Title

THE 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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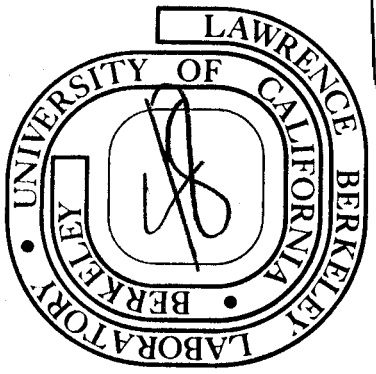

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DISCLAIMER

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ABSTRACT

Intracellular simian virus 40 (SV40) DNA in a permissive infection is complexed with histones in a repeating structure of "nucleosomes" analogous to that of eucaryotic chromatin (8,15,17,52). Derivatives of the furocoumarin, psoralen, have been shown to be useful tools for studying the in vivo structure of cellular chromatin (18,53). It has been demonstrated here that 4,5',8-trimethylpsoralen (trioxsalen) is also a valuable probe for the structure of SV40 DNA-histone complexes. Trioxsalen readily penetrates intact cells, and in the presence of 340-380 nm light, covalently cross-links 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 indicate 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.

INTRODUCTION

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 (19,33,35,36,44,47). Each nucleosome is thought to contain 140-200 base pairs of DNA wrapped around an octamer core consisting of two each of the histones H2a, H2b, H3 and H4 (1,25,46). The lysine-rich histone H1 is thought to be complexed with the "bridge" or interbead regions of the fiber (34,46,49).

Both the intracellular and viral nucleoprotein complexes of simian virus 40 (SV40) and polyoma virus have a structure similar to that of cellular chromatin. It consists of a ring of approximately 21 repeating units or nucleosomes made up of histones and DNA (5,8,14-17,22,52). Most studies report the absence of histone H1 in the viral nucleosome complex (8,13,16,17,27,31,39) although one recent report indicates that H1 may be associated with the intracellular complex when it is isolated under conditions where protein degradation is minimized (48,50). In addition, DePamphilis and his colleagues have recently observed that all five histones are associated with SV40 DNA in the same proportions as with host DNA; however, histone H1 is not as tightly bound and can be eluted at lower salt concentration from viral nucleoprotein complexes than from cellular chromatin (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 (18). 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 (6,26,32,37). 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 (7,9,10). It has been shown that the nucleosome structure is largely protected from cross-linking by 4,5'8-trimethylpsoralen (trioxsalen, Figure 1) relative to purified DNA and that the region between nucleosomes is preferentially cross-linked by the compound (53). 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's Modified Eagle's medium (DME, Grand Island Biological, New York), supplemented with 10% fetal calf serum (Grand Island Biological, New York), on 100 mm plastic tissue culture dishes (Falcon Plastics, California). The cells were infected upon reaching confluency with virus twice plaque purified from a stock of the small plaque forming strain SP12 of SV40 originally isolated by J. Robb. Radioactive label was added where indicated 24 hrs after infection.

4,5',8-Trimethylpsoralen (trioxsalen). Trioxsalen was obtained from the Paul B. Elder Co. (Bryan, Ohio) and tritiated in this laboratory (23). Stock solutions of approximately 0.9 mg/ml were made in ethanol. Specific activities were determined by counting in 1 ml water and 10 ml Omnifluor-triton scintillation fluid [2 l toluene, 1 l triton X-100, 12 g 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 (DME plus 100 mM Hepes buffer (Calbiochem) minus bicarbonate, phenol red and riboflavin) to which had been added 5 μg/ml trioxsalen. The Hepes buffer enabled the irradiation to be done at atmospheric CO_2 pressure and the absence of UV-absorbants 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/ml of trioxsalen 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 in order 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 (16,31; M. Botchan and J. Griffith, personal communications.) Cells were labeled with 2-5 μCi/ml ^{14}C-thymidine (New England Nuclear, specific activity, 50-60 mCi/mmole) for 12 hrs before isolation. Approximately 42-44 hrs after infection the cells were washed with TD (Tris-diluent: 0.137 M NaCl, 5 mM KCl, 5 mM Na_2HPO_4, and 25 mM Trizma base [Sigma, tris (hydroxymethyl) aminomethane] pH 7.5) and resuspended in approximately 0.5 ml TD/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 the Beckman J-21 centrifuge. The nuclei were resuspended by homogenization in 0.7 ml nuclei lysis buffer (10 mM Tris-hydrochloride, pH 8.0, 10 mM EDTA [ethylenediaminetetraacetate], 0.5% Triton X-100, Rohm & Haas, Pa.) 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 1500 rpm in the same rotor. The supernatant containing SV40 chromatin was then layered onto a 5-20% linear sucrose gradient [in 0.15 M NaCl, 0.01 M Tris-hydrochloride, pH 7.5, 1 mM DTT (dithiothreitol), 0.5% Triton X-100] and centrifuged for 90 min

