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Photochemical Addition of the Cross-Linking Reagent 4,5',8-Trimethylpsoralen (Trioxsalen) to Intracellular and Viral Simian Virus 40 DNA-Histone Complexes

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We demonstrated here that 4,5',8-trimethylpsoralen (trioxsalen) is a valuable probe for the structure of SV40 DNA-histone complexes. Trioxsalen readily penetrated intact cells and, in the presence of 340- to 380-nm light, covalently cross-linked DNA preferentially at the sites available for micrococcal nuclease digestion. Histograms of the lengths of the regions of SV40 DNA protected from cross-linking, as visualized by electron microscopy, indicated a repeating pattern of base pairs in DNA from both infected cells and virus particles. The ability of the trioxsalen probe to act in vivo and to map the location of protected regions may provide a powerful tool for analyzing the role of nucleosomes in the structure of the virus particle and in intracellular complexes such as transcription templates and replication intermediates.

Recent evidence from electron microscopy and endonuclease digestion has led to a model for eucaryotic chromatin that consists of a fiber of repeating "beads" or nucleosomes (20, 34, 36, 37, 46, 49). Each nucleosome is thought to contain 140 to 200 base pairs of DNA wrapped around an octamer core consisting of two each of the histones H2a, H2b, H3, and H4 (1, 26, 48). The lysine-rich histone H1 is thought to be complexed with the "bridge" or interbead regions of the fiber (35, 48, 51).

Both the intracellular and viral nucleoprotein complexes of simian virus 40 (SV40) and polyoma virus have a structure similar to that of cellular chromatin. This structure consists of a ring of approximately 21 repeating units or nucleosomes made up of histones and DNA (6, 9, 15, 18, 23, 54). Most of the original studies reported the absence of histone H1 in the viral nucleosome complex (9, 14, 17, 18, 28, 32, 40); however, recent reports indicate that histone H1 is associated with the intracellular complex when it is isolated under conditions of low ionic strength and minimal protein degradation (50, 52, 36; M. DePamphilis, personal communication). The basic structure appears to be the same as that of cellular chromatin, so it serves as a useful model system. We shall refer to this SV40 DNA-histone complex as SV40 chromatin.

It has recently been shown that derivatives of the furocoumarins (psoralens) are valuable probes for studying the structure of cellular chromatin (19). These compounds readily penetrate intact cells or viral particles, intercalate into the DNA, and, in the presence of long-wavelength UV light (365 nm), covalently add to the pyrimidines of the DNA (7, 27, 33, 38). If two pyrimidines are adjacent and on opposite strands, covalent linkages can be formed at two positions on the psoralen derivative, thus cross-linking the two strands of the double helix (8, 10, 11). It has been shown that the nucleosome structure is largely protected from cross-linking by 4,5',8-trimethylpsoralen (trioxsalen; Fig. 1) relative to purified DNA and that the region between nucleosomes is preferentially cross-linked by the compound (55). Thus, trioxsalen is a powerful probe for the intracellular location of nucleosomes.

Because the SV40 chromatin complex is small and the SV40 genome has been studied in great detail, use of trioxsalen as a probe for the role of the nucleosome structure in viral regulation was explored.

MATERIALS AND METHODS

Cells and virus stocks. TC-7 cells, a cell line derived from African green monkey kidney cells by J. Robb, were grown in Dulbecco modified Eagle medium (Grand Island Biological Co., Grand Island, N.Y.), supplemented with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), on 100-mm plastic tissue culture dishes (Falcon Plastics, Oxnard, Calif.). The cells were infected upon reaching confluency with virus twice plaque purified from a stock of the small

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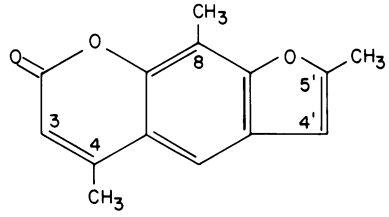


FIG. 1. *Structure of 4,5',8-trimethylpsoralen (trioxsalen).*

plaque-forming strain SP12 of SV40 originally isolated by J. Robb. Radioactive label was added where indicated 24 h after infection.

