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Nucleosome core particle catalyzed strand scission at abasic sites[&]

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Abstract

The reactivity of apurinic/aprimidinic (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 location within the nucleosome core particles, but followed a common mechanism involving Schiff base formation 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 AP₈₉ and histone H3 and H4 variants. Mutating the 5 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. AP₈₉ 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 AP₈₉ is a general phenomenon and that the lysine rich histone tails, whose modification is integrally involved in epigenetics are primarily responsible for this chemistry.

Keywords

DNA damage; abasic sites; DNA-protein cross-links; mechanism

INTRODUCTION

Abasic sites (AP) are the most common DNA lesions. They form spontaneously due to depurination and are intermediates in base excision repair (BER) (1). In total, it is estimated that at least 10,000 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

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Supporting Information. Complete sequences of all DNAs used to prepare nucleosome core particles, experimental procedures, DNase footprints of nucleosome core particles, sample reconstitution gel, representative SDS gel showing reactivity of AP₈₉, 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>.

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 carry out 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 complete ~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 regulations. The dyad axis of the crystal structures contain 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 in 5 different positions within the NCP.

AP sites are alkaline-labile lesions and are rapidly cleaved upon mild base treatment. 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 which 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 5 lysines and 3 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 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 SHL1.5) is a hot spot for molecules that bind to and damage DNA (17, 18). In addition, abasic sites specifically incorporated at this position (AP₈₉) in the α -satellite DNA sequence are cleaved as much as 60-times faster than in naked DNA and more than 100-times 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-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 and H4 variants also indicate 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 occurred at the AP site, suggesting that the cell would need to repair such species. In this manuscript 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 in order 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 Incorporated 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 done as previously described (19). Proteinase K and DNase I were obtained from New England Biolabs (NEB). Nuclease P1 (from *Penicillium citrinum*) was from Sigma and was dissolved in water (1 U/ μ L). All experiments were conducted in clear siliconized tubes (Bio Plas Incorporated). Photolyses of oligonucleotides were carried out 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 NaBH₃CN (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 μ g/ μ L sample) for 5 min at room temperature and quenched by the addition of NaBH₄ (0.1 M final concentration). These samples were analyzed by 8 % denaturing PAGE (acrylamide/bisacrylamide; 19:1, 45 % urea) in order to determine the total amount of strand cleavage. The second portion was treated directly with NaBH₄ (0.1 M final) and analyzed by 10 % native SDS PAGE (acrylamide/bisacrylamide; 29:1) as previously described (10).

General procedure for determining the protein(s) involved in cross-linking with AP with NCPs

Nucleosome core particles containing 5'-³²P-AP were incubated at 37 °C overnight in the presence of NaBH₃CN (10 mM) and were concentrated to ~30 μ L using an Amicon YM-10 (Millipore). To a portion of this solution (10 μ L) was added 1 μ L of 10 $\times$ DNase I reaction buffer (10 mM Tris-HCl, 7.5 mM MgCl₂, 0.5 mM CaCl₂, pH 7.6) and 2 U DNase I. The mixture was incubated at 37 °C for 10 min and quenched by the addition of SDS (0.5 % final). The solution was then heated at 90 °C for 3 min, cooled to room temperature, and diluted to 50 μ L using 1 X nuclease P1 buffer (50 mM Bis Tris-propane, 1 mM MgCl₂, 0.1 mM ZnCl₂, pH 6.0). Nuclease P1 (1 U) was added and the mixture was incubated at 37 °C for 2 h. An additional 1 U of Nuclease P1 was then added and the solution was incubated at 37 °C for an additional hour. The histone proteins were then precipitated by adding cold acetone (400 μ L) and incubating at -20 °C for 2 h. The proteins were pelleted by centrifugation, washed with cold acetone (100 μ L), and pelleted again. After drying, the proteins were analyzed by either SDS or triton/acid/urea (TAU) PAGE. For analysis by SDS PAGE, gels were prepared and loaded as described previously (Mini PROTEAN System, Biorad, 0.75 mm thickness) (20). Gels were comprised of a 20 % resolving layer

