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Oxidative DNA Strand Scission Induced by Peptides

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Summary

Cellular oxidative stress promotes chemical reactions causing damage to DNA, proteins, and membranes. Here, we describe experiments indicating that reactive oxygen species, in addition to degrading polypeptides and polynucleotides through direct reactions, can also promote damaging biomolecular cross reactivity by converting protein residues into peroxides that cleave the DNA backbone. The studies reported show that a variety of residues induce strand scission upon oxidation, and hydrogen abstraction occurring at the DNA backbone is responsible for the damage. The observation of peptide-promoted DNA damage suggests that crossreations within protein/DNA complexes should be considered as a significant cause of the toxicity of reactive oxygen species.

Introduction

Oxidative stress degrades the biopolymers that drive and support cellular function. Damage to DNA, proteins, and lipids is known to result from oxidative stress because radicals or reactive species generated from oxygen can react with all types of biomolecules [1–3]. DNA degradation is the most deleterious type of oxidative damage—and a contributor to human disease and aging—given the importance of maintaining the genetic information stored in this biopolymer [4]. However, protein and lipid damage can also perturb cellular function and has been implicated in several pathologies including arteriosclerosis and cataract formation [5–9].

Singlet oxygen ($^1O_2$), a cytotoxic and genotoxic species, is a potent oxidant and inducer of oxidative stress [2, 3]. This damaging species is effectively utilized in anticancer chemotherapy [10] and may also be produced at low levels during normal cellular function [11–13]. The damage of biomolecules by $^1O_2$ is attributed to direct reactions with conjugated functional groups. For example, the oxidation of the aromatic DNA bases is the main source of $^1O_2$-mediated DNA damage [14, 15]. Guanine is particularly susceptible to $^1O_2$-induced damage; the mutagenic base lesion 8-oxoguanine (along with further oxidized products) are formed at these sites and contribute to the genotoxicity of $^1O_2$ [16]. Oxidation of aromatic and sulfur-based amino acids represents the main pathway for the damage of proteins by $^1O_2$. Tyr, Trp, His, Cys, and Met are the primary sites of oxidation within proteins, with Tyr, Trp, and His forming endo- or hydroperoxides (Figure 1). In contrast, Cys and Met form zwitterionic intermediates and sulphoxides upon oxidation with $^1O_2$ [5, 17–26].

Here, we describe a series of experiments revealing an interrelationship between $^1O_2$-induced protein and DNA damage: DNA strand scission promoted by oxidized amino acids. Previous studies established that a subset of amino acids react readily with $^1O_2$ to form peroxides [17–23], and our work indicates that these species promote strand breakage when presented to DNA in the context of cationic peptides. The observation of peptide-promoted DNA damage implies that crossreactions within protein/DNA complexes should be considered as a significant cause of the toxicity of reactive oxygen species.

Results and Discussion

Investigation of Oxidative DNA/Amino Acid Crossreations

A series of DNA binding tripeptides were tested for DNA-cleavage activity with a plasmid cleavage assay to evaluate crossreactions between DNA and amino acids under oxidizing conditions (Figure 2). Rose bengal (RB) was used as a source of photosensitized $^1O_2$ [27]. The tripeptides featured glycine (G), cysteine (C), methionine (M), phenylalanine (F), histidine (H), tryptophan (W), or tyrosine (Y) flanked by two cationic lysine (K) residues that increase DNA affinity. Significant levels of strand scission were observed with KCK, KHK, KYK, and KWK in the presence of $^1O_2$, with the W-containing peptide producing the highest cleavage levels as determined by the generation of nicked circular DNA from supercoiled DNA. With extended reaction times, linear DNA was also generated by oxidized KWK. In contrast to the alkaline-labile base damage typically caused by exposure of DNA to $^1O_2$ [28], the DNA damage observed in the presence of oxidized amino acids reflects direct single-stranded breaks that do not require basic conditions or heat for visualization.

The four amino acid residues (W, Y, C, and H) that exhibited DNA-cleavage activity in the presence of $^1O_2$ are known to react efficiently with this oxidant to produce peroxides [17–23]. We used tryptophan, which produced the highest DNA-cleavage yields, to characterize the role of $^1O_2$ and probe whether peroxides were involved in the DNA-cleavage chemistry. The essential role of $^1O_2$ in the cleavage reaction was confirmed by monitoring DNA-cleavage efficiency in the presence of KWK under a variety of conditions that should alter $^1O_2$ levels (Figure 2B). Decreased cleavage was observed in the presence of NaN_3 (a singlet oxygen quencher) [29] and when O_2 levels were reduced by flushing samples with Ar. Additionally, increased cleavage was observed when the reaction was carried out in D_2O, a solvent that increases the lifetime of $^1O_2$ [30]. These results strongly suggest that the production of $^1O_2$ is required for DNA scission.

