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Repair of 8-oxoguanine in DNA. The mechanisms of enzymatic catalysis

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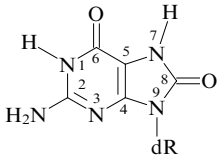
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Abstract. Data on the key enzymes involved in repair of 8-oxoguanine, one of the most abundant and mutagenic forms of oxidative in DNA damage, are summarised. The published data on the substrate specificity, active site structures and structural functional features of prokaryotic and eukaryotic 8-oxoguanine DNA N-glycosylases, adenine DNA N-glycosylases and 8-oxoguanosine triphosphatases are surveyed. Particular attention is given to chemical aspects of the mechanisms of the catalytic activity of these enzymes including stereochemistry of the catalytic processes. The bibliography includes 206 references.

I. Introduction

During functioning, cellular DNA is continually exposed to exogenous and endogenous agents, resulting in its damage. Reactive oxygen species formed as side products of cell metabolism, as a result of oxidative stress or under the action of ionising radiation are among the most aggressive factors of the environment. They induce oxidative DNA damage, which may be responsible for premature ageing, oncological and some other diseases.[1,2] More than 100 types of oxidative DNA lesions are known to date.[1,3]

An abundant oxidative DNA damage is the formation of a mutagenic nucleoside containing 7,8-dihydro-8-oxoguanine as the base (below oxoguanine, oxoG).



dR is a deoxyribose residue.

The formation of oxoG induces G:C → T:A and A:T → C:G transversions because during replication, oxoG can form a stable Hoogsteen pair with adenine.[4] Oxoguanine-containing DNA is the most stable DNA oxidation product. Oxoguanine is often used as a biomarker of the oxidative DNA damage related to carcinogenesis.[5]

During evolution, living organisms have elaborated a set of measures for preventing the genotoxic effect of DNA damages. Among these measures, mention should be made of reactivation, recombination repair, mismatch repair, excision repair, and so on. These processes differ from one another in substrates, enzymes and the mechanisms of lesion removal. Reactivation system is used by the cell to repair heterocyclic bases (cyclobutane pyrimidine dimers, alkylated bases) without DNA cleavage and subsequent synthesis.[6–8] In the case of more substantial lesions (double-stranded breaks, extended single-stranded gaps, covalent bonds between DNA strands), the recombination repair system is employed. Its functioning requires the presence of an undamaged copy of the genetic material in the cell, which is used for recombination.[6–9] Non-canonical base pairs of DNA are corrected by mismatch repair system. The mispairs in the DNA daughter chain are specifically recognised and eliminated.[6–8,10,11]

The most common way of elimination of DNA lesions is excision repair, which can be classified into two main types: nucleotide excision repair characterised by removal of short oligonucleotide DNA fragments containing a damage followed by restoration of the initial nucleotide sequence;[6,12,13] and base excision repair, which implies removal of the damaged base by specific DNA glycosylase,

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removal of the deoxyribose phosphate residue and restoration of the gap formed.

The base excision repair is the major mechanism for the elimination of oxidative damages in DNA.[7,8,14,15] In addition, the so-called SOS repair exists, which is switched on when DNA replication is required but some lesions persisted that could not be eliminated by either of the above-mentioned systems. This is an exceptionally hazardous repair mechanism, which saves the cell from destruction at a particular moment. This allows DNA polymerase to construct the DNA daughter strand with quite a few errors, which may be lethal for the cell in the future.[6,16,17]

To repair oxoG in a damaged DNA, the cell uses the so-called GO system. This system was detected in cells of almost all organisms ranging from bacteria to humans, which accounts for its significance in the retention of cell genome. The GO system of prokaryotes includes the action of a number of enzymes: 8-oxoguanine-DNA glycosylase (Fpg also known as MutM), adenine-DNA glycosylase (MutY) and 8-oxoguanosine triphosphatase (MutT)[18] (Fig. 1). The damaged base oxoG, which forms a Watson–Crick base pair with cytosine, can be removed by Fpg with subsequent reconstruction of the DNA molecule by integrated action of a number of other enzymes. If the oxoG base has not been removed before replication, the process yields DNA containing both oxoG:C and oxoG:A pairs. In this case, the MutY protein eliminates the misincorporated adenine, DNA polymerase I inserts 2′-deoxycytidine 5′-monophosphate opposite to 7,8-dihydro-8-oxo-2′-deoxyguanosine 5′-monophosphate (oxodGMP), and then DNA ligase restores the continuous DNA strand.

The action of reactive oxygen species can also induce the formation of oxidised nucleoside triphosphates. The formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine 5′-triphosphate (oxodGTP), which is a DNA polymerase substrate, results in insertion of the oxoG residue opposite to the cytosine residue in the DNA template during replication (see Fig. 1). In addition, misincorporation of oxoG residues opposite to template adenine is also possible. In this case, MutY is involved in mutagenesis because removal of adenine from the non-canonical oxoG:A pair results in A:T → C:G transversion. The MutT protein specifically hydrolyses oxodGTP to oxodGMP, thus preventing the insertion of oxoG residues into DNA.

The GO system of eukaryots is constructed in a similar way.[19,20] This includes homologues of MutT and MutY, namely, enzymes MTH and MYH, respectively, and the functional analogue of Fpg, namely, 8-oxoguanine-DNA glycosylase (Ogg1). Biochemical studies showed the presence of short-patch and long-patch pathways of DNA repair in mammalian cells after removal of oxoG residues.[21] In the cells containing DNA polymerase β in which the N-terminal domain possesses a deoxyribophosphatase activity, reconstruction follows a short-patch pathway, which comprises successive action of DNA polymerase β

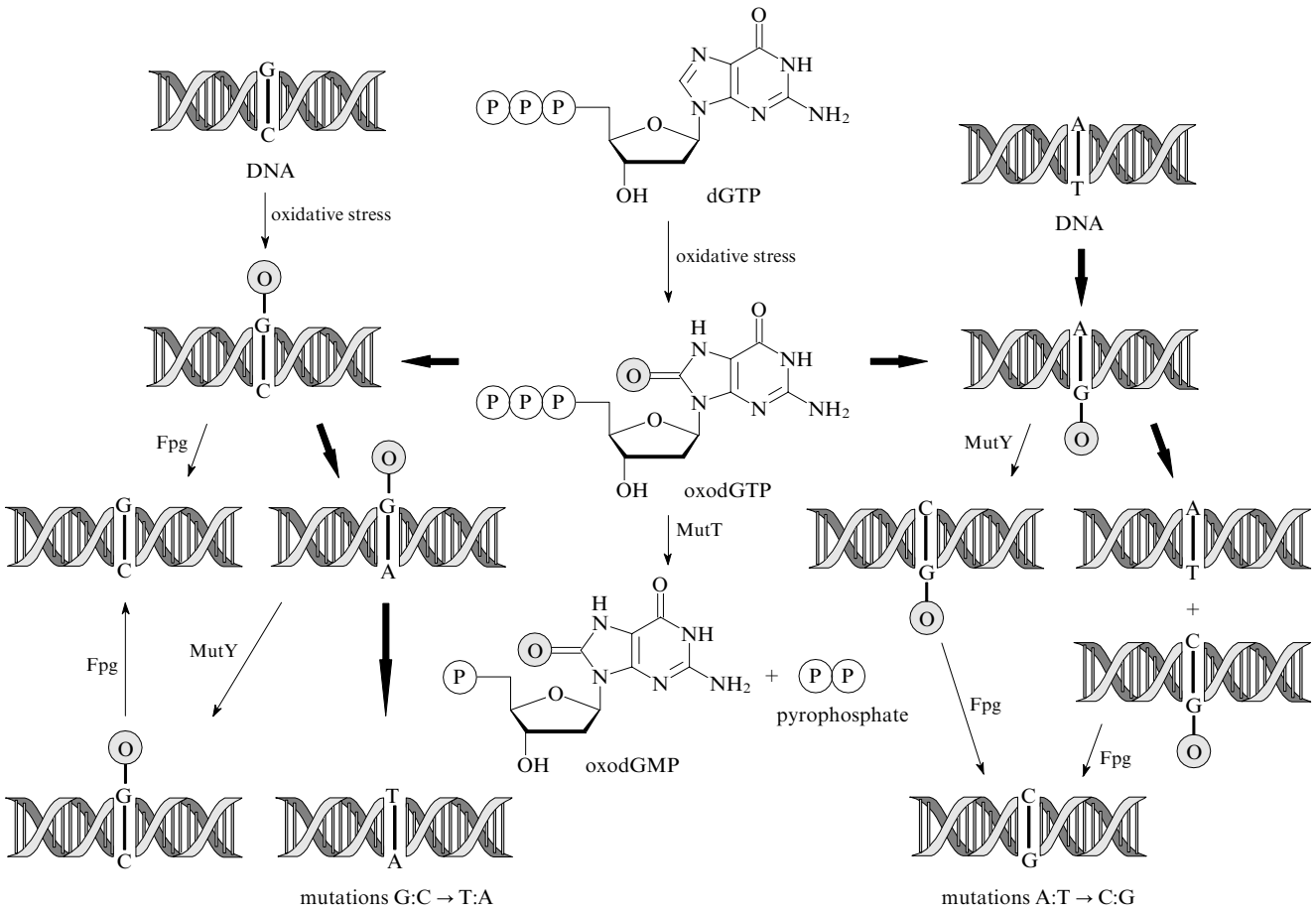


Figure 1. GO system of repair enzymes preventing the mutagenic activity of oxoG. Thick arrows mark the replication process, thin arrows denote the repair.

and DNA ligase III.[22] In the cells deficient in DNA polymerase β, this process follows a long-patch pathway, which involves gap-filling of 2–6 nucleotides in a DNA strand by DNA polymerases δ or ε together with PCNA.[23] Then flap endonuclease (FEN1) removes the displaced strand and DNA ligase I restores the covalent bond between the 5′-phosphate and 3′-OH groups.[24] Analysis of oxoG repair in the mammalian cell extracts showed that this base is removed from damaged DNA mainly along the short-patch pathway.[25]

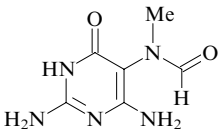
Bacteria possessing a defect in either the *mutM* or *mutY* genes display a modest increase in the rate of spontaneous mutagenesis. The *mutM*$^-$ and *mutY*$^-$ strains show a more than 100-fold increase in the rate of mutagenesis compared to the wild type strain.[18] Inactivation of the *mutT* gene is a strong mutagenic factor resulting in a 1000–10 000-fold increase in the total level of A:T → C:G transversions.[26] Studies of the eukaryotic cell lines with knockout of the corresponding genes also demonstrated an increase in the frequency of spontaneous mutations resulting in cancer development.[19, 27, 28]

Repair of DNA is a fundamental biological process ensuring the genome stability and integrity. A large number of recent publications including both original papers and reviews have been devoted to various types of DNA damage,[6, 29–33] various ways of repair;[3, 9, 11–13] biological, pharmacological and clinical aspects of repair of oxidative DNA damage;[34–36] DNA polymerases[37, 38] and DNA ligases[39, 40] involved in this process; structural, biophysical, biochemical, theoretical, physicochemical and molecular-biological investigation methods used in this field.[33, 41–43]

This review covers data published in the last decade concerning enzymes that play the key role in the protection of genetic apparatus of the cell from mutagenic action of 8-oxoguanine. The attention is concentrated on the chemical aspects of catalytic mechanisms and conformational dynamics of these enzymes and structural aspects of their interaction with DNA. Data on the latest achievements in the investigation of highly complicated search and recognition processes of 8-oxoguanine residues by specific DNA glycosylases in a genome are summarised.

II. 8-Oxoguanine-DNA glycosylases

E. coli formamidopyrimidine-DNA glycosylase (Fpg) was the first isolated and characterised protein of the DNA glycosylase class. In 1979, this enzyme was described as a DNA glycosylase specific to 2,6-diamino-4-hydroxy-5-(*N*-methylformamido)pyrimidine (N_7-Me-FapyG) residues.[44]



N_7-Me-FapyG

These residues appear in DNA upon methylation of the guanine N_7 atom followed by oxidative opening of the 5-membered ring. This lesion prevents the DNA synthesis and thus it is potentially lethal *in vivo*.[45] In 1991 it was proved that formamidopyrimidine-DNA glycosylase and 8-oxoguanine-DNA glycosylase activities are exhibited by the same enzyme present in *E. coli*.[46] Now 8-oxoguanine-DNA glycosylases have been found virtually in all pro- and eukaryotic organisms studied. These enzymes are monomeric globular proteins with molecular weight of 30–40 kDa. They are bifunctional N-glycosylases/AP lyases (AP is apurine/apyrimidine) catalysing the removal of oxoG residues and subsequent cleavage of the sugar-phosphate DNA backbone. Hydrolysis of the sugar-phosphate DNA backbone is the rate-determining step of the process.

1. Prokaryotic 8-oxoguanine-DNA glycosylase

Prokaryotic 8-oxoguanine-DNA glycosylases are highly conservative; they have similar tertiary structures, substrate specificities and catalytic mechanism. The *fpg* genes encoding the Fpg proteins of *E. coli*, *Thermus thermophilus*, *Bacillus stearothermophilus* and *Lactococcus lactis* have been cloned, their length being ~900 nucleotide pairs.[47–49]

a. Structure of prokaryotic Fpg

In 2000 the tertiary structure of the *T. thermophilus* Fpg enzyme was solved by X-ray diffraction analysis.[50] Currently this is the only structure of a free Fpg in the absence of DNA. Later, the structures of *E. coli* Fpg [51] and *B. stearothermophilus* Fpg[52] complexes and catalytically inactive P1G Fpg *L. lactis* complex[53, 54] with DNA analogues containing apurine/apyrimidine sites (AP sites) were established. The complexes formed by catalytically inactive E2Q form of *B. stearothermophilus* Fpg with DNA containing oxoG or dihydrouracil (DHU) residues were crystallised.[55] These structural studies provided valuable information about the general architecture of Fpg enzymes of prokaryotes and their complexes with DNA (Fig. 2).

It was found that prokaryotic 8-oxoguanine-DNA glycosylases comprise two different domains connected by a hinge polypeptide. The N-terminal domain is composed of eight antiparallel β-strands and two α-helices and has a structure of two-layer β-sandwich. The C-terminal domain

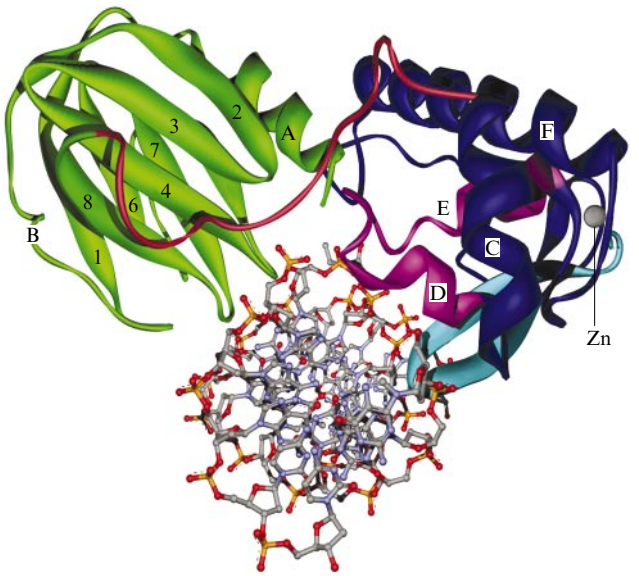


Figure 2. Structure of the covalent complex of the *E. coli* Fpg enzyme with DNA containing an AP site (PDB code 1K82).[17]
The N-terminal domain of the enzyme is shown in green, the hinge connecting the N- and C-terminal domains in red and C-terminal domain in blue, the H2TH motif in violet, 'zinc finger' motif in light blue. The zinc atom is shown by a grey ball.

includes four α-helices and two antiparallel β-strands. Two of the four α-helices form the 'helix–two turns–helix' (H2TH) motif, and β-strands form a 'zinc finger' motif. The H2TH and 'zinc finger' motifs are DNA binding.

Prokaryotic 8-oxoguanine-DNA glycosylases are metalloproteins. They contain one zinc atom in the 'zinc finger' tertiary structure motif per enzyme molecule.[50–55] The positively charged DNA-binding groove and the conservative amino acid sequence PELPEVET containing a catalytically active amino acid residue Pro-1 are located between the two main domains of the enzyme.[50–55]

In 2006, the structures of covalent complexes of the mutant form E2Q/Q166C of the *B. stearothermophilus* Fpg enzyme with DNA duplexes of different sequence containing an oxoG:C pair were established.[56] Also, the structures of *B. stearothermophilus* Q166C Fpg with DNA containing a G:C or A:T instead of oxoG:C were determined.[56] These structures allowed in-depth understanding of the processes taking place at an early stage of the search for DNA damage by prokaryotic 8-oxoguanine-DNA glycosylases.

