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Oxidation of DNA and its components with reactive oxygen species

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Abstract. The mechanisms of oxidation of nucleosides, nucleotides and DNA with reactive oxygen species including singlet oxygen, superoxide and hydroxyl radicals and high-valence metal oxo complexes are considered. The bibliography includes 137 references.

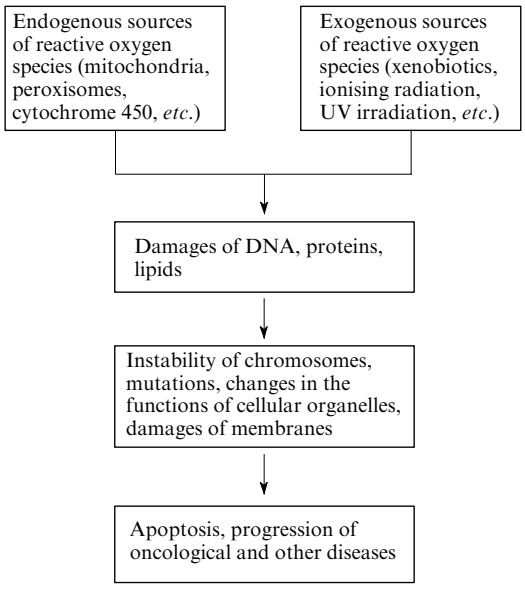
I. Introduction

Reactive oxygen species damaging various cell components including DNA can arise in some biological processes in the cells of living organisms and upon some external action. DNA damage can accelerate development of degenerative processes, aging and apoptosis (Scheme 1).[1–3] The stability of lesions in DNA, their repair within cells by DNA repair enzymes, and their consequences in terms of mutations or induction of cell death, diseases, or aging is an open field of investigation.[4,5] Every day, the number of examples illustrating the importance of DNA repair for prevention of different diseases is increased.[6–8]

However, oxidative properties of reactive oxygen species can be used for the specific degradation of nucleic acids. Thus systems generating hydroxyl radicals are widely used for the determination of protein-binding DNA sequences by the footprinting method.[9] Damage of nucleic acids can also be used for the treatment of oncological, viral and other diseases.[10–13]

Different transition-metal complexes are applied as reagents for oxidative destruction of nucleic acids. The cleavage of nucleic acids in the presence of metal complexes is directly associated with their ability to generate reactive oxygen species upon irradiation with light or in the 'dark' reaction of conjugate oxidation of some compounds such as ascorbic acid, thiols, *etc*.[14] To date, oxidation of nucleic acids with reactive oxygen species has been studied in detail. As a result of these studies, several tens of nucleic acid modification products were isolated and characterised; the mechanisms of their formation were established. The oxidative modifications of nucleic acids can involve both heterocyclic bases and the ribose residue.

Scheme 1



At present, a variety of metal complex systems for site-directed oxidative cleavage of nucleic acids has been studied, namely: 1,10-phenanthroline – copper(I),[15,16] *o*-bromobenzoic acid – copper(II),[17] EDTA – iron(II),[18,19] bleomycin – iron(II),[20] porphyrin compounds of

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iron(III),[21,22] manganese(III),[23,24] zinc(II),[25] palladium(II),[26] europium(III)[27] and many others.[28–31]

Complexes of diamagnetic metals [Zn(II), Al(III), Eu(III), Pd(II), Lu(III) *etc.*] cleave or modify DNA under aerobic conditions upon irradiation with light of definite wavelength. In this case, singlet oxygen resulting from photosensitisation is usually an oxidising species. The application of compounds causing modification of nucleic acids under irradiation is limited by the ability of light to penetrate into tissues (the range of maximum transmittance of biological tissue is 750–800 nm[32]). In this respect, significant attention has recently been directed to studies of catalysts operating under 'dark' conditions. It is known that complexes of paramagnetic metals [Fe(III), Co(II), Cu(II), Mn(III) *etc.*] catalyse oxidation of organic compounds with molecular oxygen in the presence of reductants.[33] In the course of these processes (the Fenton–Udenfriend reaction), reactive intermediate species are formed, *viz.*, superoxide and hydroxyl radicals, high-valence metal oxo complexes, which oxidise the target substrate. Complexes of porphyrins and their analogues with paramagnetic metal ions are the most efficient catalysts for the oxidation of organic substrates with molecular oxygen. It is noteworthy that investigations into porphyrins[34] allowed elucidation of the mechanisms of dioxygen activation and the formation of reactive species in detail.

Several reviews are devoted mainly to the consideration of mechanisms of oxidation of 2′-deoxyribose residue[35] or heterocyclic bases within DNA.[36–38] Oxidative degradation of nucleic acids was the topic of our review.[39] The primary attention was paid to the oxidation of nucleic acids with hydroxyl radicals generated by γ-radiolysis and to cleavage of nucleic acids by some antibiotics. Over last 15 years, many new data on oxidative DNA damage have appeared in the literature. In the present review, a wider range of oxidising species is considered, *viz.*, singlet oxygen, superoxide and hydroxyl radicals, high-valence metal oxo complexes, and, unlike other publications dealing with the mechanisms of DNA oxidation, the mechanisms of oxidation of both heterocyclic bases and 2′-deoxyribose residue are considered in detail.

II. Reactive oxygen species

The formation of reactive oxygen species from molecular oxygen has been reviewed[39]. The incomplete reduction products of molecular oxygen are referred to as the reactive oxygen species. Their reactivity is connected, as a rule, with the presence of an unpaired electron[40] (superoxide radical $O_2^{-\bullet}$, hydroxyl radical $HO^{\bullet}$, peroxides of inorganic and organic nature). The reduction of molecular oxygen in the presence of transition metal ions can occur with preliminary coordination of the oxygen molecule or the species that correspond to its incomplete reduction. As a result, high-valence metal oxo complexes are formed (for example, iron or manganese in the oxidation state 4+ or 5+, copper in the oxidation state 3+, *etc.*).[41] These complexes can directly play the role of oxidants or generate hydroxyl radicals with the reduction of the metal to a lower oxidation state. Singlet oxygen $^1O_2$ formed upon photosensitisation[42] is also referred to as reactive oxygen species.

Free hydroxyl radicals formed upon three-electron reduction of molecular oxygen, singlet oxygen and, in some systems, coordinated oxygen species in the form of high-valence metal oxo complexes are responsible for the destruction of nucleic acids under different oxidation conditions.

Superoxide radical cannot oxidise the major biomolecules, in particular, DNA.[43] It becomes an oxidant only following protonation to $HO_2^{\bullet}$ ($pK_a = 4.9$, see Ref. 44). In aqueous solutions at physiological pH values, <1% of protonated superoxide radicals are in equilibrium, which, in contrast to $O_2^{-\bullet}$, can abstract the hydrogen atom from the allylic positions of unsaturated lipids.[45] In spite of weak oxidative properties, $O_2^{-\bullet}$ readily reacts with the guanine radical, which results in the oxidation of the guanine residue in DNA.[46]

Two groups of DNA oxidation products with reactive oxygen species can be distinguished: the oxidation products of the carbohydrate fragment and of heterocyclic bases. Oxidation of carbohydrates occurs upon the action of strong oxidants, such as hydroxyl radicals and some high-valence metal oxo complexes. In most cases, oxidation of 2′-deoxyribose leads to the cleavage of the DNA chain. Oxidation of heterocyclic bases results in a variety of products, which can lead to different modifications of genome. Among heterocyclic bases, guanine is the most easily oxidisable base because it has the lowest oxidation potential.[47,48]

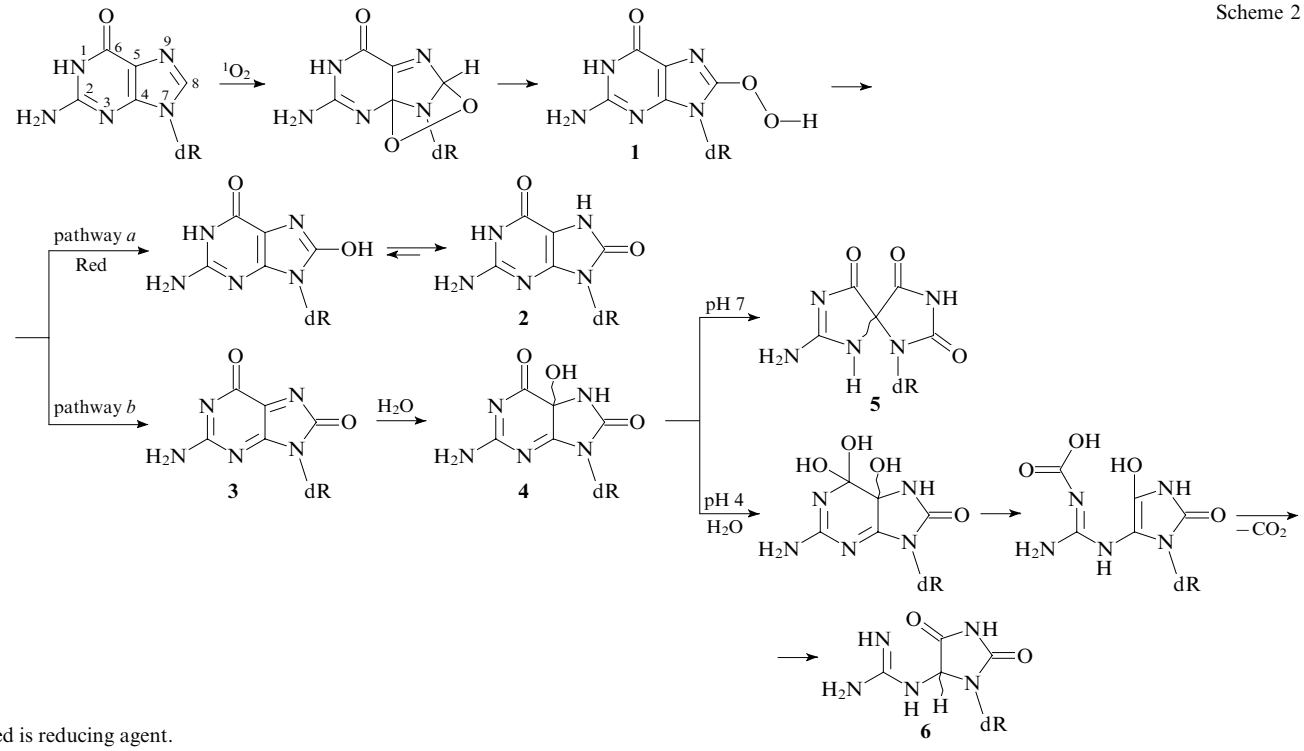
The structures of the oxidative modification products of nucleic acids were determined in experiments on oxidation of model compounds (free heterocycles, nucleosides, nucleotides or short oligonucleotides). Investigations of nucleic acids showed that the data obtained for model compounds are in most cases valid for the oxidation of polymeric molecules. The oxidation of chromatin may give rise to another type of damages, *i.e.*, covalent protein–nucleic acid complexes;[49] however, these damages are not considered in the present review.

III. Oxidation of DNA with singlet oxygen

The reaction of singlet oxygen with DNA results in selective oxidation of guanine residues.[50,51] Singlet oxygen is involved in the [4+2] cycloaddition reaction with the imidazole ring of the guanine residue (Scheme 2). This mechanism was established for the oxidation of 2′,3′,5′-tris(*tert*-butyldimethylsiloxy)guanosine with singlet oxygen.[52] However, further studies on cellular DNA showed that this mechanism is also valid for the oxidation of nucleic acids.[53] For this reason, the deoxyribose residue (dR) in Scheme 2 can represent the nucleic acid chain. Hereinafter, the oxidation products of heterocycle residues are for short termed as dR-derivatives of heterocyclic bases, while the nature of the dR residue is specified in each particular case. Following [4+2] cycloaddition, the endoperoxide formed rearranged to 8-hydroperoxyguanine derivative (**1**),[52] which is as strong oxidant as a peroxy acid and is expected to be reduced into the dR-derivative of 8-hydroxyguanine, the tautomeric form of the dR-derivative of 8-oxo-7,8-dihydroguanine (**2**) (Scheme 2, pathway *a*).[52] This mechanism is confirmed by the fact that treatment of the cellular DNA with singlet oxygen results in accumulation of **2**.[53] In addition, in the reaction with ^{18}O-enriched singlet oxygen [$^1(^{18}O)_2$], the oxygen atom in position 8 of product **2** originates directly from singlet oxygen.[53]

In the absence of a reducing agent, derivative **1** transforms to intermediate **3**, which is the oxidised form of **2** (Scheme 2, pathway *b*). Position 5 of purine in compound **3**

Scheme 2



Red is reducing agent.

is the site of easy reactions with nucleophiles. Under ordinary conditions, the water molecule reacts most often as a nucleophile giving intermediate **4**.[54] This reaction pathway is confirmed by experiments with ^{18}O-labelled water, mass spectrometry and NMR.[54] Compound **4** is a common precursor of dR-derivatives of spiroiminodihydantoin (**5**) and guanidinohydantoin (**6**).[54] The ratio **5**:**6** depends on pH of the medium and the nature of the dR residue (Scheme 2).[54] Thus the formation of the dR-derivative **5** was observed in the oxidation of 2′-deoxyguanosine at pH > 7,[54,55] while **6** was formed under oxidation of 2′-deoxyguanosine at pH < 7.[54] In the oxidation of double-stranded DNA with singlet oxygen, guanidinohydantoin was predominantly formed.[56]

