The Effect of Ionic Strength on the Photosensitized Oxidation of d(CG)$_6$

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Abstract: An oligonucleotide-based model system was used to identify the parameters that influence the variable efficiency of DNA oxidation under biomimetic photosensitization. Mechanistic study of an acetone-mediated process revealed the dominance of a single pathway for reaction under all conditions examined. Excitation of acetone initiated an electron abstraction from DNA that induced an irreversible modification by molecular oxygen. The major products formed by this mechanism were characterized by chromatographic analysis and found to be consistent with base oxidation; scission of the DNA backbone was not evident. Rate measurements, essentially monitoring the first modification event of each oligonucleotide, suggested that conformation was not an important determinant of the overall photochemical efficiency. The duplex formed by d(CG)$_6$ was oxidized 4-fold more rapidly in the presence of 4.0 M NaCl than 0.1 M NaCl, yet this enhancement was not related to the B- to Z-helical transition occurring at approximately 2.5 M NaCl. Instead, the critical feature controlling this oxidative process was likely an affinity between DNA and sensitizer based on hydrophobic interactions; the noted rate increase could be explained by a local high concentration of acetone forced by the ionic medium to surround the nonpolar regions of DNA. Replacing acetone with more hydrophobic sensitizers, 2-butanone and 2-pentanone, respectively, enhanced the efficiency of DNA oxidation by 10-fold and 5-fold under 0.1 M NaCl. The relative salt dependence exhibited by these latter compounds also indicated that their association with DNA extended over the complete range of conditions studied.

The secondary structure of DNA is extremely polymorphic despite its limited constituents of phosphate, deoxyribose, and the four bases adenine, cytosine, guanine, and thymine. The intrinsic properties that characterize the most familiar forms of duplex DNA, A-, B-, and Z-helical structures, have been identified and corroborated as a result of extensive analysis.[1,2] Investigations have also recently grown to encompass the analysis of more unusual hybridization patterns as well.[3] While many of these studies have been performed in vitro, a notable conformational diversity seems equally apparent in vivo and, moreover, is thought to play a critical role in regulation and expression of cellular functions.[4] This microheterogeneity has also been used to direct sequence-specific reactions of DNA in vitro[5] and has been held responsible for causing hypermutability of certain sequencies in vivo.[6]

Sunlight and other sources of ultraviolet (UV) light commonly induce modification of nucleic acids in vivo and produce mutations that do not appear to be randomly distributed along a genome.[7] The exact molecular mechanism for the chemical and biological selectivity of this process is not well understood but is likely influenced by the variable structure of DNA. For example, sequence-specific conformations are known to promote selective association with reagents and guide modification directly or indirectly to the vicinity of binding.[5a–c] Additionally, certain hybrid structures may be inherently more reactive because their particular geometric disposition facilitates a specific type of reaction or provides greater access for solvents and reactive intermediates.[5d,e] A number of laboratories have begun to address these questions at a molecular level by in vitro study of natural DNA fragments and synthetic oligonucleotides.[8]

Our investigations to date have focused on the conformational specificity of photosensitized oxidation by determining the relative rates of nucleic acid modification under aerobic irradiation.[9] Acetone had been considered the optimum sensitizer for our investigation, since this simple carbonyl compound was thought to display no specific affinity for DNA. Our experimental system could then evaluate the geometric requirements of DNA reactivity in the absence of any associative or proximity effects. Under weak ionic conditions, this presumption might be accurate. Associative effects, however, are inescapable and strongly dominate the rate of modification as photolysis occurs at higher ionic strength or in the presence of increasingly hydrophobic sensitizers. Unlike intercalation[10] and hydrogen bonding,[5a] a general hydrophobic

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interaction is rarely presented as the primary variable affecting reaction of DNA.[5b,11] The hyperreactivity noted in the experiments described here is best explained by the formation of a concentration gradient of sensitizer engulfing the duplex structures of DNA. This hydrophobic aggregation occurs even for the relatively featureless and water-soluble compound acetone.

