# Suppression of Cyclobutane and <6–4> Dipyrimidines Formation in Triple-Stranded H-DNA

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ABSTRACT: We have determined the effect of H-DNA formation on the distributions of two ultraviolet (UV) light induced photoproducts—cyclobutane dipyrimidines and <6–4> dipyrimidines. A region of DNA containing the sequence $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ was treated under conditions that specifically yield the triple-stranded H-y3 or H-y5 DNA structure and then irradiated with UV. The positions of cyclobutane dipyrimidines and <6–4> dipyrimidines were determined by T4 endonuclease V cleavage and by hot piperidine cleavage, respectively. Formation of H-DNA structures greatly decreased the photoproduct yield in the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ region but not elsewhere in the DNA. Suppression of photoproduct formation is greater in half of the repeat, reflecting whether the DNA is in the H-y3 or H-y5 conformation. Within the repeat, the suppression was less in the middle and toward the ends. Models for the suppression of photoproduct formation in H-DNA and the possible utility of our findings are discussed.

Under negative torsional stress or low pH, palindromic polypyrimidine–polypurine sequences such as repeating $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequences can adopt a single-stranded and triple-stranded DNA conformation, termed H-DNA. In this structure, half of the Watson–Crick duplex is disrupted, allowing a released strand to fold back into the major groove of the helical half of the repeat. This third strand, designated the donated strand, associates with purines in the helical half of the repeat (the acceptor region) by Hoogsteen base pairs giving rise to dT·dA·dT and $dC\cdot dG\cdot dC^+$ base triplets as in the H-y DNA conformers (Figure 1) [for review see Htun and Dahlberg (1989, 1990) and Lyamichev et al. (1986)].

Despite a large body of evidence supporting this model of H-DNA (Wells et al., 1988), its occurrence in vivo remains to be determined. "Single-stranded-selective" reagents react in vivo within the vicinity of sequences capable of adopting H-DNA structures (Weintraub, 1983; Kohwi-Shigematsu et al., 1983; Hoffman-Liebermann et al., 1986), and antibodies prepared against triple-stranded DNAs stain eukaryotic nuclei (Lee et al., 1987; Burkholder et al., 1988). Recently, eukaryotic nuclei have been reported to contain proteins that bind to triple-stranded DNA (Kiyama & Camerini-Otero, 1990). As such, H-DNA could be widespread in the genomes of eukaryotes (Htun & Dahlberg, 1989; Wells et al., 1988; Birnboim, 1978; Cseko et al., 1979; Manor et al., 1988).

In our attempt to develop a penetrating but not disruptive probe to detect H-DNA conformation in metabolically active cells, we have explored the usefulness of ultraviolet (UV) light to discriminate between DNAs in double-stranded and triple-stranded conformations. Pyrimidines in the H-DNA conformation should have different photoreactivity profiles than they do when in the double-stranded DNA conformation because UV photoproduct yield is sensitive to changes in the structure and flexibility of DNA, as well as the local environment about the DNA or its bases (Patrick & Rahn, 1976; Friedberg, 1985; Becker & Wang, 1984, 1989a,b; Brown et al., 1985). In this report, we analyzed the formation and distribution of the two major UV light photoproducts, cyclobutane dipyrimidines (Py<>Py) and <6–4> dipyrimidines (Py<6–4>Py), at a $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence. Recently, Lyamichev et al. (1990) demonstrated that formation of intermolecular triplexes between a $(dT\text{-}dC)_n\cdot(dA\text{-}dG)_n$ duplex and a single-stranded $(dT\text{-}dC)_n$ oligo resulted in suppression of formation of Py<6–4>Py in the duplex strand. That work left unanswered questions about distribution of protected regions when a polypyrimidine strand folds back upon itself in an intramolecular structure, as it must do in H-DNA.

We show here that production of both Py<>Py and Py<6–4>Py is considerably reduced when H-DNA is formed. This protection extends throughout the polypyrimidine repeat, regardless of which half is used as the donor or acceptor region; however, greater protection is observed for the donated strand. In contrast, protection is least in areas near the ends of the triplex. Thus, our detailed examination reveals the utility of UV light irradiation in discriminating between pyrimidines in B-DNA and H-DNA and between pyrimidines in the donor and acceptor regions.

## Materials and Methods

*Plasmid.* The plasmid pTC18tt2A DNA (Htun, 1989) consists of pGEM-2 vector DNA (Promega Biotec) fused to a 180-bp *Sac*I–*Bam*HI restriction fragment from a region 1.8 kb downstream of the human U1 RNA gene HU1-1, as previously described (Htun, 1989; Htun & Dahlberg, 1988). This plasmid contains a $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence, abbreviated as $(TC\cdot AG)_{18}$.

