DISULFIDE BONDS PROTECT THE ENCAPSIDATED CHROMOSOMES OF SIMIAN VIRUS 40

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1. Introduction

Simian virus 40 (SV40) provides an excellent system for studying different aspects of DNA packaging (reviewed in [1–3]). The structure of SV40 chromosome (or minichromosome) resembles the fundamental structure of eukaryotic chromatin [2,3]. The SV40 DNA is folded into units (nucleosome cores), each involving 146 basepairs of DNA which is wrapped about the octameric histone core (2 each of the 4 cellular histones: H2A, H2B, H3 and H4). In minichromosomes, the length of SV40 DNA is compacted ~6-folds [2]. To conform to the size of virus particles, the minichromosomes are organized into higher order structures by the virus-coded capsid proteins VP1, VP2 and VP3.

The capsid proteins, via hydrophobic interactions, pH-dependent bonds, divalent ion-mediated linkages and disulfide bridges, protect the SV40 chromosomes. Strongly alkaline conditions (pH 10.5) degrade the SV40 virions into 2 components [4]: soluble proteins containing VP1 and VP2, and a deoxynucleoprotein complex containing the viral DNA, some of the histones, and the minor capsid protein VP3. Other studies have shown that the treatment of the virions under milder pH conditions (pH 9.8), in the presence of reducing agents, results in the release of the major capsid protein (VP1).

In the biogenesis of stable polyoma and SV40 virions, disulfide bonds and Ca^{2+} play central roles [7–10]. The concomitant removal of divalent ions and reduction of S–S bonds (at pH 8.5) relaxes the compact structure of SV40 virions; some of the capsid proteins are released and the viral DNA becomes accessible to enzymes [10]. Here we have investigated the role played by disulfide bridges in the protection of encapsidated SV40 DNA to micrococcal nuclease.

2. Materials and methods

2.1. *Preparation of virus*

SV40 (strain 776) was grown on BSC1 cells, labeled with mixed [^{3}H]amino acids (Amersham), and purified as in [11].

2.2. *Nuclease digestion*

The micrococcal nuclease was purchased from Worthington. After digestion for an appropriate time under given conditions (see text for detail), the reactions (20 μl) were terminated by the addition of 2 μl 5% sodium dodecyl sulfate (SDS), 0.1 M EDTA, and 24 mM 2-mercaptoethanol. Incubation at 45°C for 15 min freed the DNA from histones and capsid proteins.

2.3. *Gel electrophoresis*

The DNA was fractionated on 1.2% agarose gels, after the addition of 5 μl 50% glycerol in 0.05% bromophenol blue. The gels, 4 mm thick and 130 mm long, were run horizontally in an apparatus from Savant for 5–8 h at 50 V. The electrophoresis buffer contained 40 mM Tris–HCl (pH 7.9), 5 mM sodium acetate and 1 mM EDTA. The gels were stained with 0.5 μg ethidium bromide/ml, and photographed under UV illumination [12].

2.4. *Isokinetic sucrose gradients*

The digestion products were characterized by sedimentation on isokinetic sucrose gradients [13] in 0.1 M NaCl and 0.05 M Tris–HCl (pH 7.5); the mixing chamber (61.2 ml) and the reservoir contained 15% and 31.5% sucrose, respectively. The gradients were centrifuged for 60 min at 40 000 rev./min and 4°C in a Beckman SW 41 rotor. Fractions were collected from the bottom of the tubes and the radioactivity was monitored by scintillation spectrometry.

3. Results and discussion

The VP1 polypeptide is the only capsid protein to contain cysteine residues [7]. The VP1 subunits cross-link by intermolecular S–S bridges and form very stable and highly polymeric structures [7]. To study the role played by disulfide bonds in the protection of encapsidated SV40 chromosomes, we tested the accessibility of viral DNA to micrococcal nuclease in the absence or presence of the reducing agent DTT. Fig.1 shows that incubation of SV40 virions with increasing concentrations of micrococcal nuclease (2–100 units) in 5 mM $CaCl_2$ did not result in the cleavage of viral DNA (lanes 2–6); the DNA comigrated with supercoiled SV40 DNA marker (lane 1). The virions were then incubated for 15 min at 33°C with 10 mM of DTT (in 0.1 M NaCl, 0.05 M Tris–HCl (pH 7.5)). Subsequently, they were treated with 2 units of micrococcal nuclease and increasing [$CaCl_2$] (for 10 min at 33°C). The DNA remained intact below 0.55 mM $CaCl_2$ (fig.2, lanes 1–5) and was digested

