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# Quadruplex DNA Formation in a Region of the tRNA Gene supF Associated with Hydrogen Peroxide Mediated Mutations†

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ABSTRACT: A hot spot for $H_2O_2$/Fe-mediated mutation has been observed between bases 154 and 170 of the supF gene in the mutation reporter plasmid pZ189 [Moraes et al. (1990) *Carcinogenesis 11*, 283; Akman et al. (1991) *Mutat. Res.* (in press)]. To further characterize this hot spot, we synthesized the 33mer d(pAAAGTGATGGTGGTGGGGGAAGGATTCGAACCT) (pZ33), which is complementary to bases 159–191 of the supF gene. pZ33 annealed spontaneously in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA–100 mM NaCl at 50 °C into two major forms, one of which migrates more slowly than does $d(pT)_{33}$ on nondenaturing 12% polyacrylamide gels. We propose that this form is a four-stranded structure stabilized by Hoogsteen-type deoxyguanosine quartets involving all deoxyguanosines of the sequence d(pGGTGGTGGGGG) because of the following. (1) pZ33 migrates as a single form that comigrates with $d(pT)_{33}$ on denaturing 20% acrylamide–8 M urea gels. (2) Annealing an equimolar mixture of 5′-$^{32}$P-labeled pZ33 and the oligodeoxynucleotide d(pTTTTTTTTpZ33TTTTTTTT) (pZ49), as well as 5′-$^{32}$P-labeled pZ49 and pZ33, caused the formation of four, discreet slowly migrating bands on nondenaturing 12% polyacrylamide gels. Mixing 5′-$^{32}$P-labeled pZ33 with 5′-$^{32}$P-labeled pZ49 resulted in five slowly migrating bands. (3) An oligodeoxynucleotide identical with pZ33 except that every deoxyguanosine has been replaced with deoxyinosine did not anneal into a slowly migrating form. (4) Dimethyl sulfate protection studies demonstrated that all deoxyguanosines of the sequence d(pGGTGGTGGGGG) were protected at N-7 in the slowly migrating form but not in single-stranded pZ33. These data suggest that a hot spot for $H_2O_2$/Fe-mediated base substitutions is located adjacent to a sequence that can spontaneously adopt a quadruplex structure in which deoxyguanosine quartets are Hoogsteen bonded.

Four-stranded complexes composed of sets of guanosine residues Hoogsteen bonded in deoxyguanosine quartets were first proposed by Gellert et al. (1962) on the basis of X-ray diffraction patterns obtained from fibers of polyguanylic acid [poly(G)][1] and gels formed by guanylic acid. These structures are remarkably stable. For poly(G) the alkali-induced helix–coil transition does not occur until pH 11.2 at 25 °C, and the thermally induced transition is not complete even at 100 °C at neutral pH (Fresco & Massoulie, 1963). This exceptional stability and the capacity of the mononucleotide to form an extended complex may be due in part to the unusual stacking energy of the guanosine quartet (Arnott et al., 1974) and in part to the capacity of O-6 of guanine to participate in a coordination complex involving either $Na^+$ or $K^+$, as subsequently suggested for the stabilization of telomeric DNA (Sundquist & Klug, 1989; Williamson et al., 1989; Sen & Gilbert, 1990). The RNA structures appear to form with all four strands in a parallel orientation (Arnott et al., 1974; Zimmerman et al., 1975).

More recently, structures containing deoxyguanosine quartets have been suggested for oligodeoxynucleotides corresponding to the immunoglobulin switch region (Sen & Gilbert, 1988), the region containing codon 12 of the human c-Ha-*ras* gene (Smith et al., 1989), and the telomeric repeat sequences of Tetrahymena (Sundquist & Klug, 1989) and *Oxytrichia* (Williamson et al., 1989). While the oligodeoxynucleotides from the immunoglobulin switch region and c-Ha-*ras* appear to form parallel quadruplexes similar to the structure proposed for poly(G), the telomeric sequences form antiparallel structures composed of sets of foldback molecules.

