The C-terminal Domain of MutY Glycosylase Determines the 7,8-Dihydro-8-oxo-guanine Specificity and Is Crucial for Mutation Avoidance

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Escherichia coli MutY is an adenine DNA glycosylase active on DNA substrates containing A/G, A/8-oxoG, or A/C mismatches and also has a weak guanine glycosylase activity on G/8-oxoG-containing DNA. The N-terminal domain of MutY, residues 1–226, has been shown to retain catalytic activity. Substrate binding, glycosylase, and Schiff base intermediate formation activities of the truncated and intact MutY were compared. MutY has high binding affinity with 8-oxoG when mispaired with A, G, T, C, or inosine. The truncated protein has more than 18-fold lower affinities for binding various 8-oxoG-containing mismatches when compared with intact MutY. MutY catalytic activity toward A/8-oxoG-containing DNA is much faster than that on A/G-containing DNA whereas deletion of the C-terminal domain reduces its catalytic preference for A/8-oxoG-DNA over A/G-DNA. MutY exerts more inhibition on the catalytic activity of MutM (Fpg) protein than does truncated MutY. The tight binding of MutY with GO mispaired with T, G, and apurinic/apyrimidinic sites may be involved in the regulation of MutM activity. An E. coli mutY strain that produces a N-terminal 249-residue truncated MutY confers a mutator phenotype. These findings strongly suggest that the C-terminal domain of MutY determines the 8-oxoG specificity and is crucial for mutation avoidance by oxidative damage.
with bound adenine shows that the adenine is buried in the active site of the catalytic domain and suggests that the mismatched adenine must flip out of the DNA helix for the glycosylase action (24). In the active site pocket, several amino acids (Glu\(^{182}\), Glu\(^{37}\), and Asp\(^{186}\)) are involved in adenine binding.

The crystal structure of the N-terminal domains of MutY also suggests some candidate residues for the recognition of the base opposite adenine. We and Clarke’s group (39, 47) have shown that the C-terminal domain of MutY plays an important role in the recognition of GO lesions while Manuel and Lloyd (37) have not drawn the same conclusion. In this paper, we further investigated the role of the C-terminal domain of MutY. The truncated MutY construct, M25, has more than 18-fold lower affinities for binding 8-oxoG-containing mismatches tested, as compared with intact MutY. Deletion of the C-terminal domain reduces its catalytic preference for A/GO-DNA over A/G-DNA. MutY also exerted more inhibition on the catalytic activity of MutM (Fpg) protein than M25. Moreover, an \(E.\) coli \textit{mutY} strain that produces an N-terminal 249-residue truncated MutY is a mutator. These findings strongly support the notion that repair of 8-oxoG is the major function of MutY and its 8-oxoG specificity is located mainly in the C-terminal domain.

**EXPERIMENTAL PROCEDURES**

\textit{E. coli} Strains—\textit{E. coli} PR8 (Su \textit{lac} X74 galU galK Sm\(^{1}\)), PR70 (like PR8 but \textit{micA68}; \textit{Trn16}Kan), and FR68 (like PR8 but \textit{micA68}; mini \textit{Trn16}Kan mutL218; \textit{X74}To) were obtained from M. S. Fox.

Cloning of the N-Terminal Domain of \textit{mutY} Gene—The N-terminal domain of the \textit{mutY} gene corresponding to Met\(^1\) to Glu\(^{296}\) (M25) was polymerase chain reaction amplified from the \textit{pTW10–12} plasmid (7) using primers Chang 222 (5\'-GCCGACCATATGCAACGTCGCAATT- TTC-3\') and Chang 223 (5\'-GCCGAGGATCCTACGTTCCGTTTGCGGC-3\'). Primer Chang 222 contains an \textit{NdeI} site at the 5' end of the coding sequence and primer Chang 223 contains a \textit{BamHI} site after the stop codon. The polymerase chain reaction product was purified from agarose gels, cut with \textit{NdeI} and \textit{BamHI}, and cloned into an \textit{NdeI}/\textit{BamHI}-digested \textit{pET11a} expression vector. The inserted \textit{mutY} sequence in the resulting clone p16-146-13 was confirmed by DNA sequencing. The expression of M25 was under the control of the T7 promoter.

