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Stability and Components of Mature Simian Virus 40[†]

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ABSTRACT: We have examined the stability of mature simian virus 40 (SV40) to changes in pH and have investigated the DNA-specific enzymatic activities that are released from SV40 virions. Our studies show that when SV40 virions are disrupted by the alkaline disruption method described by Brady et al. [Brady, J. N., Winston, V. D., & Consigli, R. A. (1977) *J. Virol.* 23, 717-724], in addition to the capsid proteins, a DNA topoisomerase and a DNA endonuclease are released. Treatment with a reducing agent and a divalent ion chelator is required for the release of both enzymes from the mature virions. Kasamatsu and Wu have previously isolated from SV40 virions a protein covalently linked to SV40 DNA under strongly denaturing conditions [Kasamatsu, H., & Wu, M. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 1945-1949]. Considering the known biochemical properties of DNA topoisomerases, we propose that the protein in Kasamatsu and Wu's isolated protein-DNA complex is the SV40-associated topoisomerase. In this work, we show that the topoisomerase

is activated by Mg²⁺ ions and does not require a high-energy cofactor to relax supercoiled DNA. On the other hand, the endonuclease is active at 37 °C only in the presence of Mg²⁺ ions. While the topoisomerase and the endonuclease activities are released from the virions near neutral conditions, the extent of dissociation of capsid proteins from SV40 chromosomes and the shape of subviral DNA-protein complexes depend on pH. At pH 10.4, the DNA-containing particles sediment at 60 S. At lower pH values, 9.8 and 9.2, the subviral nucleoprotein complexes sediment at 75 and 92 S, respectively. Below pH 9.0, the virus particles decay to a heterogeneous mixture of DNA-containing particles. The majority of the nucleoprotein complexes that result from disruption of the virions at pH 8.8 appears as highly compact SV40 chromatin. Electron microscopic observations and biochemical analysis suggest that a fraction of encapsidated SV40 chromatin seems to be deficient in histones or "normal" nucleosomes.

Simian virus 40 (SV40) is a small icosahedral nonenveloped virus. About 88% of the SV40 virion is protein, and the remaining 12% is DNA [reviewed by Tooze (1980), Nathans (1979), and Chambon (1977)]. Highly purified SV40 virions contain at least seven polypeptides. The four minor viral proteins correspond to the cellular histones H2A, H2B, H3, and H4. The three other polypeptides (VP1, VP2, and VP3) are encoded by the SV40 genome (Tooze, 1980).

Within virions and in infected cells, the SV40 DNA is folded by the cellular histones into the SV40 chromosome or mini-

chromosome (Griffith, 1975; Chambon, 1977). The structure of minichromosomes resembles the fundamental structure of eukaryotic chromatin. The DNA is organized into units (nucleosome core), each involving approximately a 146 base pair segment of DNA and an octamer of the four cellular histones (Chambon, 1977). The capsid proteins form an icosahedron of 42-nm diameter encasing the minichromosomes (Tooze, 1980).

The integrity of mature SV40 virions is maintained by pH-dependent bonds, divalent ions, and disulfide bridges. Strong alkaline conditions (pH 10.5) disrupt the SV40 virions (Huang et al., 1972). At pH 9.8 in the presence of a reducing agent, the major capsid proteins dissociate from the SV40 chromosomes; the protein components that remain bound to

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SV40 DNA are the host cellular histones and the minor capsid proteins, VP2 and VP3 (Christiansen et al., 1977). Under milder pH conditions (pH 8.5) in the presence of divalent ion chelators and reducing agents, a nucleoprotein complex can be isolated that, in addition to histones and VP3, contains the major capsid protein VP1 (Brady et al., 1980). The binding sites of the divalent ions on SV40 virions are not known. It is likely that the S-S linkages bridge the VP1 molecules, since VP1 is the only capsid protein that contains cysteine residues (Tooze, 1980). The disulfide bonds not only stabilize the structure of SV40 virions but also protect the encapsidated SV40 chromosomes. Intact SV40 virions are refractory to nucleases, but in the presence of the reducing agent DTT¹ and in the absence of any divalent ion chelator, micrococcal nuclease penetrates the shell and cleaves the viral DNA (Ng & Bina, 1981).

To obtain additional information on the components of SV40 virions, we have investigated the DNA-specific enzymatic activities that copurify with mature SV40 and are released when the virions are disrupted with a divalent ion chelator and a reducing agent. We have also followed the dissociation procedure described by Brady et al. (1977, 1978, 1980) and analyzed the structure of subviral SV40 DNA-protein complexes that are generated under constant concentrations of a monovalent ion, a divalent ion chelator, and a reducing agent, as a function of pH. This allowed a unification of the various methodologies previously used for disrupting SV40 virions under alkaline conditions (Huang et al., 1972; Christiansen et al., 1977; Brady et al., 1980).

