

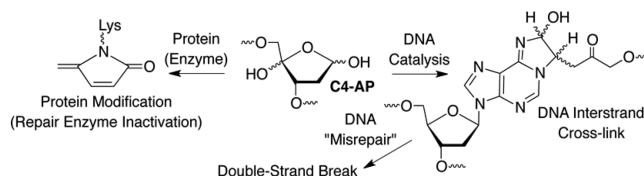
Abasic and Oxidized Abasic Site Reactivity in DNA: Enzyme Inhibition, Cross-Linking, and Nucleosome Catalyzed Reactions

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CONSPECTUS



A basic lesions are a family of DNA modifications that lack Watson–Crick bases. The parent member of this family, the apurinic/apyrimidinic lesion (AP), occurs as an intermediate during DNA repair, following nucleobase alkylation, and from random hydrolysis of native nucleotides. In a given day, each cell produces between 10000 and 50000 AP lesions. A variety of oxidants including γ -radiolysis produce oxidized abasic sites, such as C4-AP, from the deoxyribose backbone. A number of potent, cytotoxic antitumor agents, such as bleomycin and the enediynes (e.g., calicheamicin, esperamicin, and neocarzinostatin) also lead to oxidized abasic sites in DNA.

The absence of Watson–Crick bases prevents DNA polymerases from properly determining which nucleotide to incorporate opposite abasic lesions. Consequently, several studies have revealed that (oxidized) abasic sites are highly mutagenic. Abasic lesions are also chemically unstable, are prone to strand scission, and possess electrophilic carbonyl groups. However, researchers have only uncovered the consequences of the inherent reactivity of these electrophiles within the past decade. The development of solid phase synthesis methods for oligonucleotides that both place abasic sites in defined positions and circumvent their inherent alkaline lability has facilitated this research.

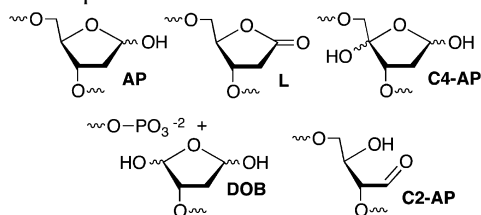
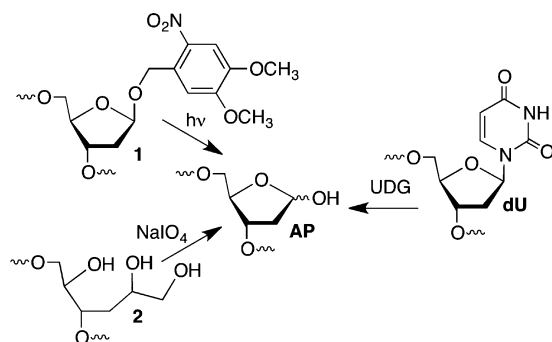
Chemically synthesized oligonucleotides containing abasic lesions provide substrates that have allowed researchers to discover a range of interesting chemical properties of potential biological importance. For instance, abasic lesions form DNA–DNA interstrand cross-links, a particularly important family of DNA damage because they block replication and transcription absolutely. In addition, bacterial repair enzymes can convert an interstrand cross-link derived from C4-AP into a double-strand break, the most deleterious form of DNA damage. Oxidized abasic lesions can also inhibit DNA repair enzymes that remove damaged nucleotides. DNA polymerase β , an enzyme that is irreversibly inactivated, is vitally important in base excision repair and is overproduced in some tumor cells. Nucleosome core particles, the monomeric components that make up chromatin, accentuate the chemical instability of abasic lesions. In experiments using synthetic nucleosome core particles containing abasic sites, the histone proteins catalyze strand cleavage at the sites that incorporate these lesions. Furthermore, in the presence of the C4-AP lesion, strand scission is accompanied by modification of the histone protein.

The reactivity of (oxidized) abasic lesions illustrates how seemingly simple nucleic acid modifications can have significant biochemical effects and may provide a chemical basis for the cytotoxicity of the chemotherapeutic agents that produce them.