4,5',8-Trimethylpsoralen (trioxsalen). Trioxsalen was obtained from the Paul B. Elder Co. (Bryan, Ohio) and tritiated in this laboratory (24). Stock solutions of approximately 0.9 mg/ml were made in ethanol. Specific activities were determined by counting in 1 ml of water and 10 ml of Omnifluor-Triton scintillation fluid (2 liters of toluene, 1 liter of Triton X-100, and 12 g of Omnifluor [New England Nuclear] on a Beckman LS-230 scintillation counter) and measuring the absorbance at 249 nm (the extinction coefficient in 100% ethanol at this wavelength is 31,008 M^{-1} cm^{-1} [J.E. Hyde, personal communication]).

Irradiation of infected cells. The medium was removed from cells at the times indicated and replaced with "albino" medium (Dulbecco modified Eagle medium plus 100 mM HEPES buffer [*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid] [Calbiochem] minus bicarbonate, phenol red, and riboflavin) to which had been added 5 μg of trioxsalen per ml. The HEPES buffer enabled the irradiation to be done at atmospheric CO_2 pressure, and the absence of UV absorbents enhanced the efficiency of the photoreaction. Cells were irradiated in closed tissue culture dishes at a distance of 2 cm from the lids by a bank of six General Electric F15T8 BLB fluorescent tubes at an incident intensity of 3 mW/cm^2. Multiple additions of trioxsalen were made by alternating the addition of 5 μg of trioxsalen per ml from an ethanol stock solution with a complete medium change such that the alcohol concentration was never greater than 1%. The solubility of trioxsalen in water is 0.6 μg/ml. Supersaturating solutions and additions of 5 μg/ml were used to assure that the solution was always saturated during the photoreaction.

Irradiation of isolated SV40 chromatin. SV40 chromatin complexes were isolated by a modification of the published procedures (17, 32; M. Botchan and J. Griffith, personal communications). Cells were labeled with 2 to 5 μCi of [^{14}C]thymidine (New England Nuclear; specific activity, 50 to 60 mCi/mmol) per ml for 12 h before isolation. Approximately 42 to 44 h after infection the cells were washed with Tris-diluent (0.137 M NaCl–5 mM KCl–5 mM Na_2HPO_4–25 mM Trizma base [Sigma], pH 7.5) and suspended in approximately 0.5 ml of Tris-diluent per 10^8 cells. The cells were lysed by the addition of 8 ml of cell lysis buffer (50 mM Tris [pH 7.5]–5 mM $MgCl_2$–25 mM KCl–0.25 M sucrose–0.5% Nonidet P-40 detergent; Shell Oil Ltd., London) and briefly mixed on a Vortex mixer. Nuclei were pelleted by centrifuging for 10 min at 2,000 rpm in the JA-21 rotor of a Beckman J-21 centrifuge. The nuclei were suspended by homogenization in 0.7 ml of nuclei lysis buffer (10 mM Tris-hydrochloride, pH 8.0–10 mM EDTA–0.5% Triton X-100; Rohm & Haas), incubated at 37°C for 5 min, and homogenized a second time by 20 strokes with the tight pestle of a Dounce homogenizer. The solution was made 0.15 M in NaCl and the cellular chromatin pellet was removed by centrifugation for 5 min at 1,500 rpm in the same rotor. The supernatant containing SV40 chromatin was then layered onto a 5 to 20% linear sucrose gradient (in 0.15 M NaCl–0.01 M Tris-hydrochloride, pH 7.5–1 mM dithiothreitol–0.5% Triton X-100) and centrifuged for 90 min at 40,000 rpm in a Beckman SW 41 rotor. The fractions containing the SV40 chromatin complex were determined by counting 25-μl sample, pooled, and irradiated at 6°C in an irradiation device that has been described elsewhere (55), with an incident intensity of 25 mW/cm^2.