Materials and Methods

Preparation of Virus. SV40 (strain 776) was grown on BSC-1 cells as described previously (Bina et al., 1979). The infected cells were labeled 48 h after infection. The virions were isolated 5–8 days after infection as described (Friedmann & Haas, 1970).

Isolation of Plasmid DNAs. The *Escherichia coli* K-12 GC 579 strain harboring the pDMS630 plasmid or HB101 cells harboring PBR322 were propagated in LB medium. The DNA was extracted by the cleared lysate technique (Kupersztoch-Portnoy et al., 1974). The superhelical form was isolated by the dye-CsCl equilibrium centrifugation method (Randolff et al., 1967).

Isolation of DNA Topoisomerase from BSC-1. Confluent BSC-1 monkey kidney cells were washed twice with cold TD buffer (0.025 M Tris-HCl, 0.136 M NaCl, 7 mM KCl, and 0.7 mM Na₂HPO₄), pH 7.4, and drained. The cells were scraped from the dishes in TD buffer, spun at 2000 rpm for 10 min, and resuspended in a 0.2 mM phosphate buffer, pH 7.5. Phenylmethanesulfonyl fluoride was added to a 1 mM final concentration, and the cells were dounced gently with 15 strokes of a dounce homogenizer. The lysed cells were spun at 2000 rpm for 10 min, and the supernatant was reserved as a cytoplasmic fraction. The pellet was suspended successively in 0.1, 0.2, 0.3, and 0.5 M phosphate buffer, pH 7.5, the nuclei were respun at 2000 rpm between washes, and the supernatants were subsequently assayed for topoisomerase activity (Germond et al., 1975; Bina et al., 1976). The reactions were conducted in 20- μ L assay mixtures and were terminated by the addition of 5 μ L of a 5% NaDodSO₄, 125 mM EDTA,

and 24 mM 2-mercaptoethanol mixture. The reaction mixtures were subsequently incubated at 50 °C for 15 min. The samples were applied to a horizontal 1% agarose gel and electrophoresed at 60 V for 16 h as described (Germond et al., 1975). The gels were stained with 0.5 μ g/mL ethidium bromide and photographed under ultraviolet light (Sharp et al., 1973).

Assay of Topoisomerase Activity Associated with SV40 Virions. The virions were incubated for 10 min at 33 °C in 10 μ L of appropriate buffers (see the text). Four microliters of plasmid DNA (0.5 μ g of DNA with or without MgCl₂) was then added, and the reactions were incubated for an additional 30 min at 33 °C. Two microliters of 5% NaDodSO₄, 0.1 M EDTA, and 24 mM 2-mercaptoethanol was added to terminate the reactions. Incubation at 50 °C for 15 min was necessary to free SV40 DNA from histones and capsid proteins. The DNA was fractionated on agarose gels (30 cm \times 30 cm \times 5 mm) after the addition of 5 μ L of 50% glycerol in 0.15% bromophenol blue. The gels were stained and photographed as above. The negatives were traced on a Joyce-Loebl microdensitometer. The relative concentrations of the various forms of DNA in Figure 7 were determined from the area under each peak.

Electron Microscopy. Samples were fixed with 2% formaldehyde and adsorbed onto carbon-coated grids as described previously (Bina & Simpson, 1977). The specimens were examined with a Philips 300 electron microscope, operated at 60 kV, after staining with 0.5% uranyl formate for 30 s.

Results

SV40 Virions Are Stabilized by pH-Dependent Bonds. There is a long history of methodology for disrupting SV40 or polyoma virions by alkali, divalent ion chelators, and reducing agents (Huang et al., 1972; Christiansen et al., 1977; Brady et al., 1977, 1978, 1980). To unify the various procedures, we have conducted a systematic study and followed the release of proteins from mature SV40 virions as a function of pH under constant concentrations of the divalent ion chelator EGTA, the reducing agent DTT, and a monovalent ion.

To monitor the DNA and proteins simultaneously, we labeled the SV40 virions in vivo with ³H-labeled amino acids and [¹⁴C]thymidine. The isolated virions were disrupted with EGTA and DTT under physiological ionic strength (0.1 M NaCl), at various pH values, at 33 °C for 15 min. The dissociation products were analyzed by centrifugation in sucrose gradients (Figure 1). Intact SV40 virions sediment at about 220 S. The extent of dissociation of proteins from SV40 chromosomes and the shape of subviral DNA-protein complexes depend on pH. At pH 10.4, the DNA-containing particles sediment at 60 S (Figure 1F). Incubation at lower pH values, 9.8 and 9.2, results in an increase of sedimentation coefficient of subviral nucleoprotein complexes to 75 and 92 S, respectively (Figure 1E,D). Below pH 9, the virus particles decay to a heterogeneous mixture of DNA-containing particles. In the range of pH 7.5–7.8, the majority of the complexes sediment between 220 and 190 S, with two minor components sedimenting at 120 and 104 S (Figure 1A). An increase in pH in the range of 8–9 results in the gradual reduction of the 220–190 S peak, with the concomitant formation of particles that sediment between 104 and 96 S (Figure 1B). The reduction of the sedimentation coefficient of the nucleoprotein complexes concurred with the appearance of free proteins on the top of the gradients. The observations described above suggest that electrostatic interactions may play a role in the formation of the virus shell and/or in the condensation of SV40 chromatin into higher order structures.