1. Introduction

A DNA abasic site (AP) results from hydrolysis of a nucleotide's glycosidic bond, which occurs 10000–50000 times per day per cell under typical aerobic conditions.¹ Oxidized abasic sites arise following hydrogen atom abstraction from the 2'-deoxyribose ring of a nucleotide (Scheme 1) and are

produced by some of the most potent chemotherapeutic agents that damage DNA.² Abasic sites are highly mutagenic, but this important, well-documented topic will not be discussed.^{3–7} In addition, although there is still much to learn about the mechanisms for oxidized abasic site formation, this topic was recently reviewed and also is not

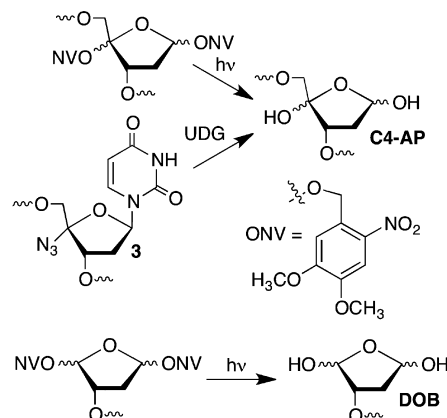
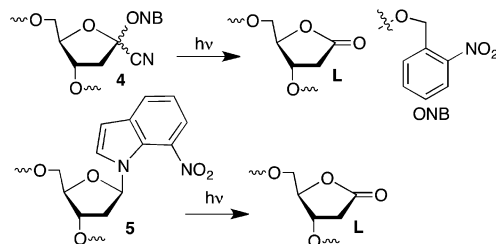
SCHEME 1. Examples of DNA Abasic Sites**SCHEME 2.** Synthetic Methods for Preparing Oligonucleotides Containing AP Sites

covered.² The chemical reactivity of various abasic sites in free DNA and as part of nucleosome core particles (NCPs), as well as their inactivation of repair enzymes, is the focus of this Account.

It is well established that abasic sites destabilize duplex DNA due to the loss of hydrogen bonding and other non-covalent interactions. Abasic sites themselves are electrophilic and chemically unstable. For instance, an AP site has a half-life on the order of weeks at pH 7.4 but is completely cleaved upon mild alkaline treatment.^{8,9} With the exception of C2-AP, DNA containing an abasic site is readily cleaved under mild alkaline conditions. Strand scission via β -elimination introduces another electrophilic site that can react with DNA and proteins. Nonetheless, abasic sites are often considered less biologically significant than other more rare families of lesions, such as interstrand cross-links (ICLs) and double-strand breaks (DSBs). This is primarily due to the efficiency by which AP is repaired by enzymes of the base excision repair (BER) pathway. However, recent discoveries indicate that abasic site electrophilicity plays an important role in biologically relevant reactions, resulting in DNA interstrand cross-links, irreversible inhibition of DNA repair enzymes, and histone modification.

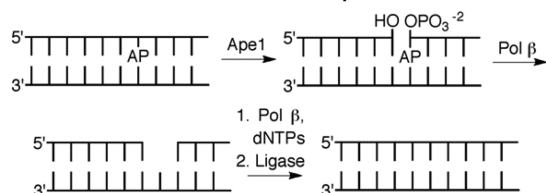
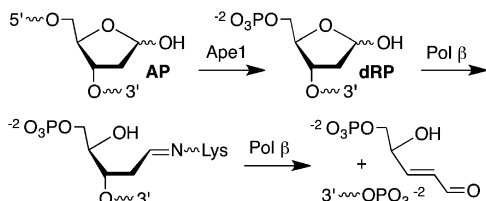
2. Synthesis of Oligonucleotides Containing Abasic Lesions at Defined Sites

Although AP and oxidized abasic sites are produced by a variety of DNA damaging agents, it is impractical to use this

SCHEME 3. Synthetic Methods for Preparing Oligonucleotides Containing C4-AP and DOB Lesions**SCHEME 4.** Synthetic Methods for Preparing Oligonucleotides Containing L Lesions

approach for preparing oligonucleotides containing a single lesion at a defined position. Studies on abasic lesions are greatly facilitated by their incorporation in oligonucleotides using solid phase synthesis methods. In order to maximize compatibility with standard solid phase synthesis protocols, the alkaline lability of these molecules requires that they be unmasked following oligonucleotide deprotection and cleavage from the solid support. Oligonucleotides containing AP are most frequently prepared by reacting an incorporated dU nucleotide with glycosylase, uracil DNA glycosylase (UDG) (Scheme 2). Alternatively, AP sites are introduced into chemically synthesized oligonucleotides as the photolabile *o*-nitrobenzyl acetal (**1**), which protects the lesion from alkaline deprotection/cleavage conditions.^{10–12} The AP site is revealed postsynthetically by photolysis at 350 nm. The syntheses of the requisite phosphoramidites are straightforward from 2-deoxyribose. AP sites are also introduced via periodate oxidation of a vicinal diol (**2**). The aldehyde produced during this reaction spontaneously cyclizes to yield the desired product.¹³ A similar approach has also been used to synthesize oligonucleotides containing C2-AP.¹⁴

