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Stable Association of Viral Protein VP_1 with Simian Virus 40 DNA

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Mild dissociation of simian virus 40 particles releases a 110S virion core nucleoprotein complex containing histones and the three viral proteins VP_1, VP_2, and VP_3. The association of viral protein VP_1 within this nucleoprotein complex is mediated at least partially through a strong interaction with the viral DNA. Treatment of the virion-derived 110S nucleoprotein complex with 0.25% Sarkosyl dissociated VP_2, VP_3, and histones, leaving a stable VP_1-DNA complex. The VP_1-DNA complex had a sedimentation value of 30S and a density of 1.460 g/cm^3. The calculated molecular weight of the complex was 7.9×10^6, with an average of 100 VP_1 molecules per DNA. Agarose gel electrophoresis of the VP_1-DNA complex demonstrated that VP_1 is associated not only with form I and form II simian virus 40 DNAs but also with form III simian virus 40 DNA generated by cleavage with *Eco*RI.

We recently reported that mature simian virus 40 (SV40) virions can be dissociated in vitro to release a compact 110S nucleoprotein core (4). The 110S nucleoprotein complex contains a fraction of viral proteins VP_1 and VP_2 in addition to the proteins found associated with the 55S viral minichromosome, i.e., VP_3 and histones H_2A, H_2B, H_3, and H_4. The 110S virion core approaches the efficiency of superhelical (form I [F_I]) SV40 DNA as a template for transcription and is four- to fivefold more efficient than the 55S minichromosome. These transcription studies suggest that the association of VP_1 or VP_2 or both with the viral minichromosome modifies the transcriptional properties of the nucleoprotein complex and makes the 110S virion core an efficient template for transcription. To determine how the viral proteins might increase transcriptional activity, it is important to understand the manner in which the proteins attach to the minichromosome. The experiments presented below demonstrate that at least a portion of the VP_1 association with the nucleoprotein complex is through a strong binding to SV40 DNA.

MATERIALS AND METHODS

Viruses and cells. SV40 (strain 776) was originally obtained from K. Takemoto, National Institute of Allergy and Infectious Diseases. Virus was grown in BSC-1 cells.

Infection and purification of SV40 virions. Confluent monolayers of BSC-1 cells maintained in Eagle minimal essential medium supplemented with 0.03% glutamine, 10% fetal calf serum, and streptomycin, penicillin, ampicillin, and mycostatin were infected with SV40 at a multiplicity of infection of 10 PFU per cell. After a 2-h adsorption at 37°C, Eagle minimal essential medium supplemented with 1 to 2% fetal calf serum was added. After 7 to 8 days, when cell lysis was complete, infected cells and supernatant were collected and the virus was purified as previously described (4).

Preparation and Sarkosyl treatment of SV40 cores and minichromosomes. Preparation of SV40 nucleoprotein cores was carried out by ethylene glycol-bis-*N,N'*-tetraacetic acid-dithiothreitol (EGTA-DTT) dissociation of SV40 virions as previously described (4). The preparation of minichromosomes from SV40 virions was carried out by the method of Christiansen et al. (5). Sarkosyl treatment was performed immediately after virion dissociation by the addition of 0.1 volume of 2.5% Sarkosyl, followed by incubation at 32°C for 15 min.

Velocity sedimentation of Sarkosyl complexes. After Sarkosyl treatment, samples were layered onto 5 to 20% sucrose gradients containing 1 mM sodium EDTA-60 mM KCl in 10 mM Tris-hydrochloride (pH 7.4). Centrifugation was performed in an SW41 rotor at 40,000 rpm (4°C) for 3 h. Isokinetic gradients were prepared by the method of McCarty et al. (12).

Agarose gel electrophoresis of SV40 DNA. Electrophoresis of DNA samples was done on a 1.5% agarose slab gel at 150 V for 4 h with a 40 mM Tris acetate (pH 7.2)-20 mM sodium acetate-1 mM sodium EDTA buffer. The gel was stained with ethidium bromide and photographed under a UV light source. SV40 DNA, treated with *Eco*RI under partial digest conditions, was used as a marker for supercoiled, re-

laxed circular, and linear SV40 DNAs (F_I, F_{II}, and F_{III}, respectively).