In light of the occurrence of these structures at sites of spontaneous gene rearrangement (Sen & Gilbert, 1988) and point mutation (Smith et al., 1989), it is important to ask whether a relationship exists between the spectrum of mutations induced by DNA-damaging agents and the potential for unusual structure formation in target DNAs. To approach this question, we prepared a map of mutations occurring in the supF gene replicating in the reporter plasmid pZ189 (Seidman et al., 1985) after exposure to hydrogen peroxide/Fe (Akman et al., 1991). This map is in agreement with that of Moraes et al. (1989) for hydrogen peroxide/Fe-mediated mutations resulting from the use of similar mutagenesis techniques. Both maps identify hot spots for mutation adjacent to sequences having the properties appropriate for the formation of unusual structures, e.g., triplexes (Beal & Dervan, 1991) or quadruplexes (Sen & Gilbert, 1990). In this report,

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[1] Abbreviations: TBE buffer, 89 mM Tris base–89 mM boric acid–2 mM EDTA, pH 8.3; poly(G), polyguanylic acid.

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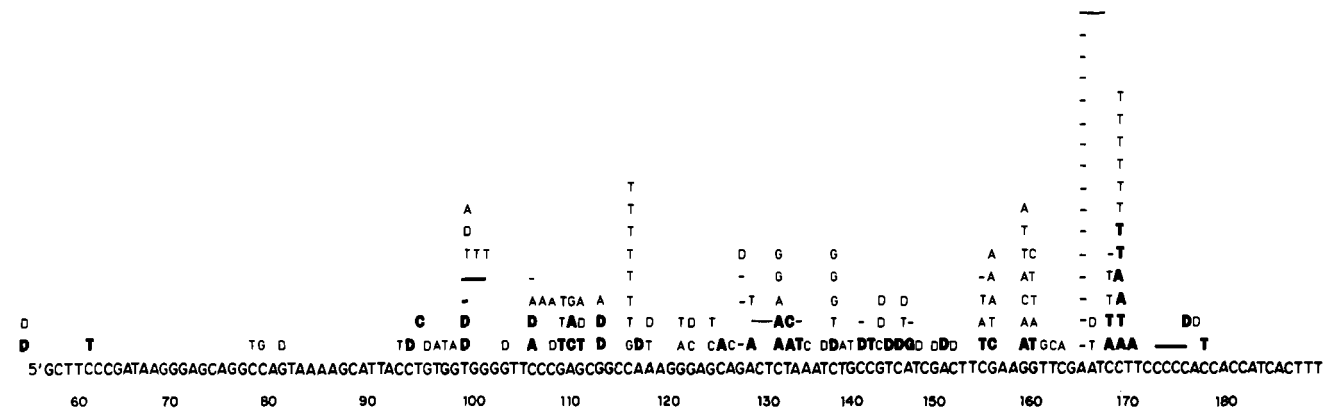


FIGURE 1: Complete sequence of the supF gene. Depicted above the wild-type sequence is a compilation of all $H_2O_2$/Fe-mediated mutations identified by Akman et al. (1991) (in boldface type) or Moraes et al. (1989). -: Deletions of less than three bases. D: Deletions of more than three bases. The two deletions listed above 5′ extended upstream of the supF gene.

we establish that oligodeoxynucleotides near one of these sequences spontaneously form a quadruplex DNA structure in vitro.

## Materials and Methods

*Oligodeoxynucleotide Synthesis and Labeling.* All of the oligodeoxynucleotides used in this report (see Figure 2) were synthesized at the core DNA synthesis facility of the City of Hope Cancer Center. Oligodeoxynucleotides were prepared from nucleotide precursors that had the β-cyanoethyl protecting group of the phosphate moiety [β-cyanoethyl phosphoramidites]. Single-stranded products were synthesized by standard techniques (Eritja et al., 1987) and purified by polyacrylamide gel electrophoresis and high-performance liquid chromatography.

Aliquots of oligodeoxynucleotide (50 pmol) were 5′-end-labeled with $^{32}P$ by incubation at 37 °C for 30 min in 50 mM Tris-HCl (pH 7.6)–10 mM $MgCl_2$–5 mM dithiothreitol–0.1 mM spermidine containing 50 pmol of [γ-$^{32}P$]ATP (New England Nuclear, Boston, MA) and 10 units of T4 polynucleotide kinase (New England Biolabs, Beverly, MA). After the labeling, reactions were stopped by adding 20 mM EDTA and heating the mixture to 68 °C for 15 min. Labeled oligodeoxynucleotides were purified from the unincorporated radiolabel and protein by chromatography through NENsorb columns (Du Pont, Wilmington, DE) according to the manufacturer's instructions. Labeled oligodeoxynucleotides were eluted in 50% ethanol and partially dried under vacuum prior to use.

