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Enhanced Cleavage at Abasic Sites Within Clustered Lesions in Nucleosome Core Particles

Kun Yang^[a] and Marc M. Greenberg^[a]

^[a]Dr. Kun Yang and Professor Dr. Marc M. Greenberg, Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218, mgreenberg@jhu.edu

Abstract

Clustered lesions are a hallmark of γ -radiolysis, and are produced by other damaging agents as well. Bistranded clustered lesions are precursors to double strand breaks and pose challenges to repair, making them an especially deleterious form of DNA damage. An abasic site (AP) is an alkaline-labile lesion frequently present in clustered lesions. Strand scission at an AP site is accelerated ~ 100 -fold in nucleosome core particles (NCPs). We examined how AP reactivity was affected within a clustered lesion in NCPs. The rate constant of strand scission is increased as much as 2.5-fold in the presence of a proximal abasic site or thymidine glycol in the complementary strand. A proximal mismatch has a similar effect on AP reactivity. Increased AP reactivity within a clustered lesion correlates with decreased UV-melting temperatures of corresponding duplexes compared to one containing an isolated abasic site. However, thermodynamics of duplex melting do not correlate with AP reactivity within different clustered lesions. Overall, increased AP reactivity within clustered lesions is attributed to greater access of the lesion to histone proteins due to decreased duplex stability.

Abstract

Histones catalyze strand scission at abasic sites (AP) within nucleosome core particles. We report that strand scission is enhanced in the presence of other proximal DNA lesions (F, Tg) in the complementary strand. Increased AP reactivity within such clustered lesions is likely due to increased DNA flexibility, which increases the histone accessibility to the lesion. Bistranded lesions are likely more prone to strand scission in chromatin than previously realized.

Abasic sites (AP) are the most ubiquitous of DNA lesions, being formed $\sim 10,000$ times/day in a single cell as a result of respiration.^[1] Although DNA is cleaved at AP sites under

alkaline conditions, the lesions are stable for weeks at pH ~7.^[2] However, AP half-life is reduced ~100-fold to as short as ~7 hours within nucleosome core particles (NCPs), the monomeric component of chromatin.^[3] Strand scission is promoted by the lysine-rich histone proteins that function as primitive lyases, despite the absence of carboxylate containing residues for catalyzing the β -elimination. Here we show that an AP site that is part of a clustered lesion, a form of DNA damage commonly generated by γ -radiolysis, is even more reactive within a NCP than an isolated lesion.

Clustered lesions are defined as two or more damaged nucleotides within 1–2 helical turns of DNA. They are especially common in DNA exposed to γ -radiolysis because the high energy source produces multiple hydroxyl radicals from the ionization of water in the vicinity of the biopolymer.^[4] Bifunctional small molecules, such as the enediyne antitumor antibiotics also produce bistranded clustered lesions.^[5] Clustered lesions are often comprised of single-strand breaks, modified nucleotides, and/or abasic sites.^[6] Although the individual forms of damage are readily repaired, base excision repair (BER) is impeded by many clustered lesions.^[7] In addition, bistranded clustered lesions are precursors to double-strand breaks (dsbs), the most deleterious form of DNA damage.^[8] Bistranded lesions can be converted to dsbs during BER, during which AP sites are generated as intermediates.^[9]

Within chromatin, AP sites are also converted to strand breaks via an enzyme independent pathway (Scheme 1).^[3] Lysine residues within the histone tails are primarily responsible for Schiff base formation and rate limiting β -elimination.^[3c] The rate constant describing AP reactivity varies with respect to position within the 145 bp of DNA that wrap ~1.7 times around the octameric core of 4 histone proteins (Figure 1A). AP reactivity within NCPs is dependent upon access that lysine residues have to it and varies less than as a function of nucleosomal position than reactions involving diffusible species.^[10] The rate constant is enhanced as much as 2.7-fold in a clustered lesion in which the complementary strand is nicked proximal to the AP site.^[11] Even larger effects are observed in NCPs when a 1–2 nucleotide gap is proximal in the complementary strand.

