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Review

Modifications of nucleosides by endogenous mutagens–DNA adducts arising from cellular processes

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ABSTRACT

DNA damage plays a significant role in mutagenesis, carcinogenesis and ageing. Chemical transformations leading to DNA damage include reactions of the base units with agents of endogenous and exogenous origin. The vast majority of damage arising from cellular processes such as metabolism and lipid peroxidation are identical or very similar to those induced by exposure to environmental agents. A detailed knowledge of the types and prevalence of endogenous DNA damage provides insight into the chemical nature of species involved in these modifications and may be of help in understanding their influence on the induction of cancer or other diseases. This knowledge may also be essential to the development of rational chemopreventive strategies directed against the initiation of oxidative stress- and lipid peroxidation-associated pathology.

The present work reviews findings regarding the interaction between DNA bases and various reactive species arising from lipid peroxidation and other cellular processes, drawing attention to the mechanism responsible for the formation of the resulted modifications. The biological consequences of these interactions are also briefly discussed.

1. Introduction

Endogenous, metabolic processes are sources of highly reactive chemicals that are implicated in DNA damage. So-called reactive oxygen species (ROS) belong to a group of these chemicals. They are formed continuously in living cells as a consequence of chronic infections that elicit an inflammatory response [1], during mitochondrial respiration [2] and as a result of other biological reactions. The ROS can attack DNA bases or deoxyribose residues to produce oxidized bases or single- and double-strand breaks [3]. Alternatively, ROS can interact with other cellular components such as lipid molecules to induce their peroxidation. Lipid peroxidation results in generating bifunctional electrophiles, mainly aldehydes and epoxides that exhibit a range of biological activities, including cytotoxicity, genotoxicity, carcinogenicity, effects of gene expression, cell proliferation and differentiation [4]. These electrophiles damage DNA by the reaction at electron rich sites of the DNA bases leading to the formation of exocyclic adducts. Beside modifications caused by ROS, exocyclic adducts are the most frequently occurring damage to DNA. These lesions may participate in the induction of mutations present in human tissues and can lead to cancer initiation. The role that endogenous products of cellular metabolism may play in the aetiology of cancer has become increasingly important over the past 10 years [5]. This is largely due to epidemiological data which show that apart from tobacco smoke and sunlight, exposure to genotoxic environmental carcinogens accounts for only a minority of human cancers [3,6].

Studies focused on the identification of reactive chemicals arising from cellular processes and on structural characterisation of the DNA modifications induced by these chemicals would provide a more complete understanding of the spectrum of DNA damage generated by endogenously formed potentially mutagenic agents. Such studies would also be helpful in establishing the role that lipid peroxidation and other biological processes being sources of DNA damaging agents play in mutagenesis and carcinogenesis.

The aim of this review is to present the recent findings regarding the interaction between DNA bases and various reactive species of endogenous origin. The work deals mainly with the chemical aspect of base modifications including mechanisms responsible for their formation however, the biological consequences of the interactions are also briefly discussed.

2. Oxidative reactions

Deleterious reactive oxygen species (ROS) are produced upon exposure of living organisms to various chemical and physical agents such as redox-cyclic drugs, UV light and ionizing radiation. But ROS might also be of endogenous origin since they are produced in cells through a series of processes including normal oxidative metabolism and inflammation.

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Among the species belonging to ROS there are free radicals: superoxide radical anion ($O_2^{\cdot -}$) and hydroxyl radical ($^{\cdot}OH$), as well as non-radical oxidants such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Singlet oxygen, because of its relatively long lifetime, may diffuse towards potential targets and react like a strong electrophile with biomolecules that contain sites of high electron density. Therefore, cellular targets may include DNA, ribosomes, mitochondria and membranes [7,8]. However, among all the ROS hydroxyl radical, ($^{\cdot}OH$) is considered to be the most reactive and, in a consequence, the most harmful. Its reactivity is so great that it does not diffuse more than one or two molecular diameters before reacting with a cellular component [9]. It must be generated in close vicinity to the nucleic acid molecule to oxidize it.

Beside ionizing radiation, superoxide radical anions are thought to be a major source of hydroxyl radicals [10]. These anions are present in cells because of leakage from the respiratory chain in mitochondria. $O_2^{\cdot -}$ may undergo conversion to hydrogen peroxide by superoxide dismutase, either spontaneously or through metal- and enzyme-catalyzed pathways [11]. Hydrogen peroxide may in turn react with reduced transition metal ions (Fenton reaction) to produce hydroxyl radical [12,13]:

$$M^{n+} + H_2O_2 \rightarrow M^{n+1} + {}^{-}OH + {}^{\cdot}OH$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$$

Moreover hydrogen peroxide can interact *via* Haber-Weiss reaction with superoxide radical anion to form hydroxyl radical as well:

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + {}^{-}OH + {}^{\cdot}OH$$

Active reduced transition metal ions that catalyse Fenton reaction are regenerated in reduction of higher oxidation stage metal ions by superoxide radical anion or another reducing agent. *In vivo*, iron and, to a lesser extent, copper are the most likely promoters of hydroxyl radicals [11].

Another source of hydroxyl radicals can be peroxynitrite (ONO_2^-), [14]. This extremely strong oxidant is the coupling product of nitric oxide and superoxide. Although peroxynitrite generates small quantities of hydroxyl radicals, its protonated form (peroxynitrous acid, ONO_2H) is capable of oxidizing DNA independently of its ability to produce hydroxyl radicals [15].

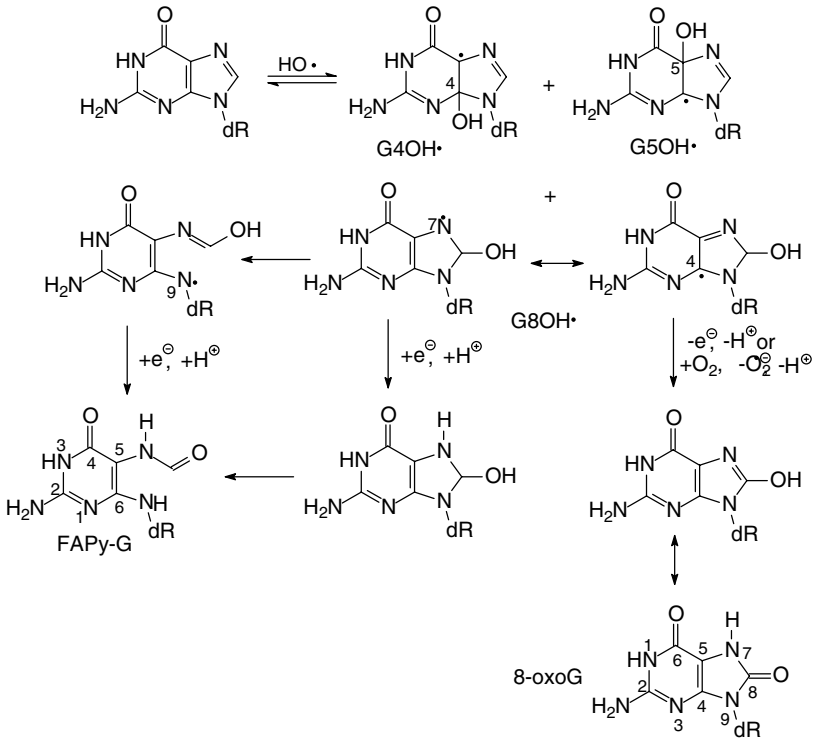
When formation of ROS exceeds the natural organism antioxidant defence mechanisms, oxidative stress occurs. The reactive species involved in metal-catalyzed endogenous oxidative stress can damage biomolecules such as DNA. There are two main modes of DNA attack: addition to the double bonds of DNA bases and/or hydrogen abstraction from the deoxyribose sugar units in DNA. The resulting lesions include strand breaks, cross-links to either, proteins or other sites within DNA, abasic sites and modified bases [16–18]. The later class of damage has been widely studied in terms of formation, mutagenesis and repair processes. The identification, determination and quantification of modified DNA bases provide insight into the chemical nature of the involved species. Hydroxyl radicals lead to the formation of a wide array of base damage. The most important modifications are presented below.

2.1. Oxidative DNA adducts

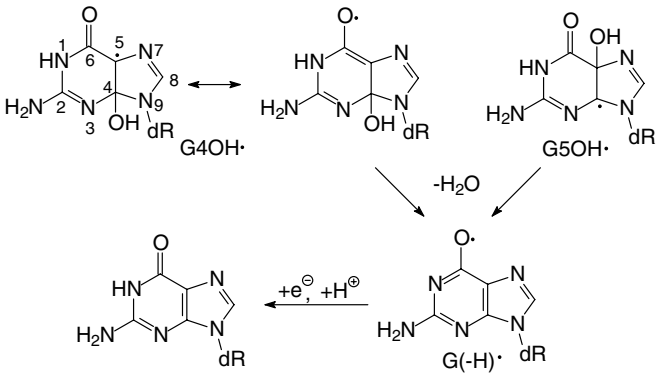
2.1.1. Oxidation of purine bases

2.1.1.1. Guanine oxidation. Due to its lowest oxidation potential guanine is the most easily oxidized among the four DNA bases [9]. Hydroxyl radical can attack at C-4, C-5 and C-8 of a guanine nucleobase giving the radicals G4OH$^{\cdot}$, G5OH$^{\cdot}$ and G8OH$^{\cdot}$, respectively, as shown in (Scheme 1) [16,19].

Radical adducts G4OH$^{\cdot}$ and G5OH$^{\cdot}$ can eliminate a molecule of water giving G(-H)$^{\cdot}$ radical which by gaining an electron and protonation is transformed back to guanine (Scheme 2).



Scheme 1. Addition of hydroxyl radical to guanine.



Scheme 2. Transformation of G4OH· and G5OH· to guanine.

On the contrary the adduct G8OH· leads to two forms of DNA damage. Reduction of the adduct's form in which the radical is localized on N-7 gives hemi-aminal which opens the imidazole ring forming 2,6-diamino-5-formamidopyrimidine (FAPy-G, Scheme 1). The same compound may also be formed by ring opening of G8OH·, followed by reduction of the resulting N-9 centred radical. Alternatively, oxidation of the C-4 and C-2 resonance forms of G8OH· following by the loss of a proton yields 7,8-dihydro-8-oxoguanine (8-oxoG), which also exists in the form of its minor tautomer 8-hydroxyguanine [20]. It has been observed that dehydration of G4OH·, G5OH· and G8OH· leads to the neutral radical intermediate (Scheme 3). In the presence of a molecule of oxygen the intermediate is converted to the ring-opened product (imidazolone) which slowly hydrolyzed to oxazolone [21].

