UV-induced Interstrand Cross-linking of d(GT)$_n$•d(CA)$_n$ Is Facilitated by a Structural Transition*

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Photochemical alterations following ultraviolet irradiation of the alternating copolymer d(GT)$_n$•d(CA)$_n$ were studied. We found that in solution conditions which produced circular dichroism spectra compatible with B-form or A-form DNA, no interstrand cross-linking or photoproduction formation could be demonstrated. Zimmer et al. (Zimmer, C., Tymen, S., Marcck, C., and Guschlbauer, W. (1982) Nucleic Acids Res. 10, 1081–1091) and Vorlickova et al. (Vorlickova, M., Kypr, J., Sotkrova, S., Sponar, J. (1982) Nucleic Acids Res. 10, 1071–1080) have reported a number of solution conditions which produce a structural transition of this polymer characterized by a negative deviation of the circular dichroism spectrum in the region of 280 nm. The nature of this transition has not yet been elucidated. Following ultraviolet irradiation of d(GT)$_n$•d(CA)$_n$ under two conditions which produce this transition (manganese solution or ethanol plus trace salt solution) we found ultraviolet dose-dependent interstrand cross-linking as well as dose-dependent formation of thymine-containing photoproduc.t Interstrand cross-linking is demonstrated by two criteria: 1) increase in polymer size as detected by alkaline agarose gel electrophoresis, and 2) generation of intermediate density material in alkaline cesium sulfate isopycnic gradients. The thymine-containing photoproduc.t was demonstrated by thin layer chromatography of acid hydrolysates of the polymer. The photoproduc.t is at least partially photoreversible. These findings suggest that the geometry of the alternative conformation is such that pyrimidines from different strands are closely approximated, allowing for photodimerization.

Ultraviolet irradiation induces occasional interstrand cross-links in DNA in vivo and in vitro (3–9). Because UV gives rise to a variety of DNA photoproducts, principally various pyrimidine cyclobutane dimers and dipyrimidine adducts (10), it has not been possible to determine which, if any, is responsible for interstrand cross-linking. To approach this problem, we are investigating the formation of interstrand cross-links in alternating purine-pyrimidine copolymers. We reasoned that in these polymers there should be a great reduction in UV photoproduction formation since there are no adjacent pyrimidines in the same strand, thus facilitating the identification of any pyrimidine-containing cross-linking photoproducts. However, in studying the alternating copolymer d(GT)$_n$•d(CA)$_n$, this approach did not appear fruitful as we found no evidence of UV-induced cross-linking or DNA photoproduction formation when the polymer was irradiated in B- or A-form.

Both Zimmer et al. (2) and Vorlickova et al. (1) have reported a transition of d(GT)$_n$•d(CA)$_n$ characterized by a negative maximum of the circular dichroism spectrum in the region of 275–280 nm. They found a variety of solution conditions which produced this spectral alteration, including high concentrations of ethanol, manganese, cesium chloride, cesium fluoride, or ammonium fluoride. These authors speculated that this transition may represent a B-DNA to Z-DNA conversion. Additional investigations of d(GT)$_n$•d(CA)$_n$, under these solution conditions have been carried out in other laboratories. Jenkins et al. using NMR and Raman analysis of the polymer at low and high cesium chloride concentrations found a conformational rearrangement: however, the high salt conditions appeared to produce a variation of B-form DNA. Sutherland and Mugavero (11) examined this transition induced by ethanolic conditions using CD and vacuum ultraviolet CD. They found that the CD alteration at 275–280 nm was not accompanied by the characteristic Z-DNA spectrum in the 180–200 nm region; both the B-form and the alternative form showed a large positive peak at 187 nm, characteristic of right-hand duplex DNA. Given these additional studies it seems likely that the transition first detected by Zimmer et al. (2) and Vorlickova et al. (1) represents a conversion of B-form polymer to a modified B-form.

We UV irradiated d(GT)$_n$•d(CA)$_n$ in this alternative conformation induced by either ethanolic solution or high manganese concentrations. Under these conditions there was a dose-dependent cross-linking of d(GT)$_n$ strands with d(CA)$_n$ strands. We have also detected a thymine containing photoproduc.t in acid hydrolysates of the polymer. These findings suggest that a property of this alternative conformation is that pyrimidines from different strands are closely approximated, allowing for photodimerization.