Expression and Purification of M25 and MutY—\textit{E. coli} strain PR70/DE3 harboring the expression plasmid containing M25 was grown in LB broth containing 50 \textmu g/ml rifampicin. The cell titer of each culture was determined by measuring the absorbance at 600 nm. Independent cultures were plated and the experiments were repeated at least three times. Bands corresponding to enzyme-bound and free DNA were quantified from PhosphorImager images and \(K_d\) values were obtained from analyses by a computer-fitted curve generated by the Enzfit program (49).

Table 1. Oligonucleotide Substrates and Enzymes—The nucleotide sequences of the oligonucleotide pairs used in this study were as follows: 19-mer 5'-CCGGAGAATTXGCCCTTCG-3' and 3'-GCTCCTTAAAYCAGGAAGC-5' and 40-mer 5'-AATTGGTCCTGAGAAATXGCTTCG-3' and 3'-CCGGAGAATTXGCCCTTCG-5'.

MutY activity with substrate DNA was measured using DNA glycosylase (UDG, Life Technologies, Inc.) at 37 °C for 1 h in MutY buffer (20 mM Tris-HCl, pH 7.6, 80 mM NaCl, 1 mM diethio-riitol, 1 mM EDTA, and 2.9% glycerol). The MutY binding reaction mixture contained 20 mM Tris-HCl, pH 7.6, 80 mM NaCl, 1 mM diethio-riitol, 1 mM EDTA, 2.9% glycerol, 20 ng of poly(dI-dC), and 1.8 fmol of labeled DNA in a total volume of 10 \mu l. After incubation at 37 °C for 30 min, an identical aliquot of MutY for each sample was supplemented with 1.5 \mu l of 50% glycerol and analyzed on 4% polyacrylamide gels in 50 mM Tris borate, pH 8.3, and 1 mM EDTA. To determine the \(K_d\) values, different MutY enzyme concentrations were used to bind DNA substrates and experiments were performed at least three times. Bands corresponding to enzyme-bound and free DNA were quantified from PhosphorImager images and the \(K_d\) values were obtained from analyses by a computer-fitted curve generated by the Enzfit program (49).

Table 2. Western Blot Analysis—Proteins were resolved on an SDS-12% polyacrylamide gel and transferred to a nitrocellulose membrane (51). The membrane was subjected to the Enhanced Chemiluminescence analysis system from Amersham Pharmacia Biotech according to the manufacturer’s protocol. The anti-MutY antibodies were affinity purified by reaction with membrane-bound MutY protein (52).

**RESULTS**

\textit{G/GO Is a Substrate for MutY}—We have shown that MutY has much tighter binding, but weaker glycosylase activity to A/GO- than A/G-containing DNA at an enzyme/DNA molar ratio of 40 at 37 °C for 30 min in a steady-state kinetic study (38). To further delineate the reactivity of MutY on different
The combined MutY DNA glycosylase/AP lyase activities can be assayed by monitoring the nicking products in a denatured sequencing gel. As shown in Fig. 1A, MutY adenine glycosylase removed adenines from A/G and A/GO mismatches (Fig. 1A, lanes 2 and 4). DNA substrates containing T/GO and C/GO were not cleaved by MutY (Fig. 1A, lanes 8 and 10). Surprisingly, the duplex containing G/GO was cleaved about 5% of the extent of DNA containing an A/GO mismatch (Fig. 1A, lane 6). A Schiff base intermediate between MutY and DNA can be trapped in a stable enzyme-DNA covalent complex in the presence of sodium borohydride. At an enzyme:DNA molar ratio of 40, MutY could trap A/G and A/GO mismatches efficiently (Fig. 1B, lanes 2 and 4) but not T/GO and C/GO (Fig. 1B, lanes 8 and 10). MutY could also trap the G/GO-containing DNA (Fig. 1B, lane 6) although its efficiency is much weaker (less than 2%) than A/G- and A/GO-DNA (Fig. 1B, lane 6). Thus, MutY is both an adenine and guanine DNA glycosylase.