It is noteworthy that 8-oxo-7,8-dihydroguanidine (**2**) readily undergoes second oxidation with singlet oxygen. The oxidation products of 3- and 15-mer oligodeoxyribonucleotides with singlet oxygen were identified based on data from ESI and MALDI-TOF mass spectrometry.[57] In the first step, the [2 + 2] cycloaddition to the C(4)–C(5) bond of the purine ring gives 5-hydroperoxy-8-oxo-7,8-dihydroguanine **7** (Scheme 3; in this scheme, dR is the 2′-deoxyribose residue in the polynucleotide chain). Then the pyrimidine ring opening with cleavage of the C(5)–C(6) bond occurs followed by decarboxylation to yield the dR-derivative of dehydroguanidinohydantoin **8**, which is further transformed to dR-derivatives of parabanic (**9**) and oxaluric (**10**) acids.[57]

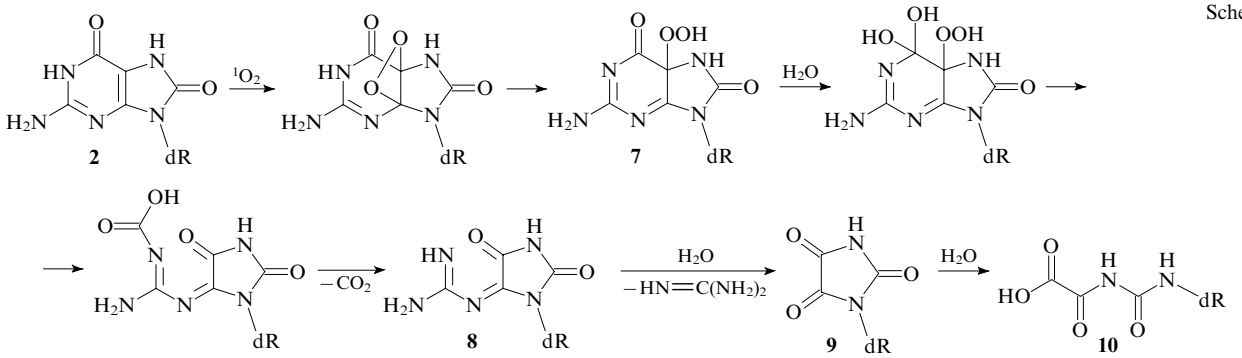
Thus, in the reaction of singlet oxygen with nucleic acids, only guanine residues are oxidised. The major oxidation products are dR-derivatives of 8-oxo-7,8-dihydroguanine, spiroiminodihydantoin, guanidinohydantoin and dehydroguanidinohydantoin.

IV. Oxidation of DNA with superoxide radicals

Superoxide radical is unable to oxidise DNA, however, it oxidises the guanine radical.

The formation of guanine radical was observed in different oxidation systems.[58] In the first step, one electron is abstracted from the guanine residue to form the radical cation of the guanine heterocycle $G^{+\bullet}$. It was found by pulse radiolysis[59] and laser photolysis[60] that the pK_a of the radical cation $G^{+\bullet}$ generated from 2′-deoxyguanosine is 3.9. At neutral pH, deprotonation from N(1) of the guanine heterocycle occurs to form the neutral radical $G^{(-H^+)\bullet}$ (Ref. 59). Unpaired electron is localised on the

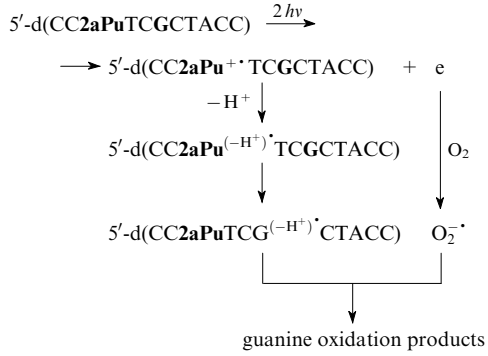
Scheme 3



O(6) or C(5) atoms of guanine.[59] EPR analysis showed[61] that in neutral aqueous solutions at room temperature the lifetime of these radicals in the DNA of calf thymus is ~5 s. Laser pulse photolysis[62] demonstrated that the lifetime of the radical $G^{(-H^+)\bullet}$ in the 16-mer duplex is ~0.3 s. This radical reacts rapidly with other radical species. Thus in single- and double-stranded DNA, it reacts with $O_2^{-\bullet}$ with the rate constant of $\sim 4.7 \times 10^8$ (Ref. 46), the reaction with $NO_2^{\bullet}$ occurs with the rate constant of $\sim 4.3 \times 10^8$ (Ref. 63), while for the reaction with the radical $Me_3C^{\bullet}$, the rate constant is $\sim 10^9$ (mol litre^{-1} s)$^{-1}$ (Ref. 62).

Oxidation of the neutral guanine radical $G^{(-H^+)\bullet}$ with the superoxide radical $O_2^{-\bullet}$ gives imidazolone derivative as the major product.[46] The superoxide radical and $G^{(-H^+)\bullet}$ were generated in the laser flash photolysis (12 ns) of oligonucleotide 5′-d(CC**2aPu**TC**G**CTACC) containing one 2-aminopurine residue (**2aPu**) and one guanine residue (**G**) (Scheme 4). In the first step, the 2-aminopurine $2aPu^{+\bullet}$ radical cation was formed in the oligonucleotide, which was rapidly deprotonated to give the neutral radical $2aPu^{(-H^+)\bullet}$. This radical is a strong one-electron oxidant and can oxidise the guanine residue to the neutral radical $G^{(-H^+)\bullet}$, even if 2aPu and G are separated in the chain by several bases,[64, 65] *e.g.*, by two nucleotides.[46] The hydrated electron formed upon photoionisation of the 2-aminopurine residue is rapidly and quantitatively caught by the O_2 molecule to give a superoxide radical (Scheme 4).

Scheme 4



The addition of superoxide radical to the neutral guanine radical $G^{(-H^+)\bullet}$ followed by protonation of the anion affords the dR-derivative of guanine 5-hydroperoxide (**11**) (Scheme 5).

After addition of two molecules of water, opening of the pyrimidine ring occurs at the C(5)−C(6) bond followed by decarboxylation. The subsequent rearrangement of the product formed, opening of the imidazole ring and elimination of formamide results in the dR-derivative of imidazolone **12**. Imidazolone undergoes hydrolysis ($t_{1/2}$ for compound **12** is ~10 h in neutral aqueous solutions at 20 °C) to give the dR-derivative of oxazolone **13**.[46]

As in the case of the guanine radical, electron abstraction from 8-oxo-7,8-dihydroguanine results in the radical cation of 8-oxo-7,8-dihydroguanine (8-oxo$G^{+\bullet}$).[66, 67] At neutral pH, deprotonation of 8-oxo-7,8-dihydroguanine occurs to form the neutral radical 8-oxo$G^{(-H^+)\bullet}$ [pK_a of this radical is 6.6 (Ref. 67)].

Oxidation of the neutral radical 8-oxo$G^{(-H^+)\bullet}$ was observed upon photooxidation of 2′,3′,5′-tri-*O*-acetyl-8-oxo-7,8-dihydroguanosine in the presence of riboflavine (Rf).[68] Riboflavine in the excited triplet state (Rf*) abstracted one electron from the 8-oxo-7,8-dihydroguanine residue to form the radical cation 8-oxo$G^{+\bullet}$ and radical anion $Rf^{-\bullet}$

$$\text{8-oxoG} + \text{Rf}^* \longrightarrow \text{8-oxoG}^{+\bullet} + \text{Rf}^{-\bullet}. \quad (1)$$

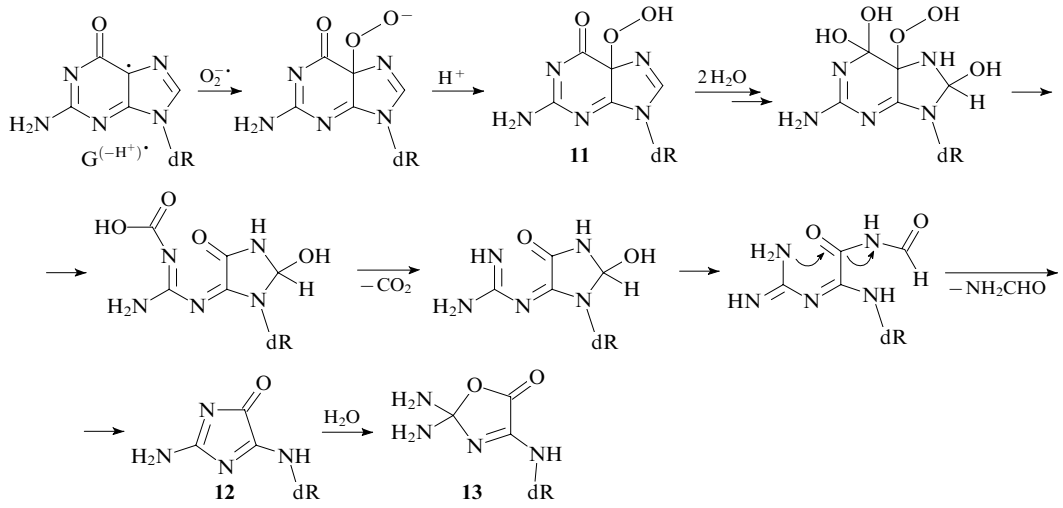
Electron transfer from the riboflavine $Rf^{-\bullet}$ radical anion to molecular oxygen resulted in the generation of the superoxide radical, and riboflavine transformed to the initial ground state.[69]

$$\text{Rf}^{-\bullet} + \text{O}_2 \longrightarrow \text{Rf} + \text{O}_2^{-\bullet}. \quad (2)$$

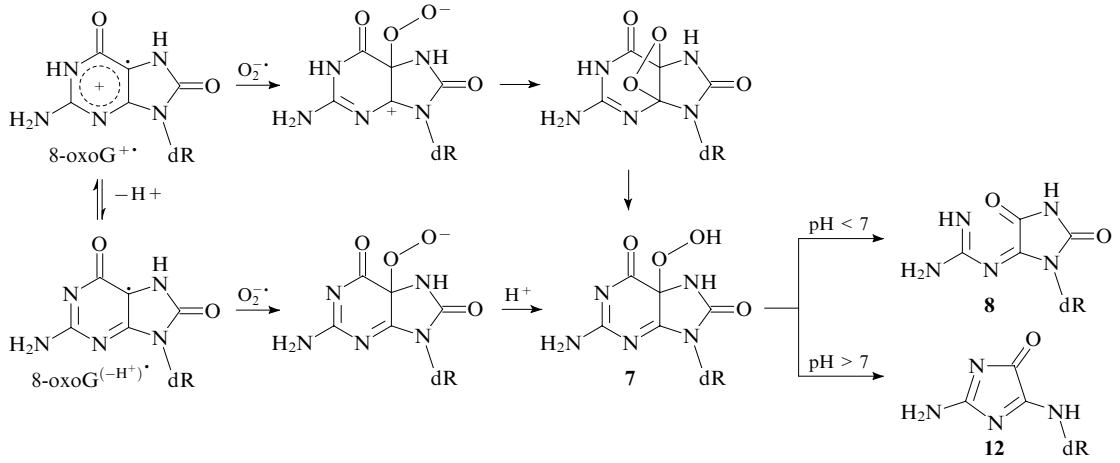
The reaction of $O_2^{-\bullet}$ with the neutral radical 8-oxo-7,8-dihydroguanine (8-oxo$G^{(-H^+)\bullet}$) affords 5-hydroperoxy-8-oxo-7,8-dihydroguanine **7** (Scheme 6, dR in this scheme is the 2′,3′,5′-tri-*O*-acetylribose residue). It was found[68] that at pH ⩽ 7 the major product of the further transformation of intermediate **7** is the dR-derivative of dehydroguanidinohydantoin **8** formed according to the mechanism described above for the reaction of 8-oxo-7,8-dihydroguanine derivatives with singlet oxygen (Scheme 3). At pH > 7, the major oxidation product is the dR-derivative of imidazolone **12**. It was suggested[68] that **12** is formed due to the intramolecular nucleophilic attack of the group O_2^- at the C(5) atom of product **7** on the C(8) atom of the purine ring, however, the detailed mechanism is not described.[68]

Thus, the reaction of the superoxide radical with the radicals $G^{(-H^+)\bullet}$ and 8-oxo$G^{(-H^+)\bullet}$ results in the formation of dR-derivatives of imidazolone and dehydroguanidinohydantoin as the major products.

Scheme 5



Scheme 6



V. Oxidation of DNA with hydroxyl radicals

Hydroxyl radical is a strong oxidant. It is able to abstract the hydrogen atom from the C−H bond or add to C=C bonds. The rate constants of the addition reactions onto the double bonds are usually higher than those of the hydrogen abstraction from the C−H bond.[70]

Radicals HO• react with nucleic acids and model compounds with the reaction rates close to diffision rates. In this case, processes of addition onto the double bond of heterocycles and hydrogen abstraction from C−H bonds of the ribose residue dominate. Radicals HO• react predominantly with heterocyclic bases: the ratio between the heterocycle-derived radicals and ribose radicals achieves 5–10.[70] Usually, the formation of heterocycle-derived radicals is followed by their irreversible modications in the course of further transformations. Some of these modifications weaken the glycoside bond and cause the formation of apyrimidine/apurine sites, which is accompanied by the cleavage of the ribose phosphate backbone in alkaline media.

