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Histone-Catalyzed Cleavage of Nucleosomal DNA Containing 2-Deoxyribonolactone

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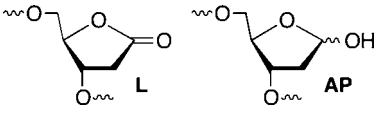
Supporting Information

ABSTRACT: Oxidized abasic sites such as 2-deoxyribonolactone (L) are produced in DNA by a variety of oxidizing agents, including potent cytotoxic antitumor natural products. 2-Deoxyribonolactone is labile under alkaline conditions, but its half-life in free DNA at pH 7.5 is approximately 1 week. Independent generation of L at defined positions within nucleosomes reveals that the histone proteins catalyze strand scission and increase the rate between 11- and ~43-fold. Mechanistic studies indicate that DNA−protein cross-links are not intermediates en route to strand scission and that C2 deprotonation is the rate-determining step. The use of mutant histone H4 proteins demonstrates that the lysine-rich tail that is often post-translationally modified in cells contributes to the cleavage of L but is not the sole source of the enhanced cleavage rates. Consideration of DNA repair in cells suggests that L formation in nucleosomal DNA as part of bistranded lesions by antitumor antibiotics results in de facto double strand breaks, the most deleterious form of DNA damage.

2-Deoxyribonolactone (L) is an alkali-labile DNA lesion that is produced by a variety of oxidizing agents, including the radiomimetic copper−phenanthroline and several potent cytotoxic antitumor antibiotics.[1,2] Irreversible inhibition of DNA repair enzymes by 2-deoxyribonolactone and its effects on replication underscore its biochemical significance.[3−5] Most of these biochemical investigations have been carried out on L in free DNA. In eukaryotes, however, nuclear DNA is found in the chromatin, of which the nucleosome is the integral component. Nucleosome core particles consist of ~146 base pairs of DNA wrapped around an octameric core of highly positively charged histone proteins. The histone proteins play an important role in regulating transcription and have been shown to affect DNA repair by altering enzyme access to damaged DNA.[6−9] We recently reported that an abasic site (AP) is significantly destabilized within a nucleosome core particle and that the histone proteins play an active role in cleaving DNA at the site of this lesion.[10] Herein we demonstrate that cleavage of 2-deoxyribonolactone is also significantly accelerated in nucleosomal DNA, albeit by a different mechanism.



Incorporating a lesion within a nucleosome core particle introduces two features not present in naked DNA that can affect the reactivity. Wrapping the DNA around the octameric core introduces heterogeneity into the duplex, resulting in regions that are bent and/or in which the base stacking is altered.[11] In addition, the large number of lysine (Lys) and arginine (Arg) residues can directly interact with the lesion. Lys side chains are directly involved in AP cleavage within nucleosomes via Schiff base formation.[10] Although 2-deoxyribonolactone was reported to cross-link with histone proteins in solution, it is less electrophilic than AP.[12] On the basis of other reports, it was uncertain whether Lys side chains would induce cleavage via nucleophilic attack on the carbonyl.[13−15]

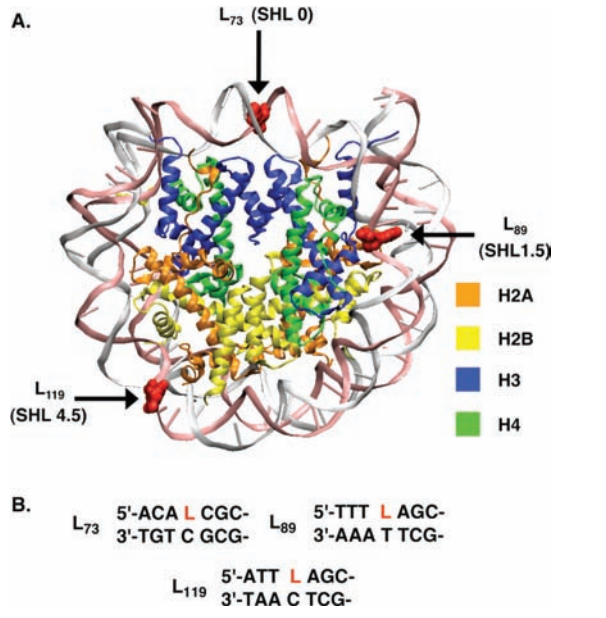


Figure 1. Independent generation of 2-deoxyribonolactone (L) in nucleosome core particles containing the 601 DNA sequence (A) at various superhelical locations (SHLs) within specific local DNA sequences (B). (See the Supporting Information for the complete DNA sequences.). Figure created using data from PDB: 3LZ0.

