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REVIEW

Mechanism of Recognition and Repair of Damaged DNA by Human 8-Oxoguanine DNA Glycosylase hOGG1

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Abstract—Recent data on structural and biochemical features of human 8-oxoguanine DNA glycosylase (hOGG1) has enabled detailed evaluation of the mechanism by which the damaged DNA bases are recognized and eliminated from the chain. Pre-steady-state kinetic studies with recording of conformational transitions of the enzyme and DNA substrate significantly contribute to understanding of this mechanism. In this review we particularly focus on the interrelationship between the conformational changes of interacting molecules and kinetics of their interaction and on the nature of each elementary step during the enzymatic process. Exhaustive analysis of these data and detailed mechanism of hOGG1-catalyzed reaction are proposed.

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Key words: conformational dynamics, pre-steady-state kinetics, human 8-oxoguanine DNA glycosylase, hOGG1

DNA of all living beings is constantly affected by various agents such as highly reactive cell metabolites, UV and ionizing radiation, etc. Oxidation, alkylation, deamination, apurination/apyrimidination, and single- and double-strand breaks are only part of the spectrum of processes leading to DNA lesions [1-4]. These impairments of the genetic apparatus exert cytotoxic and mutagenic effects and can result in various disorders [5-9]. Oxidative damage of cell macromolecules also leads to acceleration of degenerative processes in the organism [10, 11].

Most common DNA lesions are formed due to interactions with reactive oxygen species (ROS) such as $^{\cdot}O_2^-$, H_2O_2, and $^{\cdot}OH$. ROS are normally formed during cell respiration. They can also appear under the action of UV or ionizing radiation or various chemical agents. The major products of purine base modification are 7,8-dihydro-8-oxoguanine (8-oxoguanine, 8-oxoG) and 5-formamidopyrimidine derivatives of adenine (4,6-diamino-5-formamidopyrimidine; Fapy-A) and guanine (2,6-diamino-4-hydroxy-5-formamidopyrimidine; Fapy-G) [12, 13].

During replication 8-oxoG can form the Hoogsteen-type base pair with adenine (8-oxoG/A). Subsequent replication leads to G/C → T/A mutation [14, 15]. Cells have a specific defense system counteracting accumulation of 8-oxoG in DNA (GO-system) [16, 17]. This system is well characterized in the bacterium *Escherichia coli*. It is composed of three enzymes, Fpg (MutM) (specific N-glycosylase/AP-lyase removing 8-oxoguanine residues), MutY (specific N-glycosylase removing the adenine residue opposite 8-oxoguanine), and MutT (phosphatase cleaving 8-oxo-dGTP). Most eukaryotes have structural or functional homologs of these bacterial enzymes [18, 19]. In eukaryotes, 8-oxoguanine residues are removed from DNA by 8-oxoguanine DNA glycosylase (OGG1) [20]. In the human cell, only 50,000 hOGG1 molecules protect the $6 \cdot 10^9$ bases comprising the genome [21]. So, this enzyme must effectively differentiate 8-oxoG from the four normal heterocyclic bases and remove it.

The human *OGG1* gene maps to 3p25 chromosome and expresses two major mRNAs encoding proteins composed of 345 and 424 amino acid residues (α-hOGG1 and β-hOGG1, respectively) [20, 22-24]. The N-termi-

Abbreviations: AP, apurinic/apyrimidinic site; 2-aPu, 2-aminopurine; BER, DNA base excision repair; Cy3 and Cy5, cyanine dyes; Dab, DABCYL; F, 2-(hydroxymethyl)-3-hydroxytetrahydrofuran; Flu, fluorescein; FRET, fluorescence resonance energy transfer; HhH, helix-hairpin-helix; hOGG1, human 8-oxoguanine DNA glycosylase; 8-oxoG, 7,8-dihydro-8-oxoguanine; ROS, reactive oxygen species; Trp, tryptophan.

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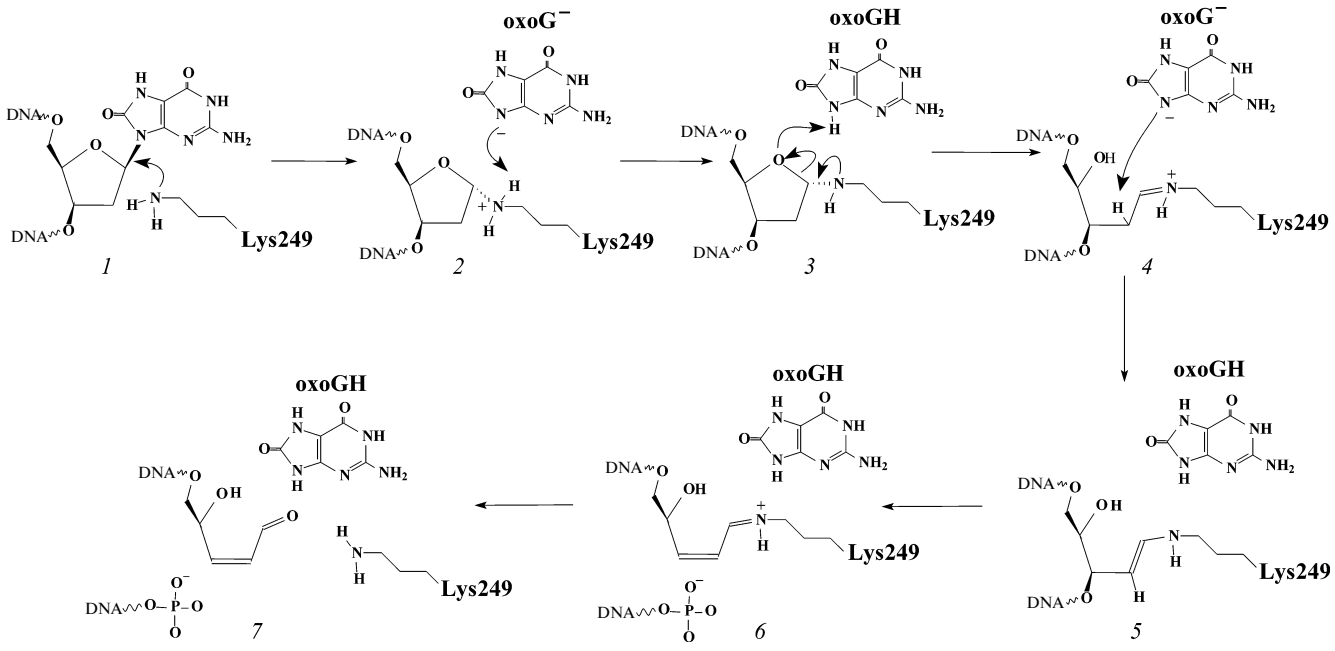


Catalytic mechanism of hOGG1 [31] (reprinted by permission from Macmillan Publishers Ltd: [Fromme, J. C., Bruner, S. D., Yang, W., Karplus, M., and Verdine, G. L. (2003) *Nat. Struct. Biol.*, **10**, 204-211], copyright 2003)

Scheme 1

nal 316 residues are the same in both hOGG1 isoforms, whereas the C-ends are completely different [22, 23, 25, 26]. The α-hOGG1 isoform is localized in nuclei, and the β-hOGG1 is in mitochondria [26]. Nuclear α-hOGG1 protein is highly conserved. Its homologs have been characterized in *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Drosophila melanogaster*, and some mammals [27, 28]. The α-hOGG1 protein has 38% homology with the yeast protein. The β-type OGG1 was only found in human cells [28], so catalytic and structural features of human OGG1 proteins were studied using exclusively α-hOGG1.