The structures and properties of heterocycle-derived radicals were studied using free heterocycles, nucleosides and nucleotides as examples.[71–75] The oxidation products of heterocyclic bases with hydroxyl radicals are established in the oxidation of model nucleosides,[76–81] nucleotides[77–79] and short oligonucleotides.[77,78] The majority of modified bases formed in the oxidation of model compounds are also formed in the oxidation of DNA.[78,79,82,83]

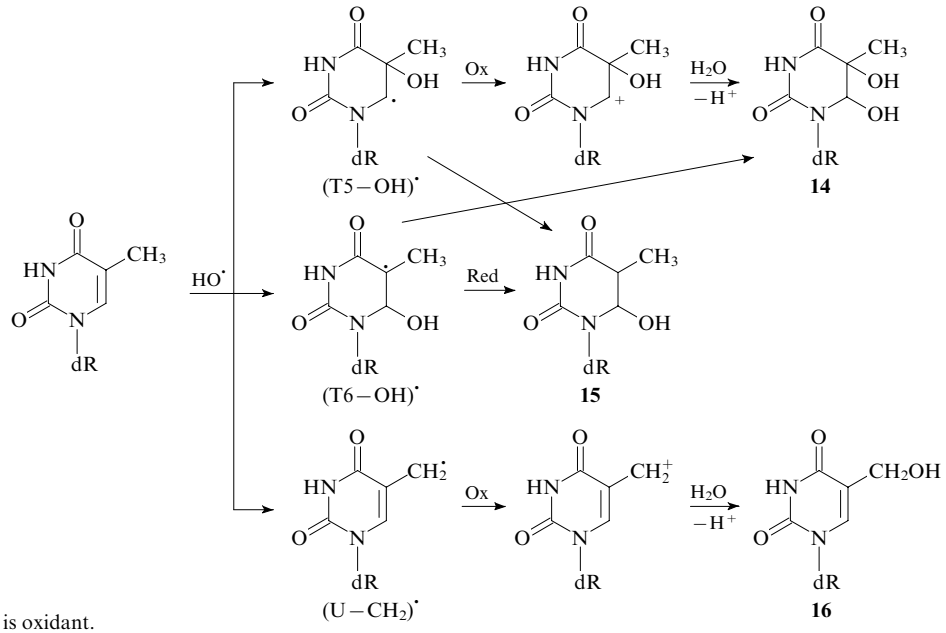
1. Oxidation of heterocycles within nucleic acids

a. Oxidation of the thymine residue

In the reaction of thymine residue with the hydroxyl radicals, the formation of three radicals with different properties was registered.[71] It was found that hydroxyl radicals add to the C(5) or C(6) carbon atoms of the nitrogen base to form the radicals (T5−OH)• and (T6−OH)•, respectively. Hydrogen abstraction from the C(5)-methyl group is also observed to form the exocyclic radical (U−CH$_2$)• (Scheme 7).† Pulse radiolysis studies in the presence of oxidants and reductants have shown that the OH radical adds predominantly to the C(5) atom of the heterocycle (~56%) due to the highest electron density on

† Remind that, if is not specially mentioned, dR in the Schemes denotes the 2′-deoxyribose residue in the polynucleotide chain.

Scheme 7



Ox is oxidant.

this atom, ~35% of the hydroxyl radicals add to the C(6) atom, whereas <9% of the radicals are involved in the hydrogen abstraction from the methyl group.[72]

The three types of radicals formed differ in their redox properties.[72] The radical (T5−OH)$^{\bullet}$ belongs to the α-aminoalkyl type and demonstrates reductive properties. The radical (U−CH_2)$^{\bullet}$ is also a reductant, but weaker than (T5−OH)$^{\bullet}$. On the contrary, the radical (T6−OH)$^{\bullet}$ manifests oxidative properties.

Oxidation of (T5−OH)$^{\bullet}$ results in a carbocation, which adds rapidly the hydroxyl anion to form quantitatively dR-derivatives of *cis*- or *trans*-5,6-dihydroxy-5,6-dihydrothymine (**14**) (Scheme 7). Reduction of the radical (T6−OH)$^{\bullet}$ gives the dR-derivative of 6-hydroxy-5,6-dihydrothymine (**15**), whereas oxidation of the radical (U−CH_2)$^{\bullet}$ gives the dR-derivative of 5-hydroxymethyluracil (**16**).

Since redox properties of thymine radicals differ, in the absence of exogenous oxidants or reductants, the radical (T5−OH)$^{\bullet}$ can be oxidised by the radical (T6−OH)$^{\bullet}$ to carbocation with subsequent formation of **14** and **15**.

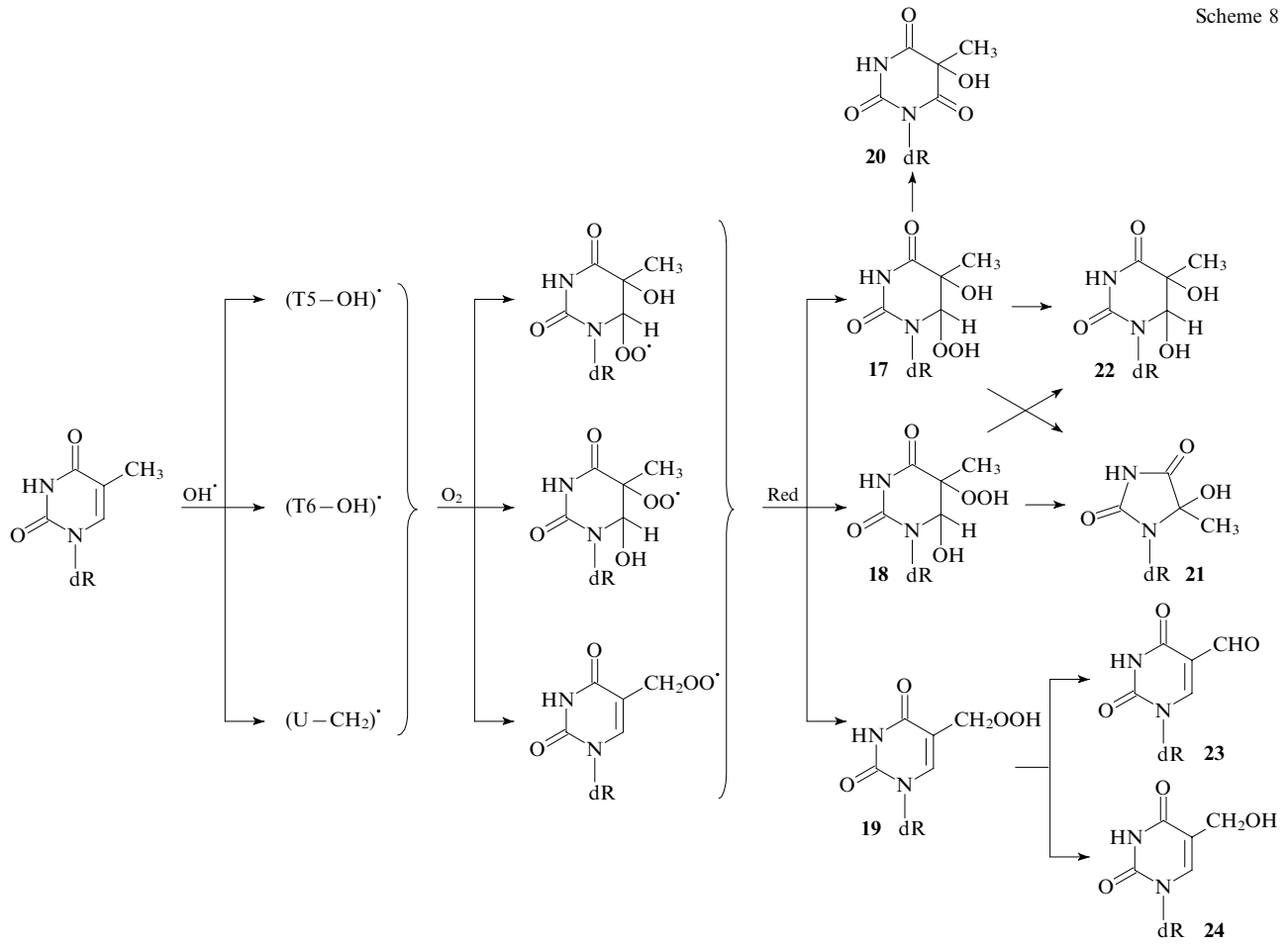
Under aerobic conditions, the additon of molecular oxygen to the radicals (T5−OH)$^{\bullet}$, (T6−OH)$^{\bullet}$ and (U−CH_2)$^{\bullet}$ proceeds with a diffusion limited rate constant. As a result, peroxide radicals are formed, which can be reduced to the corresponding dR-derivatives of hydroperoxides: *trans*- and *cis*-forms of 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine (**17**) and 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine (**18**), as well as 5-hydroxymethyl-2′-deoxyuracil (**19**) (Scheme 8). The lifetimes of hydroperoxides at 37 °C vary from several days to one week.[84]

Hydrolytic cleavage of hydroperoxides **17**–**19** results in stable products (Scheme 8).[85] The major cleavage product of thymine 6-hydroperoxide **17** is the dR-derivative of 5-hydroxy-5-methylbarbituric acid (**20**), whereas *cis*- and *trans*-isomers of thymine 5-hydroperoxide **18** are transformed predominantly to the dR-derivative of 5-hydroxy-5-methylhydantoin (**21**). The formation of *cis*- and *trans*-isomers of 5,6-dihydroxy-5,6-dihydrothymine **22** is explained by competitive dismutation reaction of the 5- and 6-peroxide radicals. As a result, highly reactive alkoxy radicals are formed, which can abstract hydrogen atoms from different compounds, which results in the accumulation of derivative **22**. In addition, the pyrimidine ring opening can occur in alkoxy radicals due to the cleavage of the C(5)−C(6) bond. Further transformations result in either fragmentation to form urea derivatives or cyclisation to give the dR-derivative of 5-hydroxy-5-methylhydantoin **21**.

Decomposition of 5-hydroxymethyl-2′-deoxyuracil **19** results in the accumulation of two products: 5-formyl-2′-deoxyuracil **23** and 5-hydroxymethyl-2′-deoxyuracil **24** (Scheme 8).

Thus, the major oxidation products of the thymine residue upon the action of hydroxyl radicals are dR-derivatives of 5,6-dihydroxy-5,6-dihydrothymine, 5-hydroxymethyluracil, 6-hydroxy-5,6-dihydrothymine, 5-hydroxy-5-methylhydantoin and 5-hydroxy-5-methylbarbituric acid.

Scheme 8



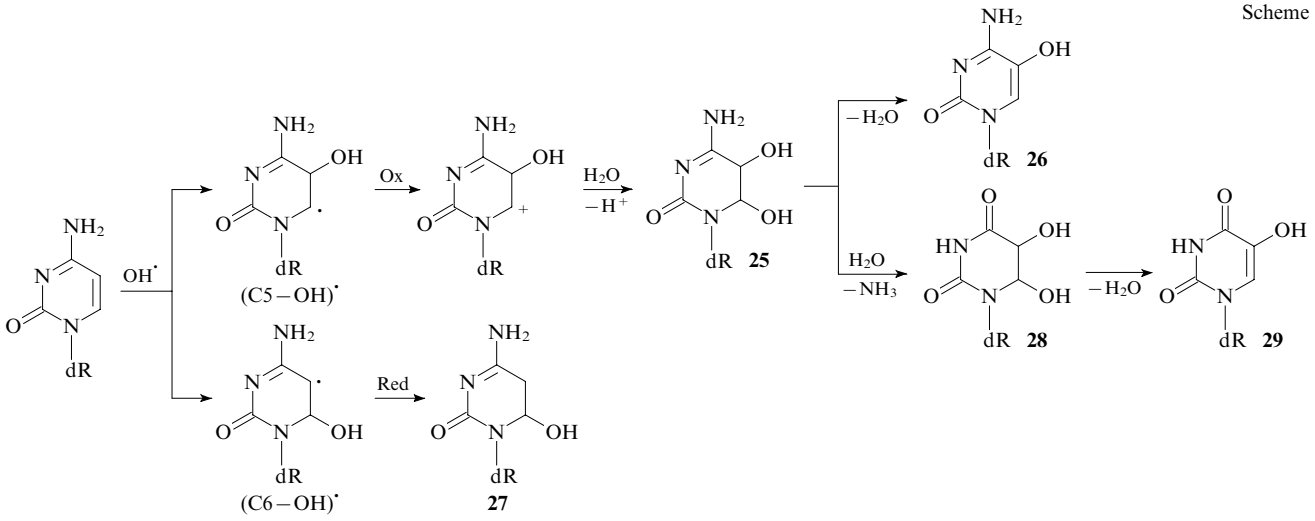
b. Oxidation of the cytosine resudue

Similarly to the reaction with the thymine residue, in the reaction of hydroxyl radicals with the cytosine residue, the addition of the OH radicals (about 87% of reactive species) to the C(5) atom of the heterocycle occurs predominantly. Only 10% of the OH radicals are added to the C(6) atom (Scheme 9).[86] Two types of radicals are formed: (C5−OH)$^{\bullet}$ and (C6−OH)$^{\bullet}$, which are similar in their redox properties to the thymine radicals (T5−OH)$^{\bullet}$ and (T6−OH)$^{\bullet}$. The radical (C5−OH)$^{\bullet}$ exhibits reductive properties, whereas the radical (C6−OH)$^{\bullet}$ is an oxidant.[86]