These issues were investigated by independently generating L at various positions within nucleosome core particles containing the 601 sequence of DNA (Figure 1).[16] The 601 DNA strongly binds the octameric histone core, and the

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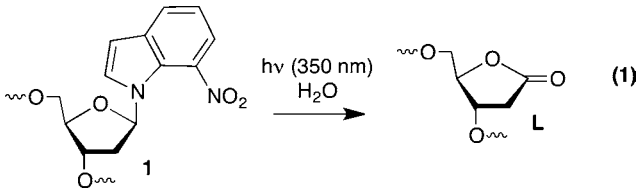
structure of the corresponding nucleosome was determined by X-ray diffraction.[17] 2-Deoxyribonolactone was produced photolytically from **1** (eq 1) at three positions chosen because of their



different environments within the nucleosome.[18] Superhelical location (SHL) 1.5 is a known hot spot for DNA-damaging molecules.[19] SHL 4.5 is a region within the 601 sequence in which base stacking is decreased because of stretching of the DNA. Of the three locations, the dyad region (SHL 0) has the weakest direct interaction with the histone core and is also furthest from the proteins' lysine-rich tails. The individual strands of DNA (145 nucleotides) were prepared via ligation (T4 DNA ligase) of the requisite chemically synthesized oligonucleotides and purified by denaturing polyacrylamide gel electrophoresis.[20] The nucleosomes were reconstituted following ^{32}P labeling of the respective strands containing **1**.

The cleavage of DNA in nucleosomes containing 2-deoxyribonolactone exhibited first-order kinetics (Table 1).

Table 1. Cleavage Kinetics of 2-Deoxyribonolactone (L) as a Function of Position in Nucleosome Core Particles and Free DNA at 37 °C

	nucleosome core particles		free DNA
position (SHL)	k_{cleave} (10^{-5} s^{-1})[a]	$t_{1/2}$ (h)	$t_{1/2}$ (h)[b]
L_{73} (0)	1.6 ± 0.2	12.5 ± 1.6	139
L_{89} (1.5)	2.3 ± 0.3	8.4 ± 1.2	165
L_{119} (4.5)	6.3 ± 1.8	3.3 ± 0.9	142

[a]Rate constants are averages ± standard deviations of at least three experiments, each consisting of three independent reactions. [b]Data were obtained in a single experiment consisting of three independent reactions in 100 mM NaCl, 1 mM EDTA, 10 mM HEPES (pH 7.5).

The half-life of the DNA ranged from 3.3 to 12.5 h, depending upon its position within the nucleosome. Although the local sequence surrounding L varied at the three sites, this is not believed to account for the differences in reactivity within the nucleosome core particle because a similar trend was not observed in the free DNA. The half-life of 2-deoxyribonolactone was shortest at SHL 4.5 (L_{119}), where X-ray crystallography revealed that the 601 DNA is stretched.[17] The rate constant for strand scission at L was smallest at the dyad axis (L_{73}), where the interaction between DNA and the proteins is weakest. The acceleration of strand scission in the nucleosome relative to that in free DNA (100 mM NaCl) ranged from 11- to 43-fold. Although the absolute rate constant for strand scission of 2-deoxyribonolactone (L_{89}) in the nucleosome is similar to that for AP at the same position, the acceleration relative to L cleavage in free DNA is smaller.[10]