Protein iodination and SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described previously (4). Proteins were labeled in vitro with ^{125}I by the method of Frost and Bourgaux (7).

RESULTS

Sedimentation analysis of Sarkosyl-treated chromatin complexes. SV40 virions were dissociated in vitro by incubation in either EGTA-DTT buffer (pH 8.6) to release 110S virion cores or glycine-DTT buffer (pH 9.85) to release the 55S virion minichromosome (5). After dissociation, Sarkosyl was added to a final concentration of 0.25% (wt/vol), and samples were incubated at 32°C for 15 min. The Sarkosyl-treated nucleoprotein complexes were then compared on parallel isokinetic sucrose gradients. Sarkosyl-treated SV40 minichromosomes cosedimented with naked 21S F_I SV40 DNA, indicating that all protein had been removed from the nucleoprotein complex. However, when Sarkosyl-treated SV40 cores were analyzed, the [^{3}H]thymidine label sedimented at 30S, indicating that proteins were still attached to the SV40 DNA. To eliminate the possibility that the 30S complex was the result of de novo binding of proteins to SV40 DNA during Sarkosyl treatment in EGTA-DTT buffer, the following experiment was performed. SV40 virions were dissociated by glycine-DTT treatment and then treated with Sarkosyl to generate naked SV40 DNA (Fig. 1). The sample was diluted in EGTA-DTT buffer containing 0.25% Sarkosyl and then incubated and sedimented on sucrose gradients. The results of this experiment demonstrate that no protein attached to the DNA in the presence of Sarkosyl, since the DNA sedimented at 21S (data not shown).

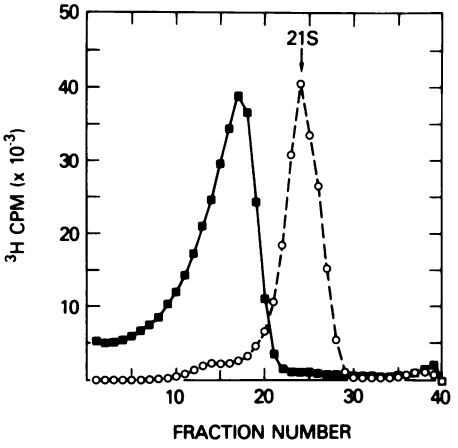

FIG. 1. *Velocity sedimentation of Sarkosyl-treated nucleoprotein complexes. [^{3}H]thymidine-labeled SV40 virions purified as described previously (4) were dissociated by treatment with 5 mM EGTA-3 mM DTT-0.15 M NaCl in 50 mM Tris (pH 8.7) for 30 min at 32°C to release the 110S virion core, or with 3 mM DTT-0.075 M NaCl in 100 mM glycine (pH 9.85) for 5 min at 37°C as described by Christiansen et al. (5) to release the 55S virion minichromosome. After dissociation, Sarkosyl was added to a final concentration of 0.25%, and samples were incubated for 15 min at 32°C. Samples were layered onto isokinetic 5 to 20% sucrose gradients (12) containing 1 mM sodium EDTA-60 mM KCl in 10 mM Tris-hydrochloride (pH 7.4) and centrifuged in an SW41 rotor at 40,000 rpm for 3 h (4°C). Arrow indicates the position of 21S marker SV40 DNA. Symbols: ■, 110S virion core, Sarkosyl treated; ○, 55S virion minichromosome, Sarkosyl treated.*

