Current Topics

# Biological and Pharmaceutical Aspects of Nucleic Acids Chemistry

## Human DNA Glycosylases Involved in the Repair of Oxidatively Damaged DNA

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**Reactive oxygen species from endogenous and environmental sources induce oxidative damage to DNA, and hence pose an enormous threat to the genetic integrity of cells. Such oxidative DNA damage is restored by the base excision repair (BER) pathway that is conserved from bacteria to humans and is initiated by DNA glycosylases, which simply remove the aberrant base from the DNA backbone by hydrolyzing the N-glycosidic bond (monofunctional DNA glycosylase), or further catalyze the incision of a resulting abasic site (bifunctional DNA glycosylase). In human cells, oxidative pyrimidine lesions are generally removed by hNTH1, hNEIL1, or hNEIL2, whereas oxidative purine lesions are removed by hOGG1. hSMUG1 excises a subset of oxidative base damage that is poorly recognized by the above enzymes. Unlike these enzymes, hMYH removes intact A misincorporated opposite template 8-oxoguanine during DNA replication. Although hNTH1, hOGG1, and hMYH account for major cellular glycosylase activity for inherent substrate lesions, mouse models deficient in the enzymes exhibit no overt phenotypes such as the development of cancer, implying backup mechanisms. Contrary to the mouse model, hMYH mutations have been shown to lead to a multiple colorectal adenoma syndrome and high colorectal cancer risk. For cleavage of the N-glycosidic bond, bifunctional DNA glycosylases (hNTH1, hNEIL1, hNEIL2, and hOGG1) use Lys or Pro for direct attack on sugar C1′, whereas monofunctional DNA glycosylases (hSMUG1 and hMYH) use an activated water molecule. DNA glycosylases for oxidative damage, if not all, are covalently trapped by DNA containing 2-deoxyribonolactone or oxanine. Thus, the depletion of functional DNA glycosylases using covalent trapping may reduce the BER capacity of cancer cells, hence potentiating the efficacy of anticancer drugs or radiation therapy.**

**Key words** oxidative DNA damage; DNA glycosylase; action mechanism; covalent trapping; anticancer drug development

### 1. INTRODUCTION

DNA molecules in cells constantly suffer from chemical decay due to exposure to endogenous and environmental agents, the immediate consequence of which is alterations to hydrogen bonding and base-stacking interactions in the DNA helix. Considering that such interactions are pivotal to the faithful expression and replication of genetic information, unrepaired DNA damage poses a substantial threat to living organisms. Reactive oxygen species (ROS) produced by aerobic metabolism or inflammatory responses are the major sources of DNA damage, and they induce strand breaks and a variety of oxidative base lesions, with the latter predominating. The steady-state levels of oxidative pyrimidine and purine lesions in the human chromosome are currently estimated as several per $10^6$ base pairs, respectively,[1] indicating frequent insult to DNA by ROS. Oxidative DNA damage is also implicated in many human diseases.[2]

Living organisms possess a variety of DNA repair mechanisms to cope with the deleterious effects of DNA damage, and these mechanisms are conserved from bacteria to humans. Oxidative base damage, characterized as structurally non-distorting and non-bulky in nature, is repaired by the base excision repair (BER) pathway. The BER pathway is initiated by a class of enzymes called DNA glycosylases that constantly monitor aberrant bases in the genome and hydrolyze the N-glycosidic bond linking sugar and base moieties. During the past decade, our understanding of a human BER process for oxidative base damage has been greatly advanced by progress in the method to prepare defined oligonucleotide substrates containing unique DNA lesions and the identification of human homologues of DNA glycosylases that were first found in bacteria. This review summarizes recent progress in the research field of human DNA glycosylases involved in the repair of oxidative base damage. For some related aspects that are not covered in this article, readers should consult recent reviews.[3—6]