The enhanced AP reactivity caused by proximal DNA nicks or gaps is likely due to the greater flexibility of DNA, which increases its accessibility to the histones that promote strand scission. Proximal modified nucleotides, which significantly destabilize the DNA duplex, may also stimulate the AP reactivity. To test this hypothesis we designed a series of NCPs (**2–5**) containing an abasic site at a common position (AP₂₀₅; position –13, superhelical location 1.2 in the nucleosome core particle based upon X-ray crystal structure PDB: 3lz0) in the presence of a DNA lesion in the complementary strand shifted 1–3 nucleotides in the 3'-direction relative to AP₂₀₅. The rate constant for disappearance (k_{Dis}) of AP₂₀₅ in the clustered lesions was compared to that of the isolated lesion (**1**). Position 205 was chosen because it is in a NCP region where the DNA is kinked and is a hot spot for molecules that bind to the nucleosome.^[12] The lesions introduced were thymidine glycol (Tg) and a chemically stable abasic site analogue (F, Scheme 2). Thymidine glycol is a common pyrimidine lesion produced as a result of oxidative stress, which destabilizes the thermal stability of duplex DNA and significantly disrupts the local duplex structure.^[13] The tetrahydrofuran abasic site analogue (F) is commonly used as a biochemical substitute of AP and has similar destabilization effects on duplex stability as AP and distorts local structure

but DNA containing it does not cleave.^[14] The required DNA (145 bp) was prepared by ligating chemically synthesized oligonucleotides using previously described methods.^[3b, 3c] Tg and F were introduced using commercially available phosphoramidites; whereas AP was generated within NCPs immediately prior to experiments from DNA containing the photochemical precursor (pAP, Scheme 3).^[3a, 15] NCPs were also prepared as previously described.^[15–16]

The half-life of AP₂₀₅ within a NCP composed of the Widom strong-positioning 601 DNA was previously reported and the value measured in this study (Table 1) is within experimental error of that.^[3c, 17] AP₂₀₅ (**1**) was purposefully chosen as the benchmark because cleavage was slow enough that we would readily be able to measure acceleration in the clustered lesions. Incorporation of either F (**2**) or Tg (**4**) one nucleotide removed (N₈₇) had the largest effect on the rate of strand scission at AP₂₀₅ in the NCPs. The rate constants for AP₂₀₅ disappearance in these NCPs were within experimental error of one another, and approximately 2.5-times faster than in a NCP containing the isolated lesion (**1**). The enhanced effect of the clustered lesion on AP₂₀₅ reactivity decreased when Tg was 2 nucleotides away (N₈₈) from the abasic lesion (**5**). Similarly, the rate constant for AP₂₀₅ disappearance was slower when F was 3 nucleotides away (**3**) than in **2**. However, the reactivity of AP₂₀₅ in **3** and **5** was still 1.4- and 2.1-fold higher respectively than in **1**. Overall, AP reactivity is enhanced within cluster lesions. The magnitude of the enhancement is greater the closer the lesion in the opposite strand is to AP₂₀₅.

We hypothesize that the correlation between AP₂₀₅ reactivity and the proximity of the other modification (F or Tg) is attributable to greater perturbation of the duplex structure in the vicinity of the abasic site the closer the lesions are to one another. This concept was tested by examining the effect of a mispair on AP₂₀₅ reactivity (**6**, **7**). Introducing a dA-dA mispair adjacent to AP₂₀₅ (N₈₇) decreased the abasic lifetime almost 2-fold. Accelerated AP₂₀₅ reactivity due to an adjacent mispair (**6**) was less than that induced by the presence of an abasic site analogue (**2**) or Tg (**4**) at the comparable position. However, the effect of introducing a T-T mispair at N₈₉ (**7**), 3 base pairs away from the abasic site, was within experimental error that resulting from incorporating F (**3**) at that site.

The kinetic data (Table 1) led us to postulate that decreased AP half-life within the NCP when part of a clustered lesion is due to perturbation of the local DNA structure. This explanation was initially probed by comparing the melting temperatures of duplexes (Table 2) containing the local sequences within **1-7** (**8b-h**) and another containing a T-A base pair (**8a**). The duplexes contain 5'-overhangs for convenience, as the oligonucleotides were the same as those used to prepare the nucleosomal DNA. Abasic sites and lesions such as Tg reduce duplex thermal stability.^[13a, 14a] Indeed, the duplex containing isolated AP₂₀₅ (**8b**) melted significantly lower than that containing a native A-T base pair (**8a**). Moreover, the T_m's of duplexes comprised of a clustered lesion (**8c-8f**) or AP₂₀₅ and a mispair (**8g, 8h**) were reduced further. The T_m for duplexes containing clustered lesions in which F (**8c**) or Tg (**8e**) were adjacent to AP₂₀₅ in the complementary strand were within experimental error of one another, which correlates well with AP₂₀₅ half-life in the respective NCPs (**2**, **4**). However, differences in the T_m's of duplexes comprised of different distributions of the same lesions (e.g. **8c** versus **8d**, **8e** versus **8f**, **8g** versus **8h**) did not correlate with AP₂₀₅