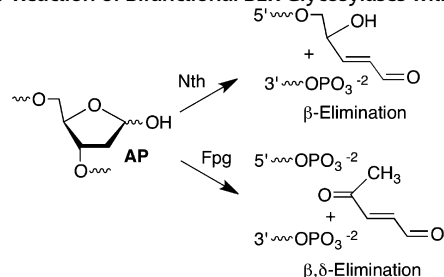
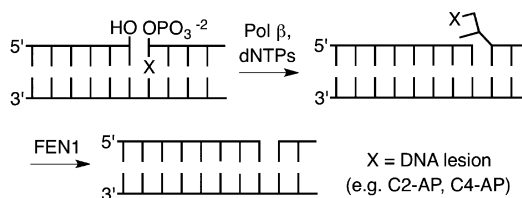
The *o*-nitrobenzyl photoredox reaction was also used to produce DNA containing the DOB, C4-AP, and L lesions

SCHEME 5. Overview of Base Excision Repair (BER) of AP Sites**SCHEME 6.** Removal of AP Lesion by BER

(Schemes 3 and 4).^{15–18} A clever combination of the *o*-nitrobenzyl photoredox reaction and a C1-cyano substituent (**4**) provided oligonucleotides containing L upon photolysis and spontaneous loss of HCN.¹⁹ Photoexcitation of the 7-nitroindole (**5**), which abstracts the C1'-hydrogen atom has also proven to be a very reliable method for generating the lactone lesion.²⁰ In addition, a derivative of 2'-deoxyadenosine that utilizes this photochemistry enables enzymatic incorporation of L at defined sites.²¹ Finally, C4-AP can be generated chemoenzymatically by subjecting oligonucleotides containing 4'-azido-2'-deoxyuridine (**3**) to UDG.²² The scalability of this method was demonstrated by producing sufficient material to characterize duplex DNA containing C4-AP by NMR.²³

3. Irreversible Inhibition of Base Excision Repair Enzymes by Oxidized Abasic Lesions

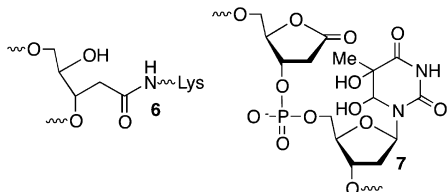
The genome is protected against AP sites by the BER pathway (Scheme 5). In mammalian cells the primary repair pathway begins with 5'-incision of the AP lesion by apurinic endonuclease 1 (Ape1).²⁴ The remnants of the AP site (dRP, Scheme 6) are removed in a β -elimination reaction that is catalyzed by the lyase domain (dRPase) of DNA polymerase β (Pol β).²⁵ The resulting single nucleotide gap is filled in by Pol β and rejoined by DNA ligase. A variety of endonucleases in other species possess the same activity as Ape1. Some BER glycosylases (e.g., Nth) also possess the ability to induce β -elimination creating cleaved DNA in which the 5'-fragment contains a 3'-pent-2-en-1-al terminus, which is subsequently removed by a nuclease such as Ape1 (Scheme 7).²⁶ One BER protein in *Escherichia coli*, formamidopyrimidine N-glycosylase (Fpg), activates AP by using a N-terminal proline

SCHEME 7. Reaction of Bifunctional BER Glycosylases with AP**SCHEME 8.** Long Patch Base Excision Repair

residue to form an iminium ion, which is followed by the β,δ -elimination reaction to fully remove the lesion.²⁷

C2-AP is a substrate for phosphodiesterases, but not for the lyase activities of glycosylase enzymes or Pol β because it lacks a β -phosphate group relative to the aldehyde.^{28,29} This obstacle to repair is overcome by Pol β and FEN1, which carry out long patch BER via strand displacement synthesis (Scheme 8). The displaced DNA containing the lesion is hydrolyzed by FEN1. Although L contains the requisite leaving group in the β -position relative to its carbonyl, it inactivates *E. coli* endonuclease III (Nth), which possesses a relatively strong lyase activity. L forms DNA–protein cross-links (DPCs) with Nth and inhibits the enzyme's activity on AP.^{30–32} DPC formation was rationalized based upon analogy to Schiff base formation with AP, which involves Lys120. However, attack by this nucleophile on the lactone carbonyl forms a stable amide (**6**). Cross-linking is eliminated when the Lys120Ala Nth variant is incubated with duplex DNA containing L.³⁰ Nth inhibition by L even extends to tandem lesions (e.g., **7**) containing the oxidized abasic site where the enzyme cannot excise a typical substrate such as thymine glycol.³³ Consequently, long patch BER is required to remove **7** from DNA. Inactivation of Nth by L was more the exception than the rule, because several other glycosylases, including Fpg, were not cross-linked. However, Fpg and Neil1 formed DNA–protein cross-links with the β -elimination product from L. Preincubation of the butenolide with thiol prevented cross-linking to Fpg and Neil1 suggesting that the products resulted from 1,4-conjugate addition. Inactivation of BER glycosylases by L and its β -elimination