Isolation of SV40 DNA. SV40 DNA was isolated from infected cells by a modification of the Hirt procedure (21). Approximately 10^8 cells were suspended in 3.6 ml of 0.01 M EDTA (pH 7.5); 0.4 ml of 10% sodium dodecyl sulfate was added, and the lysate was mixed gently and incubated at 50°C for 20 min. The solution was then made 1.0 M in NaCl by adding 1.0 ml of 5 M NaCl, mixed gently, and stored overnight at 4°C. After centrifugation at 4°C for 60 min at 15,000 rpm in a Beckman SW 50.1 rotor, the supernatant was adjusted to 25 mM EDTA (pH 8), 1.5% Sarkosyl NL-97 (Geigy), and 1 mg of Pronase (Calbiochem; grade B, nuclease-free, or grade B, preincubated at 20 mg/ml for 30 min at 37°C) per ml and incubated for 4 h at 50°C. The SV40 DNA was then banded by density equilibrium centrifugation in Cs_2SO_4 (3.37 g of Cs_2SO_4 added to 5.5 ml of DNA solution, centrifuged at 35,000 rpm and 18°C for 48 h in a Beckman 50, 65, or SW 50.1 rotor), or in ethidium bromide-CsCl (3.62 g of CsCl, 3.48 ml of DNA solution, and 50 μl of a 10-mg/ml ethidium bromide solution centrifuged for 60 to 72 h at 35,000 rpm and 18°C in a Beckman SW 50.1 rotor) to separate supercoiled SV40 DNA from nicked DNA (SV40 and cellular). The DNA was then extracted at least five times with buffer-saturated butanol to remove ethidium bromide and dialyzed against storage buffer (TE: 10 mM Tris-hydrochloride–1 mM EDTA). DNA from Cs_2SO_4 gradients was dialyzed against TE, made 1.0 M in NaCl, and extracted three times with chloroform-isoamyl alcohol (24:1) to remove unbound [^{3}H]trioxsalen (removed by butanol extraction in the case of ethidium bromide-CsCl gradients) and dialyzed against TE. Samples of irradiated SV40 chromatin complexes that had been digested with micrococcal nuclease were digested with Pronase and extracted with chloroform-isoamyl alcohol as described above, and then digested in succession at 37°C with 150 μg of RNase B (Calbiochem, grade A) per ml for 3 h, 150 μg of amylase (Calbiochem, grade B) per ml for 1 h, and 1 mg of Pronase per ml for 4 h. The DNA was again extracted three times and dialyzed into TE.

DNA concentrations were determined using an extinction coefficient of 6,000 M^{-1} phosphorous (39) (50 μg/ml per 1.0 optical density unit at 257 nm) in a cuvette with a path length of 1 cm.

Virus purification. Virus particles were purified

by a modification of method IV of Estes et al. (13). Sonically treated virus lysate was concentrated by precipitating with 6% (wt/vol) polyethylene glycol overnight at 4°C and centrifuging at 10,000 rpm for 30 min at 4°C in a Beckman JA-14 rotor. Virion pellets were eluted by stirring with TBS (0.15 M NaCl-0.025 M Tris, pH 7.4) overnight at 4°C and centrifuged at 10,000 rpm for 30 min at 4°C in a Beckman JA-20 rotor. The supernatant was brought to a density of 1.33 g/cm^3 with CsCl (Calbiochem) and was spun to isopycnic equilibrium by centrifuging at 40,000 rpm for 16 h at 4°C in an SW 50.1 rotor. Full virion bands were collected and dialyzed against TBS.

Electron microscopy. The purified DNA samples were nicked with pancreatic DNase at 0°C (according to the method of Wang [53]) or cut with RI restriction enzyme (a gift from P. Modrich) at 37°C for 30 min in 0.1 M Tris-hydrochloride (pH 7.6)-0.05 M NaCl-0.005 M $MgCl_2$-0.2 mM dithiothreitol-0.1 mM EDTA in 50- to 200-μl volumes. Under these conditions, 1 unit of RI enzyme cut 5 μg of SV40 DNA to completion (<1% circular molecules as judged by electron microscopy). The DNA was extracted as described above and dialyzed against TE. Samples were then denatured in 10% formaldehyde-0.02 M Na_2CO_3-5 mM EDTA (pH 7.0) at 70°C for 2 h (19), or in 0.5 M glyoxal-70% formamide-0.01 M sodium phosphate (pH 7.0)-1 mM EDTA for 60 min at 40°C (4) and spread in formamide according to the method of Davis et al. (12) by using a modification of the Kleinschmidt procedure (25). Grids were rotary shadowed with 80% Pt-20% Pd or with tungsten, and microscopy was carried out on a Philips 201 electron microscope.

Histograms of loop length were generated by projecting 35-mm negatives and measuring with an electronic planimeter (Numonics Corp.) interfaced to a paper-tape punch. A PDP 8/E computer was used to calculate and compile the data into the form of histograms in base-pair units.

