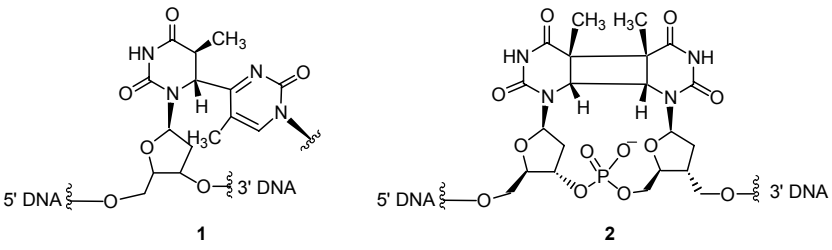
REPAIR OF DNA DAMAGE: CASES OF IRREVERSIBLE INHIBITION AND SMALL-MOLECULE ASSISTANCE

Reported by Mary Smalley October 21, 2002

INTRODUCTION TO DNA DAMAGING AGENTS AND REPAIR PATHWAYS

DNA damage is induced by a variety of agents. Exogenous agents such as sunlight,[1] tobacco smoke,[2] γ-radiolysis,[3] and tumor antibiotics[4] inflict harm on DNA. Endogenous agents such as oxygen and even water are also responsible for DNA damage.[2] The types of DNA damage are as numerous as the agents that cause them. For example, single-strand breaks are often a result of attack by reactive oxygen species.[2] Double strand breaks, which are dangerously cytotoxic but occur much less often, can be generated by ionizing radiation[2] and by the antitumor agent bleomycin.[4] Bleomycin is one of the best understood DNA-damaging agents, and it degrades DNA by causing an extraordinary large number of double strand breaks.[4,5]

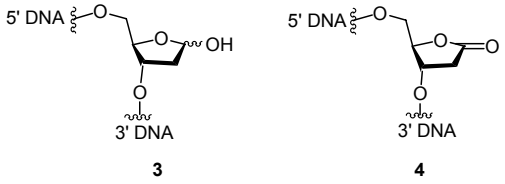
It is well known that ultraviolet light causes DNA damage.[1,6] Two frequently observed UV photoproducts are the 6-4 photoproduct **1**,[7] and the thoroughly studied thymine dimer **2** (Figure 1).



1 2

Figure 1. UV-induced photoproducts

Another type of damage is the formal hydrolysis of a nucleotide glycosidic bond, resulting in an abasic site **3** (Figure 2). Abasic sites are also formed as intermediates by many repair enzymes.[8] A deoxyribonolactone lesion (**4**) results from damage by γ-radiolysis,[3] enediyne tumor antibiotics,[9,10] and manganese porphyrins,[11] as well as other sources. These examples are illustrative not only of the scope of the types of DNA damage, but also the multitude of sources of that damage.



3 4

Figure 2. Abasic damage

In mammalian cells, damaged DNA is repaired in a variety of ways, depending on the type of damage. The base-excision repair (BER) pathway is responsible for the repair of DNA lesions and single- and double-stranded breaks.[2] A second DNA repair pathway, the nucleotide excision repair pathway (NER), removes UV-induced lesions. Due to the mutagenic and cytotoxic nature of UV photoproducts, and given the fact that humans have no secondary repair pathway[2] for them, proper functioning of the NER pathway is critical.

This review will focus on two specific types of DNA damage, the thymine dimer **2** and deoxyribonolactone lesion **4**. Formation and repair of each will be discussed. Model systems to effect remote repair of the thymine dimer, and inhibition of the enzymes involved in repair of the deoxyribonolactone lesion, as well as implications of those processes, will be presented.