at 40,000 rpm in the Beckman SW 41 rotor. The fractions containing the SV40 chromatin complex were determined by counting 25 µl aliquots, pooled and irradiated at 6° C in an irradiation device that has been described elsewhere (53) 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 (20). Approximately 10^8 cells were resuspended in 3.6 ml 0.01 M EDTA, pH 7.5; 0.4 ml of 10% SDS were 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 the Beckman SW 50.1 rotor, the supernatant was adjusted to 25 mM EDTA, pH 8, 1.5% Sarkosyl NL-97 (Geigy), and 1 mg/ml pronase (Calbiochem, grade B nuclease-free or grade B, preincubated at 20 mg/ml for 30 min at 37° C) and incubated for 4 hrs at 50° C. The SV40 DNA was then banded by density equilibrium centrifugation in Cs_2SO_4 (3.37 g Cs_2SO_4 added to 5.5 ml of DNA solution, centrifuged at 35,000 rpm and 18° C for 48 hrs in the Beckman 50, 65 or SW 50.1 rotor), or in ethidium bromide -CsCl (3.62 g CsCl, 3.48 ml DNA solution, 50 µl of 10 mg/ml ethidum bromide solution centrifuged for 60-72 hrs at 35,000 rpm and 18° C in the 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 ^3H-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, then digested in succession at 37° C with 150 µg/ml RNase B (Calbiochem, A grade) for 3 hrs, 150 µg/ml amylase (Calbiochem, B grade) for 1 hr and 1 mg/ml pronase for 4 hrs. 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 (38) (50 µg/ml/O.D.$_{257}$ 1.0) in a cuvette with a pathlength of 1 cm.

Virus Purification

Virus particles were purified using a modified Method IV of Estes et al. (12). Sonicated virus lysate was concentrated by precipitating with 6% (w/v) 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 gm/cm^3 with CsCl (Calbiochem) and was spun to isopycnic equilibrium by centrifuging at 40,000 rpm for 16 hrs at 4° C in a SW 50.1 rotor. Full viron bands were collected and dialyzed against TBS.

Electron microscopy. The purified DNA samples were nicked with pancreatic DNase at 0° C [according to the procedure of Wang (49)] 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 DTT, 0.1 mM EDTA in 50-200 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 hrs (18), 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. (11) using a modification of the Kleinschmidt procedure (24). 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 DNA in 1 ml of sample in the pooled sucrose gradient fractions (i.e., approximately 10-15% sucrose) at 37° C for 2 min. The reactions were stopped with 25 mM EDTA and the DNA purified as described. The fragments were separated according to size by electrophoresis at 40 V for 9 hrs on 0.4 x 10 cm 4% polyacrylamide slab gels (28). The bands were visualized by staining in 1 µg/ml ethidium bromide for 60 min. The gels were sliced into a 1.4 mm fractions and incubated for 6 hrs 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 mls of Spectrafluor scintillation fluid (Amersham/Searle, 6 g PPO and 75 mg POPOP/liter 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 (18,53). In order 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 340-380 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 hrs 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 (20), purified and the amount of covalently bound trioxsalen determined. It can be seen from Fig. 3 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 (53).

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

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 parlodian surface of the electron microscope grid and

appear relaxed (29). 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. Fig. 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 200-250 base pairs with a smaller peak at 350-400 base pairs. The proportion of loops in the smaller size class decreases at low doses of trioxsalen (Fig. 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 (3,21,30,40,54). 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. In order 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. As is shown in Fig. 6, histograms of two different doses of trioxsalen each show a relatively smooth curve (no "dimer") in which the maximum peak shifts as a function of the extent of cross-linking (Fig. 6 and L. Hallick, unpublished results). 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 (5,14,22). Purified virus particles were treated with

trioxsalen and irradiated in order 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 in Fig. 7. It can be seen that there is a sharp peak at 150-200 base pairs at the high dose. At the lower dose of trioxsalen, a small dimer peak can also be 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. In order to demonstrate that the cross-linked sites are the same as sites susceptible to nuclease digestion, SV40 chromatin labeled with ^{14}C-thymidine were isolated 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 purified by pronase digestion and chloroform extraction and the digested product analyzed on polyacrylamide gel electrophoresis. The results are shown in Table 1. If the trioxsalen was randomly distributed with respect to nuclease sites, the ^{3}H to ^{14}C ratio 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 ^{3}H to ^{14}C ratio decreases after digestion. The extent of this preferential digestion is actually a minimum estimate of the specificity of the trioxsalen reaction because trioxsalen containing substrates are more resistant to nuclease than untreated DNA (53).

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 40-50 base pairs) remains constant for at least 2 hrs of additional irradiation and drug addition. In addition, we have demonstrated that ^{3}H-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 (53). Approximately 25-40% of the trioxsalen adducts are covalently bound to both strands (7, L. Hallick and G. Weisehahn, unpublished observations) and there is indirect evidence that these adducts are particularly resistant to digestion (53). 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 (60-75% 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 (42) 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 (18). 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 (Ponder and Crawford, in press; DePamphilis, personal communication; and 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 make them a valuable probe for the structure of transcription complexes, replication intermediates and virus nucleoprotein cores.

