

## Review Article

# The Biological and Metabolic Fates of Endogenous DNA Damage Products

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DNA and other biomolecules are subjected to damaging chemical reactions during normal physiological processes and in states of pathophysiology caused by endogenous and exogenous mechanisms. In DNA, this damage affects both the nucleobases and 2-deoxyribose, with a host of damage products that reflect the local chemical pathology such as oxidative stress and inflammation. These damaged molecules represent a potential source of biomarkers for defining mechanisms of pathology, quantifying the risk of human disease and studying interindividual variations in cellular repair pathways. Toward the goal of developing biomarkers, significant effort has been made to detect and quantify damage biomolecules in clinically accessible compartments such as blood and urine. However, there has been little effort to define the biotransformational fate of damaged biomolecules as they move from the site of formation to excretion in clinically accessible compartments. This paper highlights examples of this important problem with DNA damage products.

## 1. Introduction

Endogenous processes of oxidative stress and inflammation cause DNA damage that is mechanistically linked to the pathophysiology of cancer and other human diseases [1]. The DNA damage is comprised of dozens of mutagenic and cytotoxic products [2–4] reflecting the full spectrum of chemical mechanisms, including oxidation, nitrosation, halogenation, and alkylation, as described in numerous published reviews [5–15]. There has been significant interest in developing DNA damage products as biomarkers of disease risk given the strong association between DNA damage and disease pathology [12, 14, 16–22]. However, there has been little consideration given to the biological fate of DNA damage products, such as release from DNA as a result of instability, repair, and reaction with local nucleophiles, and the effect of this fate on the steady-state level of DNA lesions in cells and tissues. Further, the use of tissue-derived DNA for biomarker development poses the problem of accessibility and limits clinical studies, so researchers are exploring the presence of

DNA damage products in other sampling compartments, such as urine (e.g., [16, 23]). These efforts have presumed that DNA repair or cell death leads to dissemination of DNA damage products in blood, with subsequent excretion of specific molecular forms predicted to arise from the various DNA repair or other enzymatic processes. However, one of the major drawbacks to the use of blood or urine as a sampling compartment for development of DNA damage products as biomarkers is the lack of mechanistic information about the fates of the damage products in terms of metabolism and distribution. While information about the metabolic fate and pharmacokinetics of drugs based on nucleobases has been well defined (e.g., [24, 25]), studies of the metabolism of DNA damage products have been limited to a few products such as adducts of ethylene dibromide [26], the pyrimidopurinone adduct of dG, M<sub>1</sub>dG [27–29], and the base propenal and butenedialdehyde species arising from 2-deoxyribose oxidation in DNA [30–32].

The mechanisms governing the fate of endogenous DNA damage products can be viewed from two perspectives,

the first being local reactions that lead to the release of the damage product, such as chemical instability or DNA repair, or the reaction of electrophilic damage products with local nucleophiles. The second perspective is that of drug and xenobiotic metabolism and distribution. In both cases, the release of the damage products from DNA results in their diffusion or transport into extracellular space for subsequent distribution in the blood circulation to the liver and excretory organs. Chemical stability governs the extent and form of distribution of the damage product, with electrophilic species reacting with local nucleophiles and more stable products circulating throughout the body. The damage products may be recognized as substrates for the variety of local or distant metabolic enzymes that cause oxidation, reduction, hydrolysis, and conjugation (e.g., glucuronic acid, sulphate, or glutathione), with metabolites excreted in either urine or bile [33, 34]. We can also view DNA damage products from the perspective of metabolic toxification and detoxification. Metabolic reactions are well known to either reduce the activity of reactive and toxic xenobiotics or to convert unreactive molecules to reactive intermediates that are genotoxic, hepatotoxic, or nephrotoxic [33, 34]. This paradigm applies to DNA damage products that range from relatively stable (e.g., nucleobase deamination products) to highly electrophilic (e.g., base propenals from 2-deoxyribose oxidation in DNA), with metabolic reactions occurring in cells in which the DNA damage occurs or in the liver or other metabolic tissues.

This review addresses the current state of understanding of the metabolic and biological fates of DNA damage products, with an eye on the implications of these fates for mechanisms of toxicity and for development of biomarkers of oxidative stress and inflammation.

## 2. The Spectrum of Nucleic Acid Damage Products

As a prelude to understanding the biological fate of damaged nucleic acids, we must first consider the spectrum of damage products. Nucleobases in DNA, RNA, and the nucleotide pool are subject to damage by a variety of chemical mechanisms related to normal and pathological processes. The superoxide ( $O_2^{\bullet-}$ ) and hydrogen peroxide ( $H_2O_2$ ) generated during aerobic respiration participate in Fenton chemistry to produce hydroxyl radical ( $HO^\bullet$ ), while the activated macrophages and neutrophils of chronic inflammation generate a host of chemically reactive species, including the oxidants peroxynitrite ( $ONOO^-$ ) and nitrosoperoxycarbonate ( $ONOCO_2^-$ ), hypohalous acids ( $HOCl$ ,  $HOBr$ ), and nitrosating agents ( $N_2O_3$ ) [8]. Damage to nucleic acids and nucleotides can occur by direct reaction with these agents or indirectly by reaction with electrophiles generated during oxidation of lipids, carbohydrates, and proteins. Both the nucleobase and sugar moieties are susceptible to attack, with examples of nucleobase damage products shown in Figure 1 and 2-deoxyribose oxidation products shown in Figure 2. The biological and metabolic fates of nucleobase damage products will be addressed first and that of 2-deoxyribose oxidation products later in this chapter.