**EXPERIMENTAL PROCEDURES**

**Materials**—Polymers were purchased from P-L Biochemicals. We characterized d(GT)$_n$, d(CA)$_n$, by analytic density centrifugation and were provided by Grant ES 3446 from the National Institutes of Health. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or necessarily reflecting the views of the Uniformed Services University of the Health Sciences or Department of Defense. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 The solution conditions used were 90% ethanol, 1.5 mM CsCl, 0.02 mM CaCl$_2$ (J. Sutherland, personal communication); these conditions are identical to those used by Zimmer et al. (2), and are used in the current study.
gel electrophoresis under both alkaline and neutral conditions. Neutral CsSO₄ density sedimentation yielded a single peak (Aₔ₅₀₀) at a density of 1.423, the expected value for native d(GT)ₙ, d(CA)ₙ, at neutral pH (Ref. 12). The standard was d(AT)ₙ, ρ = 1.425 (12). Alkaline CsSO₄ equilibrium sedimentation yielded two peaks (Aₔ₅₀₀), one with a density of 1.425 (d(GT)ₙ, d(CA)ₙ) and the other at 1.416 (d(AT)ₙ) (12). The standard was d(AT)ₙ, ρ = 1.416 (12). Neutral gel analysis (0.3% agarose) revealed that most of the polymer did not migrate out of the sample loading well after lengthy electrophoresis; by extrapolation from λ-HindIII markers, we conservatively estimate the size of the double-stranded polymer to exceed 100,000 base pairs. Alkaline gel electrophoresis showed fragments ranging from 200 to 4000 nucleotides with a maximum staining intensity at about 1100 bases. The polymer was visualized in the alkaline gel, following a neutralization step, with ethidium bromide (which binds to double-stranded DNA) or acridine orange (which binds to both single- and double-stranded DNA); results were identical. From this analysis, we concluded that the polymer was double-stranded d(GT)ₙ, d(CA)ₙ, with overlapping d(GT), strands and d(CA), strands forming long concatemers with intermittent single-stranded nicks or gaps. The polymer was stored at 1 mg/ml in water or 0.1 M NaCl. The order of addition of the components of the ethanolic solution used in this study was as follows: to an aliquot of polymer solution (1 mg/ml), first CaCl₂ (1 mM stock), then CsCl (100 mM stock), then water, and finally 95% ethanol was added. The reason for this protocol is that we had earlier found that addition of 60% ethanol (no salts) to the polymer resulted in a CD spectrum typical of denaturation (13). Subsequent addition of the salts did not alter this CD spectrum. 

UV-induced Cross-linking—Ultraviolet irradiation was performed at 23°C under a General Electric germicidal bulb with a maximum output at 254 nm. The dose rate was 10 J/m² s as determined by a UVX Digital Radiometer (Ultra-Violet products Inc.).

Circular Dichroism—CD spectra were measured on a Jasco J-500A spectropolarimeter at 23 °C. Polymer concentration was 10 μg/ml in all samples.

Alkaline Agarose Gel Electrophoresis—Following UV irradiation the polymer was ethanol precipitated. It was necessary to ethanol precipitate several times if the polymer had been in MnCl₂ solution, since any residual Mn²⁺ precipitated in alkali. Following precipitation the polymer was ethanol precipitated. It was necessary to ethanol precipitate the polymer at anytime prior to ultraviolet irradiation to decreased photoproduction yield. Following ultraviolet irradiation, yeast tRNA was added to a final concentration of 1 mg/ml and the sample ethanol precipitated, resuspended in 90% formic acid, and hydrolyzed in combustion vials at 180 °C for 60 min. The formic acid was then removed by vacuum centrifuge and the dried hydrolysate dissolved in a small volume of water and spotted on the TLC plates (silica gel G-60; Merck). The running solvent was 80% ethyl acetate, 20% 1-propanol (v/v) which was water saturated. Plates were developed to 12 or 17.5 cm and processed as described by Reynolds et al. (16). Fractions were 0.5 cm each.

