Correlated Cleavage of Damaged DNA by Bacterial and Human 8-Oxoguanine-DNA Glycosylases[†]

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ABSTRACT: Many enzymes acting on specific rare lesions in DNA are suggested to search for their targets by facilitated one-dimensional diffusion. We have used a recently developed correlated cleavage assay to investigate whether this mechanism operates for Fpg and OGG1, two structurally unrelated DNA glycosylases that excise an important oxidative lesion, 7,8-dihydro-8-oxoguanine (8-oxoG), from DNA. Similar to a number of other DNA glycosylases or restriction endonucleases, Fpg and OGG1 processively excised 8-oxoG from pairs with cytosine at low salt concentrations, indicating that the lesion search likely proceeds by one-dimensional diffusion. At high salt concentrations, both enzymes switched to a distributive mode of lesion search. Correlated cleavage of abasic site-containing substrates proceeded in the same manner as cleavage of 8-oxoG. Interestingly, both Fpg and especially OGG1 demonstrated higher processivity if the substrate contained 8-oxoG·A pairs, against which these enzyme discriminate. Introduction of a nick into the substrate DNA did not decrease the extent of correlated cleavage, suggesting that the search probably involves hopping between adjacent positions on DNA rather than sliding along DNA. This was further supported by the observation that mutant forms of Fpg (Fpg-F110A and Fpg-F110W) with different sizes of the side chain of the amino acid residue inserted into DNA during scanning were both less processive than the wild-type enzyme. In conclusion, processive cleavage by Fpg and OGG1 does not correlate with their substrate specificity and under nearly physiological salt conditions may be replaced with the distributive mode of action.

DNA in living cells is continually damaged by various endogenous and environmental factors, including metabolic byproducts (e.g., reactive oxygen species), ionizing radiation, ultraviolet light, etc. (*1, 2*). The possible consequences of such damage are mutagenesis, cell death, carcinogenesis, and aging. To avoid these grave outcomes, cells use several enzymatic pathways, collectively known as DNA repair (*3*). In particular, damaged DNA bases are excised by specific DNA glycosylases, which, together with AP endonucleases, DNA polymerases, DNA ligases, and several accessory proteins, are combined in the base excision repair pathway (*3, 4*).

DNA repair enzymes face a hard task of locating rare lesions among a vast excess of intact dNMP in DNA. Even the most abundant damaged bases are still encountered with the frequency of one per thousands under normal conditions (*5*), while many mutagenic or toxic lesions are much scarcer. For instance, 7,8-dihydro-8-oxoguanine (8-oxoG),[1] a significant source of mutations, including those in human cancers (*6, 7*), is found in DNA at the background level of ~1 per 10^6 guanine bases in human cells (*8*).

The repair of 8-oxoG is initiated by dedicated DNA glycosylases, formamidopyrimidine-DNA glycosylase (Fpg) in bacteria and 8-oxoguanine-DNA glycosylase (OGG1) in eukaryotes (*9–12*). These two enzymes are not homologous at the sequence level and do not share similar folds (*13, 14*). However, their substrate specificity is comparable. Both Fpg and OGG1 excise 8-oxoG and 2,6-diamino-4-oxo-5-formamidopyrimidine (Fapy-G); additionally, Fpg efficiently removes 4,6-diamino-5-formamidopyrimidine (Fapy-A) (*9, 15–18*). The guanine-derived bases are excised much more efficiently from pairs with cytosine, and both enzymes show a much lower activity if adenine is present opposite the lesion (*9, 19–21*). This opposite-base preference minimizes mutagenesis by 8-oxoG, which directs misincorporation of dAMP by replicative DNA polymerases; 8-oxoG·A pairs are repaired by another DNA glycosylase, MutY, with the initial excision of adenine (*6*). Both Fpg and OGG1 belong to the group of bifunctional DNA glycosylases, which, in addition to base excision, cleave DNA at the nascent or preformed abasic (AP) site by β-elimination mechanism; however, the activity of Fpg on the AP site is much higher than the activity of OGG1 on the same substrate (*20*).