CHEMICAL MECHANISM

The hOGG1 enzyme is a bifunctional DNA glycosylase/β-lyase that can cleave the N-glycoside bond between a lesioned base and the C1′ atom of ribose to form free 8-oxoG with subsequent cleavage of the 3′-phosphodiester bond at this ribose moiety. The catalytic mechanism of hOGG1 recruits the Lys249 side chain amino group that substitutes the 8-oxoG base at C1′ atom and facilitates elimination of the 3′-phosphodiester bond via formation of a Schiff base intermediate with the C1′ atom of ribose [29]. The K249Q mutant can recognize DNA lesions, but it does not possess catalytic activity [30, 31]. The chemical stage – cleavage of the phosphodiester bond from the side of the 3′-carbon atom of 2′-deoxyribose – occurs by the β-elimination mechanism (AP-lyase activity). This reaction is catalyzed by 8-oxoguanine that was cleaved at the first catalytic stage and is still localized in the active site of hOGG1 [31].

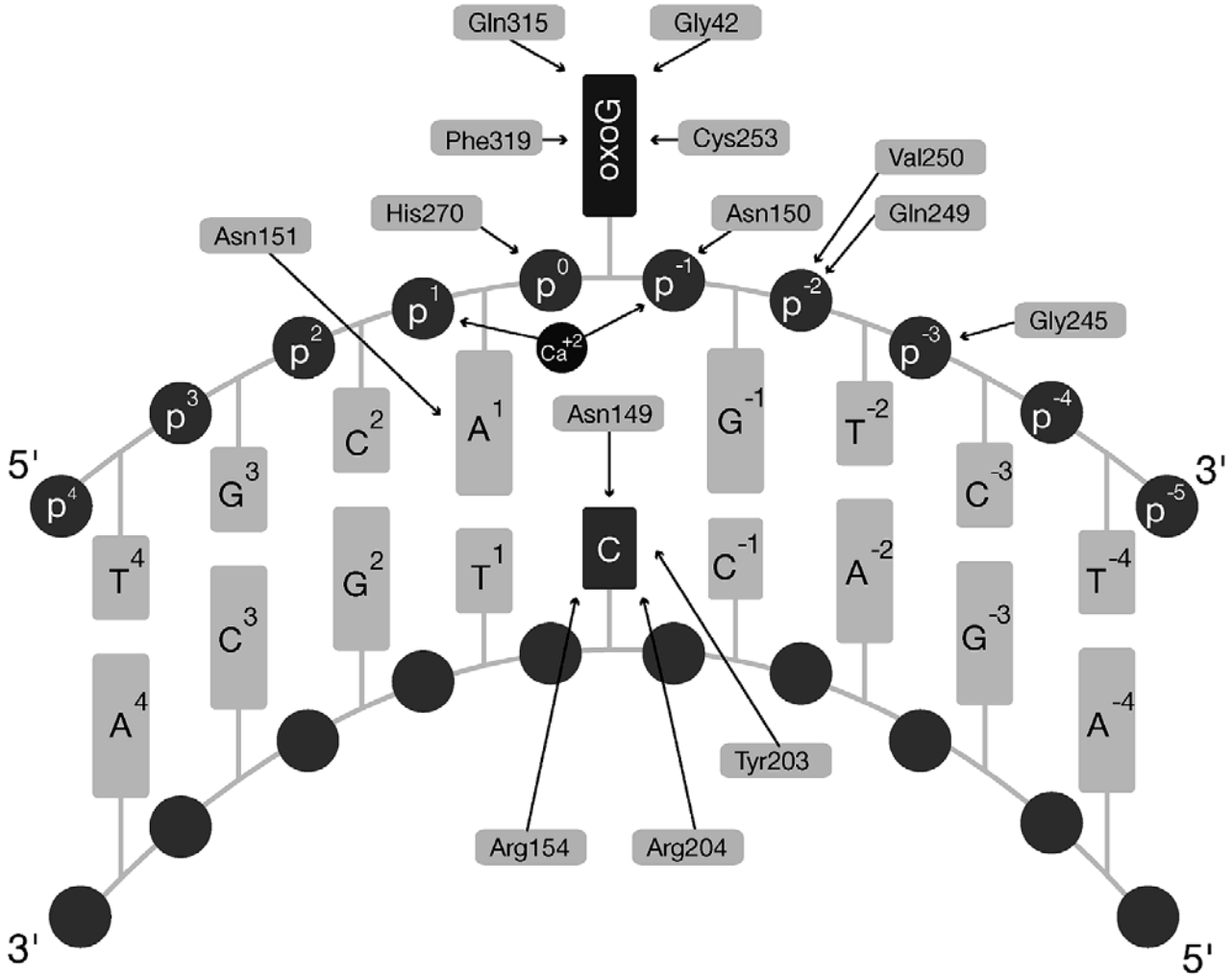
Scheme 1 presents the catalytic mechanism of hOGG1 proposed in [31]. In the first step, the ε-NH_2-group of Lys249 attacks the C1′ atom of initial substrate *1* resulting in cleavage of the glycoside bond to form 8-oxoG$^-$ anion and intermediate *2*. Then this anion abstracts a proton from the Lys249 ε-NH_2-group to form aminal *3*. The protonated 8-oxoGH transfers the proton to the ribose O4′ atom. Due to this process, aminal *3* is reorganized into a Schiff base *4* with opening of the furanose ring. Schiff base *4* has an additional proton on the N atom, which is returned to 8-oxoG$^-$. Thus, 8-oxoGH and uncharged Schiff base *5* are formed, and the latter undergoes β-elimination with cleavage of the 3′-phosphodiester bond to form DNA fragments with 5′-phosphate and intermediate *6* carrying α,β-unsaturated positively charged Schiff base at the 3′-end. Hydrolysis of intermediate *6* results in free enzyme and a DNA fragment carrying 4-hydroxy-2,3-pentenal-1 (*7*) at the 3′-end.

STRUCTURAL CHARACTERISTICS OF THE INTERACTION OF hOGG1 WITH DNA

Several hOGG1 structures are now known, such as those of the free enzyme [32] and various complexes with DNA: inactive K249Q mutant with 8-oxoG-containing DNA [33], N149C with 8-oxoG- and G-DNAs [34],

D268N with 8-oxoG- and F-DNAs [35], and hOGG1 with F ligand [36]. Structures of covalent hOGG1 adduct with AP substrate [31] prepared by Schiff base reduction with sodium borohydride have also been studied, as well as hOGG1 mutants (H270A, Q315A, Q315F, and G42A) with substituted amino acids implicated in formation of contacts with 7,8-dihydro-8-oxoguanine [37]. The structure of the last intermediate in which 8-oxoG base is almost completely submerged into the active site that has not yet gained the final catalytically active conformation has also been studied [38].

