The 2-Deoxyribonolactone Lesion Produced in DNA by Neocarzinostatin and Other Damaging Agents Forms Cross-links with the Base-Excision Repair Enzyme Endonuclease III

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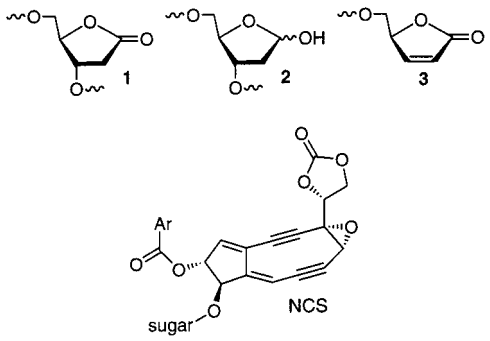
The integrity of genetic information is safeguarded by families of DNA repair enzymes. Base-excision repair (BER) enzymes incise base mispairs, altered nucleobases, and abasic sites.[1] The downside of DNA maintenance is that these enzymes can thwart the function of therapeutic agents that target DNA, such as antitumor agents. DNA lesions that are refractory to BER are potentially desirable products produced by nucleic acid damaging agents. Irreversible inhibition of BER is one means by which DNA lesions may contribute to a cell's resistance to repair. However, modified nucleotides that form cross-links to repair enzymes are extremely rare.[2] We wish to report that an oxidized abasic site, 2-deoxyribonolactone (**1**), which is produced in a variety of DNA damage processes, irreversibly inhibits the BER enzyme endonuclease III.



2-Deoxyribonolactone (**1**) is an alkali-labile lesion produced in DNA by γ-radiolysis, UV-irradiation of 5-halopyrimidine containing biopolymers, the neocarzinostatin chromophore (NCS), manganese porphyrins, and possibly the radiomimetic copper-phenanthroline.[3−8] Although the most common pathway for forming **1** involves initial C1′-hydrogen atom abstraction, the lesion is also produced in DNA via direct hydride abstraction, and monomer studies suggest that the radical pathway may be accessed by initial one-electron oxidation of the pendant nucleobase.[9,10] In contrast to abasic sites (**2**) resulting from formal hydrolysis of a nucleotide's glycosidic bond, very little is known about the susceptibility of 2-deoxyribonolactone (**1**) to repair.[11] Recent methods for generating 2-deoxyribonolactone (**1**) at defined sites in oligonucleotides have facilitated structural studies of duplexes containing the lesion and investigations into its reactivity.[4,12,13] On the basis of these results and studies on the reactivity of **2** with BER enzymes in which nucleophilic attack on the carbonyl was proposed, we suggested that 2-deoxyribonolactone (**1**) may be an irreversible inhibitor of some members of this family of repair enzymes. We wish to report evidence that one of these enzymes, endonuclease III from *E. coli*, cross-links to 2-deoxyribonolactone (**1**).

Endonuclease III (endo III) was chosen to test this proposal, because the mechanism of this enzyme is well understood, and its detailed structure has been determined.[11,14] Following formation of **1** in DNA (5′-^{32}P-**5**), as previously described by irradiation of 5′-^{32}P-**4**, addition of endo III led to the formation of a covalent adduct as evidenced by SDS-denaturing PAGE (Figure 1). On the basis of the amount of lactone present, the cross-linking efficiency was typically ∼20%.[15] Cross-linking was not observed upon reaction of comparable duplexes containing the ketone precursor of **1** (**4**), **2** (**6**), or a tetrahydrofuran analogue of **2** (**7**), supporting the proposal that 2-deoxyribonolactone was responsible for cross-linking the enzyme.[16] Cross-link formation was specific to double-stranded DNA, supporting the proposal that endo III was recognizing and binding to 2-deoxyribonolactone in a manner analogous to that used to incise an abasic site (**2**).[16] The cross-linked product was cleaved upon heating (90 °C) under alkaline conditions (Figure 2), but not in the absence of base.[16] A covalent adduct was also observed upon exposure of 3′-^{32}P-**5** to endo III, indicating that the oligonucleotide backbone remained intact upon cross-link formation.[16] Evidence that the lysine previously invoked

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(16) See Supporting Information.

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Figure 1. SDS-PAGE analysis of reaction between 5′-^{32}P-**5** and endonuclease III. [**5**] = 5 nM in all lanes. Lane 1, **5**; Lane 2, **5** + piperidine; Lane 3, **5** + endo III (wt), 20 nM; Lane 4, **5** + endo III (Asp44Ala), 20 nM; Lane 5, **5** + endo III (Lys120Ala), 20 nM. Note: The mutant proteins contain a hexa-His-tag, which gives rise to a slower moving product (higher molecular weight) than the native protein in the gel.

