
Salt and divalent cations affect the flexible nature of the natural beaded chromatin structure

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ABSTRACT

A natural chromatin containing simian virus 40 (SV40) DNA and histone has been used to examine changes in chromatin structure caused by various physical and chemical treatments. We find that histone H1 depleted chromatin is more compact in solutions of 0.15M NaCl or 2 mM MgCl₂ than in 0.01 M NaCl or 0.6M NaCl, and is compact in 0.01 M NaCl solutions if histone H1 is present. Even high concentrations of urea did not alter the fundamental beaded structure, consisting of 110Å beads of 200 base pair content, each joined by thin DNA bridges of 50 base pairs. The physical bead observed by EM therefore contains more DNA than the 140 base pair "core particle". The natural variation in the bridge length is consistent with the broad bands observed after nuclease digestion of chromatin. Chromatin prepared for EM without fixation contained long 20Å to 30Å fibers possibly complexed with protein.

INTRODUCTION

The structural model of eukaryotic chromatin based on biochemical and electron microscopic findings describes chromatin as a flexible chain of repeating globular subunits, each of about 100 Å diameter, connected by short flexible filaments (1,2). A similar model has been proposed for SV40 minichromosomes, isolated from nuclei of monkey kidney cells infected with SV40 (3). The SV40 minichromosome contains a 3×10^6 dalton covalently closed circular DNA molecule and host cell histone proteins (4,5). Each histone class is represented (4,5,6,7), it shows beaded and condensed appearance as seen by EM (3,8), and its DNA has a fragmentation pattern following micrococcal nuclease treatment (7) very similar to that of eukaryotic chromatin. These similarities allow us, we believe, to exploit the small and defined size of the SV40 minichromosome to investigate the changes in eukaryotic chromatin structure caused by various chemical and physical treatments.

In this paper we present an analysis of changes which occur when SV40 minichromosomes are exposed to various concentrations of salt, divalent cations, to high concentrations of urea and to physical shearing. In

order to directly examine the effects of these treatments by electron microscopy, we have developed a fixation procedure which retains the native sedimentation behavior of SV40 minichromosomes while rendering them resistant to the harsh forces likely present during the preparative steps. It was found that without such fixation marked changes in chromatin structure would occur.

MATERIALS AND METHODS

Preparation of SV40 minichromosomes. SV40 minichromosomes were prepared according to Griffith, 1975 (3). A CV-1 strain of African Green Monkey kidney cells was grown on 10 cm plastic plates to confluency and the cells infected with SV40 (SVS) at a multiplicity of 10 per cell (9). Thymidine (^3H), 100 $\mu\text{C}/\text{plate}$) was added 24 hrs post infection and the cells harvested at 30 to 40 hrs. Cells were removed with a rubber policeman, collected by low speed centrifugation, and suspended in 0.01 M Tris, pH 7.5, 0.5 mM MgCl_2 (1 ml/plate) for 10 min on ice. The swollen cells were lysed in a glass dounce homogenizer (10 strokes) and an equal volume of 0.5 M sucrose, 0.3 M NaCl, 0.01 M Tris pH 7.5, added immediately to protect the nuclei from lysis. The nuclei were collected by centrifugation at 5,000 g for 5 min, resuspended in 1.7 M sucrose, 0.15 M NaCl, 0.01 M Tris pH 7.5, (2 ml/plate of cells) and centrifuged for 30 min at 35,000 rpm in an SW 56 rotor at 5°. For extracting SV40 minichromosomes from the nuclei one of the two following procedures was used. 1) Nuclei were resuspended in 0.01 M Tris pH 7.5, 0.15 M NaCl, 0.25% Triton X100 (0.25 ml/plate of cells) and incubated for 10 min on ice followed by 5 min at 37°. This extract was centrifuged for 10 min at 10,000 g to remove cell chromatin. The supernatant was layered (0.5 ml/tube) over 5% to 20% sucrose gradients in 0.25% Triton X100, 0.15 M NaCl, 0.01 M Tris pH 7.5 and centrifuged for 150 min at 40,000 rpm in an SW 41 rotor (5°). Peak fractions were pooled and dialyzed into 0.01 M Tris pH 7.5, 0.01 M NaCl, 0.25% Triton X100. 2) The nuclei were resuspended in 0.4 M NaCl, 0.01 M Tris pH 7.5, 1 mM EDTA, 0.25% Triton X100 (0.25 ml/plate) and incubated 10 min on ice followed by 1 min at 37° to extract the SV40 complex (Mel Green, personal communication). The extract was centrifuged as described in 1) and the supernatant layered over a 5% to 20% sucrose gradient containing 0.6 M NaCl, 0.01 M Tris pH 7.5, 1 mM EDTA, 0.25% Triton X100 and centrifuged, fractionated and dialyzed as described in 1).