¹ Abbreviations: DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid; NaDodSO₄, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine 5'-triphosphate.

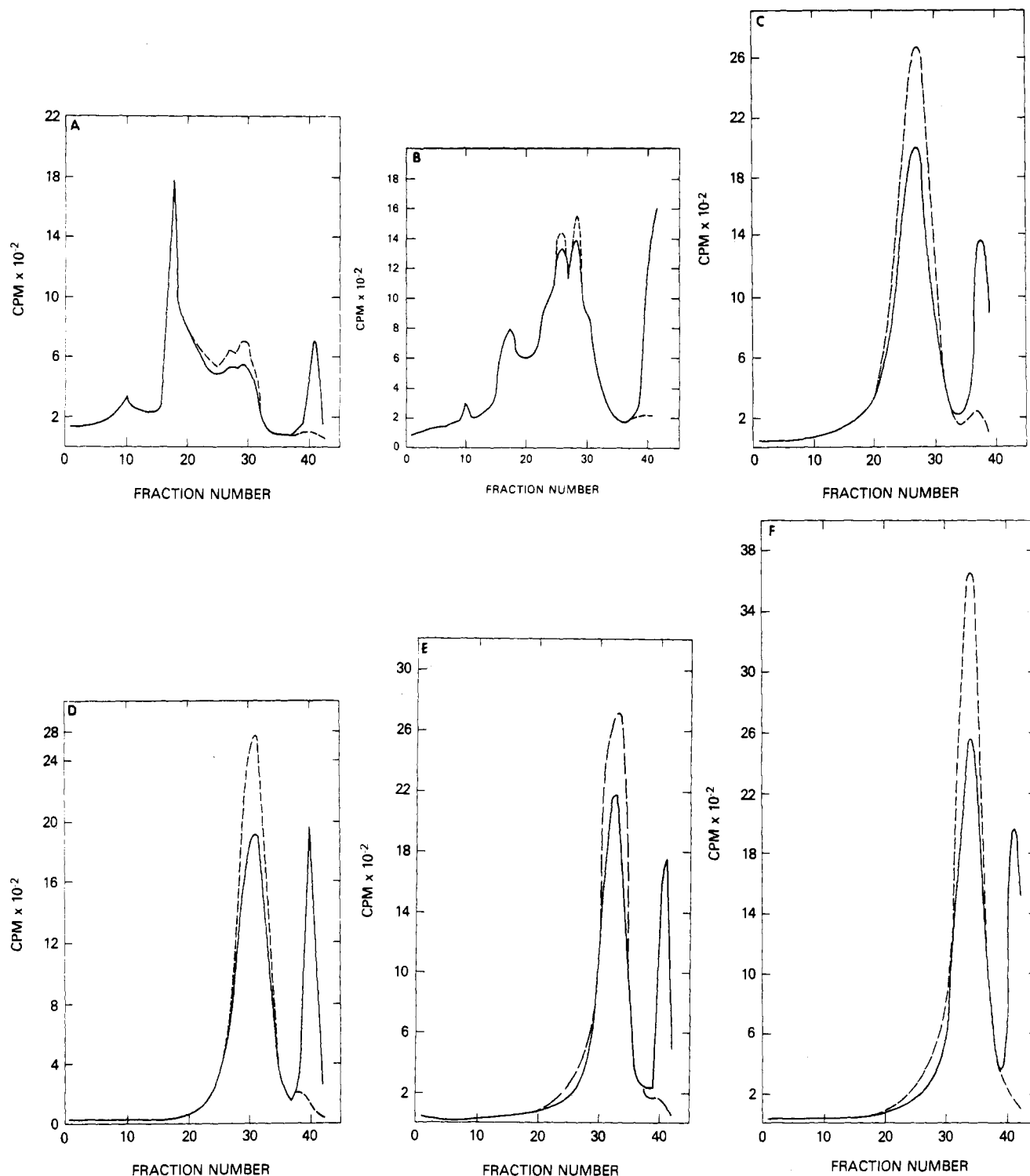


FIGURE 1: Sedimentation profile of disrupted SV40 virions in sucrose gradients. SV40 virions, labeled with ^3H -labeled amino acids and ^{14}C thymidine, were exposed to 20 mM EGTA, 10 mM DTT, and 0.1 M NaCl under various pH conditions; 0.05 M Tris-HCl was used in the pH range 7.5–9.0; 0.05 M glycine-NaOH was used for higher pH values. The reactions were incubated for 15 min at 33 °C and subsequently fractionated in a 15–30% sucrose gradient (in 0.05 M Tris-HCl, pH 8.0, 1 mM EDTA, and 0.1 M NaCl) in a SW41 rotor for 90 min at 4 °C. Fractions were collected and numbered from the bottom of the tubes. Panels A–F show the sedimentation profiles of the samples disrupted at pH values of 8.0, 8.8, 9.0, 9.2, 9.8, and 10.4, respectively. The solid curves and the dash lines denote the positions of ^3H -labeled proteins and ^{14}C DNA, respectively.

