Characterization of the Recombinant MutY Homolog, an Adenine DNA Glycosylase, from Yeast Schizosaccharomyces pombe*

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The mutY homolog (SpMYH) gene from a cDNA library of Schizosaccharomyces pombe encodes a protein of 461 amino acids that displays 28 and 31% identity to Escherichia coli MutY and human MutY homolog (MYH), respectively. Expressed SpMYH is able to complement an E. coli mutY mutant to reduce the mutation rate. Similar to E. coli MutY protein, purified recombinant SpMYH expressed in E. coli has adenine DNA glycosylase and apurinic/apyrimidinic lyase activities on A/G- and A/7,8-dihydro-8-oxoguanine (8-oxoG)-containing DNA. However, both enzymes have different salt requirements and slightly different substrate specificities. SpMYH has greater glycosylase activity on 2-aminopurine/G and A/2-aminopurine but weaker activity on A/C than E. coli MutY. Both enzymes also have different substrate binding affinity and catalytic parameters. Although SpMYH has great affinity to A/8-oxoG-containing DNA as MutY, the binding affinity to A/G-containing DNA is substantially lower for SpMYH than MutY. SpMYH has similar reactivity to both A/G- and A/8-oxoG-containing DNA; however, MutY cleaves A/G-containing DNA about 3-fold more efficiently than it does A/8-oxoG-containing DNA. Thus, SpMYH is the functional eukaryotic MutY homolog responsible for reduction of 8-oxoG mutational effect.

Cellular and organism aging have been correlated with accumulated DNA damage (1, 2). Oxygen is metabolized inside the cell by a series of one-electron reductions with the generation of reactive and potentially damaging intermediates called reactive oxygen species (3). The frequency of oxidative damage to DNA has been estimated at 10^4 lesions/cell/day in humans (4). 8-Oxo-7,8-dihydrodeoxyguanine (8-oxoG or GO) is one of the most stable products of oxidative DNA damage. The formation of GO in DNA, if not repaired, can lead to misincorporation of A opposite to the GO lesion and result in G:C to T:A transversion of GO in DNA, if not repaired, can lead to misincorporation of GO adducts and ring-opened purine lesions (13–16). The E. coli MutY is an adenine glycosylase that is responsible for the correction of A/GO as well as A/G and A/C mismatches (9, 17–22). MutY removes misincorporated adenines paired with GO lesions and reduces the GO mutational effects. Recent results show that MutY and the N-terminal catalytic domain can be trapped in a stable covalent enzyme-DNA intermediate in the presence of sodium borohydride (23–25) and support the hypothesis that MutY contains both DNA glycosylase and AP lyase activities.

MutY homologous (MYH) activities have been identified in human HeLa (26) and calf thymus (27) extracts. Both human and calf MYH systems share similar features with the E. coli mutY-dependent pathway: mismatch specificities to A/G, A/C, and A/GO, and cleavage of the A but not G strand. Recently, a human cDNA of putative hMYH was cloned and its open reading frame predicts a 60-kDa protein (28), which is in good agreement with the size of a band detected in HeLa extracts with MutY antibodies (27). hMYH shares high homology with the E. coli MutY protein (28). However, no enzyme activity has been reported for the protein encoded by this open reading frame.

Here, we report the cloning and expression of the MYH gene from Schizosaccharomyces pombe. Expression of SpMYH suppresses the spontaneous mutation rate of E. coli mutY mutant strains. Like E. coli MutY, purified recombinant SpMYH has both adenine glycosylase and AP lyase activities on A/G and A/GO mismatches. Defined oligonucleotides containing various purines were used to examine the substrate specificity. SpMYH has slightly different substrate specificity from that of E. coli MutY protein. SpMYH has greater glycosylase activity on 2-aminopurine (2AP/V and A/2AP but weaker activity on A/C than does E. coli MutY. Both enzymes also have different substrate binding affinity and catalytic parameters. These results suggest that SpMYH is a functional eukaryotic homolog of the bacterial MutY. The high homology of MutY homologs among different organisms suggests important roles in their cellular functions.