Chromatin fixation. Chromatin samples were fixed by a two step procedure following dilution or dialysis into a buffer containing 0.01 M

NaCl, 0.02 M sodium phosphate pH 7.5. One tenth volume of 10% formaldehyde (freshly diluted from Malincrodt 37% formaldehyde) was added to the sample for 15 min on ice, followed by the addition of one tenth volume of 6% glutaraldehyde (freshly diluted from Fisher 50% glutaraldehyde) for an additional 15 min on ice.

Electron microscopy. The EM techniques used in this work have been described in detail (10). Aliquots of the fixed samples (at about 1 μ g DNA/ml) were placed in 100 μ l wells of a plastic microtiter plate in an ice bath. Carbon supporting films on 400 mesh copper grids treated with a 10,000 volt discharge for 0.5 to 1 min at 200 μ m Hg pressure prior to use were immersed in each well for 5 min. Each grid was washed in 20 ml baths of 0%, 20%, 50%, 75% and 100% ethanol diluted with distilled water for 5 min each at room temperature and air dried. The grids were tungsten shadowed with rotation. Dimensions were measured from 35 mm micrographs of the Philips EM 300 using a computer-coupled tracing device. The data obtained from the measurements was stored on magnetic tape.

RESULTS

Sedimentation of native SV40 minichromosomes. SV40 minichromosomes freshly extracted from infected cells in solutions of 0.15 M NaCl were found to sediment at 80S to 90S in sucrose gradients containing 0.01 M Tris pH 7.5, 1 mM EDTA, 0.25% Triton X100 and either 0 or 0.15 M NaCl (Fig. 1A, Table 1). When the ionic strength was increased to 0.6 M NaCl, a treatment known to remove histone H1, the 0.15 M NaCl-extracted SV40 minichromosomes were in a sharp peak sedimenting at 55S with either 1 mM EDTA or 2 mM MgCl₂ present. Following dialysis into 0.01 M Tris pH 7.5, 0.25% Triton X100 and resedimentation, the 55S SV40 minichromosomes now sedimented at 55S in solutions of 0.6 M NaCl or of 0.01 M NaCl with 1 mM EDTA. The 55S sedimentation rate increased to 70S to 75S however if the gradients contained 0.15 M NaCl or 0.01 M NaCl with 2 mM MgCl₂. These changes suggest that exposure to 0.6 M NaCl caused the release of a histone H1 - like moiety producing a structure whose sedimentation behavior was now dependent on salt concentrations between 0 and 0.15 M NaCl as well as to Mg⁺⁺ ions. Prolonged exposure to low ionic strength (0.01 M NaCl) also caused the 0.15 M NaCl extracted minichromosomes to sediment at 55S.

Optimization of the fixation of chromatin for electron microscopy. The distinct sedimentation properties of the SV40 minichromosome combined with information about its structure deduced from previous EM (3,8), in