### 2. BASE EXCISION REPAIR PATHWAY

In the first step of the human BER pathway for oxidative base damage, DNA glycosylases with relatively wide damage specificity remove the aberrant base from the DNA backbone by hydrolyzing the N-glycosidic bond between sugar C1′ and the base (Fig. 1). Most DNA glycosylases involved in the repair of oxidative base damage have an associated apurinic/apyrimidinic (AP) lyase activity and further catalyze either $\beta$-elimination of the 3′ phosphodiester bond or $\beta,\delta$-elimination of the 3′ and 5′ phosphodiester bonds. These enzymes are called bifunctional DNA glycosylases (*i.e.*, N-glycosylase/AP lyase). Subsequently, the major human AP endonuclease (APE1) removes the 3′ terminal 4-hydroxypentenal phosphate (formed by $\beta$-elimination) or the 3′ terminal phosphate (formed by $\beta,\delta$-elimination) that blocks repair synthesis. The resulting single nucleotide gap with a 3′-OH terminus is filled by DNA polymerase $\beta$ using base-pair information from the complementary strand, and finally the nick is sealed by DNA ligase III/XRCC1. Another class of DNA

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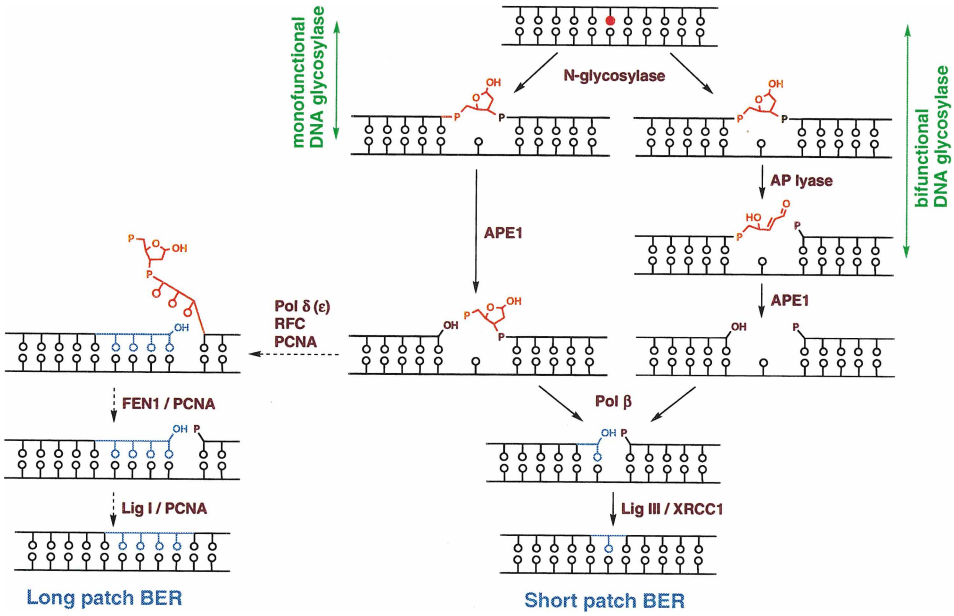


Fig. 1. Pathways of Base Excision Repair (BER)

Table 1. Human DNA Glycosylases for Oxidative Base Damage

| Type | DNA glycosylase | | Substrate | AP lyase | C1′ nucleophile | Localization |
|---|---|---|---|---|---|---|
| | Human | *E. coli* homologue | | | | |
| Bifunctional | hNTH1 | Endo III | Tg, hoU, hoC, urea, FapyG | $\beta$ | Lys212 | n, m[d] |
| | hNEIL1 | Endo VIII | Tg, hoU, hoC, urea, FapyG, FapyA | $\beta$, $\delta$ | Pro2 | n |
| | hNEIL2 | Endo VIII | AP site, hoU | $\beta$, $\delta$ | Pro2 | n |
| | hNEIL3 | Endo VIII | ? | ? | ? | n |
| | hOGG1 | Fpg[a] | 8oxoG, FapyG | $\beta$ | Lys249 | n, m |
| Monofunctional | hMYH | MutY | A:8oxoG | None[b] | $H_2O$ (Asp233)[c] | n, m |
| | hSMUG1 | None | U, hoU, hmU, fU | None | $H_2O$ (Asn85)[c] | n |

*a*) Fpg catalyzes $\beta,\delta$-elimination as AP lyase and also recognizes FapyA. *b*) Weak opportunistic AP lyase activity is observed. *c*) Amino acid residue involved in activation of a water molecule. *d*) n: nucleus, m: mitochondria.