Structure of Nucleoprotein Complexes Released from Disrupted SV40 Virions. The components released from SV40 virions under various pH conditions were isolated by sedimentation and examined under an electron microscope. Consistent with the sedimentation analysis, the structure of DNA-containing particles depends on pH. The majority of the complexes isolated after treatment at pH 7.8 (220–190 S species) resembled the intact virions; a small fraction of the

complexes consisted of SV40 chromatin attached to empty shells by filamentous material (Figure 2). The majority of the nucleoprotein complexes resulting from treatment at pH 8.8 appeared as highly compact SV40 chromatin (Figure 3); they contained closely packed globular subunits comparable in size to nucleosomes. Occasionally, we observed structures that contained a cluster of nucleosome-like particles separated by stretches of free DNA (Figure 3).

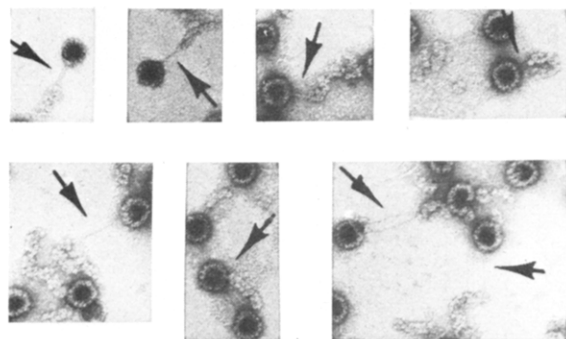


FIGURE 2: Visualization of SV40 DNA-protein complexes by electron microscopy. SV40 virions were disrupted at pH 7.8 and fractionated in sucrose gradients (as described in Figure 1). The DNA-containing particles, which sedimented between 220 and 190 S, were pooled and examined by an electron microscope. A fraction of the particles consisted of SV40 chromatin attached to empty shells by filamentous material, shown by the arrows. The majority of the complexes resembled the intact virions (not shown).

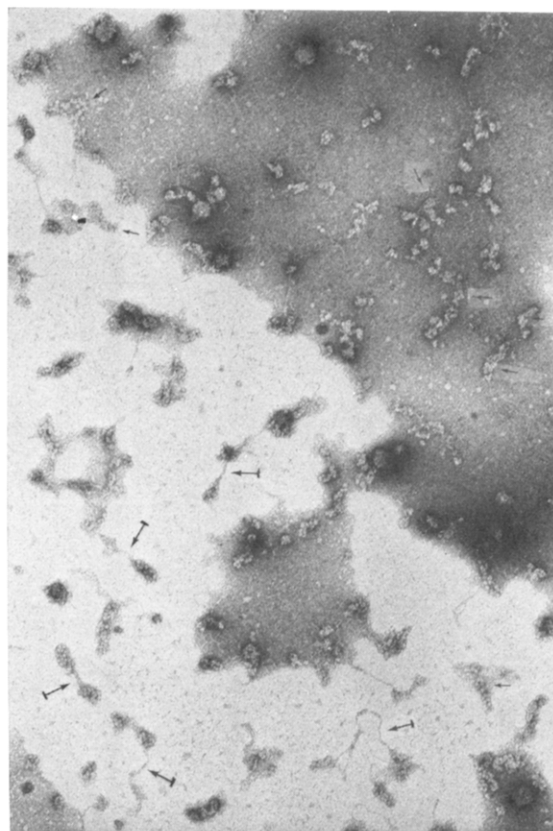


FIGURE 3: Visualization of SV40 DNA-protein complexes isolated at pH 8.8. SV40 virions were disrupted at pH 8.8 and fractionated on sucrose gradients. The component that sediments at 104–94 S was examined under an electron microscope. The majority of the complexes consists of compact SV40 chromatin (\rightarrow). A fraction of the complexes contain a stretch of free DNA (\rightarrow).