**DNA Mismatch Binding of M25 and MutY**—Previous results have shown that the N-terminal domain (M25, Met¹ to Gln²²⁶), obtained from proteolysis of MutY or expressed as a recombinant protein, retains the catalytic activity (39, 47). Thus, MutY is both catalytic substrates of MutY (Fig. 1), the tight binding of these substrates by MutY through its C-terminal domain is surprising. When an AP site was placed opposite G or GO, M25 displayed similar binding affinity to AP/G, but weaker binding affinity to AP/GO relative to the intact enzyme. M25 showed lower binding affinity to DNA substrates containing GO paired with any partner mismatches than intact MutY. Thus, the C-terminal domain of MutY contributes to the high binding affinity of MutY to GO.

**Catalytic Activities of M25**—In the trapping assay in the presence of sodium borohydride, expressed M25 protein produced about 2-fold less covalent protein-DNA complex on A/G-containing DNA than intact MutY at the protein/DNA ratios greater than 10 (Fig. 2A, lanes 1–3 and 6–8). At lower protein/DNA ratios, a 2-fold amount of M25 was needed to produce the same amount of complex as MutY. With A/GO mismatch, when M25 was present in large enzyme excess (Fig. 2B, lane 6), the extent of protein-DNA covalent complex formation was 2-fold less than that of intact MutY (Fig. 2B, lanes 1–4). However, the amount of M25 needed to produce the same amount of covalent complex was 40-fold higher than that of intact MutY with A/GO-containing DNA (Fig. 2B, compare lane 5 with lane 9).

Because of the slow turnover rate of MutY (30, 53), the steady-state kinetics of the MutY reaction, as measured at
Reactions were stopped by adding 5 mlane 6–10T/G0 than does the intact MutY (Table I), it requires a higher MutY/MutM ratios of 32 and 0.6 for C/G0 and T/G0, respectively). The times required to reach 50% of

37 °C for 30 min (33), may not reflect the true reactivity. Thus, we used single-turnover glycosylase kinetics to compare the reactivities of MutY and M25. Time course studies to determine the extent of glycosylase activity on both A/G and A/G0 substrates were performed with intact MutY and M25. As shown in Fig. 3A, the rate of cleavage of A/G-containing DNA is similar for both intact MutY (circles) and M25 (diamonds). The times required to reach 50% of V_{max} on A/G-containing DNA are 2.1 and 2.7 min for MutY and M25, respectively. However, the rate of cleavage of M25 on A/G0-containing DNA is much slower than that of MutY (Fig. 3B). The times required to reach 50% of V_{max} on A/G0-containing DNA are <0.1 and 1.9 min for MutY and M25, respectively. There is a greater than 20-fold difference in reaction rate. The rate of M25 cleavage of A/G0-containing DNA is similar to the rates of MutY and M25 cleavage of A/G-containing DNA (compare diamonds of Fig. 3B to both curves in Fig. 3A). Thus, M25 has reduced rate of glycosylase activity on A/G0-containing DNA, compared with intact MutY.

The results of Fig. 3 also showed that there was no difference between MutY and M25 when the reactions proceeded for more than 15 min with both A/G and A/G0 mismatches. At the steady-state, MutY glycosylase activity with A/G0 mismatch is weaker than with A/G-containing DNA. This result is consistent with the previous finding (33).

Inhibition of MutM (Fpg) Activity by MutY and M25—DNA substrates containing C/G0 and T/G0 are catalytic substrates of MutM (Fpg) (54) but not of MutY (Fig. 1) although they are bound tightly by MutY (Table I). To explore the significance of these properties, we tried to include MutY in the MutM glycosylase reaction. Both DNA substrates were labeled at the 5’ ends of GO-containing strands and preincubated with increasing amounts of MutY before MutM was added. As shown in Fig. 4, A and B, MutY could inhibit MutM activity on both C/G0 and T/G0 mismatches. MutM activity was reduced to 50% at MutY/MutM ratios of 32 and 0.6 for C/G0 and T/G0, respectively (Table II). Because M25 has lower affinities to C/G0 and T/G0 than does the intact MutY (Table I), it requires a higher amount of enzyme compared with MutY to achieve the same extent of inhibition on MutM activity (Fig. 4, A and B, compare circles and diamonds). MutM activity was reduced to 50% at M25/MutM ratios of 1600 and 80 for C/G0 and T/G0 mismatches, respectively (Table II). The extent of MutM activity inhibition by MutY and M25 is consistent with the K_{i} values of these proteins with these substrates.