8-OxoG is the most common oxidative lesion observed in duplex DNA and a family of DNA repair enzymes has evolved to deal with this lesion and its mismatches [19].

Whereas guanine bases have a strong preference to pair with cytosine, 8-oxoG can pair both in Watson–Crick mode with cytosine and in Hoogsteen mode with adenine. In the later pairing mode, 8-oxoG residues in DNA frequently mispair with adenine during replication *in vitro* and *in vivo*, giving rise to G·C → T·A transversion mutations [22]. These transversions are the second most common somatic mutation found in human cancers and are prevalent in the mutational spectrum of the tumour suppressor gene p53 [23].

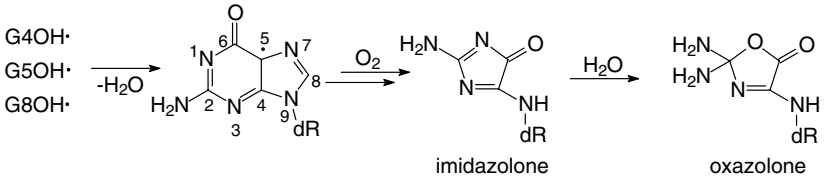
8-OxoG may also be formed in reaction of the guanine base with singlet oxygen but the mechanism for the adduct formation under these conditions remains unclear [24,25]. It might proceed *via* an unstable endoperoxide as an intermediate but this pathway requires supply of two equivalents of reducing agent (Scheme 4).

Another path for the formation of 8-oxoG is that the initial endoperoxide undergoes rearrangement leading to a very reactive 8-hydroperoxy derivative (Scheme 5). This hydroperoxide being a strong oxidant would be expected to donate an oxygen atom to guanosine or other oxidizable molecules present in the reaction medium.

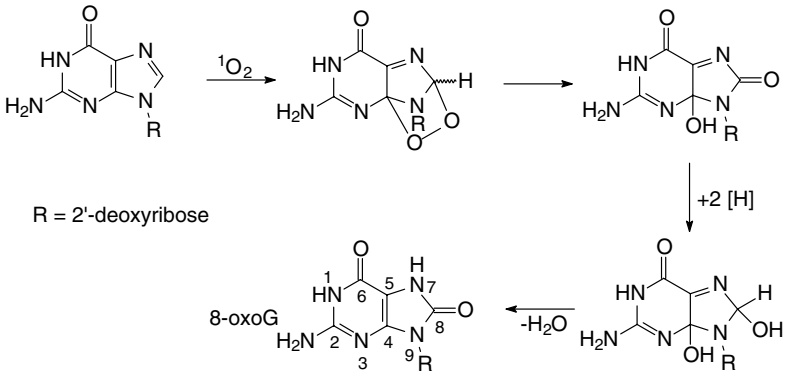
It has been observed that 8-oxoG is a better substrate than guanine for additional photooxidation. The major $^1O_2$-induced decomposition products of 8-oxoG are oxazolone, cyanuric acid derivatives and 4-hydroxy-8-oxo-4,8-dihydroguanine (Fig. 1), [26].

2.1.1.2. Adenine oxidation. The oxidation of adenine by hydroxyl radicals is analogous to that of guanine, although oxidative lesions at adenine are less prevalent in DNA damage. Hydroxyl radicals add to the C-4 and C-8 positions of adenine and its nucleosides and nucleotides, forming the A4OH· and A8OH· radical adducts [16,19,27], (Scheme 6). Adduct A4OH· loses a molecule of water giving the neutral and strongly oxidizing A(-H)· radical. Reduction of this radical regenerates adenine. The radical adduct A8OH· is less abundant and can be oxidized to 8-oxoadenine (8-oxoA), or can undergo reductive ring opening to give the FAPy-A product. 8-OxoA and FAPy-A are the adenine analogues of guanine oxidation.

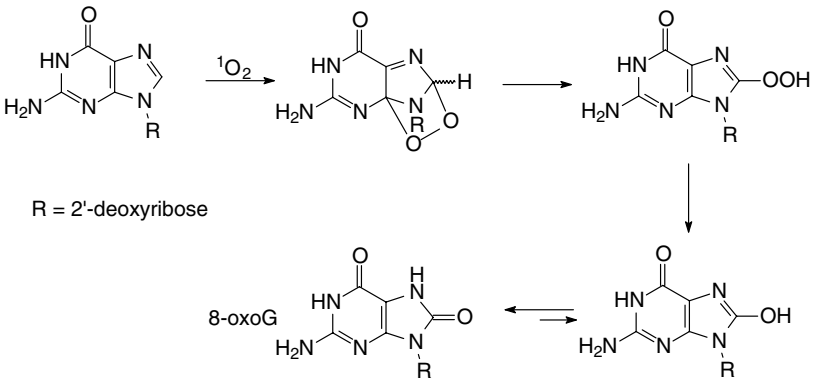
2-Hydroxyadenine has also been observed as an oxidation product of the reaction of DNA with Fe-EDTA/H_2O_2 or of human cells with H_2O_2. The yield of 2-hydroxyadenine is similar to that of 8-oxoG in monomers but in DNA its formation is less efficient. The adduct is as mutagenic as 8-oxoG in *E. coli* and mammalian cells and has been found to induce A·T → G·C transitions [28].



Scheme 3. Formation of oxazolone from G4OH·, G5OH· and G8OH·.



Scheme 4. Formation of 8-oxoG in reaction with singlet oxygen.



Scheme 5. Formation of 8-oxoG *via* 8-hydroperoxy derivative.

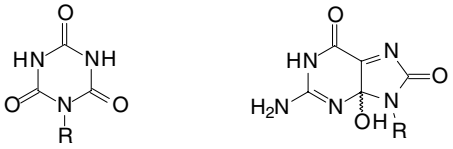
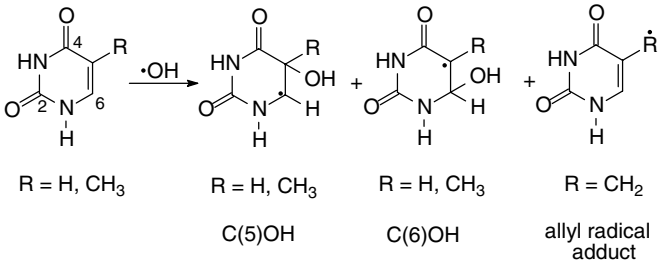


Fig. 1. Major $^1O_2$-induced decomposition products of 8-oxoG.

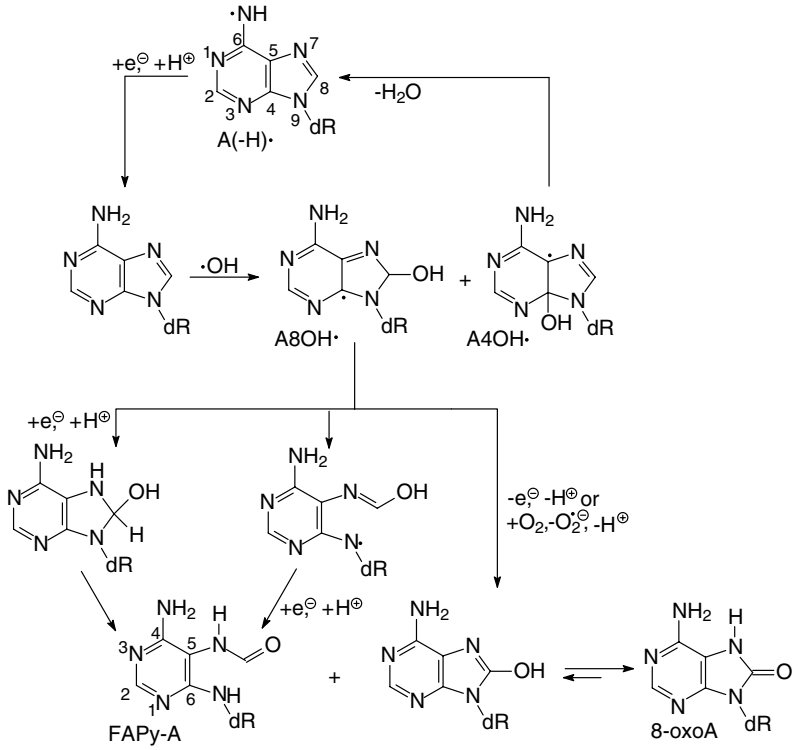


Scheme 7. Reactivity of hydroxyl radical towards pyrimidine derivatives.

2.1.2. Oxidation of pyrimidine bases

At neutral pH hydroxyl radicals react with uracil derivatives by two mechanisms. The more favoured reaction is addition to either end of the double bond that leads to the formation of the C(5)OH and C(6)OH radical adducts in which radical is placed at C-6 or C-5 position, respectively [29], (Scheme 7). The less favoured reaction is abstraction of a hydrogen atom from the methyl group of thymine (Scheme 7).

The resulting allyl radical adduct is stable and can potentially abstract hydrogen atoms from adjacent ribosyl unit leading to direct strand scission [19]. Hydroxyl radicals are highly electrophilic and preferentially attack position C-5, that is a site of highest electron density. This causes preferential formation of the



Scheme 6. Addition of hydroxyl radical to adenine.

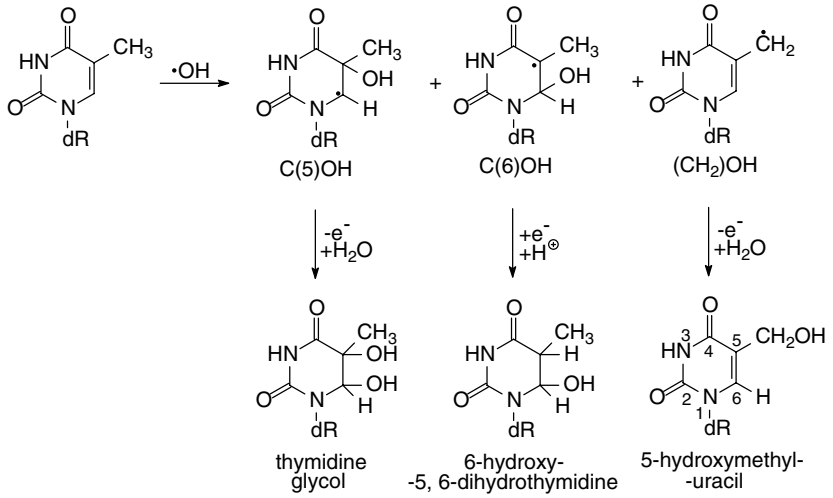
C(5)OH radical adduct *versus* the C(6)OH one. In thymine presence of the methyl group reduces an easiness of attack at C-5 by steric hindrance and stabilizes slightly the C(6)OH radical adduct.

