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Nucleosome Core Particle-Catalyzed Strand Scission at Abasic Sites

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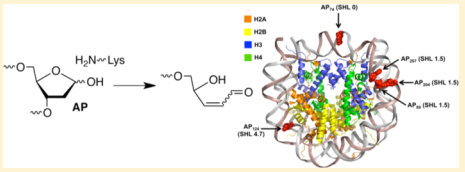
Supporting Information

ABSTRACT: The reactivity of apurinic/apyrimidinic (AP) sites at different locations within nucleosome core particles was examined. AP sites are greatly destabilized in nucleosome core particles compared to free DNA. Their reactivity varied ~5-fold with respect to the location within the nucleosome core particles but followed a common mechanism involving formation of a Schiff base between histone proteins and the lesion. The identity of the histone protein(s) involved in the reaction and the reactivity of the corresponding DNA−protein cross-links varied with the location of the abasic site, indicating that while the relative rate constants for individual steps varied in a complex manner, the overall mechanism remained the same. The source of the accelerated reactivity was probed using nucleosomes containing AP_{89} and histone H3 and H4 variants. Mutating the five lysine residues in the amino tail region of histone H4 to arginines reduced the rate constant for disappearance almost 15-fold. Replacing histidine 18 with an alanine reduced AP reactivity more than 3-fold. AP_{89} in a nucleosome core particle composed of the H4 variant containing both sets of mutations reacted only <4-fold faster than it did in naked DNA. These experiments reveal that nucleosome-catalyzed reaction at AP_{89} is a general phenomenon and that the lysine rich histone tails, whose modification is integrally involved in epigenetics, are primarily responsible for this chemistry.



A basic sites (AP) are the most common DNA lesions. They form spontaneously because of depurination and are intermediates in base excision repair (BER).[1] In total, it is estimated that at least 10000 AP sites are formed per day per cell as a result of respiration.[2] Hydrolysis of alkylated nucleobases produced in DNA by a variety of antitumor agents also yields AP sites.[3,4] In the case of leinamycin, AP sites are believed to be the source of the drug's cytotoxicity.[5] In addition to being cytotoxic, AP sites are mutagenic.[6,7] Repair systems protect cells against the effects of AP by removing them from the genome. The major pathway for AP removal in mammalian cells begins with 5′-phosphate incision by apurinic endonuclease 1 (Ape1), a phosphodiesterase.[8] In addition, some BER proteins that initiate damaged nucleotide repair by hydrolyzing the glycosidic bond are bifunctional and act on the AP product of this reaction.[9] The BER proteins conduct a lyase reaction on the AP-containing DNA via Schiff base formation. Recently, we reported that histone proteins use a similar mechanism within a nucleosome core particle to catalyze DNA cleavage at AP sites.[10,11] Herein we report on the generality of this process in nucleosome core particles and probe the source of the rate acceleration using histone H4 variants.

Nucleosomes are the monomeric component of chromatin in which nuclear DNA is condensed. Approximately 146 bp of DNA completes ~1.6 turns around the octameric core of histone proteins (Figure 1). The octameric core consists of a dimer of tetramers (H2A, H2B, H3, and H4) of histone proteins. The histones are highly positively charged and contain lysine rich amino-terminal tails that protrude from the octameric core and have a high degree of motional freedom as evidenced by their undefined electron density in X-ray crystal structures.[12,13] The lysine residues in these tail regions are often methylated or acetylated, and these chemical modifications play a large role in genetic regulation. The dyad axis of the crystal structures contains the central base pair of the DNA sequence and is defined as superhelical location (SHL) 0. The SHL increases by one with each subsequent turn of the helix. We have examined the reactivity of AP sites at five different positions within the NCP.

AP sites are alkaline-labile lesions and are rapidly cleaved upon being treated with a mild base. However, the half-life of an AP site in naked DNA is more than 3 weeks at pH 7.5 (37 °C).[10,11] Lysine rich peptides (e.g., Lys·Trp·Lys) act as lyase enzyme mimics and cleave DNA containing AP sites.[14] The lysine residues are critical in this chemistry. Their positive charge at physiological pH provides binding affinity for the DNA, and their amino groups activate AP sites for cleavage by forming Schiff bases with the lesion.[15] The N-terminal tails of the histone proteins that comprise the octameric core of nucleosome core particle are also rich in basic residues that could facilitate AP cleavage by presenting possible acid−base catalysts and positively charged amino acids that can help neutralize the phosphate leaving group in the proximity of the lesion.[12] For instance, the N-terminal tail of histone H4 contains five lysines and three arginines within the first 20 amino acids. Histone H4 also contains a histidine residue at position 18 of the tail, which can play a role in acid−base chemistry and Schiff base formation with a proximal abasic site.[16] Nucleosomal DNA is also bent and stretched in some regions, producing a heterogeneous structure wrapped around