We were concerned that the stretches of free DNA, observed in a fraction of subviral nucleoprotein complexes, could be an artifact generated during the spreading of samples for electron microscopy. Therefore, we investigated the extent of torsional constraint imposed by proteins on the DNA of disrupted virions using the topoisomerase assay (Germond et al., 1975). SV40 virions were treated with 20 mM EGTA, 10 mM DTT, 0.05 M Tris-HCl, pH 7.5, and 0.1 M NaCl at 33 °C for 15 min. Topoisomerase activity was then added, and the reactions were incubated for an additional 30 min to relax the superhelical turns that were not constrained by proteins (Germond et al.,

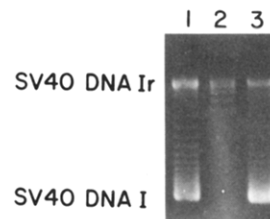


FIGURE 4: Heterogeneity of SV40 chromatin. (Lane 1) SV40 virions were disrupted in 0.05 M Tris-HCl, pH 8.5, 20 mM EGTA, 10 mM DTT, and 0.1 M NaCl. The samples were incubated at 33 °C for 15 min. Topoisomerase activity was added and the reactions were incubated for an additional 15 min at 33 °C. (Lane 2) Purified SV40 DNA was treated with topoisomerase activity for 15 min at 33 °C. (Lane 3) SV40 virions were disrupted as described for lane 1. The reactions were incubated for 30 min at 33 °C in the absence of exogenously added topoisomerase activity.

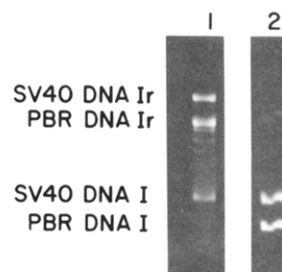


FIGURE 5: A topoisomerase activity copurifies with SV40 virions. SV40 virions were mixed with 1 μ g of purified PBR322 DNA and incubated for 30 min at 33 °C in reactions that contained 0.05 M Tris-HCl, pH 8.5, 10 mM DTT, 20 mM EGTA, and 0.1 M NaCl, with (lane 2) or without (lane 1) 1% NaDodSO₄.

1975; Bina & Singer, 1977). Subsequently, the proteins were removed by extraction with phenol, and the DNA was characterized by electrophoresis in an agarose gel. Figure 4, lane 1, shows that the resulting SV40 DNA is heterogeneous and contains topological isomers that migrate from fully supercoiled to partially relaxed forms, although the supercoiled molecules predominate. When the DNA was extracted directly from the virions, by treatment with NaDodSO₄ and phenol, it migrated as a fully supercoiled species, as previously observed (Germond et al., 1975). These experiments corroborate the electron microscopic observations shown in Figure 3 and suggest that a fraction of encapsidated SV40 chromatin may be deficient in histones or "normal" nucleosomes.

A Topoisomerase Activity Is Released from Disrupted SV40 Virions. We were puzzled to find that the same set of topoisomers, shown in Figure 4, lane 1, was also observed if the SV40 virions were simply treated with EGTA and DTT in the absence of exogenously added DNA topoisomerase (Figure 4, lane 3). It appeared that concurrent with the disruption event, a topoisomerase activity was released or activated, which then relaxed the supercoils not constrained by nucleosomes. We tested for the presence of an endogenous topoisomerase activity by incubating the mature virions, intact or disrupted with EGTA and DTT, with supercoiled PBR322 DNA. Figure 5, lane 1, shows that when incubated with disrupted virions, the PBR322 DNA becomes relaxed but not after incubation with intact virions (Figure 5, lane 2).

Since the SV40 chromatin, isolated from infected cells, contains a topoisomerase I activity (Sen & Levine, 1975; Keller et al., 1977; Hamlin & Yaniv, 1979), we investigated whether the enzymatic activity we detected with mature virions was a contaminant of the preparation. To do so, we repeated the virion isolation procedure and analyzed the topoisomerase activity at the various stages of purification as follows. SV40 virions were labeled *in vivo* with ³H-labeled amino acids. After

cell lysis, the bulk of the virions was dissociated from the cellular components by freeze-thawing and was separated from cellular chromatin by centrifugation. The supernatant was adjusted to 0.5 M NaCl, and the virions were precipitated by the addition of poly(ethylene glycol). The virions were then purified by equilibrium density centrifugation in CsCl; SV40 chromatin and previrions are not stable under high ionic strength and dissociate into soluble proteins and DNA (Fernandez-Munoz et al., 1979; Baumgartner et al., 1979; Seidman et al., 1979). Fractions were collected and dialyzed against 0.05 M Tris-HCl, pH 7.5. An aliquot of each fraction was assayed for topoisomerase activity after incubation with DTT and EGTA. We observed two peaks of topoisomerase activity. One banded at the position of mature virions ($\rho = 1.33$); the other was on the top of the gradient at the position of free proteins. We then subjected the peak that contained the mature virions to two further rounds of purification by equilibrium banding in CsCl. The latter gradient was fractionated, and after dialysis, aliquots of each fraction were assayed for protein, SV40 DNA, and topoisomerase activity. Figure 6A,B shows that the bulk of ^3H -labeled proteins and SV40 DNA band as a single homogeneous peak, as expected; the mature virions were also evident in the peak fraction (fraction 4) when we monitored the turbidity. Topoisomerase activity was assayed by following the conversion of supercoiled pDMS630 plasmid to covalently closed relaxed DNA. The pDMS630 plasmid consists of a ColE1 genome with a Tn3 insertion (Inselburg, 1977). We chose this plasmid because it is 2.4 times larger than SV40 DNA and thus easily distinguished. Figure 6B shows that the plasmid is fully relaxed only when it is incubated with the fraction that contains the SV40 virions, provided that the virions are first disrupted with EGTA and DTT. Only a trace amount of enzymatic activity can be detected if the EGTA or DTT is omitted from the reaction mixtures (Figure 6C). These experiments clearly suggest that a topoisomerase activity copurifies with mature virions and is only released when the virions are disrupted in the presence of a divalent ion chelator and a reducing agent.