EXPERIMENTAL PROCEDURES

Cloning of cDNA of S. pombe MYH—According to the published genomic sequence of S. pombe (accession no. Z69240), the putative mutY homolog (MYH) sequence contains two introns and codes for a 461-residue protein. To clone the S. pombe MYH gene, we synthesized two PCR primers, Chang 219 (5‘-GGGATATACATAGTGCGATT- AAATCATTC-3’) and Chang 220 (5‘-GCACCGGATCTCATGACTACCT- GCCGTCCTG-3’). Chang 219 and Chang 220 anneal at the first six and last six codons of the predicted coding sequence for SpMYH, respectively. DNA prepared from an S. pombe cDNA library in pGADGH (kindly provided by D. Beach, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) was used as a template for the PCR reactions. PCR
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reactions (100 μl) contained 20 ng of plasmid DNA, 100 pmol of each primer, 1.5 mM MgCl₂, and 5 units of Pfu DNA polymerase (Stratagene). The reactions were performed as follows: 95 °C (1 min), 58 °C (1.5 min) and 72 °C (1.5 min) for 30 cycles and a final cycle with extension time at 72°C for 4 min. The 1.4-kb PCR product was purified using a Wizard PCR clean-up kit (Promega), digested at 30°C with BglII, BamHI, NdeI, and HindIII, and ligated into pET11a and transformed into DH5α cells. Sequence analysis of one such clone (pSP11a-2.3) revealed that it contained several mutations.

To obtain a mutation-free SpMYH clone, the 1.4-kb PCR product was labeled and used as a probe to screen the S. pombe cDNA library in pGADGH by colony hybridization. Out of 2500 colonies screened, two contained the SpMYH sequence. Subsequent restriction and PCR analysis indicated that the MYH cDNA was within a 1.6-kb BamHI fragment in one of the clones. This fragment was isolated and transferred to pUC19 to generate pSPMYH19. Based on the restriction map of pSPMYH19, the 1.1-kb EcoRI fragment containing the C-terminal domain of SpMYH and 21 base pairs of pUC19 sequence was isolated and ligated with the 5.76-kb fragment of pSP11a-2.3 that contained the vector pET11a and the N-terminal portion of SpMYH to yield the plasmid pSPMYH11a-4. The SpMYH sequence of pSPMYH11a-4 (accession no. AF053340) was exactly the same as the predicted sequence for the S. pombe MYH cDNA.

The recombinant expressed a 52-kDa protein in GBE943/DE3 (lacIq-pgk::lacZp4008/lacIq::Zpo4008/LacIq::Zpo4017/pGADGH) (Fig. 1). The purified protein was gel filtered on a 50-ml column of Sephadex G-25 (Pharmacia) equilibrated with 0.6 M KCl in buffer A. Fractions that eluted between 0.2 and 0.4 M KCl were pooled (fraction III, 50 ml). Fraction III was loaded onto a 30-ml hydroxylapatite column equilibrated with 0.1 M NaCl in buffer A containing 0.1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF) and 50 mM KCl and disrupted with a bead beater (Biospec Products, Bartlesville, OK) using 0.1-mm glass beads. The cell debris was removed by centrifugation, and the supernatant was collected as fraction I (595 ml). Ammonium sulfate (134 g) was added to fraction I, and the protein was precipitated for 1 h. After centrifugation, 126 g of ammonium sulfate was added to the supernatant protein pellet collected by centrifugation and resuspended in 50 ml of buffer A containing 50 mM KCl and dialyzed against two changes of 3 liters of the same buffer for 4 h each. The dialyzed protein sample was diluted 2-fold with buffer A containing 50 mM KCl as fraction II (140 ml). Fraction II was loaded onto a 50-ml phosphocellulose column, which had been equilibrated with buffer A containing 0.05 M KCl. After washing with 100 ml of equilibration buffer, proteins were eluted with a 400-ml linear gradient of KCl (0.05–0.4 M) in buffer A. Fractions containing the MYH nicking activity, which eluted between 0.1 and 0.3 M KCl, were pooled (fraction V1, 8.5 ml), divided into small aliquots, and stored at −80 °C. Cleavage of A/G-containing 44-mer DNA was assayed during the purification of the recombinant SpMYH enzyme. One unit of activity is defined as that resulting in cleavage of 0.018 fmol of labeled DNA per hour per milligram of protein.