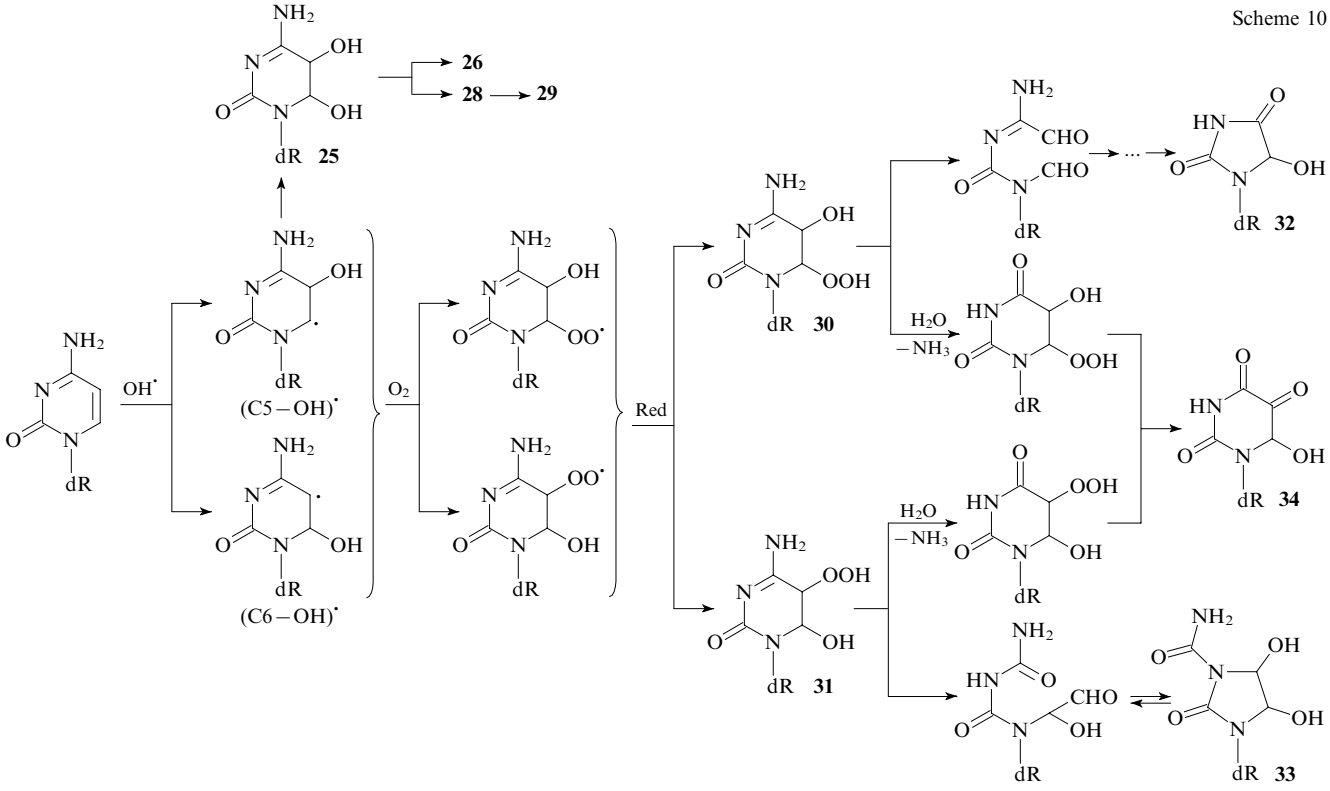
The mechanism of cytosine oxidation with hydroxyl radicals is similar to that of thymine oxidation. Oxidation of (C5−OH)$^{\bullet}$ gives the carbocation, which adds the hydroxyl anion to form the dR-derivative of *cis*- or *trans*-5,6-dihydroxy-5,6-dihydrocytosine (**25**). In turn, **25** can undergo dehydration to form dR-derivatives of 5-hydroxycytosine (**26**) (Scheme 9).[87] Reduction of the radical (C6−OH)$^{\bullet}$ results in the dR-derivative of 6-hydroxy-5,6-dihydrocytosine (**27**). In addition, deamination reaction results in dR-derivatives of uracil: *cis*- or *trans*-5,6-dihydroxy-5,6-dihydrouracil (**28**) and 5-hydroxyuracil (**29**). The mechanism of deamination has not finally been established.

Under aerobic conditions, molecular oxygen adds to the radicals (C5−OH)$^{\bullet}$ and (C6−OH)$^{\bullet}$ with the diffusion limited rate constant. As a result, peroxide radicals are formed that can be reduced to the corresponding dR-derivatives of hydroperoxides: *trans*- and *cis*-forms of 6-hydroperoxy-5-hydroxy-5,6-dihydrocytosine (**30**) and 5-hydroperoxy-6-hydroxy-5,6-dihydrocytosine (**31**) (Scheme 10).[77,80]

Scheme 9



Scheme 10



Decomposition of 5- and 6-hydroperoxycytosine **30** and **31** results in the dR-derivatives of 5-hydroxyhydantoin (**32**) and *trans*-1-carbamoyl-4,5-dihydroxyimidazolidin-2-one (**33**) as the major products (Scheme 10). Derivative **32** is formed due to the intramolecular rearrangement upon the cleavage of the C(5)–C(6) bond, whereas the formation of **33** can be explained by the cleavage of the C(4)–C(5) bond. In one of the first steps of cytosine oxidation, dR-derivatives of 5- and 6-hydroperoxides undergo deamination. The major oxidation product is dR-isodialuric acid (**34**). It is of note that compounds **25**, **26**, **28** and **29** were found among the oxidation products of the cytosine residue under aerobic conditions.[77,80] The formation of these products can be explained by the second attack of the hydroxyl radical on the radical $(C5-OH)^{\bullet}$ to result in the dR-derivative of 5,6-dihydroxy-5,6-dihydrocytosine (**25**), which undergoes dehydration and deamination according to Scheme 9. The products of more profound destruction of the cytosine residue are dR-formamide, biuret, dR-urea, oxalic acid and ureides.[39]

Thus, the major oxidation products of the cytosine residue with the radicals $HO^{\bullet}$ are dR-derivatives of 5,6-dihydroxy-5,6-dihydrocytosine, 5-hydroxycytosine, 6-hydroxy-5,6-dihydrocytosine, 5,6-dihydroxy-5,6-dihydrouracil, 5-hydroxyuracil, 5-hydroxyhydantoin, *trans*-1-carbamoyl-4,5-dihydroxyimidazolidin-2-one and dR-isodialuric acid.

c. Oxidation of the guanine residue

Among heterocyclic bases, guanine undergoes the deepest oxidation by the radicals $HO^{\bullet}$ and by other oxidants because it has the lowest oxidation potential compared with other nucleic acid components [$E^{\circ}_{dG/dG^{\bullet}} = 1.29\ V$, $E^{\circ}_{dA/dA^{\bullet}} = 1.42\ V$, $E^{\circ}_{dC/dC^{\bullet}} = 1.6\ V$, $E^{\circ}_{dT/dT^{\bullet}} = 1.7\ V$ at pH 7 (Refs 47 and 48)]. At present, the guanine oxidation products with the hydroxyl radicals and the mechanisms of their formation have been established.[78,81,85]

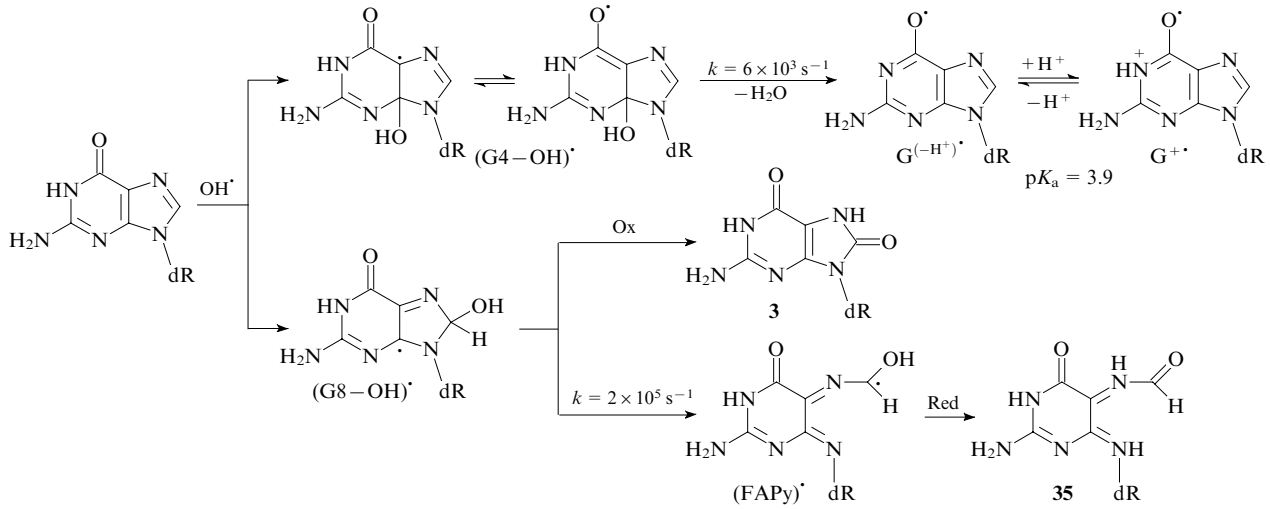
In the reaction with guanine, the hydroxyl radicals add predominantly to the C(4) (60%–75%) and, to a lower extent, to the C(8) atom (~17%) of the purine ring to form the radicals $(G4-OH)^{\bullet}$ and $(G8-OH)^{\bullet}$, respectively (Scheme 11).[74]

The radicals $(G4-OH)^{\bullet}$ and $(G8-OH)^{\bullet}$ formed in the first step have different reactivities toward molecular oxygen, oxidants and reductants.[74] Thus the radical $(G4-OH)^{\bullet}$ exhibits oxidative properties, for example, with respect to *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine and $[Fe^{II}(CN)_6]^{4-}$. Moreover, it undergoes slow dehydration to form the radical cation (or its deprotonated form) showing stronger oxidative properties. The radicals $G^{+\bullet}$ and $G^{(-H^+)\bullet}$ formed do not virtually react with molecular oxygen [$k < 10^6$ (mol litre^{-1} s)$^{-}$], but react rapidly with superoxide and other radicals. Transformations of the radical $G^{(-H^+)\bullet}$ upon the action of $O_2^{-\bullet}$ result in dR-derivatives of imidazolone and dehydroguanidinohydantoin (see Section IV).

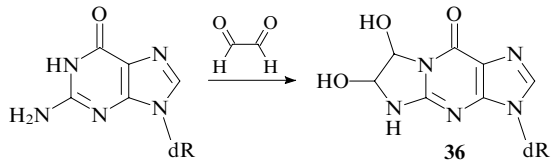
In contrast to the radical $G^{(-H^+)\bullet}$, the radical $(G8-OH)^{\bullet}$ reacts with molecular oxygen with a diffusion limited rate constant [$k = 4\times10^9$ (mol litre^{-1} s)$^{-1}$]; in the absence of molecular oxygen, it undergoes rapid imidazole ring opening ($k = 2-10^5\ s^{-1}$) to form the radical $(FAPy)^{\bullet}$. In addition, the radical $(G8-OH)^{\bullet}$ manifests reductive properties with respect to such an oxidant as $[Fe^{III}(CN)_6]^{3-}$. Transformations of the radical $(G8-OH)^{\bullet}$ result in dR-derivatives of 8-oxo-7,8-dihydroguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**35**) (Scheme 11). Most often, molecular oxygen plays the role of an oxidant in aqueous solutions; its reaction with the radical $(G8-OH)^{\bullet}$ gives the superoxide radical and the dR-derivative of 8-oxo-7,8-dihydroguanine.

The distribution of the guanine residue oxidation products depends on the nature of the substituent dR and on the method of generation of the OH radicals.[78,85] Thus the major γ-irradiation-induced oxidation products of 2′-deoxyguanosine were derivatives of oxazolone **13** and imidazolone **12**. In this case, an insignificant amount (<3%) of product **2** was formed.[85] However, when the hydroxyl radicals were generated by the Fenton reaction in the system Fe^{2+}–EDTA/H_2O_2, the yield of **2** was substantially higher and achieved 30%.[78] In the oxidation of double-stranded DNA, another distribution of the guanine modification products was observed. Upon γ-irradiation, the share of compounds **12** and **13** in the guanine oxidation products was ~30%, while the yield of compound **2** was ~50%. Moreover, **35** is generated in a significant yield (~20%).[88] Upon generation of hydroxyl radicals by the Fenton reaction, the major guanine oxidation products were **2** and **35**.[78]

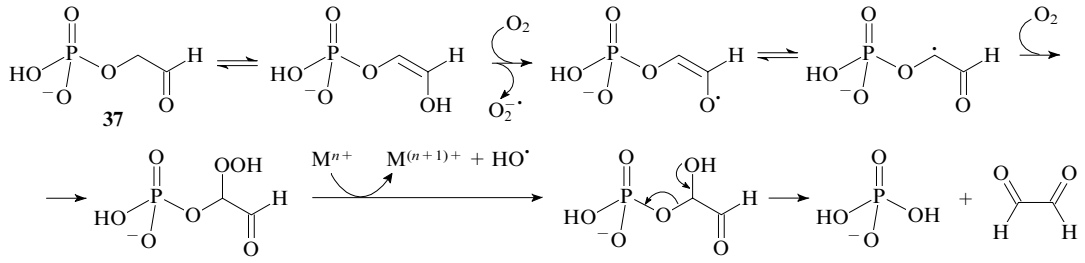
Scheme 11



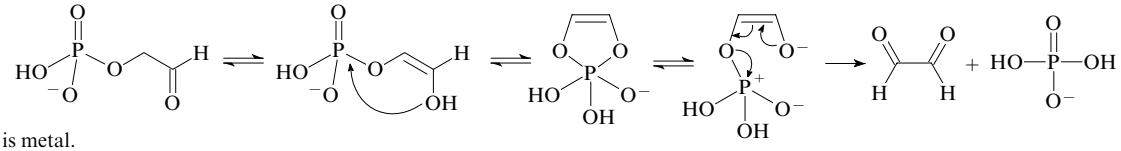
Scheme 12



Mechanism I



Mechanism II

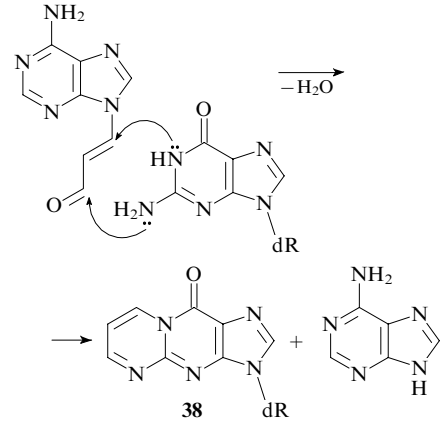


M is metal.