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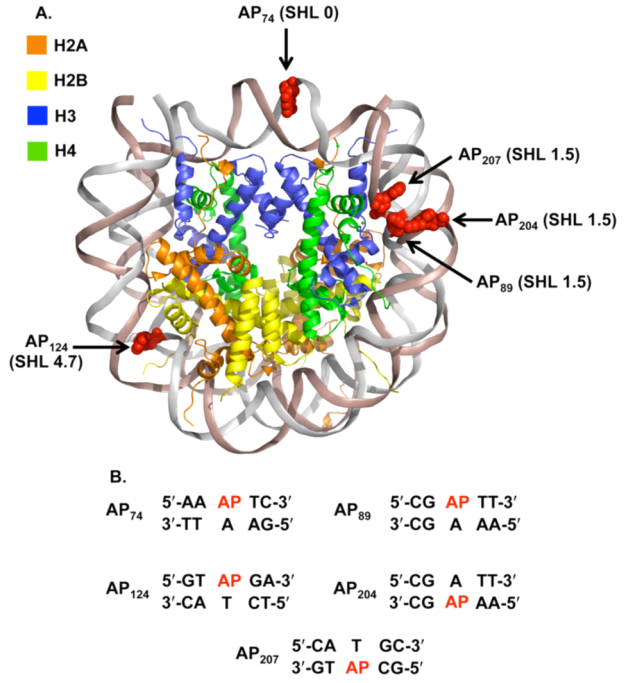
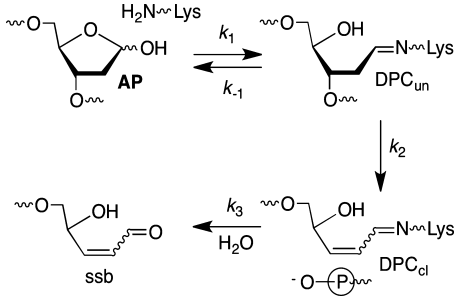


Figure 1. Nucleosome core particle (NCP) composed of α-satellite DNA. (A) Positions within NCP at which AP sites in α-satellite DNA are generated are highlighted. Structure obtained from Protein Data Bank entry 1AOI. (B) Local DNA sequences surrounding AP sites.

the positively charged protein core, which may affect nucleic acid properties, including the reactivity of alkaline-labile lesions. The portion of DNA that is positioned 1.5 helical turns from the central axis of the particle (superhelical location 1.5, or SHL 1.5) is a hot spot for molecules that bind to and damage DNA.[17,18] In addition, abasic sites specifically incorporated at this position (AP_{89}) in the α-satellite DNA sequence are cleaved as much as 60 times faster than in naked DNA and more than 100 times faster in core particles composed of the strong positioning 601 DNA.[10,11] Removing the N-terminal tail of H4 protein reduces the rate constant for AP disappearance ~3−5-fold. Trapping experiments and product identification showed that cleavage is dependent upon reversible Schiff base formation and that the H4 protein is responsible for more than 90% of the reactions of an AP site at SHL 1.5 in NCPs composed of α-satellite DNA and is the only protein trapped when the 601 DNA is present (Scheme 1). Histone H4 variants containing specific Lys to Ala mutations indicate that multiple tail lysines catalyze strand scission at AP sites and that other residues compensate for the loss of one lysine.[11] Comparison of product ratios in core particles containing wild type H4 and its

Scheme 1



variants also indicates that the lysines are involved in catalyzing β-elimination, which deuterium kinetic isotope effects reveal is the rate-determining step in strand scission, as well as Schiff base formation. The DNA−protein cross-links created by Schiff base formation persist long after strand scission occurs at the AP site, suggesting that the cell would need to repair such species. In this work, we explore the generality of AP reactivity in nucleosome core particles. AP reactivity is examined at several positions within nucleosome core particles composed of two structurally well characterized DNA sequences and in core particles containing histone H4 and H3 variants to characterize the effect of local nucleic acid structure and the proximity of lesions to histone proteins on chemistry.[12,13]

MATERIALS AND METHODS

Oligonucleotides were synthesized on an Applied Biosystems Inc. 394 oligonucleotide synthesizer, and DNA synthesis reagents were purchased from Glen Research (Sterling, VA). Expression and purification of all core histone proteins, as well as refolding and purification of the histone octamer, were conducted as previously described.[19,22] Proteinase K and DNase I were obtained from New England Biolabs (NEB). Nuclease P1 (from *Penicilium citrinum*) was from Sigma and was dissolved in water (1 unit/μL). All experiments were conducted in clear siliconized tubes (Bio Plas Inc.). Photolyses of oligonucleotides were conducted in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm.