Structural studies have shown that in complexes of bacterial Fpg proteins with undamaged DNA (devoid of oxoG), the polypeptide fragment of the enzyme comprising amino acids that interact with the damaged base is disordered.[53,54,56] However, in the structures of free Fpg enzyme of *T. thermophilus*[50] and in the complexes of catalytically inactive E2Q and E2Q/Q166C forms of *B. stearothermophilus* Fpg with DNA duplexes containing an oxoG:C pair,[55,56] this fragment is ordered.

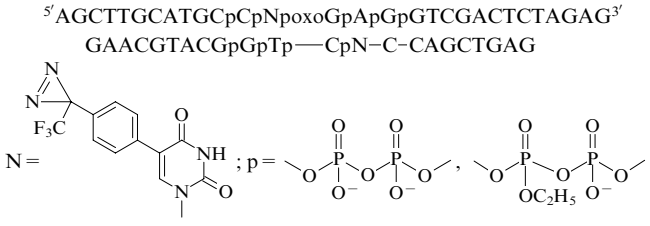
No data on the structure of complexes of catalytically active Fpg enzymes with oxoG-containing DNA are available to date. This is due to the fact that wild type DNA glycosylases cleave their substrates much faster than crystallisation occurs and data collection takes place. Owing to the progress in DNA chemical synthesis and modification, which allowed the design and synthesis of uncleavable substrate analogues, it became possible to study the interaction of DNA glycosylase with damaged bases. In 2004 the structure of the *L. lactis* Fpg complex with DNA containing a carbocyclic analogue of formamidopyrimidine was determined.[57] New analogues of 8-oxoguanine-DNA glycosylase substrates in which one natural internucleotide phosphate group in the vicinity of the oxoG residue has been replaced by a pyrophosphate or reactive substituted pyrophosphate group are promising compounds for structural studies of complexes of wild type enzymes with oxoG-containing DNA.[58,59] It was shown than DNA duplexes containing these modified groups either in the 3′-position of 7,8-dihydro-8-oxo-2′-deoxyguanosine (oxodG) or one nucleotide unit away are non-hydrolysable analogues of 8-oxoguanine-DNA glycosylase substrates.[60,61]

b. Substrate specificity of prokaryotic Fpg

There exist numerous varieties of substrates of prokaryotic 8-oxoguanine-DNA glycosylases. Apart from oxoG and N_7-Me-FapyG residues, these enzymes efficiently excise 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA) residues† and single-electron oxidation products of oxoG such as spiroiminohydantoin (Sp) and guanidinohydantoin (Gh) from DNA.[62–64] The 7,8-dihydro-8-oxo-2′-deoxyinosine (oxodI) residue is removed by these enzymes approximately twice as efficiently as the oxodG residue,[65] whereas 7,8-dihydro-8-oxo-2′-deoxynebularine (oxodN) and 7,8-dihydro-8-oxo-2′-deoxyadenosine (oxodA) residues are excised by prokaryotic Fpg proteins two orders of magnitude less efficiently (Fig. 3).[65,66] In addition, prokaryotic Fpg enzymes excise, from oligodeoxynucleotides, derivatives of 1,N^6-ethenodeoxyadenosine (εdA′ and εdA″),[67] the oxoG oxidation products — oxazolone (Z)[68,69] and oxaluric acid (oxA)[68,70] — and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy) derivatives substituted at the formamide N atom, *e.g.*, 2-(hydroxyethylthio)ethyl-(N_7-HETE-FapyG),[71] 2-{*N*-(2-chloroethyl)-*N*-[(amino)-hydroxy]phosphoryl}aminoethyl-(N_7-PM-FapyG),[57] aflatoxin B1 (N_7-AFB1-FapyG)[57] and C_8-aminofluorene guanine derivatives with an open imidazole ring: C_8-AF-iroG and C_8-AF-G.[65]

It was found that bacterial Fpg proteins are also active with respect to some oxidised pyrimidines. Thus these enzymes excise 5-hydroxycytosine (5-OH-dC),[72,73] 5-hydroxyuracil (5-OH-dU),[73] 5,6-dihydrouracil (DHdU),[57] 5,6-dihydrothymine (DHdT),[72] uracil glycol (Ug),[57,74] thymine glycol (Tg),[72,74] 5-hydroxy-5-methylhydantoin (5-OH-5-Me-dHyd),[75] 5-formyluracil (5-fodU),[76] 3-ureido-(2*R*)-hydroxy-2-methylpropionic acid (αRT) nucleosides from DNA duplexes[77] and remove *O*-methyl- and *O*-benzylhydroxylamines from the corresponding modified AP sites (Me-Am-AP, Bn-Am-AP).[78] Presumably, this broad spectrum of the substrate specificity of prokaryotic Fpg enzymes can be due to their ability to change their active sites in order to bind both oxidised pyrimidines and oxidised purines.[79,80] Note that Fpg enzymes remove a much broader spectrum of damaged bases from oligonucleotide substrates than from high-molecular-weight DNA,[62,81] in particular, DHU, 5-OH-C and 5-OH-U bases are not removed from high-molecular-weight DNA.[62]

Recently, new analogues of 8-oxoguanine-DNA glycosylase substrates containing a 8-oxoguanine residue and modified internucleotide groups in different positions have been proposed.[58,60,61] It was shown that such substrates are recognised by, and are effectively bound to, *E. coli* Fpg and its eukaryotic analogue, *viz.*, human 8-oxoguanine-DNA glycosylase (hOGG1). The efficiency of catalytic cleavage of these compounds depends on the position of the modified group with respect to the oxoG residue and can be either higher than the efficiency of cleavage of the natural substrate with a similar structure or lower down to complete inhibition of the excision of oxoG residue.[58,60] Analogues of substrates of *E. coli* Fpg containing photoactivated aryl(trifluoromethyl)diazirine groups in the nucleosides located in the near vicinity of oxodG are also documented.[82]



It was demonstrated that the proposed substrate analogues containing reactive *O*-alkyl-substituted pyrophosphate and aryl(trifluoromethyl)diazirine groups are efficient reagents for probing the active sites of *E. coli* Fpg and hOGG1.[61,82]

† OxoG, FapyG, N_5-Me-FapyG and FapyA are physiological substrates of prokaryotic Fpg.

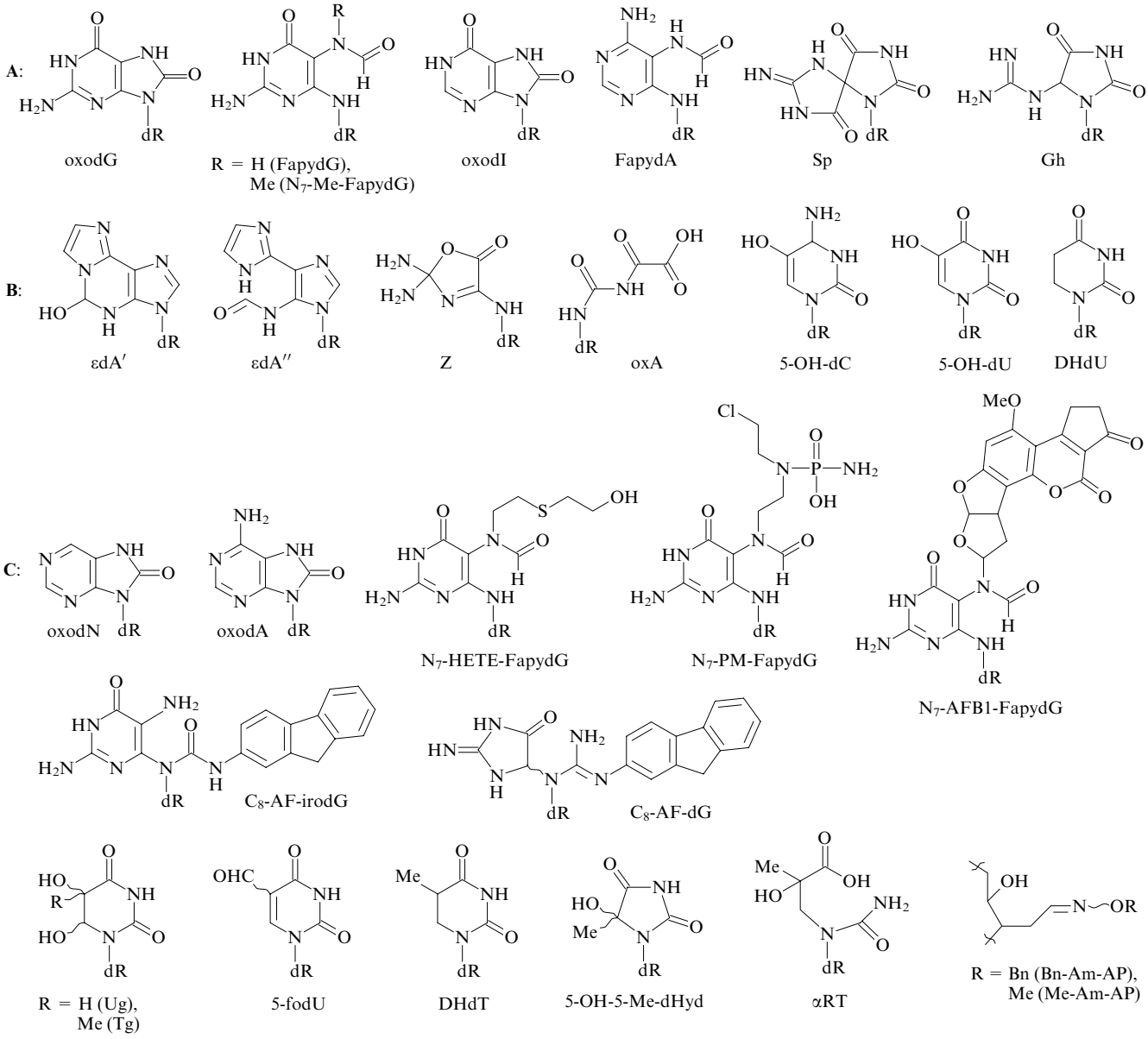


Figure 3. Structures of damaged sites removed from DNA by prokaryotic Fpg enzymes.
Fragments presented in sections **A**, **B** and **C** are removed with high, medium and low efficiency, respectively.

Yet another substrate for the prokaryotic 8-oxoguanine-DNA glycosylase is DNA that contains AP sites resulting from hydrolysis of the N-glycosidic bond.[54,83] The N-glycosidic bond can be cleaved by DNA glycosylases and as a consequence of spontaneous DNA apurinisation/apyrimidinisation. It was found that spontaneous hydrolytic processes in human cell produce about 1000 AP sited per cell per day.[29] The presence of AP sites in DNA can give rise to mutations and be lethal for the cell.[80]

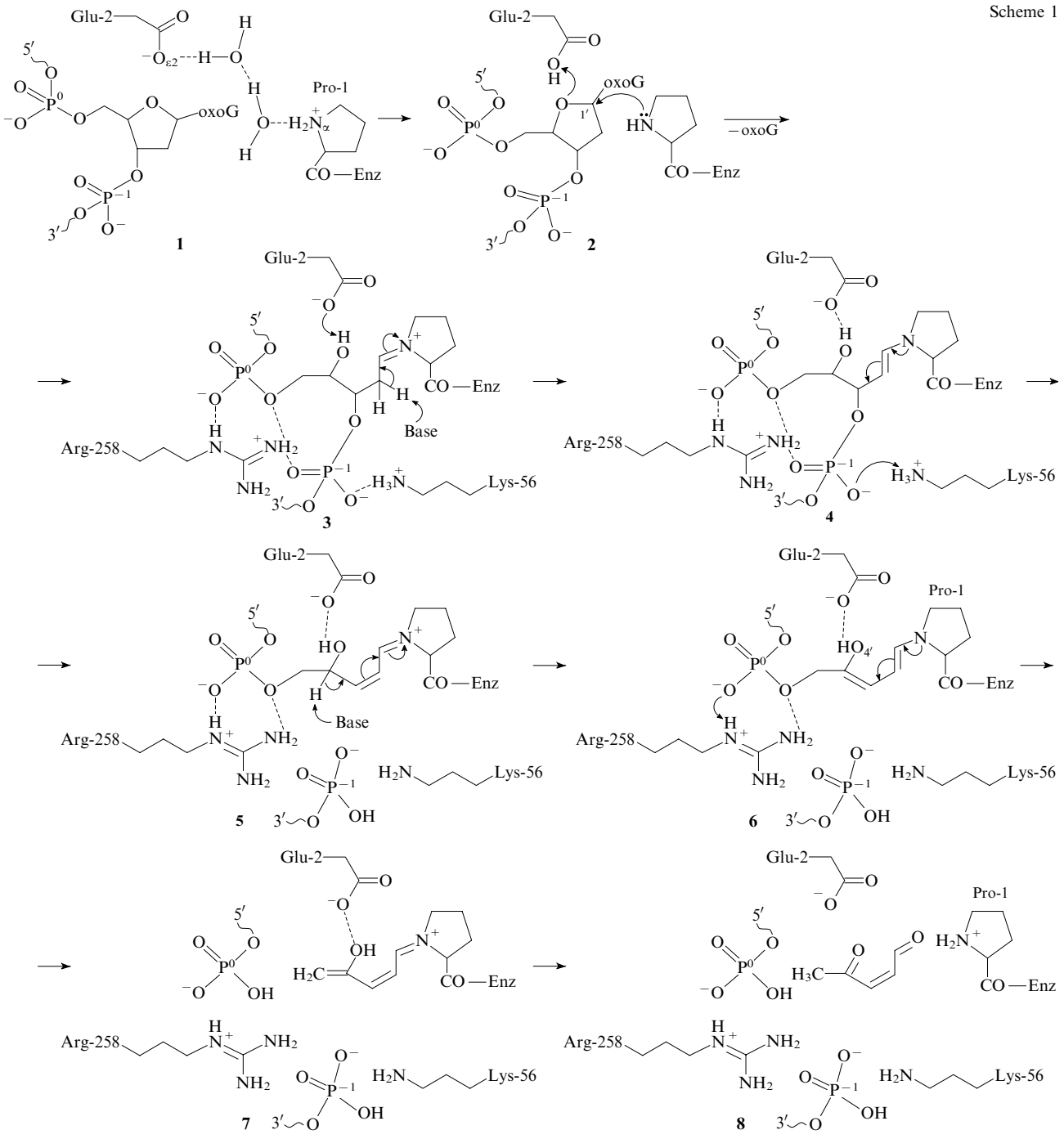
It was found that 5′-terminal deoxyribose phosphate (5′-dRp), the product of action of cell AP-endonucleases, is also a substrate of prokaryotic 8-oxoguanine-DNA glycosylase.[84] The dRp-ase activity rate of prokaryotic Fpg is higher than its DNA glycosylase activity rate and is comparable with the rate of AP site liberation.[84]

c. Catalytic mechanism of substrate cleavage by prokaryotic Fpg enzymes

The general mechanism of catalytic cleavage of a substrate by prokaryotic 8-oxoguanine-DNA glycosylases includes everting and removal of the oxoG residue from the DNA double helix by cleavage of the N-glycosidic bond. The next stage includes cleavage of the DNA sugar-phosphate backbone according to the β,δ-elimination mechanism. As a result, one-stranded single-nucleoside gap flanked by 3′- and 5′-phosphate groups is formed in DNA, the deoxyribose residue and the damaged base being removed (Scheme 1).

The N-glycosidic bond is cleaved upon nucleophilic attack of the catalytically active amino acid of the enzyme at the $C_{1'}$ atom of damaged nucleoside to give a covalent

Scheme 1



enzyme–substrate intermediate, the Schiff base (see Scheme 1). The formation of this derivative was confirmed by its reduction with sodium cyanoborohydride followed by X-ray diffraction study of the DNA–protein complex formed.[51,52] It was demonstrated by electrospray mass spectrometry[85] and X-ray diffraction[51,52] that the α-amino group of the N-terminal proline residue in the highly conserved PELPEVET sequence of prokaryotic Fpg acts as the nucleophile. In the case of DNA containing an AP site, Pro-1 is necessary for the protein to exhibit an AP-lyase activity but not for the Schiff base formation.[86] It was found that Pro-1 is not essential for the maintenance of the active site conformation of prokaryotic Fpg.[86] The orientation of the N_α and $O_{4'}$ atoms in the covalent complex of *E. coli* Fpg with AP-DNA indicate that the nucleophilic attack occurs along the $C_{1'} \rightarrow O_{4'}$ bond.[51] According to X-ray diffraction data for the non-covalent complex formed by *B. stearothermophilus* E2Q Fpg with oxoG-containing DNA,[55] the proline amino group is arranged rather closely to the $C_{1'}$ atom but rather unfavourably for the S_N2 attack.