reactivity. In each instance, the duplex with the lower T_m corresponded to the NCP in which the AP₂₀₅ half-life is longer.

A connection between thermal duplex stability and AP₂₀₅ reactivity was examined more thoroughly by carrying out Van't Hoff thermodynamic analysis of the duplexes containing bistranded lesions (AP₂₀₅ and F or Tg, Table 3). We speculated that the duplexes containing the arrangement of lesions in which AP₂₀₅ was more reactive (e.g. **8c** versus **8d**) might have smaller ΔS° for melting, indicative of a more flexible reactive site, despite having an overall higher ΔG°_{298} . However, this did not prove to be the case. For each pair of bistranded lesions the less stable duplex exhibited a smaller ΔH° and ΔS° for melting.

We propose that despite the lack of correlation between ΔG° of duplex melting and AP₂₀₅ reactivity, that increased AP reactivity in clustered lesions requires local perturbation of the duplex in the vicinity of the abasic site. The lack of correlation between duplex stability and AP₂₀₅ reactivity may be due to the latter being a consequence of local structure, while the former is a macroscopic property. Molecular dynamics studies could shed light on this issue. We suggest that local base pairing is weakened more when the destabilizing lesions and/or mispairs are closer to one another. DNA in this region is more flexible and abasic sites are more accessible to the histones that promote the reaction. Hence, within clustered lesions the closer the second lesion (or mispair) is the more reactive AP is. This overall pattern for clustered lesion reactivity is consistent with that observed for AP sites in NCPs in which a strand break is present in the opposite strand.^[11] In those constructs, the more single-strand like the environment surrounding the AP site, the more reactive it was.

In summary, these experiments reveal an additional consequence of clustered lesion formation. It is widely recognized that clustered lesions can impede base excision repair and that they are precursors to double-strand breaks. These experiments reveal that abasic sites within clustered lesions are more susceptible histone-induced strand scission within nucleosome core particles than isolated molecules. Consequently, the possibility that clustered lesions may yield single- and double-strand breaks within cellular DNA is greater than one would expect based upon studies on free DNA.

Experimental Section

Preparation of 145 nt DNA containing F, Tg or mispair.

The 145 nt DNA containing an pAP₂₀₅ was prepared by enzymatic ligation of the chemically synthesized oligonucleotides, as previously described.^[18] Similar procedures were used to prepare 145 nt DNA containing F, Tg or native nucleotides for the nucleosomal DNA in **6** and **7**. Briefly, oligonucleotides O2, S and O3 (Fig. SI1) were 5'-phosphorylated (50 μ L) in a reaction containing DNA (1.5 nmol), 1 $\times$ PNK buffer (70 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 5 mM DTT), 2 mM ATP, and 50 units T4 PNK at 37 °C for 4 h. T4 PNK was inactivated by incubating at 65 °C for 30 min. The phosphorylated oligonucleotides were combined with 1.5 nmol oligonucleotide O1 (Fig. SI1) scaffolds O4, O5 and O6 (2 nmol, Fig. SI1) in 1 $\times$ T4 DNA ligase reaction buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM ATP, 10 mM DTT). The mixture was hybridized by heating at 90 °C for 2 min, followed by cooling to room temperature. T4 DNA ligase (3600 units) was added and

the mixture (~150 μ L) was incubated at 16 °C overnight. The reaction was then phenol extracted (equal volume). The supernatant containing DNA was mixed with 95% formamide (30 μ L), heated at 90 °C for 5 min, and purified by 8% denaturing PAGE gel (20 $\times$ 16 $\times$ 0.1 cm). The gel was run under limiting power (10 W) until the xylene cyanol migrated to the bottom of the gel. The product band was excised from the gel and the DNA was eluted overnight at room temperature in elution buffer (1 mL, 0.2 M NaCl and 1 mM EDTA). The slurry was briefly centrifuged at 1000 g and the supernatant was filtered using a polyprep-column (BioRad). The DNA solution was concentrated and buffer exchanged 4 times with water using a 10K Amicon Ultra membrane. The ligated DNA products (~300 pmol) were stored in -80 °C until use.