DNA with a G/G0 mismatch is a weak substrate of MutY (Fig. 1) but is cleaved very well by MutM (54). When MutY and M25 were added to the MutM reaction with G/G0-containing DNA, strong inhibition was observed (Fig. 4C). MutM activity on G/G0 mismatch was reduced to 50% at MutY/MutM and M25/MutM ratio of 0.7 and 6, respectively (Table II). The extent of MutM activity inhibition by MutY and M25 is in agreement with the K_{i} values of these proteins with the substrate, however, M25 appears to exhibit greater inhibition of MutM activity than as predicted from its K_{i} value.

AP/G0-containing DNA is a substrate for both MutY and MutM. The AP lyase activity of MutY can cleave at the 3’ side of the AP site (37, 45). MutM can excise the GO base and cleave the AP site on both DNA strands leading to a double-strand break (54). As shown in Fig. 4D, MutY could block MutM cleavage on the GO-strand of AP/G0 mismatch, M25, which had an 18-fold lower binding affinity to AP/G0 than the intact MutY, inhibited MutM activity on AP/G0 to a lesser degree than MutY (Fig. 4D, compare circles and diamonds). MutM activity on AP/G0-containing DNA was reduced to 50% by MutY and M25 at enzyme/MutM ratios of 0.3 and 16, respectively (Table II).

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Formation of covalent complexes of MutY and M25 with A/G- and A/G0-containing DNA in the presence of NaBH4. Oligonucleotide substrates (1.8 fmol) were incubated with various amounts of MutY (lanes 1–5) or M25 (lanes 6–10) in the presence of 0.1 mM NaBH4 at 37 °C for 30 min. A, trapping reactions with A/G-containing DNA. Lanes 1–5 show results from reactions containing decreasing amounts of MutY (144, 36, 9, 1.8, 0.45 fmol from lane 1 to lane 5, respectively). Lanes 6–10 show results from reactions containing decreasing amounts of M25 (288, 72, 18, 3.6, and 0.9 fmol from lane 6 to lane 10, respectively). B, trapping reactions with A/G0-containing DNA. Lanes 1–5 show results from reactions containing decreasing amounts of MutY (36, 9, 1.8, 0.45, and 0.09 fmol from lane 1 to lane 5, respectively). Lanes 6–10 show results from reactions containing decreasing amounts of M25 (288, 72, 18, 3.6, and 0.9 fmol from lane 6 to lane 10, respectively). Reactions were stopped by adding 5 mM C-M25 or M25 (288, 72, 18, 3.6, and 0.9 fmol from lane 6 to lane 10, respectively). The positions of free DNA (Free) and covalent complexes (C-M25 and C-MutY) are indicated.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Time course studies of MutY and M25 glycosylase activities. A/G (panel A) and A/G0 (panel B) containing 20-mer oligonucleotides (1.8 fmol) were incubated at 37 °C with 72 fmol of MutY (circles) or 72 fmol of M25 (diamonds) for various times. The products, after reaction, were treated with piperidine, lyophilized, resuspended in formamide dye, heated at 90 °C for 2 min, and analyzed on a 14% denaturing sequencing gel. Data were from PhosphorImager quantitative analyses of gel images over three experiments. Percentages of DNA cleaved were plotted as a function of time.
Deletion of C-terminal Domain of MutY Causes a Mutator Phenotype—The micA (mutY) mutant PR68 was originally isolated in a mutL Ss− background and was defective in a repair pathway that removes the adenines from A/G mismatches (5). The mutY mutant strain PR70 (same as PR68 but MutL−) also displays a mutator phenotype (5).