Some recent data indicate that the damaged base is removed from DNA by S_N1 mechanism. According to this dissociative mechanism, the first stage is excision of the damaged base to give intermediate reactive oxocarbenium ion, which is attacked in the next stage by the Pro-1 α-amino group.[55] This mechanism is supported by the formation of a hydrogen bond network involving the O_6 atom of oxoG in the complex of *B. stearothermophilus* E2Q Fpg with oxoG-DNA, which activate the oxoG base as a leaving group.[55] According to X-ray diffraction data for free *T. thermophilus*

Fpg enzyme,[50] the amino acid residues Pro-1, Glu-2, Asn-161 and Ile-162 form a tight network of hydrogen bonds with several water molecules. This network could potentially enable proton transfer from Pro-1 N_{α} to Glu-2 $O_{\varepsilon 2}$ (see Scheme 1, structure **1**), which are separated by a 6.2 Å distance in the crystal structure. This activates simultaneously the Pro-1 residue towards nucleophilic attack and allows the Glu-2 residue to protonate the $O_{4'}$ atom of deoxyribose (see Scheme 1, structure **2**); this ensures deoxyribose ring opening in the damaged nucleoside.

The formation of a reversible covalent intermediate (see Scheme 1, structure **3**) is favourable for further removal of the deoxyribose residue as 4-oxopent-2-enal by the β,δ-elimination mechanism. For this process to be successful, proton acceptors should be present in the near vicinity of $C_{2'}$ and $C_{4'}$ atoms of the damaged nucleoside. Their nature has not been elucidated as yet. In the case of *B. stearothermophilus* Fpg, this function is presumably performed by the water molecule located at a 3.5 Å distance from the $C_{2'}$ atom.[52] However, this water molecule is missing in the case of *E. coli* Fpg, thus making its participation in this process less likely.

Published data on the stereochemistry of β,δ-elimination performed by prokaryotic Fpg enzyme are scarce. There are

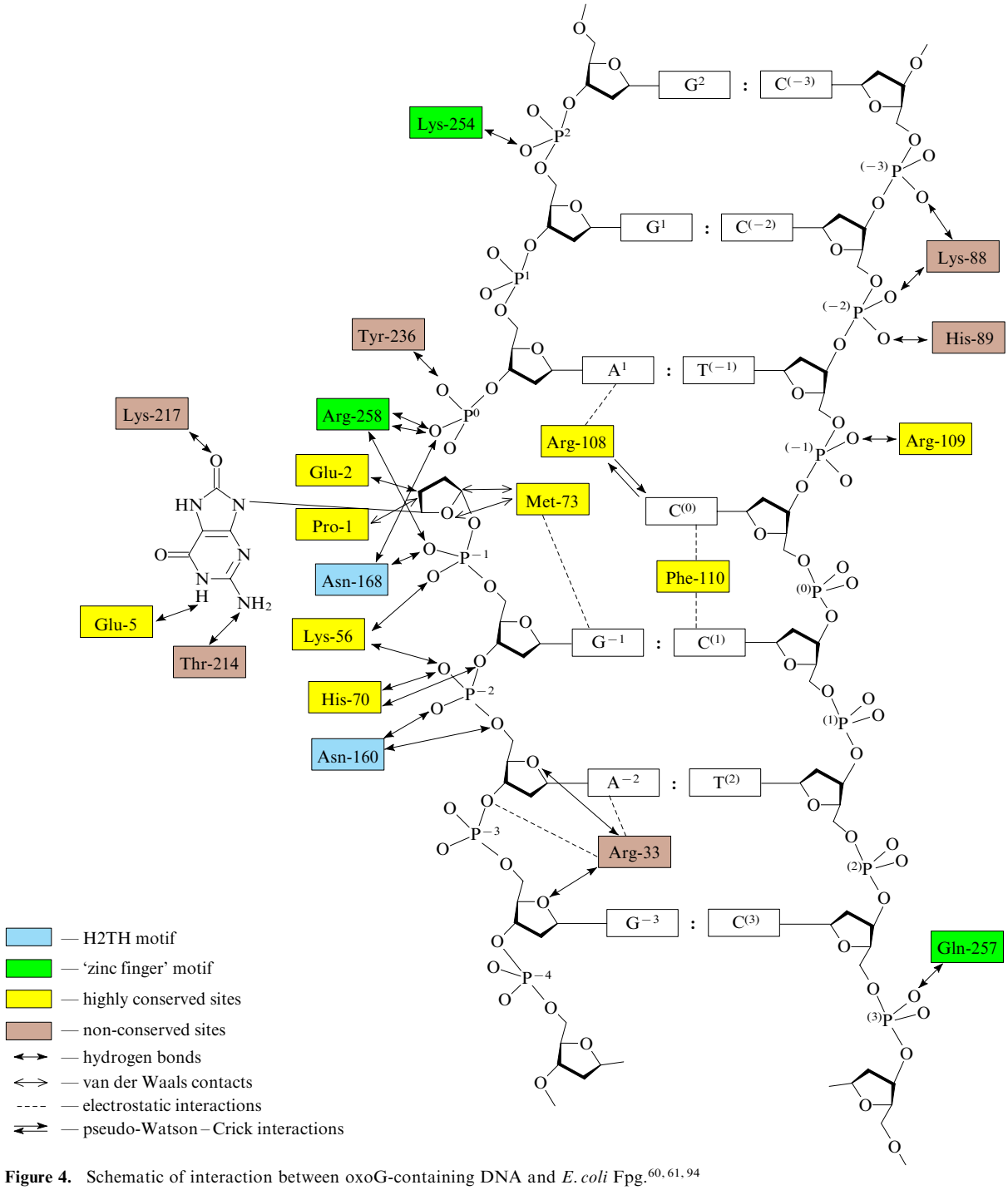


Figure 4. Schematic of interaction between oxoG-containing DNA and *E. coli* Fpg.[60, 61, 94]
The central nucleotides of the damaged and complementary DNA – $oxoG^0$ and $C^{(0)}$ strands, respectively.

four possible stereochemical pathways for β-elimination, namely, *syn*-elimination of a *pro-S*-2′ proton [corresponding to torsion angle $v_2(C_{2'}-C_{3'}) = 120°$], *syn*-elimination of the *pro-R*-2′ proton ($v_2 = 0°$), *anti*-elimination of the *pro-S*-2′ proton ($v_2 = -60°$) and *anti*-elimination of the *pro-R*-2′ proton ($v_2 = 180°$).[87] Structural data for the covalent complexes of prokaryotic Fpg with AP-containing DNA are most consistent with *anti*-elimination of the *pro-S*-2′ proton.[51,52] The average torsion angle v_2 equals $-81.6°$ for *E. coli* Fpg[51] and $-121.0°$ for *B. stearothermophilus* Fpg.[52] Note that other AP lyases abstract the *pro-S*-2′ proton from the deoxyribose residue according to *syn*-elimination mechanism.[88]

One more specific feature of β,δ-elimination is stabilisation of the leaving phosphate groups. According to X-ray diffraction, in the case of *E. coli* Fpg, phosphate groups flanking the damaged nucleoside are tightly coordinated by the absolutely conserved amino acid residues Lys-56, Asn-168, Arg-258 and Tyr-236 (Fig. 4). These residues protonate the phosphates, thus forming good leaving groups, and stabilise the negative charge formed on phosphates as a result of β-elimination (see Scheme 1, structures **4**–**7**). Thus Lys-56 protonates the P^{-1}-phosphate in the 3′-position of the oxidised nucleoside, while Arg-258 protonates both P^{-1}-phosphate in the 3′-position and P^0-phosphate in the 5′-position from the lesion (structure **4**). After cleavage of the phosphodiester bond in P^{-1}-phosphate, its bond with Arg-258 weakens, thus permitting this amino acid residue to shift toward P^0-phosphate for its more efficient protonation (structure **5**). It is noteworthy that the coordination of the leaving phosphate groups of DNA by N-glycosylases/AP lyases correlates with their β,δ-elimination activity with respect to corresponding phosphates.[87]

d. Conformational changes in the structures of prokaryotic Fpg complexes with DNA

The two-domain structure of prokaryotic 8-oxoguanine-DNA glycosylases suggests that both domains can move during DNA binding and during search and excision of the damaged base. This is in line with X-ray diffraction data, which imply that the Pro-1 and Glu-2 residues of the *T. thermophilus* Fpg enzyme are involved in direct interactions with amino acids from the H2TH motif of this protein, whereas in the *E. coli* Fpg complex with DNA, these interactions are replaced by specific contacts with DNA. In addition, changes in the intrinsic fluorescence of the tryptophan residue detected on binding of Fpg *E. coli* with DNA[89] attest to conformational changes in the enzyme structure. According to NMR data, αA- and αF-helices forming a part of the DNA-binding groove of the *E. coli* Fpg enzyme are mobile.[90,91] Zharkov *et al.*[87] suggest the existence of an open (for DNA-non-bound enzymes) and closed (for DNA-bound enzymes) conformations of *E. coli* Fpg. The open form of Fpg enzymes has a much larger angle between the N- and C-terminal domains than the closed form. Thus, the whole set of obtained data indicates that upon DNA binding, two prokaryotic 8-oxoguanine-DNA glycosylase domains can rotate with respect to each other.[87]

Koval *et al.*[92] studied the kinetics of conformational transitions in the Fpg molecule during the catalytic process. This was done using the stopped-flow method with recording the change in the fluorescence intensity of tryptophan residues after its interaction with specific oligonucleotide substrates containing an oxoG residue. It was shown that the kinetic curves comprise five conformational transitions, which correspond to five stages of catalytic removal of oxoG residues from DNA. These stages include binding of the Fpg enzyme to DNA, recognition of the damaged base and mutual adjustment of the protein and DNA structures needed for catalysis.

While recording the fluorescence of tryptophan residues in *E. coli* Fpg and 2-aminopurine in DNA by the stopped-flow technique, Kuznetsov *et al.*[93] studied the presteady-state kinetics of enzymatic reactions with participation of *E. coli* Fpg. It was found that the formation of a catalytically active complex of the Fpg enzyme with a specific DNA substrate also comprises five stages. The first stage is non-specific binding of the enzyme to DNA, giving rise to a primary collision complex. The second stage produces a secondary complex, which plays a key role in the recognition of damaged DNA site. This stage was shown to be accompanied by conformational changes in the enzyme structure about the Phe-110 residue. The third stage is eversion of the damaged base. This stage gives rise to a pocket in the DNA duplex. The fourth stage comprises insertion of amino acid residues of the enzyme into the DNA excision and adjustment of the enzyme active site conformation to the catalytic stages of the process. The enzymatic cycle is completed by an equilibrium dissociation of the enzyme–product complex.

Along with the conformational changes in the enzyme structure that accompany its interaction with damaged DNA, substantial changes in the DNA structure also take place. This is due to the fact that direct displacement of the oxidised base from the DNA double helix seems improbable, because this would require penetration of the Pro-1 inside the DNA helix. Therefore, for effective catalytic reaction, prokaryotic Fpg enzymes, like all DNA glycosylases, evert the damaged base out of the DNA double helix and bind it in a deep pocket of the enzyme active site.[51,55] Eversion of the damaged base occurs through the minor groove *via* rotation around the $P-O_{5'}$ and $O_{3'}-P$ bonds. The energy needed for extruding the damaged base from DNA is ~ 3 kcal mol^{-1}. This barrier can be easily overcome by non-covalent enzyme–substrate interactions. Prokaryotic Fpg enzymes have a relatively large contact surface area with DNA (for *E. coli* Fpg, this is 2512 Å^2).[51] The conformational rearrangement of DNA involves amino acid residues from both domains of prokaryotic Fpg.

The DNA complexed with Fpg enzymes has a classical B-form along the whole length except for the damaged section.

Enzyme-dependent changes in the DNA structure at the lesion site are mainly due to the reactions between DNA phosphate groups and Fpg amino acids (see Fig. 4).[51,60,61,94] An important role in this process is played by the Phe-110 residue from the β7–β8 loop of the N-terminal domain of the enzyme. The aromatic ring of Phe-110, wedged between $C^{(0)}$ and $C^{(1)}$ engages in face-to-face π-interactions with the $C^{(1)}$ pyrimidine ring.‡ This violates the stacking-interactions between these two bases, which in combination with specific contacts of phosphate

‡ The numbers of nucleotides of the damaged strand are given without parentheses and the numbers of nucleotides in the complementary strand are in parentheses. The damaged unit serves as the reference point for nucleotide numbering.

groups with enzyme amino acids results in considerable kink of DNA (66°) at the lesion site and extrusion of the oxidised base from the DNA helix (see Fig. 4).

Extrusion of the damaged base creates a substantial gap between opposite stands of DNA (the distance between the $C_{1'}$ atoms of deoxyriboses of damaged oxodG^0 and complementary nucleotide dC$^{(0)}$ is 14.7 Å instead of usual 10.5 Å in B-DNA).[51] The gap is filled by the hydrophobic amino acid residues of the enzyme. In the case of *E. coli* Fpg, these are Met-73, Arg-108 and Phe-110 (see Fig. 4). The bulky Met-73 residue of the β4–β5 loop is inserted into the DNA helix through the minor groove, thus occupying the position vacated by the extruded oxoG^0 base. The C$^{(0)}$ base complementary to the extruded one remains intrahelical stabilised by the formation of two hydrogen bonds with the Arg-108, which is located on the β7–β8 loop. These interactions contribute to Fpg specificity through opposite base recognition.

It is noteworthy that the interaction of prokaryotic Fpg with undamaged DNA differs considerably from enzyme interactions with damaged DNA. When the enzyme is bound to damaged DNA kinked in the oxoG region, some of the positively charged DNA-binding groove of the prokaryotic Fpg enzyme remains free. In the case of undamaged DNA, the structure is not kinked or is kinked to a lesser extent; therefore the whole enzyme groove participates in the binding of this DNA. This is accompanied by additional or alternative interactions needed for the enzyme to look for lesions.

e. Search for the oxoG residues in the genome by prokaryotic Fpg enzymes

The model for the search for oxoG residues in the genome by prokaryotic Fpg enzymes includes the following stages: (i) non-specific binding of the enzyme to DNA; (ii) scanning of DNA grooves as the enzyme diffuses through it; (iii) recognition of the damaged base; (iv) formation of an enzyme–substrate transition complex; (v) eversion of the damaged nucleoside out of the DNA helix and (vi) its binding in the enzyme active site pocket.[95]

The mechanism of lesion recognition by prokaryotic Fpg enzymes remains obscure. It is known that eversion of each base out of the DNA helix is accompanied by considerable energy expenditure. However, DNA glycosylases do not spend energy when searching for DNA lesions.[96] Structural studies of *B. stearothermophilus* Fpg complexes with various DNA duplexes indicate that interaction of the aromatic ring of the Phe-114 residue of the enzyme intercalated into the helix of undamaged DNA results only in a slight rotation of heterocyclic bases whereas the interaction of this residue with oxoG:C-DNA results in eversion of the damaged base.[56] It might happen that it is the damaged base everted spontaneously out of the DNA helix that is bound in the active site of prokaryotic Fpg enzymes. The same mechanism acts in the case of uracil-DNA glycosylase;[97] however, the mechanisms of action of other DNA glycosylases have not been proven. Currently, recognition of the oxidised heterocyclic base located within the DNA helix before eversion is considered to be most probable.[65] This mechanism of intrahelix lesion recognition is kinetically most preferred.

Important aspects in the lesion recognition process are elucidation of the mechanism of recognition of the oxidised base by prokaryotic Fpg enzymes and determination of the structural elements involved in this process. Two versions of recognition are possible: (i) direct readout comprising lesion recognition through the formation of hydrogen bonds or electrostatic interactions with the heterocyclic base of the nucleoside located in either minor or major DNA groove;[98] (ii) indirect readout representing a mechanism of recognition of the rather thermodynamically unstable DNA structure at the lesion site.[81] Both mechanisms imply the existence of a 'reading head', *i.e.*, a structural unit used by the enzyme to search for lesions and interacting with DNA before its kinking and eversion of the damaged base from the helix take place.[87]

Since the oxoG:C Watson–Crick base pair is thermodynamically stable,[99] it was considered that prokaryotic Fpg enzymes use direct readout mechanism and distinguish the structural features of the oxoG base, *i.e.*, distinguish the N_7–H and C_8=O groups exposed in the DNA major groove.[65] However, later it was found by X-ray diffraction that prokaryotic Fpg enzymes bind DNA from the minor groove side.[51,52] From the structural standpoint, only one *E. coli* Fpg amino acid residue Arg-258 in the 'zinc finger' motif can interact directly with the N_7–H and C_8=O groups of oxoG. In view of the fact that DNA is considerably kinked in a specific enzyme–substrate complex, indirect readout model seems more likely.