It is worth considering another method for guanine modification. One of the oxidation products of 2′-deoxyribose is 3′-phosphoglycolaldehyde (the mechanism of oxidation of 2′-deoxyribose with hydroxyl radicals is considered below in detail), which can react with the guanine residue to form a 1,*N*(2)- glyoxal adduct of guanine (**36**) (Scheme 12). This type of guanine modification was first established by Murata-Kamiya *et al.*[89] in studies of DNA oxidation with hydroxyl radicals generated by the Fenton reaction in the system Fe^{2+}–EDTA/O_2. It was found that DNA oxidation in this system gives the same guanine modification product as in the reaction of guanine with pure glyoxal. Later it was established that the reaction of 2-phosphoglycolic aldehyde **37** with guanine also gives the glyoxal–guanine adduct.[90]

Awada and Dedon[90] initially assumed that glyoxal formation from 2-phosphoglycolaldehyde **37** would involve an oxygen-dependent, radical-mediated pathway such as that for the glyoxal formation from glycolaldehyde[91] (Scheme 12, mechanism I). This process should lead to the formation of the hydroxyl radical. However, EPR studies revealed that transformations of **37** result in hydroxyl radicals in significantly lower yield than similar transformations of glycolaldehyde.[90] Furthermore, in contrast to glycolaldehyde, radical scavengers such as sorbitol and ethanol did not affect the formation of glyoxal-dG adducts with 2-phosphoglycolaldehyde, and the reaction was found to be independent of molecular oxygen. On the basis of these data, the non-radical mechanism of glyoxal formation from **37** was suggested, which includes the phosphate–phosphonate rearrangement (Scheme 12, mechanism II). The data on the reverse reaction are reported.[92,93] Thus the addition of glyoxal to phosphonic acid diesters gave **37**. Awada and Dedon[90] suggested that their data indicate the reversibility of the reaction of glyoxal with diphosphonates in aqueous solutions.

Another possible product of the modification of the guanine residue in the oxidation of 2′-deoxyribose is pyrimidopurinone **38**.[94]



C(4′)-Oxidation of deoxyribose with hydroxyl radicals results in heterocycle-substituted acrolein (propenal) as one of the products, which exhibits electrophilic properties. It is known that base-substituted propenals in micromolar concentrations are lethal for cells,[95] but the mechanism of their genotoxicity has been largely unexplored. It was found[94] that in the reaction of adenine-substituted propenal with DNA, **38** is selectively formed. Its accumulation was also observed in the treatment of DNA with Fe(II)-bleomycin and calicheamycin, which cause the C(4′)-oxidation of deoxyribose to form base propenals.[96,97]

Thus, the major oxidation products of the guanine residue with hydroxyl radicals are dR-derivatives of imidazolone, oxazolone, 8-oxo-7,8-dihydroguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. In addition, 1,*N*(2)-adducts of glyoxal and propene to guanine can be formed.

d. Oxidation of the adenine residue

In the reaction with adenine and its analogues, the OH radical adds predominantly to the C(4) atom of the C(4)=C(5) double bond of the purine ring (~80%) and to a lesser extent to the C(8) atom of the N(7)=C(8) double bond (~15%).[75] As a result, the radicals (A4−OH)$^{\bullet}$ and (A8−OH)$^{\bullet}$ are formed (Scheme 13).

The radical (A8−OH)$^{\bullet}$ possesses reductive properties. Its oxidation gives the dR-derivative of 8-oxo-7,8-dihydroadenine **39**. In the absence of oxidants, (A8−OH)$^{\bullet}$ undergoes intramolecular opening of the imidazole ring to form radical (FAPyA)$^{\bullet}$, which can be reduced to the dR-derivative of 4,6-diamino-5-formamidopyrimidine **40**. Both H^+ and OH^- activate the ring-opening.[75]

The radical (A4−OH)$^{\bullet}$ is a weak oxidant; however, after hydration, the radical $A^{(-H^+)\bullet}$ is formed showing substantially stronger oxidative properties. Thus it can oxidise the radical (A8−OH)$^{\bullet}$ to form non-oxidised adenine and **39**. Elimination of a water molecule and formation of $A^{(-H^+)\bullet}$ is reduced in acidic media.

It is of note that the formation of the dR-derivative of 2-hydroxyadenine or isoguanine (**41**) in the generation of the hydroxyl radicals in the system Fe^{2+}−EDTA/O_2 (Scheme 13) is reported.[76,79] However, the mechanism of this reaction has not been established.

The distribution of the products of the adenosine residue oxidation depends of the method of generation of OH radicals. In the system Fe^{2+}−EDTA/O_2, no formation of the dR-derivative **39** was observed,[76] while upon γ-irradiation, **39** and **40** are the major products of adenine oxidation.[98] The formation of **39** was observed upon treatment of 2′-deoxyadenosine or DNA with peroxynitrous acid O=N−O−OH, which decomposes to form the hydroxyl radical.[99]

Thus, oxidation of adenine with hydroxyl radicals gives dR-derivatives of 4,6-diamino-5-formamidopyrimidine, 8-oxo-7,8-dihydroadenine and, in some cases, 2-hydroxyadenine as the major products.

2. Oxidation of the carbohydrate fragments within nucleic acids

In the reaction with the ribose−phosphate backbone of DNA, hydroxyl radicals abstract the hydrogen atom from different positions of 2′-deoxyribose.[35,39] As a result, C(1′)-, C(2′)-, C(3′)-, C(4′)- and C(5′)-radicals are formed. Under anaerobic conditions, oxidation of these radicals and addition of a water molecule yield the corresponding hydroxy-derivatives of 2′-deoxyribose (Scheme 14).

Under anaerobic conditions, 1′-hydroxy-derivative is unstable and releases the heterocycle (BH) rapidly. As a result, a lactone derivative **42** is formed, which remains incorporated in the sugar−phosphate backbone of DNA. This damage can result in degradation of DNA on heating or in an acidic medium.

The final 2′ oxidation product under both anaerobic and aerobic conditions is the four-carbon fragment of deoxyribose (erythrose) **43**; however, the mechanism of its formation was suggested only for aerobic conditions.[35] In alkaline media, the retro-aldol reaction proceeds to form 3′- and 5′-phosphoglycolaldehydes **44**.

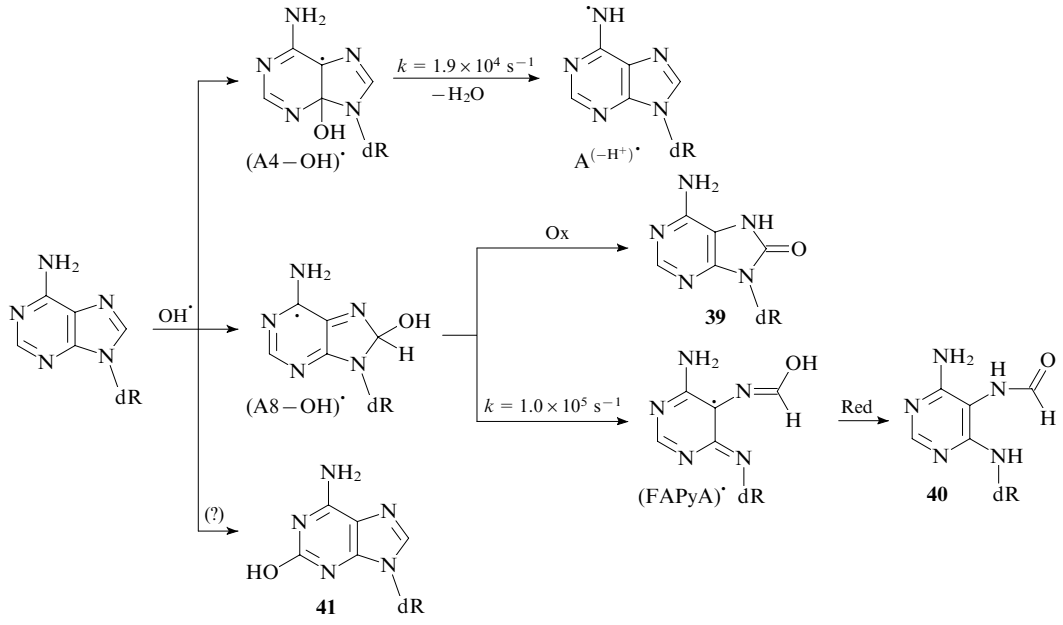
3′-Oxidation results in the corresponding 3′-hydroxy-derivative, which is unstable and undergoes several β-elimination processes to release 3′- and 5′-phosphate 'ends' and a free heterocycle. The oxidised deoxyribose residue is released as 2-methylenefuran-3(2*H*)-one (**45**).

After hydrogen abstraction from the C(4′) atom and formation of the corresponding 4′-hydroxy-derivative, the heterocyclic base is released. In this case, the 4′ oxidised deoxyribose residue **46** remains incorporated in the DNA chain. Under alkaline conditions, elimination of the 3′-terminal fragment occurs. The sugar oxidation product, *viz*., 3-hydroxy-5-oxocyclopent-1-ene (**47**), remains bound to the other fragment of the chain.

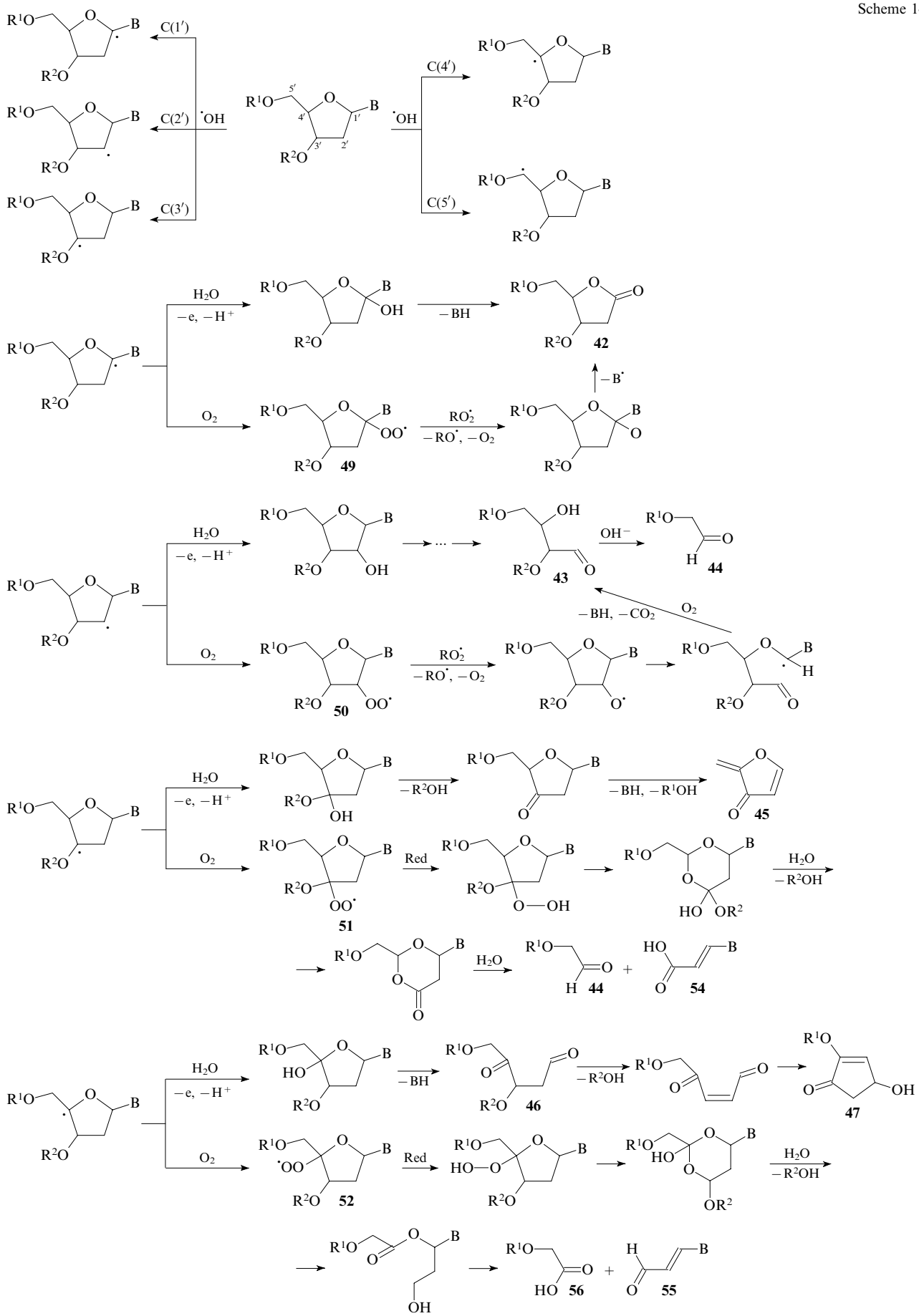
Oxidation of the 5′ carbon atom under anaerobic conditions yields 5′-aldehydonucleoside (**48**).

In the presence of molecular oxygen, O_2 adds to the pentose radical to form the corresponding peroxide radicals **49**–**53** (Scheme 14).

