

DNA-Binding Properties of the Major Structural Protein of Simian Virus 40

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We investigated whether the VP1 protein of simian virus 40 binds to DNA. In vitro DNA-binding experiments clearly indicate that VP1 bound strongly to double-stranded and single-stranded DNA, with a higher affinity for the latter; additional experiments show that VP1 did not bind to a specific sequence of simian virus 40 DNA.

Although the DNA-binding properties of simian virus 40 (SV40) large T antigen (T-Ag) are well characterized, no such properties have been described for the SV40 capsid proteins. Nevertheless, several studies have shown that dissociation of mature virions in vitro leads to the formation of a nucleoprotein complex containing the viral DNA and a fraction of the viral capsid proteins VP1 and VP2. Treatment of this complex with high-salt (4) or detergent (6) solutions has shown that the VP1-DNA interaction is rather stable. These observations led us to examine directly the DNA-binding properties of VP1.

Confluent monolayers of CV1 cells were infected with SV40 at a multiplicity of infection of 10 PFU per cell. Twenty-one hours after infection, the cells were labeled for 3 h with 300 μ Ci of [³⁵S]methionine in 2 ml of methionine-free medium per 10-cm petri dish. After labeling, nuclear proteins were extracted as described by May et al. (9) with certain modifications. Briefly, the cells were washed with phosphate-buffered saline and incubated in 0.5 ml of 0.1% Tween 80 per petri dish for 5 min. Ice-cold isotonic buffer (10 mM triethanolamine [pH 7.0], 0.25 M sucrose, 25 mM NaCl, 2 mM EDTA) was then added (3 ml per petri dish). The nuclei were pelleted at 1,500 \times g for 5 min, washed with isotonic buffer three times, and lysed in 1.0 M NaCl (1 ml/5 petri dishes) for 30 min at 4°C. The suspension obtained was centrifuged at 20,000 \times g for 60 min, and the supernatant was dialyzed overnight against column buffer (10 mM sodium phosphate [pH 7.4], 0.1 M NaCl, 10% glycerol [wt/vol], 0.5% Nonidet P-40, 1 mM EDTA) and clarified by centrifugation at 8,000 \times g for 15 min. Such extracts were stored at -70°C. All buffers contained 1 mM phenylmethylsulfonyl fluoride and 1 μ g of leupeptine per ml.

A nuclear extract prepared from SV40-infected cells as described above was passed through a double-stranded DNA (dsDNA)-Sephadex column (calf thymus DNA) (5), and bound proteins were then eluted with column buffer of increasing ionic strength. The collected fractions were immunoprecipitated with either Hamster tumor serum or anti-SV40 capsid serum and then analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Fig. 1A shows the result of immunoprecipitation of the extract before applying it to the column. At 24 h after infection, substantial quantities of VP1 protein were found in the nucleus. This is consistent with earlier findings that viral

capsid proteins are transported to the nucleus as early as 13 h after infection (2, 16). As expected, there were also large amounts of T-Ag. Analysis of the eluted fractions shows that, under our loading conditions (pH 7.4), a large portion of this T-Ag did not bind to the DNA. The bound fraction was eluted at 0.2 M NaCl (Fig. 1B). This is in good agreement with previous results described by others (13). We found that a certain fraction of VP1 bound strongly to the column under these conditions. It was eluted at 0.35 M NaCl and thus was bound to the DNA more strongly than did T-Ag (Fig. 1C). However, there was also a fraction of VP1 which did not bind to the column under these conditions (pH 7.4, 0.1 M NaCl). This nonbinding fraction of VP1 was consistently seen in several independent experiments. To verify that this was not due to saturation of the column, the 0.1, 0.2, and 0.35 M NaCl fractions were rechromatographed on dsDNA-Sephadex columns. Each fraction displayed an unchanged affinity to DNA (data not shown). It is known that a minor fraction of VP1 is phosphorylated (12). Under our experimental conditions this form of VP1 represented a very small