Presumably, during one-dimensional diffusion of enzyme along DNA, the amino acid residues of prokaryotic Fpg form weak non-specific additive bonds with the phosphate groups of undamaged DNA with participation of water molecules.[56,100,101] Transition from undamaged DNA to oxoG-containing DNA is accompanied by a decrease in the degree of solvation of the Fpg-DNA complex contact surface and by a change in the type of interaction of amino acid residues of enzyme with the DNA phosphate groups.[56] In a specific complex these contacts arising due to interaction of separate, directly contacting groups are cooperative.[100,101] This transition is accompanied by a free energy change in *E. coli* Fpg binding $\Delta\Delta G = -2.8$ kcal mol^{-1}, the contribution of Fpg to oxoG binding being -0.9 kcal mol^{-1} (Refs 100 and 101).

Five amino acid residues of *E. coli* Fpg: Lys-88, His-89, Arg-108, Arg-109 and Phe-110 cause the DNA kinking; they function together and 'try' to change the DNA structure. The key role in this process is played by the Phe-110 residue. This may cause only a local structural change in the DNA site containing the oxidised base. Although the oxoG:C pair does not introduce conformational changes in the DNA structure [99] and specific interactions between the oxoG and C are slightly weaker than those in the A:T pair,[102] the interaction of the Phe-110 residue with the oxoG:C pair differs from its interaction with A:T or G:C pairs.

When the damaged site has been found, the oxoG residue is everted into a specific pocket of Fpg.

f. Interaction of prokaryotic Fpg enzymes with the oxoG residue of damaged DNA

Molecular dynamic simulation of the interaction of *E. coli* Fpg with damaged DNA has shown [81,103] that the oxodG residue is recognised irrespective of its conformation (*syn* or *anti*) in DNA. The recognition is due to formation of a stable hydrogen bond between the O_8 atom of oxoG and the ε-amino group of Lys-217 (see Fig. 4). The length of this bond is 2.8 ± 0.1 Å and 3.1 ± 0.5 Å for *anti*- and *syn*-conformations, respectively. In addition, the ε-amino group of Lys-217 is involved in a hydrogen bond with the deoxy-

ribose $O_{4'}$ atom of the oxidised nucleoside. Apparently, the formation of these hydrogen bonds facilitates N-glycosidic bond cleavage in the damaged nucleotide unit of DNA and removal of the damaged base from DNA. It is noteworthy that Lys-217 does not form contacts with the undamaged guanine residue located in the vicinity of the *E. coli* Fpg active site irrespective of the oxoG conformation.[81] Therefore, it is assumed that contacts involving Lys-217 account for the substrate specificity of *E. coli* Fpg. This is confirmed by site-directed mutagenesis data.[81] Thus the replacement of Lys-217 by Thr resulted in about fivefold decrease in the catalytic activity of *E. coli* Fpg; the binding efficiency of oxoG-containing substrates was similar to that for the wild type enzyme.

Specific recognition of oxoG-containing DNA by *E. coli* Fpg involves also the Met-73 and Thr-214 residues of the enzyme apart from the Lys-217 residue. For *anti*-oxodG, a hydrogen bond may form between the oxodG N_7H group and the Met-73 carbonyl group rather than between the Lys-217 ε-amino group and the O_8 atom of oxodG, while the hydroxyl group of the Thr-214 may be involved in hydrogen bonding with the exocyclic amino group of oxoG.

Data[65] on the substrate specificity of *E. coli* Fpg indicate that effective removal of a damaged base from DNA requires interactions of this enzyme with the N_1 and O_6 atoms of oxoG. Despite the fact that these atoms are present in both oxidised and undamaged guanine, they form different bonds with the enzyme. Thus the N_1 atom of *anti*-oxodG is involved in electrostatic or van der Waals interaction with the Glu-5 carboxyl group of *E. coli* Fpg. The replacement of the glutamic acid residue by the glutamine residue results in a decrease in the N-glycosylase activity of *E. coli* Fpg.[104]

In the non-specific complex of undamaged DNA with *E. coli* Fpg, interaction of the N_1 atom of undamaged guanine with the Thr-214 carbonyl group takes place instead of the interaction of N_1 with the Glu-5 carboxyl group. In this case, undamaged guanine has a less favourable conformation in the *E. coli* Fpg specific pocket, which probably induces fine rearrangement of the enzyme active site and hampers the removal of guanine. The *syn*-oxodG nucleoside does not interact with Glu-5 but contacts with other amino acids of the *E. coli* Fpg active site.[81]

It follows from the foregoing that Lys-217, Met-73 and Thr-214 of *E. coli* Fpg are responsible for the recognition of a damaged oxoG base, whereas Glu-5 influences the rate of removal of oxoG from the damaged DNA. It is worth noting that Thr-214 and Lys-217, unlike Glu-5, are not highly conserved in the Fpg protein family.

The interaction of oxoG with amino acid residues of the *B. stearothermophilus* E2Q Fpg differs from its interaction with the amino acid residues of *E. coli* Fpg. According to X-ray diffraction analysis, in this case, the looped damaged nucleoside has a *syn*-conformation in the enzyme specific pocket. The N_7H group forms a hydrogen bond with the carbonyl group of Ser-220 (Thr-214 in *E. coli* Fpg). The guanidine group of the Arg-223 residue (Lys-217 in *E. coli* Fpg) does not interact with the O_8 atom of oxoG. The *B. stearothermophilus* E2Q Fpg active site recognises oxoG through hydrogen bonding between non-consered Thr-224 and Glu-78 residues and the N_1 and N_2 atoms of oxoG. In addition, *B. stearothermophilus* E2Q Fpg is involved in four hydrogen bonds with the O_6 atom of oxoG,[55] whereas *E. coli* Fpg forms only one hydrogen bond with this atom. Probably, these differences are related to a conformational change in the *B. stearothermophilus* E2Q Fpg active site caused by replacement of Glu-2 by Gln, namely, replacement of the charged carboxyl group by a neutral amide group.

g. Interaction of prokaryotic Fpg with a DNA strand complementary to the damaged strand

A distinctive feature of all DNA glycosylases is the formation of a large number of contacts with damaged DNA strand, whereas the undamaged strand is retained in the complex mainly due to specific interactions with complementary base pairs of the damaged strand. Prokaryotic Fpg enzymes form a small number of contacts with the opposite DNA strand, the amino acid residues contacting with Fpg being mainly non-conserved.[51–55]

The Fpg enzymes remove oxoG residues from DNA containing non-canonical oxoG:T and oxoG:G pairs more efficiently than from the physiological substrate containing an oxoG:C pair.[65,81] The structures of *B. stearothermophilus* Fpg complexes with DNA containing a reduced AP site opposite to cytosine, guanine or thymine[52] provide a clue for the understanding of how prokaryotic Fpg enzymes can stabilise the productive Michaelis complexes with such DNA but do not explain the order of substrate preference. Presumably, lower thermodynamic stability of oxoG:G and oxoG:T pairs is the main reason for this preference.[65,102]

Structural[51] and biochemical[81] data indicate that the Arg-108 residue of the *E. coli* Fpg enzyme is the key element discriminating the base in the DNA undamaged strand complementary to the damaged strand. The N_ξ and N_ε atoms of the guanidine group of Arg-108 intercalated within the DNA helix form pseudo-Watson–Crick hydrogen bonds with the N_3 and O_2 atoms of cytosine [$C^{(0)}$], thus holding it within the DNA helix. The formation of these hydrogen bonds is facilitated by van der Waals contacts between the Phe-110 residue intercalated between the $C^{(0)}$ and $C^{(1)}$ bases and is 5′-terminal with respect to $C^{(0)}$, which restricts the mobility of these bases.

According to structural data for the *B. stearothermophilus* Fpg complex with DNA containing a reduced AP site opposite to guanine and thymine,[52] guanine occurs there in the *syn*-conformation and forms two contacts with Arg-108 involving O_6 and N_7 atoms, whereas thymine forms only one hydrogen bond with Arg-108 with participation of the O_2 atom. In this case, Arg-108 acquires a conformation in which the repulsion forces between the $N_{\xi 1}$ and N_3 atoms are minimum. Although the structure of the complex of prokaryotic Fpg with DNA containing an oxoG:A pair is unknown so far, it is believed that this DNA is a poor substrate due to its weak binding to this enzyme, high thermodynamic stability of the oxoG:A Hoogsteen pair and the capability of adenine to form only one hydrogen bond with Arg-108.[52]

According to X-ray diffraction data for the covalent complex of *E. coli* Fpg with AP-DNA, the amino acid residues His-89 and Arg-109 of the enzyme are in contact with the phosphate group of the DNA strand complementary to the damaged strand. The replacement of His-89 reduces the specificity of enzyme, whereas the replacement of both amino acids His-89/Arg-109 results in complete loss of Fpg activity with respect to oxoG-containing DNA.[81]

2. Eukaryotic 8-oxoguanine-DNA glycosylases

Functional analogues of the prokaryotic Fpg enzyme, the eukaryotic enzymes Ogg1, were found in the cell of many

eukaryotes.[105–107] It is of interest that plants contain simultaneously the *fpg* and *ogg1* genes.[108–110] The reason for retention of both DNA glycosylase genes having similar functions in plants has not been elucidated as yet. Perhaps, they are located in different organelles or exhibit different specificities in plant cells.

The *ogg1* gene of the yeast *Saccharomyces cerevisiae* was the first to be cloned; its nucleotide sequence was established.[106] This gene encodes the yOgg1 protein comprising 376 amino acids, its molecular weight is 43 kDa.[106,111] A comparison of the amino acid sequences of *E. coli* Fpg and yOgg1 has shown the lack of homology between these enzymes. It was found that the *yogg1* gene is homologous to the gene encoding endonuclease III of *E. coli* (Endo III), and the yOgg1 enzyme is a structural analogue of the Endo III enzyme. Later, cDNA of mammalian Ogg1 homologous to the *S. cerevisiae ogg1* gene were cloned.[20,105,112,113]

In human cells, the *hogg1* gene is located on chromosome 3 (3p25).[112–115] It comprises 8 exons.[113] By alternative splicing using exons 7 or 8, two main hOGG1 isoforms were expressed; they were identical in the first 316 amino acid residues but differed in the C-terminus.[116] The hOGG1-α protein contained a nuclear localisation signal, while the hOGG1-β protein had a mitochondrial or chloroplast (for plants) localisation signals.[116,117]

The hOGG1-α protein (molecular weight 39 kDa) comprises 345 amino acid residues and hOGG1-β (47 kDa) has 424 amino acid residues.[114–116] The enzyme hOGG1-α is a bifunctional DNA glycosylase/AP lyase, which removes oxoG and FapyG from DNA and cleaves the sugar-phosphate DNA backbone according to β-elimination mechanism.[105,111–114,117] The substrate specificity and the catalytic mechanism of hOGG1-β have not been studied in detail but it has been shown that this enzyme cleaves oligonucleotide substrates containing an oxoG:C pair.[114]

Apart from the two major isoforms, hOGG1-α and hOGG1-β, several more minor hOGG1 isoforms differing in the use of alternative splicing sites were detected in the human cells at the mRNA level.[113,116] However, it remains obscure whether translation of these mRNA takes place.

The eukaryotic Ogg1-α enzyme was found and characterised in *S. cerevisiae*, *Arabidopsis thaliana*, *Drosophila melanogaster* cells and in mammalian cells, whereas Ogg1-β was found only in human cells.[117] In what follows, by hOGG1 we mean the hOGG1-α isoform because this was used in all structural and functional studies reported in the literature.

Structural analysis of the *hogg1* gene and its transcripts has shown that due to a genetic polymorphism, two proteins with different amino acids at codon 326, hOGG1-Ser-326 and hOGG1-Cys-326, are produced in human cells.[118] The repair activity towards oxoG residues was greater in hOGG1-Ser-326.

The hOGG1 enzyme belongs to the family of DNA glycosylases the typical feature of which is the presence of a 'helix–hairpin–helix' motif followed by a Gly/Pro-rich loop ending by an invariant aspartic acid residue (HhH-GPD motif) in the active site.[119] This family includes monofunctional DNA glycosylases: *E. coli* 3-methyladenine-DNA glycosylase (AlkA), *E. coli* adenine-DNA glycosylase (MutY), and bifunctional DNA glycosylases/AP lyases: the endonucleases *Micrococcus luteus* UV, Endo III (known also as Nth1), yNtg1, yNtg2 and yeast and mammalian Ogg1 enzymes.[111,119] It was found that the functional lysine residue in the HhH motif is conservated for all proteins of this family possessing lyase activity and is missing in the proteins that do not show this activity.[120–122]

a. Structure of eukaryotic 8-oxoguanine-DNA glycosylase

Of all identified eukaryotic 8-oxoguanine-DNA glycosylases, the tertiary structure has been solved only for a minimum hOGG1 fragment containing amino acid residues 12–327.[123] Attempts to obtain crystals of full-size hOGG1 and its complexes with DNA failed. The non-structured peptide segments were removed by restricted trypsinolysis of hOGG1 from N- and C-termini. This gave a minimum hOGG1 fragment, the so-called catalytic core having the same catalytic activity as the wild type protein but binding to DNA with lower affinity.[123]

In 2000, the mutant form of the minimum fragment of K249Q-hOGG1 having no catalytic activity but retaining the ability to recognise the oxoG:C pair in DNA was crystallised as a complex with oxoG-DNA and the structure of this complex was studied by X-ray diffraction.[123] Later, the structures of the hOGG1 catalytic core and its covalent complex with DNA containing uncleavable analogue of the AP site were solved.[124–126] It was found that the conformations of hOGG1 in these structures are similar, except for the active site.[123,125] Like other enzymes of this structural class, hOGG1 contains two α-helix domains and an antiparallel β-folded domain. This β-domain was found also only in AlkA. Perhaps, it participates in protein–protein interactions (Fig. 5).

The invariant amino acid residue Asp-268 located at the C-terminus of the HhH-GPD motif of hOGG1 is essential for both support and the DNA glycosylase and AP-lyase activities of the enzyme.[127] It was found that Asp-268

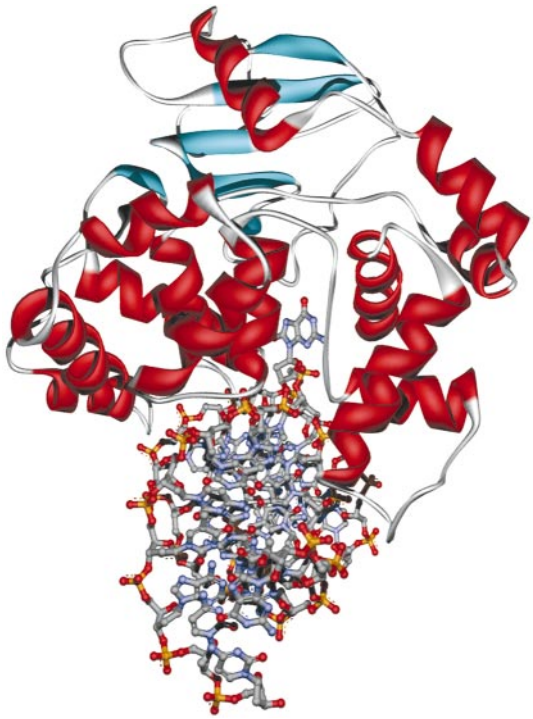


Figure 5. Structure of the K249Q-hOgg1 complex with DNA containing an oxoG:C pair (PDB code 1EBM).[123]
α-Helical domains of the enzyme are shown in red the β-folded domain is light blue.

participates in the orientation of the deoxyribose of damaged nucleoside necessary for successful catalytic cleavage of oxoG-containing DNA with the hOGG1 enzyme. The replacement of Asp-268 by asparagine does not cause a change in the protein structure but causes complete loss of the enzymatic activity.