THYMINE DIMERS

Biological Relevance of Thymine Dimers

Thymine dimers (**2**), which form between adjacent thymines on the same DNA strand, are the most frequently formed photoproduct.[12] Repair of thymine dimers is slow in both bacterial and mammalian cells. Consequently, it is the photoproduct mostly likely encountered by a polymerase during DNA repair or replication, and as mentioned, thymine dimers can be mutagenic and carcinogenic.[1] When DNA replication and repair take place on strands containing thymine dimers, mutations frequently occur.[13] Additionally, thymine dimers may block replication and transcription completely. Recent studies of Xeroderma pigmentosum (XP) link thymine dimers directly to the development of skin cancer. XP is a rare genetic disease characterized by defects in cellular repair of DNA damage by the NER pathway.[1,6] Individuals with XP experience extreme sensitivity to sunlight and have a 1000-fold increased chance of developing skin cancer. UV light produces characteristic mutations in DNA, including C→T and tandem CC→TT mutations at dipyrimidine sites.[1,6] The tumor-suppressor p53 gene of skin cancer is inactivated by multiple diverse mutations. C→T and CC→TT mutations have been found in p53 in skin cancers. The double base change CC→TT is a signature pattern known to be induced only by UV light[1,6] and thus provides a direct link from UV light to skin cancer.

Repair of Thymine Dimers

Repair of thymine dimers differs in bacterial and mammalian cells. In bacterial cells, thymine dimers can be repaired chemically or by a class of specialized repair enzymes, photolyases, that catalyze the reverse reaction[14] (Scheme 1). The light-activated enzyme induces a one-electron photoreduction of the dimer substrate, initiating repair.[14] By contrast, in mammalian cells, many enzymes are required for

Scheme 1: [2+2] cycloaddition to form thymine dimer.

thymine dimer repair by the nucleotide excision repair pathway (NER). This pathway is responsible for recognition of damage, incision of the DNA strand containing the lesion, and DNA repair synthesis and ligation.[2] Upon recognition of damage, which is usually evident from a distortion in the helix, ATP-dependent helicases form an open bubble structure. The open bubble makes specific sites, recognized as junctions between single- and double-stranded DNA, available for 3'- and 5'-cleavage. Six core factors, made up of fifteen to eighteen proteins, are required for the two cutting events. A 24-32 nucleotide fragment is released, and the gap is filled by DNA polymerase δ or ε and then sealed by DNA ligase 1.

Remote Repair of Thymine Dimers

It is known that rhodium(III) intercalating complexes bind tightly to DNA and are also strong photo-oxidants.[14] Barton and coworkers demonstrated that a Rh(III) interacalator **7**, $Rh(phi)_2DMB^{3+}$ (Figure 3), binds to a 16-base pair DNA duplex and induces a one-electron photo-oxidation, repairing a site-specifically incorporated thymine dimer. First order kinetics were observed, and nearly quantitative repair occurred with as little as 500 nM noncovalently attached Rh(III) complex. Repair occurred when **7** was covalently tethered to the 5'-end or tethered to the 3'-end of the 16-base pair duplex, though yields were lower (~50%) than with noncovalently attached **7**. Distance from the metal center to the site of damage did not matter in repair efficiency; the metallointeracalator can be as far away as 16 Å to 26 Å. However, perturbation of base stacking in the DNA duplex markedly diminished repair. Barton and coworkers established that repair occurs without direct contact of the metal center and the thymine dimer, indicating that the DNA duplex facilitates long-range electron transfer.

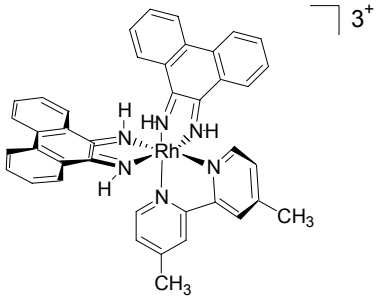


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Figure 3. $Rh(phi)_2DMB^{3+}$

Remote repair of thymine dimers in short DNA duplexes has been investigated by Barton and coworkers with **8**, an aromatic naphthalene diimide intercalator (NDI).[15] The naphthalene intercalator (Figure 4) has found therapeutic use as an anticancer and an antiviral agent[16] and is also known to be a long-range photo-oxidant. The intercalator **8** repaired site-specifically incorporated thymine dimers upon photoexictation. As with the Rh(III) complex, repair occurred with NDI noncovalently bound, but in much lower yield, only ~50%. The covalently attached NDI gave only 35% repair of the thymine dimer. In the same study, several anthroquinone intercalators were