The hOGG1 protein contains a fold that is common to members of the superfamily of base-excision DNA repair proteins [33] implicated in base excision repair (BER) typified by *E. coli* endonuclease III and alkyl-DNA glycosylase AlkA [39]. The proteins of this family are found in organisms ranging from bacteria to mammals and repair a wide variety of base lesions resulting from, for example, DNA oxidation, alkylation, hydration, and deamination. The hallmark of these proteins is a helix-hairpin-helix (HhH) structural element [29] followed by a Gly/Pro-rich loop and a conserved aspartic acid (HhH-GPD motif). The structure of hOGG1 contains, in addition to the two α-helical domains common to all known superfamily members, a third anti-parallel β-sheet domain found thus far in only the alkylation repair DNA glycosylase AlkA. The protein is firmly latched onto the DNA backbone of the 8-oxoG-containing strand, but it does not contact the backbone of the complementary strand at all (Scheme 2 [33]). The substrate 8-oxoG residue is fully flipped out of the DNA helix and deeply inserted into an extra-helical active-site pocket, consistent with similar structures of other HhH-GPD superfamily members [40-43]. Although the 8-oxo substituent in oxoG causes the nucleoside to favor the *syn*-conformer about the glycoside bond, 8-oxoG binds the active site of



Contacts between interacting hOGG1 and substrate 8-oxoG residue [33] (reprinted by permission from Macmillan Publishers Ltd: [Bruner, S. D., Norman, D. P., and Verdine, G. L. (2000) *Nature*, **403**, 859-866], copyright 2000)

Scheme 2

hOGG1 in an *anti*-conformation quite similar to that in normal duplex DNA. The flipped out DNA backbone and glycosyl conformations combine to extend the 8-oxoG far out from the DNA helix, thus enabling the nucleoside to plunge deeply into the active-site pocket of hOGG1.

Extensive interactions between hOGG1 and the phosphates of the 8-oxoG-containing strand, the substrate 8-oxoG, and the complementary cytosine altogether comprise 2.268 Å^2 of contact surface area. Whereas most DNA-binding proteins use surfaces that are rich in lysine and arginine residues to contact backbone phosphates, hOGG1 binds the DNA backbone in a channel that is nearly charge-neutral with only a single basic residue, His270. A striking feature of the complex is the large number of the α-helices in hOGG1 that have their N-terminals orientated toward the DNA. Only one of these helices, αL, actually contacts the DNA backbone. This is noteworthy because αL and the loop and helix (αK) preceding it comprise the conserved helix-hairpin-helix (HhH) element. In addition to the phosphate contacts made by the main chain of αL to p^{-2} (Val250 and Gln249), a highly conserved glycine residue of the loop (Gly245) forms hydrogen bonds to p^{-3} ("p" is an internucleotide phosphate group on Scheme 2; the upper index indicates position about the lesion nucleotide). The HhH contacts a region of the DNA substrate on the 3′ side of the lesion that has a nearly normal B-form conformation. Hence the HhH appears primarily to be involved in positioning the duplex for presentation of the lesion into the active-site cavity.

The central residue (Asn150) of a conserved NNN motif in hOGG1 also stabilizes the inward orientation of p^{-1} through hydrogen bonding. On the 5′ side of the 8-oxoG residue, His270 forms a hydrogen bond with p^0, but the protein makes no further backbone interactions.

The duplex area released due to flipping out of the 8-oxoG residue is filled with an amino acid residue of the conserved NNN motif, namely Asn149, which forms a hydrogen bond between the amide carbonyl atom of the side moiety and exocyclic NH_2-group of the opposite cytosine on the complementary strand (C^0 on Scheme 2). Besides, hOGG1 wedges the aryl ring of Tyr203 into the space between C^0 and the base on its 5′ side (C^{-1} on Scheme 2), thereby unstacking the two bases and creating a sharp kink in the DNA, which greatly improves access to the Watson–Crick edge of the base from the minor-groove side. Residues Arg154 and Arg204 converge on C^0 from the minor-groove side, one arginine above and the other below the plane of the pyrimidine ring, and simultaneously establish hydrogen-bonding interactions with the acceptor N3 and O2 atoms of cytosine C^0. The interaction between Asn149 and the amide carbonyl of C^0 completes the pentad of hydrogen bonds between the enzyme and the complementary cytosine residue.

The 8-oxoG residue in the active site is recognized by formation of specific contacts between the lesioned base and amino acids of the protein. The 8-oxoG-recognizing pocket of hOGG1 fits the lesioned base structure very well, properly positioning the Phe319, His270, and Asp268 residues [36]. The enzyme recognizes the 8-oxoG urea fragment involving the C8 carbonyl group and N7 and N9 atoms. Then the N7 atom forms a hydrogen bond with the carbonyl of Gly42. Among all of the contacts made with the 8-oxoG base, that involving Gly42 is the only one that would clearly be different with 8-oxoG versus guanine; thus, the responsibility for discriminating 8-oxoG from guanine appears to be borne by a single hydrogen bond [33]. It is noteworthy that this critically important amino acid Gly42 is located in the β-sheet domain within the hOGG1–DNA interface. The other residues in the hOGG1 8-oxoG-specificity pocket appear exquisitely capable of recognizing the lesion while excluding A, C, and T. Phe319 and Cys253 interact with opposite π-faces of the 8-oxoG, sandwiching the base in the active site. The Gln315 amide-NH_2 and a tightly bound water molecule cooperate to recognize O6, and the side-chain carbonyl of Gln315 forms hydrogen bonds with both N1 and N^2H. A second tightly bound water is hydrogen bonded to O6. Gln315, the captive water molecules, and Gly42 are not chemically matched to the hydrogen bond donor/acceptor pattern on A, C, and T.

The role of Asp268 and His270 located within the active site of the enzyme has been discussed in a series of works [31, 33-37]. It was supposed that these residues participate in protonation–deprotonation of Lys249 and formed intermediates including the Schiff base. In particular, this role of His270 well explains its invariance among members of the HhH-GPD superfamily.

The kink in the DNA helix at the locus of inversion of the nucleoside in the complexes between hOGG1 and 8-oxoG- and G-containing DNAs was found to constitute ~70° and ~80°, respectively [35]. The 8-oxoG base is deeply submerged into the enzyme pocket recognizing the lesion, and G is pushed from this pocket and lies on the enzyme surface in the extra-helical site (exo-site) located at the distance of ~5 Å from the enzyme pocket. The G base interacts with Phe319 and His270 of the active site, but these contacts differ from those established by 8-oxoG. His270 does not contact this base in the complex with the substrate 8-oxoG, but forms a hydrogen bond with its 5′-phosphate instead. This phosphate does not make contact in the complex with G, and His270 interacts with the π-system of the base.

STEADY-STATE KINETICS

Kinetics of the stationary process catalyzed by hOGG1 has been studied in a series of works [35, 44-46]. The kinetic data are represented in Table 1. The constants for glycosylase and AP-lyase reactions (k_{glyc} and k_{lyas}, respectively) were determined separately in work [35].