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Table 1

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

Micrococcal nuclease digestion of SV40 chromatin. SV40 chromatin was labeled with ^{14}C-thymidine, extracted from infected cells, photoreacted with ^{3}H-trioxsalen, and analyzed as described in the text and in the MATERIALS AND METHODS section. The ^{3}H CPM have been corrected for ^{14}C spillover. The undigested/monomer ratio is the ratio of the respective $^{3}H/^{14}C$ ratios. In Experiment I, the samples were irradiated for a total of 32 min with 8 additions of 5 µg/ml ^{3}H-trioxsalen (every 4 min) in a high intensity light source described elsewhere(53). In Experiment II, the four samples were irradiated for 1 1/2, 2 1/2, 3 1/2 and 4 1/2 hrs respectively in the fluorescent tube light source employed in all other experiments with 5 µg/ml ^{3}H-trioxsalen added every 30 min.

FIGURE LEGENDS

Figure 1. The structure of 4,5',8-trimethylpsoralen (trioxsalen).

Figure 2. Kinetics of SV40 DNA replication. Confluent TC-7 cells were infected at a multiplicity of infection of 10. Every 6 hrs the DNA from two dishes was extracted by the Hirt procedure as described in MATERIALS AND METHODS. The DNA was labeled with ^{3}H-thymidine (New England Nuclear, 20 Ci/mmole) at a concentration of 2 μCi/ml of medium for the 6 hrs immediately preceding extraction. The Hirt pellet was resuspended by homogenizing in a volume of TE equivalent to the supernatant volume and aliquots from both fractions were counted.

Figure 3. Kinetics of trioxsalen photoaddition to SV40 DNA in vitro and in vivo. The in vivo experiment was carried out as described in the RESULTS and the MATERIALS AND METHODS sections. The data from three separate experiments are shown (Δ,O,●). In the in vitro experiment (▩) 50 μg of purified SV40 DNA and 5 μg/ml ^{3}H-trioxsalen (renewed every 30 min) were irradiated in 5 mls TE in a 100 mm tissue culture dish. These conditions were chosen in order to mimic the in vivo experiment.

Figure 4. Electron micrographs of cross-linked SV40 DNA. Samples of DNA from the experiments shown in Figure 3 were either nicked (A and B) or cleaved with RI endonuclease (C), denatured and spread as described in MATERIALS AND METHODS. The three sections represent low, intermediate and high (but not saturating) levels of cross-linking: (A) approximately 450 base pairs/trioxsalen adduct, molecules are from two separate photographs;

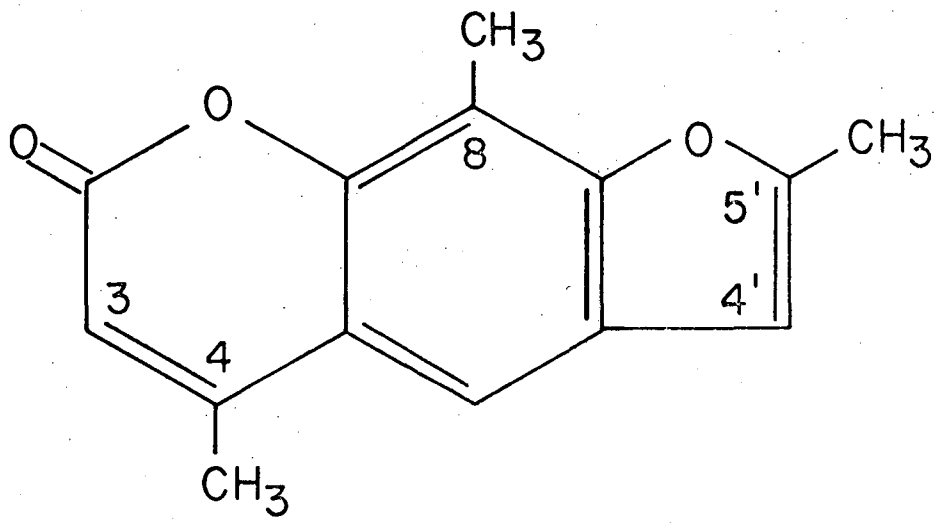
(b) 200 base pairs/trioxsalen adduct, molecules are from two separate photographs (note that one is a dimer) and (C) 120 base pairs/trioxsalen adduct. The length marker represents 500 base pairs.

Figure 5. Histogram of the loop-length of SV40 DNA cross-linked in permissively infected cells. The experiments were carried out as described in MATERIALS AND METHODS in Figure 3. (A) High dose: 75 base pairs/trioxsalen adduct; 258 loops measured. (B) Low dose: 200 base pairs/trioxsalen adduct; 856 loops measured.