Sedimentation of SV40 minichromosomes

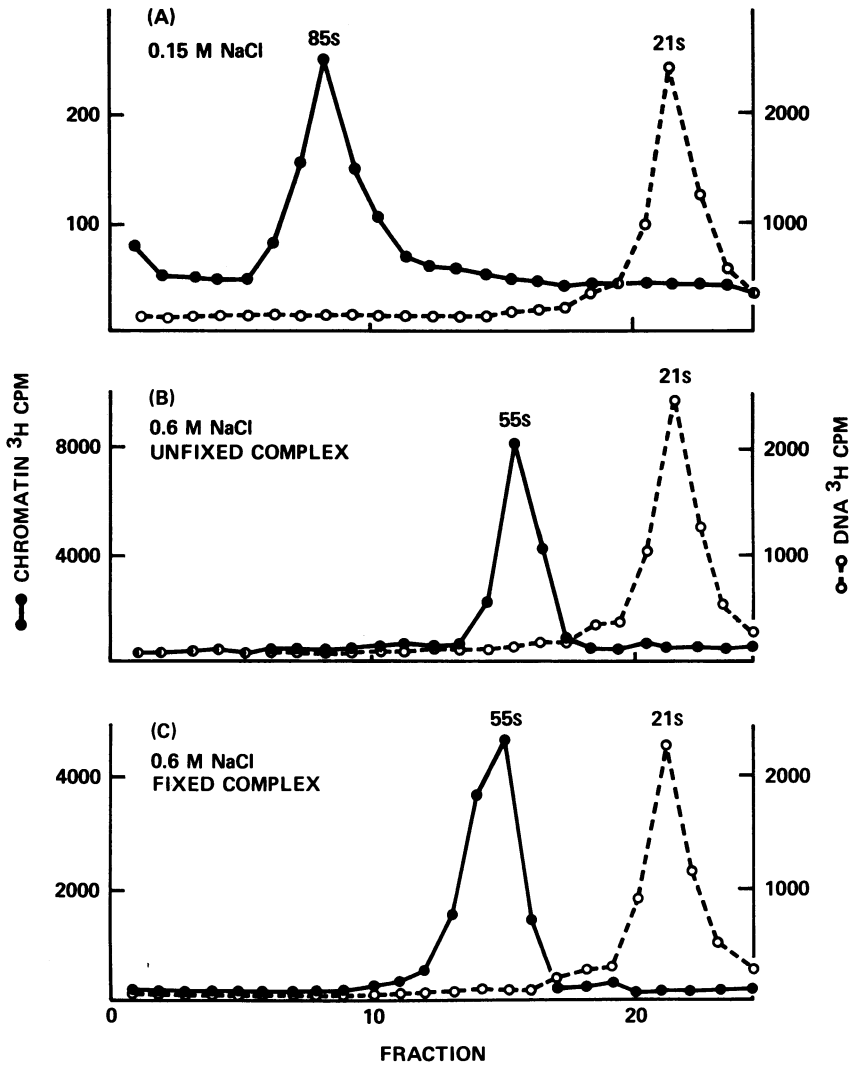


Fig. 1. SV40 minichromosomes freshly prepared in solutions of 0.15 M NaCl (materials and methods) were sedimented in 5% - 20% sucrose gradients with 0.01 M Tris pH 7.5, 1 mM EDTA, 0.25% Triton X100, and either 0.15 M NaCl (A) or 0.6 M NaCl (B). An aliquot of the peak fraction of (B) was dialyzed into 0.02 M sodium phosphate pH 7.5, 1 mM EDTA, 0.25% Triton X100, fixed by the two step procedure and sedimented as in B (C). Gradients were centrifuged for 65 min, 55,000 rpm in an SW56 rotor (5°). The DNA of the SV40 minichromosomes was labeled with (³H) thymidine for 10 hrs prior to extraction. -●-. Purified SV40 form I DNA, (³H thymidine labeled) was run in parallel gradients. -○-.

TABLE 1. Salt dependent changes in the sedimentation of SV40 minichromosomes

Salt composition of sucrose gradients*						
NaCl (M)	0.01	0.01	0.15	0.15	0.6	0.6
EDTA (mM)	1	0	1	0	1	0
MgCl ₂ (mM)	0	2	0	2	0	2
A: S _{w,20}						
0.15M NaCl freshly extracted minichromosomes	85 ₊₅	90 ₊₅	85 ₊₅	100 ₊₅	55 ₊₅	55 ₊₅
B: S _{w,20}						
0.6M NaCl treated minichromosomes	55 ₊₅	70 ₊₅	70 ₊₅	75 ₊₅	55 ₊₅	55 ₊₅

* In addition to 0.01 M Tris pH 7.5, 0.25% Triton X100

SV40 minichromosomes were sedimented in 5%-20% sucrose gradients containing the salts listed above. Samples were sedimented for 50-70 min at 50,000 rpm (5°) in an SW 56 rotor. Sedimentation values were calculated using SV40 form I DNA (21S) run in parallel gradients.

in vitro reconstitution (11), and nuclease digestion (7) studies provide a means of optimizing the conditions for chemical fixation of chromatin. Following an "optimal" fixation, the SV40 minichromosomes should 1) retain the native sedimentation rate, 2) should band in CsCl equilibrium density gradients at the density of the 1:1, DNA:protein ratio established for chromatin, and 3) remain unchanged in appearance in the EM following harsh treatments with agents such as sodium dodecyl sulfate (SDS) or high salt after fixation. Finally, the structures observed by EM after such fixation should be in harmony with the structural information deduced from other physical studies. We found these criteria best satisfied if the histone was first crosslinked to the DNA (in 0.01 M NaCl) with 1% formaldehyde for 15 min at 0°. Formaldehyde treatment has been shown to link histone to DNA; but the histones themselves are poorly crosslinked (12,13). The resulting structures were then stabilized further with 0.6% glutaraldehyde for 15 min more at 0° (glutaraldehyde will form histone polymers but few DNA-protein bonds (13)).

Following such two step fixation SV40 minichromosomes exhibited no change in the native sedimentation rate in either 0.6 M NaCl supplemented sucrose gradients (Fig 1B,C) or in the sedimentation of the 0.15 M NaCl extract in gradients with 0.15 M NaCl. Histones were fully crosslinked to the DNA after such treatment as shown by the CsCl banding profile in figure 2. Finally the resulting structures appeared to be now resistant to harsh treatments following this fixation regime (Fig 3C,D).

Electron microscopy of SV40 minichromosomes. When samples of the 55S (0.6 M NaCl treated) SV40 minichromosomes were prepared for EM without any fixation, relaxed circular fiber loops with a contour length close to that of purified SV40 DNA (1.4 to 1.6 μm) were seen (fig. 3E). The diameter of the fiber appeared slightly greater than that of protein free DNA. Because the same open fiber loops were found on examining either 55S or 80S (0.15 M NaCl treated) SV40 minichromosomes and because such relaxed fiber loops should have sedimented more like form II SV40 DNA (16S to 18S), we concluded that a marked change in structure must have occurred during the EM preparative steps. If unfixed 55S or 80S SV40 minichromosomes were

Cesium chloride equilibrium density banding of fixed SV40 minichromosomes.