General Procedure for Time Course Experiments Monitoring the Reactivity of Abasic Sites in Nucleosome Core Particles. Nucleosome core particles containing **1** were used directly in these experiments following reconstitution [10 mM HEPES, 60 mM NaCl, 1 mM EDTA, and 0.1 mM PMSF (pH 7.5)].[19] Samples were photolyzed (350 nm) for 10 min at room temperature and immediately incubated at 37 °C for the duration of the experiment. For those samples incubated in the presence of $NaBH_3CN$ (10 mM), fresh reducing reagent was added prior to photolysis. Aliquots were removed at the indicated times (Figure 2) and divided into two portions. The first portion was treated with proteinase K (2 μg/1 μL of sample) for 5 min at room temperature and quenched by the addition of $NaBH_4$ (final concentration of 0.1 M). These samples were analyzed by 8% denaturing polyacrylamide gel electrophoresis (PAGE) (19:1 acrylamide:bisacrylamide ratio, 45% urea) to determine the total amount of strand cleavage. The second portion was treated directly with $NaBH_4$ (final concentration of 0.1 M) and analyzed by 10% native sodium dodecyl sulfate (SDS)−PAGE (29:1 acrylamide:bisacrylamide ratio) as previously described.[10]

General Procedure for Determining the Protein(s) Involved in Cross-Linking with AP with NCPs. Nucleosome core particles containing [5′-^{32}P]AP were incubated at 37 °C overnight in the presence of $NaBH_3CN$ (10 mM) and were concentrated to ~30 μL using an Amicon YM-10 device (Millipore). To a portion of this solution (10 μL) were added 1 μL of 10× DNase I reaction buffer [10 mM Tris-HCl, 7.5 mM $MgCl_2$, and 0.5 mM $CaCl_2$ (pH 7.6)] and 2 units of DNase I. The mixture was incubated at 37 °C for 10 min and quenched by the addition of SDS (final concentration of 0.5%). The solution was then heated at 90 °C for 3 min, cooled to room temperature, and diluted to 50 μL using 1× nuclease P1 buffer [50 mM Bis-Tris propane, 1 mM $MgCl_2$, and 0.1 mM $ZnCl_2$ (pH 6.0)]. Nuclease P1 (1 unit) was added, and the mixture

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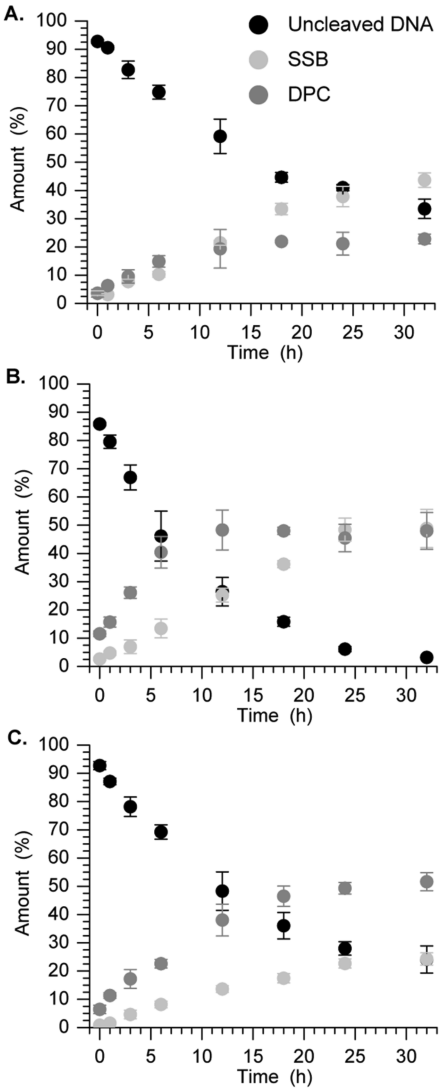


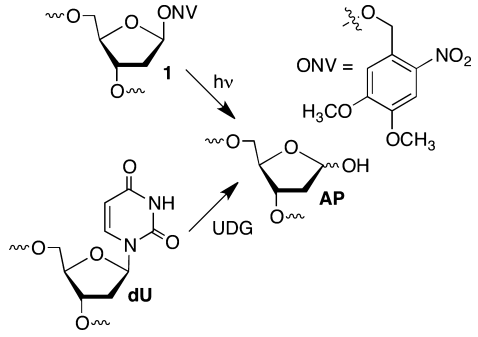
Figure 2. Representative plots of AP reactivity in α-satellite DNA as a function of time for (A) AP_{74}, (B) AP_{124}, and (C) AP_{204}. Each data point is the average ± the standard deviation of three independent measurements. Abbreviations: DPC, DNA–protein cross-links; SSB, single-strand break.