AP cleavage in the nucleosome proceeds via Schiff base formation, and DNA–protein cross-links (DPCs) at SHL 1.5 are persistent.[10] Nucleosome core particles containing L at SHL 1.5 yield only ~6% DPCs after 24 h (~3 half-lives). The DPCs with L_{89} involve exclusive reaction with the H4 protein, and unlike AP, the DNA is completely cleaved.[10,20] The lower levels of DPCs from 2-deoxyribonolactone could reflect the less electrophilic nature of its carbonyl relative to that in AP, the shorter lifetime of the cross-links, or an alternative strand scission mechanism. The mechanistic significance of the DPCs with respect to strand scission was probed by examining the effect of a competitive nucleophile, β-mercaptoethanol (BME), on their formation and on the rate constant for cleavage at L in the nucleosome (Figure 2). Increasing concentrations of BME

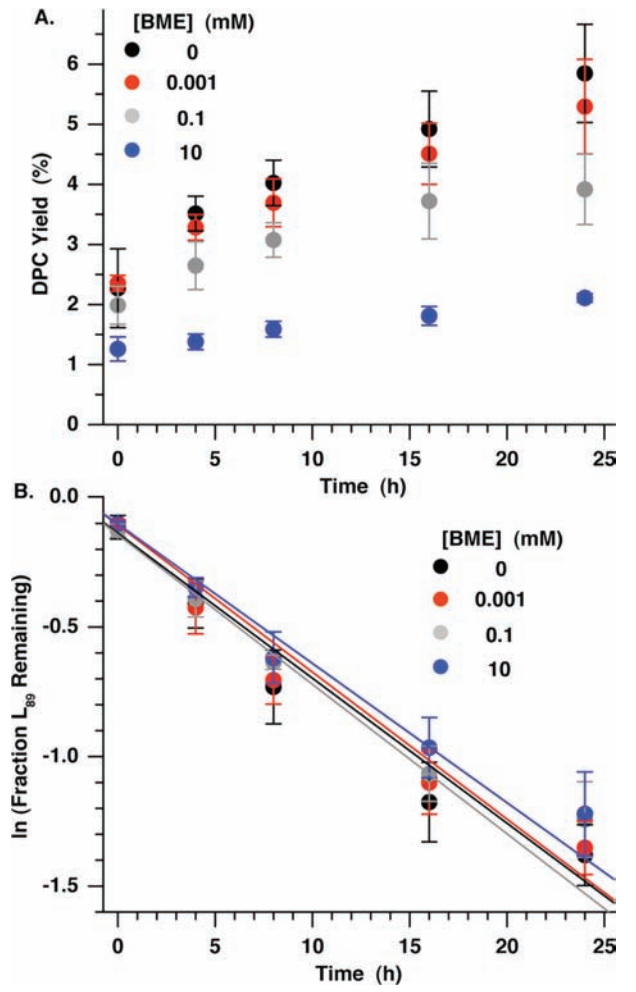


Figure 2. Effect of β-mercaptoethanol (BME) on (A) DNA–protein cross-link yield and (B) kinetics of L_{89} strand scission in nucleosome core particles.

significantly reduced the growth of DPCs (Figure 2A). However, the thiol had no effect on the rate constant for strand scission in the nucleosome containing L (Figure 2B). These data suggest that DPCs are not formed en route to strand breaks from L but instead are formed following cleavage, presumably via trapping of the butenolide intermediate **2** (Scheme 1).[14,15]

Butenolide **2** would arise via β-elimination and is known to undergo δ-elimination to produce 5′-DNA fragments containing 3′-phosphates (Scheme 1).[21] The 3′-termini of the 5′-fragments were shown to consist exclusively of phosphate end groups by examining the effect of polynucleotide T4 kinase, which removes 3′-phosphates.[20] A small decrease in migration through a denaturing gel typically accompanies dephosphorylation. ^{32}P labeling of the nucleosomal DNA at dT_{87} followed by treatment with the RsaI restriction enzyme produced a fragment short enough (10 nucleotides) to detect the change in