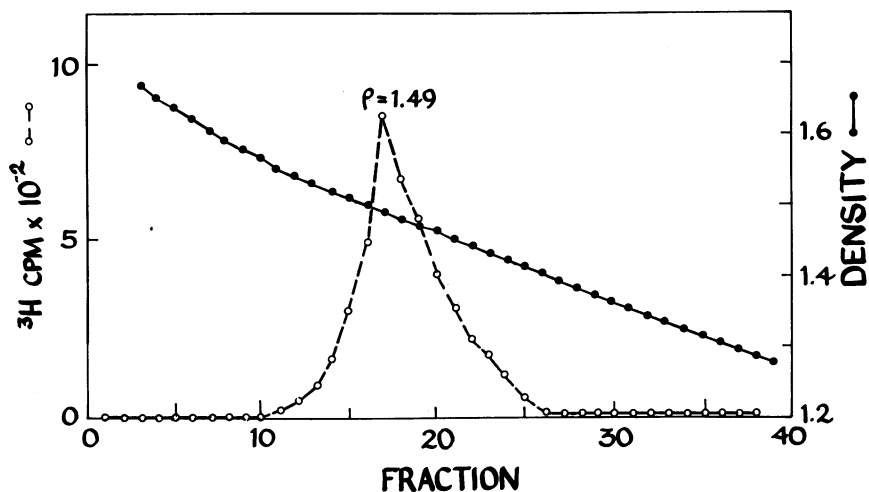


Fig. 2. SV40 minichromosomes in 0.02 M sodium phosphate, pH 7.5, 0.01 M NaCl, 0.25% Triton X100 was fixed by the two step procedure, mixed with solid CsCl and 0.01 M Tris, pH 7.5, 0.25% Triton X100 to obtain a density of 1.5 gm/cc (at 5°) and centrifuged for 48 hrs at 35,000 rpm in an SW50.1 rotor at 5°. The DNA of the SV40 chromatin had been labeled with (^3H) thymidine for the 10 hrs prior to its isolation. The density of each fraction was determined by direct weighing at 5°.

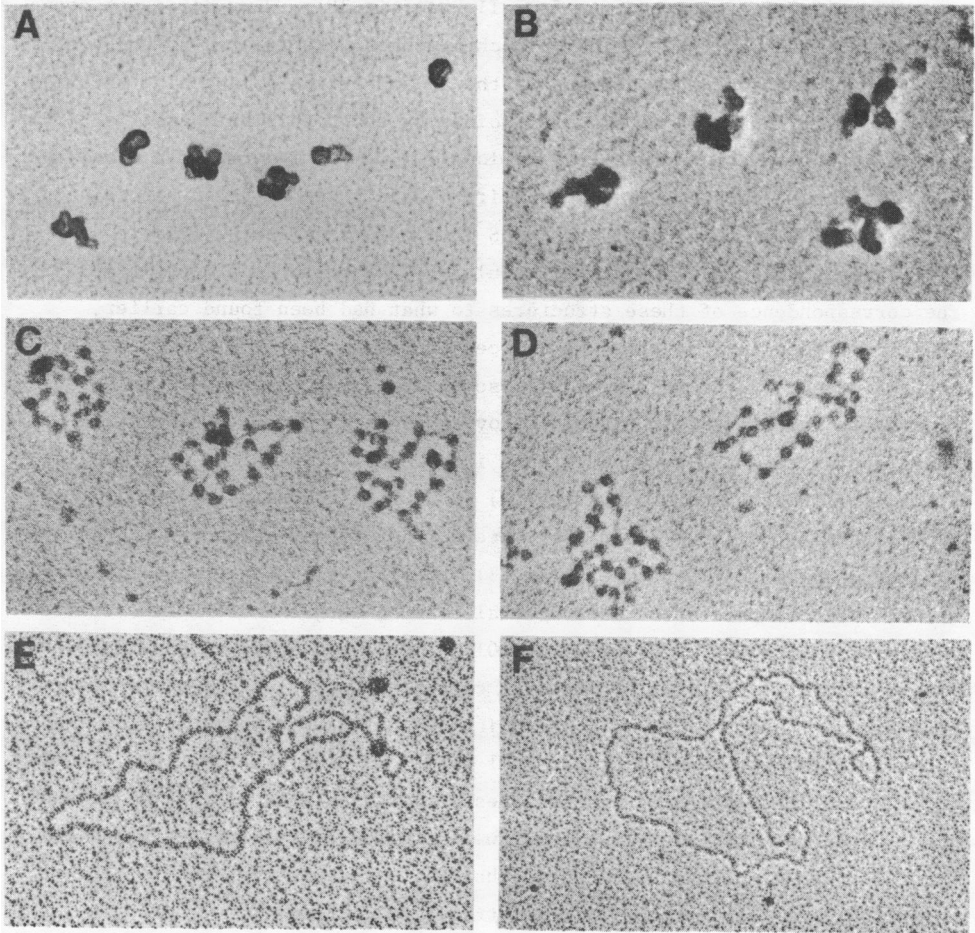
Appearance of SV40 minichromosomes after different treatments.