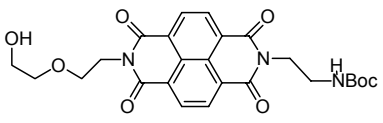

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Figure 4. Naphthalene diimide intercalator

tested, but failed to repair the dimer. Importantly, Schuster and coworkers reported that remote repair does not occur in duplexes with -GG- doublets, because oxidation of guanosine occurs in preference to thymine dimer repair.[12]

These examples of remote repair of thymine dimers suggest that DNA-targeting drugs may be designed to be activated and function from a distance. This is in contrast to current design for DNA-targeting pharmaceuticals that seek to exploit site-specific drug-DNA interactions. One complication of remote repair of thymine dimers by redox processes is that -GG- doublets are easily oxidized. Selective or tunable DNA intercalators would be required to avoid this problem.

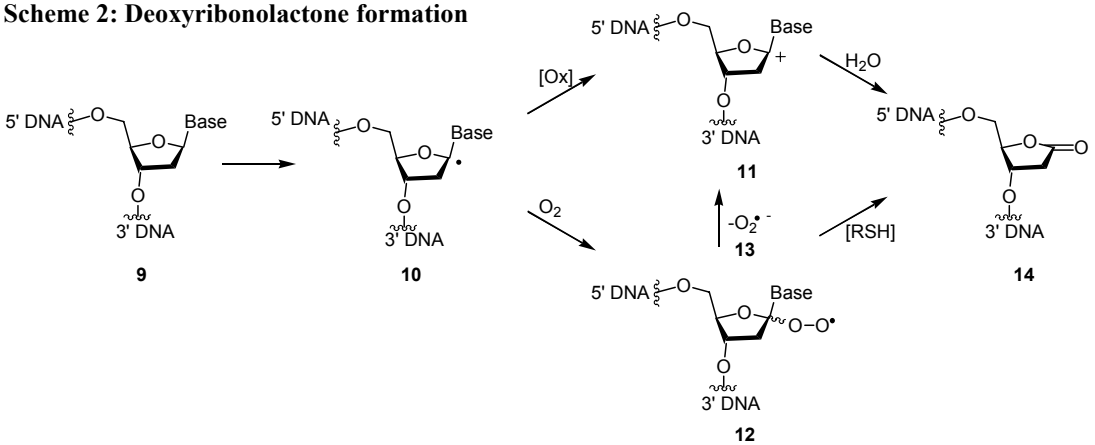
DEOXYRIBONOLACTONE LESIONS

Deoxyribonolactone-Inducing Agents and Lesion Reactivity

2-Deoxyribonolactone lesions **14**, the formal product of C1′ oxidation, are generated by many DNA-damaging sources. The lesion is often a result of γ-radiolysis[17] and enediyne tumor antibiotics such as the chromoprotein neocarzinostatin.[10] Heterocyclic N-oxides of the tirapazamine family,[18] manganese porphyrins, and artificial nucleases based on manganese porphyrins[11] also cause deoxyribonolactone lesions. The deoxyribonolactone lesion is also thought to be an intermediate in damage caused by copper phenanthroline.[19]

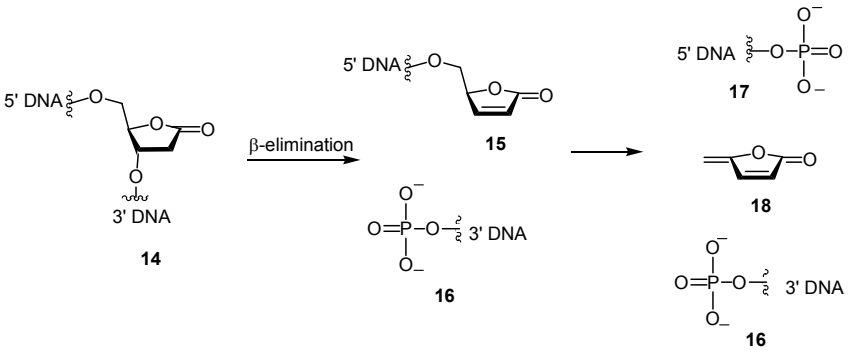
Formation of the deoxyribonolactone lesion (Scheme 2) is most commonly initiated by direct hydrogen abstraction at C1′, to form the C1′ sugar radical **10**.[20] Several oxidative pathways are possible after hydrogen abstraction, the result of which is the extrusion of the base to produce the lactone **14**. The most general oxidative pathway to the deoxyribonolactone is trapping of the C1′ radical with O_2.[3] Decomposition of the intermediate peroxyl radical (**12**) via heterolytic cleavage of the C1′-oxygen bond yields the deoxyribonolactone lesion (**14**) and a superoxide **13** ($O_2^{\cdot-}$) radical, which has the potential to amplify the damage after transformation into other radicals, such as