*H-DNA Formation.* The formation of H-DNA is affected by a number of factors, the most important being pH and level of negative supercoiling (Htun & Dahlberg, 1990; Htun, 1989). At neutral pH, a high level of negative supercoiling density ($\sigma < -0.1$) is required for formation of H-DNA at the $(TC\cdot AG)_{18}$ sequence. Under this condition, the 3′ half of the

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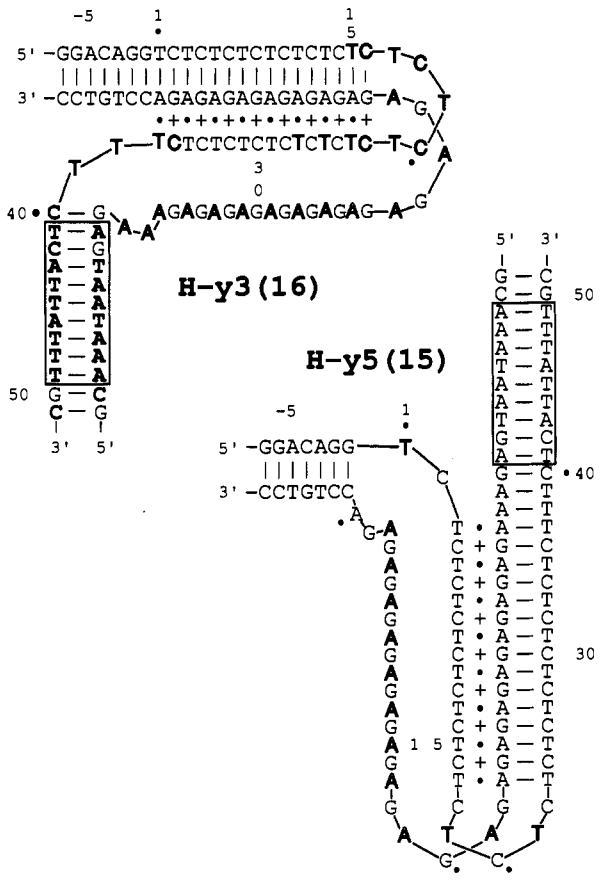


FIGURE 1: H-y conformers of H-DNA formed at the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence in negatively supercoiled and linear DNAs. Pyrimidines from the 3′ or 5′ half of the polypyrimidine tract make Hoogsteen base pairs with purines in Watson–Crick-base-paired duplex to give rise to H-y3(16) and H-y5(15) structures, respectively; the number within parentheses immediately following the designation indicate the number of base triplets within each conformer. Watson–Crick base pairs (in the flanking and acceptor helices) are shown as lines, and Hoogsteen base pairs between the acceptor purines and uncharged [T] or protonated [$C^+$] pyrimidines are shown by • and +, respectively. Residue numers and the box denoting a flanking region sequence are shown. Outlined letters indicate nucleotides that are reactive to diethyl pyrocarbonate (A), osmium tetroxide (T), or methoxyamine (C) when the DNAs are in the H-y DNA conformations. Additional chemical reactivity, indicated by bold letters, is observed within the boxed region of the H-y3 (16) DNA when the level of negative supercoiling increases from native ($\sigma \approx -0.05$) to highly supercoiled levels ($\sigma < -0.1$), resulting in further unwinding of the boxed region. [Reprinted with permission from Htun and Dahlberg (1989). Copyright 1989 American Association for the Advancement of Science.]

$(TC)_{18}$ sequence serves as the donated strand resulting in the H-y3 DNA structure (Figure 1) (Htun & Dahlberg, 1988, 1989). At pH 5, the bacterial level of the negative supercoiling density ($\sigma \approx -0.05$) suffices for formation of H-DNA resulting mostly in the H-y3 DNA structure (Htun & Dahlberg, 1988, 1989). At pH 3.85 and 100 mM [$Na^+$], a $(TC\cdot AG)_{18}$ sequence spontaneously rearranges to the H-DNA conformation in linear DNA (Htun & Dahlberg, 1988, 1990; Htun, 1989). In this conformation, the donated strand is derived from the 5′ half of the $(TC)_{18}$ sequence, resulting in the H-y5 DNA structure (Figure 1).

Highly supercoiled DNA was produced by treating the native supercoiled DNA with topoisomerase I (35 units) in the presence of 25 μg/mL ethidium bromide and bovine serum albumin (30 μg/mL) in 100 mM sodium chloride/50 mM Tris, pH 7.5/1 mM dithiothreitol for 16 h at room temperature. The reaction was terminated, and proteins and ethidium bromide were removed by phenol/chloroform/isoamyl alcohol (25:24:1) extractions followed by diethyl ether extractions. The superhelicity of the DNAs was checked by electrophoresis in 2% agarose gels containing 32 μg/mL chloroquine in TAE buffer (40 mM Tris–acetate, pH 8.0/1 mM EDTA). An equal volume of 200 mM HEPES buffer (pH 7) was added to the DNA solution, and the mixture was incubated at room temperature for 45 min before UV irradiation.

H-y5 (Figure 1) DNA was produced by treating *Eco*RI-linearized DNA with 100 mM trisodium citrate, pH 3.85, at 37 °C for 45 min. Alternatively, to produce H-y conformers containing predominantly the H-y3 conformers but also a significant fraction of H-y5 conformers, supercoiled pTC18tt2A DNA at native level of supercoiling was treated with 100 mM sodium acetate, at the indicated pH, for 45 min at room temperature. After the treatments, the DNA solutions were immediately irradiated with UV light.