2.1.2.1. Thymine oxidation. Thymine displays a rich variety of reactions induced by hydroxyl radical. Initial formation of the radical adducts C(5)OH and C(6)OH is followed under anaerobic conditions by redox reactions or by direct hydrogen abstraction from a neighbouring ribose moiety [29,30], (Scheme 8).

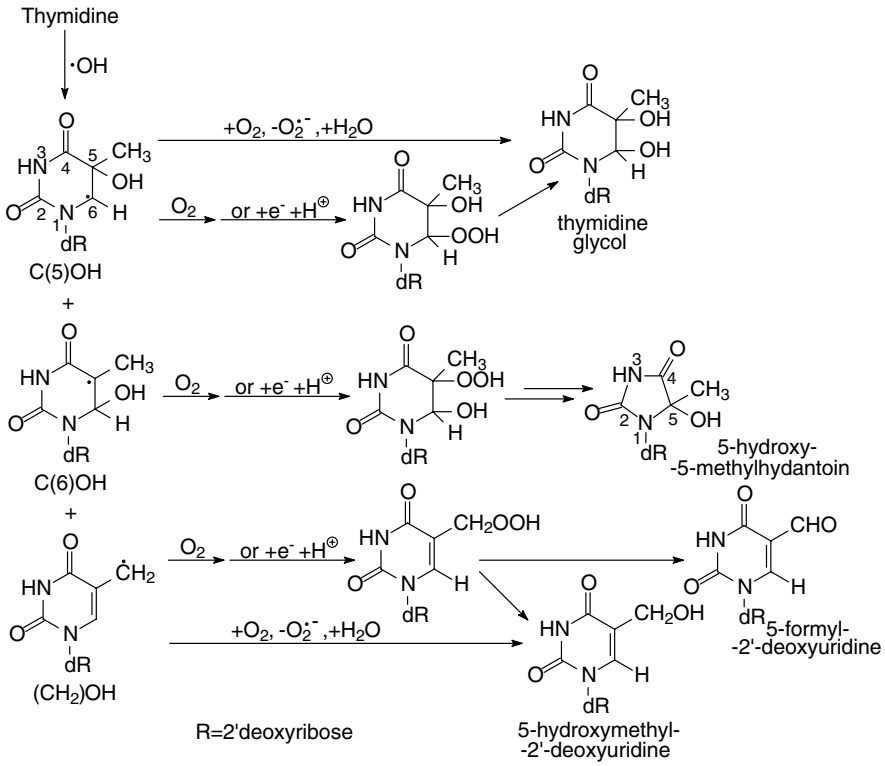
The major radical adduct, C(5)OH, undergoes oxidation that is followed by hydration of the cation and results in the formation of thymidine glycol. The minor C(6)OH radical adduct is reduced and protonated to give 6-hydroxy-5,6-dihydrothymidine. The methyleneuracil radical $(CH_2)OH$ leads to 5-hydroxymethyluracil by electron loss and subsequent water addition. Hydrogen abstraction by the C(5)OH radical adduct is thought to be formed from neighbouring sugar moiety and accounts for a major part of radiation-induced strand cleavage.

Under aerobic conditions, however, the three thymidine radical adducts trap oxygen leading to the formation of various products (Scheme 9).

Reactions of C(5)OH and allyl radical adducts give thymidine glycol and 5-hydroxymethyluracil respectively, the products that are also formed in oxygen-free conditions [31,32]. This may occur by direct electron transfer to oxygen, by addition of O_2 and elimination of $O_2^{\cdot-}$ followed by trapping by water or alternatively by decomposition of the respective hydroperoxides.



Scheme 8. Addition of hydroxyl radical to thymidine and thymidine lesions formed under anaerobic conditions.

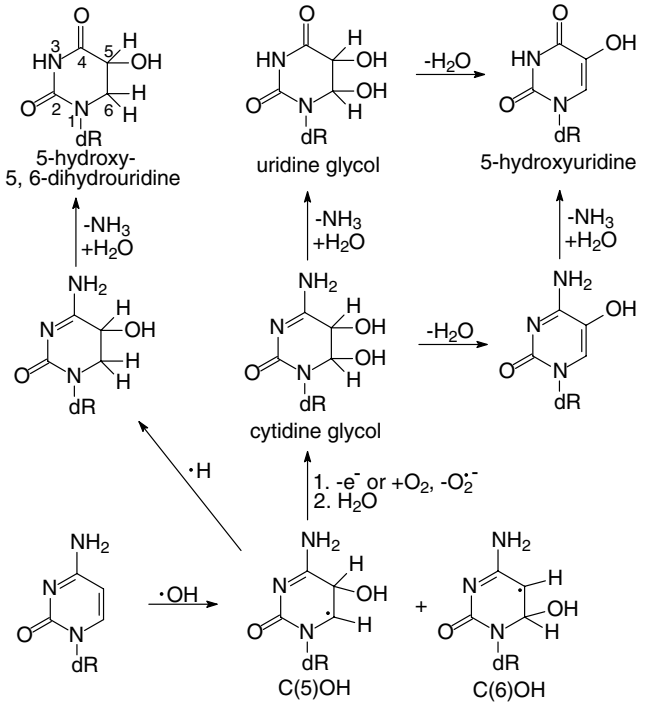


Scheme 9. Thymidine lesions formed under aerobic conditions.

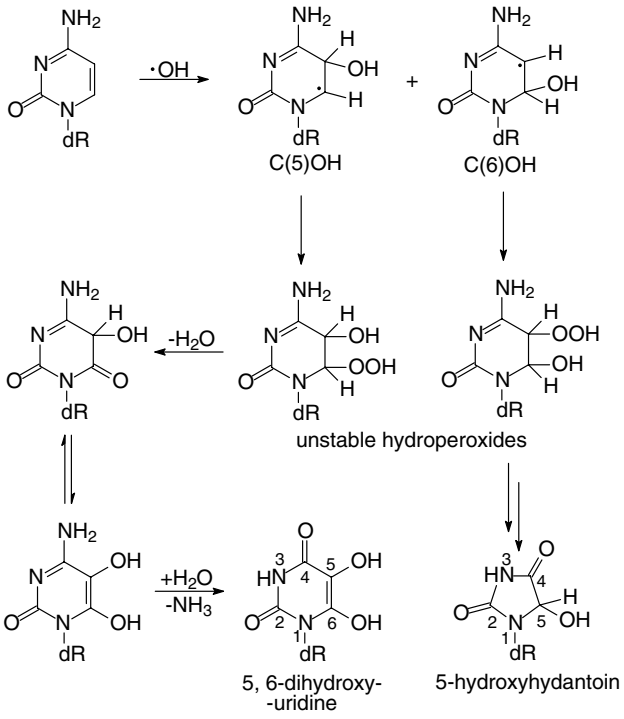
Peroxyl radicals from C(5)OH, C(6)OH and from allyl radical adducts are reduced to the labile and reactive hydroperoxides. These reactions may occur directly by electron transfer followed by protonation [33]. Alternatively the thymine peroxyl radicals may abstract a hydrogen atom from intracellular thiols or from the DNA chain to give the hydroperoxides. Some thymidine related hydroperoxides decompose slowly. 5-Hydroperoxy-6-hydroxy-5,6-dihydrothymidine undergoes conversion to 5-hydroxy-5-methylhydantoin, which has been found as a modified base retained in DNA. 6-Hydroperoxide gives thymidine glycol while 5-hydroperoxymethyl compound decomposes to 5-hydroxymethyldeoxyuridine and 5-formyldeoxyuridine derivatives (Scheme 9).

2.1.2.2. Cytosine oxidation. The chemistry of cytosine reacting with ·OH is complicated by the fact that primary products of the addition of hydroxyl radicals to the double bond in the cytosine moiety are unstable and cause the exocyclic amino group more susceptible to hydrolytic deamination [19]. As a result a complex mixture of uracil derivatives is formed (Scheme 10).

The major radical adducts of cytidine, C(5)OH formed by addition of hydroxyl radical to C-5 position, leads to cytidine glycol (5,6-dihydroxy-5,6-dihydrocytidine), by conversion to the stabilized C-6 cation followed by quenching with water. This compound undergoes further deamination to uridine glycol (5,6-dihydroxy-5,6-dihydrouridine). Next, uridine glycol can lose a molecule of water to form 5-hydroxyuridine. Alternatively these processes can occur in the reverse order; cytidine glycol can dehydrate first to 5-hydroxycytidine that regain the 5,6-double bond and then deaminate slowly to 5-hydroxyuridine. Both 5-hydroxycytidine and 5-hydroxyuridine have been identified in DNA irradiated under anaerobic as well as aerobic conditions and aerobically cultured human cells [34,35]. Under oxygen-free conditions the C(5)OH radical adduct can be quenched by some source of hydrogen to form 5-hydroxy-5,6-dihydrocytidine that after deamination gives 5-hydroxy-5,6-dihydrouridine.



Scheme 10. Cytidine hydroxyl radical adducts and degradation products of C(5)OH.



Scheme 11. Degradation products of cytidine hydroxyl radical adducts formed under oxygen conditions.

Under oxygen conditions, the peroxyl radicals from the cytidine C(5)OH and C(6)OH radical adducts could be reduced to the unstable hydroperoxides (Scheme 11).