SDS-PAGE of ^{125}I-labeled chromatin complexes. To determine which SV40 core protein remained associated with the viral DNA after Sarkosyl treatment, peak fractions from the isolated 30S complex were pooled and labeled in vitro with ^{125}I. Isolated 110S SV40 cores and 55S minichromosomes were labeled to serve as controls. Chromatin samples were iodinated as intact complexes at pH 7.0. Under these conditions, histones H_2B and H_4 are labeled extensively, whereas H_3 and H_2A label poorly (15). Previous analysis of Coomassie blue-stained gels demonstrated the presence of all four histones in 110S core and 55S minichromosome samples (data not shown). As shown in Fig. 2A, SDS-PAGE of ^{125}I-labeled SV40 cores demonstrated the presence of VP_1, a faint VP_2 band, VP_3, and histones H_2B and H_4. ^{125}I-labeled minichromosomes demonstrated the presence of histones H_2B and H_4 (Fig. 2B). VP_3, which is normally found associated with the DNA-histone complex (4), was removed by treatment with 0.5 M NaCl before iodination. ^{125}I labeling of the 30S complex revealed only one protein band which migrated in the SDS-polyacrylamide gel with VP_1 marker (Fig. 2C). Even when the autoradiogram was overexposed, only VP_1 could be detected in the 30S complex sample. ^{125}I labeling of the 21S peak of DNA in SV40 minichromosome-Sarkosyl preparations failed to label any detectable protein in a similar analysis (data not shown).

Buoyant density determination and estimation of number of VP_1 molecules bound to SV40 DNA. To estimate the amount of VP_1 associated with the 30S VP_1-DNA complex, we fixed peak fractions from the sucrose gradient with formaldehyde and centrifuged them to equilibrium in CsCl gradients. The results of this experiment were compared with similar density determinations obtained previously for SV40 virions, 110S cores, and 55S minichromosomes

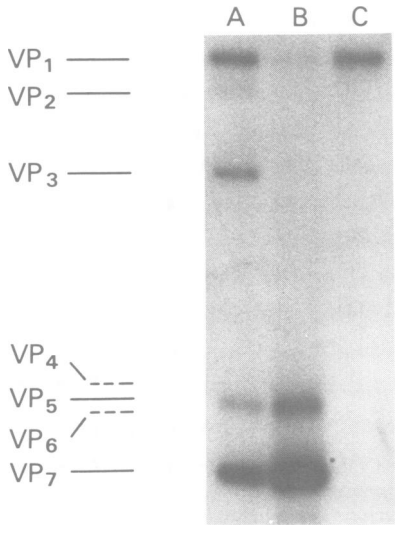

FIG. 2. *SDS-PAGE of ^{125}I-radiolabeled nucleoprotein complexes. 110S virion cores and 55S minichromosomes were prepared as described in the legend to Fig. 1 and isolated on sucrose gradients as described previously (4). The 30S core-Sarkosyl complex was isolated as described in the legend to Fig. 1. The nucleoprotein complexes were radiolabeled in vitro with ^{125}I by the chloramine T procedure as described by Frost and Bourgaux (7) and prepared for electrophoresis on a 15% acrylamide gel as described previously (4). (A) ^{125}I-labeled SV40 110S cores; (B) ^{125}I-labeled SV40 55S minichromosome; (C) ^{125}I-labeled 30S complex.*

(Table 1). The formaldehyde-fixed 30S complex banded as a homogeneous species at a density of 1.460 g/cm^3. By using a protein average density of 1.28 g/cm^3 and a viral DNA density of 1.700, it was calculated that the 30S VP_1-DNA complex consists of 57% protein and 43% DNA, with a total molecular weight of 7.9×10^6. By using the molecular weight of VP_1 (40,000) determined from DNA sequence analysis (6, 14), we calculated that there is an average of 100 molecules of VP_1 per molecule of SV40 DNA. This estimate is close to the VP_1 estimate obtained with 110S cores. Thus, we conclude that most, if not all, of the VP_1 present in the virion core remains associated with the viral DNA during Sarkosyl treatment. If similar calculations are performed on whole SV40 virions in which VP_1 contributes 75% of the total protein, 24% of the total VP_1 present in the virion is associated with the viral DNA. It is interesting to note that the 55S minichromosome has a density of 1.495 g/cm^3, with a calculated molecular weight of 6.5×10^6. Thus, although the calculated molecular weight of the VP_1-DNA complex is apparently greater than the 55S minichromosome, the sedimentation coefficient is significantly less (see below).