SpMYH Binding Assay—The binding activity of SpMYH, which is the combined action of the glycosylase and AP lyase activities, was assayed similarly as described (31). The standard reaction mixture contained 10 mM Tris-HCl (pH 7.6), 0.5 mM dithiothreitol, 0.1 mM EDTA, 1.45% glycerol, 50 μg/ml bovine serum albumin, and 1.8 fmol of labeled DNA in a total volume of 20 μl. SpMYH protein, diluted in a buffer containing 20 mM potassium phosphate (pH 7.4), 1.5 mM dithiothreitol, 0.1 mM EDTA, 50 mM KCl, 200 μg/ml bovine serum albumin, and 50% glycerol, was added to the reaction mixture and incubated at 30 °C for 30 min. The reaction products were analyzed on 8% or 14% polyacrylamide DNA sequencing gels. Kinetic analyses were performed using a concentration range of DNA substrates with 0.4 mM SpMYH. Following autoradiography, bands corresponding to cleavage products and intact DNA were excised from the gel and quantified by liquid scintillation counting. The Km values and Vmax values were obtained from results of three experiments by a computer-fitted curve generated by the Enzfitter program (32).

Purification of SpMYH—Eighteen liters of E. coli GBE943/DE3 cells harboring overproduction plasmid pSPMYH11a-4 were cultured to an OD590 of 0.7 in LB medium containing 50 mg/ml ampicillin when necessary. After 2.5 h of induction by the addition of 0.1 mM IPTG, 0.1 ml of cells from each culture was plated onto LB agar containing 0.1 mg/ml rifampicin. The cell titer of each culture was determined by plating a 10⁻⁶ dilution onto LB agar. The ratio of Rif- cells to total cells was the mutation rate.

Measurement of Mutation Rate—Independent overnight cultures of each strain were grown to an A500 of 0.7 in LB medium containing 50 mg/ml ampicillin when necessary. After 2.5 h of induction by the addition of 0.1 mM IPTG, 0.1 ml of cells from each culture was plated onto LB agar containing 0.1 mg/ml rifampicin. The cell titer of each culture was determined by plating a 10⁻⁶ dilution onto LB agar. The ratio of Rif- cells to total cells was the mutation rate.

SpMYH Nicking Assay—The nicking activity of SpMYH, which is the combined action of the glycosylase and AP lyase activities, was assayed similarly as described (31). The standard reaction mixture contained 10 mM Tris-HCl (pH 7.6), 0.5 mM dithiothreitol, 1.5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride. After washing with 100 ml of equilibration buffer, proteins were eluted with a 400-ml linear gradient of KCl (0.05–0.4 M) in buffer A. Fractions containing the MYH nicking activity, which eluted between 0.15 and 0.3 M KCl, were pooled and dialyzed against 2 liters of buffer A containing 0.05 mM KCl and 10% (v/v) glycerol for 1 h (fraction IV, 53 ml). Fraction IV was then applied to a 30-ml hydroxylapatite column equilibrated with buffer B (0.1 M potassium phosphate (pH 7.4), 1.0 mM NaCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, 1.5% glycerol, 50 μg/ml bovine serum albumin, and 5 ng of poly(dI-dC) at 30 °C for 30 min). Protein-DNA complexes were analyzed on 8% polyacrylamide gels in 50 mM Tris borate (pH 8.3) and 1 mM EDTA as described previously (19). The apparent dissociation constants (Kd values) of SpMYH and DNA were determined using a range of protein concentrations. Following autoradiography, bands corresponding to enzyme-bound and free DNA were excised from the gel and quantified by liquid scintillation counting. The Km values were obtained from results of three experiments by a computer-fitted curve generated by the Enzfitter program (32).