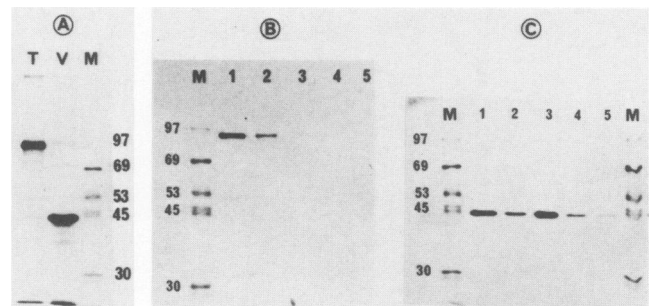


FIG. 1. Chromatographic behavior of T-Ag and VP1 on a dsDNA-Sephadex column. (A) Nuclear extract of SV40-infected cells immunoprecipitated with hamster tumor serum (lane T) or anti-SV40 capsid serum (lane V). (B and C) A 0.2-ml portion of extract in column buffer was loaded onto a 1-ml dsDNA-Sephadex column (approximately 0.5 mg of DNA). The column was washed, and proteins were eluted with buffers of increasing ionic strength, and the eluted fractions were immunoprecipitated with hamster tumor serum (B) or anti-SV40 capsid serum (C). The immunoprecipitated proteins were then analyzed by electrophoresis through an SDS-10% polyacrylamide gel, followed by fluorography of the gels. The salt concentrations of NaCl in the loading and elution buffers were 0.1 M (lane 1 [flow through]), 0.2 M NaCl (lane 2), 0.35 M (lane 3), 0.5 M (lane 4), and 1.0 M (lane 5). Lanes M contain molecular weight markers (molecular weights are indicated in thousands next to lanes).

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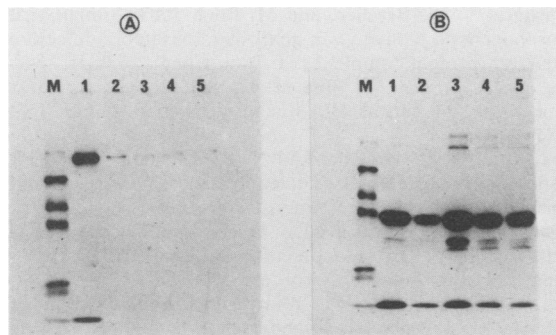


FIG. 2. Chromatographic behavior of T-Ag and VP1 on ssDNA ultrogel column. Procedures and lane designations are described in the legend to Fig. 1. (A) Eluates immunoprecipitated with hamster tumor serum. (B) Eluates immunoprecipitated with anti-SV40 capsid serum.

portion of the total VP1 population, too small to correspond to either the binding or nonbinding forms observed here (data not shown). Since VP1 contains seven cysteine residues and intermolecular disulphide bonding is known to be involved in capsid formation, it was possible that the unbound VP1 might represent aggregates of VP1 incompletely dissociated by the extraction procedure used. We therefore repeated the experiment, incubating the nuclear extract in 0.5 M β -mercaptoethanol for 30 min at 4°C to ensure the complete dissociation of all complexes. However, the results obtained (data not shown) were the same as those shown in Fig. 1C. Thus, under all conditions used, we consistently found a subpopulation of VP1 which did not bind to dsDNA. At present we cannot say whether this represents VP1 which has lost its affinity for dsDNA during the extraction procedure or whether it represents a distinct population devoid of this function.

We also examined the binding of VP1 to single-stranded DNA (ssDNA)-ultrogel column (Fig. 2B). Again, some VP1 did not bind to ssDNA. A peak of bound VP1 was eluted at 0.35 M NaCl, but a substantial portion of VP1 was not eluted until 0.5 or 1.0 M NaCl. T-Ag did not bind significantly to ssDNA under these conditions (Fig. 2A).

To verify that VP1 interacts directly with DNA (and not via another protein) we tested the binding of VP1 to dsDNA by using the Western blot technique. Nuclear extracts from infected CV1 cells were immunoprecipitated with anti-VP1 serum, separated by SDS-polyacrylamide gel electrophoresis, transferred to a nitrocellulose filter, and incubated with labeled SV40 DNA. Only one band was visible, corresponding to a protein with a molecular weight of 45,000 (Fig. 3, lane 2). When similar experiments were carried out with uninfected CV1 cells (Fig. 3, lane 1) or Cos cells (lane 3), both of which lack VP1, no bands were visible. It thus appears likely that VP1 interacts directly with DNA.

We used the McKay binding assay (10) to examine whether VP1 binds to a specific sequence of SV40 DNA. SV40 DNA was double digested with *Hind*III and *Hpa*II and end-labeled by using Klenow DNA polymerase and 32 P-labeled dATP. The conditions of binding and immunoprecipitation have already been described (7). Immunoprecipitation was performed with anti-tumor serum (Fig. 4, lane T) and anti-VP1 serum (lanes 1 through 6). Under these conditions (0.2 M NaCl; pH 7.4), even in the presence of an excess of calf thymus DNA, T-Ag bound strongly and specifically to fragment F, which contains the origin of DNA replication. In the absence of calf thymus DNA, VP1 bound equally to all