Micrococcal nuclease digestions and polyacrylamide gel electrophoresis. Samples of irradiated SV40 chromatin complex were made 1 mM in $CaCl_2$ and digested with approximately 72 units of micrococcal nuclease (Worthington) per 100 μg of DNA in 1 ml of sample in the pooled sucrose gradient fractions (i.e., approximately 10 to 15% sucrose) at 37°C for 2 min. The reactions were stopped with 25 mM EDTA, and the DNA was purified as described. The fragments were separated according to size by electrophoresis at 40 V for 9 h on 4% polyacrylamide slab gels (0.4 by 10 cm) (29). The bands were visualized by staining in 1 μg of ethidium bromide per ml for 60 min. The gels were sliced into 1.4-mm fractions and incubated for 6 h at 50°C in 1 ml of tissue solubilizer (9 parts NCS [Amersham/Searle] to 1 part water) in scintillation vials and counted by adding 10 ml of Spectrafluor scintillation fluid [Amersham/Searle; 6 g of 2,5-diphenyloxazole and 75 mg of 1,4-bis-(5-phenyloxazole)benzene per liter of toluene].

RESULTS

Protection of intracellular SV40 DNA from trioxsalen cross-linking. It has been shown that DNA in whole cells or intact nuclei of *Drosophila melanogaster* is protected from cross-linking by 4,5',8-trimethylpsoralen (trioxsalen) relative to native DNA (19, 55). To determine whether this protection extended to intracellular SV40 DNA, cells permissively infected with SV40 were treated with [^{3}H]trioxsalen and irradiated with UV light of 340 to 380 nm. Because trioxsalen had a limited solubility in aqueous solutions (0.6 μg/ml), it was added in several saturating doses of 5 μg/ml each and irradiated for 30 min upon each addition. The irradiation device is constructed such that the cells can be irradiated in closed tissue culture dishes without detachment from the plastic surface. The entire irradiation took place between 36 and 48 h after infection, during the peak of viral DNA replication (Fig. 2), and before the maximum production of completed viral particles. After irradiation, the viral DNA was extracted by the Hirt procedure (21) and purified, and the amount of covalently bound trioxsalen was determined. Figure 3 shows that the reaction with intracellular SV40 DNA reaches a plateau at approximately 1 trioxsalen per 40 base pairs of DNA. In contrast, purified DNA binds 1 trioxsalen molecule per 4 base pairs. This saturation level of protection from trioxsalen addition is the same as that seen for DNA in cellular chromatin in both whole cells and intact nuclei (55).

Size distribution of the regions of intracellular SV40 DNA protected from cross-linking. To determine the size of the regions protected from cross-linking, SV40 DNA was isolated from infected cells that had been treated with trioxsalen and irradiated with UV light at 340 to 380 nm as described above. The DNA was denatured in the presence of formaldehyde or glyoxal and spread for electron microscopy as described above. The DNA appears as a series of loops (single-stranded DNA) and bridges or crossover points (cross-links) in Fig. 4.

Supercoiled DNA was found to be unsuitable for this analysis because it would denature only to an extent sufficient to allow the molecules to lie flat on the Parlodion surface of the electron microscope grid and appear relaxed (30). Therefore, the viral DNA was either nicked with pancreatic DNase (at 0°C) or cut with RI restriction enzyme and repurified before denaturation. The viral DNA was photographed, projected, and measured; histograms of the loop sizes were generated by computer. Figure 5 illustrates the histograms from SV40 DNA cross-linked in vivo at the peak of replication in the infectious cycle. The predominant loop size class is 200 to 250 base pairs with a smaller peak at 350 to 400 base pairs. The proportion of loops in the smaller size class decreases at low doses of trioxsalen (Fig.

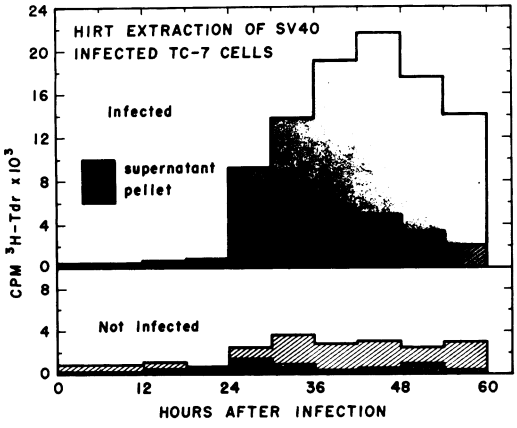


FIG. 2. *Kinetics of SV40 DNA replication. Confluent TC-7 cells were infected at a multiplicity of infection of 10. Every 6 h the DNA from two dishes was extracted by the Hirt procedure as described in the text. The DNA was labeled with [³H]thymidine (New England Nuclear; 20 Ci/mmol) at a concentration of 2 μCi/ml of medium for the 6 h immediately preceding extraction. The Hirt pellet was resuspended by homogenizing in a volume of TE equivalent to the supernatant volume, and portions from both fractions were counted.*

5B), presumably due to a decreased probability of cross-linking between each nucleosome (see below).