Analysis of X-ray diffraction data of the D268N-hOGG1 complex (hOGG1 minimum fragment) with oxoG-containing DNA [127] and complexes of D268E-hOGG1 and D268Q-hOGG1 with DNA containing a tetrahydrofuran residue revealed that Asp-268 interacts with the N-terminal amino acids of the αM-helix located directly after the HhH-GPD motif. The carbonyl group of Asp-268 participates in the formation of hydrogen bonds with the amide group of the Met-271 residue and the imidazole ring of His-270, thus ensuring the αM-helix orientation needed for the successful catalytic event. These interactions play an important role in the formation of hOGG1 tertiary structure.[127]

Note that the Glu-2 residue of prokaryotic Fpg plays the same role as the Asp-268 residue of hOGG1. By forming hydrogen bonds with the amide groups of the α-E helix backbone of DNA-binding H2TH motif, the Glu-2 residue of *B. stearothermophilus* Fpg ensures the orientation of this helix needed for the successful catalytic cleavage of damaged DNA.[55]

In 2005, the structures of hOGG1 catalytic core complexes with undamaged DNA and with oxoG-containing DNA were established.[128] For this purpose, Verdine and coworkers [128] developed and applied a strategy for covalent binding of DNA to the enzyme. To this end, two modifications were performed: (i) a disulfide linker group was introduced into DNA at the exocylic amino group of cytosine complementary to oxoG; (ii) the Asn-149 residue in hOGG1 was replaced by Cys. The enzyme N149C-hOGG1 thus produced showed a catalytic activity similar to the activity of wild type hOGG1. The reaction between thus modified enzyme and DNA gave a disulfide bond. Later (in 2006), this approach was used to determine the structure of the covalent complex of the N149C-hOGG1 enzyme with oxoG:G-DNA in which the guanine residue located directly at the 5′ to oxodG proved to be extrahelical.[129] These data shed light on a most intriguing aspect of the base excision repair related to the mechanism of the search for genome lesions by DNA glycosylases [129, 130] (see Section II.2.e).

In 2007, the structures of H270A-hOGG1, Q315A-hOGG1, Q315F-hOGG1 and G42A-hOGG1 complexes with oxoG:C-DNA were determined.[96] It was found that amino acid residues His-270, Gln-315 and Gly-42 present in the hOGG1 active site form bonds with extrahelical oxidised nucleotide. Thus His-270 forms a hydrogen bond with the 5′-terminal phosphate group relative to oxodG. This allows the hOGG1 enzyme to distinguish between oxoG and adenine, cytosine or thymine but not guanine. The only hOGG1 amino acid residue that recognises oxoguanine and guanine is Gly-42. The Gly-42 residue is a part of oxoG-recognising loop (amino acid residues 35–43) of hOGG1 the conformation of which is rigidly fixed by numerous hydrogen bonds.[96] The rigid fixing of the Gly-42 conformation is essential for differentiation of damaged and undamaged guanine by the hOGG1 enzyme. These data provided detailed understanding of the recognition process of the DNA damaged base by eukaryotic 8-oxoguanine-DNA glycosylases.

b. Substrate specificity of the eukaryotic Ogg1 enzyme

Eukaryotic Ogg1 enzymes remove oxoG residues located opposite cytosine, thymine and guanine from DNA but they are almost inactive towards duplexes containing a oxoG:A pair.[112] It was shown in *in vitro* experiments that N-glycosylase and AP-lyase reactions of Ogg1 with a DNA substrate containing an oxoG:C pair are most efficient.[105, 107, 131] In the case of formation of non-canonical pairs of oxoguanine with other bases, only glycosylase reaction takes place, its efficiency being greater for DNA containing an oxoG:T pair.[111, 132]

It was shown that eukaryotic 8-oxoguanine-DNA glycosylases split the DNA containing FapyG, N_7-Me-FapyG, oxoA, oxoI and 7,8-dihydro-8-oxo-6-*O*-methylguanine (6-OMe-oxoG) residues.[107, 131–134] Note that eukaryotic Ogg1 enzymes remove the N_7-Me-FapyG residues much less efficiently than oxoG,[105, 106] while prokaryotic Fpg enzymes remove these residues from damaged DNA with equal efficiency.[46, 47] The yOgg1 enzyme, unlike Fpg, does not remove FapyA residues,[131] while mOgg1 removes 8-oxonebularine residues from oligonucleotide substrates containing oxodN:dC and oxodN:dG pairs and 8-aminoguanine residues less efficiently than oxoG residues.[105, 132]

The efficiency of cleavage of DNA containing non-canonical base pairs, oxoG:A, oxoG:T or oxoG:G, by eukaryotic Ogg1 and prokaryotic Fpg are considerably different. Eukaryotic enzymes cleave DNA containing cytosine opposite the damaged base approximately twice as efficiently as DNA containing any other nucleoside. Conversely, prokaryotic enzymes are 10–30 times more efficient in the cleavage of DNA with guanine or thymine opposite the damaged base than DNA with cytosine.[132]

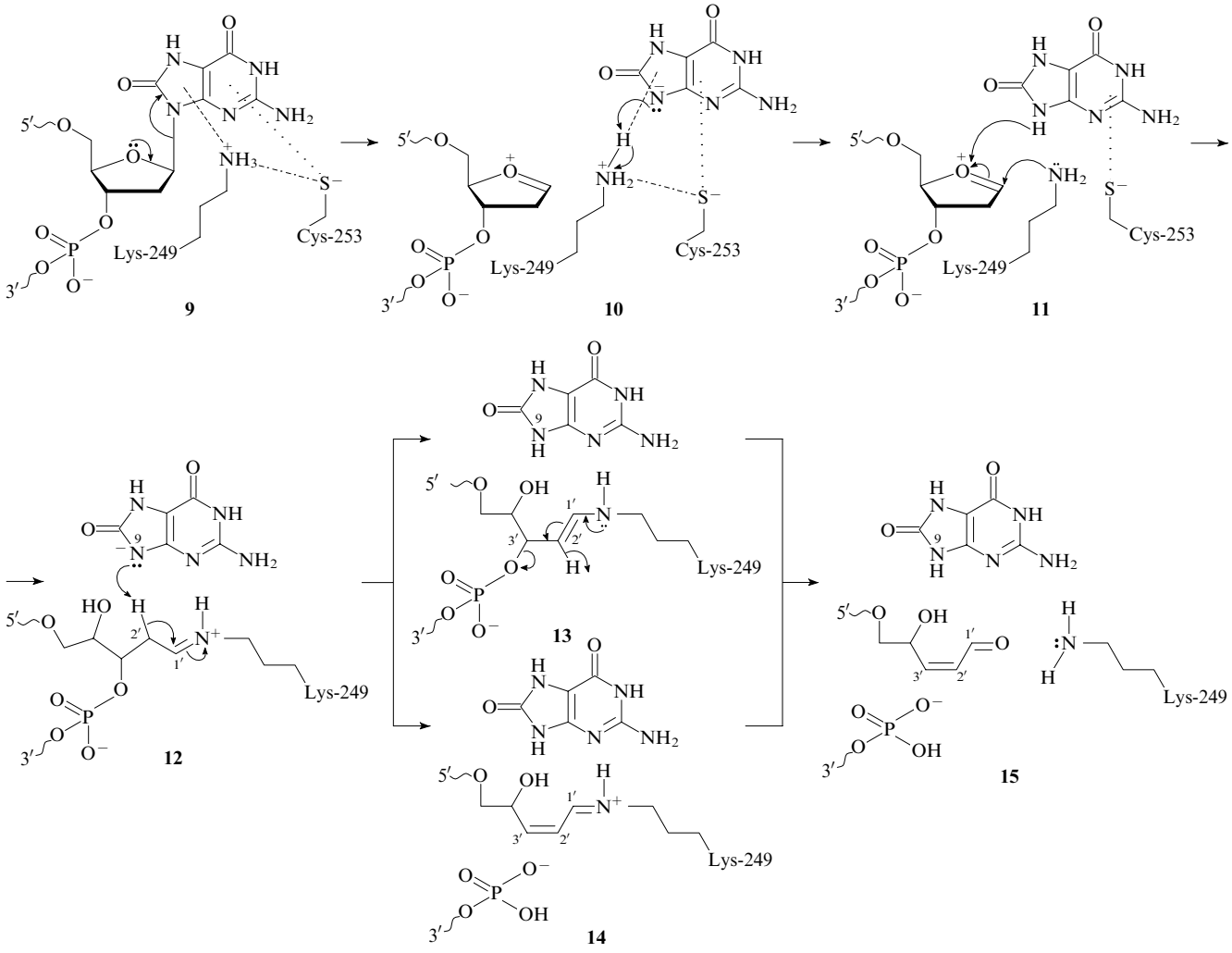
Yet another substrate of eukaryotic Ogg1 is DNA containing an AP site; the efficiency of AP site removal decreases in the series AP:C > AP:T ≫ AP:G.[135] Eukaryotic Ogg1 exhibiting AP-lyase activity cleaves DNA from the 3′-terminus relative to the lesion by β-elimination mechanism.[135–137] The AP-lyase activity of eukaryotic Ogg1 is much lower than the DNA glycosylase activity.[132]

It was found that upon interaction with DNA containing an AP site, the rOgg1 enzyme (similarly to *E. coli* Fpg) removes the deoxyribose residue by the β,δ-elimination mechanism [107] and yOgg1 has a deoxyribosophosphatase activity.[138]

Recently it has been shown that synthetic DNA duplexes containing pyrophosphate and *O*-ethyl-substituted pyrophosphate groups in different positions towards the oxoG residue are analogues of hOGG1 substrates.[60, 61]

In addition to Ogg1, the Ogg2 protein with a molecular weight of ~36 kDa, which eliminates the oxidised heterocyclic bases paired with purine bases, was found in *S. cerevisiae* and HeLa cells.[111, 137, 139] The efficiency of the removal of oxoG by hOGG2 decreases in the oxoG:G > oxoG:A > oxoG:T ≡ oxoG:C series.[111] It was shown that yOgg2 removes FapyG residues and splits DNA containing AP sites by the β-elimination mechanism.[104] It is noteworthy that the yOgg2 protein can exhibit activity only in the presence of EDTA and is fully inhibited by a 0.2 M KCl solution. The biological role of Ogg2 is to repair oxoG residues inserted in DNA during replication as oxodGMP, thus preventing T:A → G:C transversions.[102, 104]

Scheme 2



c. Catalytic mechanism of substrate cleavage by eukaryotic Ogg1 enzymes

The catalytic removal of the damaged base oxoG by eukaryotic Ogg1 enzymes occurs by S_N1 mechanism and comprises not less than five successive stages taking place in one active site (Scheme 2).[119, 126, 127, 140] In the first stage, the ε-amino group of Lys-249 and the sulfhydryl group of Cys-253 belonging to the HhH-GPD motif of hOGG1 form a π-cationic (designated by dashed line) and van der Waals (dotted line) bonds, respectively, with the damaged base (compound **9**) and a hydrogen bond between each other (dash-and-dot line). This results in elimination of oxoG as the $oxoG^-$ anion to give the oxocarbenium cation (DNA-glycosylase activity) (Scheme 2, compound **10**).[119, 126, 127] Note that the eliminated $oxoG^-$ base is not released from the DNA–protein complex but remains bound in the hOGG1 active site and is used by the enzyme as a cofactor (acid-base catalyst). Thus $oxoG^-$ abstracts the proton from the $N_ε$ atom of the Lys-249 residue, thus activating this residue toward the nucleophilic attack on the $C_{1'}$ atom of the deoxyribose residue of damaged nucleoside (Scheme 2, compound **11**), resulting in the formation of a covalent DNA–protein intermediate (compound **12**).§ During the nucleophilic attack, the hydrogen bond between the Lys-249 and Cys-253 residues is destroyed. Then oxoG participates in proton transfer to the $O_{4'}$ atom, resulting in opening of the deoxyribose ring of the damaged nucleoside. The next stage is catalysis of the cleavage of the sugar-phosphate backbone by Ogg1 at the 3′-terminus from the resulting AP site (AP-lyase activity).[119, 126] This reaction follows the β-elimination mechanism and is initiated by the removal of the proton from the $C_{2'}$ atom of the damaged nucleoside to give either enamine (Scheme 2, compound **13**) or α,β-unsaturated Schiff's base (compound **14**). If the enamine is formed, the next stage is spontaneous β-elimination resulting in Schiff's base. As a consequence, one of DNA strands is broken[91, 92, 119] (products **15**).

The stereochemistry of substituents at the C=N and C=C bonds in compounds **12**–**14** has not been determined to date. In the absence of cofactor, hOGG1 exhibits low stereospecificity and removes 65% of *pro-S* protons and 35% of *pro-R* protons. In the presence of a nucleic base, hOGG1 has a higher specificity, 75%–85% of the *pro-R* protons being removed.[126]

It is noteworthy that the use of oxoG as a cofactor for the cleavage of the DNA sugar-phosphate backbone with hOGG1 is the only currently known example of enzymatic catalysis accomplished by a catalytic cleavage product.[126] The catalytic cleavage of the AP-containing DNA with the hOGG1 enzyme is accelerated more than 75-fold in the

§ Intermediate **12**, which is the Schiff base, was detected upon sodium borohydride reduction.[126]

presence of 8-aminoguanine, more than 35-fold in the presence of 8-bromoguanine, and approximately 10-fold in the presence of guanine.[126]

As has been noted above (see Section II.1.c), the activity of DNA glycosylase in β,δ-elimination is correlated with the efficiency of coordination of the leaving phosphate groups of DNA by enzyme amino acids. Indeed, the hydrogen bonds with the 3′-phosphate relative to the modified nucleoside involves two amino acid residues (Asn-149 and Asn-150) in the case of hOGG1 enzyme and three residues (Lys-56, Arg-258 and Asn-168) in the case of *E. coli* Fpg. This accounts for weaker AP-lyase activity of the hOGG1 enzymes compared to *E. coli* Fpg.[123] The rate-determining step of the catalytic cleavage of damaged DNA by eukaryotic Ogg1 enzymes is the β-elimination. As opposed to this, for prokaryotic Fpg enzymes, cleavage of the N-glycosidic bond is the rate-determining step. Thus, the presence of proton donors in the environment of phosphate groups plays a key role in the cleavage process of damaged DNA by 8-oxoguanine-DNA glycosylases.

d. Conformational changes in the structure of hOGG1 complex with damaged DNA

Upon interaction with damaged DNA, eukaryotic Ogg1 enzymes change considerably its structure while contacting mainly with the damaged strand.[123] Thus hOGG1 'compresses' the sugar-phosphate DNA backbone twofold at the damage site as compared with the DNA B-form, thus decreasing the distance between the 8-oxoguanosine-flanking phosphates.[123] Compression is a result of formation of hydrogen bonds between the amino acid residues of the hOGG1 active site and the DNA phosphate groups, the oxoG residue being extrahelical¶ and positioned in a specific 'pocket' of the enzyme catalytic site.[123, 128]

The side chains of the hOGG1 amino acid residues Asn-149, Tyr-203 and Arg-204 are inserted into the cavity formed upon extrusion of the damaged base. Intercalation of Tyr-203 between the cytosine of oxoG:C [$C^{(0)}$] pair and a neighbouring (at the 5′-terminus) base makes a considerable contribution to the deformation of the DNA sugar-phosphate backbone and recognition of the oxoG:C pair (similarly to the recognition of Phe-110 of *E. coli* Fpg).[123] The cytosine $C^{(0)}$ remains intrahelical through the formation of five hydrogen bonds with amino acid residues Asn-149, Arg-154 and Arg-204 of hOGG1. The cytosine $C^{(0)}$ does not participate in the stacking interactions with neighbouring heterocyclic bases due to sharp bending (70°) of DNA from the plane of the oxoG:C pair. The amide oxygen of the Asn-149 residue interacts with the cytosine $C^{(0)}$ amino group. Each of the amino acid residues Arg-154 and Arg-204 located in the close vicinity of the heterocyclic ring of the cytosine $C^{(0)}$ form simultaneously two hydrogen bonds with the cytosine N_3 and O_2 atoms. These interactions are exceptionally strong and specific; they allow the enzyme to distinguish between cytidine and other nitrogen bases of DNA. The DNA segment not contacting with the enzyme occurs in the canonical B-form.