Experimental Section

Materials. All oligonucleotides were synthesized via standard solid-phase cyanoethyl phosphoramidite chemistry on Du Pont or Biosearch equipment. The self-complementary sequences with or without the terminal dimethoxytrityl group eluted anomalously under reverse-phase conditions; therefore, all DNA purification and characterization were carried out under strongly denaturing conditions (pH 12) by using anion-exchange chromatography (see below). Chelating resin, Chelex 100, was obtained from Bio-Rad and used as instructed by the manufacturer. All aqueous solutions were made with purified water (Nanopure, Sybron/Barnstead) and reagents of the highest commercial quality; NaCl, KCl, potassium phosphate, and HPLC-grade acetone (Fisher Scientific) were used without further purification, as were all other reagents not otherwise noted. 2-Butanone and 2-pentanone were distilled before use and all sensitizers were stored as aqueous solutions.

Sample Preparation and Irradiation Procedure. The oligonucleotide concentration was calculated from absorbance at 260 nm and the corresponding ϵ_{260} value estimated from the sum of nucleotide absorptivity as affected by the adjacent bases.[12] Just prior to irradiation, stock solutions of oligonucleotides were diluted to a final concentration (typically 6–7 μM) in 10 mM potassium phosphate, pH 7, and the indicated salt concentration. DNA was then annealed by placing the solutions into a water bath (Precision, Model 182) that was heated to 90 °C. After 3 min, the bath was turned off and the samples were allowed to cool along with the bath under ambient conditions. Once the samples returned to room temperature (>3 h), sensitizer was added (standard concentrations: acetone, 7.0 mM; 2-butanone, 2.0 mM; 2-pentanone, 1.4 mM). Aliquots (100 μL) were then irradiated at the focal point of a high-pressure xenon arc lamp (150 W, Photochemical Research Associates) using a simple Pyrex filter to absorb light below 290 nm. Although samples were not exposed solely to 290–310 nm, these wavelengths were crucial to the sensitization process. The light intensity in the region centering on 300 nm [200 J/(m^2 s)] was determined with a radiometer (Model 65A, Yellow Springs Instruments) placed at the exit of a monochromator (2-mm slits, Model B102, Photochemical Research Associates) that in turn was positioned equivalently to a photolysis sample.

Product and Rate Measurements. Reaction progess was measured discontinuously by quantitative anion-exchange chromatography (Mono-Q, Pharmacia) using a gradient of 0–560 mM NaCl in 11.5 mM NaOH over 55 min at 1 mL/min. Under these conditions, base oxidation and strand scission produced compounds that eluted earlier than the unmodified DNA.[9,13] Oxidized derivatives and starting material were then quantified individually by integrating their absorbance (254 nm) in the elution profile and comparing the resulting values to those produced by unirradiated samples. Consumption of starting material was monitored every 5–10 s during an irradiation period of less than 60 s and found to conform to an exponential first-order decay. This determination recorded the rate of incorporating the first oxidative hit to each oligonucleotide. To qualitatively identify the sites of DNA modification, additional samples were treated after irradiation with 0.2 M piperidine at 90 °C for 30 min. This exposure to alkaline conditions induced strand scission at the site of oxidation,[14] and the resulting series of fragments were also characterized under the same anion-exchange conditions. The heterogeneous duplex was prepared and photolyzed in a manner identical with that for d(CG)$_6$. Photochemical oxidation of this latter duplex was also monitored by the chromatographic procedure described above, since this allowed for resolution of each oligonucleotide strand and its oxidized products.[9]

Reaction Characterization and Quenching. To measure the solvent isotope effect on oxidative photolysis, standard samples containing DNA, buffer, and NaCl were dried under high vacuum (Speed-Vac concentrator, Savant). The residues were then dissolved in deuterium oxide (99.9%, Cambridge Isotope Laboratoy), and this cycle was repeated a second time before the solutions were finally annealed and photolyzed. The quenchers mannitol and glycerol were added to DNA samples after the annealing step and just prior to irradiation. The array of products formed under each condition of photolysis was also compared to the product profile formed by treatment of d(CG)$_6$ with hydroxyl radical generated by the decomposition of H_2O_2.[15]

The oxygen dependence of photolysis was measured in a parallel study using anaerobic and aerobic samples. Aliquots for both conditions were alternately placed under vacuum and argon for three cycles to remove much of the oxygen. For anaerobic analysis, irradiation proceeded directly following this treatment; for comparable aerobic study, DNA solutions were reequilibrated with air before irradiation. In each case, sample volumes were increased by 50% to adjust for solvent evaporation during this process. To retain a convenient concentration of sensitizer after the degassing procedure, only the least volatile sensitizer, 2-pentanone, was used. This sensitizer was added to produce an initial 1% solution but after exposure to high vacuum only ca. 0.01% (1 mM) remained for photosensitization.