glycosylases has no associated AP lyase activity and is called monofunctional DNA glycosylases (*i.e.*, N-glycosylase alone). This type of glycosylases mostly participates in the repair of deaminated or alkylated bases, but some are involved in the repair of oxidative base damage. The phosphodiester bond 5′ to an intact AP site generated by monofunctional DNA glycosylases is incised by APE1. The resulting 3′-OH terminus is extended by Pol $\beta$ and at the same time 5′ terminal deoxyribose phosphate (5′-dRP) is removed by AP lyase activity associated with Pol $\beta$, and finally the nick is sealed by DNA ligase III/XRCC1. The net reaction in these BER processes is the replacement of a single nucleotide unit, and is called short patch BER. The subpathway of BER is long patch BER, where Pol $\delta$ or Pol $\varepsilon$ synthesizes several nucleotides by displacing the downstream strand containing 5′-dRP. The resulting flap structure bearing 5′-dRP is incised by flap endonuclease (FEN1), and the nick is sealed by DNA ligase I. PCNA interacts with Pol $\delta/\varepsilon$, FEN1, and DNA ligase I throughout the process, supporting their functions. The long patch BER pathway appears to have a crucial role in processing oxidized or reduced AP sites that are resistant to the AP lyase activity of Pol $\beta$.

## 3. DNA GLYCOSYLASES FOR OXIDATIVE DAMAGE

The first studies into the repair mechanisms for diverse oxidative DNA lesions in *Escherichia coli* identified multiple but a limited number of DNA glycosylases, including endonuclease (Endo) III, Endo VIII, formamidopyrimidine-DNA glycosylase (Fpg/MutM), and MutY.[7] All of the human (functional) homologues of these enzymes have now been identified (Table 1). Human cells have an extra enzyme (hSMUG1) that recognizes a subset of oxidative base damage.

**hNTH1 and hNEIL1** hNTH1 and hNEIL1 are homologues of *E. coli* Endo III (Nth) and Endo VIII (Nei), respectively, and belong to Endo III and Nei/Fpg superfamilies, respectively (Table 1). They are both bifunctional DNA glycosylases, exhibiting essentially overlapping damage specificity.[8—11] hNTH1 has an associated $\beta$ AP lyase activity, while hNEIL1 has a $\beta,\delta$ AP lyase activity. Additional human Endo VIII homologues (hNEIL2 and hNEIL3) have also been identified, and are currently being characterized.[11—13] The principal substrates of hNTH1 and hNEIL1 and their mouse homologues are oxidized pyrimidines such as thymine glycol (Tg), urea, and 5-hydroxyuracil (hoU) and

certain types of oxidative purine damage [formamidopyrimidine derivatives of guanine (FapyG) and adenine (FapyA)] (Table 1).[8—11,13—17] Interestingly, *E. coli* Endo III and Endo VIII recognize FapyG poorly.[18] The analysis of cell extracts from NTH1-knockout mice indicates that mouse NTH1 (mNTH1) accounts for the major glycosylase activity toward oxidized pyrimidines such as thymine glycol and urea in cells,[19,20] and mNEIL1 (or other similar activity) appears to be responsible for relatively minor activity. Despite these observations, NTH1-knockout mice develop normally and show no overt phenotypic abnormalities such as tumor development,[19,20] suggesting redundant repair roles for NTH1 and NEIL1 *in vivo*. This has been proved the case for *E. coli*, where cells deficient in both Endo IIII and Endo VIII (*E. coli* counterparts of hNTH1 and hNEIL1) exhibit a spontaneous mutator phenotype and hypersensitivity to hydrogen peroxide and ionizing radiation.[21,22] Alternatively, both eukaryotic and prokaryotic cells may have a distinct repair pathway for oxidative base damage [nucleotide incision repair (NIR)] in which damaged DNA is specifically incised 5′ to the lesion by AP endonucleases [Endo IV (*E. coli*), Apn1 (*Saccharomyces cerevisiae*), and APE1 (human)].[23—26] The NIR pathway can work with the BER pathway in a co-operative manner to remove oxidatively damaged bases.