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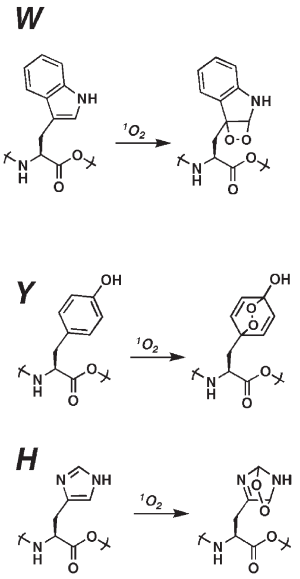

Figure 1. Examples of Amino Acid Peroxides

The amino acids W, Y, and H have been shown to react with $^1O_2$ to form dioxetane intermediates in addition to other products including peroxyl radicals and hydroperoxides [17, 19, 21, 23, 26].

Solutions containing oxidized peptide were prepared and then added to DNA to confirm that $^1O_2$ generated the reactive amino acid peroxides but did not participate in the DNA-cleavage reaction directly. As $^1O_2$ has a microsecond lifetime, there would not be any present in the solution added to induce strand scission. Indeed, the introduction of KWK subjected to peroxidation prior to incubation with plasmid DNA did cause DNA cleavage (Figure 2C). When $^1O_2$ was generated in the presence of KWK, the introduction of oxidized amino acid to DNA caused strand scission at levels that depended on the $^1O_2$ dose. More efficient DNA cleavage was observed when the peroxide was generated in methanol rather than H_2O, which is consistent with previous findings of increased levels of indole endoperoxides in methanol [31]. The addition of ascorbyl palmitate (a peroxide scavenger) [32] to the peptide solution after $^1O_2$ exposure caused the cleavage yield to decrease significantly. Taken together, these results are consistent with a mechanism in which an amino acid peroxide is formed upon reaction with $^1O_2$ and subsequently causes DNA strand scission.

Parameters Modulating Amino Acid-Promoted DNA Strand Scission

Although several amino acids produced appreciable levels of DNA cleavage when oxidized, the efficiency of strand scission caused by different side chains varied. A number of parameters could affect the cleavage reaction, including the efficiency of peroxide formation and the stability of the amino acid peroxide formed. Yields of amino acid peroxides formed upon exposure to $^1O_2$ were monitored with a modified FOX assay [33] (Figure 3A). This analysis revealed that the yields of peroxide for the different amino acids decreased in the following order: W > C $\geq$ Y >> H. W appears to produce the highest peroxide levels detectable with this assay, whereas H exhibits the lowest. However, experiments monitoring the oxidative consumption of the four amino acids after $^1O_2$ exposure showed that H and C were most efficiently oxidized, followed by W and Y (Figure 3B). These findings lead to the conclusion that H and C react with $^1O_2$ efficiently but form peroxides or other products that degrade rapidly and are not detected by the FOX assay. The lower levels of DNA cleavage observed with these residues relative to W indicate that instability of the oxidation products or existence of side products may limit reactivity with DNA. Furthermore, Y exhibits a low level of oxidative degradation but a high level of peroxide formation and moderate level of DNA cleavage, again highlighting that generation of a stable peroxide promotes DNA cleavage. These results indicate that DNA damage induced by oxidized amino acids depends strongly on the chemical properties of residues located proximal to DNA.

Mechanism of Peptide-Promoted DNA Cleavage

The reaction of an oligonucleotide duplex with oxidized KWK was monitored to obtain information concerning the mechanism of the oxidative DNA cleavage observed in the presence of peptides (Figure 4). The analysis of DNA samples exposed to $^1O_2$, $^1O_2$ + KGK, or $^1O_2$ + KWK by polyacrylamide gel electrophoresis (PAGE) revealed direct DNA damage only when the peptide containing W was present (Figure 4A, lanes 5–7 and Figure 4B), consistent with plasmid cleavage results shown in Figure 2. The oligo duplex cleavage observed by PAGE was random, occurred at all types of nucleotides, and was visible without sample workup. These observations are consistent with the occurrence of direct strand breaks resulting from scission of the sugar-phosphate backbone.