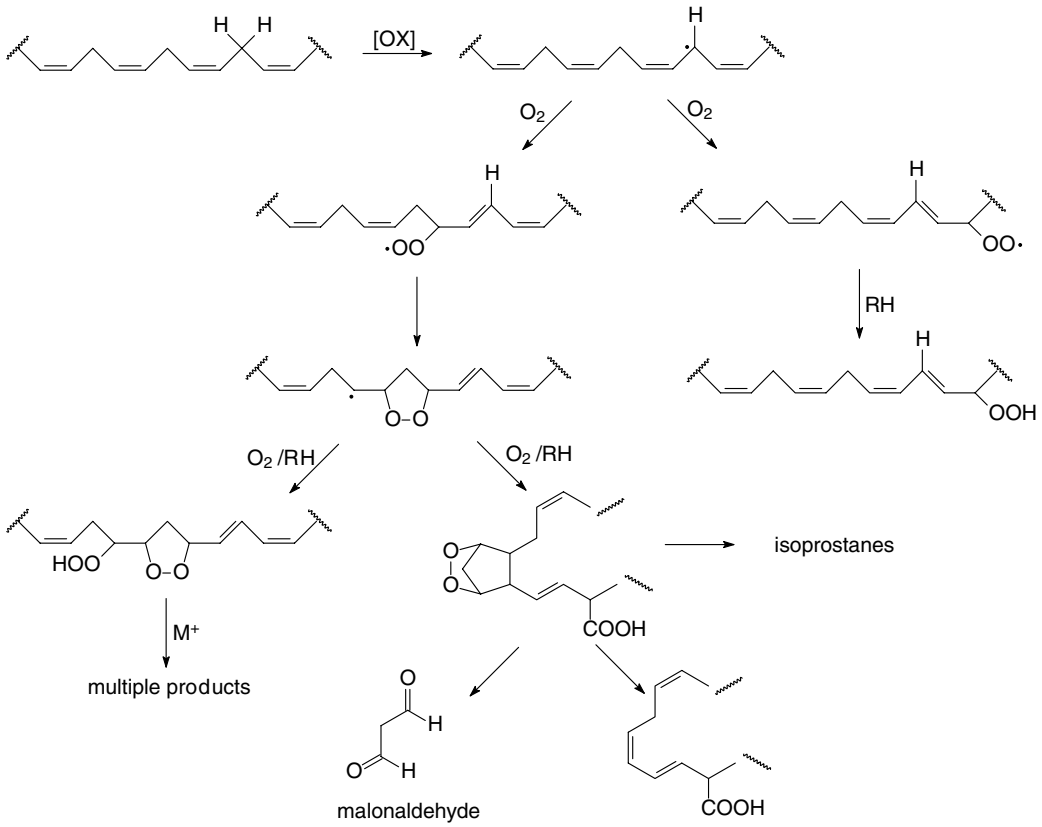
Hydroxyperoxide formed from the C(5)OH radical adduct may decompose to 5,6-dihydroxycytidine that after deamination gives 5,6-dihydroxyuridine. Hydroxyperoxide formed from the C(6)OH radical adduct would yield 5-hydroxyhydantoin. Both of these products: 5,6-dihydroxyuridine and 5-hydroxyhydantoin have been isolated from chromatin and human cells cultured under aerobic conditions [31,35].

3. Lipid peroxidation

Lipid peroxidation is a complex process that involves the participation of free-radical species. It occurs in a chain sequence characterised by classical three phases: initiation, propagation and termination [36]. The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. They contain methylene groups positioned between *cis* double bonds which are highly reactive towards oxidizing agents. Chain initiation results from the abstraction of a hydrogen atom from a methylene group (Scheme 12), [37].

This abstraction leads to the formation of carbon-centred radicals which combine with oxygen yielding peroxyl radicals. Peroxyl radicals positioned at one of two ends of the double bonds system can be reduced to hydroperoxides being the primary products of unsaturated fatty acids oxidation. These products are relatively stable in the absence of metals and can be reduced by glutathione peroxidases to unreactive fatty acid alcohols [3]. Metal cations, however, cause rapid reduction of hydroperoxides to alkoxyl radicals, which undergo multiple reactions to generate a variety of products including epoxides and carbonyl compounds [38,39].

Reduction of peroxyl radicals to hydroperoxides may occur *via* reaction with another molecule of fatty acid or with vitamin E. In the reaction with fatty acid, hydrogen atom is removed from a



Scheme 12. Pathways of lipid peroxidation.

methylene group and a new carbon-centred radical is generated which propagates the fatty acid oxidation. Quenching of peroxyl radicals by antioxidant such as vitamin E breaks the radical chain and slows the rate of lipid peroxidation.

If the peroxyl radical is situated at an internal position in the fatty acid chain, a cyclic peroxide adjacent to a carbon-centred radical is formed. This radical can react with oxygen to form a peroxyl radical which is reduced to a hydroperoxide. The carbon-centred radical can also undergo cyclization to form a bicyclic peroxide that after combination with oxygen and following reduction generates the compound which is an intermediate for the production of isoprostanes and malonaldehyde [40]. Lipid peroxides ensuing from lipid radicals can be reduced by metal ions to alkoxyl radicals that decompose to a range of products containing various oxygen functional groups. These reactive substances cause damage of DNA by the formation of exocyclic adducts that are responsible for blocking the Watson–Crick base pairing region. Many of the aldehyde products of lipid peroxidation are toxic and mutagenic. Among them malonaldehyde appears to be the most mutagenic whereas 4-hydroxynonenal is the most toxic [41].

The aldehydic products of lipid peroxidation belong to a group of bifunctional carbonyl compounds, that are important industrial chemicals, environmental contaminants but also products of human metabolism. The simplest bifunctional carbonyl compounds are α,β-unsaturated aldehydes. Due to their bifunctional character (a double bond conjugated with the formyl group), these compounds exhibit a rich chemistry of DNA modifications. One of the most important consequences of high reactivity of the α,β-unsaturated aldehydes towards nucleobases is their ability to form exocyclic DNA adducts.

The aldehydes are the most extensively studied compounds originating from peroxidized lipids. Unlike reactive free radicals, aldehydes are longer lived molecules that may diffuse from the site of their origin and may reach and attack targets which are distant from the initial free radical event. The major aldehyde products of lipid peroxidation are: malonaldehyde (MA), acrolein, crotonaldehyde, 4-hydroxynonenal (HNE) and other saturated and α,β-unsaturated aldehydes and ketones [42].

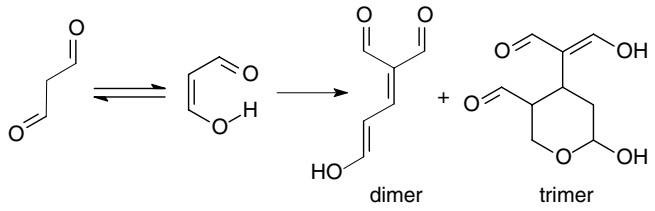
3.1. Adducts of lipid peroxidation products

3.1.1. Malonaldehyde adducts

The unique feature of the chemistry of malonaldehyde is its ability to act both as an electrophile and as a nucleophile. In polar solvents malonaldehyde undergoes tautomerisation and can exist as its enol tautomer, β-hydroxyacrolein. The presence of the nucleophilic enol group and the electrophilic aldehyde group in the same molecule is the reason why malonaldehyde is very susceptible to polymerization. The aldehyde undergoes self condensation to afford a mixture of dimers and trimers [43], (Scheme 13).

Malonaldehyde is carcinogenic in rats and mutagenic in bacteria and mammalian cells [44–47]. Malonaldehyde reacts readily with nucleic acid bases to form stable adducts of dG, dA and dC: M_1dG, M_1dA and M_1dC, respectively [48–52], (Fig. 2).

The oxopropenyl adducts (M_1dA and M_1dC) are formed by addition of exocyclic amino group of the base unit to the formyl carbon of the aldehyde followed by elimination of one molecule of water. Michael addition is also presumed to be the first stage of the condensation of malonaldehyde and 2′-deoxyguanosine leading to the formation of the pyrimidopurinone adduct (M_1dG). Unlike M_1dA and M_1dC, the pyrimidopurinone adduct is formed by elimination of two molecules of water. Aromatization provides the driving force for the dehydrations.



Scheme 13. Condensation products of malonaldehyde.

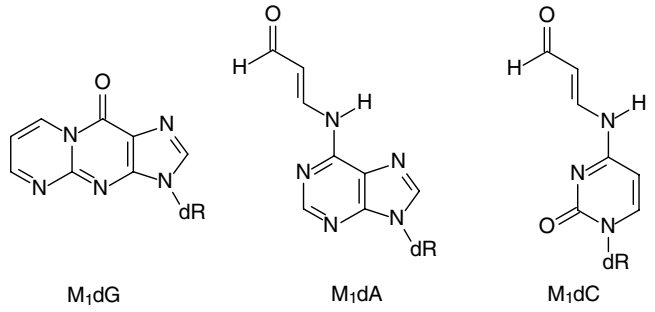


Fig. 2. Monomeric adducts of malonaldehyde.

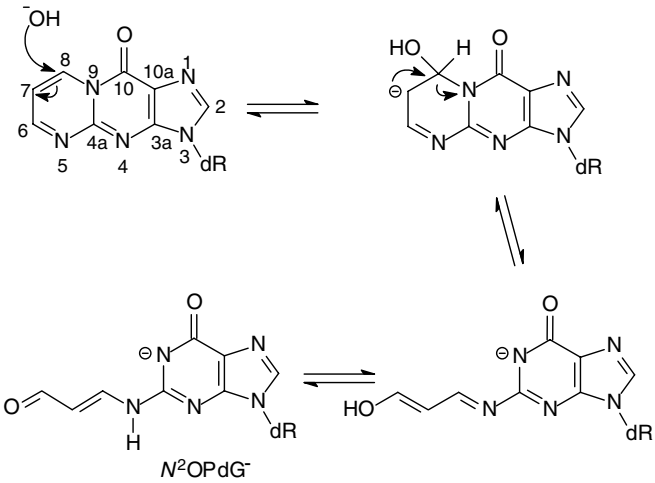
Under basic conditions M_1dG undergoes hydrolytic ring-opening to N^2-oxopropenyldeoxyguanosine anion (N^2OPdG^-, Scheme 14) [53].