*Complex Formation.* Oligodeoxynucleotides (5′-end-labeled with $^{32}P$) were dissolved at a concentration of 12 μM in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA–100 mM NaCl, heated to 95 °C for 5 min, and placed successively at 50 °C for 16 h and then at room temperature for 10 min and then stored at 4 °C. When two different oligodeoxynucleotides were mixed, each was at a concentration of 6 μM.

*Nondenaturing Polyacrylamide Gel Electrophoresis.* Oligodeoxynucleotide complexes were mixed with an equal volume of nondenaturing gel loading buffer (1× TBE buffer [89 mM Tris base, 89 mM boric acid, 4 mM EDTA; pH 8.3], 5% glycerol, 0.025% xylene cyanol, 0.025% bromophenol blue) and loaded onto 40-cm-long 12% acrylamide gels (acrylamide–bisacrylamide 29:1). Gels were run at room temperature and at 550 V for 5.5 h in 1× TBE running buffer. Following electrophoresis, wet gels were autoradiographed without intensifying screens using Kodak X-RP film (Kodak, Rochester, NY). Scanning densitometry of the autoradiograms was carried out with a Hoefer GS 300 densitometer, and the results were analyzed with GS 360 software (Hoefer Scientific Instruments, San Francisco, CA).

*Dimethyl Sulfate Protection Studies of Isolated Low-Mobility and High-Mobility Forms.* Following autoradiography of nondenaturing polyacrylamide gels, portions of the gels corresponding to the low- and high-mobility forms of pZ33 were excised. DNA was electroeluted from the excised gel fragments at 200 V for 4 h by using a Centrilutor apparatus (Amicon, Beverly, MA); 0.5× TBE was the elution buffer. Subsequent electrophoresis of the electroeluted DNA through 12% nondenaturing polyacrylamide gels confirmed that both the low-mobility and the high-mobility forms retained their original electrophoretic mobility after recovery (data not shown). The two electrophoretic forms of pZ33 were subjected to a modified Maxam–Gilbert deoxyguanosine reaction (Maxam & Gilbert, 1977) as follows. Isolated oligodeoxynucleotide ($10^5$–$10^6$ cpm) plus 3 μg of carrier DNA was incubated in 50 mM sodium cacodylate (pH 8.0)–1 mM EDTA containing 0.4% dimethyl sulfate (v/v) at 4 °C for 22 min, after which β-mercaptoethanol was added to a concentration of 0.35 M. An additional 3 μg of carrier DNA plus 1 mL of 1-butanol was then immediately added. The precipitated DNA was recovered by centrifugation at 17000*g* for 5 min, washed with 0.15 mL of distilled $H_2O$ plus 1 mL of 1-butanol, and dried under vacuum. Samples were redissolved in 0.15 mL of 1 M piperidine, reacted at 90 °C for 45 min, cooled on ice, and then precipitated by adding 0.15 mL of 70% ethanol, 2 μg of carrier RNA, and 4 volumes of 1-butanol. Precipitated samples were washed with 0.15 mL of 1% sodium dodecyl sulfate–1 mL of 1-butanol, dried under vacuum, and redissolved in denaturing gel loading buffer (80% formamide, 0.5× TBE, 0.05% xylene cyanol). Aliquots ($10^5$ cpm) were heat denatured, loaded onto 40-cm-long 20% acrylamide (acrylamide–bisacrylamide 19:1)–8 M urea gels, and electrophoresed at 54 W of constant power for 1 h. Wet gels were then autoradiographed using Kodak XR-P film and scanned as described above.

## Results

*$H_2O_2$/Fe Mutation Clustering.* Figure 1 illustrates the position of mutations generated in the supF gene of the mutation reporter plasmid pZ189 after exposure to $H_2O_2$/Fe and replication in monkey kidney CV-1 cells. In Figure 1, the data of Moraes et al. (1989) and our own data (Akman et al., 1991) have been combined. An inspection of Figure 1 reveals several regions of high mutation frequency. Runs of three or more deoxyguanosine residues [d(pGGG...)] are present near several of these hotspots. Runs of this type have been shown to provide the potential for formation of Hoogsteen-paired multistranded structures under physiological conditions (Henderson et al.,