Table 1. Rate and equilibrium constants for wild-type hOGG1 and its mutants*

Type of enzyme and DNA substrate (37°C)	$k_{cat}/k_{glyc}/k_{lyas}$, min^{-1}	K_m or K_d, nM	Reference
Wt, 8-oxoG/C	0.0246 ± 0.0018	$K_m = 3.8 \pm 1.1$	[44]
Wt, 8-oxoG/C	$k_{cat} = 0.106 \pm 0.018$ $k_{glyc} = 0.34 \pm 0.04$ $k_{lyas} = 0.106 \pm 0.018$	–	[35]
K249Q, 8-oxoG/C	–	$K_d = 14.0 \pm 0.1$	[35]
D268N, 8-oxoG/C	$k_{cat} = 0.0016 \pm 0.00027$	$K_d = 700 \pm 20$	[35]
Wt, 8-oxoG/C	$k_{cat} = 0.062$	$K_d = 8.2 \pm 0.9$	[45]
H270A, 8-oxoG/C	$k_{cat} = 3.7 \cdot 10^{-4}$	$K_d > 266$	[45]
H270R, 8-oxoG/C	$k_{cat} = 0.018$	$K_d = 36.3 \pm 4.9$	[45]
H270L, 8-oxoG/C	$k_{cat} \leqslant 4.6 \cdot 10^{-5}$	$K_d > 266$	[45]
Q315A, 8-oxoG/C	$k_{cat} = 0.038$	$K_d = 57.3 \pm 10.9$	[45]
F319A, 8-oxoG/C	$k_{cat} = 3.4 \cdot 10^{-4}$	$K_d > 266$	[45]
K249Q, 8-oxoG/C	$k_{cat} \leqslant 4.6 \cdot 10^{-5}$	$K_d = 11.8 \pm 4.3$	[45]
Wt, AP/C	$k_{cat} = 0.014$**	$K_d = 2.4 \pm 0.3$***	[45]
H270A, AP/C	$k_{cat} = 0.006$	$K_d = 4.6 \pm 0.7$***	[45]
H270R, AP/C	$k_{cat} = 0.015$	$K_d = 4.2 \pm 1.1$***	[45]
H270L, AP/C	$k_{cat} = 0.0038$	$K_d = 14.8 \pm 3.3$***	[45]
Q315A, AP/C	$k_{cat} = 0.017$	$K_d = 5.5 \pm 0.8$***	[45]
F319A, AP/C	$k_{cat} = 0.0097$	$K_d = 2.2 \pm 0.4$***	[45]
K249Q, AP/C	$k_{cat} \leqslant 4.6 \cdot 10^{-5}$	$K_d = 4.8 \pm 1.6$***	[45]
Wt, 8-oxoG/C	$k_{glyc} = 57 \pm 7$ $k_{lyas} = 0.12 \pm 0.06$	–	[46]

* All data were obtained for α-hOGG1, 345 amino acid residues, $M_r = 38$ kDa.
** Recalculated from values given in U/mg enzyme.
*** Dissociation constants were measured for F-containing DNA.

The glycosylase reaction is faster than the AP-lyase reaction (see Table 1); the data reported in [35] suggests only 3-fold difference in time, whereas a 475-fold difference was found in work [46] in which the "stopped-flow" kinetics method was used for the measurement of fast kinetics.

Table 1 shows that the rate of the AP-lyase reaction for the wild-type enzyme with 8-oxoG/C substrate [46] is one order of magnitude higher than that with the AP substrate [45], thus supporting the conclusion drawn by the authors [31] of the necessity of 8-oxoG in the enzyme active site for the AP-lyase reaction.

Data of particular interest were obtained in work [45] where hOGG1 mutants with substitutions of the active site amino acids His270 (H270A and H270L) or Phe319 (F319A) were studied. These mutants possess far less (from 50- to 1000-fold) glycosylase activity than the wild-type enzyme, but their AP-lyase activities are equal or slightly decreased (<4-fold). The affinity of hOGG1 mutants (H270A, H270L, and F319A) to 8-oxoG/C-containing DNA was also significantly decreased (>30-fold), whereas the affinity to F/C-containing DNA was only slightly decreased (<7-fold). The hOGG1 mutation at the Gln315 residue (Q315A) also had very little influence of the catalytic and DNA-binding properties of the enzyme. Therefore, only His270 and Phe319 are most important for the 8-oxoG-binding pocket, while Gln315 is less important. His270, Gln315, and Phe319 are not necessary for binding of the F/C-containing DNA and its cleavage at the AP-site. So, the authors of work [45] proposed regarding these H270A and F319A mutants as members of a new hOGG1 type deficient in DNA-glycosylase but proficient in AP-lyase activity.

The data on hOGG1–DNA structures indicate [34] that the recognition of the lesioned base and its flipping out of the DNA strand cannot occur at the same stage (in concert), but probably pass through a series of discrete intermediates.

PRE-STEADY-STATE KINETICS AND CONFORMATIONAL DYNAMICS

Approaches to investigation of conformational dynamics in hOGG1 enzyme and choice of model DNA substrates. One of the most important problems in the study of DNA repair is the understanding of the enzymatic mechanism that provides highly precise recognition of the lesioned base and its effective removal from DNA. The mechanism of the search for a single damaged DNA base among many unmodified bases remains unknown, and the role of conformational changes within the enzyme and DNA in the substrate recognition and conversion is unclear. Clarification of detailed kinetics mechanism of the hOGG1 enzymatic reaction and simultaneous analysis of conformational mobility of the enzyme structure and DNA substrate are difficult tasks requiring the use of a wide spectrum of research methods and approaches.

Knowledge of the structure of both free hOGG1 enzyme and its complexes with DNA substantially facilitates analysis and interpretation of data on conformational dynamics of the enzyme and substrate during their interaction. However, structural data represent only "snapshots" and can only provide information about definite, fixed states of the enzyme and substrate.

Optical methods enable monitoring of the structural changes in the course of the enzymatic process. Change in the structure of interacting molecules occurs within the millisecond time range, and therefore methods of fast kinetics, particularly the "stopped-flow" method, must be employed for studies of these changes.

Tryptophan (Trp) is known to be an intensively fluorescent amino acid. This characteristic can be used as a natural highly sensitive fluorescence marker of conformational changes in protein molecules. The hOGG1 molecule contains 10 Trp residues.

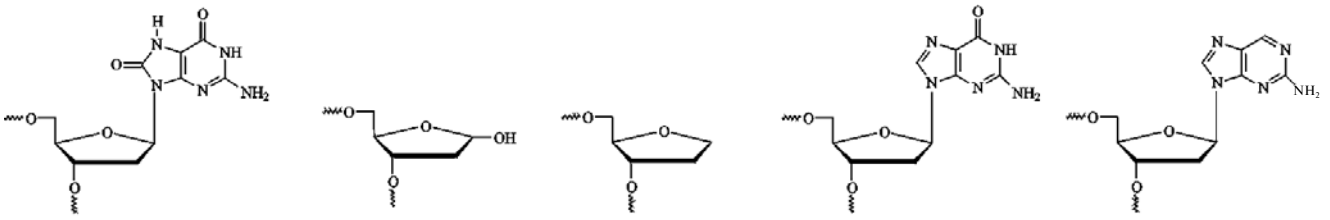
The approach involving stepwise complication of the substrate was used in works [47, 48] for the study of various stages of the enzymatic process (Table 2). Duplex containing 8-oxoguanine in one of its strands was used as a specific substrate (8-oxoG substrate). Also, duplexes were used containing either an apurinic/apyrimidinic (AP substrate), which is the reaction intermediate, or a non-reactive analog of an AP-site containing the 2-(hydroxymethyl)-3-hydroxytetrahydrofuran residue (tetrahydrofuran abasic site analog; F ligand) instead of 2′-deoxyribose. The duplex carrying this residue possesses all features of AP substrate but is not capable of being cleaved by the enzyme because it does not contain a hydroxyl group in its 1′-position. The duplex of oligonucleotides devoid of modified nucleosides was used as an unspecific substrate (G ligand).

Transition from the unspecific DNA duplex (the simplest interactions) to the specific 8-oxoG substrate (complete enzymatic reaction cycle) results in the appearance of additional interactions between the reacting molecules and, hence, to additional conformational changes in both the enzyme and substrate molecules. So, this approach enables attribution of conformational changes in the protein molecule to distinct interactions with the substrate groups during its recognition and cleavage.