## 3. The Biological and Metabolic Fates of Damaged Nucleobases

The biological fates of damaged nucleotides and nucleic acids can be viewed from the perspective of either the site of initial damage or from the final sampling compartment used for analysis of the damage products. Among the issues that arise are (1) the reactivity of a damage product and the chemical form of the lesion that is released from the site of generation; (2) the mechanism by which the released damage product reaches the systemic circulation; (3) the potential for the damage product to be chemically modified between the steps of formation and excretion; (4) the mechanism of excretion; (5) the potential for further chemical modification in the excretory compartment. The first of these issues, that of reactivity, is best illustrated by the susceptibility of 8-oxoguanine to further oxidation, as will be discussed shortly, and the deglycosylation of many damaged purines, such as 8-nitroguanine [8], and of purines subjected to  $N^7$ -nitrosation or alkylation [8], both of which have been addressed in detail in the literature. Here we will focus on the metabolic fates of nucleobase damage products.

**3.1. 8-Oxoguanine.** The first consideration of the metabolic fate of a nucleobase damage product is the well-studied 7,8-dihydro-8-oxoguanine (8-oxo-G; Figure 1) [35]. Perhaps the most comprehensive consideration of the biological fate of 8-oxo-G in terms of sources of 8-oxo-G-containing species excreted in the urine is the recent review by Cooke et al. [36], with a very recent review of the utility of 8-oxo-dG as a urinary biomarker [23]. Among the nucleobases in DNA, RNA, and the nucleotide pool, guanine is the most readily oxidized due to its favorable redox potential [35, 37–39] with the spectrum of oxidation products depending on the nature of the oxidant [8, 35] (Figure 1). 8-Oxo-G is one of the major products common to oxidation of guanine by most oxidizing agents, and it has thus been touted as a biomarker of oxidative stress (e.g., [23, 36, 40, 41]). While oxidation of G in DNA is one source of 8-oxo-G, another involves polymerase incorporation of 8-oxo-dGTP formed by oxidation of dGTP in the nucleotide pool [42]. Prokaryotes and eukaryotes are equipped with oxidized purine nucleotide di- and triphosphatases (e.g., *E. coli* MutT, 8-oxo-dGTP triphosphatase) to remove damaged nucleotides from the pool [43].

There are four fates of 8-oxoG in cellular DNA and nucleotides: further oxidation to more stable products, which will be discussed shortly, removal from DNA by repair mechanisms, removal from the nucleotide pool by nucleotide di- and triphosphatases, and eventual release from DNA following cell death. Like many nucleobase oxidation products, 8-oxo-G in DNA is removed by the base excision repair (BER) pathway [44–47], with the ultimate release of free 8-oxo-G nucleobase by N-glycosylase activity. On the other hand, dephosphorylation of 8-oxo-dGTP and dGDP ultimately releases 8-oxo-dGMP and 8-oxodG, which are also the likely forms released from DNA following cell death.