According to structural data, the formation of the oxoG-binding 'pocket' of hOGG1 involves the amino acid residues His-270, Gln-315 and Phe-319.[123, 125] The conformations of side groups in these three amino acid residues determine the two main forms of the enzyme specific 'pocket': an open form for hOGG1 linked to oxoG-containing DNA and a closed form for free enzyme.[123, 124] Transition between the closed and open forms of the oxoG-binding 'pocket' of hOGG1 is a result of a concerted change in the conformations of side groups of these three amino acids. For example, on binding of oxoG-DNA to hOGG1, the aromatic ring of Phe-319 rotates by 90°. This destroys π-stacking interactions between the amino acid residues His-270 and Phe-319, while a hydrogen bond is formed between His-270 and the 5′-phosphate group of oxodG. The Phe-319 residue, in turn, participates in the van der Waals contact with oxoG, and the Gln-315 residue forms two hydrogen bonds with N_1-imino- and N_2-amino groups of oxoG, thus stabilising it in the hOGG1 specific 'pocket'.[128]

Biochemical data also indicate that binding of damaged DNA to the hOGG1 enzyme results in a change in orientation of the amino acid residues His-270, Gln-315 and Phe-319.[141] Thus the replacements H270 → A, H270 → L, Q315 → A and F319 → A decrease substantially the efficiency of DNA-binding and N-glycosylase activities of hOGG1 but do not influence its AP-lyase activity. The H270 → R replacement results in a slight decrease in the binding and glycosylase activities of the enzyme with respect to DNA containing an oxoG:C pair.[101] The decrease in the activity of H270A-hOGG1 upon the H270 → A replacement is caused by violation of the specific interaction of His-270 with the 5′-phosphate group at oxodG.

Kuznetsov *et al.*[142, 143] studied the pre-steady-state kinetics of enzymatic reactions with participation of hOGG1 by measuring the fluorescence of tryptophan residues in proteins and 2-aminopurine in DNA using the stopped-flow method. They found that during the catalytic cycle numerous conformational changes take place both in the enzyme and in the DNA substrate. These changes result in mutual adjustment of the structures of reacting molecules for the formation of catalytically active states of enzymes and promote the transformation of the substrate into the product. The hOGG1 enzyme is linked to the oxoG substrate in three stages. First, a non-specific complex is formed, resulting in 'loosening' of the substrate double helix and fast eversion of the damaged base to the enzyme catalytic site. The second and third stages comprise successive insertion of amino acid residues into the cavity of DNA duplex formed after eversion of the oxoG residue from the chain. It was shown that discrimination of substrates in terms of the base located opposite the damaged base takes place in the second stage of the process, whereas in the third stage a catalytically competent complex is formed. The chemical stages of the enzymatic process take place consecutively in this complex. The enzymatic cycle is completed by the equilibrium dissociation of enzyme – product complex.

e. Search for oxoG residues in the genome by eukaryotic hOGG1 enzyme

The most recent data obtained using an advanced technique that allows one to detect sliding of the enzyme molecule along an extended DNA strand show that the hOGG1 enzyme binds DNA within fractions of a second under physiological conditions and slides along it at a rate higher than 2×10^7 base pairs per second.[144] Using this technique, the assumption of processivity of *E. coli* Fpg was confirmed.

The data obtained by X-ray diffraction and atomic force microscopy indicate that during the search for

¶ Eukaryotic Ogg1 enzymes, unlike prokaryotic 8-oxoguanine DNA glycosylases, extrude the oxoG residue through the DNA major groove.[128]

damaged bases, hOGG1 bends DNA.[128] Indeed, in the N149C-hOGG1 complex with undamaged DNA, the DNA duplex is bent by 80° to the 5′-terminus and is over-rotated by ~20° about the helix axis.† In the complex of this enzyme with oxoG:G-containing DNA, bending of the helix to the 5′-terminus is even greater.[129]

On the basis of X-ray diffraction data for the covalent complexes of the mutant N149C-hOGG1 enzyme with DNA duplexes containing oxoguanine or undamaged guanine, Verdine and co-workers[128] suggested that during the search for damaged bases, the hOGG1 forms complex with each guanine residue. If this is an undamaged base, it is fixed in the so-called '*exo*-site' of the enzyme active sites where it forms contacts with the amino acids Phe-319 and His-270. In a complex with the damaged base, it gets into the specific pocket located at a 5 Å distance away from the '*exo*-site' of hOGG1 through rotation by more than 110° around the $C_{4'}-C_{5'}$ and $P-O_{5'}$ bonds of the damaged nucleoside and around the $C_{4'}-C_{5'}$ bond of the nucleoside located at the 3′-terminus from the damaged one. The OxodG residue also interacts with the enzyme amino acid residues Phe-319 and His-270; however, these interactions differ appreciably from those involving guanosine. In the specific complex, His-270 is hydrogen bonded to the 5′-phosphate group of oxodG, whereas in the non-specific complex it is weakly π-bound to guanine.[128, 129]

Oxoguanine also differs from guanine in the electrostatic potential; charge inversion in positions 7 and 8 gives rise to opposite directions of the local dipoles of these bases.[128] Quantum mechanical calculations showed that an essential contribution to the preferred binding of oxoG over guanine to hOGG1 is made by the dipole–dipole interactions of this base with the Lys-249(NH_3^+)/Cys-253(S^-) pair of the hOGG1 active site. If one assumes that guanine occupies the same position as oxoG in the hOGG1 active site, then the dipole moment of the pair Lys-249(NH_3^+)/Cys-253(S^-) would be antiparallel to the dipole moment of oxoG but parallel to the guanine dipole moment. As a result, the dipole Lys-249(NH_3^+)/Cys-253(S^-) would stabilise the interaction of hOGG1 with oxoG and destabilise the interaction of hOGG1 with guanine. An important role in the specific binding of oxoG in the hOGG1 active site is played by the Gly-42 residue. On passing from oxoG to G, the hydrogen bond between the Gly-42 carbonyl group and the N_7H fragment of oxoG is replaced by strong repulsive interaction between Gly-42 and the lone pair of the N_7 atom of guanine. The hOGG1 enzyme uses these interactions for discrimination of oxoG form undamaged guanine. Despite the fact that there are no data on the rate constants for extrusion of oxoG and G from the DNA helix, it has been assumed that enzymes extrudes the oxidised base at a higher rate than the undamaged one.[144]

At early stages of base extrusion, conserved amino acids Gly-245, Lys-249 and Val-250 of the HhH motif of hOGG1 form contacts with 3′-phosphate groups of the extruded nucleoside. These contacts were detected in the mutant N149C-hOGG1 enzyme complexed with both oxoG- and oxoG:G- or G-containing DNA.[128, 129] In later stages of extrusion, after oxoG insertion into the hOGG1 specific pocket, the hOGG1 enzyme forms contacts with the 5′-phosphate group of the extrahelical nucleoside.[128, 129]

† In the N149C-hOGG1 mutant enzyme complexed with DNA containing an extrahelical oxoG, the helix kinking is smaller being equal to 70°.

III. Adenine-DNA glycosylases

Adenine-DNA glycosylases catalyse the removal of adenine residues from oxoG:A pairs, while the AP site remains in the DNA molecule.[145] A lot of knowledge has now been accumulated on the structure, substrate specificity and the catalytic mechanism of prokaryotic adenine-DNA glycosylases. Published data on the eukaryotic homologues of these enzymes are scarce. Therefore, this Section mainly summarises the data on prokaryotic adenine-DNA glycosylases.

1. Prokaryotic adenine-DNA glycosylases

Prokaryotic adenine-DNA glycosylases (MutY) belong to the family of DNA glycosylases containing an HhH-GPD motif in the structure and removing genotoxic DNA lesions.[146] Prokaryotic MutY enzymes differ from other members of this structural class in that apart from the N-terminal domain (catalytic core of the enzyme), they contain an additional C-terminal domain responsible for recognition of oxoG residues.[147] The removal of the C-terminal domain results in the loss of the ability of MutY enzymes to distinguish between A:oxoG and A:G pairs.

Analysis of amino acid sequences revealed the presence of common evolution predecessors of the C-terminal domains of *E. coli* MutY and *E. coli* MutT.[147, 148] In the sequencing of the gene *mutY*, it was found that the N-terminal domain of *E. coli* MutY is homologous to the N-terminal domain of the *E. coli* Endo III enzyme.[147]

a. Structure of prokaryotic MutY enzyme

The structures of N- and C-terminal domains of *E. coli* MutY were determined by X-ray diffraction and high-resolution NMR.[146, 148] In addition, full-size MutY enzyme (molecular weight 41 kDa) isolated from thermophilic bacteria *B. stearothermophilus* and the covalent complex of the mutant catalytically inactive form of *B. stearothermophilus* D144N-MutY with DNA containing an oxoG:A pair (Fig. 6) have been crystallised.[149]

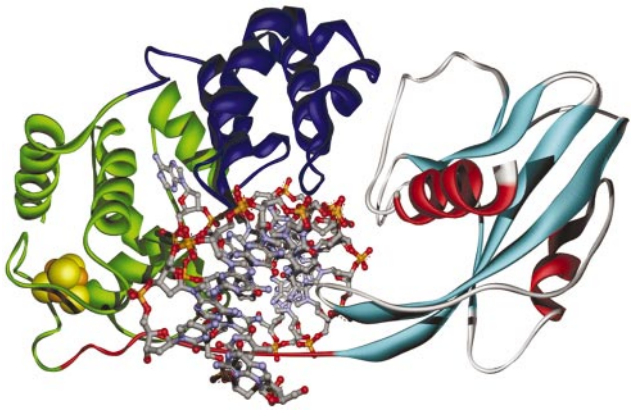


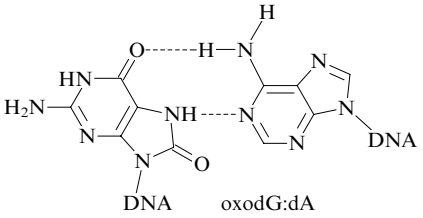
Figure 6. Structure of the *B. stearothermophilus* D144N-MutY complex with DNA, containing an oxoG:A pair (PDB code 1RRQ).[149] The enzyme [4Fe-4S]-subdomain is shown in green, the 6-helix subdomain (HhH-GPD motif) in blue, the linker connecting the N- and C-terminal domains in red. The C-terminal domain is coloured along the secondary structure (β-structures are light blue, α-helixes are red, and the loops are grey). The sulfur and iron atoms of the [4Fe-4S] cluster are shown in yellow and orange, respectively.

According to the obtained results, the catalytic core of prokaryotic adenine-DNA glycosylases consists of two α-helical subdomains: a subdomain formed by six α-helices and containing an HhH-GPD motif and a $[4Fe\text{-}4S]^{2+}$-cluster subdomain formed by five α-helices and a $[4Fe\text{-}4S]^{2+}$-cluster-binding loop. The N- and C-terminal domains are connected by a partially disordered linker comprising ten amino acids. When prokaryotic MutY interacts with DNA, this linker crosses the major groove of the duplex near the damaged site (see Fig. 6). The lysine residues present as part of the linker (in the case of *B. stearothermophilus* MutY, these are Lys-228, Lys-230, Lys-231 and Lys-235) ensure the electrostatic interactions of the enzyme with DNA.[149]

The $[4Fe\text{-}4S]^{2+}$ cluster is coordinated by four cysteine residues of the amino acid sequence Cys-192-X_6-Cys-199-X_2-Cys-202-X_5-Cys-208, where X is the residue of any amino acid.[146] Studies of its functional significance for acquiring a tertiary structure by *E. coli* MutY have shown that denatured protein can restore its structure in the absence of iron and sulfur atoms. According to circular dichroism spectroscopy data, coiling of an enzyme globule does not require a $[4Fe\text{-}4S]^{2+}$ cluster. Moreover, it does not influence the thermal stability of *E. coli* MutY.[80] However, the *E. coli* MutY enzyme devoid of the $[4Fe\text{-}4S]^{2+}$ cluster does not exhibit an adenine-DNA glycosylase activity. This enzyme is incapable of specific binding the substrate DNA but performs only non-specific binding. Thus, the presence of the $[4Fe\text{-}4S]^{2+}$ cluster in the MutY enzyme is significant not only for catalytic activity, but also for the specific recognition of substrates by this enzyme.[80, 146]

b. Substrate specificity of prokaryotic MutY enzyme

Prokaryotic adenine-DNA glycosylases have rather broad substrate specificity. The efficiency of these enzymes depends on the structure of both bases of the non-canonical oxoG:A pair of DNA but generally MutY enzymes are more sensitive to changes in the adenine structure. Below we present bases that can replace one of the components of the oxoG:A pair of DNA without a change in the catalytic activity of prokaryotic MutY proteins.[80]



oxodG:dA

Residues replacing oxodG in the non-canonical oxodG:dA pair	Residues replacing dA in the non-canonical oxodG:dA pair
oxodA, oxodN, oxodI, dG, dC, dI, 2′-*O*-methylguanosine, 8-methoxy-2′-deoxyguanosine, 2′-deoxyribosyl-2-amino-1,6-dihydropurine	oxodA, oxodN, dI, 2-hydroxy-2′-deoxyadenosine, 2′-deoxy-ribosyl-2-amino-1,6-dihydro purine, 2′-*O*-methyladenosine

The MutY enzymes remove adenine from oxoG:A, G:A and C:A pairs of DNA both *in vivo* and *in vitro*. The removal of adenine from the oxoG:A pair is sixfold faster than from the G:A pair.[150] It is worth noting that the adenine-DNA glycosylase activity of MutY with respect to DNA containing G:A pairs is 5–35 times higher than that with respect to DNA containing C:A pairs. A study of the substrate specificity of the MutY enzyme using DNA containing the 2′-deoxyformycin residue (non-cleavable analogue of 2′-deoxyadenosine) complementary to oxoG or guanine has shown that MutY binds to the oxoG-containing substrate 30 times more tightly than to the substrate containing a guanine residue. This serves as additional evidence for the fact that DNA containing an oxoG:A pair is the preferred substrate for prokaryotic MutY.[151]

In addition, it was shown that the *E. coli* MutY protein removes 2-hydroxyadenosine (2-OH-A) residues from the 2-OH-A:oxoG pair but not from the 2-OH-A:G pair.[152]

c. Catalytic mechanism of the prokaryotic MutY enzyme

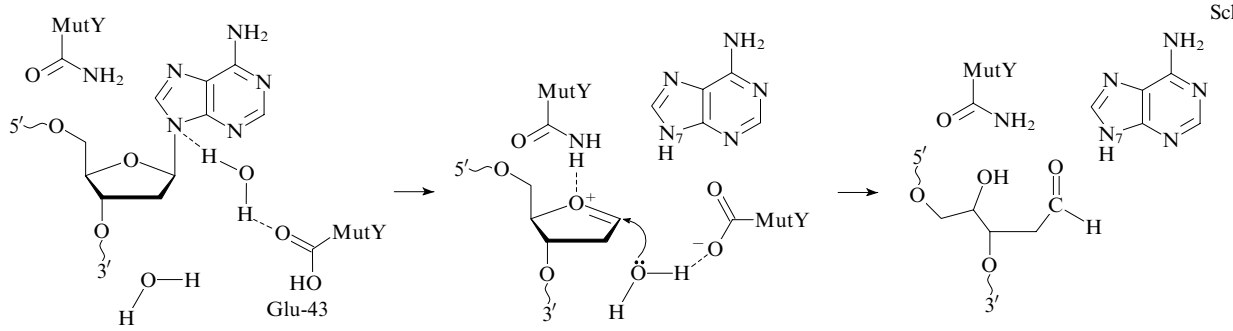
Prokaryotic MutY enzymes are monofunctional DNA glycosylases.[153] For nucleophilic attack of the damaged nucleoside, they use a water molecule located in the enzyme active site. This distinguishes monofunctional adenine-DNA glycosylases from bifunctional DNA glycosylases, which use amino acid residues from the enzyme active site for this purpose.

The mechanism of functioning of prokaryotic MutY enzymes is as follows. In the first stage, MutY enzymes bind non-specifically to DNA mainly through electrostatic and hydrophobic interactions. While diffusing along a DNA molecule, these enzymes recognise the non-canonical pair and specifically bind to it, adenine being everted into the active cleft site. The catalytic cleavage of the substrate by MutY enzyme occurs by dissociative nucleophilic substitution mechanism (S_N1) (Scheme 3).[153] Cleavage of the N-glycosidic bond yields an oxocarbenium ion; in the case of *B. stearothermophilus* MutY, the ion is stabilised by Asn-144 situated in the vicinity of the adenosine $O_{4'}$ atom. In the next stage an enzyme-activated water molecule attacks nucleophilically the deoxyribose $C_{1'}$ atom of the substrate nucleoside, which results in ring opening of deoxyribose.