Circular Dichroism Studies. Ellipticity measurements were recorded under ambient conditions on a Jasco J-20A spectropolarimeter and were used only to indicate the conformational shift of d(CG)$_6$ from a B- to Z-helical form. Solutions examined contained 9.6 μM oligonucleotide, 10 mM potassium phosphate, pH 7, and 0.1–5.0 M NaCl.

Results

Aerobic Modification of DNA. Anion-exchange chromatography, the analytical method central to this study, separates products and starting material on the basis of nucleotide length and content. Oxidative modification in the absence of strand scission causes the oligonucleotides to elute just minutes prior to the starting material.[9] When strand scission does follow oxidation, then the resulting fragments elute much earlier than the parent compounds. Under acetone sensitization, direct strand scission was evident only as a minor product of photolysis. Less than 10% of the products absorbing at 254 nm coeluted with either synthetic markers or scission products formed by reaction with the hydroxyl radical generated under Fenton chemistry.[15]

The major derivatives formed by sensitized irradiation of d-(CG)$_6$ all eluted slightly earlier than starting material. Further treatment of the photolyzed samples with piperidine demonstrated that the lesions formed by UV exposure also introduced an alkaline lability into the strands at the site of modification. The profile of fragments formed by this secondary conversion demonstrated that the photochemical oxidation did not occur at one predominant site. Instead, many sites were the target of the initial hit, as would be anticipated from the redundant nucleotide environments produced by the repetitive sequence of d(CG)$_6$.

The chemical and chromatographic characterizations of the major products are most suggestive of base oxidation, but sugar damage cannot be definitively ruled out. In a related study, the elution behavior noted above can be mimicked by the transformation of a thymidine residue within an oligonucleotide to its glycol derivative (data not shown). Furthermore, the pathway of sensitized modification described below appears to include an electron transfer from DNA to the excited carbonyl. The likely donor in this case would be the nucleotide base and not the deoxyribose moiety.

Chromatographic resolution of the unreacted starting material was crucial for measuring the consumption of the parent oligonucleotides.[16] To define this loss over time, individual samples were irradiated for specific intervals before chromatographic analysis. In every case, the concentration of starting material followed a first-order exponential decay over time of photolysis when compared to parallel determinations with unirradiated samples. Loss of the parent oligonucleotides depended strictly on continuous exposure to the xenon arc light. Oxidation of DNA also depended linearly on the concentration of sensitizer under conditions of both 0.1 and 4.0 M NaCl (range studied: acetone, 3–9 mM; 2-butanone, 0.2–3.0 mM; 2-pentanone, 0.8–2.0 mM).

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Table I. Effect of Various Additives on the Rate of $d(CG)_6$ Oxidation under Acetone-Sensitized Irradiation[a]

solvent	quencher	degradation rate[b] $k \times 10^3$, s^{-1} 0.10 M NaCl	4.0 M NaCl
H_2O		25 ± 5	75 ± 3
D_2O		11 ± 3	62 ± 10
H_2O	0.10 M mannitol	9.3 ± 2	29 ± 6
H_2O	10% glycerol	1.8 ± 0.6	1.3 ± 1

[a] All samples were annealed and photolyzed (λ >290 nm) under the standard conditions for discontinuous assay (see Experimental Section). [b] First-order rate constants for the initial oxidative modification of DNA were determined by using a linear least-squares analysis; uncertainties were based on the standard error.

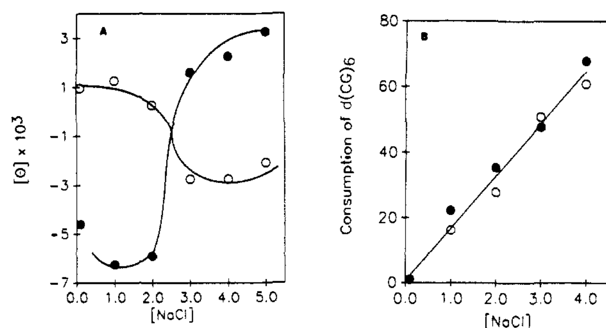

Figure 1. (A) Cooperative transition of $d(CG)_6$ between B and Z duplex structures as a function of NaCl concentration. Molar ellipticity is represented per mole of oligonucleotide[41] for measurements at 250 nm (●) and 290 nm (○). (B) Relative salt dependence of photosensitized DNA oxidation measured by using standard aliquots (100 μL) containing 6.4 μM $d(CG)_6$. Each sample was irradiated in the presence of 7.0 mM acetone for 15 s. Loss of starting material was then analyzed chromatographically as described in the Experimental Section and depicted relative to control samples detected under similar conditions.