When isolated nuclei from a wide variety of organisms are partially digested with micrococcal nuclease, the resulting DNA fragments are 200 base pairs and multiples of 200 base pairs in length (2, 22, 30, 41, 56). Thus, the pattern of cross-links in intracellular SV40 DNA is consistent with a model in which the regions between nucleosomes are particularly accessible to trioxsalen, as well as to endonucleases. To test whether this pattern of a "monomer" and a presumptive "dimer" could have been generated randomly or by some artifact due to sequence or the electron microscopy technique, purified SV40 DNA was cross-linked at several doses of trioxsalen. Histograms of two different doses of trioxsalen each showed a relatively smooth curve (no dimer) in which the maximum peak shifts as a function of the extent of cross-linking (Fig. 6; L. Hallick, unpublished data). This is a striking contrast from the pattern at different doses of trioxsalen administered in vivo.

Size distribution of the regions of SV40 DNA in virus particles protected from cross-linking. It has been reported that SV40 DNA and polyoma DNA in virus particles are also complexed with histones in a nucleosome structure (6, 15, 23). Purified virus particles were treated with trioxsalen and irradiated to determine whether the same pattern of protection exists in the virus particles as in the intracellular nucleoprotein complex. The results from two doses of trioxsalen are plotted in a histogram (Fig. 7) in which there was a sharp peak at 150 to 200 base pairs at the high dose. At the lower dose of trioxsalen, a small dimer peak was also seen. Thus, it can be concluded that approximately the same size unit is protected from cross-linking in SV40 chromatin isolated from virus particles and from infected cells.

Micrococcal nuclease digestion of SV40 chromatin complexes. To demonstrate that the cross-linked sites are the same as those susceptible to nuclease digestion, SV40 chromatin labeled with [¹⁴C]thymidine was isolated