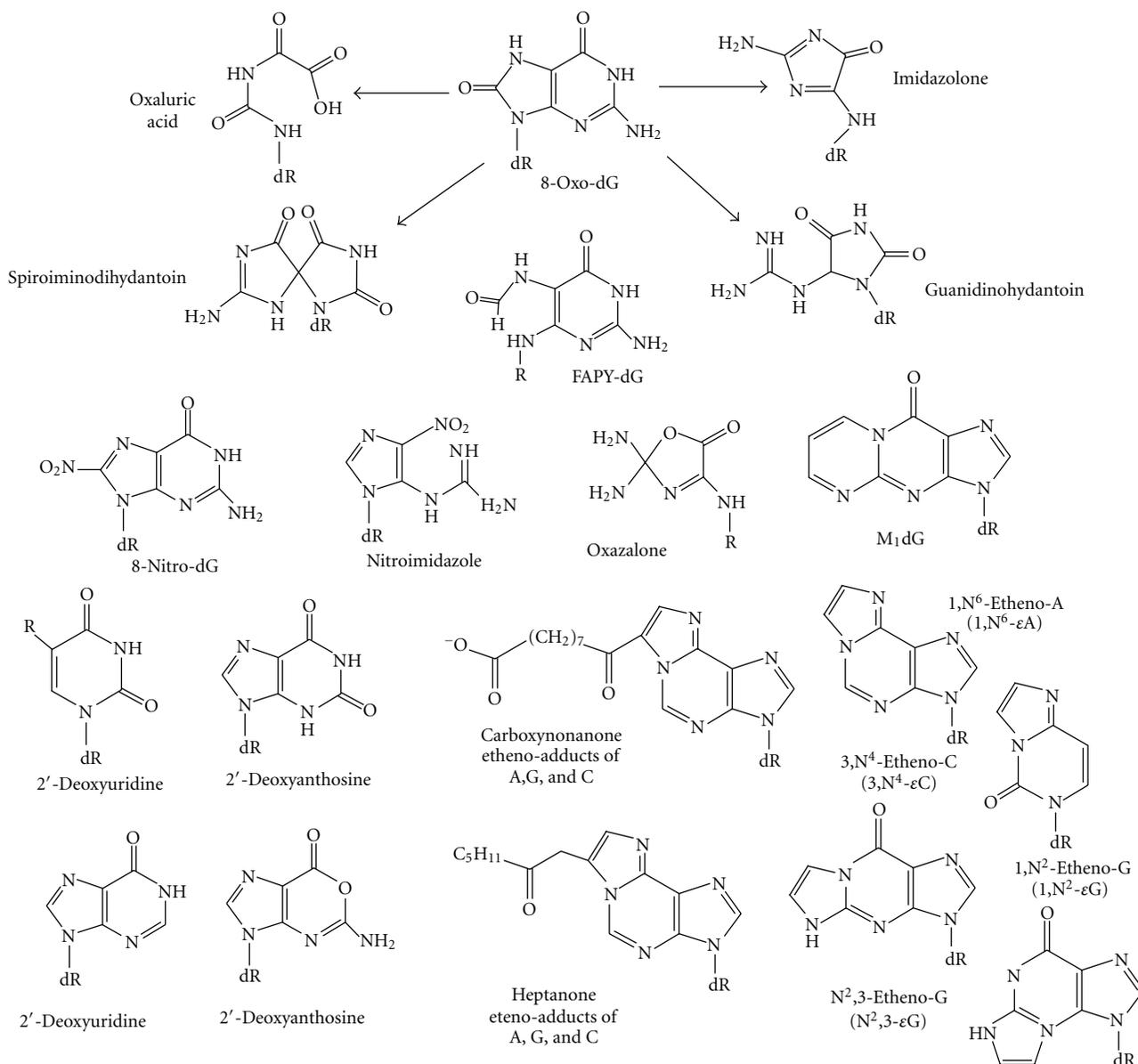


FIGURE 1: Nucleobase damage products.

So, we are faced with the choice of quantifying either 8-oxo-G, 8-oxo-dG, or 8-oxo-dGMP in sampling compartments such as blood and urine. The most abundant of these species appears to be 8-oxo-dG, which is present in human urine at concentrations in the micromolar range. 2-Deoxynucleosides are chromatographically well behaved, and this concentration is amenable to precise and accurate quantification by liquid chromatography-coupled with mass spectrometric methods. While the excretion of 8-oxo-dG may correlate well with conditions of oxidative stress and inflammation [23], the source of this 8-oxo-dG has yet to be established.

Another fate of 8-oxoG in DNA, RNA, and the nucleotide pool, as well as the fate of 8-oxo-G-containing species released from cells, is further oxidation to form a variety

of stable end products, as shown in Figure 1. 8-Oxo-G is significantly more susceptible to further oxidation than G itself (0.74 V versus 1.29 V relative to NHE [39]) and is thus susceptible to reaction with oxidants less potent than hydroxyl radical (2 V versus NHE), such as NO<sub>2</sub><sup>•</sup> (1.04 V versus NHE [48]) and alkyl hydroperoxides (~0.9 V versus NHE [49]). The oxidation of 8-oxo-dG results in the formation of several new products (Figure 1), most of which are more stable than 8-oxo-dG itself and thus potentially better candidates for biomarkers of inflammation and oxidative stress. One must again consider the roles of DNA repair, nucleotide pool cleaning activities, and excretory pathways in finalizing the fate of 8-oxo-G oxidation products.

Finally, recent studies suggest two other confounding factors in the biological fate of 8-oxo-G. The first relates to

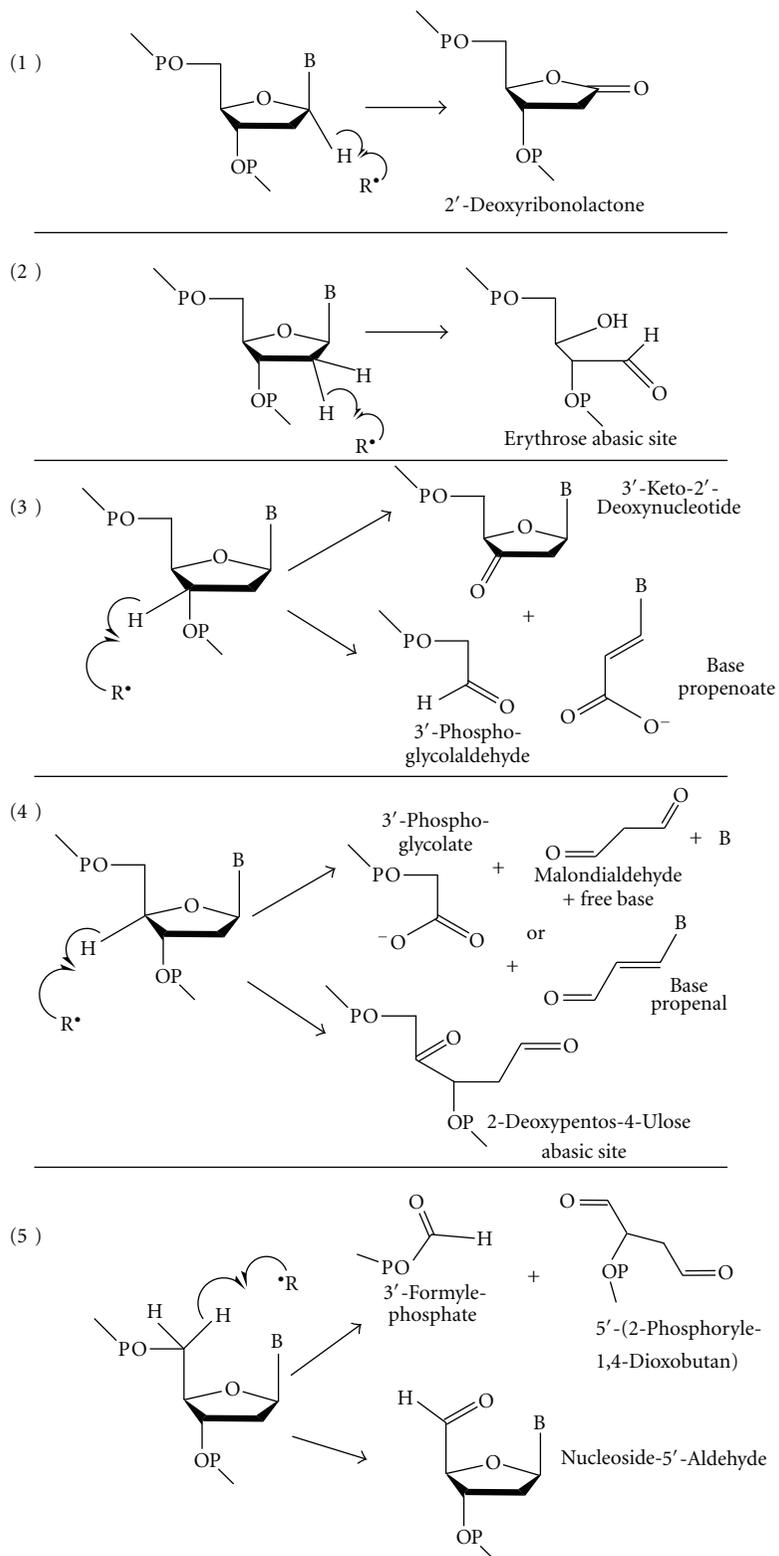


FIGURE 2: 2-Deoxyribose oxidation products.