The mechanism involves a direct attack of hydroxide anion on C8 of M_1dG leading to the transient carbinolamine carbanion which then opens *via* elimination by C8-N bond breakage [53]. Addition of hydroxide to M_1dG is a reversible reaction. Ring-closure of the N^2OPdG anion followed by elimination of hydroxide leads to the formation of M_1dG. The cyclization is slow under basic conditions but addition of acid dramatically accelerates this process [54]. Ring-opening in a nucleoside or in a single-stranded oligonucleotide remarkably differs comparing with the process occurring in duplex DNA [55]. The equilibrium between M_1dG and its ring-opened form, N^2OPdG is a hydroxide-dependent process favouring M_1dG at neutral pH in the single-stranded oligonucleotide. Under the same conditions a quantitative conversion of M_1dG to its ring-opened form takes place in the double-stranded DNA but only when M_1dG is placed opposite to dC. NMR studies of M_1dG opposite dC in oligonucleotides suggest that the adduct exists as the ring-opened form, N^2-oxopropenyloguanine [56,57]. Thus the reaction of malonaldehyde with duplex DNA results in the formation of N^2-oxopropenyloguanine adduct which upon heat denaturation cyclize to M_1dG. It has been suggested that N^2OPdG projects into the minor groove of the duplex DNA causing distortion in the DNA helix [56]. Both adducts M_1dG and N^2OPdG represent potential reactive electrophiles in the genome, and their ability to condense with nucleophiles has been reported [58,59]. This ability is important with regard to biological properties of the adducts. The electrophilic C-8 of M_1dG is positioned in the major groove of duplex DNA while the C-8 of N^2OPdG is situated in the minor groove. Both forms may react with cellular nucleophiles producing cross-linked adducts. M_1dG can also be formed by reactions of base-propenal with deoxyguanosine [60]. Base-propenal is one of the products formed during oxidation of the DNA backbone (Scheme 15) [61,62]. Base-propenal results from oxidation of the hydrogen atom combined to C-4′ of the deoxyribose moiety. Base propenals are structurally analogous to β-hydroxyacrolein (the enol tautomer of malonaldehyde) and can undergo acid-catalyzed hydrolysis to malonaldehyde.

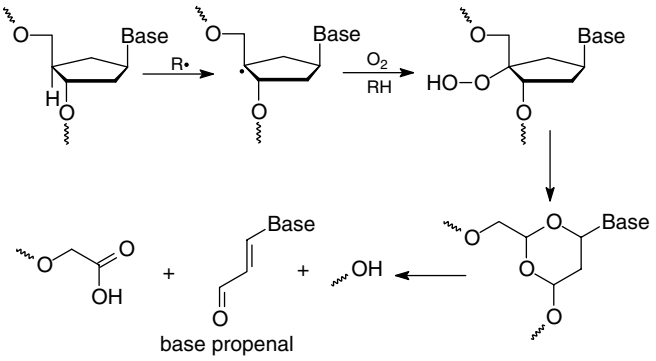
Base propenals are reactive electrophiles that are cytotoxic to human cells and mutagenic in the frameshift tester strain *Salmonella typhimurium* hisD3052 [63,64].

M_1dG can also arise from the reaction of N^{ε}-oxopropenyllysine with DNA [60]. N^{ε}-oxopropenyllysine (Fig. 3) is an other endogenously produced electrophile formed in reaction of malonaldehyde with ε-amino groups of lysine residues in protein.

Base propenal and N^{ε}-oxopropenyllysine have different leaving groups but the mechanism of their reaction with DNA is similar and probably involved initial 1,2 addition of the exocyclic amino group of guanine to the aldehyde of β-substituted acroleins which is followed by cyclization with the guanine ring nitrogen (N1), [65]. The same mechanism is most likely to be valid for transferring oxopropenyl group from malonaldehyde to guanine residues of DNA. Single-stranded DNA is more sensitive to oxopropenylation



Scheme 14. Mechanism of ring-opening in M_1dG.



Scheme 15. Formation of base propenal.

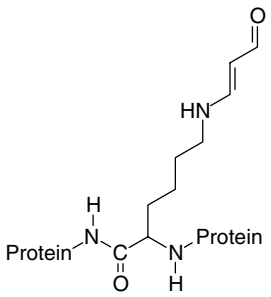


Fig. 3. N^{ε}-Oxopropenyllysine—compound related to base propenals.

comparing with double-stranded DNA [66]. The N^2 atom of guanine lies in the minor groove and is relatively hindered. In double-stranded DNA the access to the minor groove is blocked and oxopropenylation is inhibited. In single-stranded DNA position N^2 is easier accessible and the level of oxopropenylation increases [66].

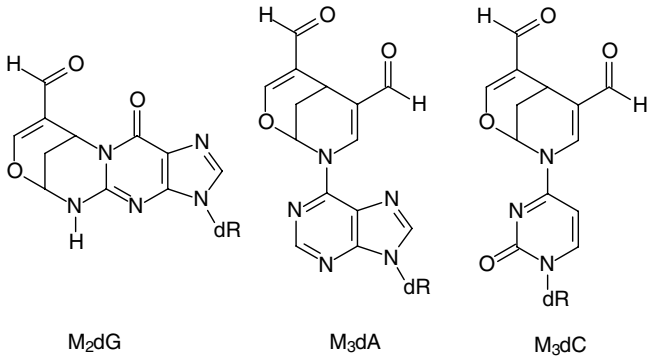
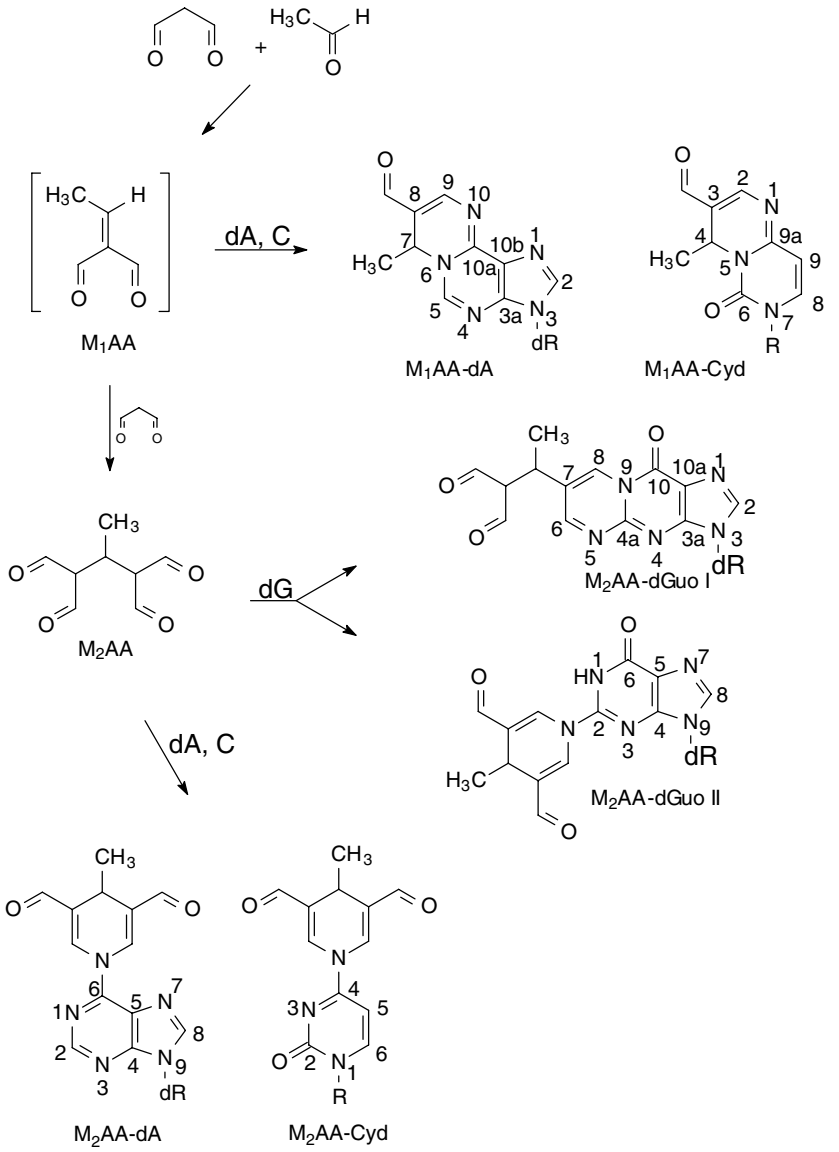


Fig. 4. Oligomeric adducts of malonaldehyde.

M_1dG appears to be the major endogenous DNA adduct in human that may contribute to cancer [67]. This adduct is mutagenic. When site-specifically incorporated into viral genomes and replicated in bacteria and mammalian cells, it induces base pair substitutions and frameshift mutations [68,69]. M_1dG has been detected in liver, white blood cells, pancreas and breast from human beings.

Products of the malonaldehyde oligomerization also react with DNA. The dimer of malonaldehyde forms with deoxyguanosine an adduct that contains an oxadiazabicyclononene group (M_2dG), whereas the trimer produces oxazocinyl adducts with deoxyadenosine and deoxycytidine (M_3dA and M_3dC, respectively, Fig. 4), [50–52].

Oligomeric adducts are not formed by sequential addition of malonaldehyde, as it was proposed earlier, but it is believed now that rather by addition of an oligomer to the deoxynucleoside bases. The oligomerization of malonaldehyde is relatively slow at neutral pH so the monomeric adducts are the major products generated under physiological conditions. However the possibility of multimeric adducts formation *in vivo* cannot be ruled out.



Scheme 16. Formation of malonaldehyde–acetaldehyde conjugate adducts.

3.1.2. Adducts of malonaldehyde and acetaldehyde

It has been shown that reactivity of malonaldehyde towards nucleosides significantly increases in the presence of acetaldehyde [70]. These two aldehydes may undergo a mixed aldol condensation and may form 1:1 or 2:1 malonaldehyde–acetaldehyde highly reactive conjugates that can be readily quenched by the nucleosides.

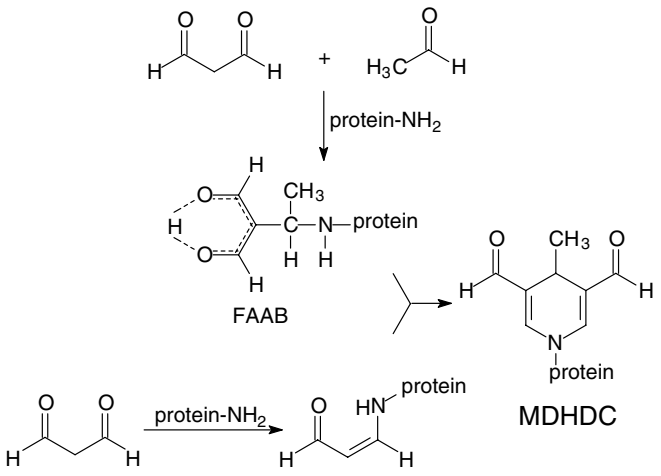
Reaction of the mixture of malonaldehyde and acetaldehyde with 2′-deoxyadenosine results in the formation of strongly fluorescent dihydropyridine (M_2AA-dA), and exocyclic propenoformyl adducts (M_1AA-dA, Scheme 16), [71–73]. Structurally related, fluorescent adducts (M_2AA-Cyd and M_1AA-Cyd, respectively, Scheme 16), are also formed when cytidine is simultaneously incubated with malonaldehyde and acetaldehyde [74].