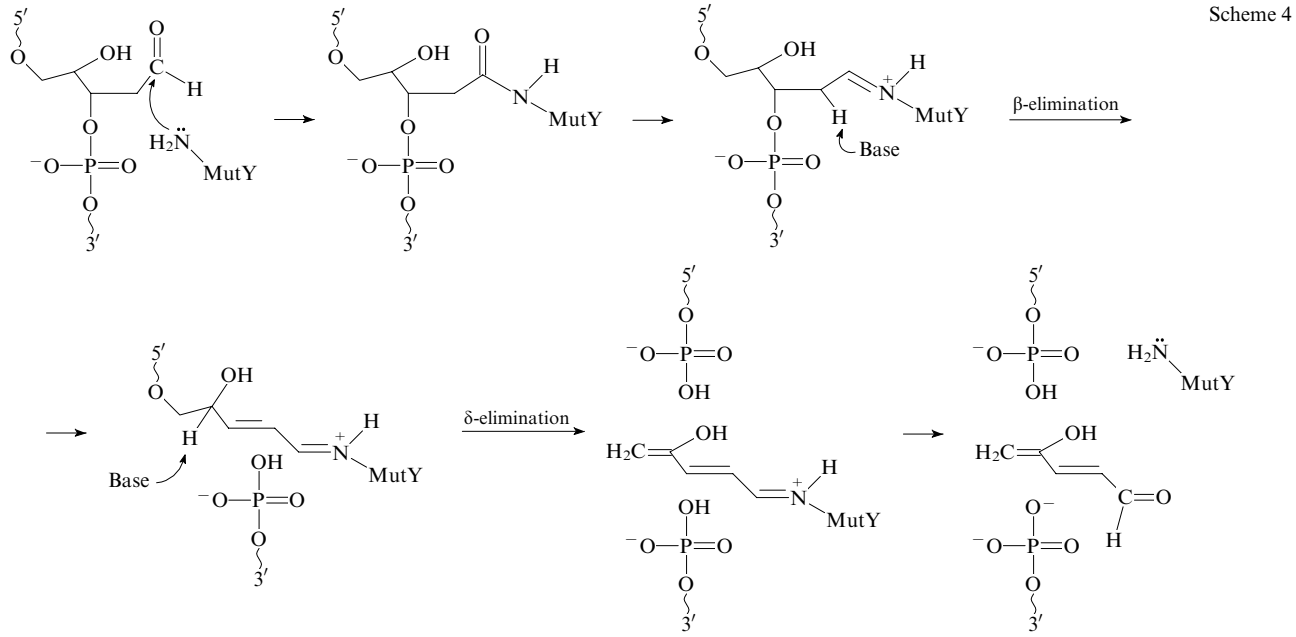
According to X-ray diffraction data for the covalent complex of *B. staerothermophilus* MutY with DNA containing an oxoG:A pair, the enzyme active site contains two oriented water molecules. One water molecule is hydrogen bonded to the N_7 atom of adenine, thus stabilising the base as the leaving group at the moment of N-glycosidic bond scission, while the other one located in the near vicinity of the deoxyribose ring of the substrate nucleoside attacks the oxocarbenium intermediate. Both water molecules form hydrogen bonds with the carboxyl group of the Glu-43 residue of *B. stearothermophilus* MutY enzyme, which behaves as an acid-base catalyst. Thus Glu-43 transfers the proton to the adenine N_7 atom through a water molecule, thus decreasing the transition state energy in the base removal reaction. The carboxylate ion of Glu-43 activates the second water molecule toward the nucleophilic attack. In the case of *E. coli* MutY, the adenine N_7 atom is protonated by the Glu-37 residue, and Asp-138 activates the water molecule for the nucleophilic attack.[146, 153]

This interaction scheme is consistent with the experimental fact, indicating that replacement of N_7 atom by a C_7H group gives a substrate analogue that is bound to but not cleaved with *E. coli* MutY.[151] In addition, the Glu-37 → Ser replacement in *E. coli* MutY results in complete loss of enzymatic activity.[146]

Scheme 3



Scheme 4



Although prokaryotic MutY enzymes are classified as monofunctional DNA glycosylases, they show AP-lyase activity, at least, *in vitro*.[154] However, the AP-lyase activity of prokaryotic MutY is not structurally or chemically related to their DNA-glycosylase activity, which distinguishes these enzymes from other bifunctional DNA glycosylases/AP-lyases. The AP-lyase activity of prokaryotic MutY is much lower than N-glycosylase activity.[153, 155] Prokaryotic adenine DNA glycosylases cleave AP-DNA according to β,δ-elimination mechanism (Scheme 4). It was found that Lys-142 initiates the AP-lyase activity of *E. coli* MutY and participates in the formation of Schiff base.[146] Note that it still remains unknown whether prokaryotic MutY enzymes exhibit AP-lyase activity *in vivo*.

d. Conformational rearrangements in the structure of prokaryotic MutY complexes with oxoG:A-containing DNA

The catalytic domain of prokaryotic MutY interacts with DNA similarly to the catalytic domain of hOGG1. Amino acids penetrate deep into the DNA helix, thus violating stacking of the bases, which results in adenine eversion from the oxoG:A pair into the specific 'pocket' located in a deep groove between the HhH-GPD motif formed by six α-helix sites and a $[4Fe\text{-}4S]^{2+}$ cluster.[112] Thus the aromatic ring of the Tyr-88 residue of *B. stearothermophilus* MutY is intercalated into DNA helix upstream of oxodG, which results in a twofold increase in the distance between oxoG and the upstream base. The amide group of the Gln-48 side chain is inserted in the cavity arising upon adenine eversion, being involved simultaneously in π-stacking interaction with the base and in hydrogen bonding with the 3′-phosphate. These interactions facilitate bending of the DNA duplex through 55° at the lesion site.

In the damaged DNA not bound to an enzyme, the oxodG residue exists in the *syn*-conformation. Being complexed with *B. stearothermophilus* MutY enzyme, oxodG acquires the *anti*-conformation.[156] This means that on binding DNA containing an oxoG:A pair to the *B. stearothermophilus* MutY enzyme, the oxoG base rotates through 180° around the N-glycosidic bond. This rotation may initiate eversion of adenine from the DNA helix.

e. Interaction of the prokaryotic MutY enzyme with the oxoG residue of damaged DNA

In the interaction of prokaryotic MutY with oxoG:A-containing DNA, the amino acid residues of the N- and C-domains of the enzyme form contacts with oxoG residue.[149] The surface area of the subdomain formed by six α-helices contacting with damaged DNA equals 444 Å^2, whereas the total contact surface area of *B. stearothermophilus* MutY with DNA is 1530 Å^2. This large contact surface area allows the enzyme to surround completely the DNA duplex at the lesion site.

According to X-ray diffraction data for *B. stearothermophilus* D144N-MutY complex with oxoG:A-containing DNA,[149] oxoG resides within the DNA helix, which is at variance with the conclusion drawn from the data on the variation of DNA fluorescence intensity induced by *E. coli* MutY.[157] However, the observed variation of fluorescence intensity may be caused by DNA bending rather than oxoG extrusion.

The oxoG residue is recognised by MutY enzyme by two hydrogen bonds formed by the N_1H group and the exocyclic amino group of oxoG with the carbonyl group of Gln-48 in the backbone and the hydroxyl group of Thr-49, respectively. These amino acid residues are hydrogen bonded to each other. The amino group of oxoG pointing to the DNA minor groove contacts with the carbonyl group of Leu-86 in the backbone. A hydrogen bond is also formed between the N_7H group and the hydroxyl group of Ser-308, the conformation of which is, in turn, stabilised by hydrogen bonding with the hydroxyl of Tyr-88. In addition, the C_8O carbonyl group of oxoG is hydrogen-bonded to the amide proton of the backbone Ser-308 residue.

The great number of hydrogen bonds formed upon interaction of prokaryotic MutY with oxoG accounts for the better substrate properties of DNA containing the oxoG:A pair compared to DNA containing the T:A pair. Note that hydrogen bonds formed by the oxoguanine N_7H group and the lone pair of guanine N_7 atom with the hydroxyl group of Ser-308 have opposite polarity. This polarity reversal results in violation of the hydrogen bonds between the functional groups of amino acid residues Ser-308, Tyr-88 and Gln-48. This difference is used by the *B. stearothermophilus* MutY enzyme to distinguish between oxoG and G bases.[149] One more possible distinguishing feature is the difference between the efficiency of stacking interactions of Tyr-88 with oxoG and G.[149] The local dipoles of groups of atoms capable of Hoogsteen interactions have opposite orientations in the five-membered rings of oxoG and G and, therefore, they can differ considerably in the mode of interaction with local dipoles of the protein amino acids.

2. Eukaryotic adenine-DNA glycosylases

Eukaryotic homologues of MutY enzymes were identified in human (MUTYH) and mouse (mMYH) cells. Genes of these proteins were cloned and sequenced.[158, 159] The protein MUTYH comprises 535 amino acid residues. Its amino acid sequence is by 41% homologous to the sequence of *E. coli* MutY. The tertiary structure of eukaryotic adenine-DNA glycosylases has not yet been solved.

The substrate specificity of MUTYH, mMYH and *E. coli* MutY enzymes is generally the same. However, MUTYH and mMYH, unlike prokaryotic MutY, show N-glycosylase activity with respect to 2-hydroxyadenine, which can be paired with either oxoG or guanine.[152, 160] It was found by site-directed mutagenesis that the amino acid residues Arg-361 and Gly-365 of mMYH play an important role for this activity to be manifested.[161] The MUTYH enzyme forms a strong complex with AP-DNA resulting from removal of adenine from the oxoG:A pair. Meanwhile the complex of this enzyme with AP-DNA resulting from removal of oxidised adenine from the pair oxoG:2-OH-A easily decomposes with enzyme release. Note that MUTYH is twice as active with respect to DNA containing a G:A pair as to DNA containing an oxoG:A pair, although binding to DNA containing oxoG:A is more effective.[80]

IV. 8-Oxoguanosine triphosphatases

A typical feature of 8-oxoguanosine triphosphatases is the presence in their structure of the so-called NudiX motif, a conservative sequence comprising 23 amino acid residues $GX_5EX_7REUXEEXGU$, where U is a bulky hydrophobic amino acid residue.[162]

1. Prokaryotic 8-oxoguanosine triphosphatases

Of all prokaryotic 8-oxoguanosine triphosphatases, *E. coli* MutT has been best studied to date. Therefore, in this Section, the structure, the substrate specificity and the catalytic mechanism of prokaryotic 8-oxoguanosine triphosphatases is mainly considered in relation to this particular enzyme, although *E. coli* GTP cyclohydrolase II, which catalyses the transformation of guanosine triphosphate into 2,5-diamino-6-oxo-4-(5-phosphoribosylamino)pyrimidine, can also exhibit an 8-oxoguanosine triphosphatase activity and efficiently hydrolyse 8-oxoG-containing deoxyribo- and ribonucleoside triphosphates to 8-oxodGMP and 8-oxoGMP, respectively.[163]

a. Structure of the *E. coli* MutT enzyme

The *E. coli* MutT enzyme is a monomeric globular protein with the molecular weight 14.9 kDa comprising 129 amino acid residues. Its structure was determined in solution;[164] later tentative data concerning the conformation of this enzyme in the crystal were obtained.[165] The *E. coli* MutT enzyme has a sandwich type structure comprising a β-sheet shielded by two α-helices (Fig. 7). The β-sheet consists of five β-elements (βA – βE) and is located on the N-terminus of the enzyme. Two turns and L3 loop connect the β-sheet to the motif 'loop L1 – αI-helix – loop L2'. The motif 'loop L4 – αII-helix' is located on the enzyme C-terminus. *E. coli* MutT is a metal-dependent enzyme, it contains a doubly charged magnesium ion.

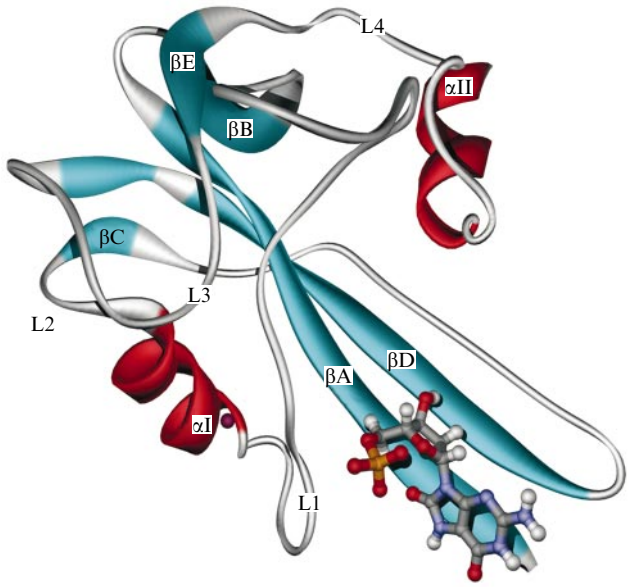


Figure 7. Structure of the *E. coli* MutT complex with oxodGMP (PDB code 1PUN).[167]
The enzyme is coloured along the secondary structure (the β-structures are blue, the α-helices are red, and the loops are grey), the magnesium ion is crimson-coloured.

For the catalytic activity of *E. coli* MutT to be manifested, it is necessary that β- and γ-phosphate groups of the nucleoside triphosphate (NTP) that is hydrolysed coordinate one more divalent metal ion.[166–168] The substrate binding site of *E. coli* MutT is mainly formed by hydrophobic amino acids (Leu-4, Leu-41, Tyr-73, Phe-75, Ile-80 and Leu-82) belonging to the β-elements βA, βC and βD, loop L1 and the N-terminus of helix L2 and by only three polar amino acid residues Lys-3, Lys-39 and Arg-78.

b. Substrate specificity of *E. coli* MutT

During the matrix biosynthesis, nucleoside triphosphates dGTP, oxodGTP and GTP, oxoGTP effectively compete with dTTP, dGTP and TTP, GTP for the insertion into DNA and RNA opposite the residues A and C, respectively.[169] *E. coli* MutT specifically hydrolyses 8-oxoG-containing deoxyribo- and ribonucleoside triphosphates, thus preventing insertion of 8-oxoguanine into DNA or RNA.

E. coli MutT also hydrolyses dGTP to dGMP and pyrophosphate.[169] However, the Michaelis constant (K_m) for the hydrolysis of oxodGTP is 10 000 times lower than K_m for dGTP hydrolysis, which indicates that oxodGTP is a more preferred substrate for *E. coli* MutT.[166]

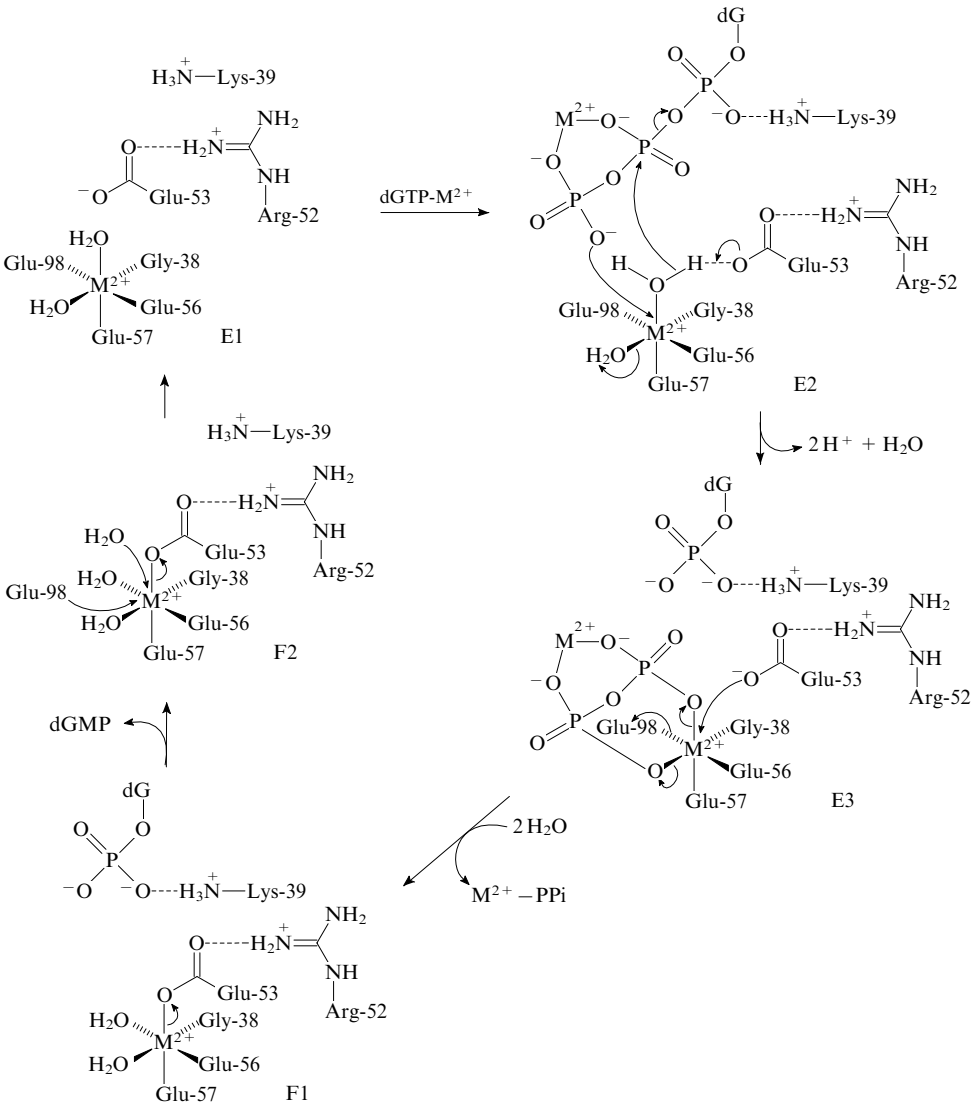
7,8-Dihydro-8-oxo-2′-deoxyguanosine 5′-triphosphate can be formed upon direct oxidation of dGTP and upon phosphorylation of oxodGDP. It was shown that *E. coli* MutT cleaves oxoG-containing deoxyribo- and ribonucleoside diphosphates with an efficiency similar to that of oxodGTP and oxoGTP cleavage.[166]