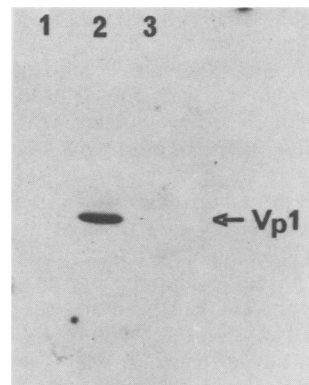


FIG. 3. Analysis of the binding of VP1 to DNA by the Western blot procedure. Nuclear extracts from uninfected CV1 cells (lane 1), infected CV1 cells (lane 2), or Cos cells (lane 3) were immunoprecipitated with anti-VP1 serum and separated by SDS-polyacrylamide gel electrophoresis. The proteins were transferred to a nitrocellulose filter as described previously (8). Prebinding was carried out for 3 h at 37°C in a buffer containing 10 mM sodium phosphate (pH 7.4), 1% (wt/vol) glycerol, 0.5% Nonidet P-40, 0.2 M NaCl, and 3% ultrapure bovine serum albumin. Binding with 50 ng of SV40 DNA labeled by nick translation (15) was performed in the same buffer for 16 h at room temperature with gentle agitation. The filter was washed four times for 15 min each at room temperature in the same buffer without bovine serum albumin.

fragments of SV40 DNA (Fig. 4, lane 1). Addition of increasing quantities of calf thymus DNA led to a parallel loss of binding to all fragments (Fig. 4, lanes 2 through 6). This indicates the absence of a strong affinity site for VP1 binding to SV40 DNA. However, with this technique we cannot exclude the presence of a specific low-affinity site.

The data presented in this communication clearly show that (i) VP1 had a high affinity for DNA, (ii) this affinity was higher for ssDNA than for dsDNA, (iii) the affinity of VP1 for dsDNA was higher than that of T-Ag, and (iv) VP1 interacted directly with the DNA but did not bind speci-

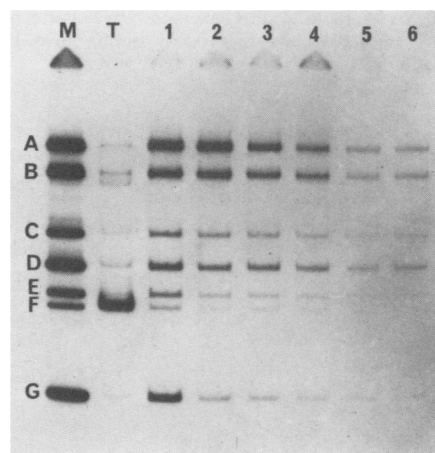


FIG. 4. Binding of T-Ag and VP1 to SV40 DNA. End-labeled SV40 DNA (100 ng) was incubated with 10 μ l of nuclear extract from infected CV1 cells in a buffer containing 10 mM sodium phosphate (pH 7.4), 200 mM NaCl, 10% (wt/vol) glycerol, 0.5% Nonidet P-40, 1 mM EDTA, and the following quantities of sheared calf thymus DNA: 5 μ g (lanes T and 6), none (lane 1), 0.5 μ g (lane 2), 1 μ g (lane 3), 2 μ g (lane 4), and 3 μ g (lane 5). Immunoprecipitation and electrophoresis were performed as described previously (7).

cally to any particular site on SV40 dsDNA. This interaction might reflect an important role for VP1 during SV40 morphogenesis. It has already been demonstrated that SV40 DNA is stably associated with capsid proteins (4, 6). This association is probably a consequence of the DNA-binding capacity of VP1 demonstrated here. Our finding supports the model of morphogenesis in which the capsid is organized by the gradual addition of capsid proteins to SV40 chromatin (3). A similar situation appears to exist in the case of the hepatitis B virus (HBV) since it has recently been shown by Petit and Pillot (14) that the major core protein p22 of HBV is a DNA-binding protein. Furthermore, the affinity of p22 is higher for HBV DNA than for plasmid DNA, and Petit and Pillot also suggest a role for p22 in HBV assembly.

Another possible role for the binding of VP1 to DNA has been suggested by Brady et al. (6), who have shown that VP1 (or VP2) increases transcription from the SV40 minichromosome *in vitro*. This stimulation is correlated with the association of VP1 to the minichromosome. A potential mechanism for the stimulation of transcription is suggested by Moyne et al. (11), who propose that VP1 can act as a nucleosome-unfolding agent. It is possible that the unfolding of nucleosomes on the SV40 minichromosome facilitates viral transcription or replication or both. We can postulate that the DNA-binding capacity of VP1 is associated with its nucleosome-unfolding activity. However our results suggest that this is not due to the binding of VP1 to any specific site on SV40 DNA.

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