Scheme 2: Deoxyribonolactone formation



hydroxyl radical. The deoxyribonolactone may also form through a C1' cation (**11**). After formation, the deoxyribonolactone (**14**) may undergo β-elimination to produce the α,β-unsaturated lactone **15**[20] and the 3'-phosporylated cleavage product (**16**) (Scheme 3). The DNA strand (**15**) may fragment to yield the 5'-phosphorylated cleavage product (**17**) and methylene furanone, **18**. When thiols are presents, the β-elimination product may be trapped by a Michael-type reaction.

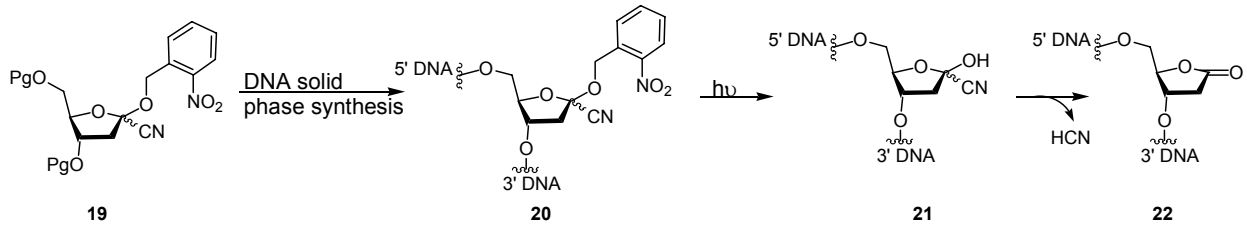
Scheme 3: Deoxyribonolactone degradation



Site-Specific Generation of Deoxyribonolactone Lesions

Several groups have recently reported the synthetic generation of the deoxyribonolactone lesion.[17,20,21] A method developed by Sheppard and coworkers[20] (Scheme 4) uses a "caged" phosphoramidite (**19**) incorporated by solid phase synthesis. The deoxyribonolactone can be generated site-specifically in DNA by this method. The *o*-nitrobenzyl ether (**20**) is cleaved by UV irradiation to produce the lactone cyanohydrin (**21**), followed by decomposition to the lactone (**22**).[20] Methods to incorporate the deoxyribonolactone are desirable because the lesion is alkaline labile and breaks down quickly *in vivo*, making it difficult to study. Using site-specifically incorporated deoxyribonolactones, Kotera and coworkers studied the stability of the deoxyribonolactone and found it to be about twenty times less stable than an abasic site.[22]

Scheme 4: Generation of Site-Specific Deoxyribonolactone Lesion



Repair of the Deoxyribonolactone Lesions

In contrast to thymine dimers, little is known about deoxyribonolactone repair by the base excision repair pathway (BER).[23] DNA glycosylases specific for a type of damage, recognize and remove damaged bases forming abasic sites. The abasic site is incised on its 5' side, followed by excision of a deoxyribose phosphate group. After the damaged base has been removed, DNA repair

synthesis takes place, followed by ligation. There are two pathways by which these events occur. One uses AP endonuclease, DNA polymerase β, and DNA ligase, with the DNA polymerase catalyzing both excision and repair synthesis. The second pathway uses proliferating cell nuclear antigen-dependent (PCNA) polymerase, DNA polymerase β, δ, or ε, FEN1, and DNA ligase, after excision of the deoxyribose phosphate group.