*UV Irradiation.* A 200-μL aliquot of DNA with the different conformers was irradiated with 0, 500, and 1000 J $m^{-2}$ of UV light with a fluence rate of 10 J $m^{-2}$ $s^{-1}$ at room temperature (Westinghouse germicidal lamp, B15T8, major emissions ~254 nm). The samples were on a rotating platform during irradiation to ensure that the DNAs were uniformly irradiated.

*$^{32}P$ Labeling.* After UV irradiation, supercoiled DNAs were linearized at a unique site by digestion with *Eco*RI and the linearized DNAs were labeled at their 5′ ends with radioactive $^{32}P$ by use of polynucleotide kinase and [$\gamma$-$^{32}P$]ATP (Maxam & Gilbert, 1980). The DNAs were then digested with *Bam*HI, and the smaller of the two resulting fragments was purified. This 190-bp fragment contains a single $^{32}P$ label at the 5′ end of the $TC_{18}$-containing strand.

*T4 Endonuclease (Endo) V and Piperidine Treatments.* Analysis of the photoproducts in the $(dT\text{-}dC)_{18}$-containing strand was essentially as described by Brash (1988). To determine the sites of Py<>Py formation, the 190-bp labeled DNA was treated with T4 endo V, which cleaves the glycosidic bond of the 5′ cyclobutane dimer and the phosphodiester bond 3′ to the apyrimidinic site, leaving a free 3′ hydroxyl end (Brash, 1988). Typically, T4 endo V (0.88–4.4 μg) prepared by using the method described by Friedberg et al. (1980) was incubated with 40 ng of labeled DNA in 100 mM NaCl/1 mM EDTA/5 mM Tris, pH 7.7 (final volume 100 μL), at 37 °C for 60 min. Reaction was stopped by phenol extraction followed by diethyl ether extractions, and the DNA was subsequently purified by ethanol precipitation.

To determine the sites of Py<6–4>Py formation, the labeled DNA was treated with hot alkali to break the labile glycosidic bond of the 3′ Py photoproduct, subsequently leading to β-elimination at the apyrimidinic site (Brash, 1988). Typically, labeled DNA (40 ng) in 0.9 M piperidine (100 μL) was incubated at 90 °C for 30 min. The DNAs were purified by ethanol precipitation following the piperidine treatment.

*Sequencing Gel Electrophoresis.* The T4 endo V and piperidine cleavage sites were determined on sequencing gels. Prior to electrophoresis, the DNA samples were dissolved in 88% formamide/10 mM NaOH with xylene cyanol and bromophenol blue as tracking dyes and heated to 90 °C for 3 min followed by quenching on ice. The DNAs were then electrophoresed in 8% (w/v) polyacrylamide with 50% urea in 50 mM Tris, pH 7.9/50 mM borate/5 mM EDTA (1/2 TBE buffer) in parallel with Maxam and Gilbert G, G+A, T, T+C reactions (Maxam & Gilbert, 1980). The intensity of each band was scanned by a soft laser densitometer (Zeineh Co.). The nucleotides in the 5′-labeled 190-bp fragment are num-