The analogous dihydropyridine adduct (M_2AA-dGuo II), in addition to the adduct marked M_2AA-dGuo I was found to be formed in the synergistic reaction of 2'-deoxyguanosine with malonaldehyde and acetaldehyde (Scheme 16), [75]. Unlike deoxyadenosine and cytidine adducts, none of the deoxyguanosine adducts exhibit fluorescent properties. The reason for this is unclear. It seems that the purine moiety affects the electron distribution of the dihydropyridine unit so that the adduct is unable to absorb or emit energy in such a way that it would have fluorescence properties [75]. The plausible mechanism for the formation of the conjugate malonaldehyde–acetaldehyde adducts implies that the nucleobases react with the initially formed aldehydes condensation products. Support for this mechanism is provided by the studies that have shown that the conjugation products are generated at acid as well as neutral conditions and these may then react with amino groups yielding dihydropyridine derivatives [76,77]. M_2AA-dGuo II can be in part formed from M_2AA-dGuo I. At basic conditions (at neutral conditions as well but at a slower rate), the transformation of M_2AA-dGuo I to M_2AA-dGuo II takes place [75]. The transformation goes most likely through a ring-opening of the propeno ring at the endocyclic nitrogen of the guanine unit followed by an attack of the guanine amino nitrogen on one of the terminal carbonyl groups. The final dihydropyridine structure is obtained by dehydration (Scheme 17), [75].

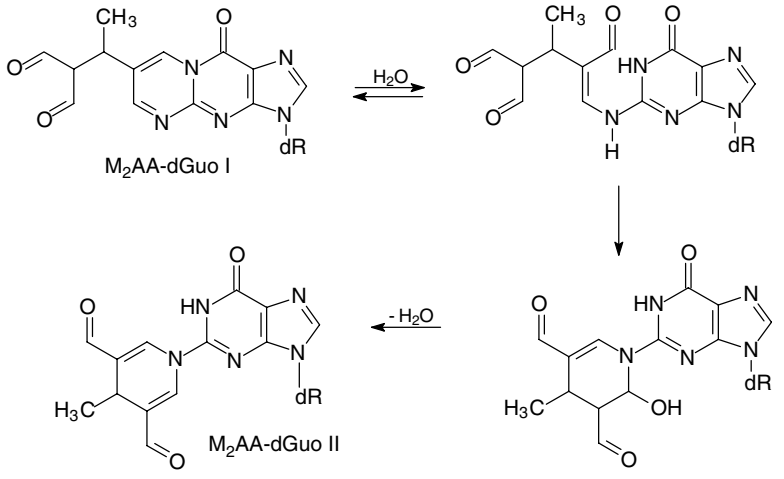
It has been found that the formation of conjugate adducts of 2'-deoxyadenosine and cytidine is very pH dependent [71–74]. High yields of the adducts were obtained at acid conditions, while at physiological pH hardly any adducts could be detected. It has also been shown that pH has only slight effect on the reactivity of 2'-deoxyguanosine towards malonaldehyde–acetaldehyde conjugates [75]. In spite of these findings, both 2′-deoxyadenosine conjugate adducts (M_2AA-dA and M_1AA-dA) in addition to 2′-deoxyguanosine ones (M_2AA-dGuo I and M_2AA-dGuo II) were shown to be formed in calf thymus DNA, when incubated simultaneously with malonaldehyde and acetaldehyde under physiological conditions [78,79]. This fact may contribute to the biological relevance of the conjugate adducts especially having in mind that malonaldehyde and acetaldehyde can coexist in biological tissues.

Malonaldehyde and acetaldehyde may react in a synergistic manner also with proteins [80–82]. The major adducts resulting from these reactions are marked FAAB and MDHDC (Scheme 18), [81].

MDHDC is a highly fluorescent compound composed of two molecules of malonadehyde and one molecule of acetaldehyde, while FAAB is an 1:1 adduct of these aldehydes. The mechanism which accounts for these adducts' formation is different from that responsible for the formation of the conjugate DNA adducts. It has been reported that FAAB may serve as a precursor for the formation of MDHDC [81]. FAAB was shown to be the prominent adduct at early reaction time of malonadehyde and acetaldehyde with proteins. It has been suggested that MDHDC is formed by dissociation of one malonaldehyde–acetaldehyde moiety from an amino acid nitrogen and transfer of this moiety to a malonaldehyde adducted amino acid nitrogen (present in the enamine form). A prerequisite for the transfer is that the enamine and the conjugate are



Scheme 18. Formation of malonaldehyde–acetaldehyde protein adducts.



Scheme 17. Transformation of M_2AA-dGuo I to M_2AA-dGuo II.

positioned on protein nitrogens of close proximity to each other [81]. FAAB and MDHDC have been identified in ethanol-fed rats and in patients with alcohol-induced liver disease [83,84] and are considered to play a significant role in the pathogenesis of alcoholic liver injury [85]. Acetaldehyde is present in liver as a metabolite of ethanol. In the early stage of ethanol consumption the FAAB adduct formation would be favoured because the level of acetaldehyde would likely exceed those of malonaldehyde. With prolonged ethanol intake, which has been shown to induce oxidative stress that elevate the level of malonaldehyde increase in the amount of MDHDC would be observed [85].

3.1.3. Adducts of acrolein

Acrolein has been shown to exhibit mutagenic activity in bacterial, mammalian and human cells and the aldehyde is carcinogenic in rats [86–88]. As a strong electrophile, acrolein may form various DNA and protein adducts. In reaction of acrolein with the guanine residue in DNA two pairs of stereomeric propano adducts (I and II) are formed [89], (Fig. 5). (The common name propano adducts comes from the observation that these adducts contain a propane fragment as a part of adducted ring.) These adducts formation proceeds *via* a Michael addition.

In the major adduct, 8-hydroxy-1,N^2-propano-2'-deoxyguanosine (I), the initial attack takes place at the position N^2, while in the minor adduct, 6-hydroxy-1,N^2-propano-2′-deoxyguanosine (II) the nucleophilic N-1 atom of the guanine unit is attacking in the first stage of reaction. The adduct I is only slightly mutagenic in *Escherichia coli* and human cells [90–92], but the adduct II displays significant mutagenicity in human cells and predominantly induced G→T transversions [93]. The adduct I may form an interchain cross-link to the N^2 position of the opposing guanine moiety in a 5′-CpG sequence [94,95], (Scheme 19), and can generate cross-links to peptides by a Schiff base linkage [96].

Four adducts of acrolein and 2′-deoxyadenosine have been identified and characterised [97,98], (Fig. 6).

The most favourable conditions for these adducts formation were found to be pH 4.6 in the case of compounds III and IV, and neutral pH for adducts V and VI [98]. Formation of the cyclic adducts III and IV proceeds *via* a Michael addition at N-1 of adenine and subsequent ring closure at the carbonyl carbon by the exocyclic NH_2 to form III (Scheme 20), [98].

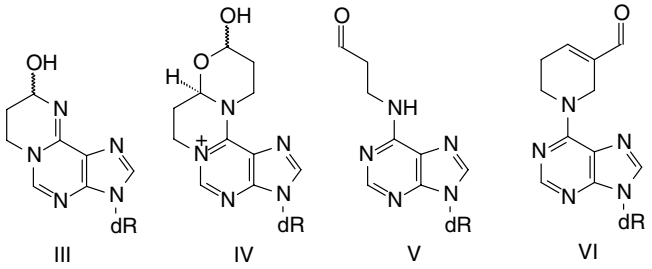
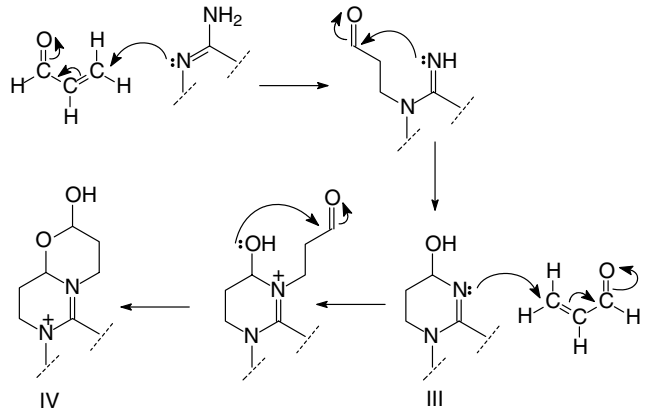


Fig. 6. Acrolein adducts with 2′-deoxyadenosine.



Scheme 20. Mechanism of the formation of cyclic acrolein and 2′-deoxyadenosine adducts.

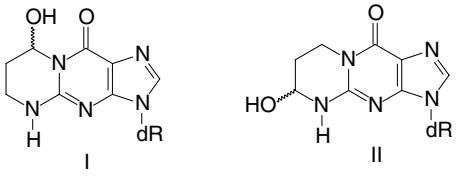
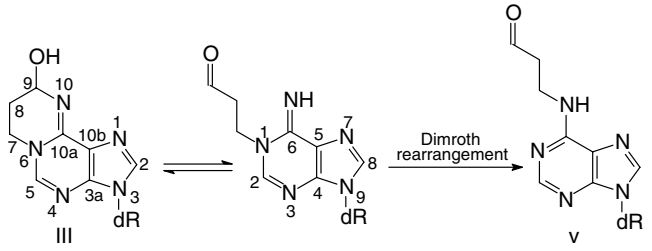
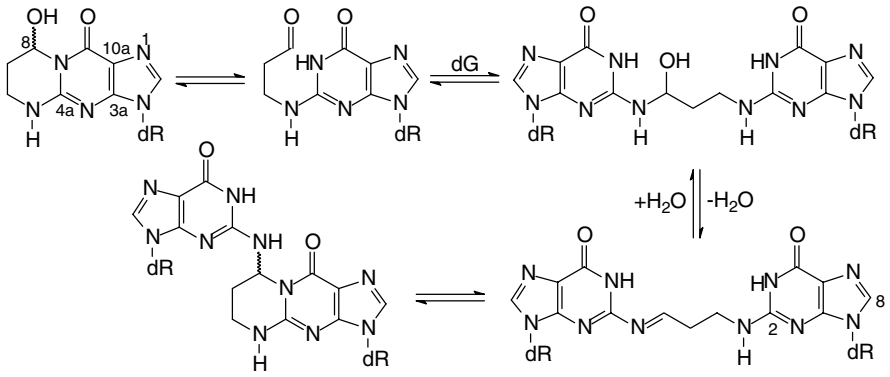


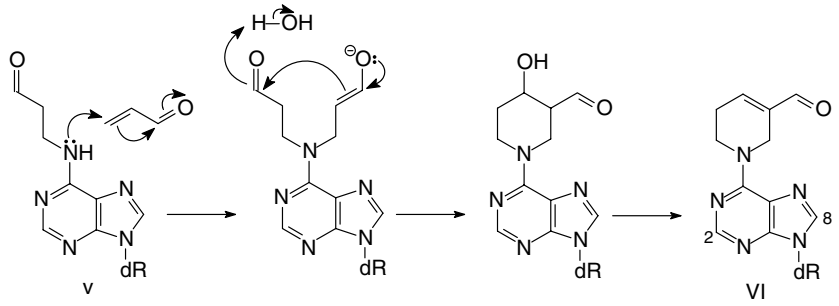
Fig. 5. Adducts of acrolein and 2′-deoxyguanosine.



