ABSTRACT

Superhelical PM2 DNA can be photochemically modified by u.v. irradiation. The variation of $S_{20,w}$ with dose shows the following characteristics. There is a linear increase from 28 to 31S produced by a low dose of u.v. irradiation (4,000 ergs/mm$^2$). A plateau in $S_{20,w}$ occurs between 4,000 and 10,000 ergs/mm$^2$. The $S_{20,w}$ then increases when irradiation is increased to 56,000 ergs/mm$^2$. Thymine dimers are introduced proportional to dose throughout the range of exposure to u.v. light. Sedimentation velocity-dye titrations reveal anomalous behavior, i.e. apparent increases in superhelix density ($\sigma$). However, the dye-buoyant density procedure showed no change in $\sigma$ under the same conditions. The most satisfactory model for the data is preferential photochemical modification of premelted (possibly hairpin) sites as a greater rate than the introduction of photoproducts into duplex sites. The origin of the anomaly in the sedimentation velocity dye titrations is still unclear.

"I ca'n't believe that!" said Alice.
"Ca'n't you?" the Queen said in a pitying tone. "Try again:
draw a long breath, and shut your eyes."
Alice laughed. "There's no use trying," she said:
"One ca'n't believe impossible things."
"I daresay you haven't had much practice," said the Queen.
"When I was your age, I always did it for half-an-hour a day.
Why, sometimes I've believed as many as six impossible things before breakfast..."

from Through the Looking-Glass
by Lewis Carroll

Dedicated to the memory of Jerry Vinograd who believed in the impossible, twisted circular DNA and made postdoctoral life like Alice in Wonderland.

INTRODUCTION

Since the original characterization of superhelical DNA from polyoma-virus by Vinograd and co-workers' the list of closed DNAs continues to grow and intensive investigations have been pursued from a number of points of view.

Our laboratory has focused considerable attention on the secondary
structure of superhelical DNA by characterizing the reactivity of several supercoiled DNAs towards reagents specific or preferential for unpaired bases. The reactivity of HCHO, CH$_3$HgOH, and a water soluble carbodiimide have been explored with the following results:

1. Chemical modification occurs far more readily with superhelical form I than with nicked circular form II.
2. The initial reactivity is generally accompanied by a sharp increase in sedimentation velocity.
3. In several cases when modified DNA is examined in a sedimentation-velocity-dye titration an anomalous behavior occurs. The minimum S$_{20,w}$ value of the titration occurs at a higher ethidium bromide concentration than the native unmodified DNA I, suggesting that reactivity has produced an apparent increase in superhelix density.

This study examines the effects of u.v. irradiation on the structure of PM2 DNA. Our rationale is as follows: 1. The analysis of hydrodynamic changes produced by irreversible photochemical modification (pyrimidine dimers) is simplified since no mass changes complicate the corrections to S$_{20,w}$ and consequently we are only dealing with structural modifications of the DNA. 2. There is neither excess reagent nor possible chemical exchanges that would interfere with an analysis of the number of superhelical turns, $\tau$. Consequently photochemical modification appeared to be a useful probe of the structure of superhelical DNA. In this paper we describe the effects of low doses of u.v. irradiation on PM2 DNA. When the data are compared to chemical modification of PM2 DNA considerable similarities can be seen, suggesting possible common features in the reaction scheme.

