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Detection by electron microscopy of photo-induced denaturation in λ DNA

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ABSTRACT

We have used an electron microscope to study localized denatured regions in ultraviolet-irradiated DNA. DNA from bacteriophage λ was UV-irradiated and then prepared for electron microscopy after fixing in buffered (pH 9.5) formaldehyde solutions at 25°C. The denatured regions observed corresponded to those described by Inman and Schnös (1) who used alkaline denaturation to preferentially destroy thymine-adenine base pairing. In UV-irradiated DNA, pairs of neighboring thymine residues are converted into photodimers; hence, loss of hydrogen bonding most likely occurs in thymine-rich regions and denaturation results. Conceivably, photo-induced denaturation may under some circumstances represent a more convenient method than alkaline denaturation for mapping thymine-rich regions in DNA.

INTRODUCTION

Inman and Schnös (1) have prepared denaturation maps of intact λ phage DNA by first denaturing the DNA in a limited fashion with either high temperature or alkaline pH in the presence of formaldehyde and then examining the partially denatured DNA in the electron microscope. Since the stability of G—C base pairs is assumed to be greater than A—T base pairs, denaturation of the DNA double helix likely occurs preferentially in the thymine-rich regions. In this paper we present similar denaturation maps obtained by using ultraviolet irradiation to preferentially modify the thymine residues in λ DNA. We conclude that ultraviolet irradiation is a suitable if not preferable method for denaturation mapping, especially since renaturation can not occur and controlled denaturation may be easily done on very small samples.

MATERIALS AND METHODS

Phage Preparation. Bacteriophage λ C_I 857 S7 was prepared, with some slight variations as previously described (2), by temperature induction of

E. coli C 607 and purification by CsCl banding (3). Following sedimentation, the phage band was collected, dialyzed two times against 10^{-2} M Tris-HCl (pH 8) and 10^{-2} M $MgSO_4$, and stored over chloroform at 4°C until used.

DNA Extraction. DNA was gently extracted from the phage by use of phenol buffered at about pH 7 with 0.01 M phosphate buffer. The extraction was performed three times using equal volumes of phenol and phage suspension. After extraction the DNA was dialyzed four times against phosphate buffer. The absorbance at 260 nm was 7.4 and the 260/280 ratio was approximately 2.

Ultraviolet Irradiation. Irradiation at 254 nm was done using two germicidal lamps at a dose rate of 90 J/m^2 per minute as determined by Jagger Meter readings (4).

Before irradiation the DNA was diluted to an absorbance (260 nm) of about 0.18 in 0.01 M PO_4. In some cases $AgNO_3$ was added in order to enhance the magnitude of thymine dimerization (5).

Electron Microscopy. After irradiation the DNA was diluted fivefold in 0.01 M PO_4 buffer and 70 µl was mixed with 30 µl of buffer containing 32% formaldehyde, 0.011 M EDTA, and 0.068 M Na_2CO_3 (pH 9.5). An equal volume of formamide (6) was added and the samples were spread on droplets of water (1), by use of the protein film technique (7). The DNA was picked up on collodion-coated copper grids, rinsed by touching the grid to the surface of 95% ethyl alcohol stained with uranyl acetate (8), and rotary-shadowed with platinum at an angle of 6°. Electron micrographs were made with a Siemens Elmiskop IA electron microscope and the magnification was calibrated with a diffraction grating replica (Fullam, 54,864 lines per inch.

Measurements. Electron micrographs were projected and the DNA molecules traced onto sheets of paper. Measurements were made of these tracings with a map measuring device, and the lengths of the native and denatured regions were recorded. The length of each molecule was normalized to 19.2 microns (1). The positions and lengths of each denatured region were either plotted in a linear representation or else summed up, and the results from 24 different molecules shown on a histogram.