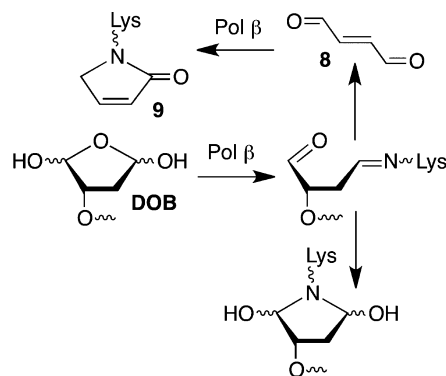
product were the first examples of irreversible inhibition of DNA repair enzymes.³⁴ However, the processes were inefficient, and it is uncertain how relevant they are biologically.



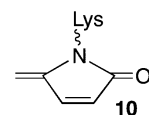
Oxidized abasic lesions L, C2-AP, and C4-AP are substrates for the first step of BER (Schemes 5 and 6), incision by 5'-phosphodiesterases, albeit poorer ones than AP.^{28,35–37} Oxidized abasic sites affect the subsequent BER step more significantly. In mammalian cells, dRP (Scheme 6) is removed by the lyase (dRPase) activity of Pol β .²⁵ Based upon its reactivity with Nth, it was not surprising that Ape1 incised L formed DNA–protein cross-links with Pol β .³⁸ Furthermore, DPC formation with incised L has a similar effect on Pol β activity as the lactone does when it is part of a tandem lesion with thymine glycol.³³ However, L cross-linking with Pol β is inefficient, possibly due to the lactone's inherently lower electrophilicity compared with the aldehyde in AP, so one must again question the biological significance.³⁸

The C4-AP and DOB lesions more closely resemble an AP site than does L because they also contain an aldehyde at what was the C1-position. However, the presence of a 1,4-dicarbonyl functional group in C4-AP and DOB, which reacts rapidly with primary amines, dramatically alter the outcomes of interactions with lyase enzymes (Scheme 9).^{18,39,40} Pol β excises on average approximately four DOB molecules before the enzyme is inactivated.^{41,42} Quantitative analysis revealed that DNA containing DOB behaved as an irreversible inhibitor ($K_i \approx 13$ nM, $k_{\text{inact}} \approx 4 \times 10^{-4}$ s⁻¹). Radioactive isotopic labeling experiments indicated that the major inhibition pathway involved DPC formation, whereas released **8** accounts for $\sim 10\%$ of the inactivation events. Subsequent MS analysis of digested Pol β provided direct evidence for covalent modification of Lys84 (**9**) and indirect evidence for reaction at Lys72. Both lysines have been implicated in Schiff base formation during 5'-dRP excision, although Lys72 is believed to be the nucleophile in the majority of reactions.^{43–46} Qualitatively similar observations were made when the 5'-phosphorylated C4-AP (pC4-AP) produced by the action of Ape1 was incubated with Pol β .³⁷ Inactivation by pC4-AP required on average between six and seven enzyme turnovers but ultimately resulted in covalent modification of the same

SCHEME 9. Inactivation of Pol β by DOB



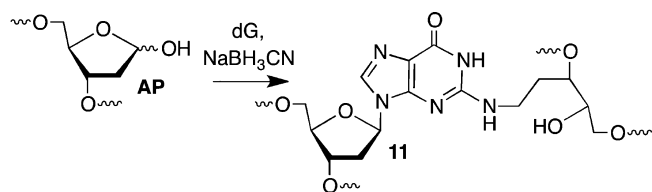
lysine residues (e.g., **10**) that were modified following reaction with DOB.



DNA polymerase λ (Pol λ), which also possesses dRPase activity, has been proposed to play a back-up role for Pol β in DNA repair.^{47,48} DOB and pC4-AP affect Pol λ similarly as they do Pol β . Approximately one out of every four interactions between DOB and Pol λ result in the enzyme's inactivation.⁴² Furthermore, mass spectral analysis indicates that DOB and pC4-AP modify homologous lysine residues in the Pol λ lyase domain (Lys312, Lys324). The efficient, irreversible inhibition of Pol β , a vital enzyme involved in BER, and its putative back-up (Pol λ) by these lesions sheds light on the mechanisms of action of the potent antitumor agents that produce them.^{2,49} Ultimately, these processes may contribute to the chemical bases for their cytotoxicity.