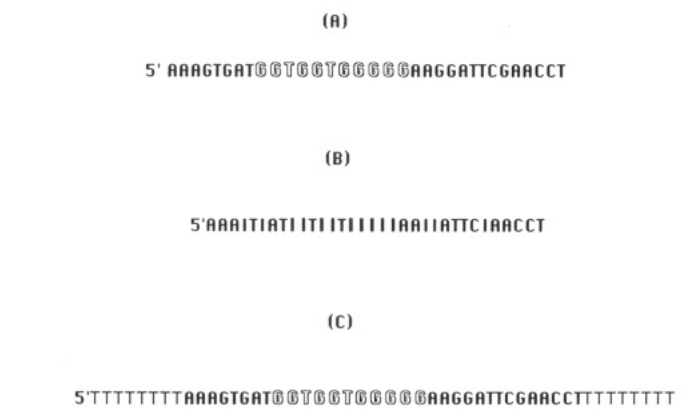


FIGURE 2: Sequences of the oligonucleotides used in these studies: (A) pZ33, a 33mer whose sequence corresponds to the portion of the noncoding strand of the supF gene identified as a hot spot for $H_2O_2$/Fe-mediated mutations; (B) pZ33-I, in which all of the deoxyguanosines of pZ33 were replaced by deoxyinosine; (C) pZ49, which contains the sequence of pZ33 flanked at both the 5′ and 3′ ends by $d(pT)_8$.

1987; Sen & Gilbert, 1988, 1990; Williamson et al., 1989; Sundquist & Klug, 1989; Beal & Dervan, 1991). In order to determine whether DNA in a run of deoxyguanosine residues in the vicinity of a mutational hot spot was capable of forming an unusual structure, we characterized the DNA near the most prominent cluster of seqeunce alterations, which was located between bases 154 and 170 of the coding strand of the supF gene. To do this, we studied the 33mer containing the deoxyguanosine-rich sequence d(pGGTGGTGGGGG) shown in Figure 2A (pZ33) as follows.

*Electrophoretic Forms of pZ33.* Electrophoretic analysis was used to determine whether pZ33 would self-associate as do other deoxyguanosine-rich sequences of this type (Henderson et al., 1987; Sen & Gilbert, 1988; Williamson et al., 1989; Sundquist & Klug, 1989). Self-annealing of pZ33 was promoted by heating and slowly cooling the oligodeoxynucleotide in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA–100 mM NaCl as described under Materials and Methods. Upon electrophoresis through a 12% nondenaturing polyacrylamide gel (Figure 3A), two major forms were observed: a high-mobility form and a low-mobility form. However, upon electrophoresis under strongly denaturing conditions (Figure 3B), only one form was observed. Under these conditions, this form had the same mobility as a 33-nucleotide oligothymidine marker. We concluded that the low- and high-mobility forms observed under nondenaturing conditions were different structural forms of the same oligodeoxynucleotide.

Self-annealing into two electrophoretic forms was not altered by varying the pH of the annealing solution from 8.0 to 12.2 (data not shown). The low-mobility form also exhibited thermal stability. No loss of the low-mobility form was observed by heating at temperatures up to 80 °C for 15 min in the annealing buffer (data not shown).

*Hoogsteen Pairing in the Low-Mobility Form.* Since previous work has shown that deoxyguanosine-rich sequences of this type adopt Hoogsteen-bonded complexes of low electrophoretic mobility (Sen & Gilbert, 1988), we investigated the possibility that the low-mobility form of pZ33 was stabilized by Hoogsteen pairing. Hoogsteen pairing between deoxyguanosine residues involves N-1–O-6 and N-2–N-7 hydrogen bonding. Deoxyinosine lacks the N-2 present in deoxyguanosine and is known to destabilize Hoogsteen bonds (Henderson et al., 1990; Arnott et al., 1974; Zimmerman et al., 1975). When each of the deoxyguanosine residues in pZ33 was replaced with deoxyinosine (pZ33-I, Figure 2B), the