Formation of Enzyme-DNA Covalent Complex—Reactions were carried out as described in the SpMYH cleavage assay except that the reactions were performed in the presence of NaBH₄. A NaBH₄ stock solution was freshly prepared immediately prior to use. After incubation at 30 °C for 30 min, SDS dye was added to the samples, which were heated at 90 °C for 2 min and separated on a 12% polyacrylamide gel in the presence of SDS according to Laemmli (33), and the gel was dried and autoradiographed.

RESULTS

Sequence Analysis of the SpMYH Gene—The SpMYH gene encodes a protein of 461 amino acid residues that displays 28 and 31% identity and 59 and 62% conservation to the E. coli MutY and human MYH, respectively, by comparison using an ALIGN program. The eukaryotic MYH sequences contain extra amino acid stretches at the N- and C-terminal regions as compared with the bacterial MutY sequences. The amino acid sequences of the N-terminal part of the SpMYH share significant homology to other DNA glycosylases including E. coli endonuclease III (endo III) (Fig. 1). Two regions of highest similarity shared among these enzymes can be identified in the known three-dimensional structure of E. coli endo III (34, 35): the helix-hairpin-helix domain (residues 147–181 of SpMYH) and the iron-sulfur domain (residues 213–242 of SpMYH). There are also distinct residues (shaded in gray boxes in Fig. 1) in the MutY family that are different from that of endo III family. Interestingly, when these residues are placed on the structure of E. coli endo III, they are located at the edge of the clef between the iron-sulfur domain and the six antiparallel α helices.

Reduction of the Mutation Rate of the E. coli mutY Mutant by S. pombe MYH—To demonstrate that the open reading frame of putative SpMYH gene encodes a functional MYH protein, we have expressed this Sp-cDNA under the control of the T7 promoter in pET11a in E. coli. E. coli GBE943 (mutY) harboring
the plasmid pSPMYH11a-4 were induced to express the Sp-MYH protein by addition of IPTG to the growth medium, and the mutation rate was measured. As shown in Table I, mutY mutant (GBE943/DE3) exhibited a 40-fold higher mutation rate than the wild-type E. coli. GBE943/DE3 cells expressing SpMYH had mutation rates almost as low as the wild-type cell, whereas the vector (pET11a) alone had no effect on the mutation rate (Table I). Expression of SpMYH in wild-type E. coli cells caused a slightly lower mutation rate than cells with vector alone (Table I).

**Purification of Recombinant SpMYH Protein**—To further demonstrate that the putative SpMYH encodes a functional MYH protein, we purified the recombinant SpMYH from the overproducing E. coli GBE943/DE3 strain harboring the plasmid pSPMYH11a-4. The SpMYH protein was purified by ammonium sulfate precipitation and phosphocellulose, hydroxylapatite, heparin-agarose, and Mono S chromatographic separation. We recovered 19 mg of SpMYH protein from 54 g of cell paste with about an 18-fold increase in specific activity. The purity of the products at different stages of the procedure is illustrated in Fig. 2. As judged on a 10% SDS-polyacrylamide gel, the protein was purified to a very high degree. The mobility of SpMYH in the gel matched the predicted size (52 kDa). SpMYH appears as a monomer because it was eluted at a molecular mass of about 45 kDa through a gel-filtration column (Superose 12) (data not shown).