Major Reaction Pathway of Photosensitized Oxidation. The chromatographic procedure above allowed for rate measurements as well as for characterization of products formed by photolysis. This analysis could not, however, be used to identify the prevailing chemical process stimulated by irradiation, since individual derivatives could be formed through multiple mechanisms. Instead, the nature of the dominant pathway was examined by a series of quenching studies. An obvious first experiment confirmed the importance of molecular oxygen in the overall process. When DNA samples were degassed prior to photolysis, the degradation of starting material slowed to less than 25% of that under related aerobic conditions. This residual rate may only reflect incomplete removal of oxygen.

Aqueous reactions that involve singlet oxygen are effectively promoted by substituting D_2O for H_2O; this change of solvent extends the lifetime of the excited-state species and increases the probability of collisions between the reactive intermediates and their targets.[17] Under acetone photosensitization, the decomposition of $d(CG)_6$ was not accelerated in the presence of D_2O (Table I). On the contrary, D_2O slightly inhibited the oxidative process. In addition, two standard free-radical traps,[18] mannitol and glycerol, significantly protected DNA from modification. These compounds are most commonly used to diagnose hydroxyl radical processes, but they may also act to inhibit a wider range of radical reactions. Any radical oxidation quenched by excess hydrogen atom donors might then also be controlled by the addition of mannitol or glycerol.

Oxidation Stimulated by DNA Conformation vs Salt Concentration. The study of a single self-complementary sequence, $d(CG)_6$, was used to assess the reactivity of both B- and Z-helical structures. This oligonucleotide spontaneously forms a B duplex structure under low salt conditions (e.g., 0.1 M NaCl) or a Z

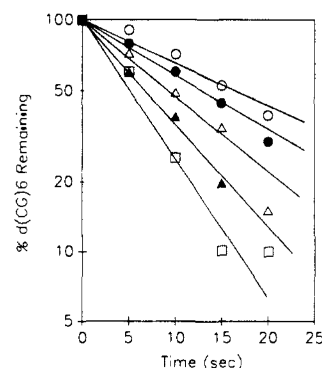

Figure 2. Initial rate of photosensitized oxidation of $d(CG)_6$ under increasing concentration of NaCl, 0.1 M (○), 1.0 M (●), 2.0 M (△), 3.0 M (▲), and 4.0 M (□), in the presence of 10 mM potassium phosphate, pH 7. Each data point is the average of two independent determinations of $d(CG)_6$ (initial concentration 6.5 μM) remaining after irradiation (λ >290 nm) in the presence of 8.4 mM acetone. All individual measurements were within 20% of the indicated average value.

duplex under high salt conditions (e.g., 4.0 M NaCl).[19] The equilibrium formed between these structures was confirmed in our laboratory by measuring the characteristic salt-dependent changes in the circular dichroic spectrum of $d(CG)_6$ (Figure 1A). The conformational shift implicit in Figure 1A is highly cooperative; the proportion of Z structure did not increase linearly with concentration of NaCl but instead exhibited a distinct transition point at ca. 2.5 M NaCl. No related pattern of reactivity was detected after photolysis (Figure 1B). Enhanced consumption of $d(CG)_6$ was noted at high salt concentrations, yet this resulted from only a linear dependence on salt concentration.

Rate studies were consequently performed to confirm and elaborate the origins of this unprecedented property of DNA photochemistry. Loss of $d(CG)_6$ over time of irradiation approximated an exponential decay under all NaCl concentrations studied (Figure 2), and again the photolytic efficiency increased significantly at high salt concentrations. Quenching studies were also repeated under 4.0 M NaCl (Table I) to check for any changes in the characteristic mechanism of oxidation. The general effects of D_2O, mannitol, and glycerol on the photochemical reaction were equivalent under both low and high concentrations of NaCl. The increased presence of salt must therefore stimulate the existing oxidative pathway and not supplement DNA modification by promoting secondary pathways.