Analysis of conformational changes in hOGG1 interacting with DNA substrates. Conformational changes in hOGG1 in the course of its binding with G and F ligands as well as with AP substrate are characterized by a weak

Table 2. Sequences of oligonucleotides comprising DNA substrates

Abbreviation	Oligonucleotide sequence
8-oxoG substrate	d(CTCTC(**8-oxoG**)CCTTCC)/d(GGAAGGCGAGAG)
8-oxoG-aPu substrate	d(CTCTC(**8-oxoG**)(2-aPu)CTTCC)/d(GGAAGCCGAGAG)
AP-aPu substrate	d(CTCTC(**AP**)(2-aPu)CTTCC)/d(GGAAGCCGAGAG)
F-aPu ligand	d(CTCTC**F**(2-aPu)CTTCC)/d(GGAAGCCGAGAG)
G-aPu ligand	d(CTCTC**G**(2-aPu)CTTCC)/d(GGAAGCCGAGAG)



change of Trp fluorescence intensity, whereas the change in intensity of Trp fluorescence was pronounced when being recorded for the oxoG substrate (Fig. 1) [47].

At least three phases are detected during lesioned base recognition and binding in the active site of the enzyme. They are characterized by difference in conformation of the protein molecule and, as a consequence, by difference in their fluorescence emission spectra. On the basis of these data, the minimal kinetic scheme (1) was proposed for the interaction between hOGG1 and 8-oxoG substrate, in which the chemical stages are represented by two consecutive reactions [47]:

$$E + OG \rightleftarrows [E \cdot OG]1 \rightleftarrows [E \cdot OG]2 \rightleftarrows [E \cdot OG]3 \downarrow$$
$$E + P \rightleftarrows E \cdot P \leftarrow [E \cdot AP] \quad (1)$$

Analysis of conformational changes in DNA substrates interacting with hOGG1. When studying enzymatic reactions involving nucleic acids, fluorescent nucleotide analogs that retain their ability to form hydrogen bonds with unmodified nucleotides are used. The 2-aminopurine residue (2-aPu) is one of the most attractive fluorescent analogs of N-heterocycles. Two hydrogen bonds are formed in the 2-aPu/T pair, like in the case of adenine. However, the formation of either one or two hydrogen bonds is possible for the 2-aPu/C pair [49, 50]. Thus, incorporation of 2-aPu opposite C and T bases leads to minimal disturbance of DNA structure.

The fluorescence intensity of 2-aPu is known to be strongly dependent on its surrounding. For instance, a drastic decrease in fluorescence intensity of 2-aPu proceeds when stacking occurs between the neighboring bases or upon the formation of duplex [51].

Conformational transitions in DNA substrate molecules induced by the interaction with hOGG1 were registered from the change in fluorescence intensity of 2-aPu [48]. DNA substrates containing 2-aPu residue were constructed using the approach of stepwise complication of substrate structure (Table 2).

Nonspecific binding of DNA duplex was shown to occur upon interaction of hOGG1 with G-aPu ligand. This process, as is obvious from the kinetics curves (Fig. 2), took 0.02 sec to complete and was accompanied by an increase in intensity of 2-aPu fluorescence. This increase in fluorescence suggested weakening of the complementary interactions in the primary unspecific complex between the DNA and enzyme.

Kinetics curves obtained for F-aPu ligand were characterized by decrease in intensity of 2-aPu fluorescence within 5 sec, suggesting the transition of 2-aPu into the hydrophobic surroundings (Fig. 2). This process is likely related to filling the gap in the DNA duplex with amino acid residues of hOGG1. These residues are Asn149, Tyr203, Arg154, and Arg204 according to the data of X-ray structural analysis [33].

Biphasic change in intensity of 2-aPu fluorescence was registered during the interaction between the enzyme and AP substrate. The decrease in intensity in the initial

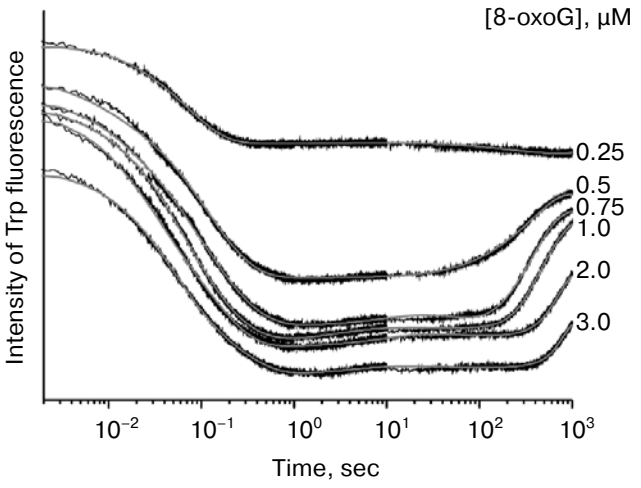


Fig. 1. Experimental kinetics curves characterizing conformational changes in hOGG1 interacting with 8-oxoG substrate and calculated curves obtained by data processing according to kinetic scheme (1) [47] (reprinted by permission from Oxford University Press: [Kuznetsov, N. A., Koval, V. V., Zharkov, D. O., Nevinsky, G. A., Douglas, K. T., and Fedorova, O. S. (2005) *Nucleic Acids Res.*, **33**, 3919-3931], copyright 2005).

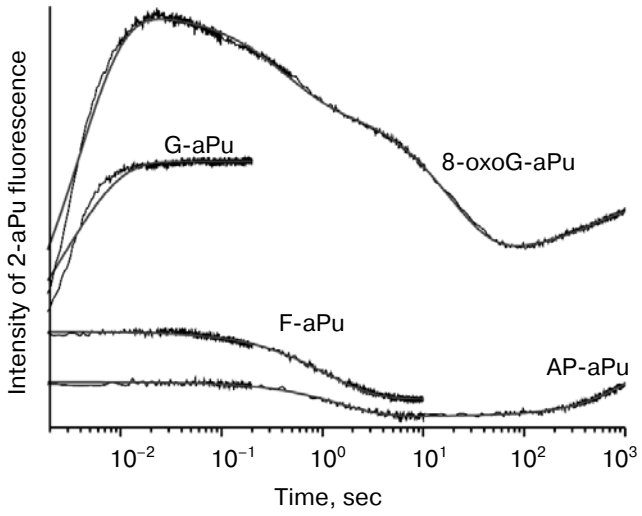


Fig. 2. Experimental kinetic curves characterizing conformational changes in DNA substrates interacting with hOGG1 and the curves calculated according with schemes proposed in work [48] (reprinted by permission from the American Society for Biochemistry and Molecular Biology: [Kuznetsov, N. A., Koval, V. V., Nevinsky, G. A., Douglas, K. T., Zharkov, D. O., and Fedorova, O. S. (2007) *J. Biol. Chem.*, **282**, 1029-1038], copyright 2007).

time range (up to 10 sec) was assigned to the stage of catalytically active complex formation. Dissociation of the enzyme–product complex led, in turn, to growth of fluorescence intensity within 10-1000 sec (Fig. 2).