The mechanism of lesion search by DNA glycosylases is subject to active studies. It is believed that these enzymes bind DNA nonspecifically and then move along it by one-dimensional diffusion until they encounter a substrate lesion

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[1] Abbreviations: 8-oxoG, 7,8-dihydro-8-oxoguanine; AP, abasic (apurinic/apyrimidinic).

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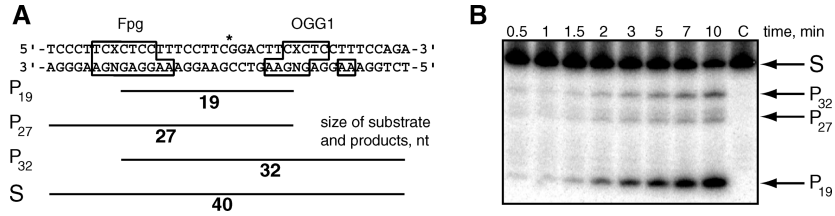

FIGURE 1: Correlated cleavage assay. (A) Scheme of the substrate for the assay. X = 8-oxoG or an AP site; N = C or A. The asterisk indicates the position of the radioactive label. Footprints of Fpg and OGG1 are boxed in the left and right part of the duplex, respectively. (B) Representative gel for the correlated cleavage assay (Fpg, 8-oxoG•A substrate, 10 mM KCl). The time of incubation is indicated. Lane C show the control reaction (no enzyme added). The arrows show the mobility of the substrate and different product oligonucleotides.

(*22*), a mechanism that was first proposed for the search of specific DNA sequences by transcription regulators (*23*). Two general mechanisms of one-dimensional diffusion are sliding and hopping. In the former case, the enzyme does not release DNA while translocating from one position to another, so the DNA base sampled by the enzyme at any moment is absolutely correlated in its position with the base sampled at the previous moment. In the hopping mechanism, the enzyme releases DNA and immediately binds it in the vicinity of its previous position; thus, although some bases may be skipped, the positions of sampled bases are still strongly correlated. On the other hand, a distributive mechanism of search implies that the enzyme releases DNA and rebinds in a place totally uncorrelated with the previously sampled position (*22, 23*).

In the case of Fpg and OGG1, the correlated translocation has been shown directly by single-molecule imaging (*24*), and the structures of both enzymes in the complex with undamaged DNA suggest that all normal base pairs are sampled during the search, albeit by different mechanisms (*25, 26*). Lesion search by one-dimensional diffusion gives rise to the phenomenon of cleavage processivity, the ability of an enzyme molecule to excise two closely spaced damaged bases without dissociating from DNA (*22, 27*). Processive cleavage has been demonstrated for Fpg (*28*); no data for OGG1 are available. In addition, the existing methods for analysis of processivity of DNA repair enzymes [damaged plasmid assay (*27*) and concatemetric oligonucleotide assay (*29*)] allow little control over the substrate DNA structure and thus hinder the analysis of the mechanisms of translocation.

Recently, we have developed a new versatile oligonucleotide-based assay, relying on correlated cleavage of two lesions in the same DNA molecule, to study the processivity of DNA glycosylases and used it to analyze lesion search by uracil-DNA glycosylase (Ung) (*30*). Here we apply this assay to investigate processive cleavage of DNA by Fpg and OGG1 on their preferred substrate base pairs (8-oxoG•C or AP•C) and on a poor substrate (8-oxoG•A).

MATERIALS AND METHODS

Enzymes. Phage T4 polynucleotide kinase, phage T4 DNA ligase, *Escherichia coli* Ung, and *Dpn*II restriction endonuclease were from New England Biolabs (Beverly, MA). *E. coli* Fpg and human OGG1 were overexpressed and purified as described (*14, 31*). Mutant forms of Fpg, Fpg-F110A and Fpg-F110W, were constructed using a QuickChange site-directed mutagenesis kit (Stratagene, Cedar Creek, TX) and purified in the same way as the wild-type enzyme.