Of course, the observed salt effect might have arisen if contaminants in the NaCl had controlled the rate of reaction. For example, traces of iron are well-known to stimulate DNA oxidation through a disproportionation of hydrogen peroxide and oxygen radicals.[20] This or other transition-metal-dependent reactions, however, could not have induced the hyperreactivity noted here. A stock solution of NaCl treated with Chelex resin to remove heavy metals induced the same rate acceleration as did untreated solutions of NaCl.

The Salt Effect Is Not Unique to $d(CG)_6$. A potential sequence or nucleotide contribution to the variable efficiency of oxidation could not be discounted merely because the specific B → Z transition did not coincide with the rate data. To extend our study beyond the limited composition of $d(CG)_6$, the photochemical oxidation of an unrelated and rather heterogeneous duplex was also measured under the same standard range of salt concentrations. The oligonucleotide d(CACGGGAACGATG) and its complement were annealed and irradiated under conditions identical with those described for $d(CG)_6$.[16] Once again, the consumption of each parent sequence over time of irradiation increased dramatically under high concentrations of NaCl. The rate constants, $k \times 10^3$, derived from the exponential loss of

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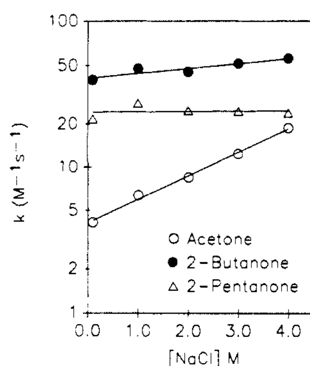

Figure 3. The photolytic efficiency for oxidative modification of $d(CG)_6$ depends on the ionic strength of the medium and the hydrophobicity of the sensitizer. First-order rate constants were measured from the exponential decrease of starting oligonucleotide [$d(CG)_6$, 6.5 μM] under conditions equivalent to those described in Figure 2. The second-order rate constants were obtained by dividing the first-order rate constants of DNA consumption by the concentration of sensitizer present: acetone, 8.40 mM; 2-butanone, 2.07 mM; 2-pentanone, 2.54 mM (determined spectrophotometrically). The logarithmic scale is used only to cover the great range in rate constants. Conversion of this figure to a linear scale or substitution of the molar values for activity values would demonstrate a similar relationship.

d(CACGGGAACGATG) rose from 8.5 s^{-1} to 35 s^{-1} when [NaCl] was increased from 0.1 to 4.0 M ([acetone] = 7 mM). The corresponding rate constants for the complementary strand grew from 13 s^{-1} to 41 s^{-1}.

The Salt Effect Is Not Unique to NaCl. Distinction between a specific effect of NaCl and the general influence of ionic strength was also necessary to determine the ultimate source for the activation of oxidative photolysis. KCl was used in place of NaCl during annealing and photolysis of $d(CG)_6$ in experiments parallel to those already described. The subsequent product profiles of sensitized irradiation ([acetone] = 7 mM) were chromatographically indistinguishable from those formed in the presence of NaCl. The first-order rate constant $k \times 10^3$ for $d(CG)_6$ consumption under 0.1 M KCl was 7.3 s^{-1} but increased to 42 s^{-1} under 3.7 M KCl. This salt once again mimicked the effects of NaCl during studies with a second sensitizer, 2-pentanone. Loss of $d(CG)_6$ in the presence of 1.4 mM 2-pentanone yielded first-order rate constants $k \times 10^3$ under the influence of 0.1 and 3.7 M KCl that were experimentally equivalent, 43 s^{-1} and 39 s^{-1}, respectively, but only coincidentally equivalent to the maximum rate of acetone.

Photochemical Efficiency Depended on a Hydrophobic Association between Sensitizer and DNA. Acetone is miscible in water and is generally described as quite hydrophilic. Under high ionic strength, however, acetone might likely be forced to seek a less polar environment, the interior surface of DNA for example. The photochemical dependence on salt could then originate from a variable association of DNA and acetone that is regulated by the ionic environment. More generally, the oxidation rate could be controlled by partitioning of sensitizer between bulk solution and nonpolar regions of DNA.