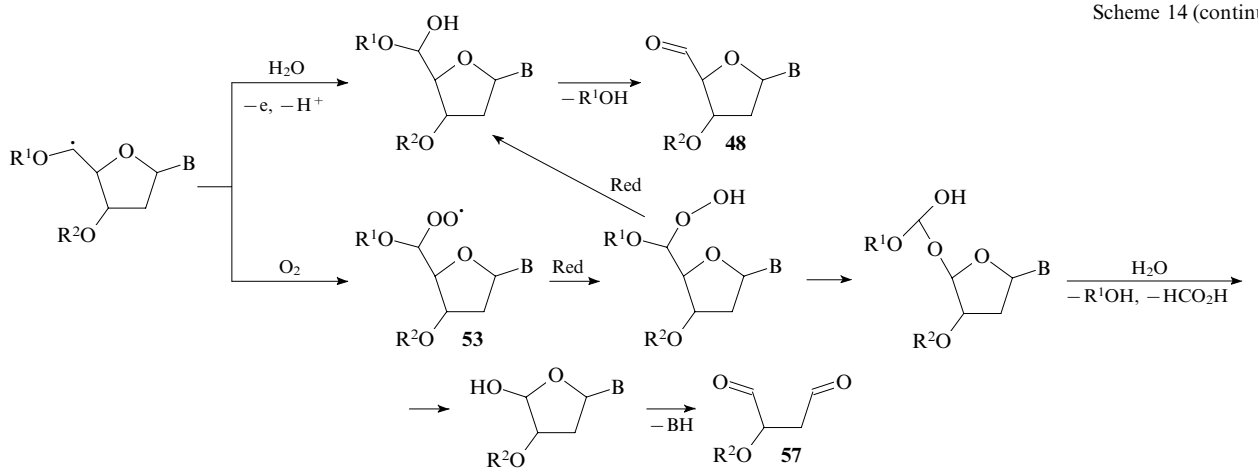
Scheme 13



Scheme 14



Scheme 14 (continued)



Reduction of radical **49** results in alkyl hydroperoxide, which transforms further (probably, according to the Russell mechanism) ‡ to give the alkoxy radical. Releasing of the heterocycle is accompanied by the formation of the lactone derivative **42** incorporated in DNA.

Upon oxidation of the C(2′) atom of deoxyribose, the peroxide radical **50** decomposes according to the Russell mechanism to form the alkoxy radical. The latter undergoes fragmentation with the cleavage of the C(1′)–C(2′) bond, which is followed by second addition of molecular oxygen and elimination of the heterocycle and a CO_2 molecule. The formation of the erythrose derivative **43** at the end is not accompanied by scission of the nucleic chain under physiological conditions.

Reduction of radical **51** results in a hydroperoxide, which undergoes the Criegee intramolecular rearrangement leading to the scission of the DNA chain to form 5′-phosphate and 3′-phosphoglycolaldehyde 'termini' **44**. Heterocycles released contain the acrylic acid residue (**54**).

Upon oxidation at the 4′ carbon atom of deoxyribose, peroxide radical **52** accepts an electron from the reductant and transforms into an unstable hydroperoxide. The latter is supposed to undergo the Criegee rearrangement. In this rearrangement, the C(3′) atom migrates from the C(4′) atom to the peroxide oxygen, which is accompanied by the heterolytic cleavage of the O–O bond. The 2′ carbon atom activated by the emergence of the carbonyl group at the C(4′) atom is deprotonated, which induces the elimination reaction of phosphate from the C(3′) atom together with the formation of heterocycle-substituted propenal **55** and phosphoglycolic acid **56** at the 5′-end upon scission of the DNA chain.

The deoxyribose C(5′)-oxidation products are 5′-aldehydonucleoside **48** and tetrodialdose **57** in the free state or DNA-bound. Tetrodialdose is formed due to the Criegee intramolecular rearrangement, elimination of the 5′-terminal fragment containing the 3′-formyl phosphate group, which is spontaneously hydrolysed to form the free 5′-phosphate end. Reduction of the initial hydroperoxide results in the 5′-hydroxy-derivative. Elimination of the phosphoester fragment from position 5′ affords a 5′-aldehydonucleoside.

It is of note that in the reaction with double-stranded DNA, the hydroxyl radicals abstract hydrogen atom from the C(1′–5′) atoms of deoxyribose with different efficiencies. This is explained by different accessibilities of different hydrogen atoms of deoxyribose in the DNA double-helix for the reactions with solvent and, hence, with hydroxyl radicals.

Thus it was found [100] that hydroxyl radicals react with double-stranded DNA with abstraction of the hydrogen atom from the C atoms of 2′-deoxyribose in the following order: 5′-H > 4′-H > 3′-H ≈ 2′-H ≈ 1′-H. The system Fe^{2+}–EDTA/H_2O_2/ascorbic acid was selected as a source of OH radicals because it is most often used in footprinting of DNA-binding proteins.[101] Synthetic DNA duplexes where one of nucleotides was specifically deuterium-labelled was used as substrates.[100] For example, all the thymidine residues in the DNA duplex contained deuterium in position 4′ of 2′-deoxyribose, whereas all the other positions of this and the other nucleotides contained the hydrogen atoms. The extent of cleavage at the deuterated nucleotides was compared with cleavage at surrounding nondeuterated nucleotides and with a control DNA molecule containing no deuterium substitution. The kinetic isotope effect ($k_H/k_D > 1$) was taken into consideration. In addition, using X-ray data for the B-form of DNA, the relative solvent accessibility of different protons of 2′-deoxyribose was calculated (Table 1).

It is seen from the data presented that the reactivities of various ribose hydrogen atoms toward the hydroxyl radicals correlate with their accessibility to solvent. The OH radicals can attack more easily the hydrogen atoms at the C(5′) and C(4′) atoms because they are the most solvent-accessible.

Table 1. Relative efficiencies of hydrogen abstraction by hydroxyl radicals from the 2′-deoxyribose residue in double-stranded DNA and solvent accessibilities of hydrogen atom.[100]

Relative parameter (%)	Position of the carbon atom				
	5′ + 5″	4′	3′	2′ + 2″	1′
Efficiency of the reaction with OH radicals	57 ± 10	22 ± 6	17 ± 5	13 ± 3	11 ± 4
Solvent accessibility	46	28	14	11	1

Note. The calculation was perfomed on the basis of X-ray data for the B-form of DNA; the second C(2) and C(5) hydrogen atoms are double primed.

‡ About this mechanism in more detail see Ref. 39.

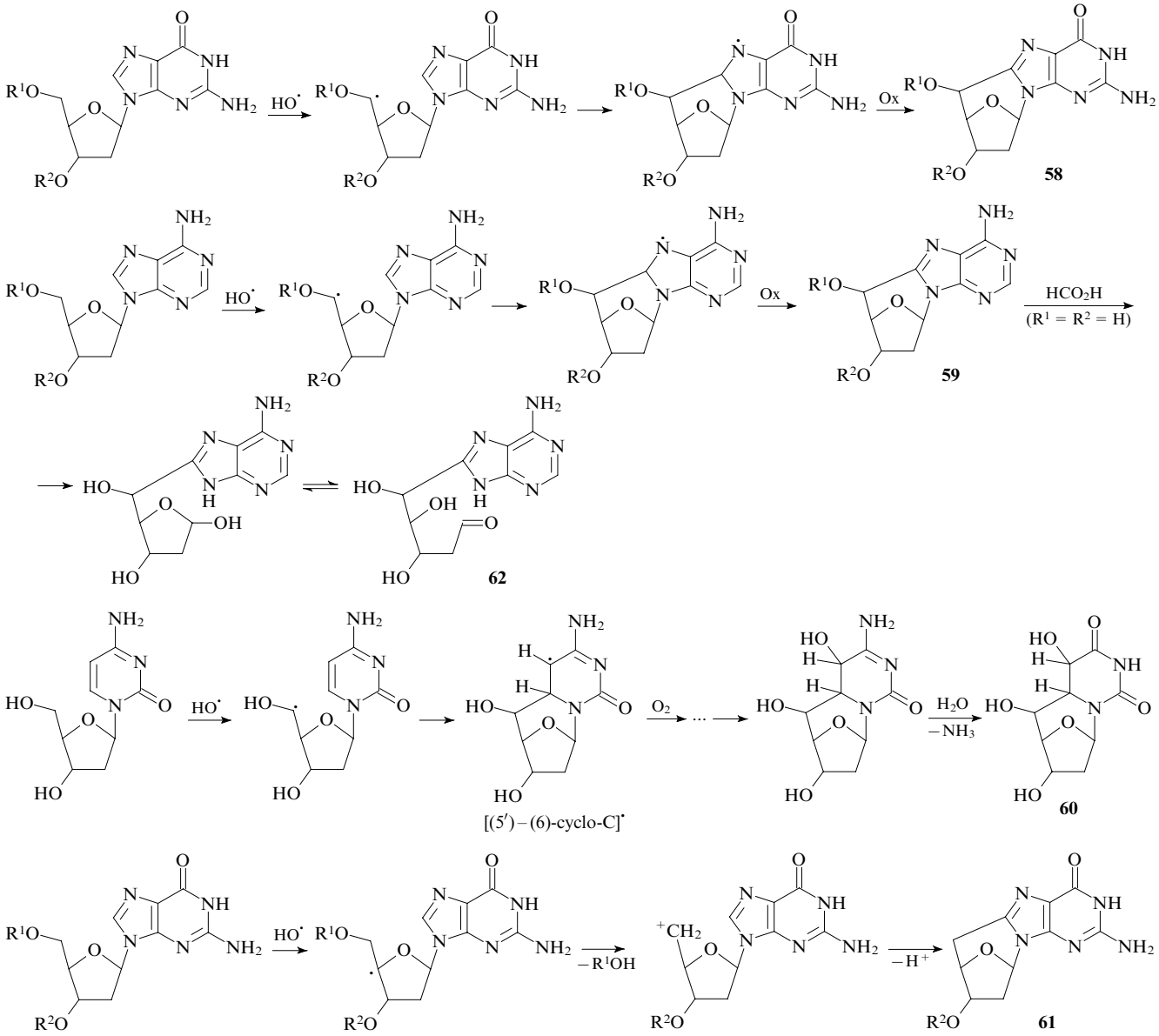
Of special interest are damages appearing upon oxidation of both the carbohydrate fragment and the heterocyclic base. Thus the C(5′) radical of deoxyribose can react with the C(8) atom of the purine base to form C(5′)—C(8)-cyclo-2′-deoxyribopurines **58** and **59** (Scheme 15). The formation of cyclopurine derivatives was observed upon γ-irradiation [102, 103] and upon generation of hydroxyl radicals by the Fenton reaction.[76, 78] The formation of cyclopyrimidine derivatives is also possible. For example, oxidation of 2′-deoxycytidine resulted in C(5′)—C(6)-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine **60**.[80] As in the case of cyclopurine derivatives, the C(5′) radical of deoxyribose attacks in the first step the C(6) atom of the pyrimidine base to form the radical (5′–6-cyclo-C)•. Addition of molecular oxygen to the C(5) atom of the pyrimidine ring results in a peroxide radical, which transforms further to compound **60** (Scheme 15).

The formation of another cyclic derivative of guanine, *viz*., C(5′)—C(8)-cyclo-2′,5′-dideoxyguanine **61**,[78] deserves mentioning (Scheme 15). This compound is formed upon abstraction of the hydrogen atom from the C(4′) atom of deoxyribose with subsequent elimination of the 5′-phosphate group.

By the example of C(5′)—C(8)-cyclo-2′-deoxyadenosine, it was found that the formation of the C(5′)—C(8) bond increases significantly the stability of the C(1′)—N(9)-glycosidic bond to acid hydrolysis (Scheme 15).[104] Thus it was noted that the rate of hydrolysis of the glycosidic bond is ~40 times lower as compared to that for 2′-deoxyadenosine. In this case, the formation of 5-(6-amino-9*H*-purin-8-yl)-2-deoxypentose **62** was observed.

Considering all the above-mentioned, a conclusion can be drawn that in the reaction of hydroxyl radicals with the ribose–phosphate backbone of DNA the abstraction of hydrogen atoms from different positions of 2′-deoxyribose occurs. In most cases the different rearrangements of the radicals formed lead to the cleavage of nucleic acid chain. Under aerobic conditions, the major oxidation products of the carbohydrate fragment are lactone and erythrose derivatives, phosphoglycolaldehyde, phosphoglycolate, base-substituted propenal and acrylate, tetrodialdose and

Scheme 15



5′-aldehydonucleosides. Cyclic derivatives can also be formed, *viz*., C(5′)–C(8)-cyclo-2′-deoxyribopurines and C(5′)–C(8)-cyclo-2′-deoxyribopyrimidines as well as C(5′)–C(8)-cyclo-2′,5′-dideoxyguanine. For the reaction of hydroxyl radicals with double-stranded DNA, the rate of hydrogen abstraction from 2′-deoxyribose decreases in the series 5′-H > 4′-H > 3′-H ≈ 2′-H ≈ 1′-H and depends directly on their solvent accessibility.

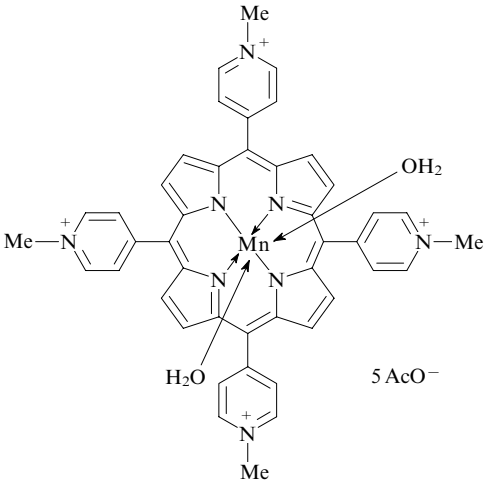
VI. Oxidation of DNA with high-valence metal oxo complexes

As was mentioned above, reduction of oxygen with transition-metal ions may be preceded by coordination of O_2 molecules or species corresponding to their partial reduction. As a result, high-valence metal oxo complexes are formed, which can oxidise selectively various organic substrates. Metal oxo complexes play very important role in enzymatic oxidation. Alkane monooxygenases such as cytochrome P450 and methane monooxygenase efficiently catalyse alkane hydroxylation under mild conditions and with high selectivity. It is even more important that this kind of enzymes perform single-step regio- and stereoselective incorporation of an oxygen atom from the oxidant into non-activated C–H bonds of different compounds. Until now, these natural enzymatic systems are the best catalysts in respect of both the rate and selectivity of oxidation.