Fig. 3. When SV40 minichromosomes freshly prepared in solutions of 0.15 M NaCl (method 1, materials and methods) are fixed by the two step procedure, structures as in (A) are most commonly seen. After exposure to 0.6 M NaCl (method 2, materials and methods), and fixation, chains of 21 chromatin beads are found (C). This appearance was not changed by exposing the fixed samples to 2 M NaCl (10 min 20°) (D). When SV40 minichromosomes prepared by method 2 were fixed in presence of 2 mM MgCl₂, compact particles were seen (B). SV40 minichromosomes (prepared by either method) exposed to the EM preparative procedure without any prior fixation yielded open fiber loops (E), similar in appearance to SV40 form II DNA prepared in the same way (F). Bar equals 0.1 micron.

treated with 2 M NaCl or 1% SDS for 5 min (20°) prior to mounting onto the EM supporting grids, very compact rods were seen whose appearance was that of SV40 form I DNA (see figure 1a, Griffith et al., (14)). Upon exposure

to X irradiation, the salt or SDS treated, unfixed SV40 minichromosomes were identical in appearance to form II DNA (fig 3F). Very different structures were found if the 55S SV40 minichromosomes were treated by the two step fixation prior to mounting onto the EM supporting grids. The relaxed strings of 21, 100 Å to 120 Å beads, joined by short thin bridges (fig 3C,D) are like those which we reported previously (3,15). The two step fixed 55S SV40 minichromosomes appeared more uniform than those we found before. Upon treatment with 2 M NaCl or 1% SDS (5 min 20°) following fixation no difference could be detected as a result of such harsh treatment (fig 3D). The correspondence of these structures to what had been found earlier, their correspondence to what was deduced from other physical studies, and the resistance of the SV40 minichromosomes to change on exposure to harsh treatments following this fixation provided confidence that these structures approximate the structure found in solution. Using the two step fixation, the 80S (0.15 M NaCl extracted) SV40 minichromosomes appeared as compact particles (fig 3A) often even more compact than the condensed SV40 minichromosomes we reported earlier (3); possible differences are discussed below. Very compact structures were also found when the 0.6 M NaCl treated SV40 minichromosomes were fixed in 0.01 M NaCl, 0.02 M sodium phosphate pH 7.5, 2 mM MgCl₂ (fig 3B). These Mg⁺⁺ compacted particles however appeared less regular than the 80S material which had not been exposed to 0.6 M NaCl. Upon prolonged exposure to 0.01 M NaCl solutions prior to fixation, the 80S (0.15 M NaCl extracted) minichromosomes appeared as open strings of 21 beads.

When the SV40 minichromosomes were treated with fixations which did not fully crosslink the histones to the DNA, structures intermediate between the open 1.5 μm loops and the strings of 21 beads were found (see below).

Bead-to-bead spacing in chromatin: changes resulting from shearing and limited fixation. Following two step fixation the bead diameter and bead to bead spacing of the 55S SV40 minichromosomes had very regular appearances. A histogram displaying the results of 700 measurements of these dimensions from the electron micrographs is in figure 4 A. To correct the bead diameter for the amount of tungsten metal deposited, we have measured the fractional increase in the diameter of tobacco mosaic virus after an identical shadowing and compared that to the diameter determined by negative staining with 1% phosphotungstic acid. The fractional increase of 60 Å to 70 Å thus obtained provides an estimate of 110 Å to 120 Å for the true bead diameter (Table 2). The mean bridge length corresponds to the bead