The chemical identity of the 3′ termini of damaged oligonucleotide fragments was investigated to elucidate the mechanism of the direct strand scission. The 3′-phosphatase activity of T4 polynucleotide kinase (PNK) was harnessed in order to test whether 3′-phosphates were present on the termini of the DNA fragments produced by oxidative cleavage. This type of analysis provides information about the cleavage mechanism because 5′-hydrogen abstraction generates free 3′-phosphates exclusively [34], whereas abstraction of the 2′-, 3′-, or 4′-hydrogen atoms from the deoxyribose units of the DNA backbone produces blocked 3′-phosphate species that are not amenable to the phosphatase activity of T4 PNK [35–37]. Both free and blocked products are observed with 1′-hydrogen abstraction [35]. However, abstraction at the 1′ position initially generates intermediates that bear sugar fragments rather than terminal phosphates. These fragments are cleaved only upon heat treatment or workup under basic conditions [35]. Typically, abstraction of the 1′-hydrogen from DNA by small molecules is not favorable because of the low solution accessibility of this position along the sugar-phosphate backbone [38].

Analysis of the oligonucleotide termini generated by the reaction between oxidized KWK and DNA yielded results consistent with the presence of a 3′ phosphate

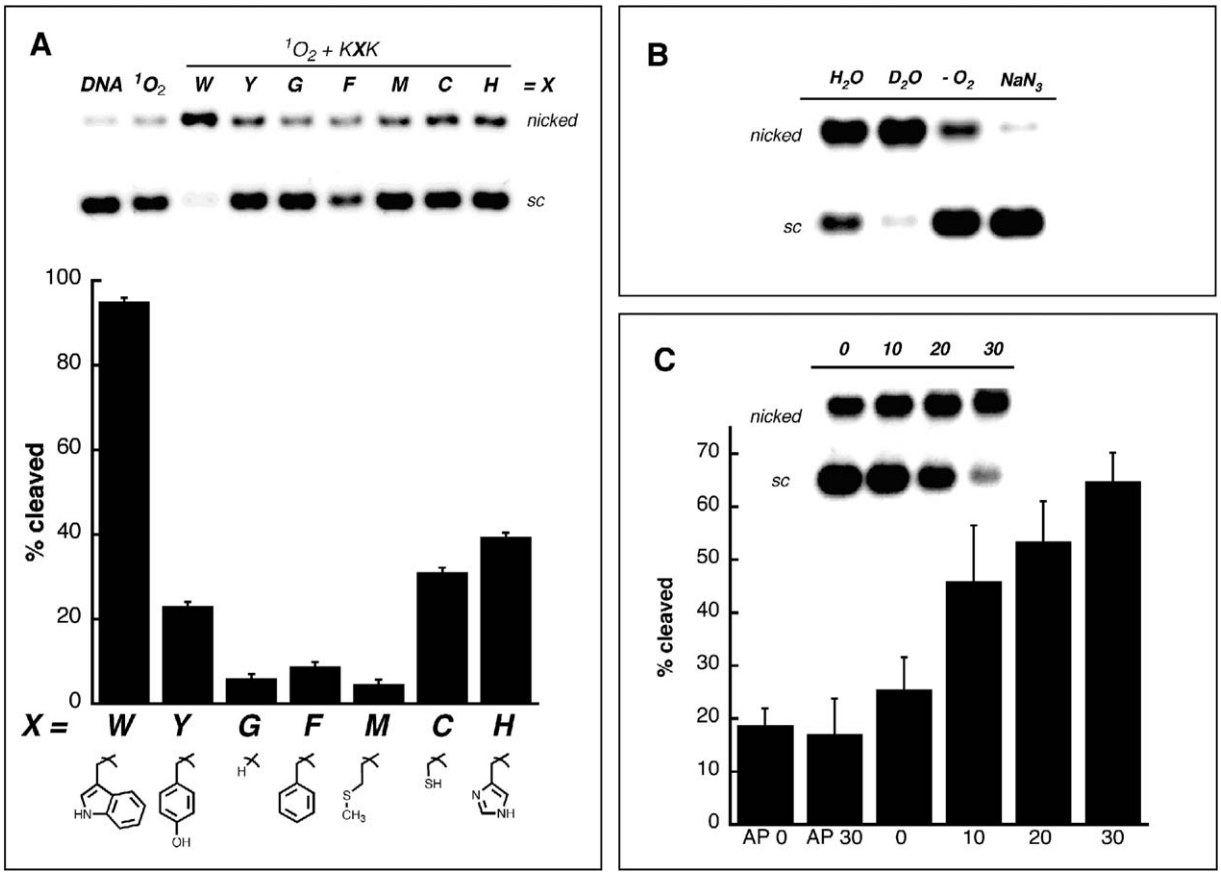

Figure 2. DNA Cleavage by Oxidized Amino Acids

(A) Cleavage efficiencies for solutions containing plasmid DNA and tripeptides (KXK, where X = G, C, M, F, H, W, or Y) after exposure to photogenerated $^1O_2$ for 4 min. Conversion of supercoiled (sc) to nicked circular plasmid is monitored by agarose gel electrophoresis as shown at the top of the figure.