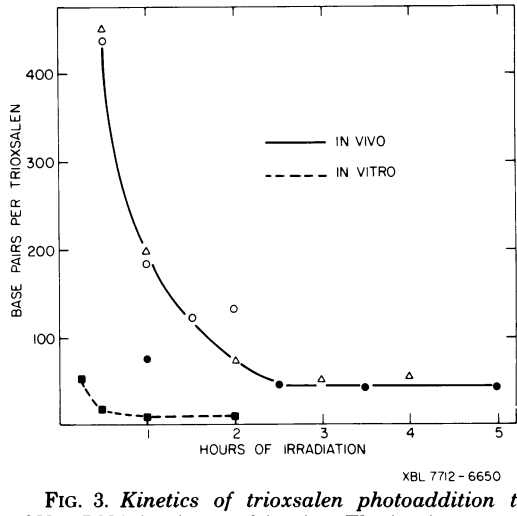
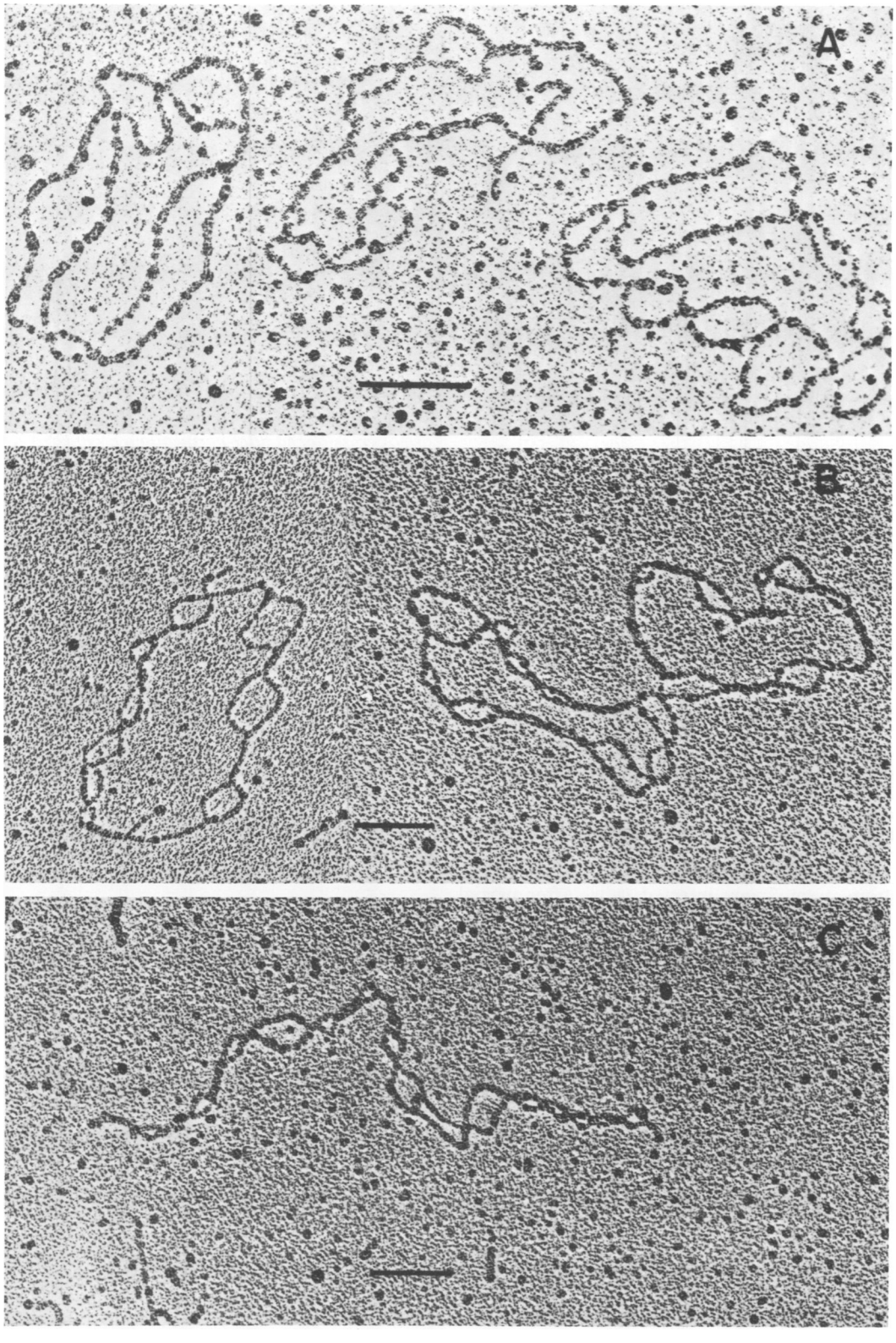


FIG. 3. *Kinetics of trioxsalen photoaddition to SV40 DNA in vitro and in vivo. The in vivo experiment was carried out as described in the text. The data from three separate experiments are shown (△, ○, ●). In the in vitro experiment (■) 50 μg of purified SV40 DNA and 5 μg of [³H]trioxsalen (renewed every 30 min) per ml were irradiated in 5 ml of TE in a 100-mm tissue culture dish. These conditions were chosen to mimic those of the in vivo experiment.*

FIG. 4. *Electron micrographs of cross-linked SV40 DNA. Samples of DNA from the experiments shown in Fig. 3 were either nicked (A and B) or cleaved with RI endonuclease (C) and denatured and spread as described in the text. The three sections represent low, intermediate, and high (but not saturating) levels of cross-linking. (A) Approximately 450 base pairs per trioxsalen adduct; molecules are from two separate photographs. (B) 200 base pairs per trioxsalen adduct; molecules are from two separate photographs (note that one is a dimer). (C) 120 base pairs per trioxsalen adduct. The length marker represents 500 base pairs.*