The mutY gene in PR70 is interrupted by a mini Tn10Kan (5) at a site about 750 base pairs downstream from the AUG start codon (22). To determine the exact insertion site of the transposon on this mutant allele, we sequenced the clone, pJTW1-1 (22), bearing micA68 with MutY its own promoter in plasmid pBR322. As shown in Fig. 5, a mini Tn10Kan interrupts at nucleotide 747 of mutY gene followed by a stop codon. PR70 cells should express a 249-residue polypeptide from the N terminus of MutY. As expected, in a Western blot with MutY antibodies, a band at approximately 27 kDa was detected in PR70 and PR70 containing plasmid pJTW1-1 (Fig. 5B, lanes 2 and 3). The 249-residue polypeptide may be less stable or less reactive to the MutY antibodies than the intact MutY as it is hardly detected in the PR70 extract and the truncated protein is present at lower levels in PR70/pJTW1-1 than MutY in PR8 (wild-type) as detected by Western blotting.

The extracts of PR68 and PR70 have been shown to be defective in the methylation-independent A/G mismatch repair on phage f1 heteroduplexes and A/G-specific cleavage activity using a 120-mer oligonucleotide substrate (22). When the PR70 extract was re-examined with A/G and A/GO 20-mer DNA substrates, no binding activity could be detected (Fig. 5C, lanes 3–4 and 9–10). Next, we examined MutY binding activity in extracts of PR70 bearing the same mutant allele in plasmid pJTW1-1. Extracts of PR70 containing pJTW1-1 had stronger binding activity with A/G-containing DNA than did PR8 extracts (Fig. 5C, compare lanes 1 and 2 with lanes 5 and 6) although MutY in the PR8 extract was present at higher levels than the truncated protein in the extract of PR70/pJTW1-1 as detected by Western blotting (Fig. 5B, lanes 1 and 3). However, the PR8 extract had much greater binding activity with A/GO-containing DNA than did PR8 extracts (Fig. 5C, compare lanes 7 and 8 with lanes 11 and 12). Intact MutY in the PR8 extract had much tighter binding with A/GO- than to A/G-containing DNA, however, the truncated protein in the PR70 extract had similar but weak binding with both A/G and A/G mismatches. Thus, the truncated 249-residue MutY polypeptide encoded by the micA68, as with M25, has lower binding affinity to GO than intact MutY.

The mutation frequency of PR70 has been determined to be more than 40-fold higher than the wild-type cells as measured by the incidence of rifampicin-resistant revertants (5, 55). As shown in Table III, the mutation frequencies of PR70 and PR70/pJTW1-1 was 75- and 32-fold higher than the wild-type cells, respectively. Although the mutation frequency of PR70/pJTW1-1 is not as high as PR70, it displays a mutator phenotype. The phenotype and the biochemical properties of the encoded protein by the micA68 allele strongly support that deletion of the GO-specific C-terminal domain of MutY could lead to a mutator phenotype. It also suggests that repair of A/GO mismatches is the primary role of MutY pathway.
A/G-containing DNA; lanes 3

MutY

B-MutY

DNA complex (DNA complex (mutY gene revealed the mini Tn insertion site at nucleotide 747 of the mutY gene and a stop codon immediately after codon 249. A 249-residue protein (lane 1) was produced as markers in lanes 4 and 5, respectively, C, gel shifting assay. A/G- and A/GO-containing 20-mer oligonucleotide was assayed for binding with 10 μg of protein (lanes 1 and 2, PR8 with A/GO-containing DNA; lanes 3 and 4, PR70 with A/GO-containing DNA; lanes 5 and 6, PR70/pJTW1-1 with A/GO-containing DNA; lanes 7 and 8, PR8 with A/GO-containing DNA; lanes 9 and 10, PR70 with A/GO-containing DNA; lanes 11 and 12, PR70/pJTW1-1 with A/GO-containing DNA. The binding products were analyzed on an 8% native gel in 50 mM Tris borate, pH 8.3, and 1 mM EDTA. Arrows indicate the positions of MutYDNA complex (B-MutY), microA68 DNA complex (B-microA68), and free DNA (Free).