Oligonucleotides. Oligonucleotides were purchased from Operon Biotechnologies (Huntsville, AL). To prepare the substrate for processivity studies, 0.6 nmol of a 20-mer d(GGACTTCXCTCCTTTCCAGA) (X = 8-oxoG or uracil) was labeled by [γ-^{32}P]ATP using 10 units of polynucleotide kinase for 40 min at 37 °C, followed by addition of the same amount of polynucleotide kinase and continuing the incubation for 40 min. In a separate mixture, an additional 0.6 nmol of the same oligonucleotide was phosphorylated at its 5′-terminus with 1 mM ATP under the same conditions. Both reactions were terminated via a 10 min heating at 95 °C, pooled, and combined with 1.8 nmol of a 20-mer d(TCCCTTCXCTCCTTTCCTTC) (X = 8-oxoG or uracil) and 1.8 nmol of d(TCTGGAAAGGAGCGAAGTCCGAAGGAAAGGAGCGAAGGGA) [the complementary 40-mer, placing C opposite both lesions (Figure 1A)]. To minimize unproductive annealing, the mixture was heated for 1 min at 95 °C, then incubated for 10 min at 57 °C (melting temperature of the 20-mers), and slowly cooled to 25 °C over the course of 2 h. After that, the mixture was supplemented with ATP (1 mM) and DNA ligase (2 Weiss units) and incubated overnight at 4 °C. The ligated 40-mer containing the internal ^{32}P label was purified by electrophoresis in an 18% polyacrylamide gel containing 7.2 M urea, eluted with 0.1× TE, precipitated with $LiClO_4$/acetone, and re-annealed to the complementary 40-mer. The correct arrangement of the ligated product was confirmed by its resistance to *Dpn*II (<1% cleaved); a recognition site of this enzyme (GATC) appears if the ligation joins the 20-mers in a reverse orientation or if concatemeric products are formed. To prepare the AP-containing substrate, the fully ligated and annealed substrate (10 pmol) was treated with 0.5 unit of Ung in the reaction buffer (see below) at 37 °C for 10 min and used immediately in the correlated cleavage assay. The conversion of U to AP sites under these conditions was nearly complete as judged by full hydrolysis of the product by 0.1 N NaOH (2 min at 95 °C).

Correlated Cleavage Assay. The reaction mixture (50 μL) included 50 nM duplex ^{32}P-labeled substrate, 25 mM sodium phosphate, and 1 mM dithiothreitol. When necessary, the mixture also contained 0−400 mM KCl. The reaction was initiated by adding the enzyme (1 nM for Fpg or Fpg-F110W and 2 nM for Fpg-F110A and OGG1) and allowed to proceed at 37 °C, and 5 μL aliquots were withdrawn at 0.5, 1, 1.5, 2, 3, 5, 7, and 10 min. In the experiments with Fpg, the aliquots were quenched by being mixed with an equal volume of formamide-containing dye (*32*) and heated for 1 min at 95 °C and kept on ice before analysis. In the reactions with OGG1, the aliquots were mixed with 1.25 μL of 0.5 M putrescine-HCl (pH 8.0), heated for 5 min at 95 °C to break DNA at the AP sites formed by the glycosylase activity, supplemented with formamide-containing dye, and kept on

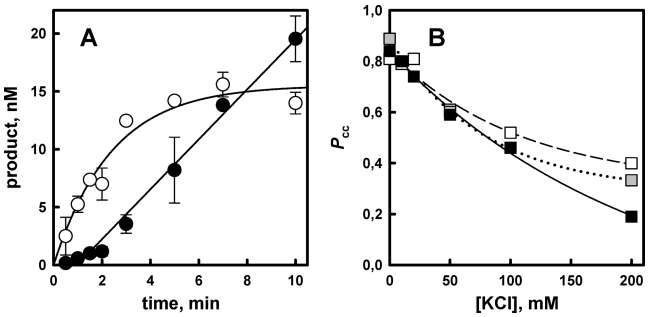

FIGURE 2: Correlated cleavage by Fpg. (A) Time course of accumulation of products of different lengths (8-oxoG·C substrate, 100 mM KCl): P_{19} (●) and $P_{27} + P_{32}$ (○; shown as a sum to avoid overlap of symbols). The mean ± the standard deviation of three independent experiments is shown; in some cases, symbols cover the error bars. (B) Salt dependence of P_{cc} of cleavage of 8-oxoG·C (black squares, solid line), AP·C (gray squares, dotted line), and 8-oxoG·A (white squares, dashed line) base pairs.

ice. Immediately before analysis, all samples were heated for 1 min at 95 °C, unless the substrate contained AP sites. The reaction products were separated by electrophoresis in an 18% polyacrylamide gel containing 7.2 M urea and quantified by phosphorimaging using a Storm 840 system and ImageQuant version 5.2 (GE Healthcare Life Sciences, Uppsala, Sweden). The initial rates were extracted from the linear parts of the time courses of product accumulation. P_{cc} values were calculated for each condition as $P_{cc} = v_{19}/(v_{19} + v_{27} + v_{32})$, where v_n is the initial rate of accumulation of an n-mer product.