The interaction between hOGG1 and 8-oxoG-aPu substrate includes additional stages within the process of catalytically active complex formation (Fig. 2). The formation of unspecific primary complex results in partial melting in the contact site, as in the case of G-aPu ligand, enhancing the 2-aPu fluorescence. However, the intensity of 2-aPu fluorescence increases to a greater extent. This fact suggests a simultaneous inversion of 8-oxoG residue into the enzyme active center, resulting in appearance of a gap in the DNA duplex. Subsequent filling of this gap with amino acid residues of hOGG1 resulted in the decrease in intensity of 2-aPu fluorescence, as in the case of the AP-site, but this decrease was 5-10 times slower than that with AP substrate, so that it was only completed in 50-100 sec. This process had at least two intermediates, as in the case of monitoring the change of hOGG1 Trp fluorescence, which corresponded to the kinetic sequence (1).

Bending of DNA in its complex with the enzyme. Fluorescence resonance energy transfer (FRET) is another additional method for the study of conformational transitions in biopolymers allowing detection of changes in distance, for instance due to DNA bending, between the fluorescence donor and acceptor located at different sites in the biopolymer. The FRET method was used for study of recognition and binding of DNA substrates with hOGG1. With this aim, the pairs of fluorescent dyes Cy3/Cy5 and Flu/Dab were linked to opposite ends of oligonucleotide duplex (Table 3).

Two types of FRET substrates were used in this work: the first contained the donor–quencher pair (Flu-G/C-Dab and Flu-F/C-Dab), the second contained the donor–acceptor pair (Cy3-8-oxoG/C-Cy5).

The spectra of FRET substrates given in Fig. 3 (a and b) show that formation of the Flu-G/C-Dab and Flu-F/C-Dab duplex compared with the single-stranded oligonucleotides Flu-G and Flu-F, respectively, leads to decrease in intensity of Flu fluorescence. This decrease is due to the fluorescence quenching by the Dab residue. The addition of hOGG1 to Flu-G/C-Dab and Flu-F/C-Dab duplexes results in further decrease in Flu fluorescence due to bending of the duplex with additional approach of the fluorophore and the quencher.

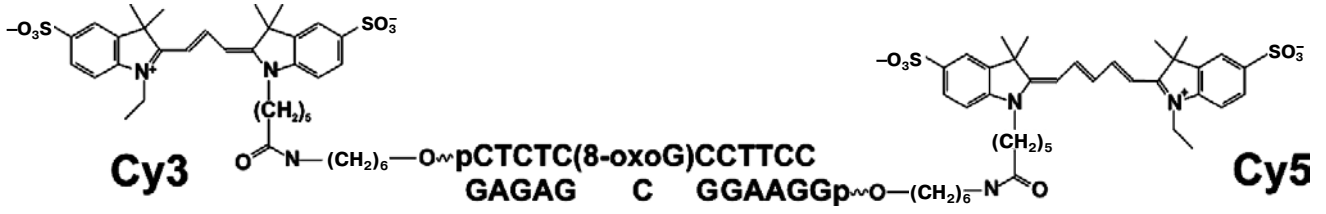
The bending kinetics of Flu-G/C-Dab duplex represented in Fig. 3c suggests fast (0.02 sec) distortion of the DNA duplex (Fig. 2 [48]) detected by 2-aPu followed by a slow bending process ($\tau_{1/2} \approx 5$ sec). It is likely that these structural changes in DNA indicate the processes of recognition of a lesioned base.

Analysis of the kinetic curve of Flu-F/C-Dab duplex demonstrated that the bending of duplex with a deleted base occurs faster ($\tau_{1/2} \approx 2$ sec) than in the case of unspecific DNA. Comparison of data obtained for F-aPu ligand (Fig. 2) with the kinetics of Flu-F/C-Dab bending (Fig. 3d) indicates that the incorporation of amino acid residues and duplex bending occurs in parallel.

The use of Cy3-8-oxoG/C-Cy5 substrate amenable to cleavage also revealed the stage of duplex bending (Fig. 4). However, in this case the formation of catalytically active complex is followed by chemical cleavage stages and the stage of complex decay between the enzyme and reaction product. Unlike Flu-F/C-Dab duplex, the bending of duplex did not result in substantial changes in fluorescence intensity in the case of Cy3-8-oxoG/C-Cy5. The kinetic curves demonstrate a slight enhancement of fluorescence intensity in the first 100 sec of the reaction.

Table 3. Oligonucleotide sequences introduced into DNA substrates

Abbreviation	Oligonucleotide sequence
Cy3-8-oxoG	Cy3-NH-$(CH_2)_6$-d(CTCTC(**8-oxoG**)CCTTCC)
C-Cy5	d(GGAAGG**C**GAGAG)-$(CH_2)_6$-NH-Cy5
Flu-G	Flu-d(CTCTC**G**CCTTCC)
Flu-F	Flu-d(CTCTC**F**CCTTCC)
C-Dab	d(GGAAGG**C**GAGAG)-Dab