RESULTS

Fig. 1A shows the CD spectrum of d(GT)ₙ, d(CA)ₙ, under conventional solution conditions (10 mM Tris-Cl (pH 7.5), 2 mM EDTA, or 0.1 M NaCl). We have employed two different solution conditions known to produce negative displacement of the CD spectrum in 275 nm region (Fig. 1, B and C) (1, 2). Fig. 1B shows the CD spectral alterations which occur with increasing MnCl₂ concentrations; Fig. 1C shows this alteration as a result of increasing ethanol concentrations in the presence of trace quantities of cesium and calcium salts. Finally, Fig. 1D shows inhibition of the ethanol-induced CD inversion when CaCl₂ or CsCl was omitted or when 0.1 M NaCl was added. This latter alteration generates a CD spectrum typical of A-form DNA (15). These various solution conditions were employed in the following experiments designed to assess UV-induced interstrand cross-links.

Alkaline agarose gel electrophoresis (Fig. 2) separates single-stranded fragments on the basis of size; the larger the fragment, the slower it migrates (17). If a double-stranded fragment becomes cross-linked, the strands will not separate.

FIG. 1. Circular dichroism studies of the effects of various solution conditions on d(GT)ₙ, d(CA)ₙ. The concentrations of monovalent and divalent salts were as indicated. All samples in Panel C contained 1.5 mM CaCl₂, 0.02 mM MnCl₂ in addition to ethanol. In Panel D, NaCl was added to, or CaCl₂ or CsCl omitted from the standard ethanolic solution conditions indicated in the panel. The CD curve obtained upon omission of CsCl was essentially the same as the CD spectrum shown for omission of CaCl₂ in Panel D.
UV-induced Cross-linking of d(GT)$_n$.d(CA)$_n$  

Polymer was UV-irradiated in the solutions indicated below, ethanol precipitated, and electrophoresis performed as described under "Experimental Procedures." The molecular weight marker DNAs (λ-Hind III and φX174-Hae III) were not UV-irradiated. Left Panel, polymer in 2 M MnCl$_2$ was UV-irradiated to the doses indicated. Center Panel, polymer in 60% ethanol, 1.5 mM CsCl, 0.02 mM CaCl$_2$, was UV-irradiated to the doses indicated. Right Panel, polymer in all lanes was UV-irradiated to 2500 J/m$^2$ with the exception of the lane labeled No UV, which serves as a negative control. Complete refers to 60% ethanol, 1.5 mM CsCl, 0.02 mM CaCl$_2$; -CaCl$_2$ and -CsCl indicate that these salts were omitted during UV irradiation; +NaCl indicates that 0.1 M NaCl was added to the complete solution prior to UV irradiation; NaCl Only and TE Only indicates that the polymer was UV-irradiated in 0.1 M NaCl or 10 mM Tris-Cl, 1 mM Na$_2$EDTA (pH 7.5).

Completely upon denaturation and consequently will migrate at the combined molecular weight. There was a dose-dependent increase in molecular weight of the polymer when irradiated in 2 M MnCl$_2$ (Fig. 2, left). Examination of the gel shows cross-linked DNA fragments in excess of the 23-kilobase λHind III marker; indeed some of the polymer was so large it did not move out of the sample loading well. This enhancement of molecular weight may be accounted for as follows: the native polymer is of high molecular weight (greater than 100 kilobase pairs; see "Experimental Procedures"); however, it contains frequent nicks or gaps on both strands yielding fragments averaging about 1100 bases upon alkaline gel electrophoresis (Fig. 2, left, track 2). Hence, as the number of covalent cross-links increases the denatured polymer approaches native size. It was possible that cross-linking observed in Fig. 2 (left) was not related to conformational alterations in the polymer (e.g., perhaps Mn$^{2+}$ acts as a catalyst). We therefore assessed UV-cross-linking when CD inversion was irradiated by another condition: 60% ethanol, 1.5 mM CsCl, 0.02 mM CaCl$_2$. Irradiation in the latter condition appeared to favor the formation of a discrete band at about 2000 bases, probably representing the cross-linking of a single fragment of d(GT)$_n$ to a single fragment of d(CA)$_n$. UV-irradiation of d(GT)$_n$.d(CA)$_n$ in B-DNA forming conditions showed no evidence of cross-linking (Fig. 2, right). We considered that the degree of cross-linking may be underestimated in alkaline gel electrophoresis due to alkaline labile sites in the polymer. However, we found that similar experiments utilizing denaturing formaldehyde gels yielded similar results (not shown).