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

Figure 7. Histograms of the loop-lengths of SV40 DNA cross-linked in the virion. SV40 virus particles prepared as described in MATERIALS AND METHODS were irradiated at a concentration of approximately 100 μg/ml of DNA equivalents with (A) 6 μg/ml or (B) 0.05 μg/ml trioxsalen for 30 min in 60 mm tissue culture dishes. The approximate extents of trioxsalen addition are one molecule per (A) 40 and (B) 200 base pairs. The histograms were based on 1415 loops and 597 loops respectively.

TRIOXSALEN (TRIMETHYLPSORALEN)



XBL 7712-6651

Fig. 1

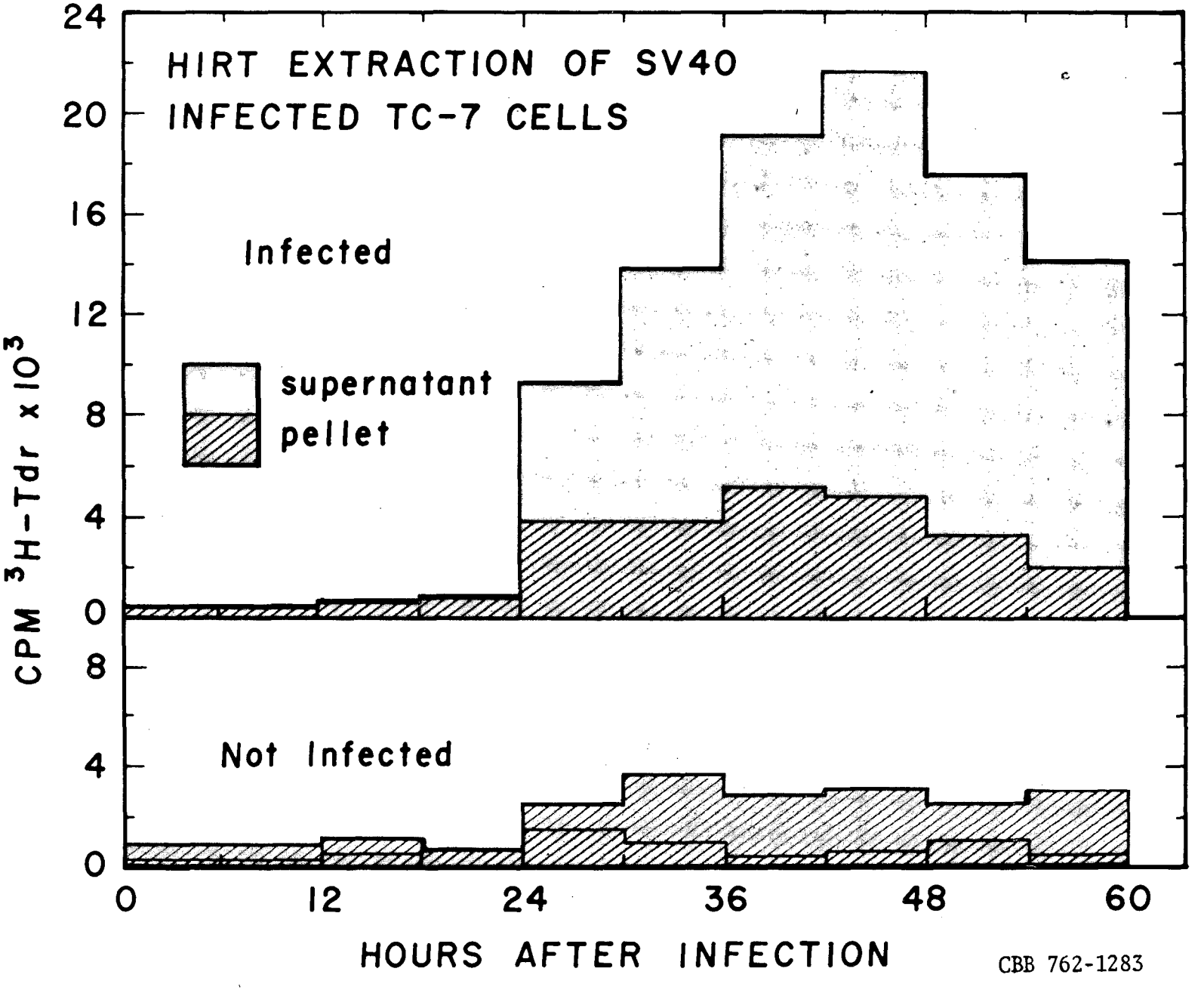

HIRT EXTRACTION OF SV40
INFECTED TC-7 CELLS
Infected
supernatant
pellet
Not Infected
CPM ^{3}H-Tdr x 10^3
24
20
16
12
8
4
0
8
4
0
0
12
24
36
48
60
HOURS AFTER INFECTION
CBB 762-1283