was incubated at 37 °C for 2 h. An additional 1 unit of nuclease P1 was then added, and the solution was incubated at 37 °C for an additional 1 h. The histone proteins were then precipitated via addition of cold acetone (400 μL) and incubation at −20 °C for 2 h. The proteins were pelleted by centrifugation, washed with cold acetone (100 μL), and pelleted again. After drying, the proteins were analyzed by either SDS–PAGE or Triton/acid/urea (TAU)–PAGE. For analysis by SDS–PAGE, gels were prepared and loaded as described previously (Mini PROTEAN System, Bio-Rad, 0.75 mm thickness).[20] Gels were comprised of a 20% resolving layer (29:1 acrylamide:bisacrylamide ratio) and a 3% stacking layer (29:1 acrylamide:bisacrylamide ratio). Samples to be analyzed by TAU–PAGE were dissolved in 8 M urea containing 10 mM DTT and 5% acetic acid. TAU gels (15%, 59:1 acrylamide:bisacrylamide ratio, 8 M urea, 5% acetic acid, 0.37% Triton X-100, 160 cm × 200 cm) were prepared and electrophoresed as described previously.[21]

RESULTS

Design and Preparation of Nucleosome Core Particles Containing AP at Defined Sites. Expression of individual histone proteins and reconstitution of nucleosome core particles were conducted using previously described methods.[22] H3 and H4 histone protein variants were also prepared as previously described.[19,23] Nucleosome core particles containing **1** at various positions were assembled using the α-satellite palindrome DNA sequence or the strong positioning 601 sequence that was selected on the basis of the stability of core particles containing it.[12,13,24] T4 DNA ligase was used to assemble the DNA from chemically synthesized oligonucleotides. Native PAGE was used to purify the final hybridized products. Following reconstitution, AP lesions were generated from **1** via a brief photolysis (350 mn) (Scheme 2). In

Scheme 2



experiments designed to identify the proteins cross-linked to AP, the lesion was generated from a specifically incorporated [5′-^{32}P]-2′-deoxyuridine (dU) residue using UDG.[19] UDG treatment was conducted prior to reconstitution to avoid the inhibitory effects of the nucleosome structure.[25,26] Correct positioning of the DNA around nucleosome core particles was verified via DNase digestion of the intact particles.[19]

Abasic sites (AP) were specifically incorporated at several positions within the nucleosome core particles (Figure 1). AP_{74} lies at the pseudo-2-fold axis of the DNA (dyad) and is termed superhelical location zero (SHL 0). SHL 0 was chosen as a region in which to study AP reactivity because the DNA and the amino-terminal histone protein tails are not as close in space as the other sites examined.[12] AP sites were also incorporated in the region of SHL 1.5 (AP_{89}, AP_{204}, and AP_{207} in α-satellite DNA and AP_{89} in 601 DNA) and SHL 4.7 (AP_{124} in α-satellite DNA) because the DNA at these positions is highly bent and in the proximity of lysine rich histone protein tails. AP_{89} and AP_{204} are at positions that oppose one another in α-satellite DNA. Positions 89 and 207 are in opposite strands, but both face inward toward the core particle containing α-satellite DNA.

Abasic Site (AP) Reactivity in Nucleosome Core Particles. The disappearance of intact α-satellite (Figure 2, uncleaved DNA) and 601 DNA containing AP was monitored using SDS–PAGE.[16] Aliquots were removed at appropriate times and quenched with $NaBH_4$ to prevent further reaction of any remaining AP sites during subsequent manipulations. The disappearance of AP at each position within the nucleosome core particle fit well to first-order kinetics (Table 1). The overall reactivity at SHL 4.7 (AP_{124}) was slightly greater than that of AP_{89} in α-satellite DNA. These two positions were modestly more reactive than AP_{204} and AP_{74}. AP_{204}, which is

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Table 1. Kinetics of Disappearance of AP at Various Positions within Nucleosome Core Particles Containing α-Satellite DNA

position	k_{Dis} (s^{-1})[a]	$t_{1/2}$ (h)
AP_{74}	$(1.0 \pm 0.3) \times 10^{-5}$	19.3
AP_{89}[b]	$(2.3 \pm 0.4) \times 10^{-5}$	8.5
AP_{124}[c]	2.8×10^{-5}	6.9
AP_{204}	$(1.5 \pm 0.1) \times 10^{-5}$	12.8
AP_{207}[b]	$(6.1 \pm 0.7) \times 10^{-6}$	31.6

[a]Rate constants are averages ± the standard deviation of at least three experiments unless otherwise noted. Each experiment was conducted in triplicate. [b]Data reported in ref 10. [c]Measurement of a single experiment conducted in triplicate.

opposite AP_{89}, was in turn more than twice as reactive as an AP site that is just three nucleotides closer to the dyad axis on the same strand (AP_{207}). Removing the N-terminal H4 protein tail (amino acids 1−19) reduced the rate constant for disappearance of AP_{204} [$k_{Dis} = (6.3 \pm 0.5) \times 10^{-6}$ s^{-1}; $t_{1/2} = 30.6$ h] approximately 2.5-fold.