**MATERIALS AND METHODS**

Preparation of viral PM2 DNA. PM2 DNA (form I) was prepared as described previously. $^6,^7,^{13}$ $^3$H-labelled PM2-DNA was prepared by growing Pseudomonas BAL-31 to a logarithmic state ($8 \times 10^8$ cells/ml), infecting with PM2 bacteriophage (MOI 15), and immediately adding deoxyadenosine (250 $\mu$g/ml) and $^3$H-thymidine (20 $\mu$Ci/ml). Growth was continued until lysis had occurred. After purification on a CsCl density gradient (0.4 g/ml), the phage were lysed with 30% Sarkosyl (5 $\mu$l/ml) and form I DNA was obtained by banding in an ethidium bromide-CsCl gradient. The ethidium bromide was removed by passing the DNA sample through a Dowex column. The DNA was dialyzed in BB buffer (0.1 M sodium borate, 0.1 M sodium chloride, pH 9.0) and stored frozen at -20°C. The specific activity of the DNA was $2.2 \times 10^5$ cpm/ug.
Chemicals. The ethidium bromide was a gift from Boots Pure Drug Co., Ltd., Nottingham, England. Optical grade cesium chloride was obtained from Harshaw Chemical Co. $^3$H-thymine was obtained from New England Nuclear Corp. Propidium diiodide was purchased from Calbiochem Corp., Dowex AG 50W-X4 was from Bio-Rad Laboratories.

Irradiation of the DNA. Aliquots (0.5 ml) of the $^3$H-PM2 DNA (35-40 μg/ml) in BB buffer were exposed to the unfiltered output of two 15 W low-pressure mercury-vapor germicidal lamps (General Electric G15 T8) at a distance of 13 cm. In order to achieve uniform exposure to ultraviolet light, each sample was placed in a small watch glass over a magnetic stirrer; the DNA samples were kept on ice during the irradiation to reduce evaporation. The incidence dose of radiation (135 to 150 ergs/mm$^2$/sec) was determined from the survival curve of φX RF DNA irradiated under identical conditions and assayed for biological activity on E. coli K12W$^5$. At 254 nm, φX RF has an inactivation cross-section of $8 \times 10^{-17}$ cm$^2$, and this value was used for calibration.

Determination of thymine dimers. Thymine dimers were detected by the method of Carrier and Setlow$^{17}$ using a two-dimensional paper chromatographic system. Samples of the DNA that had been exposed to u.v. radiation for various lengths of time were divided into two portions. One portion (50 μl) was analyzed for the presence of thymine dimers. Following acid hydrolysis, the sample was evaporated to dryness under a stream of N$_2$ gas and taken up in 30 μl of 1.0 N HCl. Carrier bases (cytosine and thymine, 5 μl of a solution of 1 mg/ml in water) were added to each sample before applying the sample to a paper chromatogram. All other procedures were conducted according to the method of Carrier and Setlow.$^{17}$ The remainder of each DNA sample was used for the determination of superhelix density by sedimentation velocity-ethidium bromide titration. In one case the buoyant density procedure of Gray et al.$^{18}$ was utilized.

Analytical centrifugation. Band sedimentation velocity experiments were performed at 20°C and 34,000 rpm in a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner. Double sector, 12 mm, type I, band-forming centerpieces were used.$^{19}$ The appropriate amount of ethidium bromide was added to the CsCl solution with a Manostat digital pipet. Ethidium bromide concentrations were determined with a Cary 15 spectrophotometer using a reciprocal extinction coefficient of 81.6 μg/ml/absorbance unit at 487 nm.$^{18}$ The centerpiece sample well was filled with 10 μl of BB buffer and 18 μl of 35 μg/ml sample of PM2 DNA (approx.
75% form I, 25% form II). Sedimentation coefficients were calculated from a least-square analysis of log distance (in cm) versus time (in minutes) using an IBM 360-50 computer. Sedimentation values were corrected to standard conditions for sodium DNA ($S_{20,w}^o$) according to the method of Bruner and Vinograd. The density of the CsCl sedimentation solvent was determined from refractive index readings taken on a Zeiss refractometer. In dye-sedimentation velocity titrations no correction was made for the buoyant density change and we adopt the symbol $S_{20,*}$ for this situation.