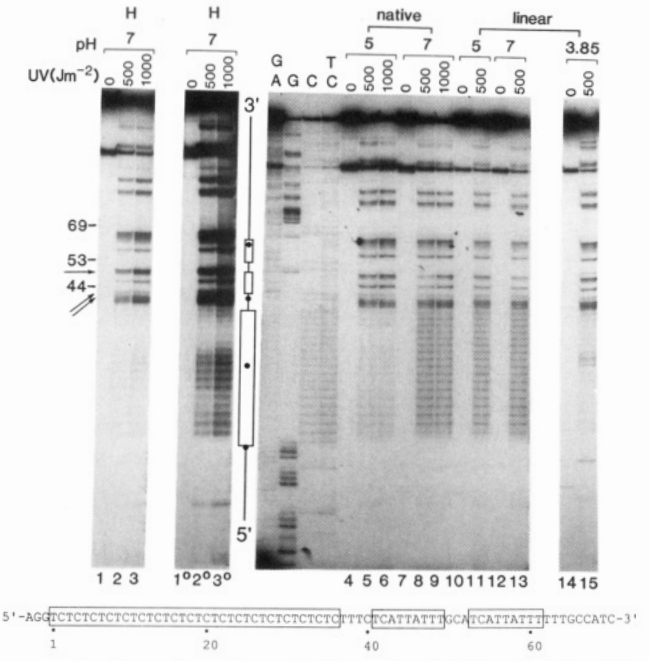


FIGURE 2: Detection of Py<>Py formation by T4 endo V in $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ region and its neighboring sequences in H-y3 conformation induced by high negative superhelicity (lanes 1–3), H-y conformation induced by pH 5 treatment for native supercoiled plasmid DNA (lanes 4–6), and H-y5 DNA conformation induced by pH 3.85 treatment of *Eco*RI-linearized plasmid DNA (lanes 14–15). Lanes 7–9 and lanes 12–13 are native supercoiled DNAs and linear DNA treated with 100 mM sodium acetate, pH 7, respectively. Lanes 10–11 are linear DNAs treated with 100 mM sodium acetate, pH 5. The details of producing H-DNA, UV irradiation, $^{32}P$ end labeling, the T4 endo V treatment, and sequencing electrophoresis were described in the Materials and Methods section. Lanes 1, 4, 7, 10, 12, and 14 are DNA without UV irradiation, lanes, 2, 5, 8, 11, 13, and 15 are DNAs irradiated with 500 J m$^{-2}$ UV, and lanes 3, 6, and 9 are DNAs with 1000 J m$^{-2}$ UV irradiation. The Maxam and Gilbert reactions are indicated by GA, G, TC and C. DNAs in lanes 1–15 were treated with T4 endo V to identify Py<>Py dimers prior to electrophoresis. Sites of interest (-$C_{40}T_{41}$-, -$T_{41}C_{42}$-, and -$T_{47}T_{48}$-) are indicated by arrows. Lanes 1°, 2°, and 3°, (overexposure) are identical with lanes 1–3. Partial sequence of this fragment is shown at the bottom. The TC repeat and -TCATTATTT- repeats are bracketed. The nucleotides are numbered from the 5′ to 3′ end with the first TC in the repeat as $T_1C_2$.

bered from 5′ to 3′ with the first TC in the $(TC)_{18}$ repeat as $T_1C_2$.

## Results

We have shown previously that a $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ polymer can assume a number of H-DNA structures (Htun & Dahlberg, 1988, 1989). To examine the UV light sensitivity of these conformers, we exposed pTC18tt2A DNA, containing a $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ copolymer, to UV light under a variety conditions. The resulting photoproducts were analyzed by labeling the 5′ end of the $(dT\text{-}dC)_{18}$-containing strand with $^{32}P$ and treating the labeled DNA with either T4 endonuclease V, which cuts at cyclobutane-linked pyrimidine dimers, or hot piperidine, which breaks the DNA chain at <6–4>-linked dipyrimidines (Brash, 1988). The yield, locations, and types of photoproducts were subsequently determined after separating the DNA fragments on sequencing gels.

*Cyclobutane Dipyrimidines Formation in H-DNA and B-DNA.* Figure 2 shows the distribution of T4 endonuclease V cleavage sites within the $(dT\text{-}dC)_{18}$-containing strand. Specific breaks were made at pyrimidine dinucleotides in the UV-irradiated DNAs (lanes 2, 3, 5, 6, 8, 9, 11, 13, and 15) but not in the unirradiated samples (lanes 1, 4, 7, 10, 12, and 14), showing that UV light can cause cyclobutane dimer formation in repeating $(dT\text{-}dC)_{18}$ (indicated by the large open box in Figure 2) when that sequence is in the B-conformation. However, not all potentially reactive 5′-PyPy-3′ dinucleotides form cyclobutane dipyrimidines; for example, dimer formation is absent at the first dT-dC repeat, $T_1C_2$ (Figure 2, lanes 2, 3, 5, 6, 8, 9, 11, 13, and 15; see bottom of Figure 2 for the numbering system).

Conversion of the dT-dC repeat from the B- to the H-conformation dramatically affected photodimerization within this sequence. In particular, photodimer yield within the alternating copolymer was greatly reduced at pH 7 for highly negative supercoiled DNAs ($\sigma < -0.1$; lanes 2 and 3) but not moderately negatively supercoiled ($\sigma = -0.05$; lanes 8 and 9) or linearized (lane 13) DNAs. A similar reduction in photodimer yield was seen at pH 5 for moderately negatively supercoiled DNAs (lanes 5 and 6) but not linearized DNAs (lane 11). With further lowering of the pH to 3.85, suppression of photodimerization was observed even within linearized DNAs (lane 15). We previously demonstrated that these conditions, which result in the suppression of photodimerization, favor the rearrangement of the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence from a double-stranded B-DNA conformation to a triple-stranded H-DNA structure (Htun & Dahlberg, 1988, 1989). Thus, pyrimidines in the H-DNA conformation are less prone to form cyclobutane dipyrimidines than those in the B-DNA conformation. The conformational specificity of the effect is illustrated by the observation that formation of cyclobutane dimers at pyrimidines elsewhere in the DNA (e.g., outside the immediate vicinity of the polypyrimidine tract) is generally not affected by changes in pH or level of negative supercoiling.

Not all pyrimidines in the H-DNA conformation show the same degree of UV suppression. This is most evident in the case of the H-DNA conformer present in highly negatively supercoiled DNAs at pH 7 (lanes 2 and 3); we showed earlier that under these conditions, the 3′ half of the copolymer serves as the donated "third" strand to form the H-y3 structure with an extended junction between the H-DNA and B-DNA that includes nucleotides in the adjacent short direct repeat (small open box). As seen in the overexposed lanes (lanes 2° and 3°), pyrimidines away from the middle of the copolymer show a greater degree of UV suppression than those in the center, the majority of which are neither Watson–Crick nor Hoogsteen base-paired [see Figure 3 of Htun and Dahlberg (1988)]. Furthermore, pyrimidines in the 3′ half are less susceptible to dimer formation than are those in the 5′ half of the copolymer. Similar results are obtained for the H-y5 conformers, formed predominantly in linearized DNAs (lane 15) except that the region of greatest UV suppression is now switched to the 5′ half of the copolymer (lane 15; also, see densitometric scan in Figure 4D). Thus, suppression of cyclobutane dipyrimidine formation is greatest in the Hoogsteen base-paired pyrimidines of the donated strand, less in the Watson–Crick base-paired pyrimidines of the acceptor helix, and least in the unpaired pyrimidines at the "fold-back" tip between the donor and acceptor regions.