alternate sources. A study by Tannenbaum and coworkers reveals that 8-oxo-G can arise by further oxidation of species such as 8-nitro-G, which arises from nitrative oxidation of G by  $\text{ONOO}^-$  and  $\text{ONOOCO}_2^-$  [50]. This and other analogous chemistries further confound the assignment of the source of 8-oxo-G-containing species as mechanistic biomarkers. The second confounder involves an alternative fate for 8-oxo-G: deamination to uric acid. Hall et al. have described 8-oxo-G deaminase activity in bacteria [51], which raises the possibility of similar activities in human cells. While we have not observed adventitious deamination of G in our studies of DNA deamination *in vitro* and *in vivo* [52–55], a G deaminase activity cannot be ruled out.

**3.2. Etheno Adducts.** Another major group of DNA lesions with a well-established association with oxidative stress and inflammation involves adducts formed in the reaction of DNA with electrophiles generated by lipid peroxidation [56–58]. This group includes the substituted and unsubstituted etheno nucleobase adducts [58–63] (Figure 1). Extensive study of the urinary excretion of unsubstituted etheno adducts has revealed a strong correlation of excretion with host of human diseases, pathologies, and environmental exposures related to oxidative stress (e.g., see recent studies in [16–21, 64]). Nonetheless, there have been few if any studies aimed at defining the source of the etheno 2-deoxynucleosides measured in these studies.

By analogy to 8-oxo-G, the fate of etheno adducts can be viewed from the perspectives of DNA repair and metabolism. Etheno adducts in DNA are presumed to be repaired by the BER pathway [65], with the release of the free-base adducts. However, biomarker studies again focus on the 2-deoxynucleoside form of the adducts [16–21, 64], which must arise from pathways other than DNA repair. The current focus on quantifying etheno adducts as 2-deoxynucleosides has recently been called into question by the Marnett group's pioneering studies of the metabolism of endogenous DNA adducts [27–29, 66]. With regard to etheno adducts, they incubated 2-deoxynucleoside forms of substituted and unsubstituted etheno adducts in rat liver cytosol and observed an initial deglycosylation of G-derived etheno adducts followed by oxidation of 1,  $N^2$ - $\epsilon$ -G to 2-oxo-1,  $N^2$ - $\epsilon$ -G and of the corresponding substituted adduct, heptanone-1,  $N^2$ - $\epsilon$ -G, to 2-oxoheptanone-1,  $N^2$ - $\epsilon$ -G (Figure 3) [66]. This raises the possibility that urinary biomarker studies may be underestimating the true level of etheno adducts as a result of loss of the 2-deoxynucleoside forms. Further, the oxidized free-base forms may also be useful as biomarkers if they are excreted at high enough levels.

**3.3.  $M_1$ dG.** This mutagenic pyrimidopurinone adduct of dG (Figure 1) forms in reactions of DNA with the lipid peroxidation product, malondialdehyde, and with base propenals derived from 4'-oxidation of 2-deoxyribose in DNA [56, 67–72]. As an endogenous DNA adduct,  $M_1$ dG has been detected at levels ranging from 1 to 1000 lesions per  $10^8$  nucleotides in a variety of organisms, including humans [67, 71, 73–79]. Recent studies suggest that the major source of  $M_1$ dG *in vivo* is base propenals from DNA oxidation [67],