Agarose gel electrophoresis of the 30S VP_1-DNA complex. Agarose gel electrophoresis of the VP_1-DNA complex was done to determine the effect of protein on the migration of DNA in the gel. Figure 3A, B, E, and F shows the results of electrophoretic analysis of the 21S minichromosome-Sarkosyl peak before and after cleavage with *Eco*RI. As was consistent with its sedimentation value and our inability to label any protein with ^{125}I, no change in DNA migration was observed with these samples after addition of SDS, indicating that all of the proteins are removed by Sarkosyl treatment. When a similar analysis was performed on the 30S VP_1-DNA complex, significant changes in migration of superhelical F_I SV40 DNA could be seen before and after SDS treatment. Figure 3C shows the electrophoretic profile of the 30S complex after SDS treatment. Only F_I and F_{II} SV40 DNA bands, which comigrated with marker DNA samples, are visible. Figure 3D reflects the migration of F_I and F_{II} SV40 DNAs with VP_1 attached. It is readily apparent that the binding of VP_1 to F_I SV40 DNA affects its migration in agarose gels. Based on several different experiments, the migration of F_I SV40 DNA was decreased by 37 to 45%. VP_1 binding was observed to have much less effect on the migration of SV40 F_{II} DNA. Even in gels run considerably longer than those shown in Fig. 3, only about a 3 to 5% decrease in mobility was detected with VP_1-F_{II} DNA complexes. To determine whether VP_1 was actually attached to F_{II} DNA, ^{125}I-labeled complexes were electrophoresed on agarose gels, and autoradiograms were performed. Radioactive bands were detected with both F_I and F_{II} DNAs before, but not after, SDS treatment, indicating that both DNA species had VP_1 attached. In addition, the buoyant density of the VP_1-F_{II} DNA complex was the same as that of the VP_1-F_I DNA complex, indicating that equal quantities of VP_1 were bound to both DNA species. Similar electrophoretic analysis on *Eco*RI-cleaved VP_1-F_{III} DNA complexes is shown in Fig. 3G and H. The SDS-treated VP_1-F_{III} DNA complex showed only one DNA band which comigrated with naked SV40 F_{III} DNA (Fig. 3G). Figure 3H reflects the migration pattern of the VP_1-F_{III} complex. The presence of VP_1 on linear DNA decreased the mobility of the DNA molecule an average of 10 to 14% compared with naked F_{III} SV40 DNA.

DISCUSSION

The results presented in this paper demonstrate that protein VP_1 associated with 110S

TABLE 1. *Estimation of protein composition of SV40 virions and nucleoprotein complexes*

Sample	Density (g/cm^3)	% Protein[a]	Mol wt (10^6)			Approx no. of VP_1 molecules[e]
			Total protein[b]	Histone[c]	Virion protein[d]	
Virions	1.330	88	25	3.3	21.7	470
110S core	1.400	71	8.4	3.3	5.1	130
30S VP_1-DNA	1.460	57	4.6	0	4.6	115
55S minichromosome	1.495	49	3.3	3.3	—[f]	—
DNA	1.700	0	—	—	—	—

[a] The percentage of protein present in nucleoprotein complexes was estimated from buoyant density measurements by using the following formula: (ρ of DNA − ρ of complex)/(ρ of DNA − ρ of protein) = % protein.

[b] The total molecular weight of the complex was determined by using the known molecular weight of SV40 DNA (3.45×10^6) and the percentage of DNA as determined in footnote *a*. The DNA molecular weight was subtracted to obtain the protein molecular weight.

[c] By using the molecular weight of histones H_3 (17,300), H_2B (15,800), H_2A (14,600), and H_4 (13,200), the nucleosome molecular weight was determined (121,600) and multiplied by 26, the upper limit of nucleosomes associated with SV40 DNA.