It is noteworthy that oxidised adenosine triphosphate is not a substrate of the *E. coli* MutT enzyme.

c. Catalytic mechanism of the *E. coli* MutT enzyme

Unlike most hydrolases, *E. coli* MutT cleaves ribo- and deoxyribonucleoside triphosphates by the nucleophilic substitution mechanism at the β- rather than α-phosphorus atom (Scheme 5).[170, 171] In the first stage of catalysis, the active form of *E. coli* MutT in which the carboxyl groups of the residues Gly-38, Glu-56, Glu-57 and Glu-98 and two water molecules coordinate a double-charged metal ion (form E1), binds the nucleoside triphosphate complexed with the metal ion, which coordinates its γ-phosphate group. Subsequently, the carboxyl group of Glu-53 in the *E. coli* MutT active site transfers a proton from the water molecule located in the coordination sphere of the enzyme metal ion, thus activating the water molecule towards

Scheme 5



nucleophilic attack on the β-phosphorus atom of nucleoside triphosphate (form E2). As a result of this attack, pyrophosphate complexed with the metal ion (M^{2+}PPi) is coordinated by the enzyme metal ion. The residue Lys-39 of the *E. coli* MutT active site stabilises the phosphate group of the leaving nucleoside monophosphate by hydrogen bonding (form E3).[171] In the next stage, pyrophosphate leaves, the enzyme metal ion is coordinated to the carboxyl group of Glu-53 of the enzyme active site; the *E. coli* MutT enzyme acquires the conformation (form F1) in which it is inactive.

E. coli MutT is inactive in conformation F1 for two reasons: first, it cannot efficiently bind the substrate; second, the Glu-53 residue bound to the enzyme metal ion cannot exhibit basic properties necessary for elimination of a proton of the catalytic water molecule. The next stage comprises dissociation of a nucleoside monophosphate, while the final stage is a change in the enzyme conformation including removal of Glu-53 from the coordination sphere of the enzyme metal ion and return of Glu-98 and a new water molecule (conformation F2). The nucleoside monophosphate formed in this way is not phosphorylated any more but is split to a nitrogen base.[172]

d. Eukaryotic 8-oxoguanosine triphosphatases

E. coli MutT homologues having oxodGTP-phosphatase activity have been found in various organisms: *Streptococcus pneumonia*, *Proteus vulgaris*,[173] in the cells of mice,[174] rats[175] and humans.[176, 177] The protein YLR151c, a functional homologue of MutT, was found in the yeast *Saccharomyces cerevisiae* cells.[178] Bacterial and mammal 8-oxoguanosine triphosphatases are similar in size. Saparbaev and co-workers[179] described an alternative way of repair of oxoG residues in the *S. cerevisiae* cells with participation of AP endonuclease (Apn1). In addition, it was shown that in the yeast cells the damaged oxoG residue can be removed by the excision nucleotide repair.[180]

Expression of 8-oxoguanosine triphosphatase from mammalian cells to the *E. coli mutT*$^-$ cells results in a decrease in the spontaneous mutations to a normal level.[181] The high activity of oxodGTP phosphatase was found in liver, thymus, colon and especially embryo cells of mice. In mammalian cells, 8-oxoguanosine triphosphatase (MTH1) is mainly concentrated in cytoplasm and mitochondria.[182] Liver cancer and, to a lesser extent, lung or gastric cancer develop in *mth1* gene knockout mice, indicating that the formation of oxodGTP can initiate malignancy *in vivo*.[6, 27]

e. Structure of eukaryotic 8-oxoguanosine triphosphatase

The structure of 8-oxoguanosine triphosphatase from human cells (NUDT1, known also as hMTH1) in solution was solved by NMR spectroscopy (see Fig. 8, p. 785).[183] This enzyme has a molecular weight of 18 kDa and comprises 156 amino acid residues. The main structural difference between eukaryotic 8-oxoguanosine triphosphatase and prokaryotic MutT is the presence of a β-hairpin consisting of βF- and βG-elements and a loop connecting them in the NUDT1 molecule.[183–185] The βF-element is bound to the βC-element by a hydrogen bond network, which gives rise to an extended β-structure comprising five elements: βA, βD, βC, βF and βG. The removal of βF- and βG-elements results in complete loss of activity by eukaryotic 8-oxoguanosine triphosphatase.[186]

The specific 'pocket' of the NUDT1 enzyme in which the damaged heterocyclic base of the substrate is bound is formed by hydrophobic amino acid residues Phe-27, Asn-33, Trp-117 and Asn-119 from loops L1 and L4. The pocket is arranged between the β-structure, αII helix and the β-hairpin loop of NUDT1. The amino acid residues Asn-33, Trp-117 and Asn-119 are used by NUDT1 to recognise the oxidised purine nucleoside triphosphates.[183, 187]

The catalytic site of NUDT1 is located within the 'NudiX' motif. The amino acid residues from this motif bind indirectly the phosphate groups of the substrate by coordinating the metal ion bound to them. The 'NudiX' motif of the NUDT1 enzyme contains a hydrophobic core, which incorporates the amino acid residues Thr-8, Val-10 and Val-12 from the βA-structure, Phe-86 from the βD-structure, Phe-35 from the loop L1, and Ala-49, Leu-53 and Leu-59 located on the inner side of the 'NudiX' helix and playing an important role in the formation of the enzyme tertiary structure (see Fig. 8). According to site-directed mutagenesis, 14 of 23 conserved amino acids of the 'NudiX' motif (residues 35, 39, 41, 43, 45, 48, 49, 51–53, 55–57 and 59) are needed to exert the catalytic activity by the enzyme.[188] The amino acid residues Glu-52, Glu-55 and Glu-56 of NUDT1 form an acid cluster and, apparently, coordinate the enzyme metal ion as in *E. coli* MutT.[166] In addition, the Glu-100 residue of NUDT1 located in the near environment of the acid cluster, similarly to Glu-98 of *E. coli* MutT, coordinates the metal ion outside the 'NudiX' motif.

For NUDT1 to exhibit the catalytic activity, significant is the conformation of the loop comprising the amino acid residues 37–45. This loop has a β-turn at Gly-42, which is stabilised by hydrogen bonds between the carbonyl group located in the side chain of Glu-43 and the amide group of Glu-40 located in the backbone and also hydrophobic contacts between Val-39 and Ile-45. The residues Gly-42 and Glu-43 are highly conserved for enzymes with the 'NudiX' motif. It was shown by site-directed mutagenesis that the replacement of Gly-37, Gly-42 and Glu-43 or Val-39 and Ile-45 results in the loss of NUDT1 activity.[181]

f. Substrate specificity of eukaryotic 8-oxoguanosine triphosphatase

Eukaryotic homologues of prokaryotic MutT enzymes are classified in two groups in terms of their substrate specificity (Fig. 9). The first group comprises MTH1[189, 190] and MTH2[191] enzymes (known also as NUDT1 and NUDT5, respectively), which cleave oxodGTP to oxodGMP by a mechanism analogous to the catalytic mechanism of substrate cleavage by *E. coli* MutT. The second-group enzymes, such as NUDT5, possess a weak activity with respect to oxodGTP but efficiently hydrolyse oxodGDP.[192, 193] Figure 9 presents schematically the mechanism of removal of oxoG-containing nucleotides from a mixture of nucleoside di- and nucleoside triphosphates.

Unlike *E. coli* MutT, NUDT1 has a broader substrate specificity, as it hydrolyses oxidised purine nucleoside di- and triphosphates such as oxodGTP, oxoGTP, 2-OH-dATP, 2-OH-ATP, 8-oxodATP and 8-oxoATP[186, 194, 195] and is responsible for preventing their insertion into nucleic acids.[177, 180]

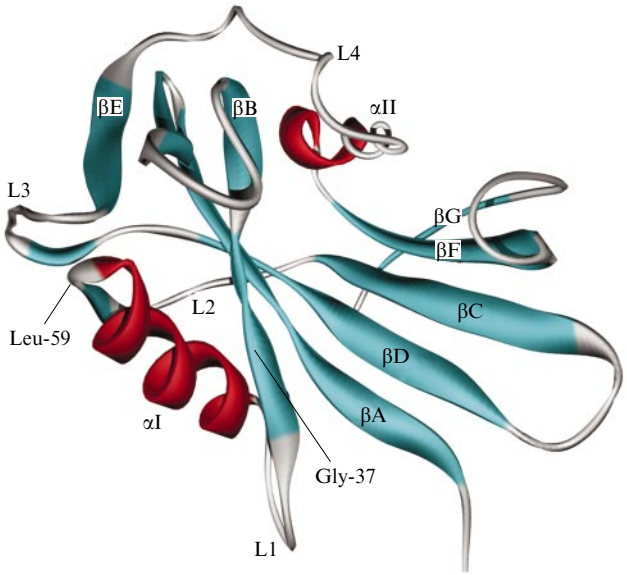


Figure 8. Structure of hMTH1 (PDB code 1IRY).[183]
The β-structures are shown in light-blue, the α-helices are red, and the loops are grey. The arrows mark the initial (Gly-37) and final (Leu-59) amino acid residues of the 'NudiX'-motif.

V. Repair system of heteroduplexes

The repair of oxoG from damaged DNA containing non-canonical base pairs formed with participation of oxoG can be accomplished by the heteroduplex repair system or, as it is also called, the mismatch repair system.[10,28] This system is used by the cell to remove unpaired bases that may appear upon direct damage of DNA bases or their precursors by the cell metabolism products, as a result of faulty insertion of a non-complementary base by DNA polymerase during replication or recombinational integration of single-stranded DNA section into non-absolutely identical DNA.

The heteroduplex repair system includes the removal of one nucleic base of a non-canonical pair in DNA followed by cleavage of phosphodiester bond, removal of the deoxyribose residue, insertion of the complementary nucleotide residue into the liberated position and reconnection of the continuous DNA strand. This process involves a group of enzymes, specifically, restriction endonucleases, DNA polymerases, DNA ligases and some other. It was shown that the heteroduplex repair system in both prokaryotic[196] and eukaryotic[197] cells can also repair DNA containing oxoG residues by removing the 8-oxoG:A base pair and thus protecting cells from the mutagenic action of oxidants at the post-replicative stage.

It was shown that oxoG is repaired not only in DNA and its precursors but also in RNA.[198,199] Thus it was found that *E. coli* cells contain proteins that bind specifically to oxoG-containing RNA.[198] The protein polynucleotide phosphorylase, which binds to oxoG-RNA and thus inhibits the cell growth was found among them. This led to elimination of the oxidised RNA from translation processes.[198]

In mammalian cells, the proteins MTH1 and NUDT5 cleave oxoGTP and oxoGDP to oxoGMP.[199] Polynucleotide phosphorylase is an analogue of these proteins functioning at the RNA level. Human polynucleotide phosphorylase binds specifically to the oxoG-containing RNA. On treatment of cells with compounds that induce oxidative stress, the amount of polynucleotide phosphorylase rapidly decreased, whereas the amount of other proteins remained invariable.[199] This indicated that human polynucleotide phosphorylase can play a role in elimination of oxidised RNA forms from translation processes.

VI. Conclusion

Oxidative damages of DNA such as oxoG induce somatic mutations, which are among the causes for carcinogenesis. It was found that the number of oxoG residues formed depends on the nature of the organism, type of cells, DNA localisation site in the cells, and on the nature of the

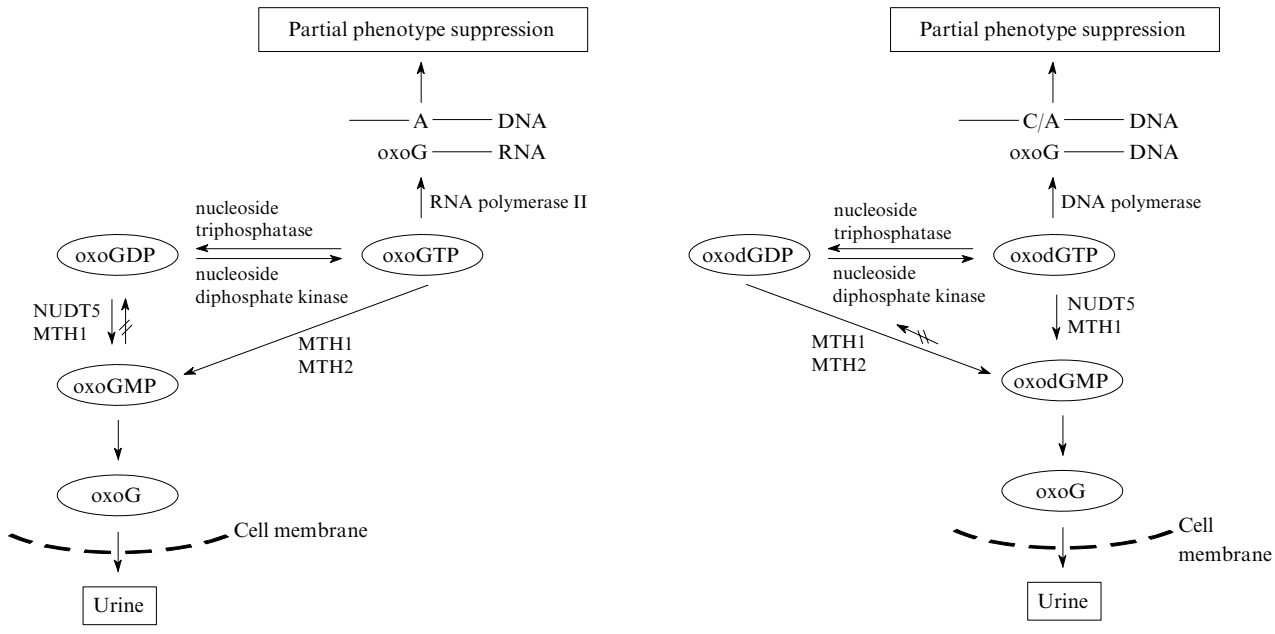


Figure 9. Model of the removal of oxoG-containing nucleoside triphosphates from a dNTP and NTP mixture in mammalian cells.

mutagen and intensity and duration of its action.[200–202] The contents of oxoG residues in DNA in the human tumour tissue is increased 2–3-fold with respect to their content in the DNA of healthy humans where it amounts to 10^5 damaged bases per cell.[203] Initiation of tumour growth requires a number of mutations inactivating tumour suppressor genes and activating proto-oncogenes. Oxoguanine causes G:C → T:A transversions, which are often encountered in mutant alleles of genes p53 and *ras* present in many human tumours.[204] The ability of oxoG to initiate the tumour growth was demonstrated most vividly in the cell transfection by a plasmid containing the gene c-Ha-*ras* with the only specifically located oxoG residue, which resulted in a considerable increase in the number of transformation foci.[205]

Numerous correlations are known between the organism age and the content of DNA oxidative damages including oxoG and between the life time of particular organisms and their capability for DNA repair. It has been hypothesised that damaged DNA is accumulated with age mainly in the mitochondrial genome. Direct measurements of oxoG level in mtDNA give most often greater values than those obtained for nuclear DNA, although experimental difficulties related to measurement of the damage level produce a broad scatter of the results.[206]

New investigations dealing with DNA repair combined with the data obtained to date would allow one to elucidate the nature of repair mechanisms at a molecular level. In the future, this would enable targeted influence on these processes to fight against many dangerous diseases.

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