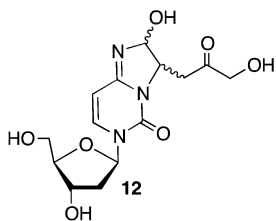
4. DNA Interstrand Cross-Link Formation by Abasic Sites

Interstrand cross-links (ICLs) are a biologically important family of DNA lesions, because they prohibit the dehybridization necessary for replication and transcription to proceed.^{50,51} ICLs are produced by a number of antitumor agents and also result indirectly from lipid oxidation.⁵² Although it had previously been proposed, Gates was the first to unambiguously characterize a DNA ICL that did not involve an exogenous molecule.⁵³ AP forms ICLs selectively with the dG in 5'-C-AP (**11**) sequences under mild reducing conditions (NaBH₃CN) (Scheme 10). In some sequences, ICL yields approach 20% in ~ 12 h.⁵⁴ Mass spectrometric analysis, varying the local sequence, and methoxyamine competition experiments all supported cross-linking

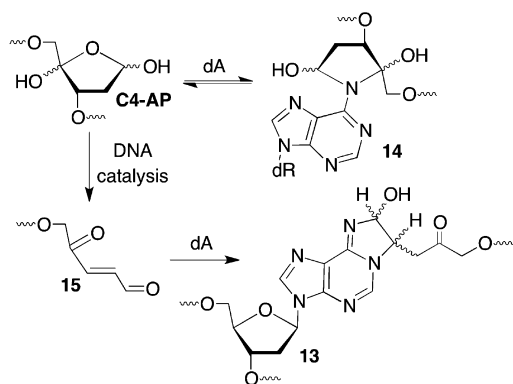
SCHEME 10. DNA Interstrand Cross-Link Formation by AP

between the AP aldehyde and 2-amino group of dG. This connectivity pattern also explains why ICL formation occurs preferentially in the 5'-C-AP sequence. The guanine 2-amino group at this position is in closer proximity (~ 3.7 Å) than when it is either opposite AP (~ 5.3 Å) or present in the 5'-AP-C (~ 8.6 Å) sequence.

In 2007, C4-AP was also proposed to form ICLs with dC in cellular DNA.⁵⁵ Ravanat detected a diastereomeric mixture of **12** by LC/MS following enzyme digestion of cellular DNA. The yield of **12** detected in human lymphocyte DNA showed a dependence on bleomycin or ionizing radiation dose, both of which are known to produce C4-AP. This was the first evidence for the formation of ICLs from an abasic site in cells.



C4-AP reactivity was investigated further using synthetic duplexes, which provided complete control over the local sequence.⁵⁶ These experiments confirmed the formation of **12** and revealed that the yield of cross-link with dC was highly sequence dependent. The preferred reactive positions did not strictly depend upon the distance between the C1-aldehyde of the lesion and cytosine-N4. Reaction occurred preferentially with dC that was either opposite C4-AP or a 5'- or 3'-flanking dG. In contrast to AP, no cross-linking was detected between C4-AP and dG, although iso-dG paired with iso-dC did yield ICLs.⁵⁷ C4-AP also cross-linked with dA in a variety of sequence contexts (Scheme 11).^{17,56} 2'-Deoxyadenosine formed two types of cross-links with C4-AP. One was structurally analogous to **12** (**13**). The other consisted of an intact duplex in which C4-AP remained uncleaved (**14**). The latter cross-link only formed with a dA opposite thymidine in duplexes containing 5'-C4-AP-T sequences, whereas the location of **13** showed variability similar to **12** with respect to changes in local sequence. In addition to its more exacting structural requirements, **14** formed almost 10-times more rapidly than **13** but proved to

SCHEME 11. DNA Interstrand Cross-Link Formation by C4-AP

be unstable and reverted ($t_{1/2} \approx 3.2$ h) to C4-AP with a rate constant that is twice as fast as that at which it is formed.

Although **13** formed more slowly than **14**, like **12**, it was chemically stable. Interestingly, even though no cross-link formed with the dA opposite C4-AP, this nucleotide catalyzed ICL formation at a dA opposite the 3'-adjacent thymidine.⁵⁶ For instance, DNA containing dA opposite C4-AP was 5-fold more reactive than when thymidine was opposite the abasic site. In addition, cross-linking was rescued upon addition of adenine to **16** (Figure 1A). The efficacy of other purines at rescuing cross-linking correlated with their electron density (Figure 1B). Finally, mechanistic studies indicated that the β -elimination from C4-AP (**15**) was the rate-determining step.^{56,57}

Catalysis of ICL formation by the local sequence is an unusual instance in which DNA promotes a process to its own detriment.⁵⁸ The outcome of nucleotide excision repair (NER) of the cross-link makes the process all the more intriguing. DNA damage is removed as part of an oligonucleotide during NER. ICL repair by NER is a multistep process, which may occur by incising or "unhooking" one strand, followed by a DNA polymerase filling in the gap produced (Scheme 12).⁵⁹ It is not understood how the NER system chooses which strand to incise first, and for most ICLs the order is unimportant. Because one strand is already nicked in DNA containing **12** or **13**, incision of the uncleaved strand first creates a double-strand break (DSB), the most deleterious form of DNA damage (Scheme 13). Indeed DSBs are produced $\sim 15\%$ of the time when ICL containing **12** is subjected to NER.⁶⁰ This is the first example in which an ICL is misrepaired and converted into a DSB, but since this report two other instances of NER misrepair have been reported.^{61,62} NER misrepair is the culmination of a series of chemical events that illustrate how an apparently simple

abasic lesion (C4-AP) can be transformed into ones that have greater biological impact.