**Effects of Salt and EDTA on SpMYH Activities**—When SpMYH was initially assayed in the MutY buffer (20 mM Tris-HCl, pH 7.6, 1 mM dithiothreitol, 1 mM EDTA, 80 mM NaCl, and 2.9% glycerol), there was little cleavage activity (Fig. 3, lane 4). This prompted us to find the optimal conditions for SpMYH. The SpMYH glycosylase activity was reduced by adding 40 mM NaCl to the reaction buffer (Fig. 3, lane 8) and was abolished at NaCl concentration higher than 80 mM (Fig. 3, lanes 9 and 10). SpMYH cleavage activity was not inhibited by 8 mM EDTA (Fig. 3, lane 13) but was abolished by 32 mM EDTA (Fig. 3, lane 15). The effects of salt and EDTA on the SpMYH binding to...
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Lysase Activities—As shown in Fig. 4, SpMYH can cleave a 20-mer oligonucleotide containing an A/G mismatch in a dose-dependent manner. Heating the samples at 90 °C for 2 min before loading to the sequencing gel enhanced the cleavage activity (Fig. 4, compare A and B). However, further treatment of the products with piperidine at 90 °C (a condition promotes β-elimination) did not significantly increase the extent of cleavage (Fig. 4, compare B and C). Thus, SpMYH does contain intrinsic AP lyase activity, although it is not strictly coupled to the glycosylase activity. These properties of SpMYH are similar to the E. coli MutY protein, which contains both DNA glycosylase and AP lyase activities.

If SpMYH has an AP lyase activity and uses the similar mechanism like MutY (23–25), an imino intermediate should be reduced by NaBH₄ to form a stable covalent protein-DNA complex. Thus, DNA containing an A/G mismatch was incubated with SpMYH in the presence of different concentrations of NaBH₄. As shown in Fig. 5, SpMYH can be trapped in two covalently linked protein-DNA complexes as a doublet in the presence of NaBH₄ although not as efficiently as MutY. The reason for the formation of two complexes is not quite clear. The optimal trapping concentration of NaBH₄ is about 20–30 mM (Fig. 5A) (less trapping is observed at 10 mM NaBH₄ with data not shown). The covalent complexes of SpMYH and DNA migrated slower than the MutY-DNA complex due to the larger size of SpMYH protein (the molecular mass of SpMYH is 52 kDa and that of MutY is 39 kDa). At 100 mM NaBH₄, E. coli MutY can be trapped efficiently but the trapping ability of SpMYH is minimal (data not shown). The effect of NaBH₄ is consistent with the inhibition of SpMYH cleavage activity by 80 mM NaCl that is present in the MutY reaction (Fig. 3). In the presence of 30 mM NaBH₄, DNA containing an A/G mismatch was tested in the trapping assay with increasing amount of SpMYH protein (Fig. 5B). At enzyme:DNA molar ratios ranging from 10 (Fig. 5B, lane 3) to 320 (Fig. 5B, lane 8), covalent complexes were detected. Because Schiff’s base formation is an important criteria for the class of DNA glycosylase with AP lyase activity, our results strongly suggest that SpMYH protein possesses AP lyase activity.

SpMYH and MutY Have Different Substrate Specificities—We have shown that E. coli MutY can cleave several mismatches with different efficiencies (30). To compare the SpMYH with MutY, we tested the glycosylase activity on different mismatches. As shown in Fig. 6, both enzymes cleave A/G, N/G, and A/GO in a similar order and have very weak or no cleavage on I/G, A/I, C/GO, and C/G. However, SpMYH has greater activity on 2AP/G and A/2AP but weaker activity on A/C than E. coli MutY.