[d] The virion protein molecular weight was determined by subtracting the histone molecular weight from the total protein.

[e] The total number of VP_1 molecules was estimated by using the VP_1 molecular weight (40,000) as deduced from the SV40 DNA sequence. The number of VP_1 molecules in 110S core is overestimated since the contribution of VP_2 and VP_3 was neglected.

[f] —, Not present.

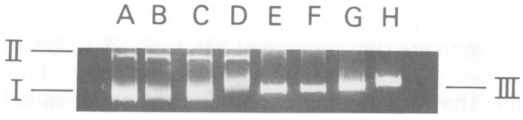

FIG. 3. *Agarose gel electrophoresis of VP_1-DNA nucleoprotein complex. SV40 virions were dissociated, treated with Sarkosyl, and isolated on sucrose gradients as described in the legend to Fig. 1. The 21S (Sarkosyl-treated minichromosome) and 30S (Sarkosyl-treated core) peaks were collected. A fraction of each peak was cleaved with EcoRI. Samples of both the uncleaved and cleaved material were then treated further with 0.5% SDS. Electrophoresis was performed on a 1.5% agarose gel with 40 mM Tris acetate (pH 7.2)-20 mM sodium acetate-1 mM sodium EDTA buffer at 150 V for 4 h. The gel was stained with ethidium bromide and photographed under a UV light source. (A) 21S plus 0.5% SDS; (B) 21S untreated; (C) 30S plus 0.5% SDS; (D) 30S untreated; (E) 21S plus EcoRI plus 0.5% SDS; (F) 21S plus EcoRI; (G) 30S plus EcoRI plus 0.5% SDS; (H) 30S plus EcoRI. SV40 DNA, treated with EcoRI under partial digest conditions, was used as a marker for supercoiled, relaxed circular, and linear SV40 DNAs (F_I, F_{II}, F_{III}, respectively).*

virion cores is firmly bound to the viral DNA and is resistant to treatment with 0.25% Sarkosyl. Velocity sedimentation, SDS-PAGE, density gradient analysis, and agarose gel electrophoresis all demonstrate the association of VP_1 with the viral DNA. Control experiments showed that only VP_1 that is bound to the viral DNA before Sarkosyl treatment remains associated; i.e., no VP_1 binds to the DNA in the presence of Sarkosyl. Agarose gel electrophoresis of the VP_1-DNA complex demonstrated that VP_1 may be associated with either F_I, F_{II}, or F_{III} SV40 DNA.

The presence of a virion or virion-associated protein firmly bound to SV40 viral DNA has been reported previously. Griffith et al. (9) have shown that treatment of viral chromatin with 1 M NaCl results in the dissociation of the bulk of the protein from the DNA molecule, releasing a high-salt-stable DNA-protein complex. High-resolution electron microscopy of circularized SV40 DNA molecules demonstrated a single "knob" of protein, which mapped at position 0.7 ± 0.05 on the *Eco*RI SV40 DNA physical map. Kasamatsu and Wu (11) reported that disruption of purified SV40 virions with 5% SDS resulted in the release of F_I and F_{II} SV40 DNAs. Electron microscopy of the isolated DNA demonstrated that F_{II}, but not F_I, SV40 DNA had a protein moiety attached to the viral genome at or near 0.67 on the *Eco*RI SV40 DNA physical map. Identification of the protein by SDS-PAGE was not presented in either case. Whether in fact this protein is VP_1 and, if so, whether it is a particular subspecies of VP_1 which is also present in the 30S complex remains to be determined. The association of the majority of VP_1 with SV40 DNA in the 30S complex apparently is different from that described above. As shown by agarose gel electrophoresis, VP_1 is associated with F_I and F_{II} SV40 DNAs. Furthermore, our

estimate of 4.6×10^6 daltons of VP_1 on the viral DNA is approximately 15 times more than that calculated by Kasamatsu and Wu (11).