Additional experiments were performed in an effort to correlate CD alteration with susceptibility to UV cross-linking. The omission of MnCl$_2$ or CsCl from the ethanolic solution greatly diminished the ethanol-induced CD spectrum inversion (Fig. 1D). UV irradiation of polymer in the solutions lacking CaCl$_2$ did not result in detectable cross-linking; however, when CsCl was omitted there was detectable, although diminished cross-linking. Finally, when 0.1 M NaCl was added to polymer in the complete ethanolic solution there was a reversal of the CD spectrum with a large positive Δε (270 nm) (Fig. 1D). When we UV-irradiated the polymer in this environment, we did not detect any cross-linking (Fig. 2, right).

The degree of CD inversion could be controlled by the concentration of ethanol added to the polymer and trace salt solution (see Fig. 1C). The polymer, exhibiting various degrees of CD inversion was irradiated with 2500 J/m$^2$ and subjected to alkaline gel electrophoresis (Fig. 3, left). Below about 45% ethanol there is little or no cross-linking, correlating closely with the lack of CD inversion. This suggests that the effect of ethanol is not that of a reactant or catalyst, otherwise one might expect that the reduction in ethanol concentration from 60 to 40% would have produced only a modest reduction in UV cross-linking. Fig. 3 (right) shows the results of irradiation at the relatively low dose of 500 J/m$^2$ in the presence of varying concentrations of MnCl$_2$. The degree of cross-linking reached maximum at 1.0 M MnCl$_2$ and did not increase at higher concentrations of MnCl$_2$ (including 2 M MnCl$_2$, not shown). Note that the corresponding CD achieves maximal inversion by 1.0 M (Fig. 1B). Is the reduced cross-linking seen at low manganese concentrations overcome at higher UV doses? Fig. 4 shows the dose dependence of cross-linking in 0.5 MnCl$_2$ solution. Extensive cross-linking occurs at high doses; however, the maximal degree of cross-linking achieved (2000 and 5000 J/m$^2$ lanes) is less than that seen in 2 M MnCl$_2$ (Fig. 2, left).

Since d(GT)$_n$ and d(CA)$_n$ have different buoyant densities...
UV-induced Cross-linking of d(GT)_n·d(CA)_n

Fig. 4. UV dose dependence of cross-linking of d(GT)_n·d(CA)_n. Alkaline agarose gel electrophoresis of d(GT)_n·d(CA)_n which was UV irradiated in 0.5 M MnCl_2 to the doses shown.

Fig. 5. Alkaline Cs_2SO_4 density gradient centrifugation. Sedimentation was performed as described under "Experimental Procedures." Panel A, UV irradiation of d(GT)_n·d(CA)_n in 60% ethanol, 1.5 mM CaCl_2; mock irradiated (---); 500 J/m^2 (----). Panel B, UV irradiation in 2 M MnCl_2; mock irradiated (---); 500 J/m^2 (----); 1000 J/m^2 (-----). Panel C, UV irradiation in 0.1 M NaCl; mock irradiated (---); 500 J/m^2 (-----). Following irradiation or mock irradiation samples were ethanol precipitated 1 to 2 times and then resuspended in alkaline Cs_2SO_4 solution for centrifugation. Because of tube to tube variation in polymer content the optical scans are normalized in each panel such that total area under the curves are equal.

UV of the polymer in B-form did not show any evidence of cross-link formation (Fig. 5C). Fig. 6 shows a representative thin layer chromatography profile of polymer which had been [3H]-labeled in thymine by nick translation, then irradiated, and formic acid hydrolyzed. The hydrolysis degrades DNA to its constituent bases and other by-products (17). The large peak is [3H]thymine; the small peak is evident when irradiation occurred in the setting of inverted CD spectrum. This peak co-migrated with thymine-containing dimer marker obtained from Escherichia coli DNA (irradiated under conventional solution conditions). This TLC system has a low resolution for different dimeric photoproducts, and the presence of a single photoproduct peak does not imply that the novel photoproduct is homogeneous. Fig. 7 shows a typical experiment measuring photoproduct as a function of UV dose using the TLC system. The maximal level of photoproduct varied from experiment to experiment, within the range of 0.8-1.8%. Polymer irradiated in MnCl_2 did not consistently show greater photoproduct formation than polymer irradiated in the ethanol solution. By 5000 J/m^2 the formation of photoproduct had consistently achieved maximal values; irradiation up to 25,000 J/m^2 did not result in increased photoproduct formation (not shown).