FIG. 5. Characterization of microA68 mutation. A, map of microA68. DNA sequencing of plasmid pJTW1-1 (22) bearing the microA68 mutant mutY gene revealed the mini Tn10 insertion site at nucleotide 747 of the mutY gene and a stop codon immediately after codon 249. A 249-residue polypeptide from the N terminus of MutY was expected to be produced from the interrupted mutY gene. B, Western blotting with MutY antibodies. Extract of PR8 (80 μg) expressed the intact 39-kDa MutY protein (lane 1). A band about 27 kDa was detected in cell extracts (80 μg) from PR70 (lane 2) and PR70 containing pJTW1-1 (lane 3). Purified M25 and MutY proteins were included as markers in lanes 4 and 5, respectively, C, gel shifting assay. A/G- and A/GO-containing 20-mer oligonucleotide was assayed for binding with 10 μg (odd lanes) or 5 μg (even lanes) of cell extracts at 37 °C for 30 min. Lanes 1 and 2, PR8 with A/GO-containing DNA; lanes 3 and 4, PR70 with A/GO-containing DNA; lanes 5 and 6, PR70/pJTW1-1 with A/GO-containing DNA; lanes 7 and 8, PR8 with A/GO-containing DNA; lanes 9 and 10, PR70 with A/GO-containing DNA; lanes 11 and 12, PR70/pJTW1-1 with A/GO-containing DNA. The binding products were analyzed on an 8% native gel in 50 mM Tris borate, pH 8.3, and 1 mM EDTA. Arrows indicate the positions of MutYDNA complex (B-MutY), microA68 DNA complex (B-microA68), and free DNA (Free).

TABLE III

| Strain          | Mutation frequency Rif+ colonies/108 cells | -Fold |
|-----------------|------------------------------------------|-------|
| PR8 (wt)        | 0.7 ± 0.2                                 | 1     |
| PR70 (mutY)     | 49.2 ± 4.6                                | 75    |
| PR70/pJTW1-1    | 40.7 ± 3.4                                | 32    |

DISCUSSION

MutY has been identified as an adenine glycosylase that repairs A/G, A/C, and A/GO mismatches (3, 4, 7, 35). Here, we showed that MutY is also a guanine glycosylase repairing G/G-containing DNA. The binding affinity of MutY with G/G is slightly lower than that with A/GO-containing DNA but higher than that with A/G-containing DNA. However, the catalytic activity of MutY to G/G-containing DNA is much lower than that to A/G- and A/GO-containing substrates. The

adenine specificity to A/G and A/GO is consistent with the mutation phenotype of mutY mutants for G:C to T:A transversions (3, 5, 56). The weak in vitro A/C and G/GO repair activities of MutY may remove the misincorporated A and G from C and G on the template strands, respectively. Thus, one may predict that mutY mutants have higher mutation frequencies for C:G to T:A and G:C to C:G. Nghiem et al. (56) and Radicella et al. (5) found that the mutY mutants had higher mutation rates for G:C to T:A transversions but not other types of mutations compared with the wild-type cells. It is possible that A/C and G/GO mismatches are repaired by other pathways such as the MutHLS system. Recently, Zhang et al. (57) found that an E. coli mutY mutant also has an increased mutation rate for G:C to C:G transversions and that MutY has guanine glycosylase activity on G/GO but not G/G mismatches. Their experimental design is similar to that of Nghiem et al. (56) except that the E. coli cells were incubated on glucose minimal media for 10 days instead of 3 days. The delayed mutation accumulation of G:C to C:G transversions may be due to the inefficiency of MutY repair to G/GO mismatches or miscoding of an oxidized form of GO that appeared at the stationary phase (58).