5. Abasic Site Reactivity in Nucleosome Core Particles

In the nucleus, DNA is condensed into chromatin, which is composed of monomeric nucleosome units. In each nucleosome, DNA (~145 bp) wraps around an octameric core of

5'-d(CGA CCG GCT CGT ATG C4-AP TGT GTG GAG CTG TGG)
3'-d(GCT GGC CGA GCA TAC T ACA CAC CTC GAC ACC)

16

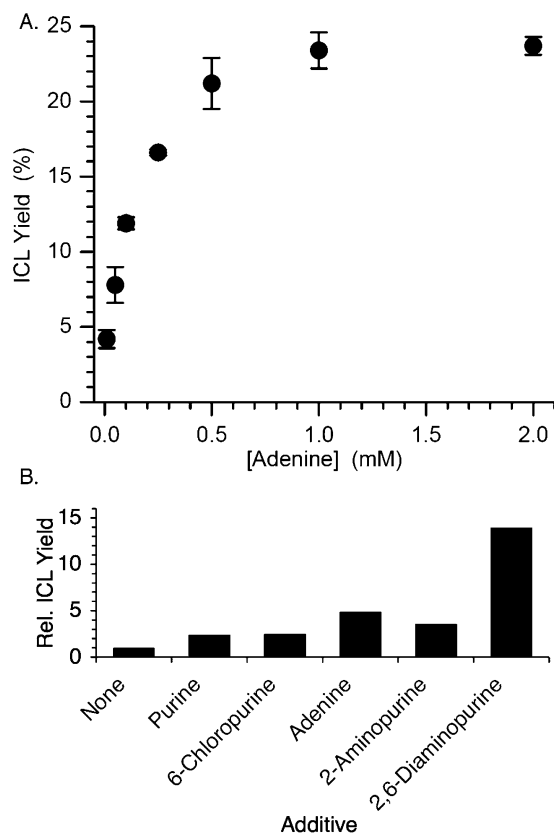


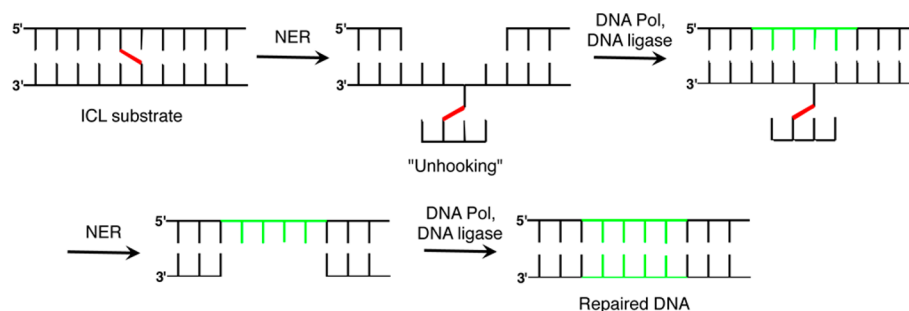
FIGURE 1. Effects on ICL formation when C4-AP is opposite thymidine (16). (A) ICL rescue as a function of adenine concentration. (B) Effects of various purines on ICL yield.

four different highly positively charged histone proteins. The histones also contain lysine-rich termini ("tails") that protrude through the nucleosome core particle (NCP). Post-translational modification of the lysines in the conformationally mobile tails plays an important role in gene regulation. Abasic sites in nucleosomal DNA are in close proximity to the lysine-rich tails and other nucleophiles within the octameric core. In studies on the reaction of bleomycin and neocarzinostatin with chromatin, Povirk was the first to observe that the histone proteins induce DNA strand scission at C4-AP and L lesions.⁶³