Reconstitution of nucleosome core particles.

The single stranded 145 nt oligonucleotide containing AP₂₀₅^[3c] was 5'-³²P labeled by incubating at 37 °C for 3 h in a reaction (10 μ L) containing DNA (20 pmol), 1 $\times$ PNK buffer (70 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 5 mM DTT), 30 μ Ci γ -³²P ATP and 15 U T4 PNK. Unincorporated γ -³²P ATP was removed using a G-50 sephadex column. The labeled DNA was mixed with the appropriate complementary strand (25 pmol). The mixture was heated at 95 °C for 3 min and cooled to room temperature to generate the 145 bp DNA. Salmon sperm DNA (~20 μ g, 31 nmol bp) and 5'-³²P labeled 145 bp DNA (4 pmol) were combined in a small siliconized tube to a final volume of 20 μ L containing 2 M NaCl and 0.1 mg/ml BSA. Various amounts of histone octamer (1.0 or 1.3 equivalents relative to total DNA) were added to the sample. The sample was incubated at room temperature for 30 min before beginning a series of dilutions using nucleosome buffer (10 mM HEPES, pH 7.5, 1 mM EDTA, 0.1 mg/ml BSA. Dilution # (volume of buffer added in μ L, incubation time in minutes): 1: 24, 60; 2: 12, 60; 3: 12, 60; 4: 20, 30; 5: 20, 30; 6: 40, 30; 7: 100, 30; 8: 200, 30. After the final dilution (total volume ~448 μ L), any precipitate was pelleted via a brief (3 min) spin at 13,000 g at 4 °C. The solution was then transferred to a fresh siliconized tube and incubated at 37 °C for 30 min. A small aliquot (~5 μ L) was removed, mixed with 40 % sucrose (2 μ L) and analyzed on a 5% native PAGE gel (10 $\times$ 8 $\times$ 0.15 cm) to determine the reconstitution efficiency. The gel was run at 4 °C under limiting power (3 W) until the bromophenol blue band migrated to the bottom.

Determination of strand cleavage.

NCPs (70 μ L, ~0.88 million cpm) were photolyzed at 350 nm for 15 min. A portion of the NCPs (20 μ L) were removed to determine the fraction of pAP converted to AP. The remainder of the NCPs (50 μ L) were incubated at 37 °C. Aliquots (5 μ L) were removed at appropriate times, immediately frozen in dry ice, and stored at -80 °C until the final time point. To determine the fraction of pAP converted to AP, the NCPs (5 μ L) were treated with 100 mM NaOH at 37 °C for 30 min, followed by neutralization with HCl and treatment of proteinase K (8 units) at room temperature for 30 min. To determine the amounts of strand cleavage in NCPs, the aliquots (5 μ L) were treated with 100 mM fresh NaBH₄ at 4 °C for 60 min, followed by treatment with proteinase K (8 units) at room temperature for 30 min. All samples were analyzed by 10 % denaturing PAGE gel (20 $\times$ 16 $\times$ 0.1 cm). The gels were run under limiting power (15 W) until the bromophenol blue migrated to the bottom of the gel.

The yield of cleaved DNA (DNA_{cl}) as a function of time was calculated using the equation below.

$$normalized\ DNA_{cl} = DNA_{cl} / ((DNA_{cl} + DNA_{un}) \times photolysis\ efficiency \times reconstitution\ efficiency)$$

$$normalized\ DNA_{cl} / normalized\ DNA_{un} = 1 - normalized\ DNA_{cl}$$

DNA_{un} represents the uncleaved DNA. The rate constant for AP reaction (k_{Dis}) was calculated by fitting the disappearance of DNA_{un} to a first order reaction.

Determination of duplex (8a-h) melting temperatures (T_m).