(B) Involvement of $^1O_2$ in W-promoted DNA cleavage. Lane 1 contains a sample of plasmid DNA and KWK incubated under standard conditions, and lanes 2–4 are identical samples containing D_2O (lane 2), reduced O_2 level (lane 3), and NaN_3 (lane 4).

(C) DNA cleavage because of preformed KWK peroxides. Samples were irradiated for varying amounts of time (see Supplemental Data for plot of peroxide levels at the same time intervals) and subsequently incubated with DNA. Ascorbyl palmitate (AP) was added after irradiation to scavenge peroxides.

generated by 5′-hydrogen abstraction. When DNA oligonucleotide samples were exposed to $^1O_2$ + KWK and directly analyzed or treated with T4 PNK, shifts in the gel mobilities of the fragments were observed (Figure 4C). To assist in the assignment of the products observed, we also treated control samples of the same DNA duplex with either DNase I [39] or bleomycin [40] to create 3′-OH or 3′-phosphoglycolate termini, respectively. Damaged fragments produced from the reaction of DNA with oxidized KWK did not migrate with the faster-

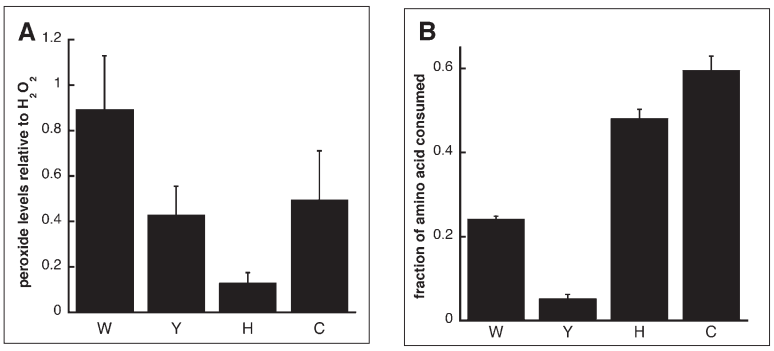

Figure 3. Analysis of Oxidative Degradation and Peroxidation of Amino Acids Displaying DNA-Cleavage Activity

(A) Levels of amino acid peroxides as detected with a modified FOX assay. Control samples containing FOX reagents were used for background correction. Results are reported relative to peroxide levels detected in a solution containing 200 μM H_2O_2.

(B) Oxidative degradation of amino acids upon exposure to $^1O_2$. Amino acid consumption was analyzed by HPLC after peptide samples were exposed to $^1O_2$.