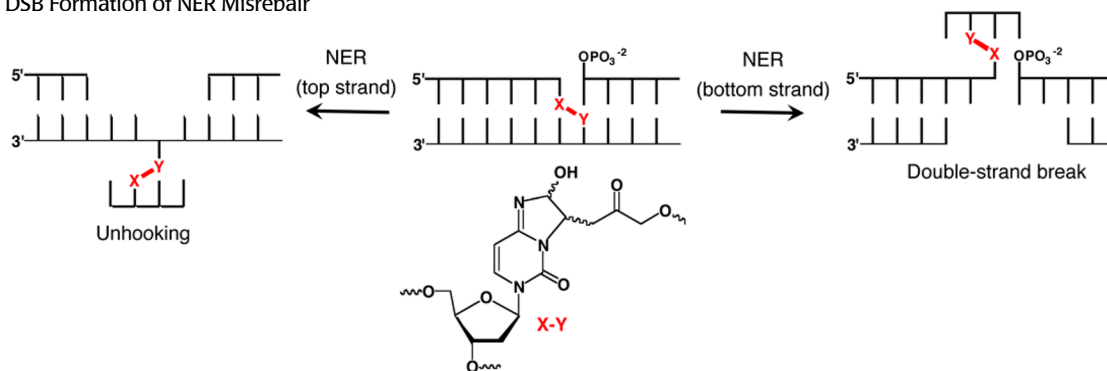
Elucidating the reactivity between abasic lesions and histone proteins in chromatin was greatly facilitated using nucleosome core particles (NCPs) prepared by combining the synthesis of 145 bp DNA containing appropriate precursors at defined sites with histone protein expression in *E. coli*. The requisite DNA molecules were prepared by enzymatically ligating chemically synthesized oligonucleotides. Histone expression and assembly of the octameric core enabled exploration of the roles of specific amino acids by preparing variants of the wild type proteins. As anticipated by Povirk's report, strand scission at AP, L, and C4-AP are all accelerated in NCPs. However, the reaction mechanisms and possible consequences are different for each.^{9,11,64–66}

Strand scission at AP sites is accelerated as much as 100-fold in nucleosome core particles.^{9,11,65} Mechanistic studies, including trapping by NaBH₃CN, and deuterium KIEs revealed that reversibly formed DNA–protein cross-links (DPCs) are intermediates en route to strand breaks and that β -elimination is the rate-determining step (Scheme 14). DPC persistence varied depending upon the location of AP within the NCP. For instance, DPCs persisted for more than 24 h at the site 1.5 helical turns from the center of the DNA sequence (SHL 1.5), which is a hot spot for DNA damaging agents.^{67,68} An important unresolved question is how these persistent DPCs are repaired.

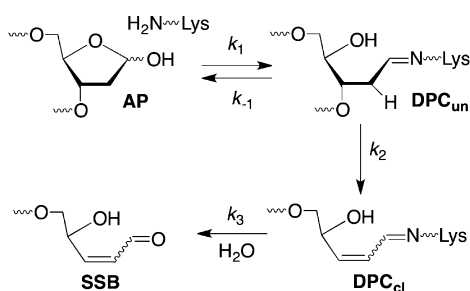
SCHEME 12. NER of an ICL



SCHEME 13. DSB Formation of NER Misrepair



SCHEME 14. AP Cleavage in a Nucleosome Core Particle



The identities of proteins cross-linked to the AP sites were determined using an assay that relied on ^{32}P -transfer from the 5'-adjacent phosphate to the protein.¹¹ DPC formation correlated with the proximity of the AP site with the lysine-rich amino terminal tail. For instance, histone H4 accounted for more than 95% of the DPCs with the AP site at SHL 1.5. Mutagenesis experiments confirmed that the histone tails played a vital role in catalyzing strand scission at AP sites.^{9,65} Approximately 95% of the accelerated cleavage of an AP site at SHL 1.5 is accounted for by mutating the five lysines in the H4 tail to arginine, and substituting alanine for histidine 18. In addition, substituting alanines for lysines in the H4 tail affects the ratio of cross-linked products (Table 1), suggesting that the proteins also play a role in the β -elimination step (Scheme 14). Overall, the histone proteins within the NCP behave like lyase enzymes.²⁵ Recently, there have been a number other reports of proteins exhibiting unexpected lyase activity.^{69,70} For instance, Ku70 was previously thought to play a role as a structural protein in double strand break repair by nonhomologous end joining, possesses dRPase activity (Scheme 6).⁷⁰

Another interesting effect was observed when two AP sites were introduced on opposite strands three nucleotides apart at SHL 1.5.¹¹ Such clustered lesions are produced by ionizing radiation and are an important family of DNA