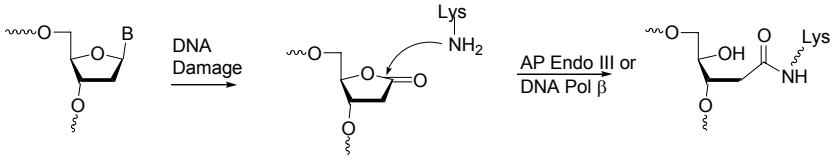
Repair Inhibition

The base excision repair enzymes can thwart the function of therapeutic agents that target DNA, such as antitumor agents, by repairing damage that is therapeutically desired. In this context, DNA lesions that *cannot* be repaired via the base excision repair pathway and its enzymes are the desired products of the action of therapeutic nucleic acid damaging agents. One way to block repair is to inhibit an enzyme in the repair pathway. One form of irreversible inhibition occurs when the modified nucleotide crosslinks to an enzyme that attempts to repair it. This is very rare, but recent evidence indicates that the deoxyribonolactone can crosslink to the enzymes in the base excision pathway.[13]

Crosslinking to AP Endonuclease III. In 1999, Kotera presented a refined solution structure of the deoxyribonolactone based on NMR and molecular modeling.[24] Destabilization and flexibility at the damage sites were noted as potentially important in recognition by repair enzymes. Later that year, Greenberg, noting that very little is actually known about the enzymatic repair of deoxyribonolactone lesion, proposed that the deoxyribonolactone acts as a suicide substrate that generates a covalent crosslink between the α,β-unsaturated lactone and a nucleophile in the enzyme binding site.[8] This hypothesis was also based on studies of the reactivity of abasic sites with BER enzymes, in which nucleophilic attack on the carbonyl was proposed.

Bacterial endonuclease III (AP endo III) was used to test the crosslinking proposal because its mechanism is well understood and a detailed structure has been published. Greenberg showed that crosslinking of the deoxyribonolactone lesion to endo III does occur in double-stranded DNA with an efficiency of ~20% (Scheme 5).[23] Crosslinking was not observed in single-stranded DNA.[23] This supports the proposal that the enzyme recognizes and binds to the lesion in a similar way as in abasic site repair. Substitutions of the active site lysine by alanine eliminated crosslinking. However, substitution of alanine for an

Scheme 5: Crosslinking of repair enzyme to deoxyribonolactone



aspartic acid thought to be important for binding damaged nucleotides decreased but did not eliminate crosslinking.[23] Greenberg concluded that the deoxyribonolactone lesion irreversibly inhibits the lyase step of endo III in double-stranded DNA by crosslinking.[23]

Crosslinking by DNA Polymerase β. In an effort to determine whether other repair enzymes in the base excision repair pathway would suffer a similar fate, Greenberg sought to test DNA polymerase β for crosslinking. DNA pol β is a α-helical, multifunctional enzyme containing an N-terminal domain with 5'-2-deoxyribose-5-phosphate lyase activity and a C-terminal domain with nucleotidyltransferase activity.[25] It has been proposed that DNA pol β catalyzes the removal of a damaged residue from an abasic site by a reaction involving an imine or Schiff base. Since these proposals, it has been shown that a Schiff base intermediate forms between the abasic site and an active site lysine residue. It has been suggested, based on site-directed mutagenesis, that the Schiff base nucleophile in the N-terminal domain is Lys^{72}.

In a recent paper, Greenberg demonstrates that DNA pol β crosslinks to an abasic site in reactions with DNA pol β and undamaged DNA (Scheme 5).[26] Crosslinks were not observed in reactions with DNA pol β and undamaged DNA, and crosslinking was dependent on Lys^{72}. The mechanism of crosslinking is suicide inhibition of DNA pol β by the deoxyribonolactone.[26]

The potential for crosslinking to repair enzymes makes the deoxyribonolactone a very dangerous DNA lesion. Because the lesion is blocked by the covalently-attached enzyme, a loss of genetic information and ability to replicate may result. The crosslinked enzyme also blocks the lesion from potential repair activities by other enzymes. Finally, the crosslink itself constitutes another type of damage.

FUTURE CHALLENGES

DNA damage presents an interesting dichotomy. In the context of cancer, damage can cause disease, as demonstrated with thymine dimers and skin cancer. By contrast, DNA damage can also be therapeutic as in the case of bleomycin and ionizing radiation. Work will continue to emerge on both fronts, examining damage in the context of other disease conditions and utilizing it for treatment.

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