While this pattern of UV suppression is reproducibly observed for the H-DNA structure formed in linearized DNAs at pH 3.85 (Figure 2, lane 15; Figure 4D) and in highly negatively supercoiled DNAs at pH 7 (Figure 2, lanes 2 and 3; Figure 4B), the extent of suppression of photoproduct production at the fold-back tip is variable for the H-DNA structure formed in moderately supercoiled DNAs at pH 5. In some cases this region shows very low suppression (data not shown), whereas in others it shows a high degree of UV

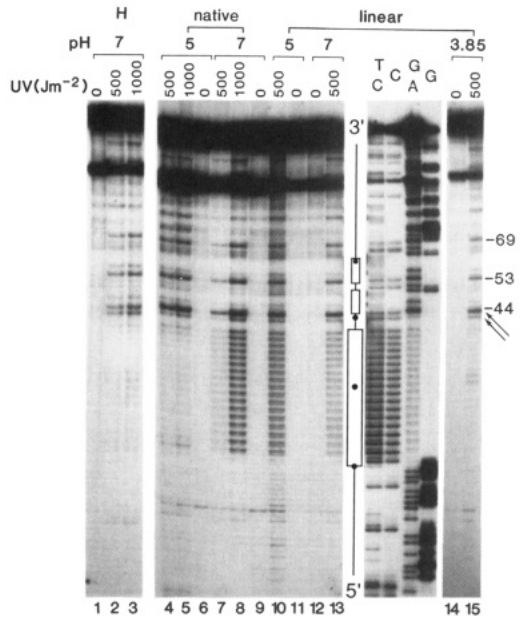


FIGURE 3: Detection of Py<6–4>Py formation by hot piperidine treatment in $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ region and its neighboring sequences in H-y3 conformation induced by high superhelicity (lanes 1–3); H-y conformation induced by pH 5 treatment for native supercoiled plasmid DNA (lanes 4–6); and H-y5 DNA conformation induced by pH 3.85 treatment of *Eco*RI-linearized plasmid DNA (lanes 14–15). Lanes 7–9 and lanes 12–13 are native supercoiled DNAs and linear DNA treated with 100 mM sodium acetate, pH 7, respectively. Lanes 10–11 are linear DNAs treated with 100 mM sodium acetate, pH 5. The details of producing H-DNA, UV irradiation, $^{32}P$ end labeling, piperidine treatment, and sequencing electrophoresis were described in the Materials and Methods section. Lanes 1, 6, 9, 11, 12, and 14 are DNA without UV irradiation, lanes 2, 4, 7, 10, 13, and 15 are DNAs irradiated with 500 J $m^{-2}$ UV, lanes 3, 5, and 8 are DNAs with 1000 J $m^{-2}$ UV irradiation. Sites of interest ($-T_{39}C_{40}$-, $-T_{41}C_{42}$-) are indicated by arrows. The Maxam and Gilbert reactions are indicated by GA, G, TC, and C. DNAs in lanes 1–15 were treated with hot piperidine to induce cleavage at Py<6–4>Py, prior to electrophoresis.

suppression (Figure 2, lanes 5 and 6; Figure 4C).

Finally, pyrimidines in the immediate vicinity of the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence can have altered photoreactivity where they become part of the H-DNA and B-DNA junction. As noted earlier for the highly negatively supercoiled DNAs at pH 7, the junction of the H-y3 structure extends from the end of the $(dT\text{-}dC)_{18}$ sequence to the proximal short direct repeat [from nucleotides $T_{41}$ to $T_{49}$ of Figure 2; also, see Figure 3 of Htun and Dahlberg (1988)]. Consequently, upon UV irradiation, this region forms a previously unobserved cyclobutane dimer at $C_{40}T_{41}$ with a concomitant loss of the dimer at $T_{41}C_{42}$ (compare lanes 2 and 3 with lanes 5, 6, 8, 9, 11, 13, and 15 of Figure 2 at the locations marked by arrows, also see Figure 4A–D).

*Py<6–4>Py Dimer Formation in H-DNA and B-DNA.* Treatment with hot piperidine reveals the presence of the other major photoproduct, the <6–4> dipyrimidines, in the $(dT\text{-}dC)_{18}$-containing strand (Figure 3). Dimer formation occurs primarily at the 5′-TC-3′ dinucleotides. As in the case of the cyclobutane dimers, not all 5′-PyPy-3′ dinucleotides readily form <6–4> photoproducts. Notably, the first dT-dC repeat, $T_1C_2$, again fails to photodimerize (Figure 3, lanes 2, 3, 4, 5, 7, 8, 10, 13, and 15).