At present, studies of different catalysts based on metal oxo complexes are in progress.[105] Oxidation of DNA with complexes porphyrin–manganese(III), bleomycin–iron(II) and 1,10-phenanthroline–copper(I) has been studied in most detail.

1. Oxidation of DNA with manganese(III) meso-tetrakis(*N*-methylpyridin-4-yl)pophyrin

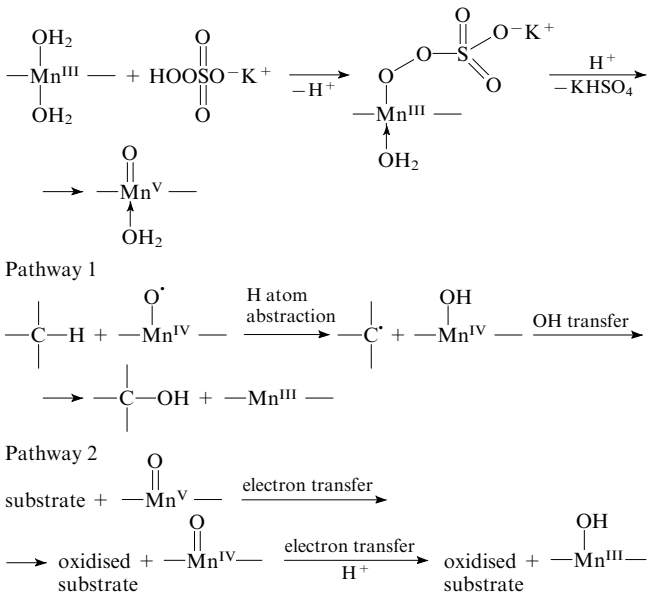
Meunier *et al*.[23,24,106–118] used manganese(III) *meso*-tetrakis(*N*-methylpyridin-4-yl)pophyrin dihydrate (Mn-TMPyP) as an oxidant.



Mn-TMPyP was activated with $KHSO_5$ into the high-valence $Mn^V{=}O$ complex (Scheme 16).

Oxidation of substrates with this form of manganese can proceed according to two pathways (Scheme 16), *viz*., oxygen insertion into the C–H bond (deoxyribose residue in the case of DNA) and electron transfer from substrates (the guanine residue in DNA).[107]

Scheme 16



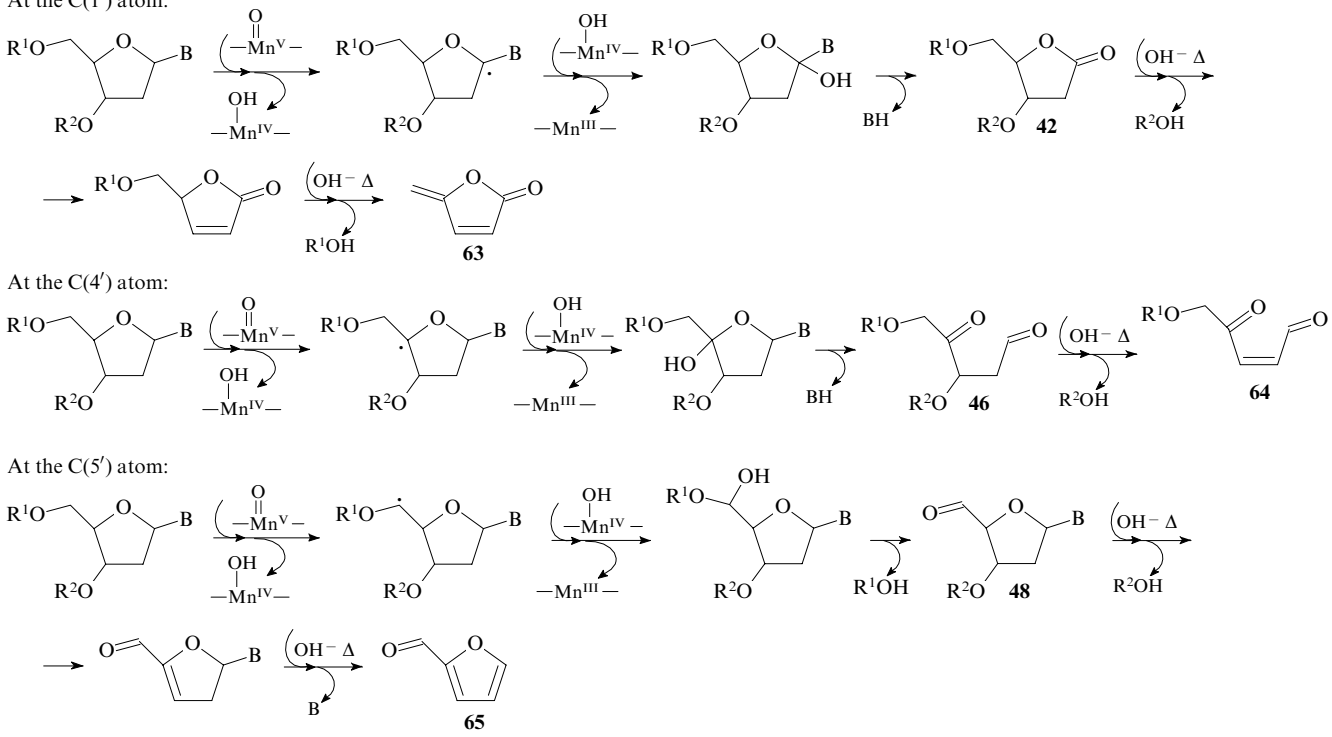
Oxidation of DNA with the oxo complex $Mn^V{=}O$ depends on the nucleotide sequence of DNA and is determined by the binding mode of DNA with porphyrin. Three oxidative degradation mechanisms are possible[107]:

1) deoxyribose C(5′)-hydroxylation;
2) deoxyribose C(1′) and C(4′)-hydroxylation;
3) guanine oxidation.

Deoxyribose C(5′)-hydroxylation occurs in A/T-rich regions of DNA, when porphyrin is located in the minor groove of DNA. It was shown that Mn-TMPyP has a high affinity to the trinucleotide sequence containing only A·T pairs, *i.e*., an $(A\cdot T)_3$ sequence.[108,109] The binding of the manganese porphyrin in the minor groove of an $(A\cdot T)_3$ site puts the $Mn^V{=}O$ centre in the right position to perform hydroxylation at the C(5′) carbon atom of the 3′-terminal deoxyribose unit of the $(A\cdot T)_3$ sequence. Thus, this reaction is extremely efficient due to the high affinity of the manganese porphyrin for that site and to the perfect match between the $Mn^V{=}O$ position and the target C–H bond.[110–112]

In the G/C-rich regions of DNA, binding of Mn-TMPyP to the minor groove becomes impossible, because amino groups of heterocycles located in the minor groove prevent packing of the porphyrin molecules. The presence of the oxo group in the axial position of the porphyrin macrocycle also hinders its intercalation between base pairs of in DNA. Therefore, in the G/C-rich regions of DNA, C(1′)-hydroxylation of deoxyribose was observed.[113] The possibility for the C(1′)-oxidation in double-stranded DNA has not been elucidated finally because the C(1′) atom is located deep into the minor groove and its spatial approach to the $Mn^V{=}O$ group is limited. It is of note that significant C(1′)-hydroxylation of the terminal nucleotide was observed in the oxidation of short DNA duplexes.[107] In addition, in this case, C(4′)-hydroxylation of the terminal nucleotide was also observed. Such an 'end effect' can be due to partial stacking of the terminal base pair of a short duplex with the porphyrin macrocycle, *i.e*., result from spatial approach of the oxidising group to the substrate.[107]

Scheme 17



The mechanisms of C(1′)-, C(4′)- and C(5′)-hydroxylation of deoxyribose are presented in Scheme 17. The C(1′)-oxidation followed by treatment with OH^- resulted in 5-methylenefuran-2(5*H*)-one (**63**), the C(4′)-oxidation afforded compound **64** and the C(5′)-oxidation gave furfural **65**.

The third type of DNA oxidation with a high-valence manganese complex includes the electron abstraction from the guanine residue.[23, 24, 114] In order to elucidate the mechanism of guanine oxidation, Meunier *et al.* used substrates with gradual complication of the structures, *viz.*, deoxyguanosine,[115] dinucleoside monophosphate[116, 117] and short double-stranded oligodeoxyribonucleotides.[118] The data from mass spectrometry, NMR spectroscopy and labelling experiments with $H_2{}^{18}O$ allowed suggesting the following mechanism of guanine oxidation with the manganese oxo complex.

In the first step, abstraction of two electrons and a proton from guanine occurs to give the guanine cation $\left(G^{(-H^+)}\right)^+$. The positive charge is located on the C(5) atom of the heterocycle. The guanine cation formed undergoes the nucleophilic attack by either a water molecule or a peroxosulfate molecule. This results in two intermediates, *viz.*, 5-hydroxyguanine (G5−OH) and guanine 5-peroxysulfate. The second attack by water on the C(6) and C(8) atoms of the heterocycle results in the pyrimidine ring opening at the C(5)−C(6) bond and subsequent decarboxylation. The second oxidation with the complex $Mn^V{=}O$ gives the dR-derivatives of guanidinohydantoin **6** and dehydroguanidinohydantoin **8**. Hydrolysis of guanidinohydantoin leads to the dR-derivative of imidazolone **12**. The next oxidation of the dR-derivative of dehydroguanidinohydantoin **8** with the complex $Mn^V{=}O$ results in the dR-derivative of parabanic acid **9** (Scheme 18).

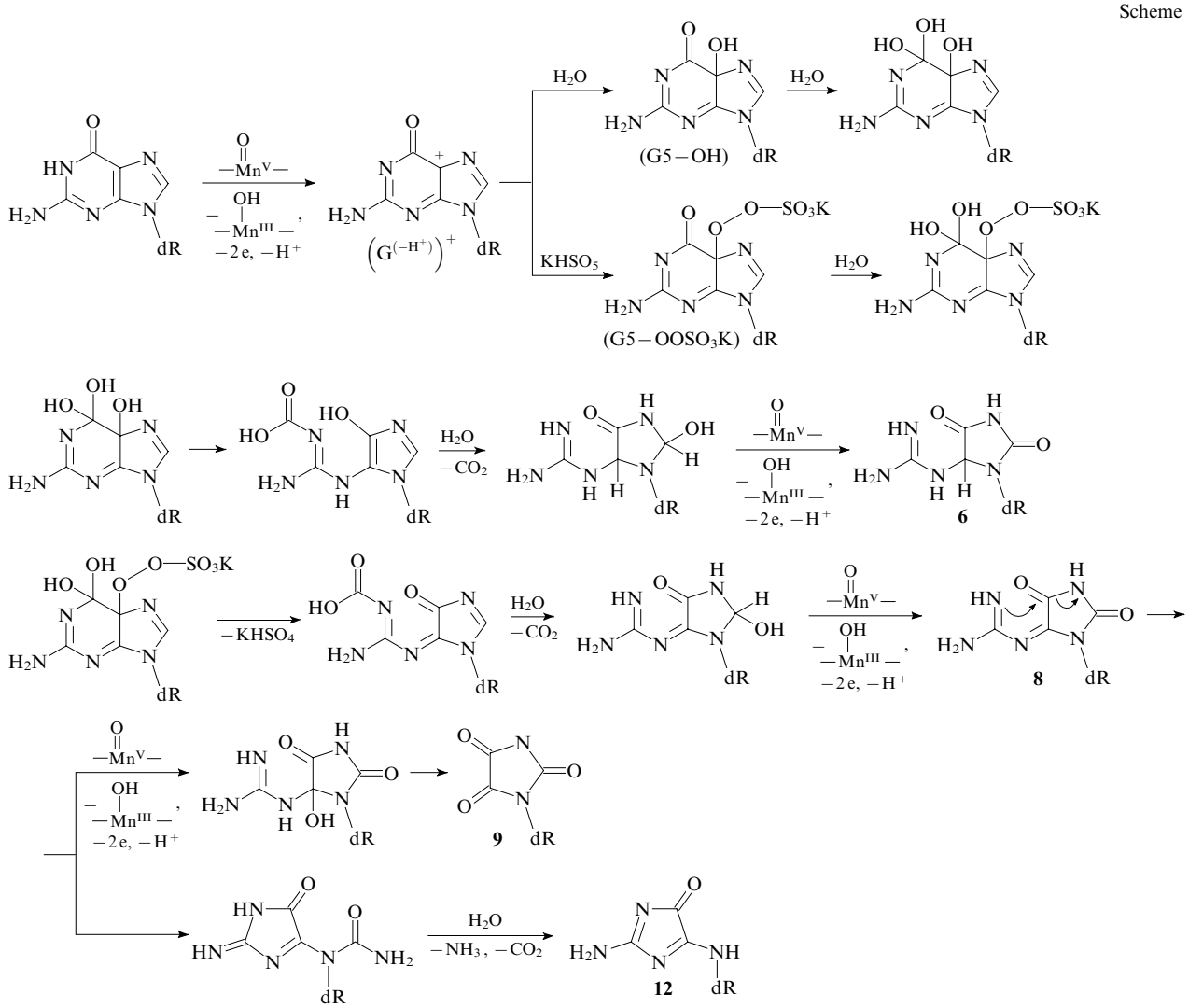
It is noteworthy that oxidation of the terminal guanine in a short DNA duplex affords dR-derivative of spiroiminodihydantoin **6** (Scheme 19).[107] This damage of guanine was not detected in the oxidation of intrastrand guanine residues. As in the case of the C(1′)- and C(4′)-hydroxylation of deoxyribose, similar 'end effect' is explained by spatial approach of oxidisable and oxidising groups; for this reason, guanine oxidation occurs by another pathway.

