sensitive enough to detect small differences in protein composition (e.g., 1 to 10%), so it is conceivable that purification, in addition to removing contaminating cellular proteins, may also remove small amounts of protein which may be adventitiously associated with complexes in Triton lysates.

In this regard we found no evidence for an activity which unwinds exogenous supercoiled DNA in purified complex, although such an activity can be detected in complexes partially purified by sucrose gradients (24). Our analysis of partially purified complexes from sucrose gradients revealed that these fractions were grossly contaminated with ribonucleoproteins having buoyant densities and sedimentation rates similar to those of complexes. Furthermore, the greatest amount of "unwindase" activity was found in parts of the sucrose gradient not containing complex (our unpublished results). Thus, it is likely that "unwindase" enzyme is not associated with complexes, but rather with some other macromolecular species, and even this association may be fortuitous.

The most abundant species of protein in SV40 complexes are core-like proteins, and, from buoyant density measurements, it is estimated that there are about 200 to 400 molecules of protein bound to each SV40 DNA molecule. Small amounts of other proteins may be associated with these complexes, but at the level of purification and sensitivity achieved in the present study it was impossible to arrive at any conclusions concerning the nature of these proteins. However, we found no evidence from acrylamide gels for association of coat protein in purified complexes although association has been reported by others (24).

Tryptophan labeling showed that this amino acid was not present in either core proteins or in

the major proteins of complexes, but the finding that one of the viral nonhistone proteins is relatively rich in tryptophan is of considerable interest. As shown in Table 1, tryptophan in the major capsid peaks VP1 and VP2 is about 2.7 times that in VP3, whereas in virus labeled with mixed amino acids, this ratio is about 6 to 1. If it is assumed that there is a uniform distribution of the two amino acids in the polypeptides, this result makes it unlikely that VP3 represents some type of cleavage product of the principal capsid protein as has been proposed as the origin for some minor polypeptides of polyoma virus (8).

Although at least three of the major protein peaks from complexes comigrated with internal histones from virus, namely, VP4, VP6, and VP7, the resolution in these gels was insufficient to make a positive identification. It was clear from several experiments that the major protein fraction in complexes was always one or two fractions displaced from the peak of viral histone, namely VP5 (see Fig. 5). Assuming that viral cores are related to complexes, it is possible that this protein may be partially degraded upon isolation or, alternatively, modified upon encapsidation. In this regard, Tan and Sokol (27) reported that all major SV40 structural polypeptides are phosphorylated including the histones. Since SV40 does not contain protein kinase, these workers proposed that phosphorylation of viral proteins occurs before maturation. Comparison of phosphorylation of complexes to cores might provide some insight into whether phosphorylation plays a role in the maturation process.

The fact that (i) the major protein constituents of both intracellular nucleoproteins and cores comigrate in SDS polyacrylamide gels and (ii) both complexes and cores have identical sedimentation rates and buoyant densities after treatment with mild alkali (our unpublished results) indicates that viral internal nucleoprotein cores may be derived from intracellular pools of complexes. Complexes contain 30 to 40% more protein than cores obtained by alkaline degradation of virions (Fig. 8, bottom), but this additional protein can be stripped from complexes by alkali treatment. This raises the possibility that intracellular complexes might be more representative of the actual internal viral nucleoprotein than the core obtained by alkali degradation of virions and that this latter nucleoprotein may represent partially degraded internal viral nucleoprotein.

That mild alkali treatment alters the electrophoretic pattern of viral proteins in SDS gels has been reported by others (4, 27) as well as ourselves (Fig. 11, bottom). The explanation for this may lie in the fact that viral proteins are phosphorylated (27). The principal phosphorylated polypeptides in SV40 proteins are believed to contain O-phosphoserine, O-phosphothreonine, or both (27). The specific alkali lability of peptides containing these amino acids has been well documented (21, 26). In addition to elimination of phosphate, mild alkali treatment can result in cleavage of the adjacent peptide bond (26). To establish the relationship of complex to cores with greater certainty would require comparison of complexes to cores prepared by gentle degradation of the virions by procedures other than alkali treatment.

The fact that SV40 DNA-protein complexes are highly compact structures and consist of protein and DNA in about equal proportions (14) suggests that SV40 complexes may have structural similarities with cellular chromatin. Indeed, from high resolution electron microscopy studies, J. Griffith (submitted for publication) has proposed that SV40 nucleoprotein complexes may represent "mini-chromosomes". This proposal was based on the findings that SV40 complexes consist of circular structures with diameters and packing ratios like that of chromatin observed under similar conditions.

Recently, in studies involving biosynthesis of polyoma nucleoproteins, we found a correlation between the amount of protein associated with complexes made after inhibition of protein synthesis and the superhelix density of the DNA extracted from the complexes (D. A. Goldstein et al., submitted for publication). The similarity to chromatin, which is probably condensed by means of supercoiling (20), led us to propose that protein binding may cause viral DNA to be supercoiled at time of ring closure. Thus, protein binding may provide a partial explanation for the apparent deficiency in duplex turns in naked SV40 and polyoma DNA. Undoubtedly, the interaction between proteins and closed circular DNA will prove to be valuable as a model system for understanding the structural and functional significance of nucleoproteins.

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