TABLE 1. Effect of Mutations in Histone H4 on the Ratio of DNA-Protein Cross-Links at AP₈₉ in a NCP⁹

| histone H4 | DPC _{un} /DPC _{cl} |
|---------------|--------------------------------------|
| wild-type | 0.15 |
| Lys5,8,12Ala | 0.12 |
| Lys16Ala | 0.26 |
| Lys20Ala | 0.64 |
| Lys16,20Ala | 1.22 |
| 1–20 deletion | 10.3 |

damage because they are recalcitrant to repair.^{71,72} Based upon the reactivity of isolated AP lesions at the respective positions, an induction period was expected for double-strand break (DSB) formation, but little or none was observed. Kinetic analysis indicated that following reaction at one AP site, the reactivity of the remaining lesion in the cluster was accelerated 10–15-fold compared with when it was the sole damage site incorporated in the NCP. Initial experiments suggested that the formation of a DPC with an AP on one strand resulted in a further acceleration of AP cleavage on the opposing strand by increasing the effective molarity of nucleophilic lysines.¹¹ However, independent synthesis of an appropriate NCP revealed that merely the presence of a proximal strand break in the complementary strand was sufficient to induce the additional acceleration in strand scission at an AP site observed during double strand break formation.⁹ Although the physical reason behind this acceleration is unknown at this point, given the cytotoxicity of DSBs, their NCP catalyzed formation from proximal AP lesions suggests a chemical basis for the biological significance of clustered lesions.

The acceleration of cleavage at L in the NCP is more modest than that of an AP site.⁶⁴ Strand scission increases between 11- and ~43-fold compared with free DNA and deprotonation at C2 is involved in the rate-determining step. In contrast to AP cleavage, small amounts of DNA-protein cross-links are formed, and they result from trapping of the

cleaved product rather than as an intermediate en route to DNA cleavage. 2-Deoxyribonolactone is often formed as part of a bistranded lesion in which the other component is a strand break.⁴⁹ Consequently, accelerated L cleavage rate in NCPs indicates that these bistranded lesions are de facto DSBs in chromatin.

C4-AP is the most reactive abasic lesion reported on as yet in NCPs, where its lifetime (as short as 14 min) is reduced as much as 550-fold compared with that in naked DNA.⁶⁶ As is the case for AP, DPC formation is necessary for strand scission, and incubation of the NCP containing C4-AP results in stable cross-links at the expense of strand breaks (Scheme 15). Compared with AP, C4-AP DPCs form more transiently and in lower yields. Furthermore, only those containing uncleaved DNA (DPC_{un}, Scheme 15) are detected. Both DNA cleavage fragments contain phosphate termini indicating that C4-AP is eliminated in its entirety. Fluorescent tagging experiments and MS analysis of digested proteins reveal that C4-AP is transferred to the lysine-rich amino terminal tail of the histone protein. The lactam modification of the histone (**10**) is analogous to the product formed when C4-AP inactivates Pol β and represents an unnatural post-translational histone modification, akin to formylation by formyl phosphate DNA.⁷³ Its biochemical effects are unknown, and it has not been detected in cellular DNA as yet.

Finally, ICLs involving C4-AP or AP were not observed in NCPs. This is not inconsistent with reports on cross-linking summarized above. The rates for AP and C4-AP ICL formation are considerably slower than the lesions' reactivity in NCPs where the limit of detection is $\sim 1\%$. Lower yields are

more readily detected by MS, which was used to detect ICLs from C4-AP in cellular DNA.⁵⁵

6. Conclusions

AP and related oxidized abasic sites are some of the most commonly observed DNA lesions. Until recently, they were thought of as mutagenic lesions that were readily repairable. The above examples illustrate that the reactivity and biochemical effects are broad, extending to the formation of the most deleterious forms of DNA damage, interstrand cross-links and double-strand breaks. Abasic site reactivity provides chemical insight into the mechanisms of action of the chemotherapeutics that produce them and presents new questions and opportunities for research at the chemistry–biology interface.

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BIOGRAPHICAL INFORMATION

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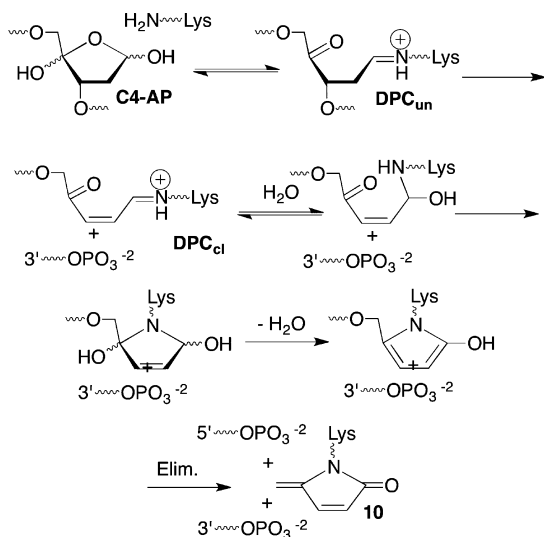
FOOTNOTES

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SCHEME 15. C4-AP Cleavage in a Nucleosome Core Particle



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