A
B
C

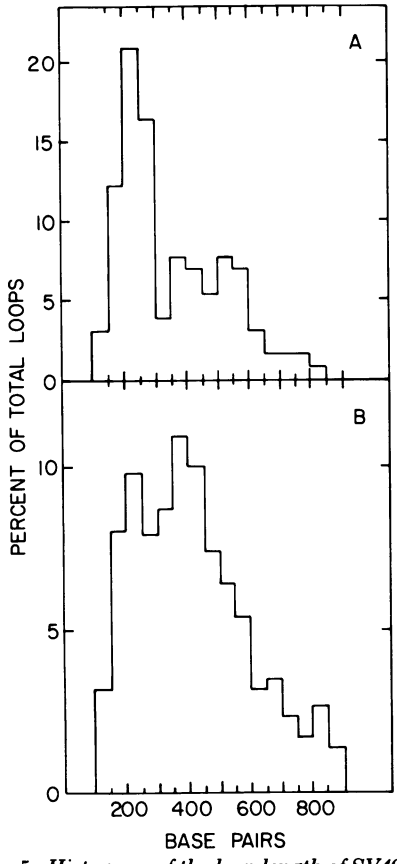


FIG. 5. *Histogram of the loop length of SV40 DNA cross-linked in permissively infected cells. The experiments were carried out as described in the text. (A) High dose: 75 base pairs per trioxsalen adduct; 258 loops measured. (B) Low dose: 200 base pairs per trioxsalen adduct; 856 loops measured.*

from permissively infected cells. After partial purification by sucrose gradient velocity sedimentation, the peaks were pooled, treated with [^{3}H]trioxsalen, and irradiated. The complex was then digested with micrococcal nuclease, the DNA was purified by Pronase digestion and chloroform extraction, and the digested product was analyzed on polyacrylamide gel electrophoresis (Table 1). If the trioxsalen were randomly distributed with respect to nuclease sites, the ratio of ^{3}H to ^{14}C would be expected to remain constant. On the other hand, if most of the trioxsalen adducts were located in regions of the DNA susceptible to nuclease, the digestion would preferentially remove ^{3}H counts relative to ^{14}C. The results clearly indicate that the ratio of ^{3}H to ^{14}C decreases after digestion. The extent of this preferential digestion is actually a minimum estimate of the specificity of the trioxsalen reaction because trioxsalen-containing sub-

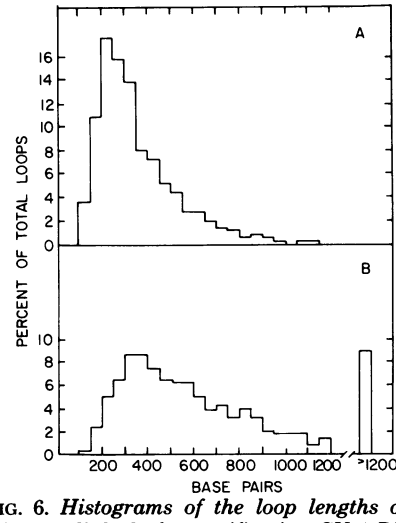


FIG. 6. *Histograms of the loop lengths of SV40 DNA cross-linked after purification. SV40 DNA was purified as described in the text and irradiated at a DNA concentration of 2 μg/ml of (A) 0.05 μg of trioxsalen per ml or (B) 0.015 μg of trioxsalen per ml (B) in 60-mm tissue culture dishes for 10 min. The approximate extents of trioxsalen addition are one molecule per 50 to 75 (A) and 250 to 300 (B) base pairs. The histograms were based on 850 loops and 782 loops, respectively.*

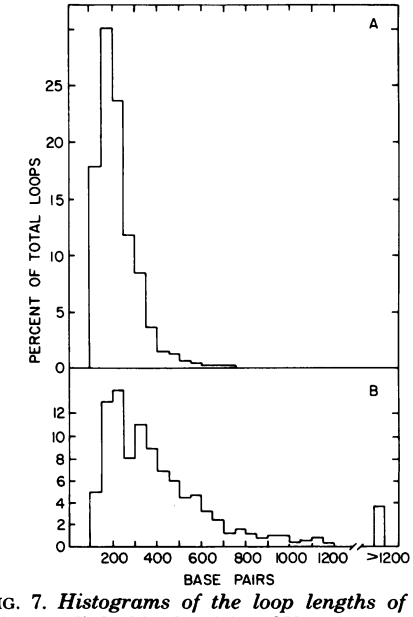


FIG. 7. *Histograms of the loop lengths of SV40 DNA cross-linked in the virion. SV40 virus particles, prepared as described in the text, were irradiated at a concentration of approximately 100 μg/ml of DNA equivalents with 6 μg (A) or 0.05 μg (B) of trioxsalen per ml for 30 min in 60-mm tissue culture dishes. The approximate extents of trioxsalen addition are one molecule per 40 (A) and 200 (B) base pairs. The histograms were based on 1,415 loops and 597 loops, respectively.*