Scheme 21. Formation of V from III by Dimroth rearrangement.



Scheme 19. Cross-linking reaction of the adduct I with guanine residue in DNA.



Scheme 22. Formation of the adduct VI.

The adduct III undergoes a second type Michael addition with acrolein at the formerly exocyclic nitrogen of adenine unit. The adduct IV is obtained by a second cyclization involving the reaction of the hydroxyl group of the initially formed ring with the formyl carbon of the second acrolein unit (Scheme 20). The compound IV is the major acrolein–2′-deoxyadenosine adduct. This is due to higher reactivity towards acrolein of the adduct III than of 2′-deoxyadenosine [98]. The adduct V was found to be generated upon storage of III at neutral pH. At these conditions, the adduct III may undergo ring opening. The open form of the adduct is susceptible to the Dimroth rearrangement yielding compound V (Scheme 21), [99].

A plausible mechanism for the formation of VI is that the two terminal aldehyde groups undergo an intramolecular aldol condensation yielding the tetrahydropyridine ring (Scheme 22) [100]. Additional proof for this mechanism is provided by the finding that in the reaction of pure V with acrolein, the adduct VI was obtained. A structurally analogous adduct has been reported to be formed in the reaction of acrolein with the lysine residue in proteins [101,102]. All adducts identified in the reaction of acrolein with 2′-deoxyadenosine were shown to be formed also in calf thymus DNA incubated with the aldehyde under physiological conditions.

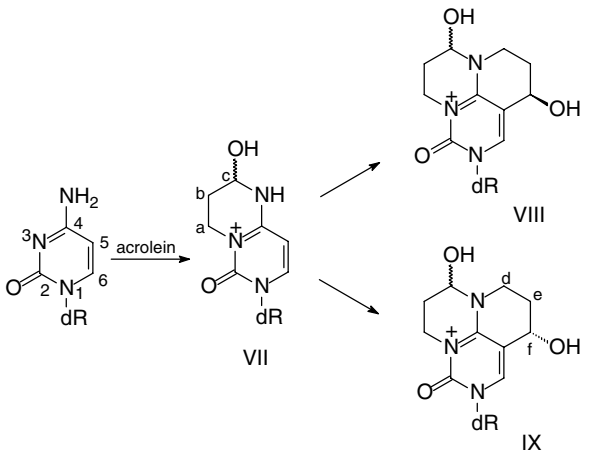
In the reaction of acrolein with 2′-deoxycytidine three major adducts have been reported to be formed [103–105]. The adducts were characterised as the cyclic products comprised of one or two units derived from acrolein (Scheme 23). All adducts were isolated as protonated species with the charge formally located on N-3 nitrogen atom of the pyrimidine ring.

Adduct VII is formed by a Michael addition at N-3 followed by cyclization at the exocyclic amine N^4. The adducts VIII and IX are likely to be generated by a second Michael addition followed by the nucleophilic attack of the pyrimidine C-5 carbon onto the residual carbonyl group of the acrolein unit (Scheme 24). Nucleophilicity of the position C-5 is enhanced by the participation of N-1 through its ion electron pair. It is known that C-5 carbon of the cytosine moiety may display reactivity towards electrophiles [106,107].

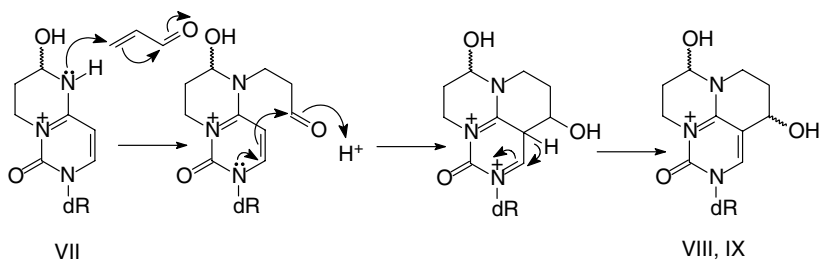
Under physiological conditions the reactivity of adduct VII towards acrolein is higher than that of 2′-deoxycytidine. Thus, it seems that in VII the former exocyclic amino nitrogen of cytosine unit is more susceptible to a Michael addition than the one in the unmodified nucleoside. This property of the nitrogen atom in VII is most likely responsible for the formation of IX in calf thymus DNA when incubated with acrolein under physiological conditions [105]. VII is also formed in the reaction and in a slightly higher yield than the adduct IX.

In the reaction of acrolein with thymidine five structurally different adducts were shown to be formed (Fig. 7).

X was reported to be the adduct formed in the highest yield [108]. It was found that the adducts XI–XIV were generated from X, when X was allowed to react with acrolein. In a water solution, acrolein may at least partially exist in the form of a hydrate. The C-2 carbon in the hydrate may attack the formyl carbon in X, leading to the formation of an intermediate which may then lose water. Either the terminal hydroxyl group or the hydroxyl group at the secondary carbon can be lost yielding the adducts XII and XIII, respectively. The enolate ion of X may be sufficiently nucleophilic to attack the C-1 carbon of the hydrate providing an intermediate



Scheme 23. Adducts of acrolein and 2′-deoxycytidine.



Scheme 24. Mechanism for the formation of the deoxycytidine adducts VIII and IX.

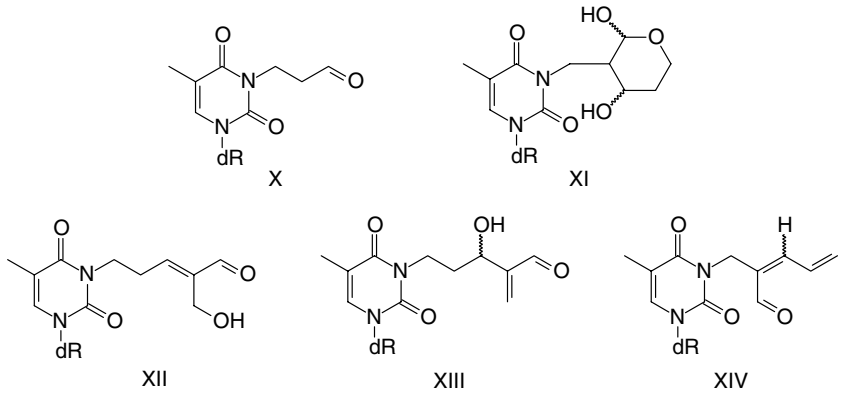


Fig. 7. Structures of acrolein adduct with thymidine.

that either can be transformed to the ring leading to hydrate X or may lose two molecule of water yielding the adduct XIV (Scheme 25), [108].

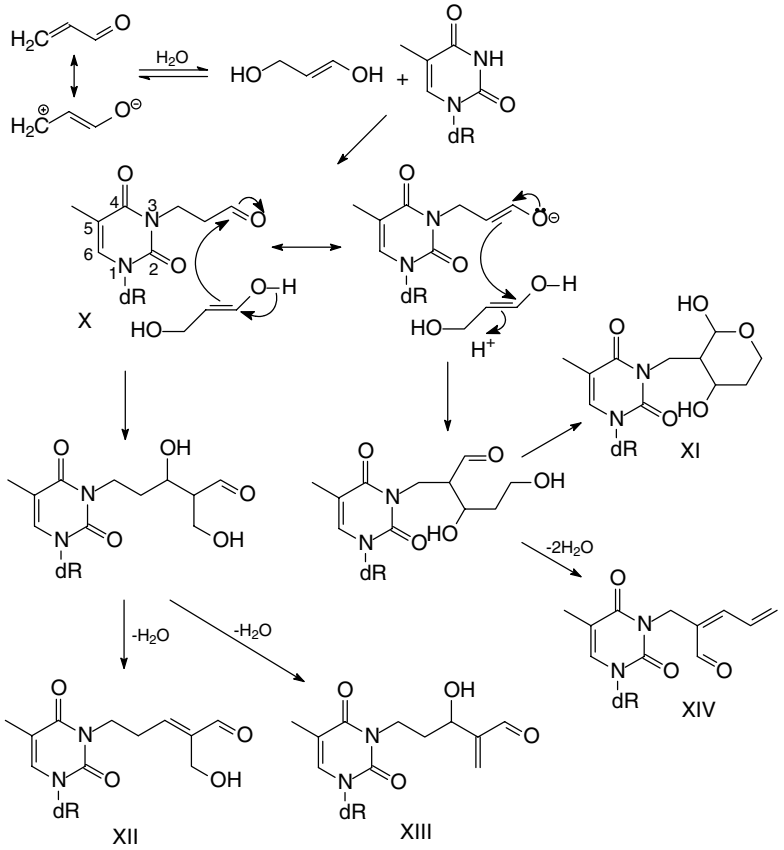
From the thymidine-acrolein adducts only X was found to be formed in calf thymus DNA.

3.1.4. Adducts of crotonaldehyde

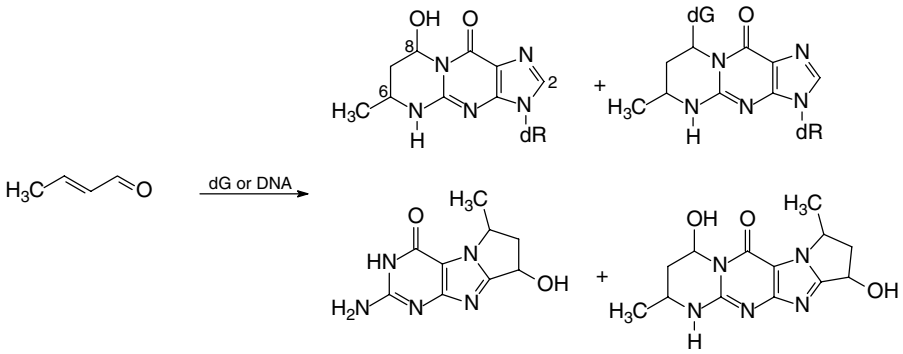
Like other α,β-unsaturated aldehydes, crotonaldehyde reacts with DNA to form adducts which are believed to be fundamental for genotoxicity. Reaction of crotonaldehyde with 2′-deoxyguanosine results in the formation of diastereomeric mixture of 8-hydroxy-6-methyl-1,N^2-propano-2′-deoxyguanosine adducts [89,109,110], (Scheme 26). The orientation of the hydroxyl and methyl groups is mainly *trans* (94%), and only a small amount of a diastereomeric mixture exhibits the orientation *cis* [109].