Buoyant density experiments were done in a Beckman Model E ultracentrifuge equipped with a u.v. scanner. Double sector 12 mm type I band centerpieces were used. The runs were made at 44,000 revs/min for 18-20 hours at 20°C. The sample well was filled with 10 µl BB buffer and 10 µl of a sample containing 35 µg/ml PM2 DNA. The sedimentation solvent contained CsCl (density 1.739 g/ml), 0.04 M Tris-HCl, pH 8.0, and M. luteus DNA (2 µg/ml) as a marker (density 1.724 g/ml). The buoyant densities were calculated using the equation of Vinograd and Hearst:

$$e - p = a\omega^2 \frac{(r^2 - r_e^2)}{2}$$

(1)

where $e$ is the buoyant density, $r$ and $r_e$ are the distances from the center of rotation to band center for PM2 DNA and marker DNA (M. luteus) respectively, $\omega$ is the angular velocity, and $p_e$ is the density of M. luteus DNA. The value of $a$ for CsCl gradient was taken to be $8.4 \times 10^{-10}$ cgs units.

Relative buoyant separations at high dye concentrations were determined in the Beckman Model E ultracentrifuge using 2 mm centerpieces fabricated from commercial 12 mm Epon centerpieces. DNA samples (0.7 µg) in 0.125 ml CsCl solutions, density 1.492-1.497 g/ml, containing 316 µg/ml propidium diiodide were centrifuged for 26 hours at 40,000 rpm at 20°C.

RESULTS

Dependence of sedimentation velocity and thymine dimer content on the dose of u.v. radiation. The sedimentation velocity of PM2 DNA form I increases substantially after irradiation with low doses (2000-4000 ergs/mm²) of u.v. light (Fig. 1a); a further 6000 ergs/mm² of radiation has little additional effect on the sedimentation velocity. In contrast, the sedimentation velocity of form II DNA remains constant as the DNA is exposed to u.v. radiation (up to 10,000 ergs/mm²). At high doses of u.v. radiation (56,000 ergs/mm²) increases in the $S_{20,w}^o$ for both the superhelical (form I and nicked circular (form II) DNAs can be detected.

Figure 1b summarizes the effect of u.v. radiation on the thymine dimer
Figure 1. The effect of u.v. irradiation on (a) sedimentation coefficient, $S_0^{20,W}$, and (b) thymine dimer formation. (a) PM2 DNA was irradiated and centrifuged in 2.83 M CsCl, 0.1 M Tris-HCl (pH 8.0) at 34,000 rpm. (•—•) PM2 DNA form I; (0---0) PM2 DNA form II. (b) $^3$H-labelled PM2 DNA I was irradiated, hydrolyzed and chromatographed on a two-dimensional paper chromatographic system.

content of PM2 DNA (form I and II). Increasing doses of ultraviolet light result in an increase of thymine dimers as well as an increase in sedimentation coefficient. The dimer analyses were done on samples of irradiated form I which contained small amounts of form II DNA that were present initially and which were produced as the result of low level nicking by u.v. For the purposes of this work, we assume that the quantum yield of dimers is the same in both form I and form II DNA.

Ultraviolet radiation of up to 8000 ergs/mm$^2$ does not affect the buoyant density of the DNA molecule (Table 1). Higher doses of u.v. light (56,000 ergs/mm$^2$) cause an increase in the buoyant density of the irradiated DNA, as also shown by Denhardt and Kato for φX-RF.

Apparent superhelical density as a function of u.v. irradiation. Since modified superhelical DNAs have shown anomalous behavior in sedimentation velocity-dye titrations in a variety of cases it was of con-
The effects of u.v. radiation on sedimentation velocity ($S_{20w}^o$), apparent superhelical density ($26\sigma$) and superhelical turns ($26\tau$), buoyant density ($\theta$) and thymine dimers.