Sarkosyl-resistant binding of cellular RNA polymerase B to F_I SV40 DNA was originally demonstrated by Gariglio and Mousset (8) and extended by several investigators (1, 2). Late in the SV40 infection cycle, viral transcriptional complexes can be isolated by Sarkosyl extraction of infected cell nuclei. With the exception of RNA polymerase B, all other chromatin proteins apparently are released by Sarkosyl treatment. Free RNA polymerase B molecules are unable to initiate transcription in the presence of Sarkosyl. Since only RNA polymerase B molecules in the process of RNA chain elongation stay bound to the DNA, one explanation for the stable association is that the protein interacts with the single-stranded DNA region generated by the opening of the double helix at the site where the mononucleotides are linked to the nascent RNA chain. Further investigation is required to determine whether the Sarkosyl-resistant binding of VP_1 to F_I SV40 DNA involves the interaction of the protein with single-stranded-like regions which have been demonstrated in the superhelical DNA molecule (10).

In a recent report on isoelectric focusing of polyoma viral protein VP_1, Bolen et al. (3) have demonstrated that six distinct species of VP_1 are present in complete polyoma virions. The VP_1 species appear to be generated by posttranslational modification of the initial VP_1 gene product. VP_1 species A (VP_1-A), pI 6.75, was found to be the only VP_1 species firmly associated with the 48S DNA-protein complex. VP_1-A could not be iodinated by the Enzymobead technique and was not present in polyoma capsids, indicating that VP_1-A was an internal virion protein associated exclusively with the DNA-protein complex. In collaboration with these investigators, we have recently begun a similar analysis of SV40 VP_1 to determine which species of VP_1 are present on the mature SV40 virion chromatin complex. As was previously observed by O'Farrell and Goodman (13), we found that six species of VP_1 can be identified by isoelectric focusing. Preliminary isoelectric focusing analysis of VP_1 associated with the SV40 110S nucleoprotein complex indicates that, of the six VP_1 species, only two are present, one of which is the most basic VP_1 species and has the same pI as the polyoma VP_1-A protein. Since experiments from this laboratory have demonstrated that the association of VP_1 or VP_2 or both with 55S virion minichromosomes enhances transcriptional activity, it is plausible that interaction of these specific VP_1 species with virion chromatin regulates transcriptional activity. Sarkosyl treatment of 110S virion cores allows us to isolate these specific species of VP_1 with a high degree of purity, and studies involving DNA binding, histone binding, and posttranslational modifications of VP_1 in relation to transcription are now possible.

It is of interest that VP_1, but not VP_2, VP_3, or histones, is resistant to Sarkosyl treatment. This observation is in contrast to the pH dependence of VP_1 binding, where we observe that as the pH of dissociation is increased to pH 9.8, VP_1, as well as VP_2, is released from the 110S core, leaving the 55S minichromosome with VP_3 and histones attached. Since alkaline pH and SDS release most, if not all, of the VP_1 from SV40 DNA, it is evident that no covalent bonds exist between the two. The role of hydrogen, ionic, and hydrophobic bonds in the interaction between VP_1 and SV40 DNA requires further investigation.

There is a differential effect of VP_1 on the mobility of F_I, F_{II}, and F_{III} SV40 DNAs in agarose gels. Since the separation of the three forms of SV40 DNA is based purely on configuration and not on molecular weight, the results probably indicate that binding of VP_1 to F_I SV40 DNA causes a relaxation of the DNA molecule. The fact that the VP_1-DNA complex sediments more slowly in sucrose gradients than its calculated molecular weight would predict may also indicate that the structure is more extended, giving it a higher frictional coefficient.

These studies represent the first step in an analysis designed to determine how VP_1 acts to stimulate transcription of the 110S virion core. Though these studies suggest that at least a portion of the VP_1 molecule is firmly attached to the viral DNA, it is not known whether VP_1 also interacts with the histone nucleosome structure. Protein cross-linking should allow us to determine whether any interaction occurs between VP_1 and histones and, if so, which histone species are involved.

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