Participation of the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence within the H-DNA conformation results again in partial protection of the pyrimidines from UV light. The <6–4> dipyrimidine yield is significantly reduced within the $(dT\text{-}dC)_{18}$ sequence (compare lanes 2 and 3 with 7, 8, and 13, and lanes 4, 5, and 15 with 10). As previously noted, dimer yield is lower for pyrimidines in Hoogsteen base pairs than for those in Watson–Crick base pairs (Figures 4, panels F or H), whereas, the yield of <6–4> dimers in the center of the copolymer can exceed that of pyrimidines involved in Watson–Crick or Hoogsteen base pairs (Figure 4H). Thus, the overall pattern of <6–4> dipyrimidine formation roughly resembles that of the cyclobutane dipyrimidines (compare panels B–D with F–H in Figure 4, respectively).

Pyrimidines in the immediate vicinity of the $(dT\text{-}dC)_{18}\cdot(dA\text{-}dG)_{18}$ sequence can also have altered photoreactivity when they become part of the H-DNA and B-DNA junction. This is most evident for the H-y3 structure formed in highly negatively supercoiled DNA at pH 7, where the H-DNA and B-DNA junction extends from $T_{38}$ to $T_{49}$. The <6–4> dimer yield increases significantly at $T_{39}C_{40}$, whereas that at $T_{41}C_{42}$ decreased (indicated by arrowheads in Figure 3; compare lanes 2 and 3 with 4, 5, 7, 8, 10, 13, and 15; also, panels E–H in Figure 4).

## Discussion

We have shown that the UV-light-induced formation of both cyclobutane and <6–4> dipyrimidine photoproducts is reduced when DNA is in the H-DNA conformation. This suppression of photoproduct formation occurs in both halves of the polypyrimidine chain, but the extent of suppression is not uniform throughout the entire region. This is the first demonstration that intramolecular H-DNA, containing a pyrimidine strand looped back on itself, alters the efficiency of creation of these photoproducts.

The reduction in photoproduct formation was reduced for pyrimidines in both the donor (in Hoogsteen pairs) and acceptor (in Watson–Crick pairs) strands and also in the single-stranded (but probably structured) central region that comprises the tip of the H-DNA (Figure 2, lanes 2, 3, 5, 6, and 15). Characteristically, accumulation of photoproducts was lower for Hoogsteen pyrimidines of the donor strand than for Watson–Crick-paired pyrimidines of the acceptor helix. The photodimer yield of the unpaired pyrimidines in the central region that forms the "fold-back" tip of the structure is variable, depending on conditions.

Suppression of dimer formation within the triple-stranded region probably results from a combination of factors. In Py·Pu·Py triple helices of the H-y DNAs, the pyrimidine sugars exist in a C3′-endo conformation (Rajagopal & Feignon, 1989a,b; de los Santos et al., 1989); A-DNA is significantly less sensitive to photodimerization than is B-DNA (Patrick & Rahn, 1976; Becker & Wang, 1989a,b). Furthermore, the presence of a third strand within an otherwise double-helical molecule imposes an additional level of rigidity, as reflected in part by increased thermal stability of triplex over duplex DNA at low pH (Rajagopal & Feignon, 1989b; Lee et al., 1979) and by hysteresis of long $(dT\text{-}dC)_n\cdot(dA\text{-}dG)_n\cdot(dT\text{-}dC^+)_n$ triple-stranded structures toward the loss of protons (Lee et al., 1984). This increased rigidity would interfere with photodimerization, perhaps by preventing optimal alignment of activated bonds. Finally, protonation of cytosines in the donor strand might reduce the yield of photoproducts (Patrick & Rahn, 1976; Brown et al., 1985).

Suppression of photoproduct formation tends to be lower at the ends of the $(dT\text{-}dC)_{18}$ sequence, near the junction of H-DNA and B-DNA (for example, see the overexposed lanes 2° and 3° in Figure 2). Suppression at the 5′ end of the repeat (T1, C2, T3) probably results from nucleotide sequences in this region rather than H-DNA conformation since it is also apparent in double-stranded B-DNA (Figure 4A,E). Near