RESULTS

Correlated Cleavage by Fpg and Its Specificity. To investigate the correlation between cleavage of DNA at two 8-oxoG residues, we have used the same type of substrate as we did before for the analysis of Ung processivity (*30*). Briefly, the substrate contains two lesions (8-oxoG or AP in the case of Fpg) separated by a stretch of DNA long enough to avoid their simultaneous coverage by a single protein footprint, and a radioactive label between the damaged sites (Figure 1A). Both lesions are placed in the same sequence context; the length of the identical sequences is chosen to exceed the enzyme's footprint so that the kinetics of cleavage at both sites are identical. Crystallographically defined footprints of Fpg and OGG1 (*13, 14*) are indicated in Figure 1A. Under Michaelis−Menten conditions, when the concentration of the enzyme is much lower than the concentration of the substrate, the enzyme is unlikely to rebind to the singly cleaved DNA molecule after full dissociation of the enzyme−product complex but can be transferred to the second damaged site in the same DNA molecule with a certain rate constant and then cleave it. Thus, the ratio of the rate of accumulation of the 19-mer product of double cleavage (P_{19}) to the combined rate of accumulation of all products [P_{19} and products of cleavage at a single lesion, P_{27} and P_{32} (Figure 1A,B)] gives the probability of correlated cleavage (P_{cc}), i.e., the probability that an enzyme molecule, after nicking DNA at one lesion, will introduce the second nick into the same substrate molecule (discussed in ref *30*).

A typical time course of accumulation of different products of cleavage by Fpg is shown in Figure 2A. The products of cleavage at a single site began to appear immediately, but eventually the rate of their accumulation decreased. Accumulation of the double cleavage product started after a small lag and in most cases proceeded linearly up to 10 min. From the linear parts of these time courses, steady-state rates could be extracted and converted to P_{cc} values ($P_{cc} = 0.46 \pm 0.04$ for the reaction in Figure 2A).

Processive cleavage of DNA by DNA glycosylases and other enzymes (e.g., restriction endonucleases) is less efficient at higher salt concentrations due to nonspecific protein−DNA interactions, which are mostly electrostatic in nature, being screened out by ions in the reaction mixture. Therefore, any quantitative measure of processivity should decrease with an increase in salt concentration. We have determined P_{cc} values for Fpg-catalyzed cleavage of 8-oxoG·C base pairs at different concentrations of KCl in the reaction mixture (Figure 2B). As expected, P_{cc} was reduced with an increase in ionic strength in the range of 0−200 mM KCl, with the decrease in the level of correlated cleavage being nearly linear. At 400 mM KCl, there was very little cleavage and no double cleavage at all (not shown). Thus, measurements of P_{cc} support the idea that lesion search by Fpg proceeds by correlated translocation at low ionic strengths but may switch to a distributive mode at higher salt concentrations.

We have also inquired about whether the nature of the substrate influences the P_{cc} of Fpg cleavage. To do this, we have measured P_{cc} in the same range of KCl concentrations for two other substrates, AP·C and 8-oxoG·A. Fpg cleaves AP·C substrates equally well or even better than 8-oxoG·C substrates (*19, 33*); however, it is possible that the lack of the damaged base in the AP·C substrate influences the processes of product release by Fpg and thus affects correlated cleavage. Of the 8-oxoG-containing substrates of Fpg, the 8-oxoG·A substrate is the least preferred [~20−100-fold worse than 8-oxoG·C in terms of k_{cat}/K_M (*19, 34*)], but nevertheless, its cleavage is detectable. The P_{cc} values for cleavage of AP·C by Fpg were nearly indistinguishable from those for 8-oxoG·C at 0−100 mM KCl (in Figure 2B, compare black and gray squares), but at 200 mM KCl, the level of correlated cleavage of AP·C remained higher than that of 8-oxoG·C. In the case of 8-oxoG·A, P_{cc} tended to be higher that for 8-oxoG·C, with the effect especially pronounced at higher salt concentrations (Figure 2B). Thus, although the differences between various substrates were not spectacular, the correlated cleavage of the physiological substrate 8-oxoG·C was the most sensitive to electrostatic effects.