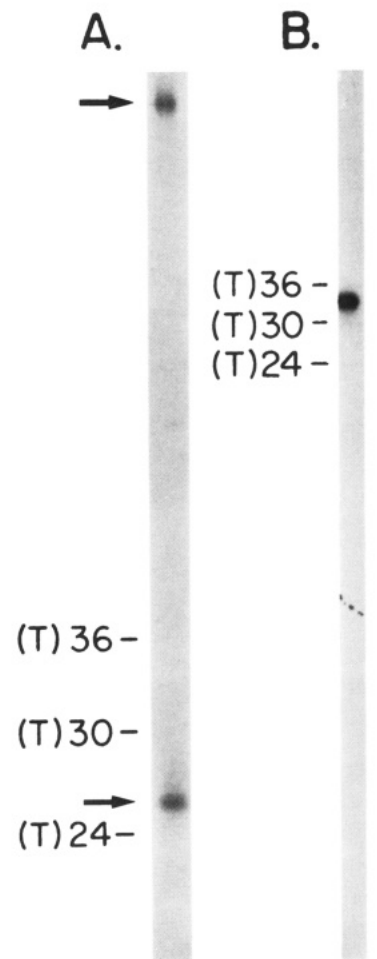


FIGURE 3: (A) Autoradiogram of 5′-$^{32}$P-labeled pZ33 that had been heated and cooled in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA–100 mM NaCl according to the protocol described under Materials and Methods and then electrophoresed through a 12% nondenaturing polyacrylamide gel. The arrows point to high- and low-mobility forms. (B) Autoradiogram of 5′-$^{32}$P-labeled pZ33 that had been dissolved in denaturing gel loading buffer (see Materials and Methods) and electrophoresed through a 20% acrylamide–8 M urea gel. The positions of a series of oligothymidine markers are shown to the left of the gel.

low-mobility form was never observed after electrophoresis under nondenaturing conditions (Figure 4, lane 3). On the other hand, a low-mobility form was always observed after self-annealing of pZ33, although the relative yield of the low-mobility form was somewhat variable (compare Figure 3A with Figure 4, lane 2). This result suggested that the low-mobility form was stabilized by Hoogsteen pairing.

As noted above, N-2–N-7 hydrogen bonding occurs in Hoogsteen-paired deoxyguanosine residues. Cleavage of DNA at these residues by the Maxam–Gilbert procedure requires methylation of N-7 by dimethyl sulfate. Thus, Hoogsteen-paired deoxyguanosines are protected from methylation by this reagent and therefore cannot be cleaved by the Maxam–Gilbert procedure (Maxam & Gilbert, 1977). For this reason, putative Hoogsteen-paired deoxyguanosine residues in the low-mobility form of pZ33 should be detected as dimethyl sulfate protected sites.

pZ33 was inspected for dimethyl sulfate protected deoxyguanosine residues as follows. Both forms of the 5′-$^{32}$P-end-labeled oligodeoxynucleotide were isolated from 12% nondenaturing polyacrylamide gels and subjected to a modified Maxam–Gilbert deoxyguanosine-sequencing procedure (Maxam & Gilbert, 1977). In this procedure, cleavage products are separated by strand length with use of electro-

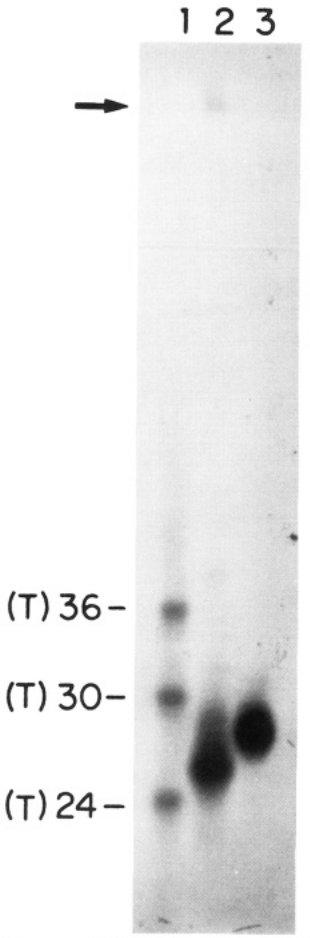


FIGURE 4: Autoradiogram of 5′-$^{32}$P-labeled pZ33 (lane 2) and 5′-$^{32}$P-labeled pZ33-I (lane 3) heated and cooled as described under Materials and Methods and electrophoresed through a 12% nondenaturing polyacrylamide gel. The arrow indicates the position of the low-mobility form. Lane 1 is a series of 5′-$^{32}$P-labeled oligothymidine markers.