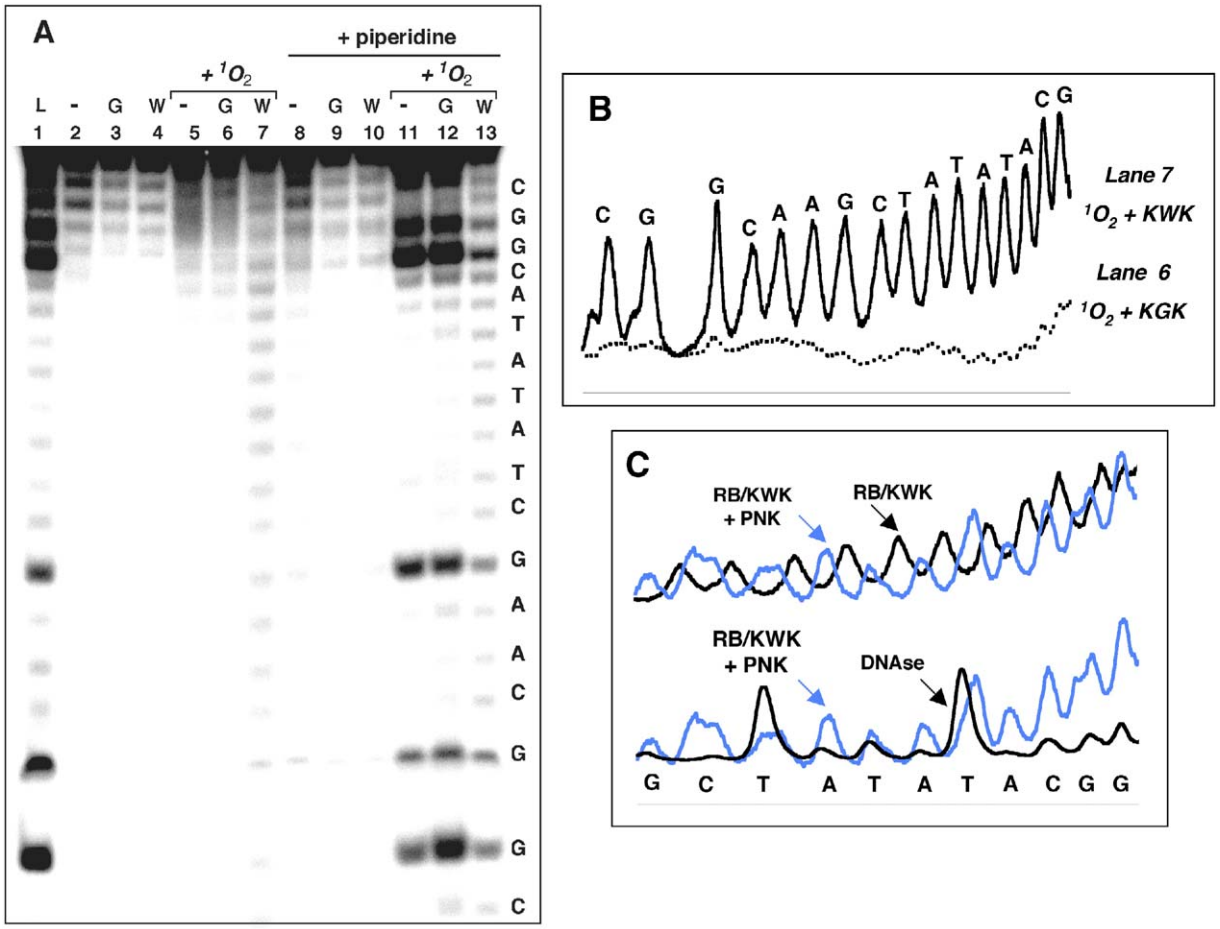

Figure 4. PAGE Analysis of DNA Oligonucleotide Cleavage by $^1O_2$ Alone and in the Presence of KWK or KGK

(A) 20% denaturing PAGE of a DNA oligonucleotide duplex (^{32}P-5′-ATAGTACGGCAAGCTATATACGGCTCGT-3′) exposed to $^1O_2$ only (lanes 5 and 11), $^1O_2$ + KGK (lanes 6 and 12), or $^1O_2$ + KWK (lanes 7 and 13). Lanes 2–4 and 8–10 contain controls samples that were identical to 5–7 and 11–12, respectively, but were not exposed to light and do not contain $^1O_2$. Lanes 8–13 were treated with piperidine. Lane 1 is a sequencing ladder (L) showing the locations of guanines within the sequence.

(B) Histograms illustrating direct cleavage of DNA oligonucleotide by oxidized KWK versus KGK extracted from gel shown in Figure 3A.

(C) Histograms representative of PAGE experiments investigating the 3′-termini of DNA oligonucleotide cleavage by $^1O_2$ and KWK, both with and without T4 PNK (top). Histograms obtained from DNA/KWK/$^1O_2$ samples show retardation of fragment mobility after T4 PNK treatment, a result of the loss of terminal 3′-phosphate. (Bottom) T4 PNK-treated DNA/KWK/$^1O_2$ samples comigrate with DNA digested with DNase I, illustrating the presence of a 3′-hydroxyl on the samples exposed to kinase. A representative gel showing the data obtained from these experiments is provided as Supplemental Data.