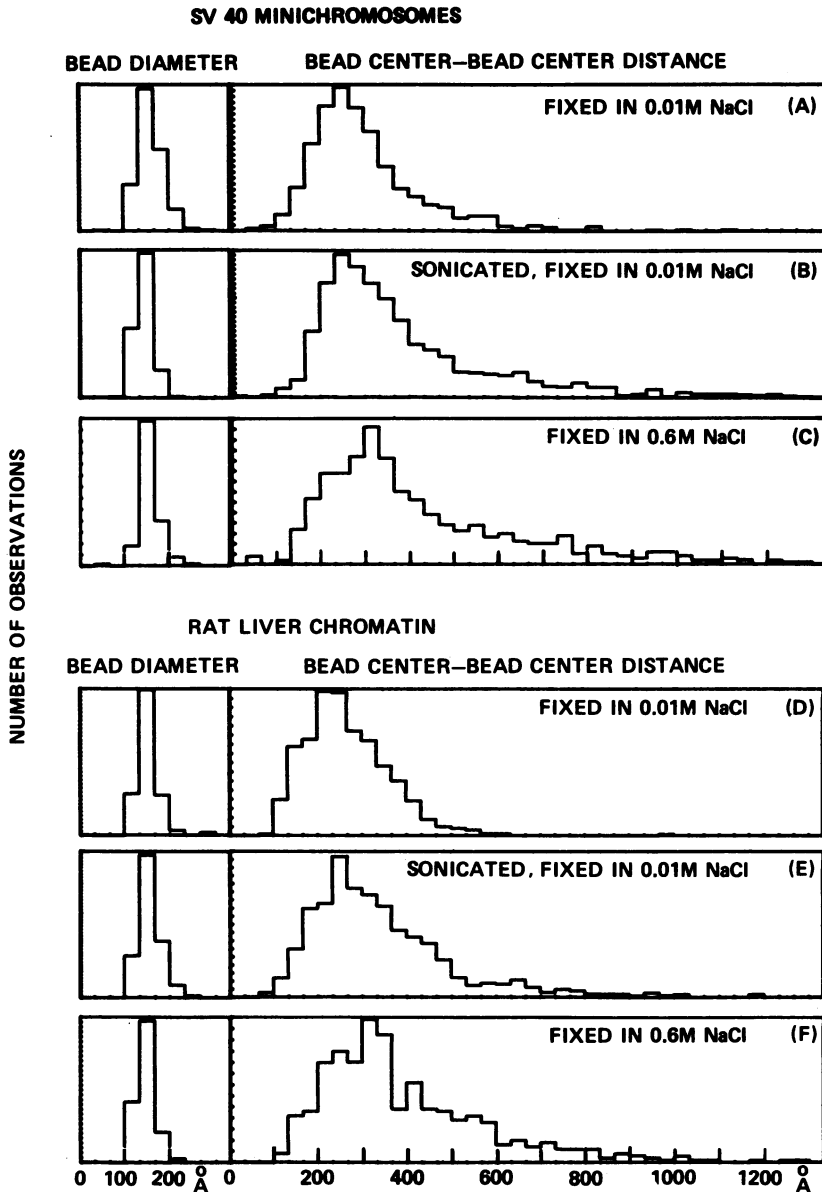


Fig. 4. Histograms showing the variation in bead diameter and distance between the centers of two adjacent beads determined directly from electron micrographs. SV40 minichromosomes depleted of histone H1, (method 2) were: Fixed by two step procedure (A). Treated with mild sonication (B) or prepared after partial fixation (C).

Purified rat liver nuclei (17) were briefly treated with micrococcal nuclease (18). Chromatin molecules of about $2 - 20 \times 10^3$ base pairs of DNA were taken from 5% - 20% sucrose gradients (containing 0.6M NaCl) following osmotic shock of the nuclei. Samples were diluted into 0.01M NaCl, 0.02M sodium phosphate pH 7.5 and treated as in A (D), in B (E) or in C (F) above.

Table 2 Dimensions of chromatin subunits.

Chromatin	Treatment	Bead center to Bead center (Å)	No of Measurements	Bead- [*] diameter (Å)	No of Measurements
SV40 Mini- chromosomes	optimal fixation	300 ± 125	774	110 ± 15	396
	partial fixation	400 ± 200	1215	100 ± 15	400
	mild son- ication	430 ± 210	580	110 ± 15	99
Rat liver chromatin	optimal fixation	275 ± 100	500	110 ± 15	300
	partial fixation	400 ± 200	544	110 ± 15	250
	mild son- ication	350 ± 175	645	110 ± 15	400

* Bead diameter corrected for deposit of metal after shadowing (see text)

center to bead center value (300Å) less one bead diameter (110Å). This value (190 ± 80Å or 55 ± 25 base pairs) and the bead diameter are in good agreement with previously reported results (3,8).

When 55S SV40 minichromosomes were mildly sonicated (5 sec at step 2 with a Heat Systems microtip) fixed, and examined by EM, the resulting structures appeared coiled about themselves. Upon X irradiation, open beaded chains like those obtained without sonication were found, but the bead to bead spacing was increased (fig 4b) and the number of beads per circle reduced. Upon more vigorous sonication, linear fragments with even greater bead to bead spacings were found. No change in the bead diameter however was detected as a result of sonication (fig 4b).

Similar changes were observed when SV40 minichromosomes were examined by EM after limited fixation (e.g. two step fixation but in 0.6 M NaCl solutions, or fixation for much shorter times) (fig 4c). With even less fixation, bead to bead spacings much greater than 200 base pairs were commonly observed.

Freshly prepared rat liver chromatin depleted of histone H1 was examined after optimal and limited fixations and after sonication followed by optimal fixation to determine whether the bead diameters and bead to bead spacings would have values similar to those found for SV40 minichromosomes. The data shown in figure 4 and Table 2 indicate that within the numerical limits given these values are indeed indistinguishable from

the SV40 minichromosome values.