The quantitative contribution of each of the three possible pathways to the oxidation with the $Mn^V{=}O$ complex was estimated using short oligonucleotide duplexes with different sequences.[107] It was found that the C(5′) hydroxylation at three contiguous A or T bases was 1000 times more efficient than guanine oxidation by electron transfer at an isolated guanine residue. The oxidation of an intrastrand GG sequence appeared to be 10 times more reactive than at a single guanine residue but still 100 times less reactive than at the $(A\cdot T)_3$ site. However, when a guanine residue was located at the terminal base pair of the short duplex, its reactivity reached that of the $(A\cdot T)_3$ site.

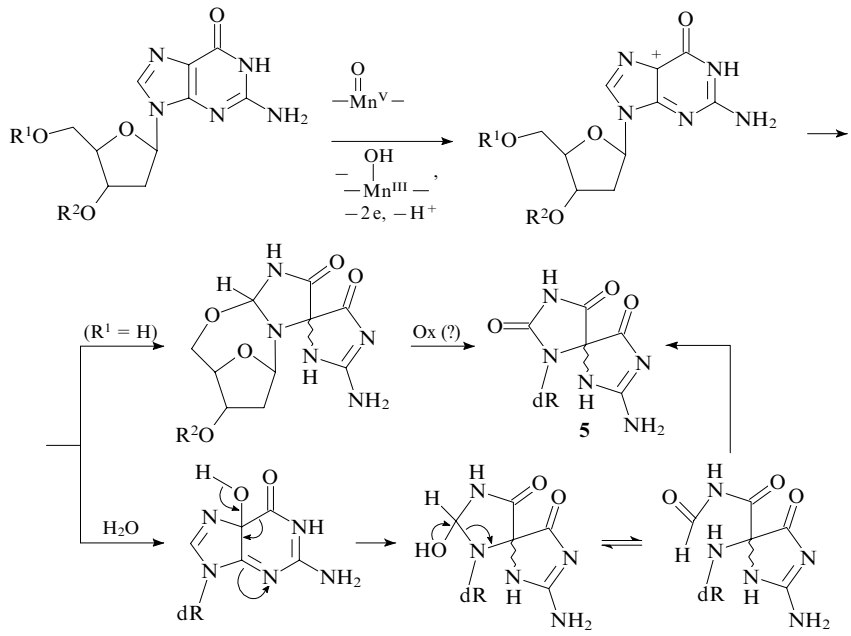
The necessary condition for the incorporation of the oxygen atom in the C−H bond is close spatial arrangement of the oxidisable bond and the metal oxo complex. This tight interaction can occur upon binding of porphyrin to the $(A\cdot T)_3$-site resulting in efficient C(5′)-hydroxylation of deoxyribose. The guanine oxidation by electron transfer is also dependent on the distance between the metal-oxo entity and the nucleobase and is most efficient in the case of their close contact.[107]

Thus, the electron abstraction from the guanine residue or deoxyribose hydroxylation are not competitive processes in the DNA oxidation with manganese oxo complex. The guanine oxidation appeared to be possible only when guanine was accessible to the active $Mn^V{=}O$ species, for example, in the case of oxidation of guanine located at the end of duplex. The major oxidation products of the sugar fragment are 5′-aldehydonucleosides and derivatives of lactone and 4′-oxo-2′-deoxyribose; in the guanine oxidation, these are dR-derivatives of guanidinohydantoin, dehydroguanidinohydantoin and imidazolone.

Scheme 18

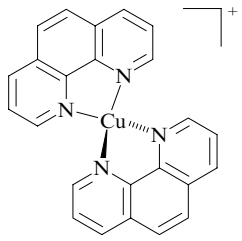


Scheme 19



2. Oxidation of DNA with copper(I) bis(1,10-phenanthroline) complex

Selective C(1′)-oxidation of deoxyribose is typical of complex Cu – (1,10-phenanthroline) [$Cu(Phen)_2^+$].

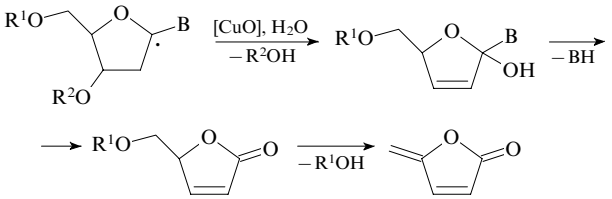


$Cu(Phen)_2^+$

The nuclease activity of complex $Cu(Phen)_2^+$ was first discovered by Sigman *et al.*[119] This complex is bound to the minor groove[120] and brings about cleavage of double-stranded DNA in the presence of hydrogen peroxide.[121]

In this case, trivalent copper oxo complexes $[CuO]^+$, $[CuOH]^{2+}$ and CuO_2H are, most likely, the oxidising species.[122, 123] The suggested mechanism of DNA scission under the action of the copper phenanthroline complex including deoxyribose C(1′)-hydroxylation[124] is depicted in Scheme 20.

Scheme 20



63

The final oxidation products are the free base, 3′- and 5′-phosphate ends and 5-methylenefuran-2(5*H*)-one **63**.[120, 125, 126] The labelling experiments with $H_2{}^{18}O$ revealed that the oxygen atom of the carbonyl group in 5-methylenefuran-2(5*H*)-one is derived from water.[124] Taking this fact into consideration, the conclusion was drawn that in one of the first steps, the deoxyribose C(1′) radical is oxidised to the corresponding carbocation. The nucleophilic attack by the water molecule results in the 1′-hydroxy-derivative; it is further transformations of this species that result in the DNA chain scission (Scheme 20). The main feature of the oxidation process is that scission occurs at room temperature and does not require additional heating or alkali treatment. Another interesting peculiarity is that in this case, unlike other processes of DNA scission, no opening of the furanose ring of 2′-deoxyribose occurs.

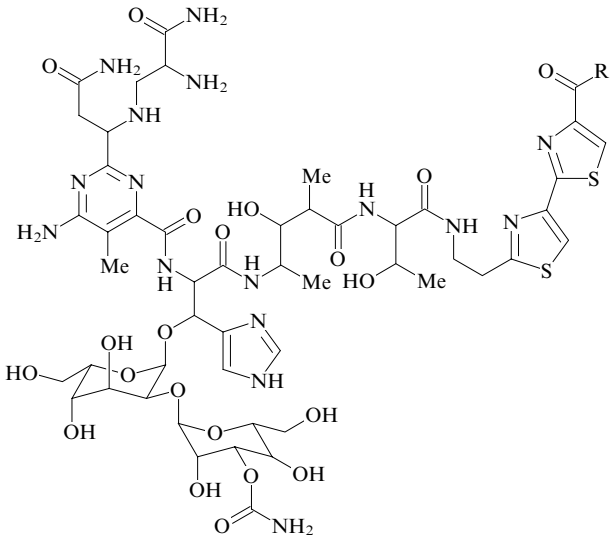
The complex $Cu(Phen)_2^+$ abstracts also a hydrogen atom from the C(4′) atom, which also results in DNA scission. However, this is a minor process as compared with hydrogen abstraction from the C(1′) atom.[127] In this case, the final products are similar to those in aerobic oxidation of 2′-deoxyribose with hydroxyl radicals: heterocycle-substituted propenal and phosphoglycolate (Scheme 14). Since in the B-form of DNA, the hydrogen atom at C(4′) is solvent-accessible,[100] the contribution of this process can be more significant in the oxidation of long DNA duplexes.

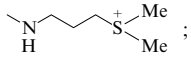
It can be noted that the spatial structure of DNA influences the oxidation process with copper(I) phenanthroline complex. Thus the cleavage of the A-form of DNA occurs less efficiently than that of the B-form. It can probably be associated with less favourable contacts between the complex and the widened minor groove of the A-form double helix.[128] In the reaction of the complex $Cu(Phen)_2^+$ with the Z-form of DNA where the minor groove is either absent or small, no cleavage of the DNA chain was observed.[128] Upon oxidation of single-stranded DNA, the cleavage was less efficient than that upon oxidation of double-stranded DNA;[129] in the former case, the process can proceed in a site-specific manner.[130] The reverse situation was observed in the case of RNA oxidation: single-stranded RNA loops were cleaved more efficiently than double-stranded regions.[129] Therefore, the spatial structure of nucleic acid has great effect on the oxidation efficiency.

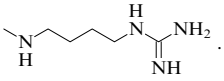
Thus, in the DNA oxidation with copper phenanthroline complex, the DNA chain is cleaved and 5-methylenefuran-2(5*H*)-one appears as a 2′-deoxyribose oxidation product.

3. Oxidation of DNA with an iron(II) bleomycin complex

Bleomycins (Blm) comprise a group of glycopeptide antitumour antibiotics that bring about scission of DNA in the presence of the Fe^{3+} ions and molecular oxygen.[131]

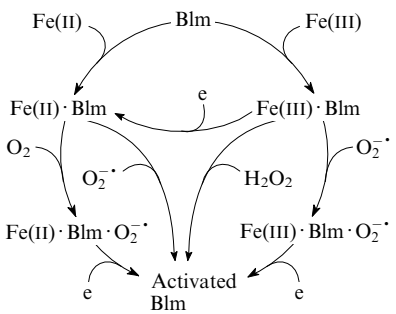


Bleomycin A_2: R =  ;

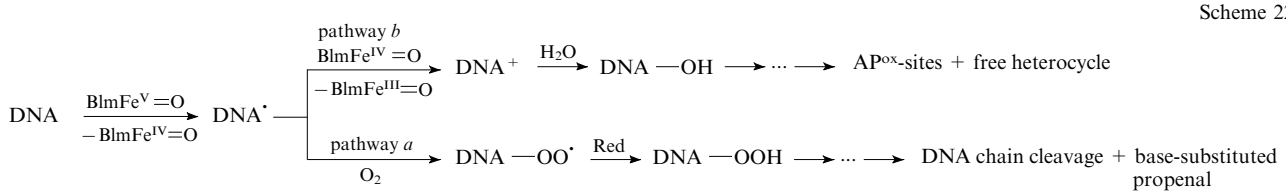
Bleomycin B_2: R =  .

So-called ‘activated bleomycin’ resulted from two-electron reduction of a triple complex $Blm \cdot Fe^{3+} \cdot O_2$ plays the role of an oxidising species. Bleomycin can also be activated by hydrogen peroxide (Scheme 21).[132, 133] The nature of the oxidising species in activated bleomycin has intensively been studied. According to the data from the ESI mass spec-

Scheme 21



Scheme 22



trometry, the oxidising species is most probably iron peroxide or high-valent iron oxo complex $Fe^{V}{=}O \leftrightarrow Fe^{IV}-O^{\bullet}$ that can abstract a hydrogen atom from the deoxyribose molecule.[132, 134]

A conclusion on C(4′)-hydrogen abstraction from deoxyribose as the rate-determining step was inferred from studies on the cleavage of different DNAs containing specifically tritiated and deuterated deoxyribose and on the isotope effect.[135–137]

The mechanism of DNA oxidation with activated bleomycin is similar to that of C(4′)-oxidation of deoxyribose with hydroxyl radicals. In the first step, the pentose C(4′) radical is formed further transformations of which depend on the presence of free molecular oxygen in the nearest environment (Scheme 22).

If the O_2 molecules are present in the nearest environment (pathway *a*), heterocycle-susbstitited *trans*-propenals **55** are formed. This process is accompanied by scission of DNA into two fragments, one of which contains 5′-terminal phosphate, whereas the second one contains 3′-terminal phosphoglycolate **56** (Scheme 14). In the absence of O_2 (pathway *b*), heterocyclic base is released to form apyrimidine/apurine sites at the oxidised residue of 2′-deoxyribose **46** (AP^{ox}-sites), which are cleaved only under alkaline conditions (Scheme 14). Under usual molecular oxygen concentrations, the amount of free heterocycles released is virtually equal to that of heterocycle-substituted propenals. At increased oxygen pressure, the reaction proceeds preferably by the pathway *a*, while in the deficiency of O_2 [the system Fe(III)–H_2O_2], pathway *b* was observed exclusively.[96, 135]

Thus, upon DNA oxidation with the activated iron bleomycin complex, selective C(4′)-oxidation of the 2′-deoxyribose fragment occurs. Among oxidation products, apyrimidine/apurine sites at the oxidised deoxyribose residue, free heterocycles, heterocycle-substituted *trans*-propenals and 3′-terminal phosphoglycolate were detected.

VII. Conclusion

The literature data presented demonstrate a great interest in the oxidation of nucleic acids with active oxygen species. As a result of these studies, various products of modification of nucleic acids were isolated and characterised and the mechanisms of their formation were established. Different transition metal complexes play the most important role in the oxidation of nucleic acids. Degradation of nucleic acids under the action of metal complexes is associated with their ability to generate active oxygen species. In this case, the main species responsible for destruction of nucleic acids in different oxidative systems are free hydroxyl radicals first of all. Singlet oxygen and coordinated oxygen particles in the form of high-valence metal oxo complexes can also oxidise nucleic acids.

Hydroxyl radicals react with nucleic acids and model compounds with diffusion-dependent rate constants. Dominating processes are addition to the double bonds of heterocycles and the hydrogen abstraction from the C–H bonds of the ribose residue. Hydroxyl radicals react predominantly with heterocycles undergoing irreversible modification. Oxidation of the ribose residue leads most often to cleavage of the DNA chain.

The efficiency of oxidation of nucleic acids with high-valence metal oxo complexes depends directly on the formation of the complex between oxidising agent and nucleic acid. Thus it was found that Mn(III) porphyrin complex activated by $KHSO_5$ to the high-valence form $Mn^{V}{=}O$ can either oxidise guanine selectively or oxidise the 2′-deoxyribose residue at the C(1′), C(4′) and C(5′) atoms depending on the nucleic acid structure. Analogously, the copper phenanthroline complex is bound to the minor groove of DNA and can cleave the DNA chain in the presence of hydrogen peroxide. The spatial structure of nucleic acid is one of the key factors influencing the efficiency of oxidation.

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