DNA−Protein Cross-Links. The yield of DNA−protein cross-links (DPCs, Figure 3) roughly correlated with the overall

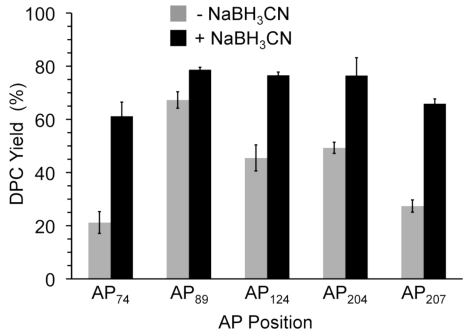

Figure 3. Effect of $NaBH_3CN$ on DNA−protein cross-link formation (24 h incubation) in α-satellite DNA. Each data point is the average ± the standard deviation of three independent measurements.

disappearance kinetics (Figure 2, Table 1).[10] The DNA−protein cross-links contained either uncleaved (DPC_{un}) or cleaved (DPC_{cl}) (Scheme 1) DNA.[11] The yields were highest for the faster reacting abasic sites (AP_{89} and AP_{124}) and lower for the slowest reacting position (AP_{207}). The lower total DPC yield [DPC_{cl} + DPC_{un} (Scheme 1 and Figure 3)] from AP_{204} (25.7 ± 0.6%) in the core particle lacking the H4 protein tail was commensurate with the overall slower rate constant for reaction. AP_{74} was the outlier in this regard. Of the AP sites examined, AP_{74} at SHL 0 is farthest from the histone tails. Although AP_{74} reacted more rapidly than AP_{207} in the nucleosome core particle, this site produced lower yields of DPCs (Figure 2A),[10] yet the yields of single-strand breaks detected by SDS−PAGE from AP_{74} were among the highest of any position examined (Figures 2 and 3). The extent of DNA cleavage within the DPCs formed with AP_{74} was also different from that at the four other positions examined (Table 2). DNA cleavage within DPCs [DPC_{cl} (Scheme 1)] at AP_{89} was previously directly determined by isolating the cross-links and subsequently separating cleaved and uncleaved DNA by denaturing gel, following proteinase K digestion.[10] The yield of cleaved DNA in DPCs at the other AP incorporation sites was determined more easily, albeit less precisely, by subtracting

Table 2. Percent Cleavage in DNA−Protein Cross-Links (DPC_{cl}) after a 24 h AP Incubation in Nucleosome Core Particles Containing α-Satellite DNA[a]

position	% DPC_{cl}[b]
AP_{74}	42.1 ± 16.5
AP_{89}[c]	93.0 ± 0.6
AP_{124}	85.1 ± 14.5
AP_{204}	98.2 ± 5.7
AP_{207}	82.1 ± 15.2

[a]Experiments were conducted in α-satellite DNA containing nucleosome core particles. [b]Yields are averages ± the standard deviation of three experiments. See the text for a description of the method used to assess cleavage in DPCs. [c]Measured directly. See ref 10.

the amount of strand scission detected by SDS gel electrophoresis from the total amount of cleaved DNA observed by denaturing PAGE following proteinase K treatment. The latter allows quantification of all cleaved molecules, whereas DPC_{cl} were not separated from DPC_{un} (detected) in the former. The DPCs detected at AP_{74} were composed of significantly greater amounts of uncleaved DNA than those formed with abasic sites at other positions within the core particle (Table 2). Less than one-half of the DPCs present after incubation of nucleosome core particles containing AP_{74} for 24 h consisted of cleaved DNA, whereas strand scission in the comparable products involving AP sites at the four other positions examined in this study ranged from >82 to ∼100%. Incubating the nucleosome core particles with $NaBH_3CN$ trapped the DPC intermediates, increasing the cross-link yields at all positions to >60%. The presence of $NaBH_3CN$ significantly reduced the total amount of strand scission [ssb + DPC_{cl} (Scheme 1)] in each nucleosome core particle (Figure 4). These observations are consistent with the mechanism proposed in Scheme 1.