The effect of high concentrations of urea on chromatin structure.

When chromatin is exposed to 4 to 6 M urea a marked increase in the intrinsic viscosity is observed (16). Such an increase could result from a reorganization of the beaded structure which retains each bead intact; possibly however the change in viscosity may signal some fundamental change in the structure of the chromatin beads themselves. It should be possible to distinguish these by examining the sedimentation behavior of SV40 minichromosomes in solutions of increasing urea concentration. SV40 minichromosomes purified through 0.6M NaCl and dialyzed into 0.01 M NaCl, 0.01 M Tris pH 7.5 (55S form), and purified SV40 DNA were sedimented in 5% to 20% sucrose gradients containing in addition to 0.01 M Tris pH 7.5, 1mM EDTA, from 0 to 5 M urea. The distance sedimented in each gradient was

Sedimentation of SV40 minichromosomes and SV40 form I DNA as a function of urea concentration.

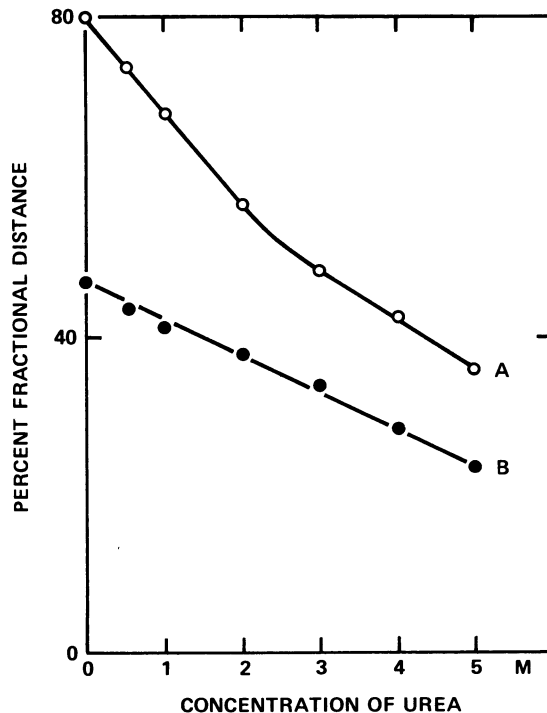


Fig. 5. SV40 minichromosomes (prepared by method 2) (A) and purified SV40 form I DNA (B) were sedimented in parallel 5% - 20% sucrose gradients with increasing concentrations of urea. Sedimentation was for 150 min, 55,000 rpm at 5° (SW56 rotor). The fractional distance each sample moved across the gradient is shown as a function of urea concentration.

plotted as a function of urea concentration (fig. 5). With increasing urea concentration, the distance the SV40 minichromosomes or DNA penetrated the gradients in a given time decreased. The curves for both DNA and the SV40 minichromosomes are roughly parallel above 2M urea. A uniform decrease in sedimentation would be expected from an increase in the density and viscosity of the sucrose solutions caused by increasing urea concentrations. A disruption of the chromatin bead should result in an abrupt drop in the sedimentation rate toward that of an extended DNA fiber. Because no such change was observed in the range of 2 to 5 M urea (where the viscosity increase occurs) a disruption of the fundamental chromatin bead itself by high concentrations of urea seems unlikely. To further examine the stability of the beaded structure in concentrated urea solutions, two step fixed samples were sedimented in gradients containing 4,5 and 6 M urea; they were found to cosediment with the unfixed samples (data not shown). Because the beaded structure appeared resistant by EM to 1% SDS, 2M NaCl, and to 6M urea following fixation, this test also argues for the stability of the beads in high concentrations of urea.

DISCUSSION

In these studies we have examined the effect of different physical and chemical treatments on the structure of SV40 minichromosomes. Following treatment with 0.6 M NaCl, they were found to behave as relaxed chains of 21 beaded chromatin subunits joined by 40 to 60 base pair bridges. These structures were in more compact forms in solutions of 0.15 M NaCl or 2 mM MgCl₂. Without this treatment, the beads appeared to be held in a condensed arrangement and this was not greatly changed by addition of divalent cations. High concentrations of urea did not disrupt the basic chromatin beads, sonication and exposure to drying during the EM preparative steps without proper fixation did however.

These conclusions are strongly supported by the EM data. The fixation procedure developed here is based on preservation of the native sedimentation behavior of the SV40 minichromosomes which also renders them resistant to harsh treatments. Other fixation schemes may equally preserve chromatin structure and different preparative techniques may possibly present less disruptive environments. However we believe that it is essential to show that the fixation procedure used for EM does preserve the physical properties of the sample. In studies not detailed here we found that chromatin would change to thin smooth contoured fibers during

the final drying steps used in a wide variety of EM preparative techniques we explored and that this change was stimulated by exposing the chromatin to very low salt, to urea, or to solutions of EDTA. The change was partially inhibited by dehydration schemes which began with 50% ethanol, by drying in the presence of 2 mM $MgCl_2$ and by the presence of histone H1. Thus when we examined poorly fixed SV40 minichromosomes, it was possible to observe a wide spectrum of structures depending on the details of the preparative procedure we used. Because thin smooth contoured chromatin fibers may possibly be a component of chromatin structure, their investigation by EM must be carried out with carefully controlled fixation.