<table>
<thead>
<tr>
<th>Dose (Ergs/mm$^2$)</th>
<th>$S_{20w}^o$</th>
<th>Apparent ($26\sigma$)</th>
<th>Apparent $26\tau$</th>
<th>$\theta$ gm/ml</th>
<th>Thymine Dimers</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 0</td>
<td>27.8</td>
<td>-.116</td>
<td>-106</td>
<td>1.6950</td>
<td>0</td>
</tr>
<tr>
<td>1350</td>
<td>29.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* 2000</td>
<td>29.9</td>
<td>-.116</td>
<td>-106</td>
<td>1.6955</td>
<td>40</td>
</tr>
<tr>
<td>2700</td>
<td>30.1</td>
<td>-.130</td>
<td>-119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* 4000</td>
<td>31.2</td>
<td>-.125</td>
<td>-115</td>
<td>1.6955</td>
<td>66</td>
</tr>
<tr>
<td>5400</td>
<td>31.3</td>
<td>-.116</td>
<td>-106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* 8000</td>
<td>30.6</td>
<td>-.116</td>
<td>-106</td>
<td></td>
<td>107</td>
</tr>
<tr>
<td>10,800</td>
<td>31.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.3</td>
<td>31.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* 56,000</td>
<td>35.2</td>
<td>-.106</td>
<td>-97</td>
<td>1.6982</td>
<td>239</td>
</tr>
<tr>
<td>56,700</td>
<td>35.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indicates those experiments where DNA was labelled with $^3$H.

It is of considerable interest to examine if this occurs for u.v. irradiated DNA. Although superhelix density will be measured by finding the dye concentration at the minimum in $S_{20w}^o$, we shall call this the apparent superhelix density.

The apparent superhelix density, $26\sigma$, was determined by the band sedimentation velocity-ethidium bromide titration procedure. $^3$H-labelled PM2 DNA was used in those experiments that included thymine dimer analysis. Determination of the superhelical density by titration of ultraviolet-irradiated DNAs are shown in Figure 2. The minimum in the curves for DNAs irradiated with 2000, 54000 and 8000 ergs/mm$^2$ of ultraviolet light occurs at the same free dye concentration (8 $\mu$g/ml) as the unirradiated DNA. However, at 2700-4000 ergs/mm$^2$ of u.v. light, higher free dye concentrations of ethidium bromide (9.0 - 9.5 $\mu$g/ml) are needed to titrate these DNAs to a minimum sedimentation coefficient. After prolonged u.v. treatment (56,000 ergs/mm$^2$) the minimum $S_{20w}^o$ occurs at a much lower free dye concentration, 7 $\mu$g/ml. The apparent superhelical densities according to the above free dye concentrations are -.116, -.130, -.125 and -.106 respectively, (Table 1). These values were determined by the equation of Bauer and Vinograd, modified for an angle of unwinding of 26° for ethidium bromide.

\[ 26\sigma_0 = \tau/\beta^o = -1.45v_c \]
Figure 2. Sedimentation velocity-ethidium bromide (EB) titrations of seven PM2 DNAs after increasing amounts of u.v. radiation. The DNAs were centrifuged at 34,000 rpm in 2.83 M CsCl, 0.01 M Tris-HCl (pH 8.0) at different dye concentrations. Each plot shows the change in $S_0^{20} \times$ versus $\mu g$ EB/ml at the following doses of u.v. light:

(a) $^3$H-PM2 DNA, unirradiated; (b) $^3$H-PM2 DNA, 2000 ergs/mm$^2$; (c) Unlabelled PM2 DNA, 2700 ergs/mm$^2$; (d) $^3$H-PM2 DNA, 4000 ergs/mm$^2$; (e) Unlabelled PM2 DNA, 5400 ergs/mm$^2$; (d) $^3$H-PM2 DNA, 8000 ergs/mm$^2$; (g) Unlabelled PM2 DNA, 56,000 ergs/mm$^2$.

An arrow indicates the ethidium bromide concentration at the minimum $S_0^{20} \times$ for each plot and is shown in order to compare the amount of EMB needed to remove superhelical turns at different doses of u.v. radiation relative to native DNA dashed line. The lower curve in each plot is for PM2 DNA form II.

The binding data for ethidium bromide to DNA for the above solvent has been determined by Gray et al. and utilized to evaluate the EB bound at the minimum ($v_c$) for each curve.

where $^{26}v_0$ is the superhelix density in the absence of dye, $\tau$ is the number of superhelical turns, $\rho^0$ is one-tenth the number of base pairs in the molecule, and $v_c$ is the number of moles of ethidium bromide bound per
mole nucleotide at the minimum in the sedimentation velocity-dye titration curve.