The finding of a plateau value for photoproduct formation, well below the expected available number of potential dimer forming sites, suggests the possibility that UV not only induces photoproduct, but also induces photoreversal of the photoproduct to monomers. This is a typical feature of cyclobutane dimer formation in wild-type DNA (19). We tested this hypothesis by eluting the photoproduct containing TLC fractions in water and then irradiating the photoproduct in alkaline Cs_2SO_4 (12), interstrand cross-linking should result in intermediate density material. Fig. 5 shows the results of experiments similar to previous ones, but utilizing alkaline Cs_2SO_4 gradients for analysis of cross-links. d(CA)_n bands near the top of the gradient (to the left as shown) and d(GT)_n bands near the bottom (right). When we UV irradiated the polymer in 60% ethanol, 1.5 mM CaCl_2, 0.02 mM CaCl_2 (Fig. 5A), or in 2 M MnCl_2 (Fig. 5B), we saw an intermediate density peak that was dose dependent. The intermediate density peak, indicating the presence of cross-linked polymer, showed slight run to run variation in density, falling within the range of ρ = 1.460–1.468. The cross-linked polymer in alkaline gradients banded at a greater density than the native polymer in neutral gradients (ρ = 1.425). We attribute this difference to the known enhancement of buoyant density that occurs upon alkaline titration of G and T residues (12, 18).
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Fig. 7. Dose dependence of photoproduct. Fraction of counts in dimer peak as assessed by the TLC system shown in Fig. 6. Samples were UV irradiated in 2 M MnCl₂ (O); 60% ethanol, 1.5 mM CsCl, 0.02 mM CaCl₂ (●); or 0.1 M NaCl (▲).

Fig. 8. Photoreversal of photoproduct. Photoproduct was generated by UV irradiation of d(GT)ₙ·d(CA)ₙ in 2 M MnCl₂ (O); or 60% ethanol, 1.5 mM CsCl, 0.02 mM CaCl₂ (●). The photoproduct was eluted in water from dimer peak fractions of the TLC plate. The water solution of photoproduct was then irradiated for doses shown, the sample dried down in the vacuum centrifuge and rechromatographed using the same TLC system.

solution for doses up to 24,000 J/m². This irradiation condition strongly favors the photomonomerization reaction, since two bases, once cleaved by UV irradiation rapidly diffuse away from each other in solution. We rechromatographed the photoreversal material. Fig. 8 shows an experiment assessing photoreversal of photoproduct originally generated in polymer under MnCl₂ or ethanol conditions. As with photoproduct formation there was experimental variation, but photoreversal was always greater than 50%. In all experiments photonomerization was accompanied by quantitative regeneration of the thymine peak (not shown).

We investigated the possibility of cross-link formation in the self-complementary Z-DNA forming polymers d(GC)ₙ and d(GmeC)ₙ. Fig. 9 (left and middle) shows alkaline agarose gel electrophoresis of d(GC)ₙ after UV irradiation in the following solution conditions: 0.1 M NaCl; 4 M NaCl; 1.5 mM CsCl, 0.02 mM CaCl₂; and 2 M MnCl₂. CD spectra of d(GC)ₙ was typical of Z-DNA when in 4 M NaCl or 2 M MnCl₂ (e.g. see Ref. 20). Several different solutions of ethanol with various salts have been reported to induce Z-conformation of d(GC)ₙ (12, 20); however, we found that the ethanolic solution employed in these studies (0.1 M NaCl, 1.5 mM CsCl, 0.02 mM CaCl₂) resulted in a CD spectrum of d(GC)ₙ typical of B-DNA and identical with the CD spectrum found in 0.1 M NaCl. In all solution conditions there was no increase in molecular weight of the d(GC)ₙ polymer following irradiation of 1500 or 5000 J/m². It is possible that lack of enhanced molecular weight might be due to hairpin conformation of all the d(GC)ₙ strands; however, this possibility was excluded by a comparison of alkaline (Fig. 9, left and middle) and neutral (Fig. 9, right) agarose gel electrophoresis of unirradiated polymer. If the polymer is hairpinned there should be an increase in size (relative to markers) upon alkaline denaturation (e.g. a 500-base pair double-stranded fragment would denature to become a 1000 base single-stranded fragment). Instead of an increase in size we found a large decrease in the size of the polymer upon denaturation, suggesting that the d(GC)ₙ polymer was a concatemer under neutral conditions, similar to the d(GT)ₙ·d(CA)ₙ polymer (see “Experimental Procedures”). This experiment suggests that d(GC)ₙ in Z-conformation (4 M NaCl, 2 M MnCl₂) or in our standard ethanolic solution conditions is not subject to cross-linking by ultraviolet irradiation.