TABLE 1. *Micrococcal nuclease digestion of SV40 chromatin*[a]

Expt	cpm		Ratio	
	^3H	^{14}C	$^3H/^{14}C$	Total/monomer
I				
Total DNA	900	1,050	0.9	3.5
Monomer	255	1,260	0.2	
II				
Total DNA	6,580	2,630	2.5	2.8
Monomer	2,150	2,300	0.9	
Total DNA	7,640	2,800	2.7	1.6
Monomer	5,640	3,330	1.7	
Total DNA	16,240	4,090	4.0	1.7
Monomer	4,590	1,880	2.4	
Total DNA	35,440	6,540	5.4	1.7
Monomer	6,460	2,080	3.1	

[a] SV40 chromatin was labeled with [^{14}C]thymidine, extracted from infected cells, photoreacted with [^3H]trioxsalen, and analyzed as described in the text. The ^3H counts per minute have been corrected for ^{14}C spillover. The ratio of undigested to monomer DNA is the ratio of the respective ^3H to ^{14}C. In experiment I, the samples were irradiated for a total of 32 min with 8 additions of 5 μg of [^3H]trioxsalen (every 4 min) per ml in a high-intensity light source described elsewhere (55). In experiment II, the four samples were irradiated for 1.5, 2.5, 3.5, and 4.5 h, respectively, in the fluorescent tube light source employed in all other experiments with 5 μg of [^3H]trioxsalen per ml added every 30 min.

strates are more resistant to nuclease than are untreated DNA (55).

DISCUSSION

It has been shown that intracellular SV40 chromatin is protected from photochemical cross-linking by trioxsalen to the same extent as cellular chromatin. The level of addition at saturation (1 trioxsalen molecule per 40 to 50 base pairs) remains constant for at least 2 h of additional irradiation and drug addition. In addition, we have demonstrated that [^3H]trioxsalen is preferentially added to intracellular SV40 chromatin at sites susceptible to micrococcal nuclease digestion, presumably the regions between nucleosomes. This preference is far from absolute; however, the extent of digestion of cross-linked regions is underestimated by at least a factor of two due to the resistance of these regions to micrococcal nuclease digestion (55). Approximately 25 to 40% of the trioxsalen adducts are covalently bound to both strands (8; L. Hallick and G. Weisehahn, unpublished data), and there is indirect evidence that these adducts are particularly resistant to digestion (55). An alternative explanation that cannot be totally ruled out at this time is that the nucleosomes of the isolated SV40 chromatin undergo rearrangement during the irradiation or digestion procedures.

Histograms of the length of the loops generated by denaturing SV40 DNA cross-linked intracellularly or in virus particles indicate a preferential spacing between cross-links of approximately 200 base pairs. These histograms do not rule out a different (perhaps random) pattern for the addition of monoadducts of trioxsalen (60 to 75% of the trioxsalen). However, the results of micrococcal nuclease digestion make this possibility seem unlikely.

Preliminary analysis of those samples of SV40 DNA which had been cross-linked in vivo and cleaved by RI endonuclease did not reveal one unique loop pattern with respect to the RI cleavage site. However, this does not preclude the possibility that there may be a finite number of possible nucleosome arrangements, as recently reported by Ponders and Crawford (44), for both SV40 and polyoma DNA.

The appearance of dimer (approximately 400 base pairs) and even higher-order multimers is less obvious from histograms of SV40 DNA than it is from cellular DNA (19). This suggests that the spacing between SV40 nucleosomes is more heterogeneous than that observed for cellular chromatin. This observation has been made in at least three other laboratories by different approaches (43; M. DePamphilis, personal communication; E. Daniell, personal communication). Perhaps this apparent variability in spacing is due to the constraints of a small covalently closed circle, to the instability (or lack) of the H1 association, to the relatively "active" state of the SV40 chromatin with respect to replication and translation, or to some combination of these factors.

The ease with which psoralen derivatives enter cells, nuclei, virus particles, and nucleoprotein complexes coupled with their specificity for specific sites on the DNA molecule makes them a valuable probe for the structure of transcription complexes, replication intermediates, and virus nucleoprotein cores.

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