It is evident from Fig. 2 and Table 1 that the apparent \( \lambda_0 \) remains constant at the low initial u.v. dose (2,000 ergs/mm\(^2\)). In the titrations of samples irradiated at 2,700 and 4,000 ergs/mm\(^2\) we observed an increase in breadth of the minimum region. This is particularly evident for the latter data. The selection of the minimum values were all performed by visual inspections. We realize that the uncertainty of selecting the exact minimum value increases with the breadth of the minimum region. Consequently, although the values in Table 1 indicate an apparent increase in superhelix density the magnitude of the change could be lower. The apparent increase in superhelix density is this study and previous reports\(^6,7,26\) continues to be puzzling and former interpretations have not been satisfactory.\(^30\) Consequently, we decided to examine an alternative approach for the determination of any change in \( \lambda \). The reason for the breadth of the minimum region is not yet understood but might reflect heterogeneity in superhelix density.\(^28,29\)

**Buoyant density analysis of a ultraviolet-irradiated DNA sample.**

Another means of examining changes in superhelical content of DNA is to compare the relative buoyant density separation of form I and II DNAs in the saturating amounts of intercalating drug, ethidium bromide or propidium diiodide. The corrected relative buoyant separation, \( \Omega_c \), is calculated by the equation of Bauer and Vinograd\(^22\)

\[
\Omega_c = f^{-1} r^* \frac{\Delta r}{\Delta r^*}
\]

where \( r/r^* \) is the ratio of the average distances from the axis of rotation for the unknown and reference DNAs respectively; \( \Delta r/\Delta r^* \) is the ratio of the separation between I and II DNA for the unknown and reference DNAs respectively, and \( f^{-1} \) corrects for any difference in the buoyant densities of the unknown and references DNAs in the absence of dye. The relative buoyant separation of DNA as a function of superhelical density is shown in Figure 3. **In vitro** ligase-closed PM2 DNAs were used to establish the dependence of relative buoyant separation of superhelical density which were determined by sedimentation velocity dye titrations. The superhelical density of the DNA molecule irradiated with 2700 ergs/mm\(^2\) of u.v. light is the same as the native molecule when determined by comparing their relative buoyant separation. It is clear that the buoyant dye measurement does not show any apparent increase in \( \lambda \).
Figure 3. The corrected relative buoyant separation, \( \Omega_c \), in CsCl solutions (density 1.492-1.497) containing 316 \( \mu \)g/ml propidium diiodide as a function of superhelical density, \( \sigma \). The DNAs were centrifuged at 40,000 rpm for 36 hours. (■,●) in vitro ligase closed DNAs; (▲) native PM2 DNA; (○) PM2 DNA irradiated with 2700 ergs/mm\(^2\) u.v. light.

DISCUSSION

Ultraviolet irradiation produces a linear increase in thymine dimers as shown in Fig. 1. This photochemical modification of PM2 DNA I is first accompanied by a linear increase in \( S_{20,\text{w}} \) of 3.5S units. A plateau in sedimentation velocity occurs between 5,400 and 10,000 ergs/mm\(^2\). The same dose range does not alter the \( S_{20,\text{w}} \) of the nicked form II significantly. A substantial further increase in dose of 56,000 ergs/mm\(^2\) causes an increase in \( S_{20,\text{w}} \) for both forms of DNA (Figure 1). Sedimentation velocity-dye titrations revealed anomalous behavior, i.e. apparent increases in superhelix density. Examination, in one case, of superhelix density by saturated propidium diiodide-buoyant density analysis did not reveal anomalous behavior and no change in \( \sigma \) was detected.