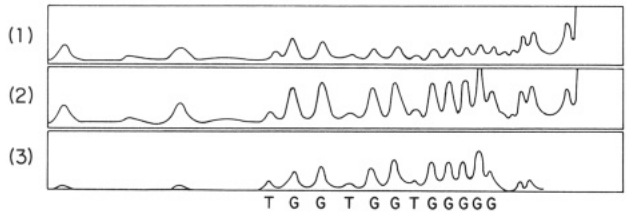


FIGURE 5: Densitograms of isolated 5′-$^{32}$P-labeled pZ33 low-mobility (panel 1) and high-mobility forms (panel 2) subjected to a modified Maxam–Gilbert deoxyguanosine reaction and electrophoresed through a 20% acrylamide–8 M urea gel, as described under Materials and Methods. Panel 3 is a tracing of panel 1 subtracted from panel 2. Subtraction was done using GS 360 data system software provided for the Hoefer GS 300 scanning densitometer. Panel 3 illustrates that all deoxyguanosines in the sequence d(pGGGGGTGGTGG) are protected from methylation at N-7.

phoresis through 20% polyacrylamide gels under conditions that suppress DNA–DNA interaction. After separation, fragments were detected by autoradiography and quantified by densitometry. Representative densitometric tracings are given in Figure 5. A comparison of the tracing from the low-mobility form (Figure 5, panel 1) with that of the high-mobility form (Figure 5, panel 2) demonstrates significant protection of the deoxyguanosine residues in the low-mobility form. The positions of protected residues in the low-mobility form are identified as d(pGGTGGTGGGGG) by the difference between the two tracings (Figure 5, panel 3). Taken together, these studies (Figures 4 and 5) demonstrate that the

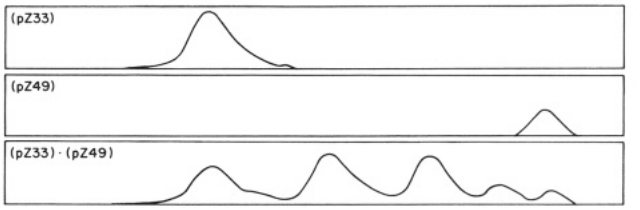


FIGURE 6: Densitogram obtained by scanning the low-mobility region of an autoradiogram of self-annealed 5′-$^{32}$P-labeled pZ33 (top), self-annealed 5′-$^{32}$P-labeled pZ49 (middle), and 5′-$^{32}$P-labeled pZ33 annealed with equimolar 5′-$^{32}$P-labeled pZ49 (bottom). The annealing solutions were heated and cooled as described under Materials and Methods and electrophoresed through a 12% nondenaturing polyacrylamide gel.

N-2–N-7 hydrogen bond is present in the low-mobility form of pZ33 and strongly suggest that Hoogsteen pairs are required for the stability of this form.

*Strand Stoichiometry of the Low-Mobility Form.* Structures of low electrophoretic mobility that contain Hoogsteen-paired deoxyguanosine residues from the immunoglobulin switch region (Sen & Gilbert, 1988) and c-Ha-*ras* (Smith et al., 1989) are composed of four strands. The strand stoichiometry of the low-mobility form of pZ33 was investigated by mixing oligodeoxynucleotides of different lengths (Sen & Gilbert, 1988). One of the sequences in the mixture was pZ33; the other was pZ49. pZ49 retained the complete sequence of pZ33 but was flanked on either end by octameric dT extensions (Figure 2C). Two major electrophoretic forms were also observed with self-annealed pZ49. When electrophoresed in adjacent lanes, the low-mobility forms of pZ33 and pZ49 were well separated (Figure 6, top and middle panels). The low-mobility form of pZ49 was easily detected. However, when these oligodeoxynucleotides were compared under identical conditions, the relative yield of low-mobility form pZ49 was consistently lower than the corresponding yield of low-mobility form pZ33, suggesting that the octameric tails lowered the efficiency of formation of the low-mobility form.

When end-labeled pZ33 was mixed with an equimolar amount of end-labeled pZ49, three intermediate-mobility forms were observed between the low-mobility forms of pZ33 and pZ49 (Figure 6, bottom panel). If the intermediate-mobility forms contain both types of oligodeoxynucleotide, then the low-mobility forms must be composed of four strands. The mixed nature of the intermediate forms was demonstrated by performing the same experiment with end-labeled pZ33 and unlabeled pZ49 (Figure 7, lane 1) or end-labeled pZ49 and unlabeled pZ33 (Figure 7, lane 2). We conclude that the low-mobility forms are quadruplexes containing Hoogsteen-paired deoxyguanosines. If the strands of pZ33 and pZ49 had combined randomly to form the quadruplexes observed in Figure 6, we would have expected the molar ratio of $(pZ33)_4$:$(pZ33)_3(pZ49)_1$:$(pZ33)_2(pZ49)_2$:$(pZ33)_1(pZ49)_3$:$(pZ49)_4$ to have been 1:4:6:4:1. Inspection of Figure 6 shows that this is clearly not the case. Instead, the more pZ49 is admixed into the quadruplex, the less efficiently it is formed. This nonrandom pattern of mixed quadruplex formation reinforces the suggestion that the octameric tails of pZ49 lowered the efficiency of quadruplex formation. The data do not allow us to address the question of strand polarity.