**hOGG1** hOGG1 is a functional homologue of *E. coli* Fpg (MutM) and is a bifunctional DNA glycosylase with an associated $\beta$ AP lyase activity (Table 1). Structurally, it belongs to the Endo III superfamily rather than the Endo VIII/Fpg family, and is thus a "functional" homologue of Fpg. The principal substrates of hOGG1 are oxidative purine damage including 8-oxoguanine (8oxoG) and FapyG. Unlike Fpg, the activity toward FapyA has not been demonstrated for hOGG1. According to the structure of the binary complex of hOGG1 and DNA containing an 8oxoG:C pair, the enzyme has two pockets in the active site: one accepting 8oxoG extruded from the helix and the other accommodating C in the opposite strand.[27] Considering that T is a physiological pairing base for FapyA, the discrimination of T in the second pocket may make a FapyA:T pair a poor substrate. The second pocket also prevents erroneous excision of 8oxoG from an 8oxoG:A pair that is formed by misincorporation opposite template A of 8oxodGTP from the cellular nucleotide pool. Consistent with these notions, urea, hoU, and 5,6-dihydrothymine (dhT) are excised when they are placed opposite C but not other bases (A, G, T) (Table 2) (ref. 28 and Odawara *et al.*, unpublished results). A similar observation is reported for AP sites.[29] The cell extracts from OGG1-knockout mice show virtually no detectable 8oxoG-excising activity.[30,31] Despite the loss of the crucial DNA glycosylase for mutagenic 8oxoG, the knockout mice, like *E. coli* Fpg mutants, show only a mild spontaneous mutator phenotype in some tissues but no increased incidence of cancer.[30,31] However, in cells lacking mOGG1, 8oxoG is removed efficiently from transcribed genes, implying additional cryptic glycosylase(s) for 8oxoG.[32] This may explain, at least partly, the lack of obvious phenotypes of OGG1-knockout mice. Another possibility is that 8oxoG is differentially tolerated in mammalian and *E. coli* cells during replication or transcription. OGG1-knockout mice treated with the ROS-generating agent $KBrO_3$ accumulate a high level of 8oxoG in the genome (200-fold relative to wild-type mice without $KBrO_3$), but survive normally with some accumulation of mutations.[33] Positive associations between genetic polymorphisms in hOGG1 and cancer risk have been implicated, but additional large and well-designed epidemiological studies are necessary to confirm the relation.[34]

Table 2. Activity of hOGG1 for Base Lesions

| Substrate | Relative activity |
|---|---|
| 8oxoG:C | 1.0 |
| Urea:C[a)] | 0.91 |
| hoU:C[a)] | 0.17 |
| dhT:C[a)] | 0.077 |
| Tg:C[a)] | $5.7\times10^{-4}$ |

*a*) Activity for other bases (A, G, T) was negligible.

**hMYH** hMYH is a homologue of *E. coli* MutY and a monofunctional DNA glycosylase with no associated AP lyase activity (Table 1). hMYH removes A that is misincorporated opposite 8oxoG in the template, hence preventing G:C→T:A transversions. hMYH also excises 2-hydroxyadenine paired with G.[35] *E. coli* uses three enzymes to cope with genotoxic effects of 8oxoG: Fpg/MutM (excising mutagenic 8oxoG from 8oxoG:C in DNA), MutY (excising misincorporated A from A:8oxoG in DNA), and MutT (hydrolyzing a mutagenic DNA precursor 8oxodGTP in the cellular dNTP pool). Although the mutations of the individual genes result in mild-to-severe spontaneous mutator phenotypes in *E. coli* (*mutT*>*mutY*>*fpg*/*mutM*), both MYH- and OGG1-knockout mice exhibit no overt phenotypic abnormalities.[36] Only mice defective in the MutT homologue (mMTH) develop tumors in lung, liver, and stomach with frequencies greater than wild-type mice.[37] However, in contrast to the results from mouse models, it has been recently discovered that biallelic mutations in hMYH lead to an autosomal recessive syndrome of adenomatous colorectal polyposis and high colorectal cancer risk.[38,39] This is the first demonstration of a link between the inherited disorder of a DNA glycosylase for oxidative DNA damage and human cancer.