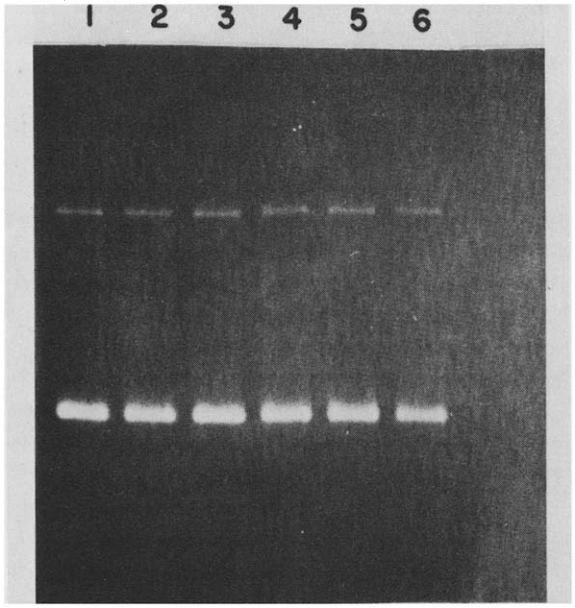

Fig.1. Digestion of SV40 virions with micrococcal nuclease in the absence of DTT. SV40 virions were incubated for 15 min in 0.1 M NaCl, 0.05 M Tris–HCl (pH 7.5) at 33°C. Increasing concentrations of micrococcal nuclease in $CaCl_2$ (final conc. 1 mM) was added and the samples were incubated for an additional 15 min at 33°C. The reactions in lanes 1–6 contained 0,10,20,30,50 and 90 units of micrococcal nuclease, respectively.

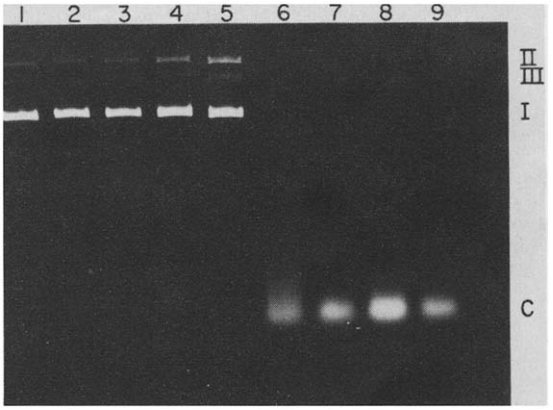

Fig.2. Effect of Ca^{2+} on the digestion of DTT-treated virions with micrococcal nuclease. SV40 virions were incubated for 15 min at 33°C in 0.05 M Tris–HCl (pH 7.5), 0.1 M NaCl and 10 mM DTT. Two units of micrococcal nuclease, in solutions containing various concentrations of $CaCl_2$, was then added and the reactions were incubated for an additional 10 min at 33°C. The final concentrations of $CaCl_2$ were:0, 0.011 mM, 0.114 mM, 0.23 mM, 0.55 mM, 1.14 mM, 2.3 mM, 5 mM and 10 mM; lanes 1–9, respectively. II, III and I denote the mobilities of nicked, linear and supercoil SV40 DNA, respectively; C corresponds to the mobility of 146 basepair DNA.