Evidence for this effect was gathered by repeating the photolysis of $d(CG)_6$ in the presence of alternative sensitizers, 2-butanone and 2-pentanone, that are closely related yet less polar than acetone. Product profiles induced by these compounds were invariant and equivalent to those dependent on acetone. Photochemical oxidation in the presence of 2-pentanone was also insensitive to the substitution of D_2O for H_2O at all NaCl concentrations studied, 0.1–4.0 M. Only the relative rate of DNA consumption was affected by the sensitizing species; this can be illustrated in a comparison of the second-order rate constants depicting sensitizer efficiency (Figure 3). These values were determined by measuring the first-order rate constant of $d(CG)_6$ consumption and dividing this by the sensitizer concentration. Even a slight increase in the sensitizer's hydrophobicity remarkably enhanced the rate of DNA oxidation. In the presence of 0.1 M NaCl, 2-butanone was almost 10-fold more active than acetone. The influence of ionic strength concurrently diminished for 2-butanone and was undetectable for 2-pentanone.

Discussion

Model for in Vivo Photochemical Oxidation. Oligonucleotide structures have been the focus of our continuing effort to determine the parameters affecting nucleic acid modification. Sequences 10–20 nucleotides in length form many of the same conformations found in polynucleotides but retain a minimum of the heterogeneity associated with larger fragments. Still, redundant sequences such as $d(CG)_6$ may react from an equilibrium of hybridized species.[13] The polymorphic nature of $d(CG)_6$ proved advantageous for the photochemistry described here, since this sequence could form two of the most divergent secondary structures of DNA, B and Z helixes.[19] Correlations between conformation and reactivity could therefore be drawn from a single sequence; only a change in the ionic strength of the medium was needed to control the duplex structure.

Irradiation conditions were chosen to model and, moreover, to accentuate the possible geometric requirements of forming a premutagenic lesion under natural conditions. Ultraviolet exposure was limited to wavelengths of greater than 290 nm to mimic the terrestrial exposure from sunlight. In addition, oxidative photosensitization was studied, since this has been shown to play an increasing role in cellular photochemistry as incident light shifts away from the absorbance maximum of DNA.[21] The photochemical properties of the model sensitizer, acetone, could exhibit a composite of the activities found in vivo; its excited state can transfer energy directly to the aromatic bases of DNA or to oxygen and can also participate in electron-transfer reactions.[8b,22] Most importantly, this small and hydrophilic compound was expected to minimize any specific association with DNA and thus allow for modification to be potentially controlled by the geometry of the helix.

Single-Hit Analysis for Comparing DNA Reactivity. Numerous unique and redundant sites for photochemical oxidation occur along each DNA strand. Significant enhancement in the reactivity of any one of these sites might alone cause the hypermutability of an entire gene in vivo; modification is naturally a very infrequent event when normalized against the total nucleotides available for reaction. Conditions affecting only this initial process were highlighted here by measuring the primary rate of oxidative modification. This study, essentially a single-hit analysis, assured a proper comparison between the structures of interest and avoided potential complications that would arise from the heterogeneity of DNA once modified by an initial reaction.

The geometric requirements for a variety of DNA reactions have been traced in a complementary approach by identifying the exact structures of the major products.[23] This type of determination, however, necessitates extensive conversion at many sites along the target sequence, and therefore the relative reactivity of a sequence can only be described in average or overall terms. Our measurements record the first detectable modification precisely yet provide only general information on the nature of the products formed. Under irradiation conditions outlined here, oxidative base modification appeared to completely dominate the reaction profiles, much as it had for oligonucleotides of more diverse sequence.[9] In addition, the exact array of products formed during irradiation of $d(CG)_6$ was equivalent regardless of the ionic conditions or whether photolysis was dependent on acetone, 2-butanone, or 2-pentanone. Only a kinetic analysis could then have uncovered the salt dependence of sensitized oxidation, and this was only possible by quantifying the remaining starting material once separated from oxidized products.

Dominant Mechanism of DNA Oxidation. The criteria used to distinguish a hyperreactive target were set necessarily by the

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specific reactions generated in our modification study. The overall degradation of DNA was found to be highly dependent on molecular oxygen. This in turn suggested the importance of one or more of the following mechanisms:[24] (1) excitation of oxygen to its singlet state for subsequent cycloaddition to guanine residues, (2) formation of oxygen radicals for attack on DNA, or (3) generation of radicals at the nucleotide bases for coupling to molecular oxygen. Singlet oxygen was not a likely participant in the oxidation of DNA, since the presence of D_2O did not facilitate the loss of $d(CG)_6$ (Table I). A small decrease was noted instead for the sensitized oxidation in D_2O vs that in H_2O. The basis of this isotope effect is not well understood but was also evident during the photolysis[9] and radiolysis[24b] of other nucleic acids.