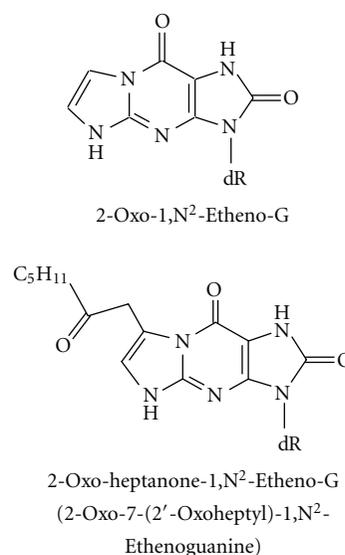


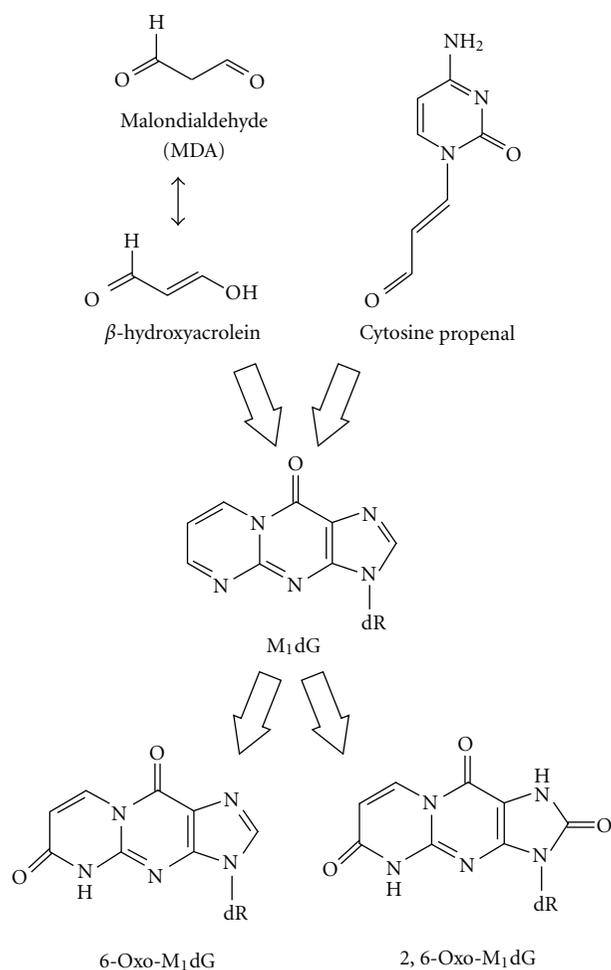
FIGURE 3: Oxidation of substituted etheno adducts.

which is consistent with the higher reactivity of base propenals than malondialdehyde [68, 69] and the proximity of base propenals to dG in DNA. However, contributions from both malondialdehyde and base propenals are likely to occur in an oxidant-, cell-, and tissue-dependent manner [72].

In terms of the biological fate of  $M_1$ dG, the adduct has been demonstrated to be a substrate for nucleotide excision repair (NER) [80, 81], which may explain the appearance of  $M_1$ dG in human and rodent urine [27–29, 79]. However,  $M_1$ dG was detectable in the human urine at levels of 10–20 fmol per kg per 24 h [79], which is a significantly lower excretion rate than other DNA lesions such as 8-oxo-dG (400 pmol per kg per 24 h) [82]. To explore the basis for this low rate of excretion, Marnett and coworkers undertook metabolic and pharmacokinetic studies of  $M_1$ dG in rats [27]. When intravenously administered to rats,  $M_1$ dG was rapidly eliminated from the plasma with a half-life of 10 min [27]. In contrast to the rapid clearance from blood,  $M_1$ dG was found in the urine for more than 24 hr after dosing, which suggested a rapid distribution to tissue followed by slower phase of excretion. Analysis of the urine revealed a metabolite of  $M_1$ dG, 6-oxo- $M_1$ dG, likely derived from hepatic xanthine oxidase activity [27]. Studies in rat liver extracts revealed further oxidation of 6-oxo- $M_1$ dG on the imidazole ring to give 2,6-dioxo- $M_1$ G (Figure 4) [28]. While most of the  $M_1$ dG was excreted unchanged in the urine and the problem of low levels of excretion remains unsolved, these studies point to the importance of defining the biological and metabolic fate of damaged biomolecules in efforts to develop biomarkers of inflammation and oxidative stress.

#### 4. The Biological and Metabolic Fates of 2-Deoxyribose Oxidation Products

In addition to the nucleobases in DNA, the 2-deoxyribose moiety is also subjected to oxidative damage that merits consideration of biological fate and metabolism [9]. As opposed

FIGURE 4: Formation and metabolism of M<sub>1</sub>dG.

to the concept of simple “strand breaks,” growing evidence points to 2-deoxyribose oxidation in DNA as a critical determinant of the toxicity of oxidative stress [9]. Oxidation of each of the five positions in 2-deoxyribose in DNA occurs with an initial hydrogen atom abstraction to form a carbon-centered radical that rapidly adds molecular oxygen to form an unstable peroxy radical. The resulting product spectra for 2-deoxyribose oxidation under aerobic conditions are shown in Figure 2 [9]. Many of these oxidation products are highly electrophilic, with  $\alpha,\beta$ -unsaturated carbonyl motifs, and are thus capable of reacting with proximate nucleophilic sites in DNA, RNA, and proteins to form adducts [9]. This section of the paper will focus on the biological and metabolic or, more broadly, biotransformational fates of 2-deoxyribose oxidation products.

**4.1. DNA Adducts of 2-Deoxyribose Oxidation Products.** One fate of DNA oxidation products is reaction with local electrophiles to form protein and nucleic acids adducts. In this regard, oxidation of 2-deoxyribose in DNA produces a variety of reactive electrophilic species (Figure 2) that readily form adducts with neighboring DNA bases. Oxidation of both the 2'- and 3'-positions of 2-deoxyribose can lead to the formation of the 2-phosphoglycolaldehyde residue

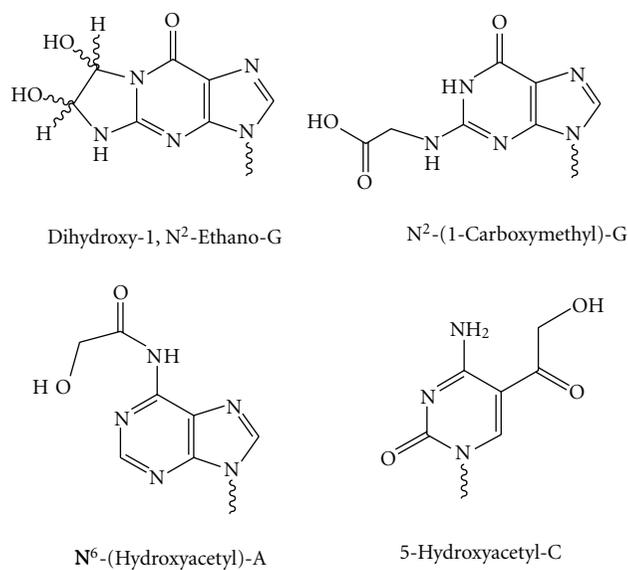


FIGURE 5: Glyoxal adducts of DNA.