Correlated Cleavage by OGG1 and Its Specificity. Eukaryotic 8-oxoguanine-DNA glycosylase OGG1 is different from Fpg in sequence, three-dimensional structure, and kinetic mechanism, although the two enzymes partially overlap in their substrate specificity and share similar chemistry of base excision, which involves a nucleophilic attack by the enzyme's amino group at C1′ of the damaged deoxynucleotide. It was therefore interesting to compare the processivity of OGG1 and Fpg in the correlated cleavage assay. The overall time courses of product accumulation for OGG1 resembled those observed with Fpg (Figure 3A), with one exception that a burst phase was seen in the appearance of products of a single cleavage, in agreement with multiple published reports (*35–38*). We have determined the dependence of P_{cc} on the ionic strength for both 8-oxoG·C and 8-oxoG·A substrates. The salt concentration in these experi-

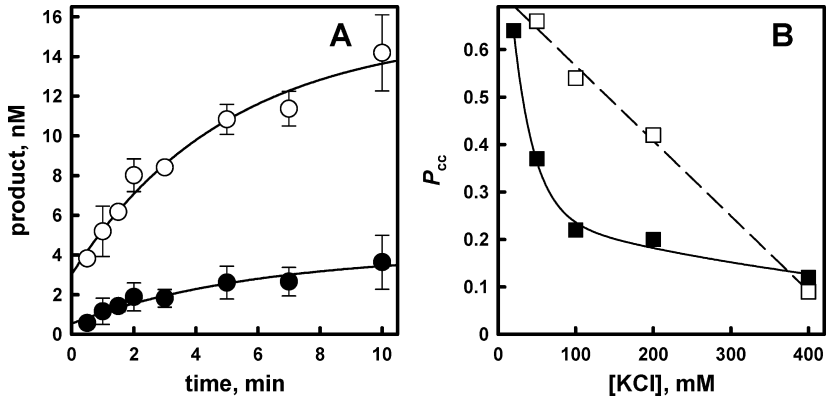

FIGURE 3: Correlated cleavage by OGG1. (A) Time course of accumulation of products of different lengths (8-oxoG·C substrate, 100 mM KCl): P_{19} (●) and $P_{27} + P_{32}$ (○; shown as a sum to avoid overlap of symbols). The mean ± standard deviation of three independent experiments is shown. (B) Salt dependence of P_{cc} of cleavage of 8-oxoG·C (■, solid line) and 8-oxoG·A (□, dashed line).

ments varied from 20 to 400 mM for 8-oxoG·C and from 50 to 400 mM for 8-oxoG·A; the P_{cc} value for 8-oxoG·C at 0−10 mM and for 8-oxoG·A at 0−20 mM KCl was impossible to measure due to a very low turnover number of the enzyme. As the activity of mammalian OGG1 on AP substrates is much lower than on 8-oxoG substrates (*20*), the study was limited to 8-oxoG substrates. Overall, the processivity of OGG1 measured by correlated cleavage of 8-oxoG·C was lower than the processivity of Fpg at salt concentrations up to 100 mM. OGG1 appeared to tolerate a higher ionic strength better than Fpg did, with noticeable correlated cleavage retained at 200 mM KCl and a P_{cc} of ~0.1 even at 400 mM KCl (Figure 3B). Interestingly, at most salt concentrations, P_{cc} for 8-oxoG·A was significantly higher than for 8-oxoG·C (Figure 3B), indicating that with the physiologically relevant substrate the probability of dissociation from DNA after base excision is higher than with the premutagenic substrate. In addition, while P_{cc} for 8-oxoG·C dropped sharply between 20 and 100 mM KCl and then decreased much more slowly, P_{cc} for 8-oxoG·A exhibited a nearly linear dependence on ionic strength (Figure 3B). Taken together, these data suggest that both OGG1 and Fpg tend to release the product of cleavage of the physiologically relevant substrate 8-oxoG·C, especially at an ionic strength close to the physiological value (>150 mM), while for the 8-oxoG·A substrate, the probability of the enzyme retained on DNA after the reaction is higher.