(acrylamide/bisacrylamide; 29:1) and a 3 % stacking layer (acrylamide/bisacrylamide; 29:1). Samples to be analyzed by TAU PAGE were dissolved in 8 M urea containing 10 mM DTT and 5 % acetic acid. TAU gels (15 %, acrylamide/bisacrylamide; 59:1, 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 particle was carried out 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 based upon 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 nm) (Scheme 2). In experiments designed to identify the proteins cross-linked to AP, the lesion was generated from a specifically incorporated 5'-³²P-2'-deoxyuridine (dU) residue using UDG (19). UDG treatment was carried out prior to reconstitution in order 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). AP₇₄ lies at the pseudo-two fold axis of the DNA (dyad) and is referred to as superhelical location zero (SHL 0). SHL 0 was chosen as a region to study AP reactivity because the DNA and the amino terminal histone protein tails are not in as close proximity as the other sites examined (12). AP sites were also incorporated in the region of SHL 1.5 (AP₈₉, AP₂₀₄, AP₂₀₇ in α -satellite DNA and AP₈₉ in 601 DNA) and SHL 4.7 (AP₁₂₄ in α -satellite DNA) because the DNA at these positions is highly bent and in close proximity to lysine rich histone protein tails. AP₈₉ and AP₂₀₄ are at positions that oppose one another in α -satellite. Positions 89 and 207 are in opposite strands but both face inwards towards 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 NaBH₄ to prevent further reaction of any remaining AP sites during subsequent manipulations. AP disappearance at each position within the nucleosome core particle fit well to 1st order kinetics (Table 1). Overall reactivity at SHL 4.7 (AP₁₂₄) was slightly greater than that of AP₈₉ in α -satellite DNA. These two positions were modestly more reactive than AP₂₀₄ and AP₇₄. AP₂₀₄, which is opposite AP₈₉ was in turn more than twice as reactive as an AP site that is just 3 nucleotides closer to the dyad axis on the same strand (AP₂₀₇). Removing the N-terminal H4 protein tail (amino acids 1–19) reduced the rate constant for AP₂₀₄ disappearance ($k_{\text{Dis}} = 6.3 \pm 0.5 \times 10^{-6} \text{ s}^{-1}$, $t_{1/2} = 30.6 \text{ h}$) approximately 2.5-fold.

DNA-protein cross-links

The yield of DNA-protein cross-links (DPCs, Figure 3) roughly correlated with the overall disappearance kinetics (Figure 2) (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 (AP₈₉, AP₁₂₄) and lower for the slowest reacting position (AP₂₀₇). The lower total DPC yield (DPC_{cl} + DPC_{un}, Scheme 1, Figure 3) from AP₂₀₄ (25.7 ± 0.6 %) in the core particle lacking the H4 protein tail was commensurate with the overall slower rate constant for reaction. AP₇₄ was the outlier in this regard. Of the AP sites examined, AP₇₄ at SHL 0 is furthest removed from the histone tails. Although AP₇₄ reacted more rapidly than AP₂₀₇ 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 AP₇₄ were among the highest of any position examined (Figures 2 and 3). The extent of DNA cleavage within the DPCs formed with AP₇₄ was also different than at the other 4 positions examined (Table 2). DNA cleavage within DPCs (DPC_{cl}, Scheme 1) at AP₈₉ 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 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 enables quantification of all cleaved molecules, whereas DPC_{cl} were not separated from DPC_{un} (detected) in the former. The DPCs detected at AP₇₄ 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 incubating nucleosome core particles containing AP₇₄ for 24 h consisted of cleaved DNA, whereas strand scission in the comparable products involving AP sites at the other 4 positions examined in this study ranged from more than 82% to almost 100%. Incubating the nucleosome core particles with NaBH₃CN trapped the DPC inter-mediate, increasing the cross-link yields at all positions to >60%. The presence of NaBH₃CN 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.

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 NaBH₃CN (Scheme 3). The gel purified DPCs were digested with DNase I and nuclease P1, and the protein products separated by triton/acid/urea polyacrylamide gel electrophoresis (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, AP was generated by hydrolysis of specifically incorporated 5'-³²P-dU residues using uracil DNA glycosylase (UDG).

AP₁₂₄ is located at SHL 4.7 in the α-satellite DNA containing NCP, which is in close proximity to the H2A protein. Indeed, H2A accounts for more than 75% of the DPCs to this abasic site (Table 3). Together with H2B these two proteins account for almost 90% of the DPCs involving AP₁₂₄. In contrast, the abasic site (AP₇₄) 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 close proximity to the dyad. Abasic sites in the region of SHL 1.5 are in close proximity to 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 AP₈₉ reactivity

Previous investigations revealed that the H4 protein was primarily responsible for Schiff base formation at AP₈₉ in core particles composed of 601 or α-satellite DNA (10, 11). Subsequent experiments involving AP₈₉ 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 overall kinetics. However, the distribution of products (DPCs and SSBs) were 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 only reduced AP₈₉ reactivity ~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 AP₈₉ 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 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 AP₈₉ reactivity in the 601 NCPs (Table 4). The histone H3 tail, which is further from AP₈₉ 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 AP₈₉ half-life. Furthermore, AP₈₉ 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 was removed. These observations strongly suggest that the histone H3 tail does not contribute to AP₈₉ reactivity in NCPs composed of the 601 DNA. Due to the possibility that the amino terminus of the 20 amino acid deleted H4 protein was catalyzing AP₈₉ 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 AP₈₉ 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 NaBH₃CN trapping shows that the H4 protein is still solely responsible for Schiff base formation (19). 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 AP₈₉ reactivity ~3.5-fold. Finally, incorporating a H4 variant that combines these mutations (Lys5,8,12,16,20Arg + His18Ala) increased the half-life of AP₈₉ to 219 h. AP₈₉ is almost 25 $\times$ longer lived in this environment than in a NCP containing wild type H4 and < 5-times shorter than the lifetime of the lesion in naked DNA.