In addition, we assessed cross-linking of d(GmeC)ₙ (not shown) in experiments identical to those in Fig. 9, except that 1.2 M NaCl was substituted for 4 M NaCl. In no case is there
any indication of increased molecular weight of polymer upon ultraviolet irradiation. As with d(GC)n, comparison of alkaline and neutral agarose gel electrophoresis did not show evidence of hairpin conformation of d(GmeC)n.

**DISCUSSION**

We have investigated the UV photochemistry of d(GT)n.d(CA)n under a variety of solution conditions. In B-form the polymer is not subject to UV cross-linking or production of photoproduct. However, in ethanolic or manganese solutions that produce a negative peak in the 275 nm region of the CD spectrum, UV produces both interstrand cross-linking and thymine-containing photoproduct detectable in acid hydrolysates of the polymer. Cross-linking is demonstrated by two different criteria: 1) an increase in polymer size as detected in alkaline agarose gels, and 2) the production of intermediate density material in alkaline cesium sulfate isopycnic gradients. The degree of cross-linking, assessed by alkaline gel electrophoresis, correlates with the degree of CD peak inversion at 275 nm in ethanol and manganese solutions. The CD alterations in ethanolic solution suggest a cooperative transition between 40 and 60% ethanol (1); this is accompanied by susceptibility to UV cross-linking (Fig. 3, left). Alterations in the CD spectrum due to the addition of NaCl or the omission of CaCl₂ or CaCl₃ from the ethanolic solution are also accompanied by decreased susceptibility to cross-linking (Fig. 2, right). It is noteworthy that although omission of either CaCl₂ or CaCl₃ from the ethanolic solution produces a similar diminution of negative peak height at 275 nm, the omission of CaCl₂ reproducibly induces a greater inhibition of cross-linking than omission of CaCl₃. The concentration of MnCl₂ in the polymer solution renders the polymer susceptible to UV cross-linking in a fashion that also correlates with CD spectral alterations (Figs. 3, right, and 4). Finally, when the polymer is UV irradiated in B-form no photoproduct is detectable in acid hydrolysates; however, when irradiated in the alternative conformation there is a dose-dependent formation of thymine-containing photoproduct (Fig. 7). A substantial fraction of the photoproduct is photoreversible (Fig. 8).

The correspondence between UV cross-linking and the production of thymine-containing photoproduct in the alternative solution conditions suggests that the photoproduct may be the cross-link. However, the alternative DNA structure may permit formation of multiple photoproducts, one or more of which may be cross-links. In preliminary studies (data not shown) we have chromatographically resolved the photoproduct, as found in trifluoroacetic acid hydrolysates of the polymer, into three peaks. One of these peaks, comprising about 15% of the overall photoproduct, has been tentatively identified as thymine-cytosine cyclobutane dimer. This assignment is based on the following: 1) migration of the photoproduct on two paper chromatographic systems capable of resolving the thymine-cytosine cyclobutane dimer from other photoproducts; and 2) detection of this photoproduct in polymer which has been labeled by nick translation either in [methyl-³H]thymine or [U-¹⁴C]cytosine. This photoproduct should be a covalent cross-link between a d(GT)n strand and a d(CA)n strand. One may speculate that its isomeric conformation may be other than the usual cis-syn type (10). The other two peaks have not yet been assigned.

A variety of dipyrimidine photoproducts other than cross-links of d(GT)n to d(CA)n are possible. Although the isopycnic gradients (Fig. 5) indicate that substantial cross-linking of d(GT)n to d(CA)n occurs, cross-linking of polymer strands to like polymer strands may also occur. Even more interesting is the possibility of dimerization of nonadjacent pyrimidines within the same strand. This phenomenon was recently discovered by Brown et al. (21) who showed that cytosines, separated by an intervening thymine may be dimerized. This finding supports a model these authors previously proposed for a self-complementary double-stranded conformation of the polymer d(CT)n in which there is a core of stacked protonated C-C' base pairs with thymidyl residues being looped out into the solvent. Obviously, the presence of a similar photoproduct in the current studies would have implications for the novel d(GT)n.d(CA)n structure occurring in the alternative solution conditions.