**hSMUG1** hSMUG1 is a relatively new member of DNA glycosylases that repair oxidative damage (Table 1). It was originally identified as a single-strand-selective monofunctional uracil-DNA glycosylase (SMUG1) belonging to the UNG superfamily,[40] but later the enzyme was found to be active for both single- and double-stranded DNA. The putative SMUG1 gene is present in most eukaryotes except for *Caenorhabditis elegans*, *Arabidopsis thaliana*, and yeast.[41] Uracil arises in DNA either by incorporation of dUTP instead of dTTP during DNA synthesis or by hydrolytic deamination of C, and the latter event can induce mutations. The *E. coli* mutant deficient in uracil-DNA glycosylase (UNG) exhibits a mild spontaneous mutator phenotype, but UNG-knockout mice show only a marginal increase in mutation frequency and no overt phenotypes, suggesting the presence of a backup enzyme.[42] hSMUG1 is the most likely backup enzyme since it accounts for the major uracil-excising activity in UNG-deficient mice.[41] In other studies, the excision activity for the methyl oxidation products of T [5-formyluracil (fU) and 5-hydroxymethyluracil (hmU)] in mammalian cells has been shown to be related to SMUG1.[43—45] hSMUG1 acts as a monofunctional DNA glycosylase for fU, hmU, hoU and U (relative activity: U, hoU>hmU, fU) in

both single- and double-stranded DNA, but does not recognize analogous cytosine damage such as 5-formylcytosine and 5-hydroxycytosine.[45] The key features for damage recognition appear to be the uracil structure and the polar group attached to the C5 position that likely interacts with SMUG1 *via* hydrophilic interactions.[45,46] The activity for fU, hmU, and hoU in HeLa cells are neutralized almost completely by hSMUG1 antibodies, indicating a major repair role for a subset of oxidized pyrimidines that are inefficiently removed by cellular hNTH1, hNEIL1, and hOGG1 (refs. 47, 48, and Katafuchi *et al.*, unpublished results). This could also prevent the genotoxic effects of fU and hoU.[47,49] So far no mice deficient in mSMUG1 have been generated by the gene-targeting method, but a hamster V79 cell line deficient in hmU-DNA glycosylase activity has been isolated by mutagenesis.[50] These cells (V79mut1) show neither hypersensitivity nor increased mutation frequencies to ROS-generating agents.[51] More detailed assessment of the *in vivo* role of SMUG1 must await generation of SMUG1-knockout mice.

## 4. ACTION MECHANISMS

Among the DNA glycosylases listed in Table 1, the structures of hOGG1[27] and *Xenopus* SMUG1[46] complexed with DNA are available, though the latter is not a productive complex that represents a snapshot of the enzyme bound to the damaged site of DNA. However, together with these data, X-ray crystallographic analyses of prokaryotic and some eukaryotic DNA glycosylases complexed with DNA reveal that a general strategy called nucleotide flipping is used to remove the aberrant base from DNA.[3] When DNA glycosylases bind to damaged DNA, the target nucleotide flips out of the double helix into a specific pocket around the enzyme active site. An amino acid side chain is concertedly inserted from the minor groove and occupies the resulting void space in the helix. These DNA-protein interactions together with other ones result in significant kinks of DNA. The conformational changes in DNA make the target sugar C1′ accessible by the nucleophile (see below), which is otherwise buried in the double helix. The damage specificity of DNA glycosylases depends on whether the everted base remains in the damage-accepting pocket to give way to the transition state. Unlike UNG and hSMUG1 that exhibit a stringent damage specificity, hNTH1, hNEIL1, and hNEIL2 recognize structurally diverse base lesions that share a common feature: the lack of an aromatic character in the pyrimidine ring or the imidazole ring of purine. In light of their wide damage specificity, the damage-specific hydrogen bonds, if any, between the aberrant base and enzyme should be considered the result of an induced-fit rather than as a common damage recognition mechanism. The same precaution applies to hOGG1 (and Fpg). hOGG1 discriminates 8oxoG and FapyG from intact G only through a single hydrogen bond to N7,[27] but N7-methylated FapyG[52] and other lesions (Table 2) that cannot form the hydrogen bond are still good-to-fair substrates when paired with C. As mentioned in Section 3, some DNA glycosylases utilize extra interactions to recognize the orphan base opposite damage, thereby distinguishing target and non-target base pairs, for example, 8oxoG:C (substrate) *versus* 8oxoG:A (not substrate) for hOGG1.