Correlated Cleavage of Nicked Substrates by Fpg and OGG1. Although the experiments described above indicate that lesion search by Fpg and OGG1 may partially proceed by correlated translocation, it was still unclear whether the translocation involves sliding, hopping, or a combination of both. A possible way of addressing this question is by introducing a small gap into DNA between the lesions, which will presumably affect sliding but have less or no influence on hopping. For instance, a one-phosphate gap in the undamaged strand decreases the P_{cc} of Ung by ~25% (*30*). Therefore, we have tested the ability of Fpg and OGG1 to exercise correlated cleavage of 8-oxoG·C substrates, in which the complementary strand was nicked, lacking the phosphate opposite the radioactively labeled one in the damaged strand (see Figure 1A). For Fpg, the comparison was conducted at 0 mM KCl, the highest-processivity conditions, whereas OGG1 was initially investigated at 50 mM KCl, where the processivity was not as high as at lower salt concentrations but the overall turnover number was higher. The results are summarized in Table 1. Only a marginal and statistically insignificant decrease in the level of correlated cleavage was observed for Fpg when the nick was introduced. For OGG1, unexpectedly, correlated cleavage of the nicked substrate was better than of the intact substrate. For this reason, the experiments were repeated at another salt concentration (100 mM), with the same result that the nick enhanced the correlated cleavage (Table 1). We infer that the contribution of sliding into one-dimensional diffusion by Fpg and OGG1 is relatively minor, and hopping is likely to be the dominating way of correlated translocation. The increase in OGG1 processivity on nicked DNA may possibly be due to the increased flexibility of the nicked substrate, bringing two halves of the substrate molecule closer together and allowing the enzyme, after dissociating from one half of the substrate DNA, to rebind faster to the other half. The apparent differences in the behavior of Fpg and OGG1 most probably ultimately stem from the differences in their structure.

Table 1: P_{cc} Values for Cleavage of Various substrates by Fpg (wild-type and mutants) and OGG1

enzyme	substrate	[KCl] (mM)	P_{cc}[a]
Fpg	8-oxoG·C	0	0.84 ± 0.03
Fpg	8-oxoG·C(nick)[b]	0	0.78 ± 0.06
OGG1	8-oxoG·C	50	0.37 ± 0.11
OGG1	8-oxoG·C(nick)	50	0.48 ± 0.16
OGG1	8-oxoG·C	100	0.22 ± 0.06
OGG1	8-oxoG·C(nick)	100	0.39 ± 0.13
Fpg	AP·C	0	0.89 ± 0.10
Fpg-F110A	AP·C	0	0.65 ± 0.08
Fpg-F110W	AP·C	0	0.63 ± 0.15

[a] Mean ± SD (n = 3). [b] Nicked complementary strand.

Correlated Cleavage by Fpg Wedge Mutants. The structure of Fpg from *Bacillus stearothermophilus* bound to undamaged DNA (*26*) indicates that scanning of normal DNA by Fpg involves wedging of Phe-114 (Phe-110 in *E. coli* Fpg) next to the sampled base. Therefore, insertion and withdrawal of this Phe residue may represent a rate-limiting step in diffusion of Fpg along DNA, if it occurs by sliding. To test whether correlated cleavage is affected by insertion or withdrawal of the intercalating Phe residue, we have constructed site-directed mutants, of *E. coli* Fpg, in which Phe-110 was replaced with either Ala (Fpg-F110A) or Trp (Fpg-F110W). Molecular modeling suggests that the former mutant should experience a lower sliding translocation