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).

L and C4-AP structures

The initial report on 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 AP positioning 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 varies 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, AP₇₄ is more reactive than AP₂₀₇ 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 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 AP₁₂₄, which is located near SHL 4.7 (Table 3). Cross-linking with AP₇₄ 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).

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 AP₇₄ in the absence of NaBH₃CN than any other position, including the slowest reacting abasic site examined, AP₂₀₇. Yet, incubating nucleosome core particles with NaBH₃CN, which traps the inter-mediate DNA-protein Schiff bases, increases the yield of DPCs at AP₇₄ (Figure 3) and reduces the amount of strand cleavage (Figure 4) to levels comparable to any other position. These data indicate that Schiff base formation is as important for strand scission at AP₇₄ 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 AP₇₄ consist of considerably lower percentages of cleaved DNA (Table 2). The combination of lower overall DPC yield and smaller percentage of DPC_{cl} (Scheme 1) at AP₇₄ suggest that Schiff base hydrolysis of DPC_{cl} (k_3 , Scheme 1) is faster than 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 AP₇₄ and histone protein tails, which introduces 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 only reduced reactivity at AP₈₉ 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 AP₈₉, 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 AP₈₉. The bulk of the rate ~100-fold 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 acceleration in the NCP compared to naked DNA to ~5-fold. We believe that the remaining acceleration is due to arginine and perhaps the N-terminal α-amino group reactivity with AP₈₉ that is inferentially supported by the NaBH₃CN 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 AP₈₉ 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, which have been observed involving AP sites were not observed (33, 34). This suggests that such cross-links are either 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, make 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 due to the importance of the proteins' post-translational modification in regulating transcription (35, 36).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

Figure 2. Representative plots of AP reactivity as a function of time (A) AP₇₄ (B) AP₁₂₄ (C) AP₂₀₄.^a Each data point is the average $\pm$ std. dev. of 3 independent measurements. (DPC, DNA-protein cross-links; SSB, single-strand break).

Figure 3. Effect of NaBH₃CN on DNA-protein cross-link formation (24 h incubation). Each data point is the average $\pm$ std. dev. of 3 independent measurements.

Figure 4. Effect of NaBH₃CN on AP cleavage (24 h incubation). Each data point is the average $\pm$ std. dev. of 3 independent measurements.

Scheme 1.

Scheme 2.

Scheme 3.

Table 1

Kinetics of AP disappearance at various positions within nucleosome core particles.

Position	$k_{\text{Dis}} \text{ (s}^{-1}\text{)}^a$	$t_{1/2} \text{ (h)}$
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 the average $\pm$ std. dev. of at least 3 experiments unless otherwise noted. Each experiment was carried out in triplicate.

^b Data reported in reference 10.

^c Measurement of a single experiment carried out in triplicate.

Table 2

Percent cleavage in DNA-protein cross-links (DPC_{cl}) after a 24 hour AP incubation.^a

Position	% DPC _{cl} ^{b,c}
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

^aExperiments were carried out in α-satellite DNA containing nucleosome core particles.

^bYields are the average ± std. dev. of 3 experiments. ^cSee text for description of method used to determine cleavage in DPCs.

^cMeasured directly. See reference 10.

Table 3

Distribution of DNA-protein cross-links with AP in nucleosome core particles.

Protein	& Cross-linked ^a	
	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

^aPercentages are the average ± std. dev. of an experiment comprised of 3 replicates.

Table 4

Effects of histone H4 variants on reactivity of AP₈₉ in NCPs containing 601 DNA.

Histone H4	$k_{\text{Dis}} \text{ (s}^{-1}\text{)}^a$	$t_{1/2} \text{ (h)}$
wild type ^b	$1.0 \pm 0.3 \times 10^{-5}$	8.9
H4 del. ^b	need data	45.4
H3 del.	$2.3 \pm 0.4 \times 10^{-5}$	9.9
H3, H4 del.	2.8×10^{-5}	45
Lys5,8,12,16,20R	$1.5 \pm 0.1 \times 10^{-6}$	128
H18A	$6.1 \pm 0.3 \times 10^{-6}$	32
Lys5,8,12,16,20R/H18A	$8.8 \pm 0.1 \times 10^{-7}$	219

^a Rate constants are the average $\pm$ std. dev. of at least 2 experiments. Each experiment was carried out in triplicate.

^b Data reported in reference 11.