The conformational changes induced upon binding of DNA glycosylases make the sugar C1′ accessible by a nucleophile for cleavage of the N-glycosidic bond linking deoxyribose and base moieties. Bifunctional DNA glycosylases use Lys [hNTH1(Lys212) and hOGG1 (Lys249)] or Pro [hNEIL1 (Pro2) and hNEIL2 (Pro2)] for direct attacks on C1′ (Fig. 2A), whereas monofunctional DNA glycosylases use a hydroxide anion that is formed by deprotonation of a water molecule by Asn [hSMUG1 (Asn85)] or Asp [hMYH (Asp233)] (Fig. 2B).

## 5. COVALENT TRAPPING OF REACTION INTERMEDIATES

The bifunctional DNA glycosylases (Table 1) use the SN2 mechanism to excise the aberrant base. When the base is displaced by the attack of a nucleophile (Lys or Pro), a Schiff base intermediate is transiently formed between DNA and enzyme (Fig. 2A). The $\beta$-elimination of the 3′ phosphodiester bond in the intermediate results in strand scission, leaving 3′ terminal 4-hydroxypentenal phosphate in the upstream strand and 5′ terminal phosphate in the downstream strand. For some DNA glycosylases with a $\beta,\delta$ AP lyase activity (hNEIL1, hNEIL2), concomitant $\delta$-elimination of the 5′ phosphodiester bond takes place in a Schiff base intermediate, generating 3′ terminal phosphate in the upstream strand. The transiently formed Schiff base intermediate can be transformed into a stable DNA-protein complex by the reduction of imine with $NaBH_4$ or $NaCNBH_3$ (Fig. 2A).[53] The covalent trapping of DNA glycosylases is used to probe the catalytic mechanism and to identify the nucleophilic amino acid, and has been also successfully applied to the preparation of glycosylase-DNA complexes, which are otherwise difficult to obtain, for X-ray crystallographic studies.[53]

It has been shown recently that Endo III and Pol $\beta$ that has an AP lyase activity are covalently cross-linked when they are incubated with DNA containing 2-deoxyribonolactone (dL),[54,55] which is a sugar lesion produced by ROS and impedes DNA replication.[56] Subsequent studies in our laboratory (Kuwahara *et al.*, unpublished results) and those of others[57] with varieties of *E. coli* and human enzymes have shown that bifunctional but not monofunctional DNA glyco-

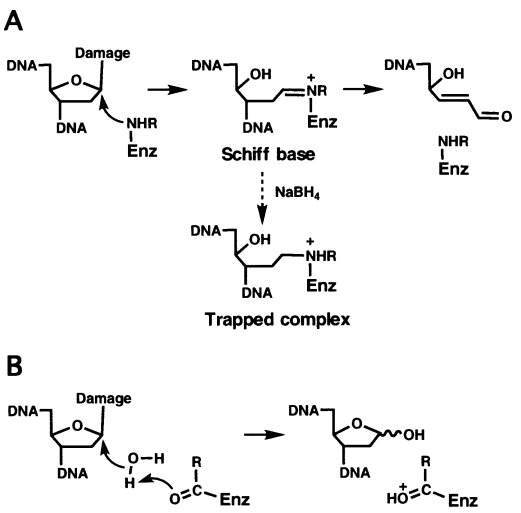


Fig. 2. Catalytic Mechanisms of (A) Bifunctional and (B) Monofunctional DNA Glycosylases

sylases can be efficiently trapped with dL *via* the formation of a stable amide bond (Table 3, Fig. 3A). Oxanine (Oxa) is a G-derived base lesion produced by reactive nitrogen oxide species such as NO and $HNO_2$,[58,59] and has a similar lactone (or O-acylisourea) structure. Incubation of DNA containing Oxa with DNA glycosylases leads to the formation of covalently trapped complexes.[60] However, the spectrum of these trapped enzymes is different from that for dL (Table 3). Interestingly, Endo III and hNTH1, which are bifunctional glycosylases and are trapped by dL, are not cross-linked by Oxa. Conversely, AlkA, a monofunctional glycosylase that is not trapped by dL, is cross-linked by Oxa. Thus, the amino acid involved in covalent trapping for Oxa appears different from that involved in borohydride trapping and cross-linking with dL (*i.e.*, the nucleophile to sugar C1′). The enzymes covalently trapped by Oxa share a common intrinsic (Fpg, AlkA, and hOGG1) or latent (Endo VIII, hNEIL1, and hNEIL2) capacity to recognize purine damage, though mMPG is an exception (ref. 60 and Nakano *et al*. unpublished results). The analysis of the reactivity of free amino acids toward Oxa suggests that side chains of Lys or Arg are responsible for the cross-link reaction with Oxa. It is probable that Lys or Arg in the active site acts as a nucleophile to Oxa, forming a stable amide bond (Fig. 3B).