## Discussion

Considerable evidence has accrued supporting the hypothesis that reactive oxygen species may be an important cause of DNA damage leading to mutation (Weitzman & Stossel, 1982; Levin et al., 1982; Moody & Hassan, 1982; Storz et al.,

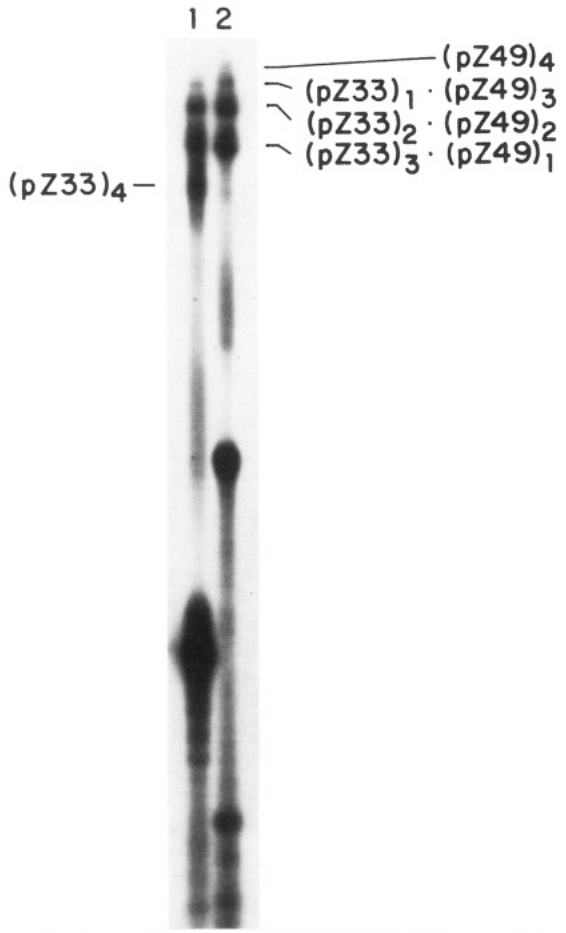


FIGURE 7: Autoradiogram of 5′-$^{32}$P-labeled pZ33 annealed with equimolar unlabeled pZ49 (lane 1) and 5′-$^{32}$P-labeled pZ49 annealed with equimolar unlabeled pZ33 (lane 2). The solutions were heated and cooled as described under Materials and Methods and electrophoresed through a 12% nondenaturing polyacrylamide gel.

1987; Ziegler-Skylakakis & Andrae, 1987; Loeb et al., 1988; Moraes et al., 1989, 1990). Several groups have identified $H_2O_2$/Fe, which generates hydroxyl radical (Cohen, 1985) and ferryl-oxo intermediates (Imlay et al., 1988), as a mutagen (Ziegler-Skylakakis & Andrae, 1987; Moraes et al., 1989; Akman et al., 1991). Our data (Akman et al., 1991) and those of Moraes et al. (1989) have demonstrated a mutational hot spot adjacent to bases 173–183 of the supF gene produced by the hydroxyl radical induced damage (see Figure 1). The mutations that clustered in this region included base substitutions and small deletions. Under the conditions we have employed, DNA strand cleavage by the hydroxyl radical has been shown to be randomly distributed in purified DNA (Tullius & Dombroski, 1986; Celander & Cech, 1990). If one assumes that this reflects a random distribution of hydroxyl radical induced damage in nuclear DNA, then the possibility that the hot spot for mutation reflects a local difference in susceptibility of DNA to damage induced by the hydroxyl radical must be considered unlikely. In fact, duplex DNA is known to be less susceptible to iron-induced free radical damage than single-stranded DNA (Massie et al., 1972). For this reason, one would expect that quadruplex DNA would offer similar protection against initial base damage induced by the hydroxyl radical. Therefore, the position of the hot-spot region more likely reflects a regional difference in the efficiency of one of the steps in the repair pathway initiated by hydroxyl radical induced damage. The hydroxyl radical associated hot-spot region has also been identified as a focus of UV light induced mutations in human fibroblasts and lymphoblasts (Brash et al., 1987; Seetharam et al., 1990) as well as in the CV-1 cell line (Hauser et al., 1986). This overlap suggests that the repair pathways for UV light induced, and hydroxyl radical induced promutagenic damage share one or more steps that can be perturbed in a sequence-specific fashion.