moving fragments generated by bleomycin that represent 3′-phosphoglycolate products of 4′-hydrogen abstraction. However, the fragments produced by the reaction with oxidized KWK migrated faster than fragments generated by DNase I treatment, suggesting that the termini of the damaged products carry an additional phosphate. Indeed, upon treatment with T4 PNK, the mobility of these same fragments was comparable to that generated by DNase I treatment (Figure 4C). Therefore, these results confirming the presence of a 3′-phosphate present two possible reaction mechanisms: oxidative DNA-cleavage activity promoted by KWK by either 1′- or 5′-hydrogen abstraction. Abstraction at the 1′ position is less likely considering that the complete conversion of the initial product of this reaction pathway would require heat or basic conditions in order to generate free 3′-phosphates. Our results are consistent with the presence free 3′-phosphates in the absence of sample workup or basic conditions, and, therefore, it is most probable that oxidized KWK induces DNA damage through initial 5′-hydrogen abstraction.

Backbone-Cleaving Peptides Suppress DNA Base Damage

Although the KWK tripeptide induces DNA damage by reacting with the DNA backbone upon oxidation, it also suppresses levels of base damage caused by direct reaction of $^1O_2$ with DNA (Table 1). Levels of guanine oxidation in DNA duplexes exposed to $^1O_2$ and KGK or KWK were analyzed by treatment of these samples with piperidine before gel electrophoresis analysis (Figure 4A, lanes 11–13). The presence of the KGK peptide had no effect on the damage produced by $^1O_2$, with high

Table 1. Quantitation of Amino Acid-Induced DNA Cleavage

	% DNA Cleaved[a] (Direct Strand Breaks)	% DNA Cleaved[b] (Piperidine Labile)	% Damage Represented by G Oxidation
RB + KGK	0 ± 1	29 ± 3	21 ± 4
RB + KWK	5 ± 2	14 ± 8	9 ± 3

[a] DNA cleavage visualized by PAGE (see e.g., Figure 4A) that resulted from direct strand breaks was calculated by determining the percent of fragmented DNA in comparison to the total amount of DNA in each sample lane after background subtraction.
[b] Damage represented by guanine oxidation was determined for piperidine-treated samples by calculating the percent of damage associated with scission at guanine residues in comparison to the total damage for each sample. Results represent > 3 sets of data from individual trials.

levels of damage apparent at all guanine nucleotides. In contrast, samples containing KWK displayed levels of guanine-specific damage that were lower relative to those observed with $^1O_2$ or $^1O_2$ + KGK (Table 1). Therefore, amino acids like tryptophan may constitute a molecular "double-edged sword" that can both suppress and induce DNA damage. The fact that the spectrum of oxidative DNA damage produced is strongly affected by the presence of a bound peptide presents the possibility that damage patterns for DNA-protein complexes will deviate significantly from those obtained with free DNA molecules.

Significance

The experiments described here are the first to demonstrate that a variety of natural amino acids can promote DNA strand scission upon oxidation. These results build upon our prior studies of a family of peptide-intercalator conjugates that exhibited DNA photocleavage activity when amino acid residues susceptible to peroxidation were present [41]. Reports of DNA cleavage induced by other types of peroxides, e.g., the hydrodioxyl radical [34] or thermally generated amidinopropane-derived peroxide radicals [34, 42], provide precedents for direct strand scission by these chemical species. Our results are also consistent with the prior observation of protein-induced DNA damage promoted by γ-irradiation [43–45]. In this case, protein peroxides were also implicated in strand scission. Although the pathways leading to oxidative DNA damage have been characterized thoroughly [46], damaging reactions are typically considered for uncomplexed molecules, i.e., DNA without associated proteins. In the cell, the majority of DNA is protein bound; therefore, possible crossreations between amino acids and DNA under conditions of oxidative stress are a relevant consideration. Nucleic acids and protein damage may therefore be interrelated, with oxidative reactions converting specific amino acids into species capable of severing the DNA backbone.

Experimental Procedures

Materials

Rose bengal, xylenol orange, and ammonium iron(II) sulfate hexahydrate were purchased from Aldrich Chemical. Sodium azide was purchased from Sigma. Hydrogen peroxide was purchased from Acros. Peptide reagents were purchased from Advanced Chem Tech.