The beaded chromatin structure appears to be remarkably stable. The resistance of the fundamental (H1-depleted) beaded structure to disruption by 5M urea was also reflected in our finding that H1 depleted SV40 minichromosomes or rat liver chromatin depleted of H1 could be heated to 65° (in 0.01M NaCl) without any apparent change in structure as detected by EM (data not shown). Although shearing was disruptive, the change was that of a slight pulling of the DNA from the histone cluster and not a disruption of the cluster itself. Although not investigated here, any of these treatments might be found to have a marked effect on histone H1 complexed chromatin.

We have confirmed (data not shown) the report that histone H1 is a component of the 0.15M NaCl extracted minichromosomes (7). The change which we observed from compact 80S structures to relaxed 55S strings of beads with short DNA bridges after exposure to 0.6 M NaCl (known to remove histone H1) showed that the SV40 minichromosomes prepared with 0.15 M NaCl solutions did contain proteins behaving as histone H1. The 80S SV40 minichromosomes were somewhat more compact than the doughnut-like SV40 minichromosomes we reported earlier. This may be simply due to a further twisting of the doughnut loop about itself, or to a cross bridging of nonadjacent beads by histone H1; also the doughnut-like minichromosomes were found using a much earlier infection time (20 to 25 hrs) with a different virus strain (RH 911). Furthermore, we have shown elsewhere that the form of the SV40 minichromosome which becomes packaged into the virus particle closely resembles the 55S, 0.6 M NaCl treated form in sedimentation behavior and appearance by EM, that it lacks histone H1, but apparently contains the virus specified protein VP3 (15). The change of the condensed, 0.15M NaCl extracted SV40 minichromosomes into relaxed strings of 21 beads upon prolonged exposure to low ionic strength had been noted before (3), and

was accompanied by a parallel decrease in the sedimentation coefficient. This change may be due to a loss of histone H1 from the structure, or merely to a rearrangement of histone H1. Similar changes in 0.15M NaCl extracted rat liver chromatin were observed following dialysis into low salt buffers. The progress of the SV40 minichromosome through replication, deposition of the histone proteins including H1, to removal of H1, its possible replacement by VP3 and final encapsidation will require a coordinated analysis of the protein composition and appearance by EM at each stage in the SV40 minichromosome life cycle.

Our original study of the SV40 minichromosome (3) provided the first proof that the beaded subunits seen by EM correspond to the repeating unit detected by nuclease digestion and that the natural supertwisting of purified SV40 DNA results from the removal of histone assembled onto the DNA in vivo. In the original study, the SV40 genome size was estimated by EM. It is instructive to recalculate the number of base pairs in each bead using the value of 5200 ± 160 base pairs (L. Sompayrac and K. Danna, personal communication) determined from gel analysis of SV40 DNA restriction fragments of which several have known sequences. A 5200 base pair genome would divide into 21 blocks of each of 250 base pairs of which 55 ± 25 would reside in the bridges. The value of 200 base pairs per bead is in agreement with another recent estimate of Bellard et al. (17) who, however, used a smaller value for the total genome size. The true SV40 genome size now appears to be well known. The number of beads in each SV40 minichromosome has been independently counted in several laboratories (3,8,17) and with different EM techniques to be between 20 and 22. There is also agreement that the average bridge size is close to 50 base pairs but with a broad distribution. We have observed that minichromosomes with 24 to 25 beads have bridges of average size much less than 50 base pairs, and minichromosomes with 18 beads have bridges of average length greater than 50 base pairs. On the basis of these numbers it appears that the bead seen by EM contains somewhat more DNA than the 140 base pair "core particle" which results from extensive nuclease digestion of various eukaryotic chromatins (18). The most plausible model is that each bead contains a rather exact amount of DNA (about 200 base pairs), and is joined to the next by DNA bridges of variable length, about a mean of 50 base pairs. Thus the average bridge length in any given SV40 minichromosome is determined by the exact number of beads in that complex. This model is in agreement with the observation that the multimer bands in the nuclease digests of natural chromatins are quite broad by comparison to that of the 140 base

pair core particle.

In this work we have exploited the small size of the SV40 minichromosome to examine the effect of different physical and chemical treatments on the basic chromatin structure. Although the only direct comparison between SV40 minichromosomes and a eukaryotic chromatin (rat liver) made here was to show the identical dimensions of the beads and bridges, we believe that the results of the urea treatment, of shearing and of conformational changes due to salt and divalent cation environment can be extended to similarly prepared eukaryotic chromatins.

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