Table 3. Covalent Trapping of DNA Glycosylases by 2-Deoxyribonolactone (dL) and Oxanine (Oxa)

| Enzyme | | Covalent trapping | |
|---|---|---|---|
| | | dL | Oxa |
| *E. coli* | Endo III | + | − |
| | Endo VIII | + | + |
| | Fpg | + | + |
| | AlkA | − | + |
| | Ung | Weak | ND[a] |
| T4 phage | Endo V | + | + |
| Human | hNTH1 | + | − |
| | hNEIL1 | + | + |
| | hNEIL2 | Weak | + |
| | hOGG1 | + | + |
| | hSMUG1 | Weak | − |
| Mouse | mMPG | − | − |

*a*) ND: not determined.

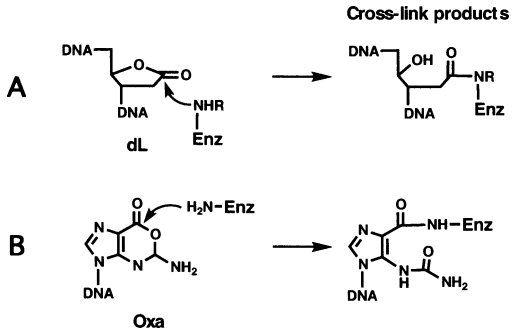


Fig. 3. Mechanisms of Covalent Trapping of DNA Glycosylases by (A) Deoxyribonolactone (dL) and (B) Oxanine (Oxa)

## 6. BER IN CARCINOGENESIS AND ANTICANCER DRUG DEVELOPMENT

It may be simply assumed that individuals carrying a defect in DNA repair pathways accumulate mutations and become more susceptible to cancer, but only a few defects in nucleotide excision repair and mismatch repair have been directly linked to human cancer syndromes to date.[61] This could be explained by defects in repair genes being incompatible with early embryonic development or by redundancy between different repair pathways or repair proteins. The BER pathway crucial for restoring DNA damage generated by ROS, alkylation, and deamination is not an exception to this case. However, the recent finding of an association between the defect in hMYH and carcinogenesis[38,39] underscores the importance of the BER pathway for oxidative DNA damage in the maintenance of the genetic integrity.

The BER pathway is considered a target of anticancer drug development. Methoxyamine potentiates the anticancer efficacy of temozolomide (TMZ) and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU)[62—65] by binding to AP sites, which are formed following the removal of TMZ- or BCNU-induced methylated purines by DNA glycosylase. Also, inhibition of BER by methoxyamine increases 5-iodo-2′-deoxyuridine-induced cytotoxicity as well as its incorporation into DNA, leading to radiosensitization.[65] Considering that AP sites are common BER intermediates formed upon the processing of not only alkylated bases but also of oxidized bases, compounds that react with AP sites and block the BER process could sensitize the therapeutic efficacy of anticancer drugs and/or radiation.

Another approach to the development of anticancer drugs is based on direct inhibition of DNA glycosylases. As mentioned in Section 5, dL and Oxa in DNA covalently trap a variety of DNA glycosylases. If oligonucleotides containing dL or Oxa are delivered to cancer cells, the BER capacity of the cells could be reduced by depletion of functional DNA glycosylases due to covalent trapping, hence potentiating the efficacy of anticancer drugs or radiation therapy. Although they do not form covalent complexes, inhibitors of DNA glycosylases have also been designed and synthesized.[3] These are transition-state analogues or stable AP sites, and both of which bind to DNA glycosylases *in vitro* and trap DNA glycosylases as non-covalent complexes. Future studies should examine whether the covalent or non-covalent trapping of DNA glycosylases in cells can potentiate the efficacy of anticancer drugs or radiation therapy.

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