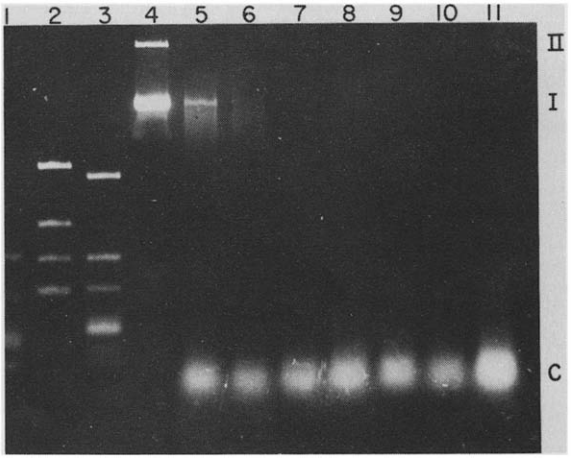

Fig.3. Effect of DTT on the digestion of SV40 virions with micrococcal nuclease. SV40 virions were treated with increasing concentrations of DTT for 15 min in 0.05 M Tris–HCl (pH 7.5) and 0.1 M NaCl at 33°C. Subsequently, 2 units of micrococcal nuclease and $CaCl_2$ (final conc. 1.1 mM) was added and the reactions were incubated for an additional 10 min at 33°C. The final concentration of the reducing agent was 0 mM, 0.1 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM and 15 mM (lanes 4–11, respectively.) Lanes 1,2 and 3 contained SV40 DNA digested with the *Alu*I, *Hin*f, or *Hae*III. II, I and C denote the mobilities of nicked circular SV40 DNA, supercoil SV40 DNA and 146 basepair length DNA, respectively.

at >1 mM (fig.2, lanes 6–9). $CaCl_2$ was only required for the activation of micrococcal nuclease; in the presence of DTT and $CaCl_2$ and the absence of the nuclease, the DNA remained intact (not shown). The DTT dependence of the digestion of viral DNA with micrococcal nuclease is further documented in fig.3. SV40 virions were incubated with increasing concentrations of the reducing agent and then treated with 2 units of the nuclease in 1 mM $CaCl_2$. In the presence of as low as 0.1 mM DTT, the viral DNA was cleaved to a core size of 146 basepairs.

We further characterized the DTT-treated virions, intact or digested with micrococcal nuclease, by sedimentation analysis on sucrose gradients. Fig.4 shows that the digested particles sedimented like intact virions (220 S). A similar phenomenon had been observed in the micrococcal nuclease digests of chromatin isolated from chicken erythrocytes [14]. Stable folded chromatin structures which contained cleaved DNA were isolated in [14]. Histones H1 and H5 stabilized the above superstructures and detain the release of oligonucleosomes. The case of SV40 is more complex; the SV40 virions do not contain histones H1 or H5 [15,16]. Therefore, it is likely that specific types of interactions (mediated by divalent ions, protein–protein and protein–DNA) prevent the release of nucleosome cores generated by the action of micrococcal nuclease on DTT–treated virions.

The SV40 virions could serve as a model system to study how a string of nucleosomes are coiled and maintained into higher order structures by non-histone structural proteins. The reduction of disulfide bonds, which link the VP1 subunits, provides a new way to penetrate the virus shell without extensive structural perturbations.

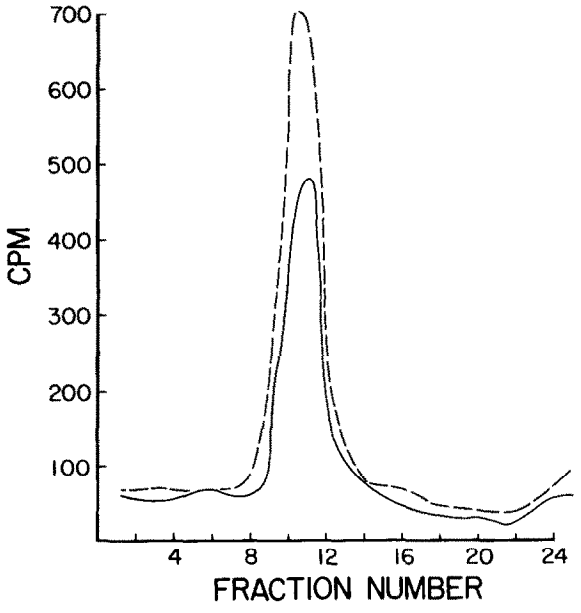

Fig.4. Sucrose gradient analysis of SV40 virions treated with DTT (——) or treated with DTT and digested with micrococcal nuclease (– – –).

Acknowledgements

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