Previous analyses of the behavior of superhelical DNA has centered on the utilization of two models. The first views the DNA with all base pairs intact and interprets enhanced reactivity as resulting solely from the free energy of superhelix formation; i.e. the binding of reagents or enzymes is driven by this free energy in order to reduce the number of physical
superhelical turns (τ) in the DNA\(^{25,30-33}\). Another model that has been proposed is that supercoiling can produce interrupted secondary structure which may have the capacity of forming intrastrand hairpins with varying degrees of complementarity\(^{6-10}\). The stability and localization of hairpins will be dependent on sequence relationships\(^{10}\).

It should be emphasized that these models are not mutually exclusive since all reagents that are capable of reacting at single strand sites can also react at duplex sites if these latter are transiently open. Thus, the reaction of any chemical probe will be a competition between premelted regions and duplex sites that become available for modification due to a local transient unwinding of the intact duplex, i.e. breathing of the secondary structure. Consequently, two reaction paths exist. Path A is the reaction with any premelted site, e.g. hairpin or alternative structure with exposed bases. Path B is the reaction which occurs as transient unwinding takes place at low frequency. The apparent equilibrium constant, \(K_{\text{conf}}\), for the conformational opening and closing reaction of basepairs along the duplex has been measured by equilibrium analysis of HCHO reactivity and the value is 0.003 at 50°C in 0.15 M NaCl\(^{34}\). Naturally, \(K_{\text{conf}}\) would be much lower at 25°C. An estimate for \(K_{\text{conf}}\) of \(10^{-4}\) has been made from theoretical considerations\(^{35}\). In addition, tritium exchange of PM2 DNA I does not reveal an enhanced \(K_{\text{conf}}\) for superhelical DNA relative to form II\(^{36}\). The bulk of the hydrogen atoms involved in basepairing exchange slowly for DNA I. However, a very fast exchanging group of \(^3\)H atoms is revealed from an extrapolation of the rate data\(^{36}\). With the above considerations in mind, we propose that reaction path A is highly favored over reaction path B and reagents specific or preferential for unpaired bases will react with premelted sites. These defective sites may act as nucleation sites for further unwinding. This unwinding will be assisted by the free energy of supercoiling.

The question that now arises is: can the above considerations be used to explain the behavior of PM2 DNA I upon u.v. irradiation? A review\(^{37}\) of part of the u.v. literature shows that the major photoproduct is the cis-syn form of the cyclobutane dimer of thymine (TT\(_1\)). This amounts to 60% of the photoproducts. The relative efficiency of formation of various photoproducts shows that TT\(_1\) is introduced into folded denatured DNA 1.3 times more than native DNA. In addition, another photoproduct the TT\(_2\) trans-syn thymine dimer is also favored 10 fold in the denatured form and this is also true of cytosine hydrates. If supercoiling generates
interrupted secondary structure we would anticipate the following: AT rich regions would be preferentially disrupted and this is supported by gene 32 protein and HCHO electron microscopy studies\textsuperscript{38,39}. Consequently, there should be a greater probability of finding intrastrand TT sites in pre-melted regions. This coupled with a higher efficiency of forming TT dimers and cytosine hydrates in folded denatured regions strongly suggests that any hairpin site could be preferentially disrupted. It should be emphasized that we are not proposing exclusive formation of photoproducts at hairpin sites but simply a greater rate due to structural features and composition of these sites. Naturally, photoproducts will form in duplex regions. Consequently, we have a situation that is analogous to the reaction of reagents which are highly preferential for single stranded DNA but in time will react with native DNA due to transient unwinding. Hence, we propose that low doses of u.v. irradiation will first disrupt hairpin regions. Woodworth-Gutai and Lebowitz\textsuperscript{8} have proposed that the localized helix-coil melting transition of a hairpin produces a new point of flexibility in the DNA with a corresponding increase in $S_{20, w}$. If superhelical DNA behaves like a flexible rod it should be possible to decrease the persistence length by the introduction of points of flexibility at modified sites. In addition localized melting of hairpins will not produce a loss of superhelical turns. The introduction of photoproducts at duplex sites would produce a coupled unwinding of duplex and superhelical turns. However, the unwinding appears to be small; Camerman and Camerman\textsuperscript{40} showed that the separation between pyrimidines is decreased and the bases are rotated by some 8° to allow the formation of the cyclobutane ring. Consequently, the introduction of photoproducts at duplex sites does not create any significant changes in $S_{20, w}$ until we reach moderate to high dose levels. This is supported by the behavior of form II in which no change in $S_{20, w}$ is apparent until moderate to high dose levels are reached.