Full-length MutY binds to A/GO-containing DNA 80-fold better than it does to A/G-containing DNA (20). We have shown that the specificity for A/G binding can be ascribed mainly to the C-terminal domain of MutY, the non-endo III like domain (39). Here, we showed that M25 and MutY have similar binding affinity with an A/G mismatch, however, M25 has much lower affinity to DNA substrates containing GO when paired with any partner mismatches than intact MutY. Thus, the high binding affinity of MutY to GO-containing DNA is contributed mainly by its C-terminal domain. Because MutY has different catalytic activities (Fig. 1) and low turnover rate (30, 53) with the mismatches, the Kd measurement for these substrates may not reflect true equilibrium values. In the case of A/G, A/GO, and A/C, MutY may bind to either the base-base mismatch substrates or the processed products such as AP site or cleaved site. The specific GO-binding role of C-terminal domain of MutY was also described by Noll et al. (47) but not found by Manuel and Lloyd (37). The N-terminal domain (p26) studied by Manuel and Lloyd (37) consists of residues 1–225 which is 1 amino acid shorter than M25 used in this study as well as by Noll et al. (47) and Gogos et al. (39). However, the functional difference between M25 and P26 is probably not derived from 1 amino acid difference.

M25 also has weaker trapping and slower glycosylase activities than MutY with A/GO-containing DNA. When M25 is present in large enzyme excess, the extent of protein-DNA covalent complex formation is about 2-fold less than that of intact MutY (Fig. 1). However, a 40-fold higher amount of M25 is needed to produce the same amount of covalent complex as that of intact MutY with A/GO-containing DNA. At the steady-state, there was no difference in glycosylase activity between MutY and M25 when the reactions proceeded for more than 15 min for both A/G and A/GO. At the pre-steady-state, M25 and MutY have similar rates of glycosylase activity with an A/G mismatch, however, M25 reacts much slower than MutY to an A/GO mismatch. Thus, both the high binding affinity and fast catalytic activity of MutY to GO-containing DNA are contributed mainly by its C-terminal domain. Furthermore, an E. coli mutY strain that produces an N-terminal 249-residue truncated MutY confers a mutator phenotype. The C-terminal MutY deletion allele encodes a polypeptide whose activity is similar to M25. These findings strongly support that the C-terminal domain of MutY is critical for mutation avoidance of oxidative damage. Thus, in vivo, the A/G mismatches may be
the most important substrates of MutY because both prokaryotic and eukaryotic DNA polymerases misinsert A opposite GO at high frequencies (9, 15, 16, 58).

M25, spanning the region homologous with *E. coli* endo III, is the catalytic domain of MutY (cdMutY) (37, 39, 40). Several DNA binding motifs have been identified in the x-ray crystal structure: a helix-hairpin-helix, a pseudo-helix-hairpin-helix, a positively charged groove with the adenine binding pocket, and an iron-sulfur cluster loop (24). These motifs are responsible for interaction with the DNA phosphates and the mismatched adenine. The crystal structure of the cdMutY also suggests some candidate residues for the recognition of the base opposite the adenine. These include the α2-α3 motif, particularly the conserved Gln**α**4, for the specific recognition of G or GO at a syn configuration through the DNA minor groove. Our results show that the major determinants of the mismatched GO reside in the C-terminal domain of MutY. We propose that the DNA is embedded between the catalytic and C-terminal domains of MutY. In this model, the C-terminal domain functions like a clamp to hold the GO-containing strand. This C-terminal clamp is more open with an A/G mismatch than with an A/GO mismatch. The contacts of MutY with the GO-containing strand may be important for promoting base-flipping of the mismatch A. The residues of the C-terminal domain of MutY involved in this GO specificity remain undetermined. It would be valuable to have the detailed structure of the intact MutY-DNA complex.

Noll et al. (47) pointed out an interesting sequence homology between the C-terminal domain of MutY and MutT. The MutT protein (14 kDa in size) hydrolyzes nucleoside triphosphates, with 8-oxo-dGTP as the best substrate, to nucleoside monophosphates and pyrophosphates (10–12). Both polypeptides are similar in size and can recognize the GO nucleoside. However, the C-terminal domain of MutY by itself has no detectable function (data not shown), unlike MutT. Thus, the C-terminal domain of MutY has evolved to function differently from MutT and it may enhance the N-terminal domain to interact with the phosphate backbone and GO base in DNA.