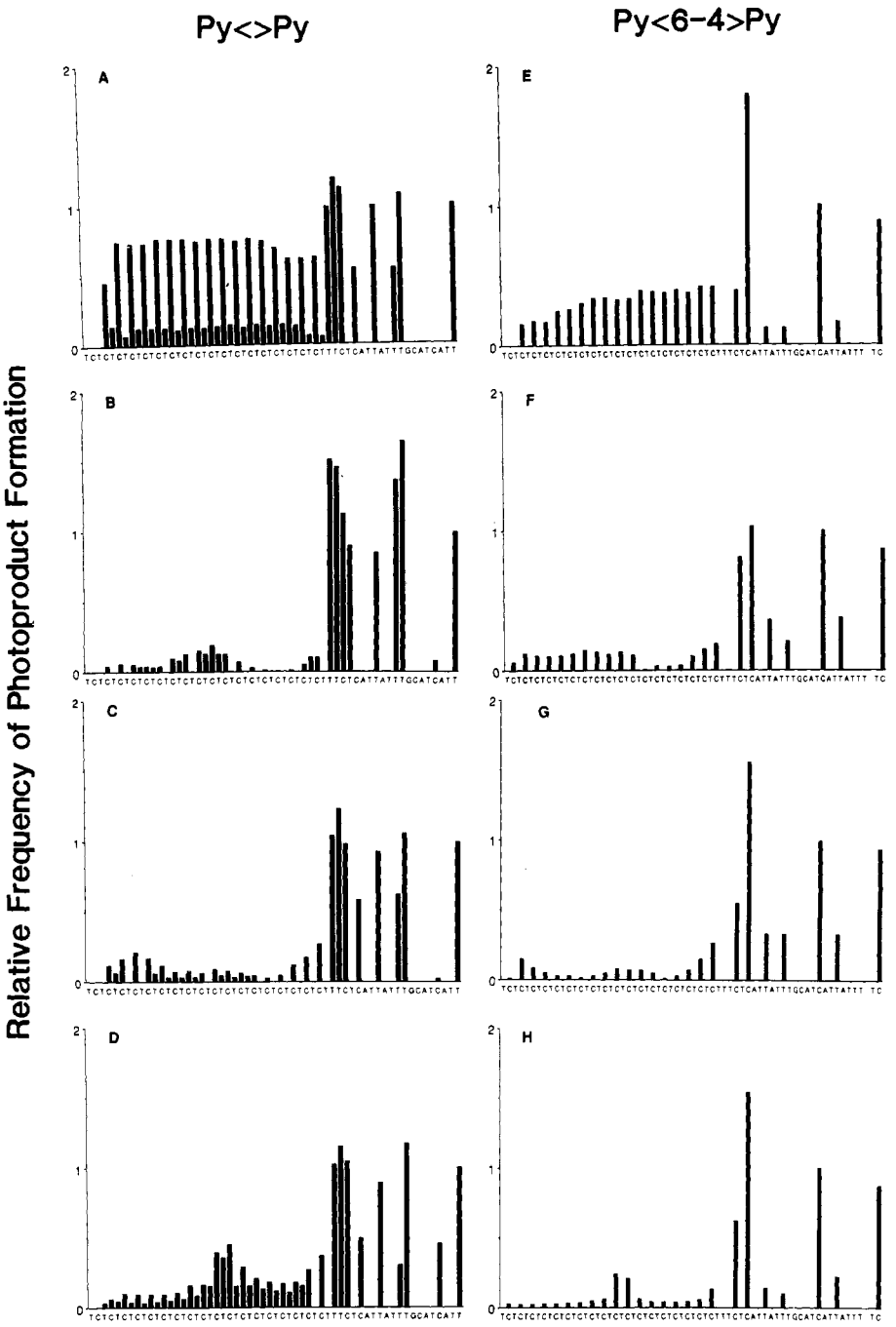


FIGURE 4: Relative frequency of Py<>Py and Py<6–4>Py formation in $(dT\text{-}dC)_{18}$·$(dA\text{-}dG)_{18}$ region in B-DNA conformation (A and E); H-y3 conformation induced by high superhelicity (B and F); H-y conformation induced by native superhelicity in 100 mM sodium acetate (pH 5) buffer (C and G); and H-y5 conformation induced in linear DNA by 100 mM trisodium citrate (pH 3.85) (D and H). The relative frequency by Py<>Py and Py<6–4>Py formation was calculated from densitometric scans by taking the ratio of Py<>Py and Py<6–4>Py formed in the $(dT\text{-}dC)_{18}$ strand relative to the Py<>Py formed at position $-T_{56}T_{57}-$ and the Py<6–4>Py formed at position $-T_{69}C_{70}-$, respectively. The sequence of $(TC)_{18}$ is written from 5′ to 3′ (left to right). The -TT- and -TC- in the far right of each panel represent position $-T_{56}T_{57}-$ and position $-T_{69}C_{70}-$, respectively.

the 3′ end of the repeat the extent of suppression varies with changes in the environment (Figures 4, panels B–D and F–H), presumably reflecting "breathing" at the ends of the triple helix.

Despite the presumed single-strandedness of the fold-back region at the center of the $(dT\text{-}dC)_{18}$ sequence, photodimerization is lower than when this sequence is in the double-helical form (compare panels A with B–D and E with F–H in Figure 4); this difference indicates that the polypyrimidine loop is highly constrained. However, variations in the relative photoproduct yield in this loop region suggest that the unpaired pyrimidines may exist in several distinct states (compare panels B and D with C and panels H with F and G in Figure 4).

To a large extent, the photoreactivity of pyrimidines immediately adjacent to the 3′ side of the dT-dC repeat is unaffected by the formation of H-DNA. The one exception is the case in which H-DNA formation was promoted by high levels of negative supercoiling. Here, the increased torsional stress fostered further unwinding of the donor helix, causing denaturation of an A-T-rich duplex region adjacent to the repeat (Htun & Dahlberg, 1988). Pyrimidines in this underwound region showed altered photoreactivity (Figure 4, compare panel B with A, C, and D and panel F with E, G, and H). Particularly noteworthy is pyrimidine T41, which

forms a cyclobutane dimer with C40 rather than C42, indicating substantial perturbation of its normal base-stacking interaction.

When formation of H-DNA is promoted by high levels of negative supercoiling, the H-y3 conformer predominates, but when low pH is the driving force in the absence of negative supercoiling, the region adopts the H-y5 conformation (Htun & Dahlberg, 1988, 1989, 1990; Htun, 1989). This change in structure is also reflected by a switch in the relative levels of formation of cyclobutane dipyrimidines. In both cases, the donated region is less reactive, probably as a result of protonating every other base in this region.