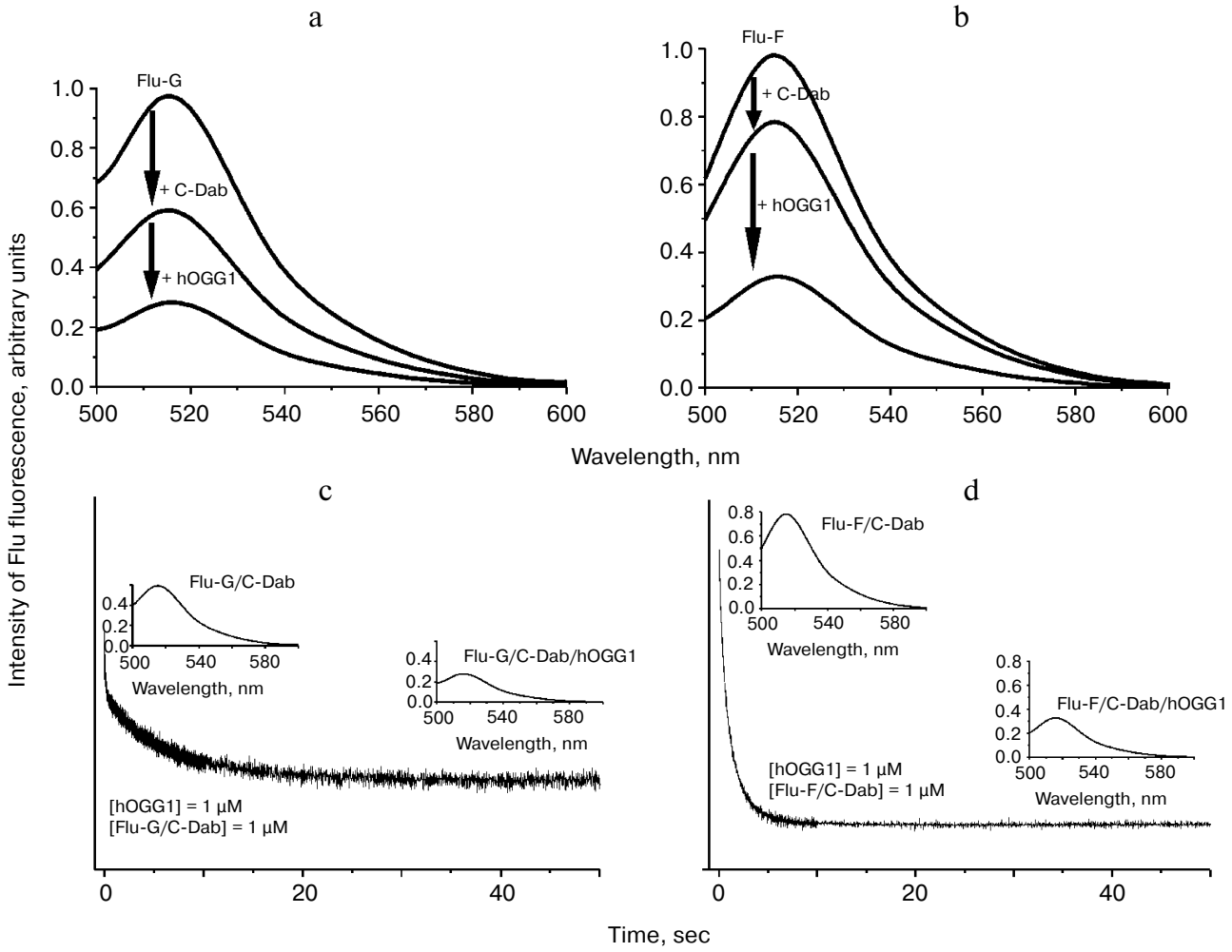


Fig. 3. Fluorescence spectra of Flu-G (a) and Flu-F (b) oligonucleotides, their duplexes with C-Dab, and complexes with hOGG1. Bending kinetics of duplexes Flu-G/C-Dab (c) and Flu-F/C-Dab (d) interacting with hOGG1.

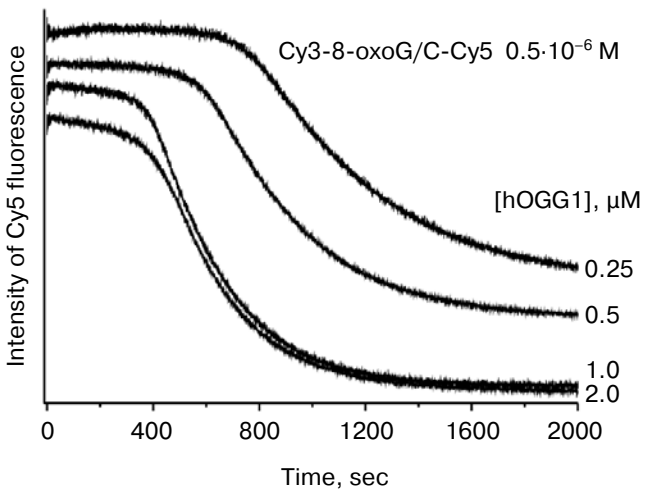


Fig. 4. Interaction between hOGG1 and Cy3-8-oxoG/C-Cy5 duplex and kinetic curves of fluorescence intensity.

It is worth noting that, consistent with earlier data, the formation of catalytically active complex occurs within 100 sec. Decay of the enzyme–product complex was detected thereafter.

MASS SPECTROMETRY OF INTERMEDIATES FORMED DURING CATALYSIS BY hOGG1

We also periodically recorded the MS/ESI-mass-spectra of the reaction mixture (Fig. 5). The N-glycosylation reaction was shown to occur within the first 30 sec to form intermediate 4, when the amount of 8-oxoG substrate twofold exceeded that of enzyme (Scheme 1). The β-elimination reaction is slow and terminates only within 1000 sec, as shown by the mass spectrometry. These data are consistent with the data of steady-state kinetics (Table 1), suggesting β-elimination to be the rate-limiting stage of hOGG1 catalysis.

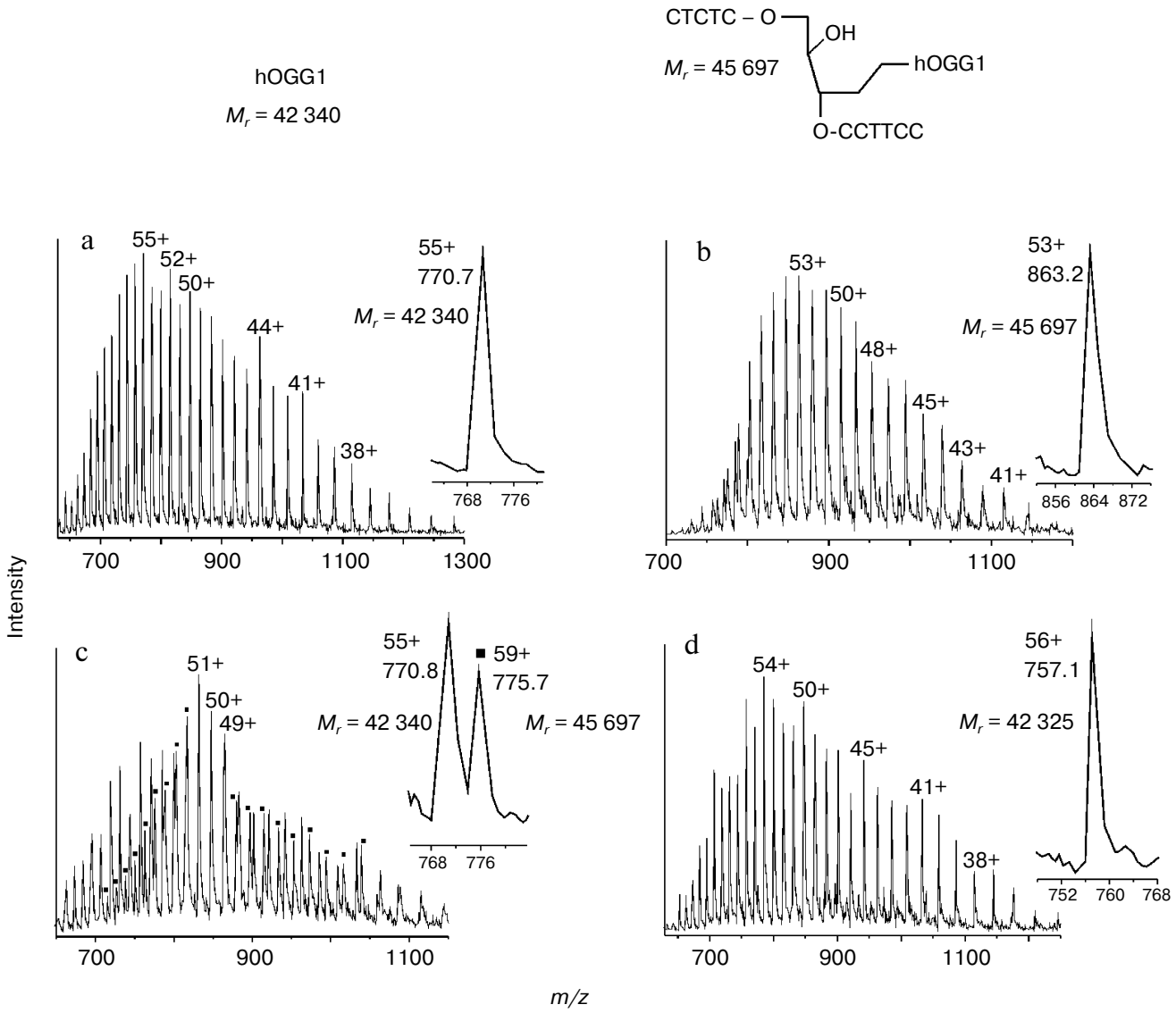
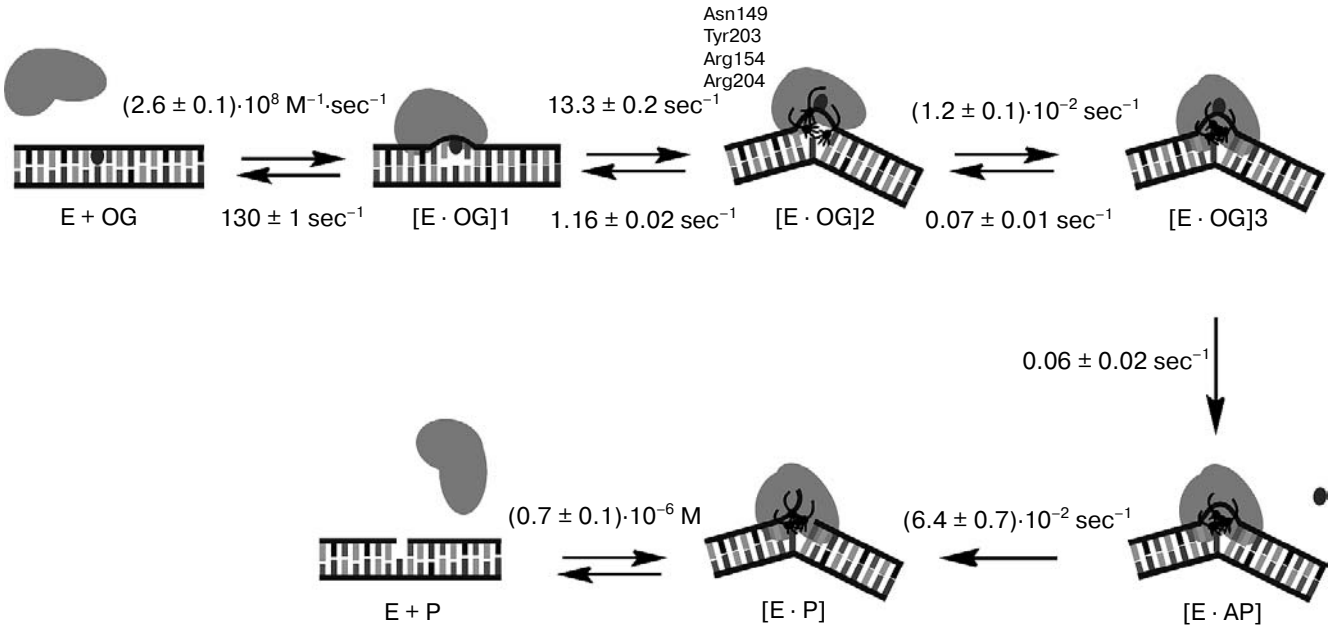