Based upon a recent carbodiimide study\textsuperscript{12} and a review of HCHO data\textsuperscript{6}, we interpret the plateau regions as simply the accumulation of photoproducts into duplex regions in a random manner. The defects created by this process are not sufficient to change the flexibility of the DNA I until significant denaturation occurs, i.e., a cluster or clusters of u.v. damage. In addition modified regions could promote enhanced potential for photoproducts by disrupting adjacent regions. The $S_{20, w}$ and buoyant density show increases between 10,000 and 56,000 ergs/mm\(^2\) in correspondence to changes in form II. Before this occurs there is con-
Nucleic Acids Research

siderable similarity in the transitions produced by chemical modification and u.v. irradiation\textsuperscript{6,12}. Therefore, we believe that the same structural considerations are applicable for an explanation of the initial $S_{20,w}$ increase and plateau. These are reviewed briefly.

In the study\textsuperscript{12} of the carbodiimide modification of PM2 DNA I it was observed that 1\% reactivity produced a sharp increase in $S_{20,*}$ of 5 S units which was followed by a plateau region where additional reactivity did not affect $S_{20,*}$. The initial modification was interpreted as causing a transition of the DNA from a rod-like structure to a wormlike coil. The latter appears insensitive hydrodynamically until further propagation of unwinding and duplex disruption finally convert the wormlike superhelical DNA to an open circle. This produces the characteristic dip for the loss of the majority of superhelical turns. It takes 16\% reactivity to remove the supercoils. This is reasonably close to the $2\sigma$ value for the DNA.

Hence it is conceivable that we could not fully unwind the superhelical turns in PM2 DNA by u.v. irradiation if we increased the dose further. In addition propagation of unwinding may not occur in the same manner or extent with u.v. photoproducts as it can with carbodiimide or other denaturants. Consequently, we might anticipate a departure in behavior as we continue to increase the dose of u.v. irradiation. Clearly, there is insufficient data concerning the accumulation of photoproducts at high doses to pursue the analogy with chemically induced unwinding further.

The similarity in the hydrodynamic behavior of PM2 DNA I produced by chemical modification and u.v. irradiation suggests a general model for the reactivity of superhelical DNA. The initial reaction is a preferential modification of premelted regions producing an increase in flexibility in the DNA. Further reaction unwinds DNA by propagation from modified premelted sites and additional duplex disruption occurs as the secondary structure undergoes transient opening. The latter events are rate limiting and a hydrodynamic balance occurs between the loss of supercoils and defects in the DNA. This is seen as a plateau in sedimentation velocity followed by a dip region when the wormlike DNA is converted to an open circle. Apparently, under our conditions, u.v. photoproducts cannot introduce sufficient coupled unwinding to accomplish the latter.

Naturally, other models can be developed to account for the observations discussed in this paper. Although we cannot exclude a model of superhelical DNA with all bases paired, we believe it is insufficient to account for the effects produced by chemical modification. Alternative
proposals that would account for the variety of observations on the reactivity of superhelical DNA and structural changes would certainly aid in the formulation of new experimental approaches for further study of supercoiled DNA.

ACKNOWLEDGEMENTS

This research was supported by National Science Foundation grant PCM 75-02156A01 (J.L.) and the Medical Research Council of Canada (D.T.D.). J.L., is currently a recipient of Public Health Service Career Development Award CA-00141-05 from the National Cancer Institute. We thank Dr. Tom Tice and Richard Woodward for helpful discussions. It is a great pleasure to thank Bonnie McLay for help in the preparation of this manuscript.

REFERENCES


1255