barrier, since the Ala residue would hardly be inserted into DNA, while the latter should face a higher sliding translocation barrier with a larger stacking area of Trp and the adjacent nucleobases (C. Simmerling, personal communication). Therefore, if correlated cleavage involves sliding, one might expect a higher P_{cc} for Fpg-F110A and a lower P_{cc} for Fpg-F110W in comparison with that of wild-type Fpg. The mutants were compared with the wild-type enzyme in their ability to catalyze correlated cleavage of AP substrates, since Fpg-F110A does not excise 8-oxoG; kinetics of Fpg-F110W on isolated AP and 8-oxoG lesions is similar to that of wild-type Fpg (D. O. Zharkov and C. Simmerling, manuscript in preparation). As shown in Table 1, the P_{cc} values for both Fpg-F110A and Fpg-F110W were similar to each other and were ~25−30% lower than the P_{cc} for wild-type Fpg. It can be concluded that wedge insertion or withdrawal does not limit the rate of translocation, at least over the distances used in our experiments. This conclusion is also in agreement with the hopping mechanism suggested above, as its rate may be dominated by processes other than the Phe wedge movement.

DISCUSSION

In this work, we have analyzed correlated cleavage of various substrates by two structurally different 8-oxoguanine-DNA glycosylases, *E. coli* Fpg and human OGG1. Through quantitative measurements of the enzyme activity on specially constructed substrates, the correlated cleavage assay provides a way to address a more fundamental question about the mechanism of lesion search by DNA repair enzymes. Currently, two general methods are used to investigate the processivity of cleavage by DNA repair enzymes. In one of them (damaged plasmid assay), a plasmid is randomly damaged so that sites appear that by chance contain two lesions in opposite strands separated by <12−16 bp, the shortest length of sticky DNA ends at physiological temperatures. The rates of accumulation of linear DNA and nicked DNA during enzymatic treatment are then compared, with the processivity showing as the preferred accumulation of linear DNA due to correlated cleavage at these adjacent damaged sites (*27, 39–41*). The other technique, concatemetric oligonucleotide assay, involves construction of a long DNA molecule with repeating damaged oligonucleotide units (monomers). Processive cleavage by a repair enzyme in this case is seen as a preferred appearance of monomer units during the reaction as compared with dimers and longer oligomers (*28, 29, 42, 43*). In comparison with these two assays, our oligonucleotide-based assay is more convenient and allows almost unlimited control of the substrate structure. Both in this work and in an earlier investigation of Ung (*30*), we have shown that P_{cc}, the main quantitative measure of correlated cleavage in this assay, behaves as expected for a measure of processivity, decreasing with increasing salt concentrations.

Our results indicate that, like several other DNA repair enzymes, such as phage T4 endonuclease V (DenV), *E. coli* and rat Ung, human AP endonuclease, and *E. coli* MutY (*27, 28, 39–43*), Fpg and OGG1 use correlated translocation to search for DNA lesions at low salt concentrations and act more distributively at higher salt concentrations. The information on processivity and lesion search by Fpg and OGG1 is presently limited to two studies, one employing a concatemetric oligonucleotide assay to address cleavage of 8-oxoG·C and 8-oxoG·A by Fpg (*28*) and the other using single-molecule fluorescence microscopy to follow movement of Fpg and OGG1 along undamaged DNA (*24*). The latter study thus cannot be directly compared to our results except the observation of the expected salt dependence of translocation efficiency, shown in ref *24* to be influenced by mean binding lifetime rather than by a diffusion constant. The data for Fpg reported in ref *28* show highly processive cleavage at lower salt concentrations for both 8-oxoG·C and 8-oxoG·A, again in agreement with our results. The only point of discrepancy seen between the findings reported here and in ref *28* is that we have observed the correlated cleavage of 8-oxoG·A by Fpg being somewhat more resistant to increasing salt concentrations, while the data in ref *28* suggest that the processivity of Fpg at 50 and 100 mM salt (NaCl in this case) is higher for 8-oxoG·C. Again in agreement with our observation, 8-oxoG·A retained more processivity at still higher salt concentrations, and the processivities on both substrates were nearly equal at <50 mM NaCl. The differences between the relative efficiencies of correlated cleavage at intermediate salt concentrations are most probably explained by variations in the assay, reaction conditions, and the sequences used; the influence of sequence and reaction components other than salt on the processivity of Fpg and OGG1 deserves further investigation.