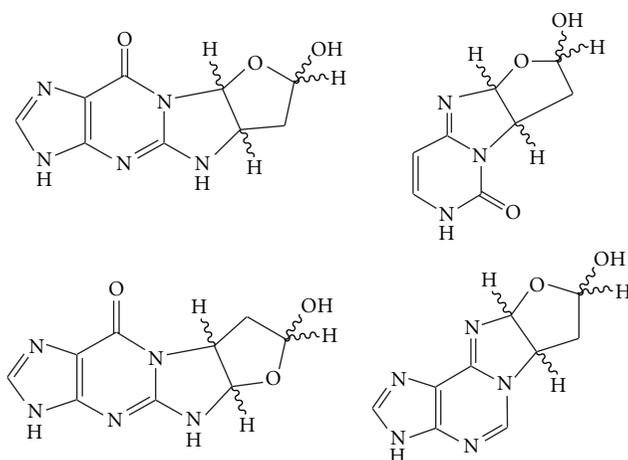


FIGURE 6: Reaction of 1,4-dioxo-2-butene to form bicyclic oxadiazabicyclo-(3.3.0) octaimine adducts.

(Figure 2), the latter directly from the oxidation [83, 84] and the former by an induced and indirect oxidation mechanism involving an erythrose intermediate [85, 86]. By either mechanism, 2-phosphoglycolaldehyde undergoes a relatively slow phosphate-phosphonate rearrangement to generate the ubiquitous lipid and carbohydrate oxidation product, glyoxal, that reacts with dG and DNA to form diastereomeric 1,N<sup>2</sup>-glyoxal adducts of dG (Figure 5) [83].

At the 4'- and 5'-positions, 4'-oxidation generates base propenals that readily react with neighboring dG to form the pyrimidopurinone adduct, M<sub>1</sub>dG, as described earlier [67–69]. Oxidation of the 5'-position leads to formation of a 2-phosphoryldioxobutane residue that, possibly following  $\beta$ -elimination to form an  $\alpha,\beta$ -unsaturated *trans*-dioxobutene species, reacts with dC ≫ dG > dA to form bicyclic oxadiazabicyclo-(3.3.0) octaimine adducts (Figure 6) [87–91].

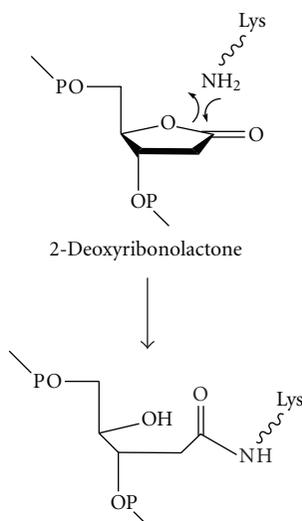


FIGURE 7: Formation of DNA-protein cross-links during repair of 2-deoxyribonolactone abasic sites in DNA.

#### 4.2. Protein Adducts of 2-Deoxyribose Oxidation Products.

In addition to DNA adducts, the electrophiles derived from 2-deoxyribose oxidation react with amino acid side chains in proteins to form a variety of adducts, some with functional consequences. One of the earliest examples of protein adducts from 2-deoxyribose oxidation involves the 1'-position. The 2-deoxyribonolactone abasic site resulting from 1'-oxidation in DNA reacts with DNA repair proteins to form stable protein-DNA cross-links [92, 93]. This phenomenon was first demonstrated by Hashimoto et al. with the *E. coli* DNA BER enzyme endonuclease III [92]. This enzyme normally functions in base excision repair pathways with both an initial N-glycosylase activity against oxidized pyrimidines and a subsequent incision of the resulting abasic site by a lyase activity [94]. Upon binding to the 2-deoxyribonolactone abasic site, however, the active site (lysine 120), which normally forms a Schiff base with the 1'-aldehyde in the ring-opened form of the native abasic site, performs a nucleophilic attack on the carbonyl group of the lactone ring (Figure 7). Unlike a Schiff base, the resulting cross-link is irreversible and complicates the DNA repair process [92]. DeMott et al. observed similar results in which a covalent amide bond was formed by the 1'-carbon of the lactone and the lysine 72 in human polymerase  $\beta$  [93]. Additionally, the 2-deoxyribonolactone undergoes a rate-limiting  $\beta$ -elimination reaction to form a butenolide species with a half-life of 20 h in single-stranded DNA (32–54 h in duplex DNA), followed by a rapid  $\delta$ -elimination to release 5-methylene-2(5H)-furanone [95]. Both the intermediate butenolide and the product methylenefuranone are electrophilic species capable of reaction with nucleophilic sites in DNA and protein, and possibly subject to metabolic reactions such as glutathione conjugation.

Another potential source of protein adducts arises from the variety of  $\alpha,\beta$ -unsaturated carbonyl and dicarbonyl products of 2-deoxyribose oxidation in DNA. The potential here lies in the high concentration of nucleophilic lysine

and arginine residues in histone proteins proximate to the sites of DNA damage and in the well-established reactivity of  $\alpha,\beta$ -unsaturated carbonyl and dicarbonyl species with nucleophilic amino acids, which is perhaps best illustrated by lipid peroxidation products (e.g., [96–103]). Several recent studies have identified specific lysine and histidine adducts of well-defined lipid peroxidation products such as malondialdehyde [100], 4-hydroxynonenal [99], and its oxidation product, 4-oxononenal [97] (Figure 8). The reactions forming these adducts are highly analogous to reactions that could occur with 2-deoxyribose oxidation products, as illustrated in Figure 8. For example, the unsaturated  $\beta$ -elimination product of the 2-deoxypentose-4-ulose product of 4'-oxidation of deoxyribose is a chemical analog of 4-oxononenal derived from lipid peroxidation. It would thus be expected to react with lysines and histidines in histone and other chromatin proteins to form the *bis*-adduct or cross-link observed by observed by Sayre and coworkers [104] and the stable furan derivative observed by observed by Blair and coworkers [97], respectively (Figure 8). Indeed, histones 2A, 2B, and 3 contain 3–5 histidines that have been exploited to cross-link histones to DNA in the classic studies of Mirzabekov and coworkers [105, 106].