Mannitol and glycerol unequivocally reduced the efficiency of DNA degradation and thereby demonstrated the importance of free-radical processes. Even though these reagents are thought to be diagnostic of hydroxyl radical intermediates, they are believed to deactivate other radical species during the acetone sensitization described here. The photolysis was inconsistent with hydroxyl radical reaction, since product profiles formed after UV exposure did not resemble those following treatment with hydroxyl radical generated under ground-state conditions. Strand scission was not even detected directly after sensitized irradiation. The most likely path of initial DNA modification then involves a reversible electron transfer from the nucleotide bases to an excited state of acetone, and this is followed by an irreversible modification of the DNA radical with molecular oxygen.[24a] Mannitol quenching, for example, could be expected to inhibit this mechanism by back-donating a hydrogen to prevent the nucleotide base from coupling with oxygen.

As long as the electron-transfer process remained dominant, the specific requirements of this pathway would command the overall response to variations in solvent and DNA structure. Otherwise, a rate enhancement could result from the acceleration of supplementary or competing reactions. These latter alternatives were unlikely in this study, since the general effect of quenchers on the rate of oxidation remained constant for irradiation under both 0.1 and 4.0 M NaCl (Table I). Furthermore, the array of compounds identified chromatographically did not indicate a change in the reaction characteristics. The accelerated loss of DNA under high salt concentrations likely illustrates only intrinsic gains in the efficiency of the predominant mechanism for photosensitized oxidation.

Salt Concentration and Not DNA Conformation Mediated the Rate of Photochemical Modification. Crystallographic analysis of B and Z helixes have clearly defined the atomic details of these contrasting structures.[1,25] This information has been used to both predict and explain the different propensity of these forms to undergo covalent modification under a variety of conditions.[5d,e,26] The photochemical oxidation of these structures was examined here with a model oligonucleotide, $d(CG)_6$, that forms a B or Z helix depending on the ionic conditions of the medium. As illustrated in Figure 1A, the B to Z transition of the duplex formed by $d(CG)_6$ was nonlinear and highly cooperative over the range of 0.1–5.0 M NaCl. The photochemical reactivity of this sequence, however, increased linearly over the same conditions and did not manifest any of the sigmoidal behavior of the conformational transition. In addition, acetone-sensitized oxidation of the duplex formed by d(CACGGGAACGATG) and its complement was regulated by NaCl concentration in a manner similar to that exhibited by $d(CG)_6$. The salt effect must then be based on properties other than symmetry, composition, or structure of duplex oligonucleotides.

An Ionic Basis for the Effect of NaCl. Transition-metal contaminants could not have regulated the photochemical oxidation, since purification of commercial NaCl did not diminish the salt dependence of photolysis. Instead, the linear correlation between NaCl concentration and photochemical efficiency assured a role for salt as a determinant of oxidation. The ionic basis of this effect was only demonstrated once further structural effects were discounted. Na^+ and K^+ differentially affect both the properties of solvent[27] and the structure of DNA,[28] yet their contribution to the overall ionic strength of the medium is equivalent. An increase in the concentration of KCl from 0.1 to 3.7 M stimulated the consumption of $d(CG)_6$ by over 4-fold, and KCl behaved equivalently to NaCl in every way. These similarities suggest that ionic strength, the common variable controlled by each salt, is the critical parameter controlling the photolytic efficiency of DNA oxidation.

Ionic strength is still not expected to affect most of the processes that contribute to the overall rate of sensitized reaction. For example, the light energy absorbed during each irradiation should remain constant, since the absorptivity of the sensitizer is unlikely to change under the conditions studied. Furthermore, the dissolved ions cannot substantially enhance the overall reaction by promoting the intersystem crossing of photoexcited acetone; the quantum yield of this process already approaches unity.[29] Finally, the ambient concentration of oxygen in air-saturated buffer decreases under high salt concentrations,[30] and this should tend to decrease rather than increase the rate of oxidation. The only salt effect likely to facilitate photolysis would be the maintenance of a local high concentration of sensitizer around DNA. NaCl or KCl may salt out acetone and force this normally hydrophilic sensitizer to partition more readily into the hydrophobic regions of DNA.