A mixture (200 μ L) containing two oligonucleotides (1:1, 100 pmol) in phosphate buffer (10 mM, pH 7.2 and 100 mM NaCl) was hybridized by heating at 90 °C for 2 min, followed by slowly cooling to room temperature. Van't Hoff analyses were carried out over a range of duplex concentrations (0.2 – 0.8 μ M). The DNA containing pAP was photolyzed at 350 nm for 15 min. Thermal denaturation profiles were measured with a Beckman DU 640 spectrophotometer equipped with a thermoelectrically controlled cell holder in 1 cm path length cuvettes. The absorbance at 260 nm was measured between 20 and 75 °C. The temperature was increased at a rate of 0.5 °C/min. The absorbance (A) as a function of temperature (T) was fitted to the sigmoidal equation below using Prism 5.0 where a, b, c and d were varied until the best fit of the data was achieved. The T_m value was then calculated as the temperature corresponding to the maximum of the first derivative.

$$A = a / (1 + e^{b - c(T)}) + d$$

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Nucleosome core particle structures. A) Partial structure showing AP₂₀₅ (red spheres) and proximity to histone tails B) Zoomed in region showing AP₂₀₅ and positions N₈₇-N₈₉. Taken from PDB: 1kx5.

Scheme 1.
Strand break formation from AP in a NCP.

Scheme 2.
DNA modifications included with AP in NCPs.

Scheme 3.
Photochemical generation of AP.

Table 1.Abasic site (AP₂₀₅) reactivity in nucleosome core particles.

NCP	$k_{\text{Dis}} (10^6 \text{ s}^{-1})^{[a]}$	AP ₂₀₅ $t_{1/2}$ (h) ^[a]	Rel. Rate ^[b]
1	6.3 ± 0.2	30.5 ± 1.1	-
2	14.4 ± 2.0	13.4 ± 1.8	2.3
3	8.9 ± 0.4	21.6 ± 1.0	1.4
4	15.8 ± 2.0	12.2 ± 1.6	2.5
5	13.5 ± 0.8	14.3 ± 0.7	2.1
6	11.4 ± 0.8	16.9 ± 1.1	1.8
7	9.6 ± 0.7	20.0 ± 1.4	1.5

^[a]Values presented are the ave. ± std. dev. from 2 experiments, each in triplicate.^[b]Rel. Rate = $k_{\text{Dis}}(\mathbf{x}) / k_{\text{Dis}}(\mathbf{1})$, where $\mathbf{x} = \mathbf{2-7}$.

Table 2.Duplex melting temperatures (T_m).

DNA ^[a]	N ₂₀₅	N ₈₇	N ₈₈	N ₈₉	T_m (°C) ^[b]	T_m (°C) ^[c]
8a	A	T	T	A	62.6 ± 0.1	-
8b	AP	T	T	A	50.6 ± 0.2	12.0
8c	AP	F	T	A	48.5 ± 0.2	14.1
8d	AP	T	T	F	45.2 ± 0.6	17.4
8e	AP	Tg	T	A	48.7 ± 0.1	13.9
8f	AP	T	Tg	A	47.0 ± 0.3	15.6
8g	AP	A	T	A	49.8 ± 0.4	12.8
8h	AP	T	T	T	47.2 ± 0.1	15.4

^[a] [DNA] = 0.5 μM, 10 mM phosphate buffer, pH 7.2, 100 mM NaCl.

^[b] T_m values are the ave. ± std. dev. from two experiments.

^[c] T_m = ave. T_m (**8a**) – ave. T_m (**8x**) where **x** = **b–h**.

Table 3.

Van't Hoff analysis of duplexes containing bistranded lesions.

DNA ^[a]	H° (kcal/mol) ^b	S° (cal/mol•K) ^{b]}	G° ₂₉₈ (kcal/mol) ^{c]}
8c	66.0 ± 9.5	177.7 ± 28.3	13.0
8d	57.4 ± 5.4	155.4 ± 16.7	11.1
8e	63.9 ± 6.2	182.5 ± 19.2	9.5
8f	60.0 ± 4.9	173.3 ± 13.6	8.4

^[a][DNA] = 0.2–0.8 μM, 10 mM phosphate buffer, pH 7.2, 100 mM NaCl.^[b]Values are the ave. ± std. dev. from two experiments.