Fig. 2

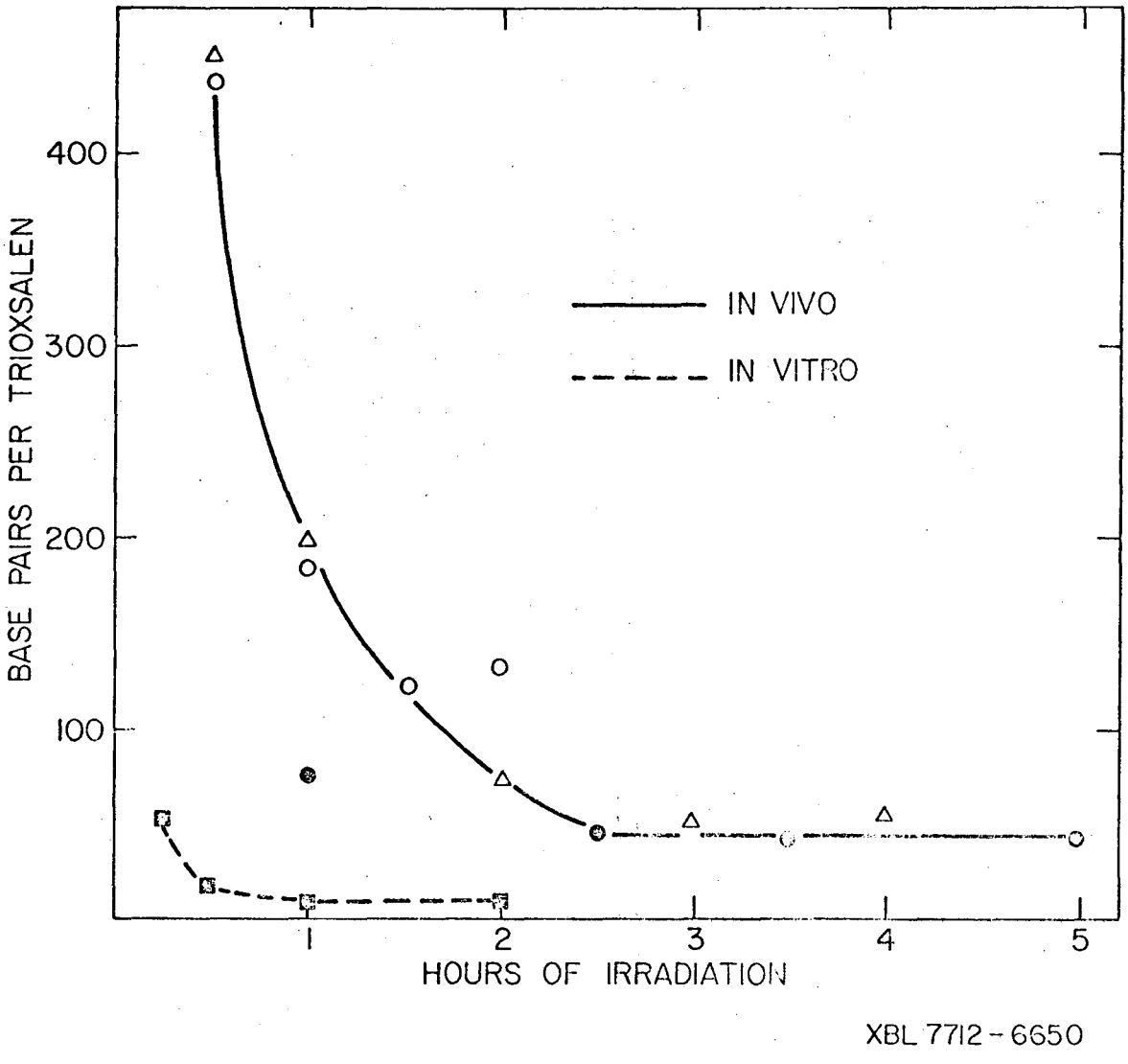

BASE PAIRS PER TRIOXSALEN
400
300
200
100
IN VIVO
IN VITRO
1
2
3
4
5
HOURS OF IRRADIATION
XBL 7712-6650

Fig. 3

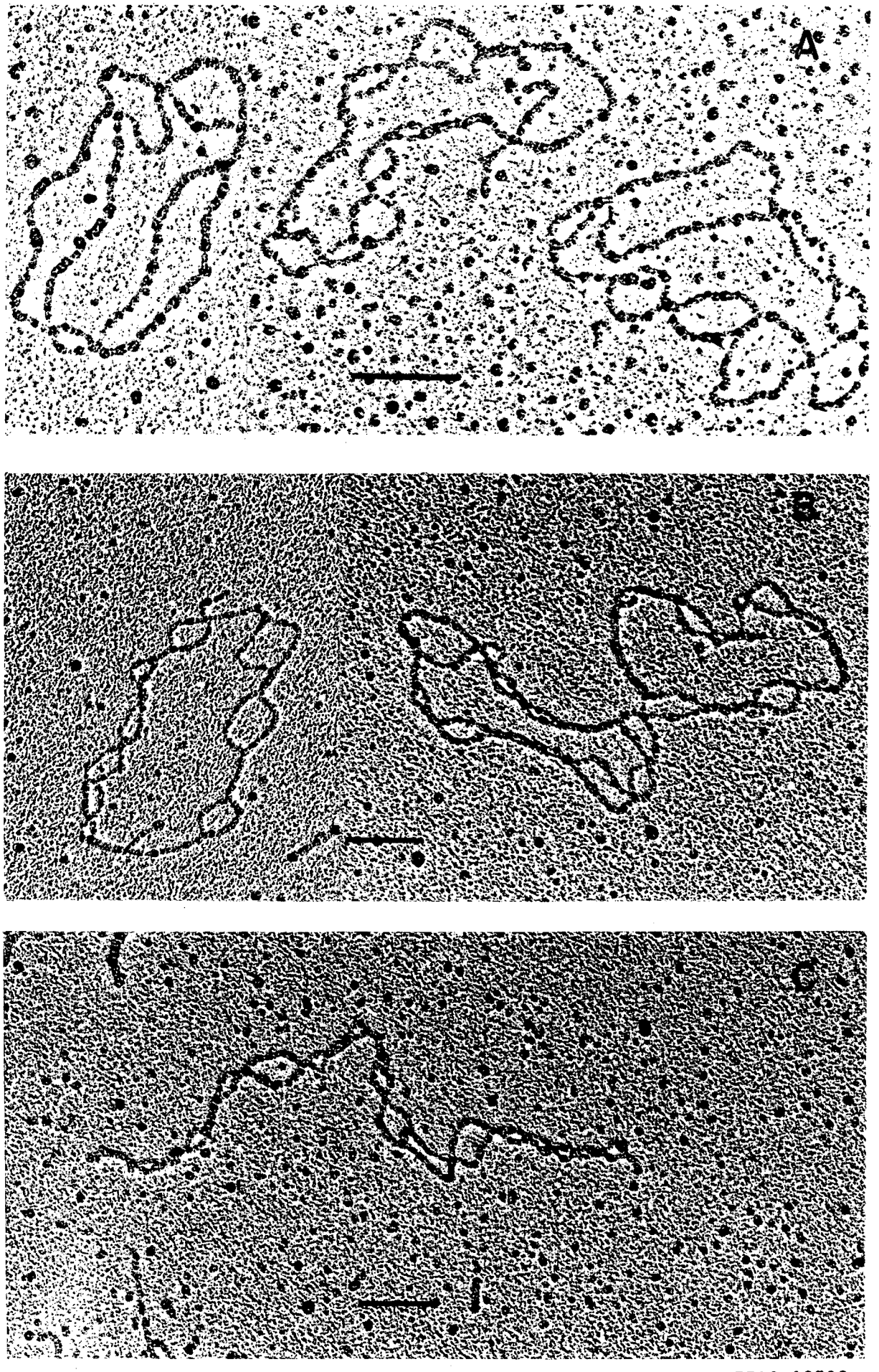