We have considered the hypothesis that the formation of UV-induced interstrand cross-links in d(GT)n.d(CA)n may result in stabilization of the alternative conformation, the cross-links serving to "lock" this conformation into place. As a test of this hypothesis we UV irradiated d(GT)n.d(CA)n up to 5000 J/m² in the ethanolic or manganese solution conditions, and then dialyzed the polymer into 0.1 M NaCl. CD spectroscopy was then performed. The CD spectra did not suggest retention of the alternative conformation, and in all cases approached similar to B conformation spectra (data not shown). We speculate that the frequency of cross-links within the polymer is insufficient to restrict the transition to B-form DNA. If one assumes that all photoproduct detected in the thin layer chromatographic assay is indeed cross-link (see Fig. 7), then one would expect only about one cross-link/100 base pairs at saturating doses of UV.

The current findings suggest that the geometry of the alternative d(GT)n.d(CA)n structure is such that pyrimidines from the d(GT)n strand overlap pyrimidines from the d(CA)n strand. One structure that allows for the approximation of pyrimidine residues from different strands is Z-DNA, in which there is partial base stacking of pyrimidines from opposite strands (22). As Zimmer et al. (2) and Vorlickova et al. (1) point out, the negative CD peak at 275 nm is reminiscent of the CD spectrum of Z-DNA. However, the lack of a large positive deflection in the 260 nm region suggests that the structure is other than Z-DNA (20). Also, the studies discussed in the Introduction suggest that the transition is to a variant of B-DNA. In the current work we have investigated the possibility of cross-link formation in the self-complementary Z-DNA forming polymers d(GC)n and d(GmeC)n (Fig. 9). In a variety of solution conditions producing either B-DNA or Z-DNA conformations, we UV irradiated these polymers up to doses of 5000 J/m². In no case did we see any increase in molecular weight upon alkaline gel electrophoresis. It occurred to us that this might be due to a hairpin conformation of all the d(GC)n and d(GmeC)n strands; however, this latter possibility was excluded by a comparison of neutral and alkaline agarose gel electrophoresis, which showed a large decrease in the size of both polymers upon alkaline denaturation. These experiments suggest that Z-DNA conformation does not predispose to UV cross-linking. Consistent with this is work by Doetsch 2 who found that Z-conformation d(GC)n did not predispose to formation of UV-induced pyrimidine-pyrimidone(6-4) products using the hot alkali assay (24).

A possible tertiary structural change of d(GT)n.d(CA)n is aggregation, perhaps predisposing towards pyrimidine dimer formation between strands of different helices. However, both Zimmer et al. (2) and Vorlickova et al. (1) argue against aggregation of d(GT)n.d(CA)n under these conditions based on light scattering data. In addition we have noted in the current work that the ethanol conditions do not lead to

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8 P. Doetsch, personal communication.
sedimentability of the polymer. At our usual working concent-
trations of polymer, 10 μg/ml, manganese chloride solution
conditions also did not yield detectable precipitable polymer.
Higher concentrations of polymer (25–50 μg/ml) in man-
ganese solutions of concentrations ≥1 M resulted in both
turbidity and precipitable material; however, the UV dose
dependence of both interstrand cross-linking and photopro-
duct formation was independent of polymer concentration
(data not shown). In addition, at 0.5 M MnCl₂, in which we
never detected precipitable material, the polymer was easily
cross-linked (Fig. 4). These findings argue against, but do not
exclude, a role for aggregation in cross-link and photoproduct
formation.

Another structure which would facilitate cross-linking of
pyrimidines from opposite strands is the intercalation of
bases, rather than Watson-Crick base pairing. Viswamitra
and Pandit (25) have proposed that such a structure is ster-
eochemically reasonable and may occur in antiparallel DNA
in stretches of base mismatch. It is possible that the solution
conditions employed in the current study permit small regions
of intercalation without a large rearrangement of the B-DNA
conformation. This hypothesis is consistent with the current
structural findings about d(GT)ₐ–d(CA)ₐ, in alternative con-
formation (see Introduction), and may also be consistent with
the findings of Glisin and Doty (7) that focal denaturation
(but not complete denaturation) of wild-type DNA enhances
UV cross-link formation. However, these latter findings have
been difficult to reproduce. Resolution of the structures of
the two unidentified photoproducts mentioned above may
provide further insight concerning the structural alterations
of d(GT)ₐ–d(CA)ₐ.

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