The malondialdehyde adducts of lysine, arginine, and histidine represent another protein adduct chemistry with potential parallels between 2-deoxyribose oxidation and lipid peroxidation. The reaction of lysine by nucleophilic substitution yields a moderately stable N-propenal-lysine species (Figure 8) that can react with another lysine to form a propyl-bridged cross-link [107], while the reaction of malondialdehyde with arginine has been shown to produce a stable pyrimidyl-ornithine species (Figure 8) [107]. In both cases, the proportions of modified amino acids are high [108]. Given the analogous reactions of malondialdehyde and base propenals from 4'-oxidation, it is reasonable to expect the formation of propyl-bridged cross-links and pyrimidyl-ornithine species in histone proteins in cells subjected to oxidative stresses.

A final example of protein adducts derived from 2-deoxyribose oxidation products involves N-formylation of lysine by transfer of formyl groups from 3'-formylphosphate residues (Figure 9) [109], among other possible sources such as oxidation of formaldehyde adducts of lysine. N<sup>6</sup>-formyllysine was detected in histone proteins from a variety of sources to the extent of 0.04%–0.1% of all lysines in acid-soluble chromatin proteins including histones, which suggests that the adduct represents an endogenous secondary modification of histones [109]. The chemical analogy of the N-formyl modification to the physiologically important lysine N-acetylation and N-methylation suggests that lysine N-formylation may interfere with signaling mediated by histone and other chromatin protein modifications (e.g., [110, 111]).

In all of these cases, the adducted proteins are subject to degradation, with the potential for the release and excretion of adducted peptides or amino acids. Their potential as biomarkers warrants further study of DNA-derived protein adducts.

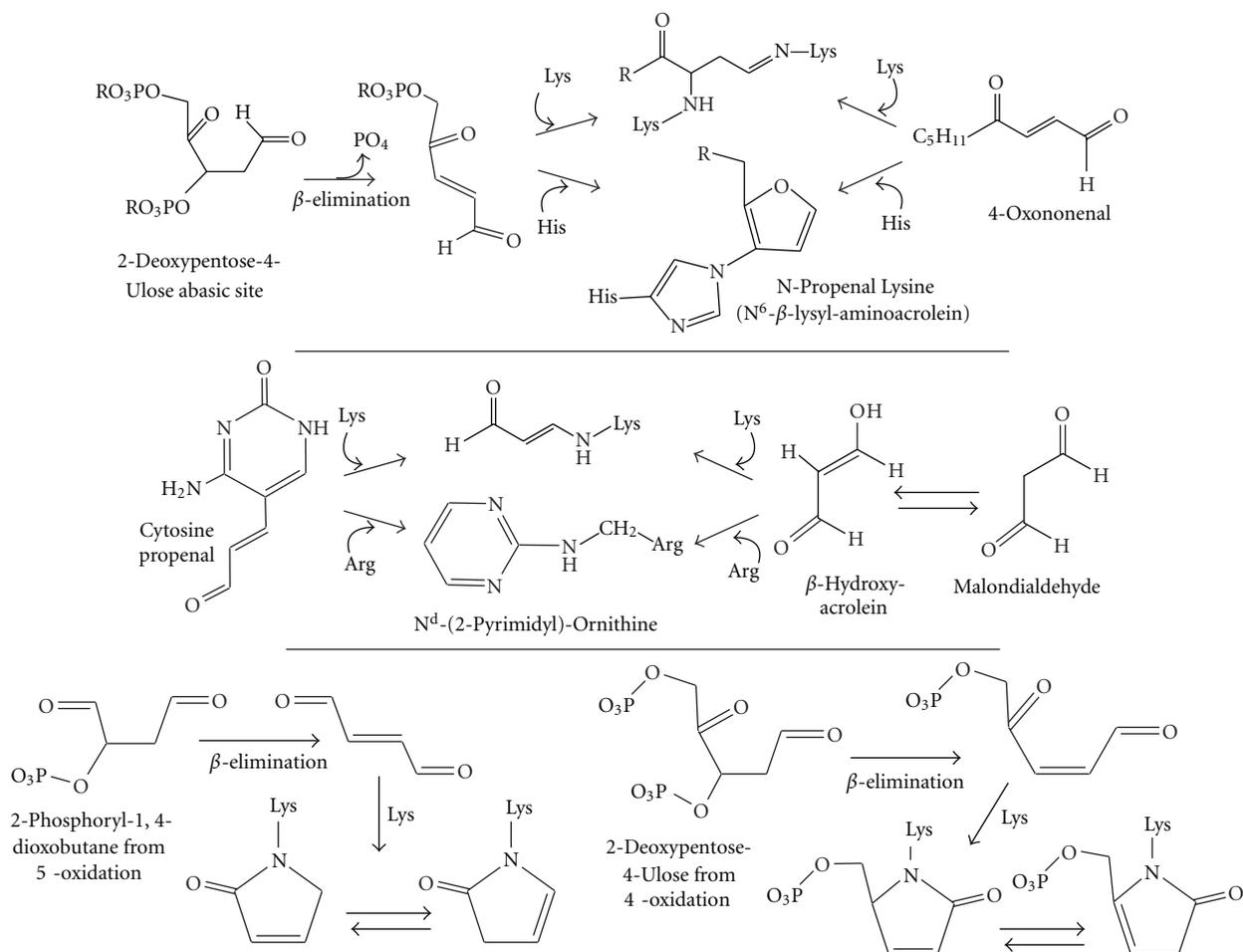


FIGURE 8: Reaction of lipid peroxidation products with lysine.