Fig. 5. Mass spectra of intermediates formed during interaction between hOGG1 and 8-oxoG within t = 0, 30, 600, and 1000 sec (a-d, respectively).

MECHANISM OF LESION RECOGNITION DURING "SLIDING"

The problem of enzymatic search for damage can be divided into the fast search for a lesioned nucleotide and its site-specific binding [52]. There are several models [53] explaining the rapid movement of proteins along unspecific DNA to specific sites. The model of one-dimensional diffusion includes two variants of enzyme transfer along DNA: "sliding", representing free transfer of the protein along the DNA strand without dissociation, and "hopping" characterized by a series of "microscopic" acts of dissociation/association resulting in lineal transfer of the protein along unspecific DNA without a complete dissociation of the complex. A model exists for dissociation/association ("jumping") assuming protein dissociation followed by its transfer to a substantial distance from the initial location and rebinding with DNA.

It is worth noting that the search for damage in chromatin probably occurs by several mechanisms simultaneously [54]. The ribose phosphate backbone was shown by atomic force microscopy to bend in the complex between hOGG1 and unspecific DNA duplex [55]. From the crystal structure of such a complex [34], it was concluded that not only the bending of DNA strand occurs within the complex, but also guanine flipping out of the double helix into the "exo-site". The authors of that work supposed that the main function of this center is discrimination between damaged and non-damaged bases at the thermodynamics level. A process of hOGG1 "sliding" along the



Kinetic mechanism proposed for interaction between hOGG1 and 8-oxoG substrate

Scheme 3

DNA molecule with very high speed providing a rapid search for DNA damage was detected in a later work [56] using fluorescence microscopy. The authors calculated the upper limit of diffusion coefficient at 25°C to be $1.2 \cdot 10^7$ $(bp)^2$/sec, according to which the enzyme can scan $2.4 \cdot 10^7$ bp per second. It should be noted, however, that this scanning rate corresponds to the transfer of the enzyme in both directions along the DNA strand. It has been shown that the enzyme only shifts by 1000 nucleotides on average from its initial localization after the scanning of 10^6 bp. Recent studies on protein sliding along DNA [57] have shown that hOGG1 makes circular motions around the double helix with one of its sides being in continuous contact with DNA. The authors of work [58] showed that the induction of a DNA lesion *in vivo* results in accumulation of hOGG1 in chromatin. These data confirm the ability of the repair enzyme to search for damages under "hard" conditions of DNA packed in nucleosomes.

Thus, this suggests that hOGG1, being accumulated in chromatin, is bound with free DNA to slide along it in search for lesions. If the enzyme comes up against an obstacle in the form of chromatin proteins, a "jumping" or dissociation/association of the enzyme occurs. When the enzyme meets a lesion, this lesion first enters the "pre-catalytic" and then the catalytic site of the enzyme. Since the repair enzymes execute the search for DNA damage via "jumping" of the enzyme molecule between DNA sites and "sliding" along the DNA strand within the range of tens of nucleotide pairs [54], the data on conformational dynamics of hOGG1 and DNA obtained in works [47, 48] are of interest for solution of the problem concerning finding of DNA damage.

We have proposed a kinetics mechanism for the interaction between 8-oxoguanine DNA glycosylase and a damaged DNA site on the basis of presently known crystal structure data on various enzyme–substrate complexes [31, 33-37] and the data presented in works [47, 48].

Formation of a nonspecific complex is the first stage of interaction between hOGG1 and DNA. The bending of the DNA molecule and insertion of each base into the "pre-catalytic" site for checking for its structure occurs in such a complex. This process is very fast and is accompanied by "distortion" of the DNA structure. The DNA duplex also bends the unspecific complex. The interaction between hOGG1 and F ligand has enabled, detecting the stage of filling the cavity in DNA with amino acid residues of the enzyme (Asn149, Tyr203, Arg154, and Arg204). Analysis of kinetic curves obtained for AP substrate demonstrated that β-elimination is very slow and is a rate-limiting stage of the overall enzymatic process.

The combined data characterizing the interaction between hOGG1 and 8-oxoG substrate suggest multiple conformational changes in both molecules (Scheme 3). The primary binding of oxoG substrate results in fast 8-oxoG flipping out of the double helix, first into the "pre-catalytic" and then into the catalytic site of the enzyme. The formed void in the DNA duplex is filled by specific amino acid residues, and the kinked ribose-phosphate

backbone of the substrate begins the second stage of the binding process. However, as we have demonstrated using 8-oxoG-aPu substrate, the third stage also reflects the incorporation of amino acid residues into the DNA duplex. Apparently, the bending of the duplex and formation of the complete contact system between the enzyme and DNA substrate, which is necessary for the catalytic stage of the process, only ends in the third complex.

Thus, the results of analysis of hOGG1 and DNA conformational dynamics by monitoring the change in Trp and 2-aPu fluorescence intensity [47, 48] as well as the data presented in the present work, in addition to the X-ray structural analysis available from the literature, have enabled evaluation of a detailed molecular kinetic mechanism of the reaction catalyzed by hOGG1, the sequence of events during the enzymatic cycle, and the role of conformational transitions associated with the catalytic process.

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