Cofactors Required for Topoisomerase and Endonuclease Associated with SV40 Virions. Although the topoisomerase activity associated with SV40 virions is active in the absence of divalent ions, the addition of Mg^{2+} enhances the enzymatic activity. The Mg^{2+} ion requirement can be best detected at low concentrations of the virus. The addition of ATP to the reactions does not enhance the enzymatic activity (not shown).

For the assays done without Mg^{2+} , there was little endonuclease activity. For the assays done with Mg^{2+} , some nuclease activity was observed at 33 °C and was increased appreciably when the incubation temperature was raised to 37 °C. Therefore, we determined the time course of the endonuclease and topoisomerase activities associated with SV40 virions, disrupted with DTT and EGTA or intact, at 37 °C in the presence of Mg^{2+} . We distinguished the nicked (DNA II) and relaxed species (DNA Ir) by adding the intercalating agent ethidium bromide to the assay mixture after stopping the reactions with NaDodSO₄ and EDTA and heating at 50 °C. Ethidium bromide separates DNA Ir and DNA II by supercoiling DNA Ir as it intercalates but not DNA II (Keller, 1975). Thus DNA Ir, which ordinarily would move with DNA II, moves faster than DNA II at low concentrations of ethidium bromide in the mixture. By this approach, we can distinguish how much of the DNA Ir/DNA II band is DNA Ir because of topoisomerase activity and how much is DNA II because of nuclease activity. We observed that in the case of disrupted virions at early phases of reaction, topological

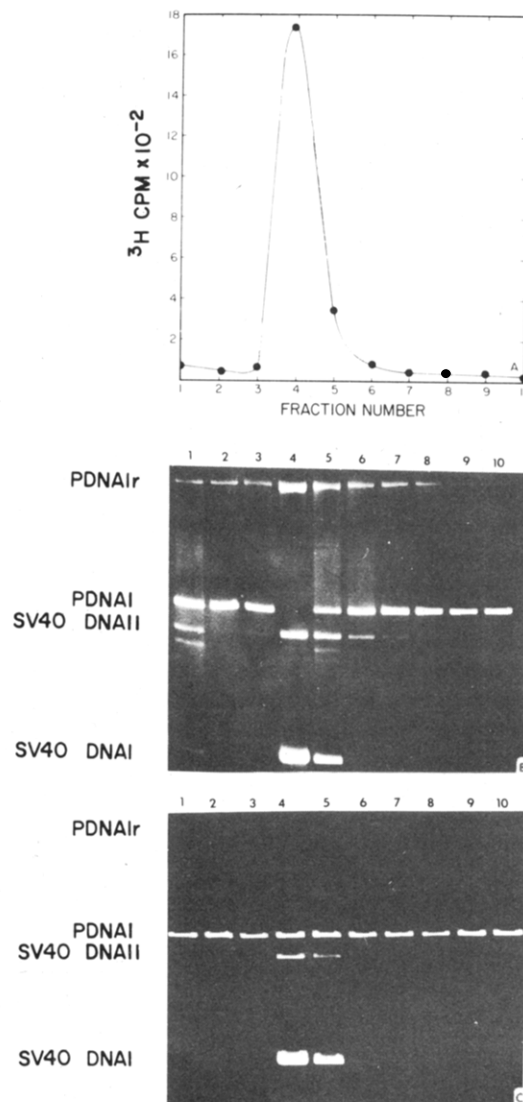


FIGURE 6: Topoisomerase activity bands with SV40 virions. Purified SV40 virions, labeled with ^3H -labeled amino acids, were centrifuged to equilibrium in CsCl solutions. Ten equal fractions were collected from the bottom of the tube. The virus was evident as an opalescent band, which was collected in fraction 4. The CsCl was removed by dialysis. (A) The fractions were monitored for proteins by counting an aliquot in a liquid scintillation counter. (B) 5 μL of each fraction was treated with 0.05 M Tris-HCl, pH 8.5, 0.1 M NaCl, 10 mM DTT, and 20 mM EGTA to disrupt SV40 virions. The topoisomerase activity was measured by following the conversion of supercoiled pDMS630 plasmid (PDNA I) to the relaxed form (PDNA Ir). (C) A similar assay to (B) was performed in the absence of EGTA.