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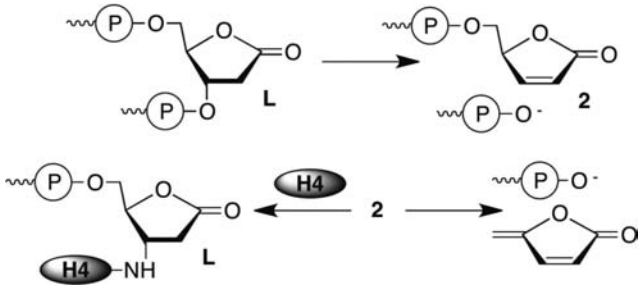
Scheme 1



migration and confirmed that 3′-phosphate groups were exclusively formed upon L cleavage. A similar procedure using DNA ^{32}P-labeled at dA_{101} and treatment of the cleaved DNA with shrimp alkaline phosphatase and AluI restriction endonuclease revealed that the 3′-fragments contained 5′-phosphates exclusively.[20] The observed end groups in the DNA fragments were consistent with β,δ-elimination of L. The elimination mechanism was probed using dideuterated L.[20] Deuterium was incorporated (94%) at C2 of the photochemical L precursor, and nucleosome core particles containing L_{89} were prepared as described above. The rate constant for disappearance of the starting nucleosome was $(5.3 \pm 0.3) \times 10^{-6}$ s^{-1} ($t_{1/2}$ = 36.3 h), yielding a kinetic isotope effect of 4.3, which indicates that deprotonation is the rate-determining step.[20]

Previous studies of AP_{89} cleavage in nucleosomes showed that the histone H4 protein is involved in ∼95% of the strand scission reactions.[10] Deleting the 19 N-terminal amino acids reduced the rate constant ∼3-fold. The N-terminal tail of histone H4 is rich in Lys residues, and their post-translational modification is important in the regulation of transcription.[6,7] If they are involved in DNA lesion chemistry, their post-translational modification could affect the stability of damaged DNA. Consequently, we probed the role of Lys residues in the N-terminal 20 amino acids of histone H4 on 2-deoxyribonolactone cleavage by preparing a series of nucleosomes containing mutant proteins (Table 2). Replacing individual or

Table 2. Effects of Histone H4 Mutations on 2-Deoxyribonolactone Cleavage at SHL 1.5 (L_{89})

H4 variant	k_{cleave} (10^{-5} s^{-1})[a]	$t_{1/2}$ (h)
wild type	2.3 ± 0.3	8.4 ± 1.2
Lys5,8,12Ala	1.2 ± 0.1	15.7 ± 1.5
Lys16Ala	1.6 ± 0.3	12.1 ± 2.0
Lys20Ala	1.5 ± 0.2	13.4 ± 1.6
Lys16,20Ala	1.4 ± 0.2	14.5 ± 2.2
deletion 1−19	1.0 ± 0.1	19.8 ± 2.8
deletion 1−20	0.76 ± 0.11	25.8 ± 3.8

[a]Rate constants are averages ± standard deviations of at least four experiments, each consisting of three independent reactions.

even two or three Lys residues with Ala decreased the rate constant for 2-deoxyribonolactone cleavage by less than 2-fold. Deleting the 19−20 N-terminal amino acids of histone H4 containing four or five Lys residues reduced the cleavage rate constant as much as 3-fold. However, L is still ∼6 times more labile in these nucleosomes than in naked DNA. These data indicate that multiple Lys in the H4 protein tail contribute to 2-deoxyribonolactone cleavage in the nucleosome. However, the histone tail is not solely responsible for the enhanced strand scission rate constant.

This is the second example in which the octameric histone core catalyzes cleavage of a DNA lesion within a nucleosome. The implications of accelerated L cleavage within nucleosomal DNA are significant with respect to the mechanism of action of antitumor agents such as neocarzinostatin (NCS), C1027, and other enediyne agents.[22−24] For instance, NCS produces 2-deoxyribonolactone as part of a bistranded ("clustered") lesion in which the complementary strand is directly cleaved within 2−3 nucleotides of L. Although isolated abasic sites such as L are efficiently incised during base excision repair, clustered lesions are much less efficiently repaired.[25,26] AP sites with clustered lesions have been suggested to exist for as long as 1 day in mammalian cells.[27] When considered together, these data suggest that formation of 2-deoxyribonolactone as part of a clustered lesion in cellular DNA results in de facto double strand scission, the most deleterious of DNA lesions, and provides additional chemical foundation to explain why antitumor agents that produce it are such potent cytotoxins.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for all experiments, complete sequences of all DNAs used to prepare nucleosome core particles, representative autoradiograms and kinetic plots, and mass spectra of oligonucleotides containing **1** and mutated histone H4 proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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