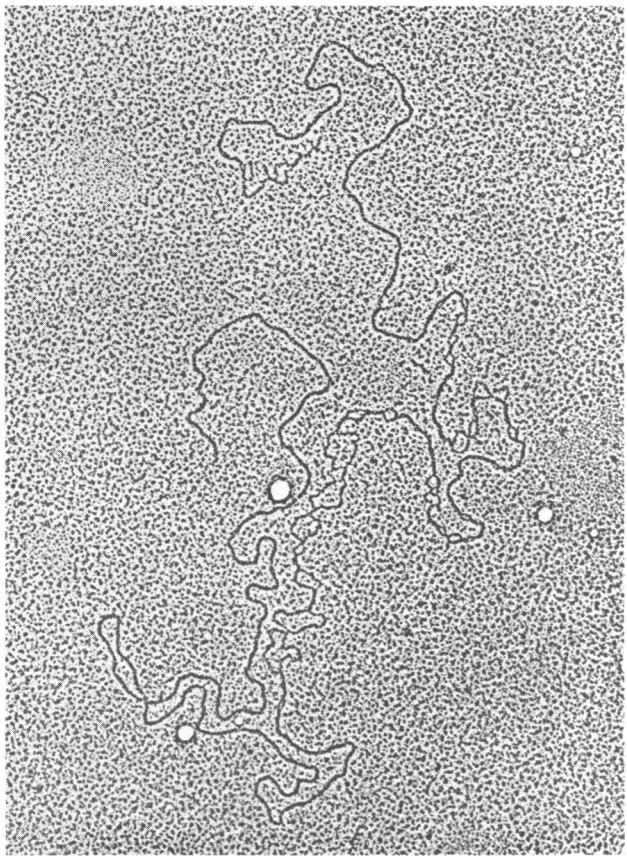

Figure 1. Electron micrograph of a λ DNA molecule irradiated (280 nm) with 1000 J/m^2 in the presence of Ag^+.

Table 1: Extent of denaturation of λ DNA UV-irradiated in the presence or absence of Ag^+

Fluence (J/m^2)	Ag^+ treatment	Percent of thymine as T<>T*	Average denaturation per molecule (%)	Range of denaturation (%)	Number of molecules observed
0	- Ag^+	0	< 0.1	—	10
0	+ Ag^+	0	< 0.1	—	10
1000	- Ag^+	2.2	3.1	1.7—4.4	10
1000	+ Ag^+	18.0	12.3	7.2—18.2	24

*Estimated from data using radioactively labeled E. coli DNA (5).

RESULTS AND DISCUSSION

Electron micrographs of UV-irradiated λ DNA were obtained as described in Methods. The irradiated molecules contained loops corresponding to locally denatured regions as indicated in Figure 1. Such loops were absent in the nonirradiated DNA (Table 1). Linear representations of a selection of DNA molecules irradiated in the presence or absence of Ag^+ are shown in Figure 2. The percentage of DNA in the denatured regions is fourfold greater for irradiation in the presence of Ag^+ (Table 1), although the yield of T<>T (9) is eightfold greater,

A composite of 24 maps of DNA irradiated in the presence of Ag^+ is presented in Figure 3 in the form of a histogram. Three major areas of denaturation occur. Their positions are in general agreement with those found by Inman and Schnös (1), whose map for pH-denatured λ DNA is included in Figure 3 for comparison. The relative differences between the two maps may be due to a smaller number of disrupted hydrogen bonds under our conditions of denaturation. Another possibility, however, is that regions along the DNA molecule containing a high proportion of alternating A—T sequences would denature readily upon raising the pH, but would not denature upon UV irradiation since no pyrimidine dimers would be formed. (Thymine dimer formation requires the presence of adjacent thymines on the same DNA strand.) Hence the absence of a peak at 15.5 microns in the UV-denatured sample may suggest that this region contains alternating A—T sequences. Thus, UV-induced denaturation combined with the pH method has the possibility of yielding information on the nature of the A—T-rich region.

In order to specifically enhance UV-induced denaturation in the thymine-rich regions, DNA was irradiated with Ag^+ complexed to the bases. Under these conditions thymine-thymine dimerization is greatly enhanced without any concomitant effect on cytosine-cytosine dimerization (5). We have avoided the use of triplet sensitizers such as acetophenone and benzophenone, which specifically enhance thymine dimerization, but which also introduce single-strand chain breaks (10).