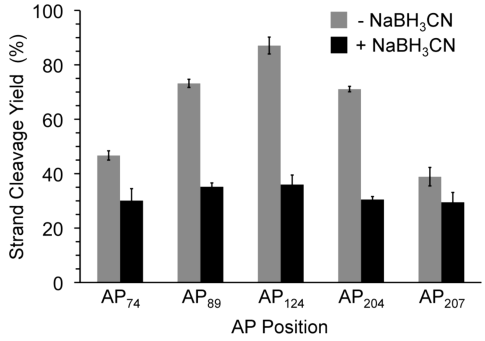

Figure 4. Effect of $NaBH_3CN$ on AP cleavage (24 h incubation) in α-satellite DNA. Each data point is the average ± the standard deviation of three independent measurements.

Identification of Protein(s) Involved in Cross-Linking. The identity of the proteins involved in DPC formation was established by digesting the isolated cross-links following incubation of the nucleosome core particle in the presence of $NaBH_3CN$ (Scheme 3). The gel-purified DPCs were digested with DNase I and nuclease P1, and the protein products were separated by TAU−PAGE. Visualization of the formerly cross-linked proteins was accomplished by radiolabeling the 5′-phosphate of the AP site. Because the photochemical precursor (**1**) to AP was not phosphorylated by polynucleotide T4 kinase,

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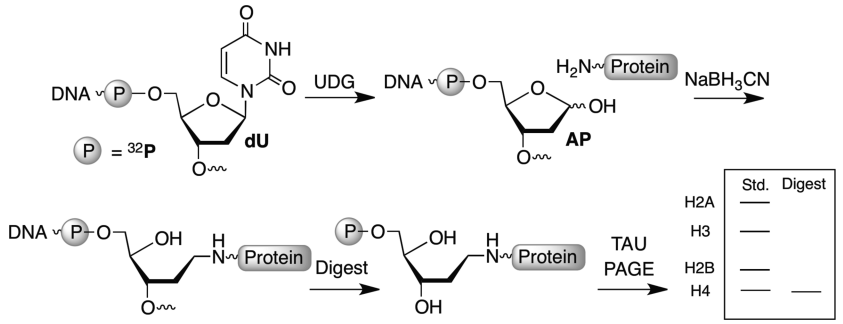
Scheme 3



AP was generated by hydrolysis of specifically incorporated [5′-^{32}P]dU residues using uracil DNA glycosylase (UDG).

AP_{124} is located at SHL 4.7 in the α-satellite DNA containing NCP, which is in the proximity of the H2A protein. Indeed, H2A accounts for >75% of the DPCs to this abasic site (Table 3). Together with H2B, these two proteins account for almost

Table 3. Distribution of DNA−Protein Cross-Links with AP in Nucleosome Core Particles

	% cross-linked[a]	
protein	AP_{74}	AP_{124}
H2A	26.1 ± 2.1	76.8 ± 0.5
H2B	7.9 ± 6.2	11.8 ± 2.9
H3	18.9 ± 13.6	6.4 ± 3.4
H4	48.6 ± 3.2	5.0 ± 0.1

[a]Percentages are averages ± the standard deviation of an experiment comprised of three replicates.

90% of the DPCs involving AP_{124}. In contrast, the abasic site (AP_{74}) located at the dyad (SHL 0) exhibits a broader distribution of proteins with which it cross-links. This observation is consistent with the lack of histone tails in the proximity of the dyad. In contrast, abasic sites in the region of SHL 1.5 are in the proximity of H4. Previous experiments showed that this protein is responsible for more than 90% of the trapped DPCs in this region of the NCP.[10]

Examining the Role of the Histone Tails in Catalyzing AP_{89} Reactivity. Previous investigations revealed that the H4 protein was primarily responsible for Schiff base formation at AP_{89} in core particles composed of 601 or α-satellite DNA.[10,11] Subsequent experiments involving AP_{89} within the 601 NCPs in which lysines in the tail region of histone H4 were mutated to alanine showed that other residues compensated for the loss of one or more lysines, resulting in little if any change in the overall kinetics. However, the distribution of products (DPCs and SSBs) was dependent on the presence of specific lysines, indicating that the protein catalyzed β-elimination in addition to activating the lesion via Schiff base formation. In related experiments, removing the entire 20-amino acid H4 tail reduced AP_{89} reactivity only ∼5-fold, leaving a large amount of the enhancement in the NCP unaccounted for. We postulated that the proximity of the amino terminus in the H4 deletion protein to AP_{89} compensates for the loss of the nucleophilic tail residues. The amino termini are more reactive than the ε-amino group in lysines.[27] Identification of the histone H4 deletion mutant as the species still being responsible for 100% of the Schiff base formation is consistent with this hypothesis.

Consequently, we sought to probe this and other sources of protein catalysis of AP_{89} reactivity in the 601 NCPs (Table 4).