The differences between the absolute values of P_{cc} for Fpg and OGG1 and the relative efficiency of correlated cleavage of 8-oxoG·C and 8-oxoG·A by these two enzymes may in part be due to their different kinetics on individual lesions. OGG1 dissociates from the AP·C product slowly; the steady-state rate of 8-oxoG·C processing is limited by product release (*35–37*), whereas the rate of 8-oxoG·A cleavage by OGG1 is likely limited by the binding or chemical step (*38, 44*). The high affinity of OGG1 for its AP·C product may lead to an apparent decrease in the rate of the second incision and thus of total correlated cleavage. Product inhibition of Fpg on either 8-oxoG·C or 8-oxoG·A is much less pronounced (*33, 45*), and the P_{cc} values for these two substrates are generally closer to each other in the case of Fpg. However, in neither Fpg nor OGG1 does the processive mode of DNA cleavage seem to contribute to the preference for correct 8-oxoG·C versus premutagenic 8-oxoG·A substrate.

Although several mutations in Fpg and OGG1 are known to affect the enzymes' substrate specificity to some degree (*18, 34, 46–49*), our finding that the replacement of the intercalating Phe-110 residue in Fpg with either Ala or Trp causes a decrease in processivity provides the first example of a mutation that inhibits the lesion search capability of this enzyme. This situation parallels the effect of an R26Q mutation in DenV pyrimidine dimer glycosylase, which also modifies the intercalating residue and greatly reduces the processivity of the enzyme (*50*). In addition, DenV processivity can be reduced by a number of mutations that reduce the level of nontarget DNA binding, mainly by eliminating positively charged side chains from the protein−DNA interface (*51–53*). It is tempting to speculate that some conserved basic residues of Fpg that contact DNA (Lys-56, His-70, Arg-109, and Arg-258) can also be involved in lesion location by one-dimensional diffusion, and that changes in

the lesion specificity observed for these mutants (*34, 49*) may be partially due to their altered DNA scanning properties.

It is worth noting that Fpg and OGG1 excise many oxidized bases from oligonucleotide substrates but not from oxidatively damaged genomic DNA (*15, 17, 18, 20, 54*). It has been hypothesized that these enzymes can bind the lesions in two modes, the jump-on mode (direct bimolecular association of the enzyme's damaged base-binding site with the lesion) and the run-on mode (finding the lesion during one-dimensional search), with the former predominating for the short oligonucleotide substrates with a very high effective lesion frequency and the latter prevailing in the more realistic model of long DNA with sparse lesions (*34*). A structural "saddle" motif in Fpg, including Arg-109, is probably necessary for the lesion location in the run-on mode (*34*). Therefore, the possibility that some specific features of correlated search may distinguish true biological substrates of Fpg and OGG1 such as 8-oxoG and Fapy lesions from "unspecific" substrates such as dihydrouracil (*34*) and some other lesions cannot be excluded. It remains to be investigated whether the processivity of OGG1 and Fpg in locating Fapy lesions (Fapy-G for OGG1 and Fapy-G and Fapy-A for Fpg) and dihydrouracil is the same as that for 8-oxoG and AP sites.

The processivity of Fpg and OGG1 under the conditions of high ionic strength, close to the concentration of salts in the intracellular environment, was rather low. It may be expected that other proteins present in the cell will further reduce it by competing with the glycosylase for nonspecific DNA binding, which was shown for *E. coli* Ung (*30*). Another factor favoring distributive lesion search would be a high intracellular concentration of DNA, which would facilitate intersegment transfer of the enzyme (*23*). Overall, it is still unclear whether processive search operates in vivo as a general mechanism, although there is evidence of that for phage T4 endonuclease V (*55*) and restriction endonuclease *Eco*RV (*56*). Nevertheless, given the relative paucity of the lesions compared with normal DNA, it is certain that most of the individual glycosylase binding events will be with a stretch of undamaged DNA. Thus, under the conditions with a high DNA concentration it may be advantageous for the enzyme to act more distributively. Our results suggest that with the relatively low DNA concentration (50 nM) Fpg and OGG1 may survey DNA by hopping rather by sliding; if the local DNA concentration increases, a fraction of the enzyme molecules would bind to another DNA segment instead of rebinding to the same segment in the hopping mode, switching from the processive to the distributive search. Therefore, even if the glycosylase cleaves DNA processively in vitro, the hopping mechanism of translocation may be easily adjusted to allow both processive and distributive searches in the cell.

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