These adducts are the expected products of Michael addition of the guanine moiety nitrogen atom N1 or N^2 to crotonaldehyde followed by ring closure. The adducts have been detected in DNA of various human tissues and may inhibit DNA synthesis and induce miscoding in human cells [111–113]. The diastereomer 6S, 8S of 8-hydroxy-6-methyl-1,N^2-propano-2′-deoxyguanosine causes miscoding at higher frequency than the diastereomer 6R, 8R and leads mainly to G→T transversions.

Crotonaldehyde also forms adducts to deoxyguanosine residues by reaction at N7 and C8 [109], (Scheme 26). These cyclic compounds in which the methyl and hydroxyl groups can be either *cis* or *trans*, are unstable in DNA and undergo spontaneous depurination. Bis-crotonaldehyde–deoxyguanosine adducts formed by reaction at N1,N^2 and N7,C8 have also been detected [109].



Scheme 25. Formation of acrolein adducts with thymidine.



Scheme 26. Crotonaldehyde adducts formed by Michael addition pathway.

Upon enzymatic or neutral, thermal hydrolysis of DNA earlier exposed to crotonaldehyde, substantial amount of 2-(2-hydroxypropyl)-4-hydroxy-6-methyl-1,3-dioxane (paraldol) is formed [114]. It has been demonstrated that the released paraldol reacts with 2'-deoxyguanosine in the DNA to form three adducts: N^2-paraldol-dG, N^2-paraldol-dG-(5′–3′) thymidine and N^2-(3-hydroxybutylidene)dG [115,116], (Scheme 27). Four diastereomers of N^2-paraldol-dG, and four of N^2-paraldol-dG-(5′–3′) thymidine were observed and structurally characterised. N^2-(3-hydroxybutylidene)dG has been identified as the major paraldol-releasing DNA adduct of crotonaldehyde [116]. This adduct accounts for more than 90% of the paraldol released from crotonaldehyde-treated DNA [116].

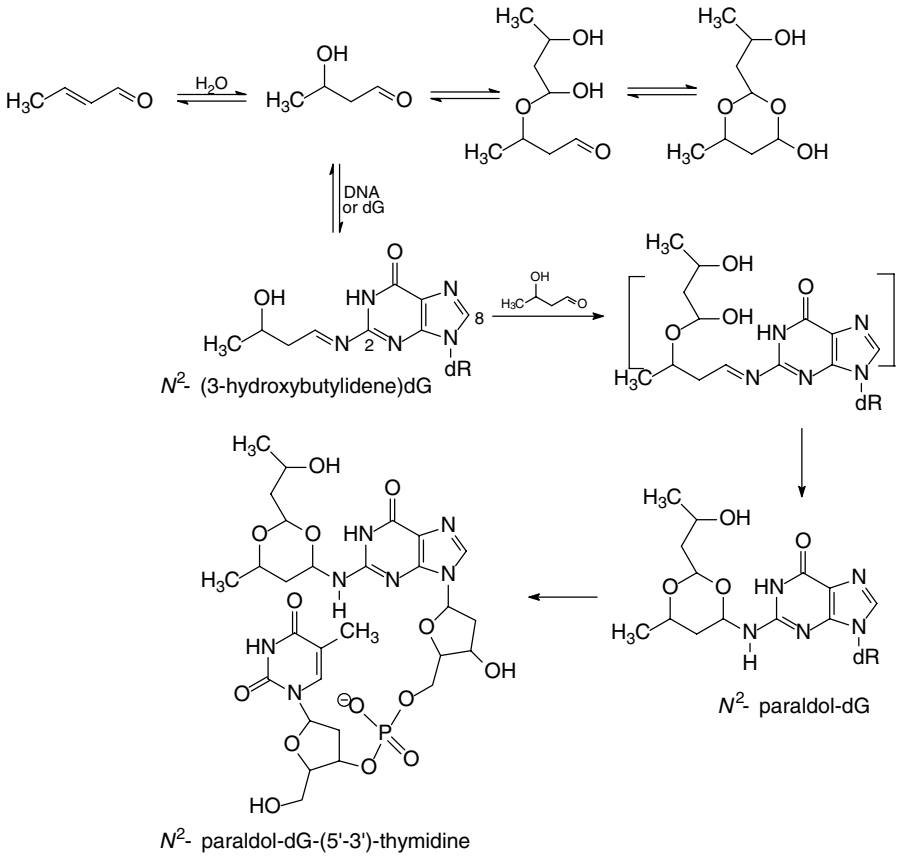
All the three paraldol-releasing adducts were found to be stable in DNA and partially resistant to enzymatic hydrolysis. This indicates that the adducts may be persistent and may play more important role in mutagenesis and carcinogenesis of crotonaldehyde than the adducts arising directly from Michael addition of 2′-deoxyguanosine to the aldehyde.

3.1.5. Adducts of trans-4-hydroxy-2-nonenal (HNE)

trans-4-Hydroxy-2-nonenal (HNE) may conjugate with cellular proteins but may also react with DNA bases to form adducts. The direct addition of HNE to 2'-deoxyguanosine leads to the formation of two pairs of diastereomeric substituted 1,N^2-propano adducts [96,117,118], (Fig. 8).

The adducts are thought to arise through Michael addition of an exocyclic amino group of 2′-deoxyguanosine and subsequent ring closure at N1.

The second pathway by which HNE may react with DNA bases involves the reaction with its epoxide, 2,3-epoxy-4-hydroxynonenal [119]. HNE can be oxidized to the reactive epoxide derivative



Scheme 27. Formation of paraldol-releasing DNA adducts.

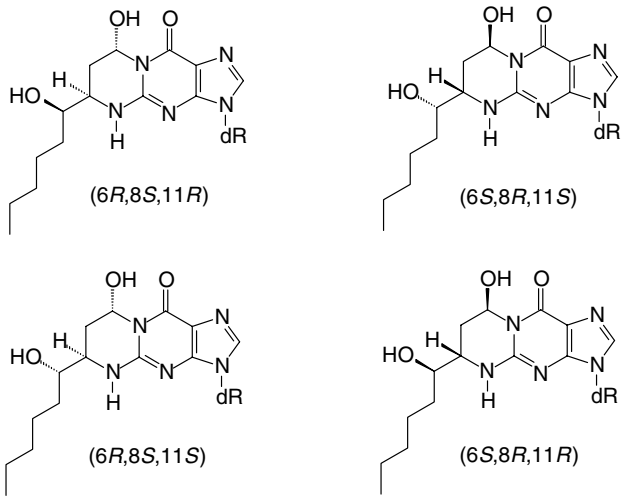


Fig. 8. 1,N^2-Deoxyguanosine adducts of *trans*-4-hydroxy-2-nonenal.

by biological oxidants such as linoleic acid hydroperoxide and hydrogen peroxide or through an autoxidation mechanism [120,121]. The epoxide appears to be more reactive towards DNA bases than the parent aldehyde. The epoxide reacts with 2′-deoxyguanosine to give several adducts, including 1,N^2-etheno-2′-deoxyguanosine, a substituted 1,N^2-etheno adduct and a tetracyclic adduct containing two five-membered rings fused at the N1 and N^2 atoms of a guanine moiety [122]. The epoxide can structurally modify also 2′-deoxyadenosine to form the analogous etheno adduct [123]. HNE has been found in both rodent and human tissues and it is considered to be the most cytotoxic lipid peroxidation product [120]. Consequently HNE is suspected to be associated with a wide variety of toxicological effects [124–127].

The main target in reactions between nucleosides and α,β-unsaturated aldehydes such as malonaldehyde, acrolein, crotonaldehyde and *trans*-4-hydroxy-2-nonenal (HNE) is the guanine moiety. Generally, exocyclic propano adduct formation in reactions between these aldehydes and 2′-deoxyguanosine are thought to take place through a two-step mechanism [128]. Michael-type addition occurs at the β-carbon by N^2 or N1 of 2'-deoxyguanosine followed by nucleophilic addition of N1 or N^2 of 2'-deoxyguanosine at the carbonyl carbon [129]. When the α,β-unsaturated aldehyde has a substituent at the β-carbon, the resulting steric hindrance precludes initial nucleophilic attack by N1 [129]. Kinetic control of the reaction favours the formation of a regioisomer in which N^2 is attached to the β-carbon and N1 is attached to the carbonyl carbon [129]. On the contrary, when 2′-deoxyguanosine reacts with substituted epoxy aldehydes, nucleophilic addition of N^2 occurs at the carbonyl carbon [122]. Subsequent reaction of N1 at C2 of the epoxide results in the formation of etheno adducts.

4. Conclusion

Oxidative damage to DNA is thought to be an important factor of carcinogenesis and other age-related diseases such as Alzheimer's, Huntington's and Parkinson's diseases [130–132]. Such damage can occur through a direct interaction with reactive oxygen species or by reaction with bifunctional electrophiles that are generated as a result of lipid peroxidation. One consequence of oxidative base modifications persisting in DNA is mutation. DNA mutation is a crucial step in carcinogenesis and elevated levels of DNA modifications have been noted in many tumors, strongly implicating such damage in the aetiology of cancer. Although the role of oxidative stress and lipid peroxidation in carcinogenesis appears well established, the extent to which oxidative DNA damage contributes has not been precisely defined. It seems that the DNA damage can be more closely associated with initiation events that with promotion and this may be due to the potential for a multiplicity of mutagenic lesions to be formed [133].

Lesions produced in cellular DNA are subjected to cellular repair processes and unless repaired may have detrimental biological consequences. For an understanding of the biological significance of the DNA lesions originating from the reactions between DNA bases and ROS as well as final products of lipid peroxide breakdown, it is essential to identify and structurally characterised such lesions.

So far our expertise is gaining more and more examples of such modifications, however, it is still far not enough to get complete insight into these chemical and biological processes.

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