Table 4. Effects of Histone H4 Variants on the Reactivity of AP_{89} in NCPs Containing 601 DNA

histone	k_{Dis} (s^{-1})[a]	$t_{1/2}$ (h)
wild type[b]	(1.0 ± 0.3) × 10^{-5}	8.9
H4 del.[b]	(4.2 ± 0.6) × 10^{-6}	45.4
H3 del.	(2.3 ± 0.4) × 10^{-5}	9.9
H3, H4 del.	(4.2 ± 0.4) × 10^{-6}	45
Lys5,8,12,16,20R	(1.5 ± 0.1) × 10^{-6}	128
H18A	(6.1 ± 0.3) × 10^{-6}	32
Lys5,8,12,16,20R/H18A	(8.8 ± 0.1) × 10^{-7}	219

[a]Rate constants are averages ± the standard deviation of at least two experiments. Each experiment was conducted in triplicate. [b]Data reported in ref 11.

The histone H3 tail, which is farther from AP_{89} than is H4, is a minor contributor to Schiff base formation at this site in the NCP-containing α-satellite DNA and not at all in the 601 DNA containing NCPs.[10,11] The H3 tail's possible involvement in subsequent steps involving the abasic site is unknown. Removing the H3 histone's 37-amino acid tail had essentially no effect on AP_{89} half-life. Furthermore, AP_{89} reacted with the same rate constant in the NCPs lacking the H3 and H4 tails as it did in that in which only the H4 tail had been removed. These observations strongly suggest that the histone H3 tail does not contribute to AP_{89} reactivity in NCPs composed of the 601 DNA. Because of the possibility that the amino terminus of the H4 protein from which 20 amino acids had been deleted was catalyzing AP_{89} reactivity, we pursued an alternate approach to probe the contribution of the multiple lysine residues in the protein's tail. The lysines were substituted with arginines, which retain the overall charge of the tail but were expected to reduce its nucleophilicity. Indeed, the rate constant for AP_{89} disappearance was more than 14 times slower when the core particle was composed of the Lys5,8,12,16,20Arg variant (Table 4) even though $NaBH_3CN$ trapping shows that the H4 protein is still solely responsible for Schiff base formation. The histone H4 tail also contains a single histidine residue (His18) that was previously shown to attack abasic sites.[16] Substituting this potential nucleophile with alanine (His18Ala) reduced the rate constant for AP_{89} reactivity ∼3.5-fold. Finally, incorporating an H4 variant that combines these mutations (Lys5,8,12,16,20Arg/His18Ala) increased the half-life of AP_{89} to 219 h. AP_{89} lives almost 25 times longer in this environment than in a NCP-containing wild-type H4 and <5 times shorter than the lesion in naked DNA.

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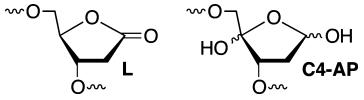
DISCUSSION

Lysine-containing peptides are well-known to act as lyase enzyme mimics.[14,15,28] Recently, lyase activity has been ascribed to DNA binding proteins that were not previously known to act as enzymes. For instance, Ku70 removes AP sites present at or near the DNA termini at double-strand breaks.[29,30] The Alk B DNA repair enzyme family has also been reported to possess lyase activity.[31] Our group reported that the histone proteins within nucleosome core particles catalyze the cleavage of DNA containing AP sites and the oxidized abasic site, 2-deoxyribonolactone (L), which is formed by a variety of DNA-damaging agents.[10,11,23] These studies indicated that abasic site cleavage is accelerated as much as 100-fold in a nucleosome core particle compared to free DNA. In addition, there is evidence from the effects of the potent antitumor agent bleomycin on chromatin that DNA cleavage at the C4′-oxidized abasic (C4-AP) is also accelerated by the histone proteins.[32]



The initial report of AP cleavage in a nucleosome core particle examined reactivity in the region of SHL 1.5, which is a known hot spot for molecules that damage DNA.[17,18] We considered whether positioning AP within the nucleosome core particle affected the cleavage chemistry and have now characterized AP reactivity further in this region and others within the nucleosome core particle. The half-life for AP disappearance spanned less than 5-fold (Table 1). The bending and base stacking of the DNA vary as it wraps around the octameric core, and it is likely that multiple properties contribute to variations in the increased AP lability within nucleosome core particles. For instance, AP_{74} is more reactive than AP_{207} despite the fact that the former is in a region that is further removed from the lysine rich histone protein tails. The overall disappearance of AP sites (Scheme 1) is dependent on reversible DPC_{un} formation and not just the irreversible cleavage to produce DPC_{cl} and ultimately SSBs.[11] The position of the AP site within the NCP could affect the individual steps differently, making it difficult to predict the overall reactivity at any particular site. The effects of AP position on the individual steps were examined using product analysis and kinetics.