Photochemical Efficiency Mediated by Hydrophobic Association. Most discussions concerning the effect of various salts on the nature of DNA have rightly focused on the polyanionic character of the phosphate–deoxyribose backbone.[31] For example, ionic interactions have been used to build a theoretical basis for understanding the relative stability of B and Z helixes.[32] A second and equally important property of DNA relies on the hydrophobic regions formed by base stacking within duplex structures. A wide variety of large planar compounds intercalate between bases quite readily, and some may react selectively once bound in this lattice.[33] Small molecules may also seek out the hydrophobic recesses of DNA.

The critical determinant of DNA photosensitization was most apparent when the photochemical efficiencies of acetone, 2-butanone, and 2-pentanone were compared. These sensitizers were equivalent in almost every way[34] except for their hydrophobic nature. A single additional methylene unit drastically affects the sensitizers' polarity. The octanol/water partition ratio is 1.0/1.7

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for acetone but quickly rises to 1.8/1.0 for 2-butanone.[35] If indeed the photochemical process is driven by a hydrophobic association between sensitizer and DNA, then a sensitizer of lower polarity should induce a higher rate of oxidation concomitant with the increase in its local concentration around the target. Furthermore, the overall salt dependence of photolysis should decrease as more sensitizer associates with DNA under low ionic strength conditions.

Both predictions were confirmed by the action of 2-butanone and 2-pentanone (Figure 3). 2-Butanone was approximately 10-fold more efficient as a photosensitizer than acetone under 0.1 M NaCl, and this may suggest that the local concentration of 2-butanone surrounding DNA was also 10-fold greater. In addition, the aggregation of 2-butanone around DNA was likely close to a maximum under low salt conditions, since additional salt only facilitated DNA oxidation by 40%. This relative activation is only a fraction of that displayed by acetone. The lack of salt dependence for 2-pentanone consequently suggested that a locally high concentration of this sensitizer was established under all salt concentrations studied.[36] The photochemical efficiency lost in the substitution of 2-pentanone for 2-butanone was likely unrelated to an association phenomenon and more probably due to a quenching mechanism available in the presence of a γ-hydrogen proximal to the excited carbonyl.[37]

Rate accelerations driven by hydrophobic effects have been noted previously,[38] but these have rarely been considered for reactions between DNA and compounds as small and nondescript as acetone. From our photosensitization experiments, this compound might have exhibited little affinity for DNA under minimal salt concentrations; however, a hydrophobic interaction between acetone and DNA quickly came to dominate the photochemical process at higher ionic strength. The magnitude of this effect is particularly striking when considered against the gradient of charge density that simultaneously builds in the vicinity of DNA as the salt concentration increases.[39] The local regions along the groove surface of the DNA, however, may still offer the organic compounds their only refuge from the highly charged environment.[40]

Conclusion

Photochemical oxidation of DNA described in this study was rather insensitive to the geometric constraints imposed by oligonucleotide conformations in solution. The leading parameter affecting the efficiency of photolysis originated instead from an association between DNA and sensitizer. An increase in the ionic strength conditions of photolysis or a decrease in the polarity of the sensitizer enhanced the rate of reaction through the formation of a local high concentration of sensitizer around the hydrophobic regions of DNA. The fundamental importance of this aggregation was demonstrated by its occurrence with the extremely nonspecific sensitizers acetone, 2-butanone, and 2-pentanone. If 2-butanone truly acted from a complex with DNA as suggested here, then perhaps most uncharged organic compounds may react with DNA once accommodated on the target surface.

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Supplementary Material Available: Sample chromatograms used to monitor the time-dependent photosensitized oxidation of duplexes formed by $d(CG)_6$ and by the heterogeneous sequences and first-order kinetic analysis for oxidation of the heterogeneous duplex (3 pages). Ordering information is given on any current masthead page.

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(36) Neither acetone nor 2-pentanone demonstrated any dependence on NaCl when parallel photochemical experiments were repeated with d(CG). In addition, the photochemical efficiency of d(CG) oxidation in the presence of 2-butanone (5 mM) decreased to less than 5-fold that in the presence of acetone (5 mM) (S. Dobbins and S. E. Rokita, unpublished observation). These data suggest that the association of sensitizer and DNA described in the text requires at least some degree of organized secondary structure.

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