Fig. 4

XBB 7712-12739

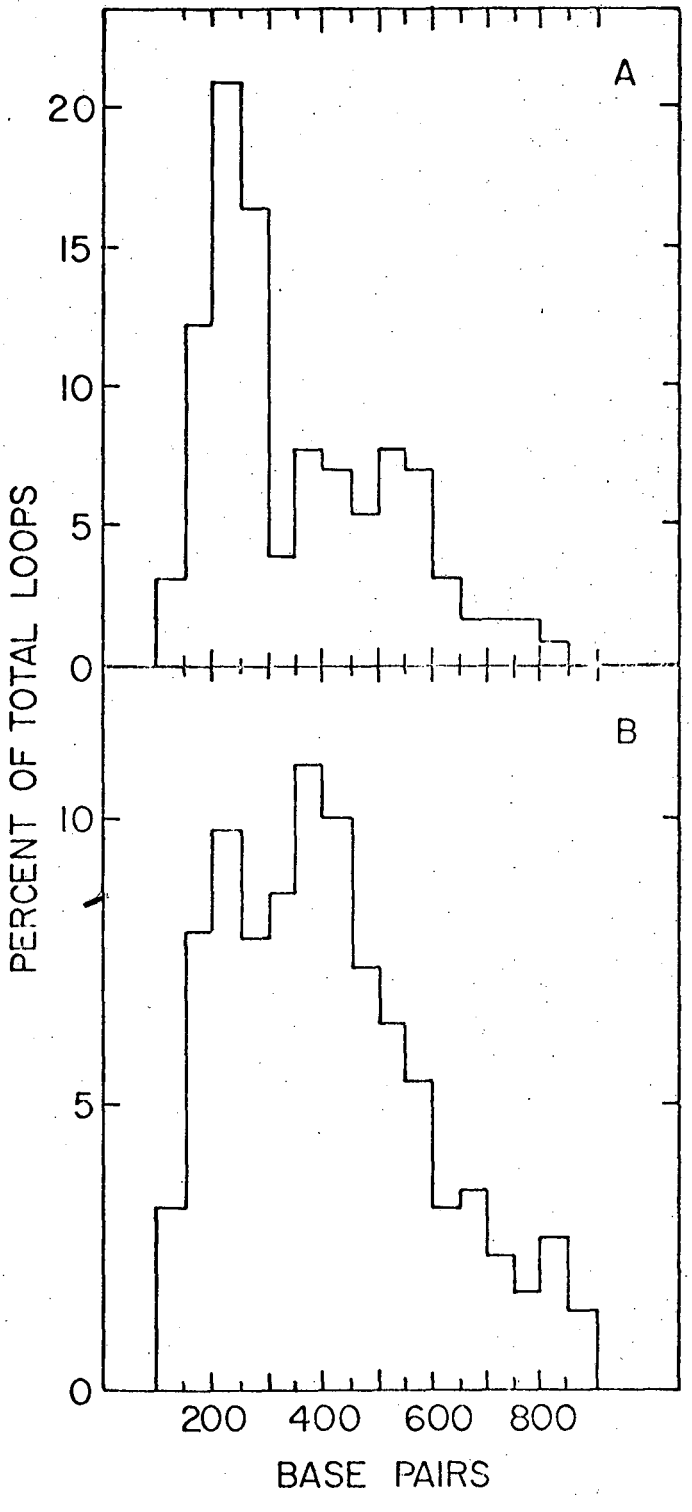

A
B
20
15
10
5
0
10
5
0
PERCENT OF TOTAL LOOPS
200
400
600
800
BASE PAIRS
XBL 7712-4795

Fig. 5