It is estimated that each dimer in a DNA strand causes disruption of the hydrogen bonding for four base pairs, two for each dimer and one on either side of the dimer (11). Since 25% of the bases in λ DNA are thymine (12), it is estimated that for DNA containing 18% of its thymine as thymine dimer, as in the Table, 18% of its length will be denatured (13). From Figure 3 we estimate that, on the average, 12% of each molecule is denatured. Obviously a portion of the thymine dimers exist in regions which are too

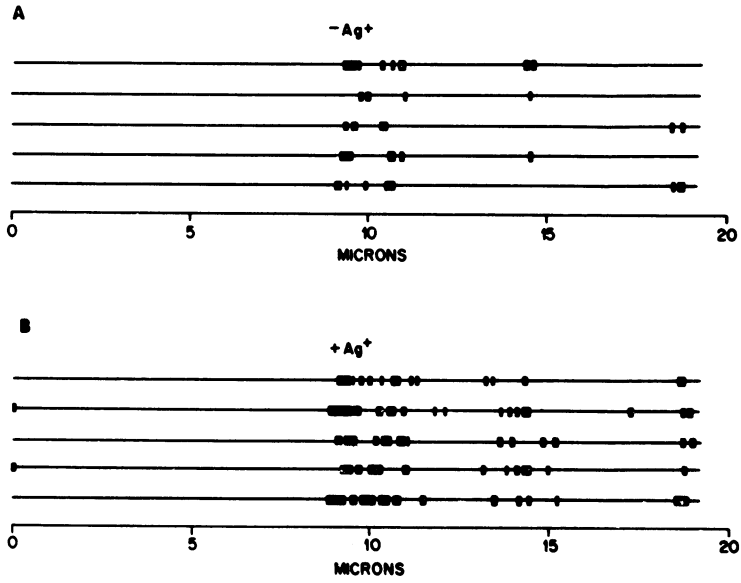

Figure 2. Linear denaturation maps for a selection of λ DNA molecules which were irradiated with a dose of 1000 J/m^2 at 254 nm in the absence (A) or presence (B) of Ag^+. The shaded portions correspond to single-stranded looped regions, and the total length of each molecule was normalized to a length of 19.2 microns. The 19.2 micron end was arbitrarily chosen as the end exhibiting the greatest extent of denaturation.

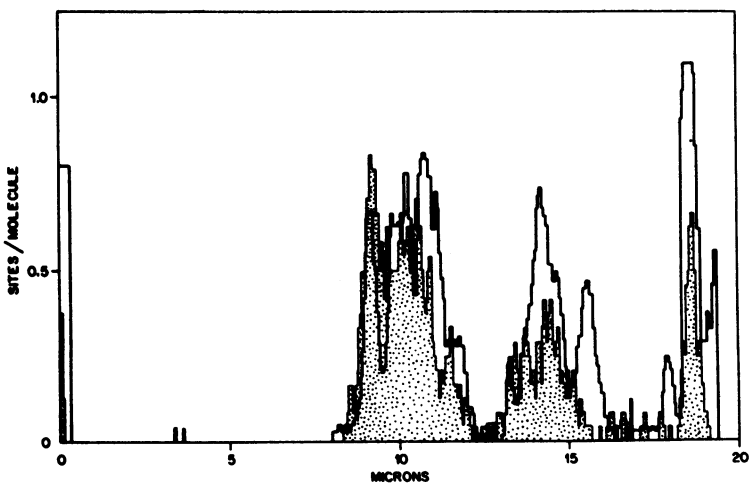

Figure 3. Histograms of partially denatured λ DNA. The shaded area is a histogram resulting from UV irradiation of DNA at 254 nm (1000 J/m^2) in the presence of Ag^+. The clear area is the histogram of alkaline pH denatured λ DNA obtained by Inman and Schnös (1). For the irradiated DNA, 24 molecules were measured and normalized to a length of 19.2 microns.

small to be measured by our method. It is important to remember that formaldehyde could react with additional bases in the vicinity of the dimer and thereby increase the size of the observed denatured region. It is estimated that a region of ~100 disrupted base pairs can be observed by this technique.

Although formaldehyde was used to fix the denatured regions of the DNA prior to electron microscopy, it is expected that thymine dimers would maintain the disruption of the secondary structure and eliminate the need for formaldehyde. Hopefully, by avoiding formaldehyde treatment, differences in the individual denaturation maps between molecules similarly treated, as in Figure 2 for example, will be minimized. In that case, fewer molecules would be needed in order to compose an accurate histogram. Future experiments are needed to establish whether UV-induced denaturation minus formaldehyde will result in less variation of the individual denaturation maps.

ACKNOWLEDGMENT

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13 Calculation:

Let P = percent denaturation due to T<>T = (fractional A+T content of DNA) X (base pairs disrupted per T<>T) X (moles of T<>T per mole of thymine as dimer X (percent thymine present as dimer).

Then, for T<>T = 18%, P = 0.5 x 4 x 0.5 x 18% = 18%.