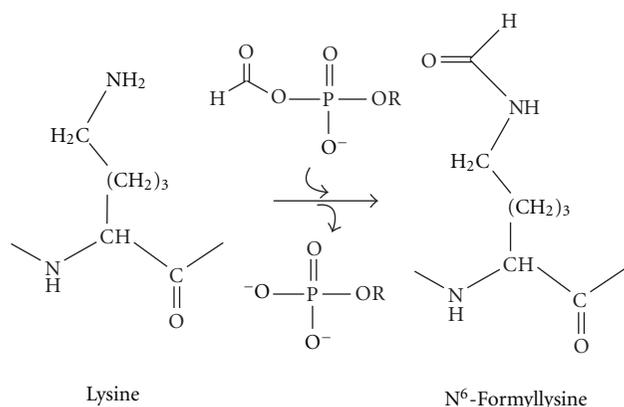


FIGURE 9: Lysine N-formylation by 3'-formylphosphate from 5'-oxidation of 2-deoxyribose.

4.3. *Metabolism of 2-Deoxyribose Oxidation Products.* As in the case of nucleobase lesions, the products of 2-deoxyribose oxidation of DNA must also be considered as substrates for metabolic enzymes and biotransformational reactions. This is all the more apparent given the electrophilic nature of the products, which points to glutathione (GSH) adduct

formation, and the  $\alpha,\beta$ -unsaturated carbonyl structure of many of the products, which makes them ideal substrates for glutathione S-transferases (GST) [34]. Indeed, GSTs have been shown to react with  $\alpha,\beta$ -unsaturated aldehyde-containing lipid peroxidation products, many of which are chemical analogues of 2-deoxyribose oxidation products [9, 68]. Two examples of GST reactions with 2-deoxyribose oxidation products illustrate this biotransformation concept.

The first example involves GSH conjugation of base propenals. One of the classic definitions of GST substrates is that they must react directly with GSH to a measurable extent [34]. This is indeed the case with base propenals, as demonstrated in studies by Berhane et al. in which GSH added to give a Michael adduct and a substitution product with loss of the nucleobase (Figure 10) [30]. In addition, base propenals were found to be among the best substrates for the Pi class of GSTs, producing a single GSH conjugate (Figure 10).

GSH conjugates have also been identified for furan metabolite *cis*-1,4-dioxo-2-butene [31, 32], the conformational isomer of the *trans*-1,4-dioxo-2-butene product of 5'-oxidation (Figure 2). Given the similarity in the reactivity of *cis*- and *trans*-1,4-dioxo-2-butene toward DNA adduct formation [9], it would not be surprising to identify

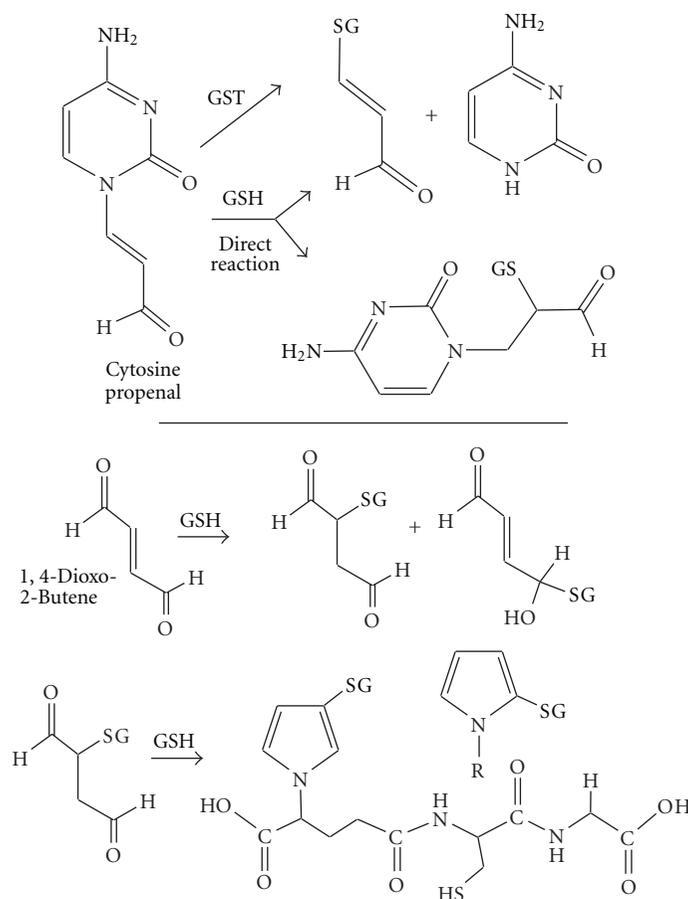


FIGURE 10: Formation of glutathione adducts of 2-deoxyribose oxidation products.

GSH adducts of the *trans*-isomer product of 2-deoxyribose oxidation, as has been observed *in vitro* and *in vivo* with the *cis*-isomer derivative of furan metabolism [31, 32, 112].

## 5. Prospects

Molecules damaged during normal physiological processes and in states of pathology represent a large source of biomarkers with potential clinical utility in defining etiological mechanisms, quantifying the risk of human disease and studying interindividual variations in cellular repair pathways. In spite of this potential, there has been little effort to define the biotransformational fate of damaged biomolecules as they move from the site of formation to excretion in clinically accessible compartments. This paper has highlighted examples of this important problem with DNA damage products. Coupled with the development of more sensitive and specific analytical technologies, there are likely to be major advancements in defining the metabolism of DNA damage products and other damaged biomolecules in the coming years.

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