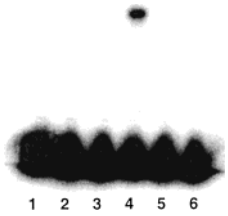
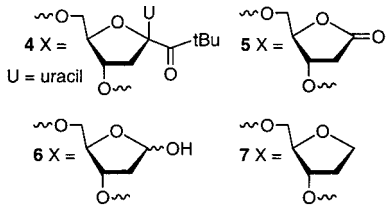


Figure 2. SDS-PAGE analysis of reaction between 5′-^{32}P-**5** and endonuclease III. [**5**] = 5 nM in all lanes. Lane 1, **5**; Lane 2, **5** + (0.1 M) piperidine; Lane 3, **5** + (0.1 M) NaOH; Lane 4, **5** + endo III (wt), 20 nM; Lane 5, **5** + endo III (wt), 20 nM, followed by (0.1 M) piperidine; Lane 6, **5** + endo III (wt), 20 nM, followed by (0.1 M) NaOH.

in Schiff base formation (Lys120) forms the cross-link to **1** was gleaned from comparable experiments with mutants of endo III.[11,17] Substitution of alanine for the aspartic acid (Asp44Ala) believed to be involved in binding damaged nucleotides reduces the amount of cross-linking observed, whereas replacement of Lys120 with alanine (Lys120Ala) eliminates cross-linking (Figure 1).[17]

5′-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)
(CAG TGC ACG ACG TAT GCT GCA CGA CTC GGA)

4 - 7



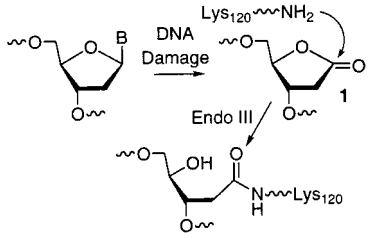
All of these observations are consistent with covalent linkage of endo III to **1**. However, structural information was lacking, and we could not rule out the possibility that cross-linking resulted from reaction of the enzyme with an artifact produced upon UV-irradiation of **4**. Consequently, we sought corroboration that **1** cross-links to endonuclease III by examining the reaction between DNA treated with the neocarzinostatin chromophore and enzyme.[16] Duplex **8** was designed so as to contain three contiguous

5′-^{32}P-d(CTT AGG ACG AGC GAG CGA GCG GCA GGC ACA)
(GAA TCC TGC TCG CTC GCT CGC CGT CCG TGT)

8

5′-dAGCG sequences, which have been shown by Goldberg and Kappen to result in significant formation of **1** at the deoxycytidines

Scheme 1



contained therein.[7a−c] Reaction of 5′-^{32}P-**8** with NCS results in 2-deoxyribonolactone (**1**) formation, as indicated by the increased yield of alkali-labile lesions at these nucleotides.[16,18] Subsequent reaction of the NCS-treated duplex with wild-type endo III or its mutant lacking Lys120 (Lys120Ala) produces cross-link from the wild-type enzyme, but not the mutant.[16] Contribution of other lesions produced by NCS to cross-link formation cannot be ruled out by these data. However, cross-link formation between endo III and DNA containing 2-deoxyribonolactone (**1**) produced by two independent methods strongly imply that the lactone must at least be partially responsible for the observed covalent adduct.

On the basis of these observations, we propose that 2-deoxyribonolactone (**1**) irreversibly inhibits the lyase step normally effected by endo III during the incision of abasic sites (**2**) in duplex DNA.[11] Inhibition results from formation of a cross-link with Lys120 via nucleophilic attack on the carbonyl group of **1** (Scheme 1). To our knowledge, **1** is the first lesion produced by an antitumor agent shown to irreversibly inhibit a DNA repair enzyme. We predict that further investigation will reveal that 2-deoxyribonolactone forms cross-links to other biological molecules, and propose that these processes contribute to the resistance of cells treated with neocarzinostatin to repair.[19,20] If so, the formation of 2-deoxyribonolactone (**1**) in DNA should be a desirable goal in therapeutic applications for which this biopolymer is a target.

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Supporting Information Available: SDS-PAGE analysis of the effect of alkaline treatment on the cross-link formed between **1** and endonuclease III, the reaction between **4**, **6**, and **7** with this enzyme, and the reaction with single- and double-stranded DNA containing **1**; denaturing PAGE and SDS-denaturing PAGE analysis of the reaction of **8** with NCS and subsequent reaction with endo III (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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