The tight binding of MutY to T/GO, G/GO, and AP/GO may have biological significance. MutM is able to remove GO from T/GO and G/GO mismatches. However, MutY glycosylase then excises the base (Fig. 6). When MutY encounters a G/GO mismatch, the base flipping and repair are very slow; MutY can block the MutM binding because it remains bound to this substrate.

**Fig. 6.** Model for MutY blockage of MutM activity. DNA strands at the replication fork are represented as lines. The N-terminal and C-terminal domains of MutY are shown in large and small oval-shaped circles, respectively. The gray-filled multi-pointed oval inside the large oval marks the adenine binding pocket. A, MutY binding with A/GO mismatch. When MutY binds A/GO or A/G, the adenine is flipped out of the DNA helix into the binding pocket and the MutY glycosylase then excises the base for repair. B, MutY binding with T/GO mismatch. When MutY binds T/GO, the T base is not flipped out, no repair occurs, and MutY blocks the MutM binding to this substrate. C, MutY binding with G/GO mismatch. When MutY encounters a G/GO mismatch, the base flipping and repair are very slow; MutY can block MutM binding because it remains bound to this substrate.

| **A** | **B** | **C** |
|---|---|---|
| Binds | No Repair | Binds |
| Base Flipped | Blocks MutM | Slow Repair |
| Repairs | | Blocks MutM |

The most significant contribution is that MutY continues to bind AP/GO mismatches after its glycosylase action in order to prevent removal of GO or cleavage at the AP site by MutM before MutY initiated repair is complete. The inhibition of MutM activity is especially important if T/GO and G/GO mismatches arise from misincorporation of T and G opposite oxidized template guanines as well as if T/GO mismatches are derived from deamination of 5-methylcytosine opposite GO. DNA polymerases may incorporate G or T opposite the GO lesions as suggested by Braun et al. (59). An oxidized form of GO, possibly guanidinohydantoin, may direct misincorporation of dNTPs during DNA synthesis (58). The removal of GO from T/GO and G/GO mismatches when GO is on the parental strands will lead to G:C to A:T transitions and G:C to C:G transversions. Hence, it is reasonable that MutM activity on these unfavorable mismatches is inhibited before other repair pathways remove the base opposite GO. The possible pathways employed to repair these unfavorable mismatches are the long-patch MutHLS mismatch repair and *usr*-dependent very short-patch repair (60). Recently, yeast MSH2/MSH6 heterodimer (*E. coli* MutS homologs) has been shown to bind A/GO mismatches and be involved in GO repair (61). Thus, it is possible that MutS homologs may be involved in repair of T/GO and G/GO mismatches. Our data in Fig. 4 indicate that the MutY protein is involved in this regulation of MutM activity through its C-terminal domain. It has been suggested by Bridges et al. (62) that MutY may regulate MutM activity in resting cells.

Our model for MutY attenuation of MutM activity is shown in Fig. 6. When MutY binds A/G and A/GO, the adenine is flipped out of the DNA helix into the binding pocket and the MutY glycosylase then excises the base (Fig. 6A). When MutY binds T/GO, the T base is not flipped out, no repair occurs, and MutY blocks the MutM binding to this substrate (Fig. 6B). When MutY encounters a G/GO mismatch, the base flipping and repair are very slow; MutY can block the MutM binding when it remains bound to this substrate (Fig. 6C). This additional role of MutY in blocking MutM activity is applicable only when GO is on the parental strands. It is possible that MutY is oriented at the replication fork with the N-terminal domain on the daughter strand and C-terminal domain on the parental strand by an unknown mechanism. With this physical polarity, MutY may repair the misincorporated A opposite template GO but not repair template A opposite misinserted GO.

**REFERENCES**

1. Au, K. G., Cabrera, M., Müller, J. H., and Modrich, P. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 9163–9166
2. Lu, A.-L., and Chang, D.-Y. (1988) *Genetics* 118, 593–600
3. Michaels, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 7022–7025
4. Michaels, M. L., Tchou, J., Grollman, A. P., and Miller, J. H. (1992) *Biochem.
The C-terminal Domain of MutY Glycosylase Determines the 7,8-Dihydro-8-oxo-guanine Specificity and Is Crucial for Mutation Avoidance
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