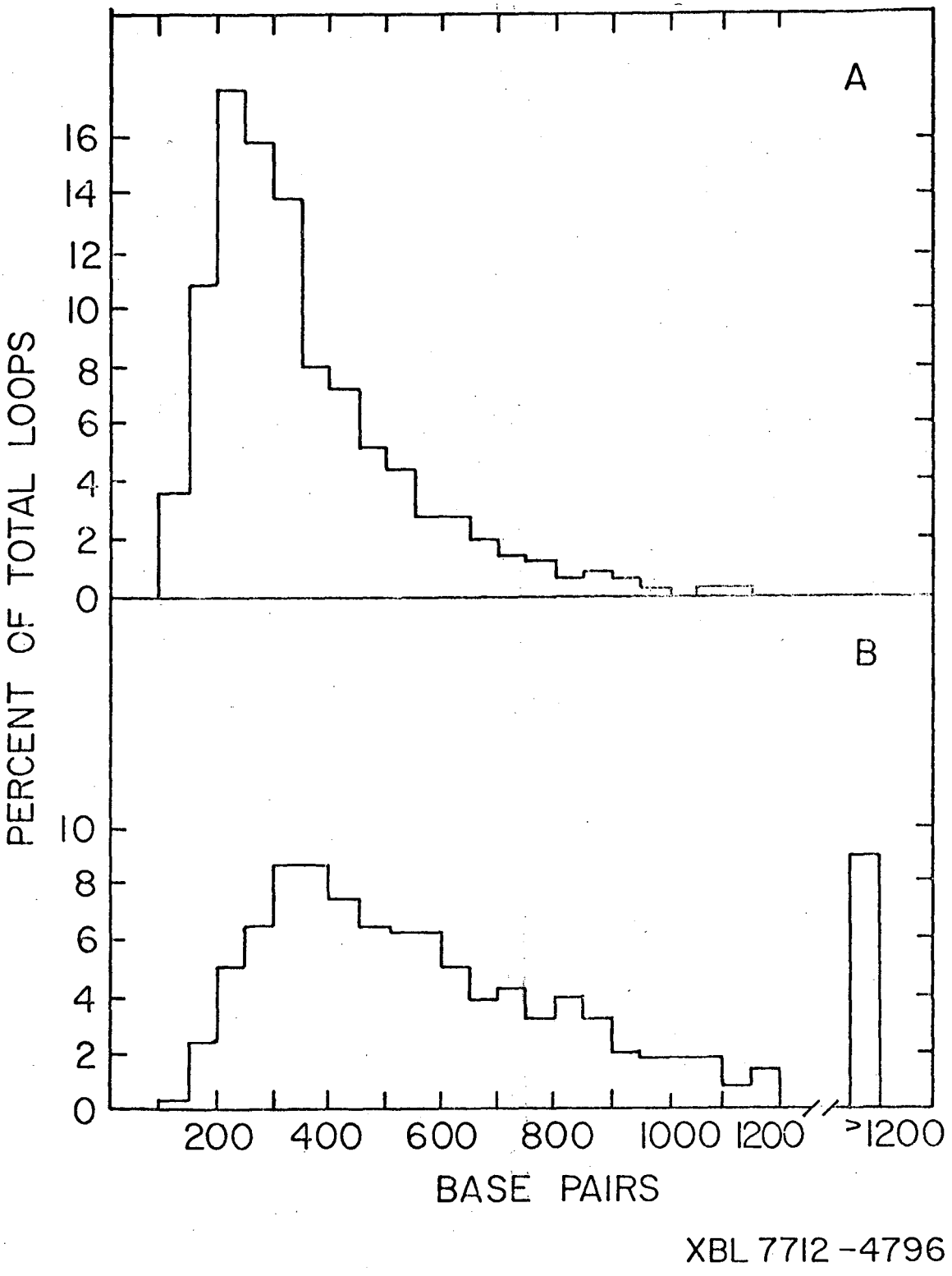

A
B
PERCENT OF TOTAL LOOPS
16
14
12
10
8
6
4
2
0
10
8
6
4
2
0
200
400
600
800
1000
1200
>1200
BASE PAIRS
XBL 7712-4796

Fig. 6

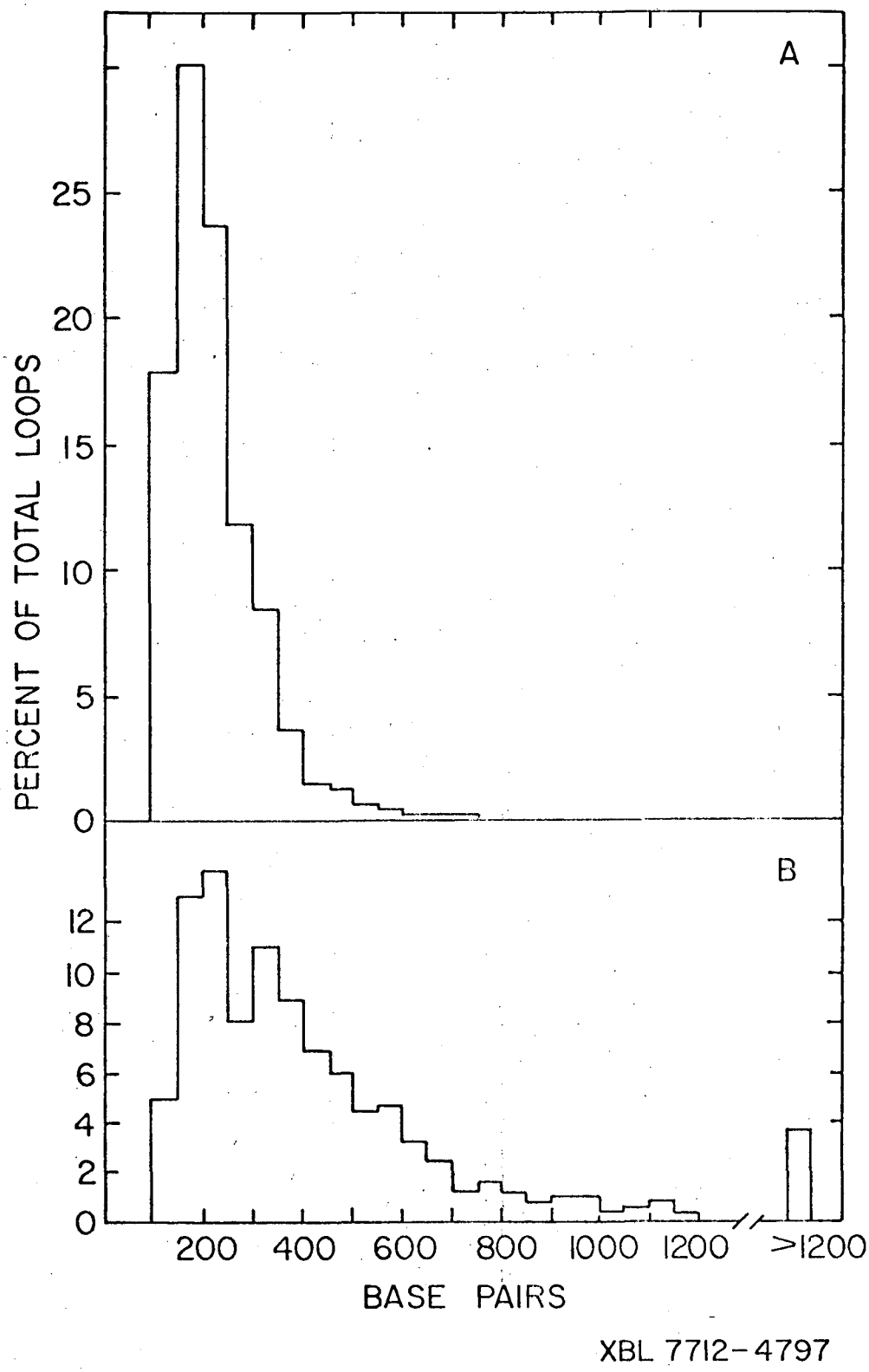

A
B
PERCENT OF TOTAL LOOPS
0
5
10
15
20
25
0
2
4
6
8
10
12
200
400
600
800
1000
1200
>1200
BASE PAIRS
XBL 7712-4797

Fig. 7

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