Peptides were synthesized on solid support with commercially available Wang-Fmoc-Lys(Boc) (0.7 mmol/g, Advanced ChemTech) or Wang-Fmoc-Ala (0.7 mmol/g, Advanced ChemTech). Couplings were performed with four equivalents of Fmoc protected amino acid, four equivalents of HBTU, and eight equivalents of diisopropylethylamine in DMF for 3 hr. Deprotection of the Fmoc group was achieved with 20% piperidine in dimethylformamide (DMF) for 30 min (after the coupling of the first amino acid to the resin, Fmoc deprotection was achieved with 50% piperidine in DMF for 5 min in order to minimize diketopiperazine formation). The peptides were cleaved from the resin and deprotected with a solution of trifluoroacetic acid (TFA):H_2O:triisopropylsilane: triethylsilane 9:1:1:1 for 30 min. The solution was concentrated under reduced pressure in the presence of toluene in order to remove any residual TFA. Alanine-containing peptides were purified by reversed-phase HPLC with an aqueous solution buffered with 0.1% TFA and linear gradients of a mixture of 80:20:0.1 acetonitrile:water:TFA. The identities of the peptides were confirmed by electrospray ionization mass spectrometry performed at the Boston College mass spectrometry facility.

Preparation and Purification of Oligonucleotides

DNA oligonucleotides (5′-ACAGTACGGCAAGCTATATACGGCTCGT-3′ and 5′-ACGAGCCGTATATAGCTTGCC GTACTGT-3′) were synthesized with an ABI 394 DNA/RNA synthesizer. Solid support, phosphoramidites, and synthesis reagents were purchased from Glen Research (Sterling, VA). Oligonucleotides were purified by reversed-phase HPLC with a C-18 stationary phase and a NH_4OAc/CH_3CN gradient.

Cleavage of Plasmid DNA

Samples contained 75 μM (bp) pUC18 plasmid DNA, 100 μM RB, and 200 μM tripeptide in 10 mM sodium cacodylate buffer (pH 7). Samples were exposed to $^1O_2$ generated by excitation of RB at 557 nm with an Oriel Spectral Illuminator (150 W Xe; power, 1.3 mW). Comparable results were obtained with a Nd:Yag laser as a light source (λ_{exc} = 532 nm). For gel analysis, samples were prepared, irradiated, and directly loaded into a 1% agarose gel buffered with 1× Tris-acetate-EDTA (TAE) in the dark. Gels were visualized with ethidium staining and imaged on a UVP BioChemi system. Cleavage yield values represent averages of > 3 trials for each peptide.

For the samples shown in Figure 2B, 200 μM NaN_3 was added. This did not cause quenching of *RB fluorescence. The sample with reduced oxygen was bubbled with argon for 10 min prior to irradiation and was irradiated under Ar. Samples were otherwise treated as described above.

For the samples and data shown in Figure 2C, 2 mM KWK and 200 μM RB were irradiated in methanol for varying lengths of time with a 532 nm Nd:Yag laser (power, 11.5 mW). Aliquots were added to solutions containing 75 μM bp and 10 mM sodium cacodylate to give a final concentration of 200 μM KWK and 20 μM RB. In some cases 2 mM ascorbyl palmitate was added as a peroxide scavenger. Samples were incubated for 30 min and put on ice until they were loaded on a 1% agarose gel and treated as above.

Preparation of 5′-[^{32}P]-Labeled DNA

Purified 5′-ACAGTACGGCAAGCTATATACG GCTCGT-3′ (500 pmoles) was 5′-radiolabeled with 100 pmoles of γ-^{32}P ATP (MP Biomedicals, 7000 Ci/mmole) and 100 units of T4 polynucleotide kinase (New England Biolabs) in 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, and 5 mM dithiothreitol. The sample was incubated at 37°C for 30 min and was then passed through a G-25 column (Amersham Biosciences). The labeled sample was then treated with 1 M piperidine at 90°C for 30 min, diluted with 100 μl of denaturing PAGE buffer (8 M urea, 45 mM Tris base, 45 mM boric acid, and 1 mM EDTA), and purified on a 20% denaturing polyacrylamide gel with a 0.5× TBE buffer. The gel-purified oligomer was electroeluted and ethanol precipitated. The precipitated sample was resuspended in H_2O.

Cleavage of Linear Oligonucleotides

Samples contained ^{32}P-labeled oligonucleotide duplex (1 μM, ~2 × 10^4 cpm), 100 μM RB, and 800 μM tripeptide in 10 mM sodium cacodylate buffer (pH 6.5). Samples were exposed to $^1O_2$ generated by excitation of RB at 532 nm (30 min) with a Nd:Yag laser (power, 11.5 mW). Piperidine-treated samples were incubated with 1 M piperidine for 30 min at 90°C. Samples were phenol/chloroform extracted, ethanol precipitated, and resuspended in 10 μl of denaturing PAGE buffer.