The proximity to histone protein tails affects the selectivity of proteins involved (Table 3). We previously reported that abasic sites in the vicinity of SHL 1.5 react predominantly with histone H4 whose lysine rich tail extends from the core in that region.[10,12] Similarly, histones H2A and H2B account for almost 90% of the DPCs with AP_{124}, which is located near SHL 4.7 (Table 3). Cross-linking with AP_{74} is considerably less selective (Table 3), and this is consistent with the greater distance between the lesion and any of the histone protein tails (Figure 1).

The location of the abasic site within the nucleosome core particle also affects the yields of DNA−protein cross-links (DPCs) (Figure 3) and their lifetime or reactivity (Table 2). For instance, the DPC yields are lower at AP_{74} in the absence of $NaBH_3CN$ than any other position, including the slowest reacting abasic site examined, AP_{207}, yet incubating nucleosome core particles with $NaBH_3CN$, which traps the intermediate DNA−protein Schiff bases, increases the yield of DPCs at AP_{74} (Figure 3) and reduces the amount of strand cleavage (Figure 4) to levels comparable to that of any other position. These data indicate that Schiff base formation is as important for strand scission at AP_{74} as any other abasic site studied. The majority (if not all) of strand breaks are believed to proceed through Schiff base formation and that the residual strand scission in the presence of the reducing agent is due to incomplete trapping of the DNA−protein cross-links. However, the reactivity of the individual Schiff bases (Scheme 1) is dependent upon the local environment, which varies throughout the NCP. In addition to producing the lowest DPC yields, the cross-links at AP_{74} consist of considerably lower percentages of cleaved DNA (Table 2). The combination of a lower overall DPC yield and a smaller percentage of DPC_{cl} (Scheme 1) at AP_{74} suggests that Schiff base hydrolysis of DPC_{cl} [k_3 (Scheme 1)] is faster than at the other abasic sites. It is also possible that k_{-1} is greater at this position. The more rapid Schiff base hydrolysis could be a consequence of the greater distance between AP_{74} and histone protein tails, which may introduce strain in this functional group.

In addition to establishing the necessity for Schiff base formation to cleave AP sites, previous experiments with histone variants revealed that the protein(s) played a role in subsequent steps.[11] However, these experiments were unable to account for the majority of the observed acceleration in NCPs compared to naked DNA. Removing the entire H4 tail reduced the reactivity at AP_{89} only approximately 5-fold, leaving approximately 20-fold acceleration unaccounted for. We speculated that the amino terminus of the truncated H4 protein, which should be near AP_{89}, was undermining the effects of the deletion and clouding the picture. The situation was clarified by examining the effects of additional histone variants on the reactivity of AP_{89}. The bulk of the ∼100-fold rate acceleration observed in NCPs was accounted for by substituting arginines for the lysines in the H4 tail. Combining this mutation with the substitution of alanine for histidine at position 18 reduced the overall rate of acceleration in the NCP compared to naked DNA to ∼5-fold. We believe that the remaining acceleration is due to arginine and perhaps the reactivity of the N-terminal α-amino group with AP_{89} that is inferentially supported by the $NaBH_3CN$ trapping studies with the Lys5,8,12,16,20Arg/His18Ala variant, in which histone H4 is still solely responsible for Schiff base formation. Hence, we believe that at least for AP_{89}, the histone H4 tail is responsible for the large acceleration of cleavage of this alkaline-labile lesion.

SUMMARY

The formation of abasic sites at various positions in nucleosome core particles significantly increases the rate of DNA strand scission via a common mechanism that involves DNA−protein cross-linking via Schiff base formation. Interestingly, DNA−DNA interstrand cross-links, involving AP sites which have been reported, were not observed.[33,34] This suggests that such cross-links either are formed in <1% yields in NCPs or are preferentially formed in the linker regions of chromatin between nucleosomes. The DNA−protein cross-links vary in stability in a manner that correlates with an AP site's proximity to histone tails that are believed to be largely responsible for catalysis of strand scission. The formation of persistent DNA−protein cross-links raises questions concerning whether and how such lesions are repaired. The mobile nature of the histone tail combined with the requisite, reversible Schiff base formation for accelerating strand scission makes it difficult to assign roles to specific amino acids. However, the general involvement of nucleophilic amino acids in histone tails in this

chemistry may have ramifications beyond DNA cleavage because of the importance of the proteins' post-translational modification in regulating transcription.[35,36]

■ ASSOCIATED CONTENT

Supporting Information

Complete sequences of all DNAs used to prepare nucleosome core particles, experimental procedures, DNase footprints of nucleosome core particles, a sample reconstitution gel, a representative SDS gel showing reactivity of AP_{89}, and mass spectra of oligonucleotides containing **1** and histone variants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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