isomers migrating between the substrate and the relaxed products are generated (Figure 7A, lanes 1–3). After a long incubation (90 min), the DNA is converted to a mixture of nicked DNA (55%), covalently closed relaxed circles (40%), and 5% of unit-length linear molecules (Figure 7A, lane 6). If incubated with intact virions, the plasmid is nicked at a slower rate; after 90 min of incubation, 25% of the DNA is nicked, and 5% is linearized (Figure 7B). These experiments suggest that when disrupted, the virions seem to release, in addition to the topoisomerase, an endonuclease that is active at 37 °C in the presence of Mg^{2+} . However, intact virions contain a low level of endonuclease and no detectable topoisomerase activities in the presence of divalent ions. The presence of an endonuclease in isolated SV40 and polyoma virions has been previously observed (Cuzin et al., 1971; Kaplan et al., 1972; Kidwell et al., 1972; Kasamatsu & Wu, 1976). However, later studies raised the possibility that the

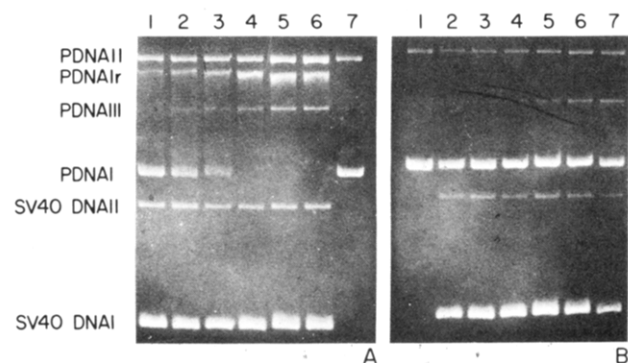


FIGURE 7: Time course of endonuclease and topoisomerase activities associated with SV40. (A) 0.5 μ g of supercoiled pDMS630 plasmid was incubated with 1.3 μ g of disrupted SV40 virions in the presence of MgCl_2 at 37 $^\circ\text{C}$. The reactions were terminated after 3.5, 7, 15, 30, 60, and 90 min (lanes 1–6). Lane 7 is the same as lane 6, except that SV40 virions were omitted. (B) 0.5 μ g of pDMS630 plasmid (lane 1). In lanes 2–7, the plasmid was incubated with 1.3 μ g of intact virions in the presence of MgCl_2 at 37 $^\circ\text{C}$ for the same length of time as in (A). In agarose gels, the nicked circular DNA comigrates with the covalently closed relaxed DNA. Addition of 0.5 μ g of ethidium bromide to the samples shifted the mobility of the relaxed plasmid (PDNA Ir) from the nicked species (PDNA II). PDNA III denotes the unit-length linear plasmid DNA molecules.

endonuclease activity is not an integral part of the virions but is contributed by the serum used in the maintenance of virus-infected cells (Dooley et al., 1976; McMillen et al., 1976). We have observed that although a trace amount of endonuclease activity is associated with intact virions, the level of the enzymatic activity is enhanced if the virions are disrupted. Our findings are in excellent agreement with those reported by Kaplan et al., who released the endonuclease activity by rupturing the virions with repeated rounds of freezing and thawing (Kaplan et al., 1972). The properties of the endonuclease that is released from virions disrupted with EGTA and DTT resemble those obtained from freeze-thawed virions.

Since the SV40 isolation procedures relied on centrifugation in CsCl (high ionic strength), we determined the ionic conditions whereby topoisomerase and endonuclease activities are extracted from cellular chromatin prepared from uninfected BSC-1 cells. BSC-1 cells were harvested, and the cytoplasmic and nuclear fractions were separated (see Materials and Methods). The nuclear fraction was suspended in increasing concentrations of phosphate buffer. The cytoplasmic and the nuclear supernatant fractions were then assayed for topoisomerase and endonuclease activities. The assays were done in 50 mM Tris-HCl, pH 8.5, and 0.1 M NaCl in the absence or presence of 8 mM MgCl_2 . For assays done without Mg^{2+} , there was little nuclease activity (Figure 8, lane 2). A topoisomerase activity was detected in both cytoplasmic and nuclear fractions (Figure 8); a concentration dependence study showed that the nuclear fractions, eluted with the 0.3 M phosphate buffer, had the highest topoisomerase activity. The 0.5 M phosphate extract had the lowest level of topoisomerase activity, as previously observed (Bina et al., 1976). For the assays done with Mg^{2+} , maximum levels of nuclease activity were observed in the cytoplasmic fraction and the nuclear extract isolated with 0.1 M phosphate (Figure 8). Only a trace amount of nuclease activity was observed in nuclear extracts isolated with 0.2 and 0.3 M phosphate buffers. Concentration dependence studies showed that the presence of the divalent ions enhanced the topoisomerase activity 5-fold, in a manner similar to that of the enzyme associated with mature SV40 virions. The cellular enzyme does not require DTT or EGTA for activity.