The predisposition to unusual secondary structure in DNA has been proposed as an important factor that might contribute to this sequence specificity (Todd & Glickman, 1982; Brash et al., 1987; Moraes et al., 1990). Previously suggested structures include regions of DNA predisposed to bending (Brash & Haseltine, 1982) or to the formation of hairpin loops or cruciforms (Todd & Glickman, 1982; Brash et al., 1987; Wells et al., 1988). However, enthusiasm for a role for these structures in hindering DNA repair has been tempered by the nonphysiologic conditions often required to form them in vitro and the limited data supporting their occurrence in vivo.

Our data do not demonstrate that quadruplex formation occurs in vivo. However, we have demonstrated here that quadruplex formation occurs at physiologic salt concentration and pH. More recently, we have observed quadruplex formation at room temperature (unpublished observations), as previously shown for telomeric DNA quadruplexes (Sundquist & Klug, 1989). Thus, the principal constraint on quadruplex formation in vivo would appear to be the local concentration of DNA, since the kinetics of formation would be expected to be fourth order in single-stranded DNA. This constraint may be satisfied during chromosome pairing at meiosis (Sen & Gilbert, 1988). If quadruplex formation were to hinder the repair process in pZ189, as suggested above, the local concentration of single strands present during the repair process would have to be sufficiently high to promote quadruplex formation. It is possible to envision the occurrence of high concentrations of single-stranded DNA during transfection of heavily damaged DNA and during plasmid replication in the nuclear compartment. Further experimentation will be necessary to address this question.

As noted above, runs of d(pGGG...) also occur near several other mutational hot spots in the supF gene. Other hot spots for mutation in human genomic DNA are also characterized by these runs. A number of these have been shown experimentally to adopt quadruplex conformation in vitro. For example, the site of point mutation at codon 12 of c-Ha-*ras* is adjacent to a sequence that is capable of forming a quadruplex (Smith et al., 1989). Sequences from the immunoglobulin switch region are also well-documented examples of quadruplex structure (Sen & Gilbert, 1988). While immunoglobulin switching involves recombination, the process is also known to generate microdeletions (Akira et al., 1987). Moreover, Chandley has speculated that hypervariability at sites of VNTR's (variable number of tandem repeats) may be attributed to their potential for quadruplex formation during meiosis (Chandley, 1989). The documented occurrence of quadruplex DNA in sequences corresponding to these sites of genetic activity suggests that experiments aimed at demonstrating the occurrence of quadruplex DNA during the process of mutagenesis in vivo will be informative.

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# Photooxidation of Specific Residues in α-Crystallin Polypeptides†

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ABSTRACT: Singlet oxygen is a biologically important, photochemically generated species that preferentially oxidizes His, Trp, and Met residues of protein molecules. Calf α-crystallin was photooxidized with use of *meso*-tetra(*p*-sulfonatophenyl)porphyrin (TPPS) and uroporphyrin (UP) as singlet oxygen generators. The effects of photooxidation were monitored by analyzing the changes in α-crystallin peptide maps obtained by reversed-phase HPLC using a photodiode array absorbance detector. The reaction led to the loss of six specific peptides, five of which contained photooxidizable residues. Peptides containing His-97 and His-154 from the A chain and Met-68 from the B chain are preferentially photooxidized, suggesting that those residues have access to singlet oxygen. Trp residues in the N-terminal region are converted to NFK, whereas Trp-60 in the B chain is not photooxidized strongly suggesting that the former are close to the surface of α-crystallin while the latter Trp residue is buried. Only one peptide that is lost from the peptide maps does not contain a photooxidizable group; however, this peptide does contain an apparently undigested Lys residue. It is suggested that it forms a cross-link with a photooxidized His residue.

Wavelengths of light greater than 293 nm are transmitted through the cornea, exposing the vertebrate eye lens to considerable, potentially deleterious far-UV irradiation (Taylor et al., 1988; Urbach, 1989; Lee, 1989; Frederick et al., 1989). It is well documented that such exposure initiates significant structural alterations to the lens proteins, including subtle conformational changes (Andley et al., 1984; Mandal et al., 1988; Andley, 1988) and photooxidation of reactive amino acid residues (Garcia-Castineiras et al., 1978; Inoue et al., 1982; Fujimori, 1982; Dillon et al., 1987; Tallmadge & Borkman,

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