By driving triplex formation to completion with an excess of oligopyrimidines, Lyamichev et al. (1990) demonstrated that pyrimidines in the acceptor region of a triplex do not form <6–4> dipyrimidines upon UV irradiation. However, those workers did not assay for cyclobutane dipyrimidines, nor did they analyze the UV sensitivity of the donated polypyrimidine strand. Also, the use of an intermolecular triplex precluded analysis of the structures at the ends of the intramolecular triplex, which are unique to the H-DNA structure.

Our work demonstrates that UV-promoted formation of both cyclobutane and <6–4> dipyrimidines is greatly reduced in the H-y class of intramolecular triple-stranded structures at low pH and at neutral pH. In both cases, the levels of photodimer yields can be used to distinguish between pyrimidines participating in Hoogsteen versus Watson–Crick base pairs; suppression of dimer formation can even be an indicator of pyrimidines in the fold-back loop, in some cases. Thus, the profiles of UV irradiation products can be used to discriminate between intermolecular and intramolecular DNA triplexes. Finally, the higher yield of cyclobutane dipyrimidines over <6–4> dipyrimidines indicates that the former are probably better products to analyze when studying the formation of triple-stranded H-DNA in vivo.

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## References

Becker, M. M., & Wang, J. C. (1984) *Nature (London), 309*, 682–687.

Becker, M. M., & Wang, Z. (1989a) *J. Mol. Biol. 210*, 429–438.

Becker, M. M., & Wang, Z. (1989b) *J. Biol. Chem. 264*, 4163–4167.

Birnboim, H. C. (1978) *J. Mol. Biol. 121*, 541–559.

Brash, D. E. (1988) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C. Eds.) Vol. 3, pp 327–345, Marcel Bekker, New York.

Brown, D. M., Gray, D. M., Patrick, M. H., & Ratliff, R. L. (1985) *Biochemistry 24*, 1676–1683.

Burkholder, G. D., Latimer, L. J. P., & Lee, J. S. (1988) *Chromosoma (Berlin), 97*, 185–192.

Cseko, Y. M. T., Dower, N. A., Minoo, P., Lowenstein, L., Smith, G. R., Stone, J., & Sederoff, R. (1979) *Genetics 92*, 459–484.

de los Santos, C., Rosen, M., & Patel, D. (1989) *Biochemistry 28*, 7282–7289.

Friedberg, E. C. (1985) *DNA Repair*, W. H. Freeman and Co., New York.

Friedberg, E. C., Ganesan, A. K., & Seawell, P. C. (1980) *Methods Enzymol. 65*, 191–201.

Hoffman-Liebermann, B., Liebermann, D., Troutt, A., Kedes, L. L., & Cohen, S. N. (1986) *Mol. Cell. Biol. 6*, 3632–3642.

Htun, H. (1989) *H-DNA: Its Structure and Formation*, Ph.D. Dissertation, University of Wisconsin—Madison, Madison, WI.

Htun, H., & Dahlberg, J. E. (1988) *Science 241*, 1791–1796.

Htun, H., & Dahlberg, J. E. (1989) *Science 243*, 1571–1576.

Htun, H., & Dahlberg, J. E. (1990) in *Structure & Methods, Vol. 3, DNA & RNA* (Sarma, R. H., & Sarma, M. H., Eds.) pp 185–205, Adenine Press, Schenectady, NY.

Kiyama, R., & Camerini-Otero, D. (1990) *Biophy. J. 58*, 65a.

Kohwi-Shigematsu, T., Gelinas, R., & Weintraub, H. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4389–4393.

Lee, J. S., Johnson, D. A., & Morgan, A. R. (1979) *Nucleic Acids Res. 6*, 3073–3092.

Lee, J. S., Woodsworth, M. L., Latimer, L. J. P., & Morgan, A. R. (1984) *Nucleic Acids Res. 12*, 6603–6614.

Lee, J. S., Burkholder, G. D., Latimer, L. J. P., Haug, B. L., & Braun, R. P. (1987) *Nucleic Acids Res. 15*, 1047–1061.

Lyamichev, V. I., Mirkin, S. M., & Frank-Kamenetskii, M. D. (1986) *J. Biomol. Struct. Dyn. 3*, 667–669.

Lyamichev, V. I., Frank-Kamenetskii, M. D., & Soyfer, V. N. (1990) *Nature (London) 344*, 568–570.

Manor, H., Sridhara, B., & Martin, R. G. (1988) *J. Mol. Evol. 27*, 96–101.

Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.

Patrick, M. H., & Rahn, R. O. (1976) in *Photochemistry and Photobiology of Nucleic Acids, Vol. II. Biology* (Wang, S. Y., Ed.) pp 35–95, Academic Press, New York.

Rajagopal, P., & Feigon, J. (1989a) *Nature (London) 339*, 637–640.

Rajagopal, P., & Feigon, J. (1989b) *Biochemistry 28*, 7859–7870.

Weintraub, H. (1983) *Cell 32*, 1191–1203.

Wells, R. D., Collier, D. A., Hanvey, J. C., Shimizu, M., & Wohlrab, F. (1988) *FASEB J. 2*, 2939–2949.