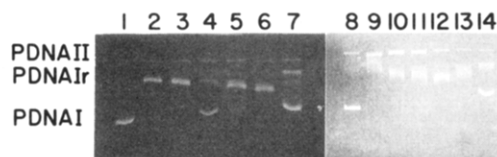


FIGURE 8: Assay of cytoplasmic and nuclear fractions of BSC-1 cells for topoisomerase and endonuclease activities. BSC-1 cells were harvested and the cytoplasmic fraction was separated from the nuclear fraction. The nuclear fraction was suspended successively in increasing concentrations of phosphate buffer (0.1, 0.2, 0.3, and 0.5 M) at pH 7.5. Two cytoplasmic and the four nuclear supernatant fractions were then assayed for topoisomerase and endonuclease activities. The assays were done in 50 mM Tris-HCl, pH 8.5, and 0.1 M NaCl in the absence (lanes 2–7) or presence (lanes 9–14) of 8 mM MgCl_2 . Lanes 1 and 8 contain the plasmid DNA, which was incubated in the absence of any cellular extracts.

Discussion

Our studies show that in addition to capsid proteins, two enzymatic activities, a topoisomerase and an endonuclease, are released when SV40 virions are treated with a divalent ion chelator and a reducing agent. Thus, exogenously added supercoiled DNA is converted to a mixture of covalently relaxed DNA and nicked DNA species when incubated with disrupted SV40 virions.

Kasamatsu and Wu have isolated a fraction of nicked SV40 DNA covalently attached to a protein by treating the virions with NaDodSO_4 in the presence of EDTA and DTT; all three reagents are essential both for the introduction of the nicks on the DNA and for the formation of the protein–DNA linkage (Kasamatsu & Wu, 1976). These observations, taken together with the general properties of DNA topoisomerases (Champoux, 1978; Wang & Liu, 1979) and our results, strongly suggest that Kasamatsu and Wu might have trapped and characterized the covalent linkage of the SV40-associated topoisomerase with the viral DNA. Kasamatsu and Wu further showed that the protein–DNA linkage is located in the genomic region, which encompasses the replication origin of SV40 DNA and the transcription initiation sites of both early and late genes (Kasamatsu & Wu, 1976).

The release of a topoisomerase activity from highly purified SV40 virions provides a strong support for the interaction of this enzyme with SV40 chromatin *in vivo*. The association of DNA topoisomerase activity with SV40 chromatin in the course of the lytic cycle has been previously reported (Sen & Levine, 1974; Keller et al., 1977; Hamelin & Yaniv, 1979). This observation, however, has not been unequivocal since the enzyme could have associated with the accessible segments of the viral chromatin during the isolation procedure.

SV40 chromatin *in vivo* contains a region accessible to regulatory proteins [reviewed by Das & Niyogi (1981)]; this “open” region contains the origin of DNA replication, the promoters of early and late genes, and a 72 base pair tandem repeat that is required for lytic growth (Gruss et al., 1981). It is tempting to speculate that the 72 base pair repeat might serve as the binding site for the SV40-associated DNA topoisomerase. This is consistent with the observation that the repeat can be transposed into any part of the SV40 genome (P. Berg, personal communication) and yet exert long-range effects.

Our electron microscopic observation suggests that a fraction of the SV40 chromatin that are released from SV40 virions under mild conditions contains stretches of free DNA. Thus, the SV40 DNA that remains fully supercoiled if isolated under more stringent conditions (in the presence of NaDodSO_4) becomes partially relaxed under milder disruption conditions. Moyne and co-workers have also observed that a fraction of

SV40 chromatin released from disrupted virions has a "highly relaxed" configuration (Moyne et al., 1981). The DNA of the latter complexes, on the average, appears to be compacted only half as much as SV40 minichromosomes isolated from infected cells (Griffith, 1975; Moyne et al., 1981) or reconstituted in vitro (Bina & Simpson, 1977; Stein et al., 1977). These observations can be explained in two ways: (i) once the virions have been purified through high salt and disrupted by EGTA and DTT, the normal nucleosome structure is not present in all the DNA molecules and (ii) a fraction of encapsidated SV40 DNA is truly deficient in histones or normal nucleosomes. The second explanation raises the interesting possibility that a fraction of encapsidated SV40 DNA is perhaps not constrained by histones but by the other SV40-associated proteins.

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