Guanine-Sequencing Reaction

Samples contained ^{32}P-labeled oligonucleotide (1 μM, ~2 × 10^4 cpm). Reaction was prepared in 200 μl of 50 mM sodium cacodylate (pH 8.0) and 1 mM EDTA and contained 3 μg carrier DNA. Neat (2 μl) dimethyl sulfate (Aldrich) was added, and the sample was vortexed and incubated on ice for 5 min. The reaction was quenched by adding 50 μl of 1.5 M sodium acetate/1M 2-mercaptoethanol. The sample was ethanol precipitated and treated with 1 M piperidine for 30 min at 90°C and ethanol precipitated and resuspended in 10 μl denaturing PAGE buffer.

3′ Termini Analysis

All samples contained ^{32}P-labeled oligonucleotide duplex (1 μM, ~2 × 10^4 cpm). Samples were treated as stated above to generate cleavage. They were then treated with T4 PNK after ethanol precipitation. Samples were resuspended in 50 μl of 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, and 50 U T4 PNK (New England Bio Labs). Samples were incubated at 37°C for 4 hr. DNase I-treated standards were prepared in 20 μl solution containing 10 mM Tris-HCl (pH 7.5), 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 1 × 10^{-3} U DNase I (Takara). Samples were incubated at 37°C for 10 min. Bleomycin-treated samples were prepared in a 200 μl solution containing 50 mM Tris-HCl (pH 7.5), 3.5 μM bleomycin, 200 μg/ml carrier DNA, and 9 μM freshly prepared $FeSO_4$. The samples were incubated at room temperature for 10 min. All samples were ethanol precipitated and resuspended in 10 μl of denaturing PAGE buffer.

PAGE Analysis

The samples were analyzed on 21 cm (w) × 40 cm (h) × 0.4 mm (d) 20% denaturing polyacrylamide gels in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, and 1 mM EDTA). Gels were electrophoresed for 1.3 hr at 25 mA and were then exposed on a Kodak K-plate for 2 hr. Imaging was performed on a Bio-Rad Molecular Imager FX Pro Plus phosphor imager.

Modified FOX Assay

The procedure for the modified FOX assay was based on that developed by Gebicki and coworkers [33]. Solutions were comprised of 100 μM RB and 200 μM amino acid in 10 mM sodium cacodylate (pH 7). Samples were irradiated for 5 min with a 532 nm Nd:Yag laser (power, 11.5 mW). After irradiation, one volume of glacial acetic acid was added, followed by 100 μM xylenol orange and 200 μM ammonium iron(II) sulfate hexahydrate. Samples were thoroughly mixed after adding FOX reagents and then incubated for 20 min in the dark. Samples were diluted with 1.5 volumes of water and mixed, and the absorbance at 595 nm was measured with a Agilent 8453 UV-Vis spectrophotometer. The background FOX reagent absorbance was subtracted from each sample. Yields of peroxide are reported relative to 200 μM H_2O_2. It is noteworthy that because cysteine can act as a reductant, the amount of Fe(III) generated by the peroxide may be underestimated.

Peptide Decomposition Assay

Solutions containing 100 μM RB, 1 mM alanine tripeptide (AXA, X = W, Y, H, C) and 10 mM sodium cacodylate (pH 7) were made in triplicate and irradiated for 10 min with a 532 nm Nd:Yag laser (power, 11.5 mW), and controls were incubated in the dark. HPLC analysis was performed on a Varian 250 × 4.6 mm stainless steel column packed with Microsorb-MV 300 C18 (5 μm) on an Agilent 1100 HPLC. A flow rate of 1.0 ml/min was used with an aqueous solution buffered with 0.1% TFA and linear gradients of a mixture of 80:20:0.1 acetonitrile:water:TFA. The decomposition trend was consistent over > 5 trials.

Supplemental Data

Supplemental Data consist of PAGE analysis of the 3′-termini of DNA damaged by the oxidized tripeptide KWK, with and without T4 PNK treatment. The gel analysis also includes DNase I and bleomycin standards that generate 3′-OH and 3′-phosphoglycolate termini, respectively. Supplemental Data can be found with this article online at http://www.chembiol.com/cgi/content/full/12/6/695/DC1/.

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