Binding Affinity of SpMYH and MutY for Different Mismatches—Because SpMYH and MutY have different glycosylase activities on different substrates, we then determined the apparent dissociation constants (Kₐ) of SpMYH from different mismatches. The apparent dissociation constants (Kₐ values) of SpMYH and DNAs were determined using a range of protein concentrations with a fixed DNA concentration (90 pm). Representative autoradiograms of the binding assays and the corresponding binding curves for SpMYH to A/G and A/GO-containing 44-mer DNA are shown in Fig. 7. When the concentration of SpMYH protein was higher than 50 nM, an extra slower migrating complex was observed in binding assays with A/G-44 DNA (Fig. 7A). SpMYH binding is saturated below 70% of A/GO-44 DNA at the highest MutY concentration tested (Fig. 7D).

As shown in Table II, SpMYH has great affinity to A/GO-containing DNA as does MutY, but the binding affinity to A/G-containing DNA is substantially lower for SpMYH than MutY. The difference in the binding affinity of SpMYH with
A/G and A/GO-44 is 155-fold and with A/G-20 and A/GO-20 is 700-fold. The difference in the binding affinity of MutY with A/G-44 and A/GO-44 is 13-fold and with A/G-20 and A/GO-20 is 80-fold. SpMYH has higher nonspecific binding to 44-mer homoduplex than MutY. Therefore, SpMYH has only a 4–7-fold higher binding affinity to A/G mismatch than C/G pair, whereas MutY has a 70–175-fold higher binding affinity to A/G mismatch than C/G pair. As shown above, SpMYH has greater cleavage activity on 2AP/G and A/2AP than MutY; however, the binding affinities of SpMYH to these two mismatches were not greater than that of MutY.

**Kinetic Parameters of SpMYH**—The cleavage efficiencies of 20-mer oligonucleotide containing A/G or A/GO by SpMYH and MutY were compared (Table III). As measured at 0.4 mM protein concentration, the $K_m$ values for SpMYH on both substrates were slightly higher than that of MutY. The turnover number ($K_{cat}$) for SpMYH on an A/G 20-mer is 2 times lower than that of the MutY. SpMYH has similar reactivity ($K_{cat}/K_m$) to both A/G- and A/GO-containing DNA; however, MutY cleaves A/G-containing DNA about 3-fold more efficiently than it does A/GO-containing DNA. When 44-mer DNA substrates were tested for cleavage, SpMYH also displays similar specificity constants ($K_{cat}/K_m$) with both A/G and A/GO mismatches. The specificity constants of SpMYH with 44-mer is 3–4 times higher than with 20-mer DNA substrates.

**DISCUSSION**

As part of the genome project, the genomic sequence of mutY homolog of S. pombe was obtained (SPAC26A3.02 in entry Z69240). To confirm that this sequence codes for a functional MutY-like protein, we expressed the SpMYH cDNA in E. coli and assayed the functions of the recombinant protein. SpMYH is shown to be the functional MutY homolog by its ability to complement the mutator phenotype of an E. coli mutY mutant containing expressed SpMYH (Table I). This proves the conservation of the repair function in these organisms. The SpMYH protein displays similar extent of identity and conservation to both E. coli MutY and human MYH. SpMYH belongs to a superfamly of base-excision DNA repair proteins that recognize diverse lesions such as oxidized purines, fragmented and oxidized pyrimidines, UV-cross-linked bases and base-base mismatches (36). In addition to the MutY and endo III families, this superfamily also includes the OGG1 (8-oxoG glycosylase) family (37–40) and AlkA family (41, 42). As shown in Fig. 1, MutY and endo III families share significant homology. Two regions of highest similarity can be identified in the known
three-dimensional structure of *E. coli* endo III (34, 35): the helix-hairpin-helix (HhH) domain (residues 147–181 of SpMYH) and the iron-sulfur domain (residues 213–242 of SpMYH). The HhH motif with a β-hairpin structure was first identified as the binding site for thymine glycol in crystals of endo III (34). Due to the diverse substrate specificities, binding to HhH presumably reflects interactions that are common to all substrates. Residues in the vicinity of the iron-sulfur cluster have also been proposed to be involved in DNA binding, and the conservation of residue spacing for the cysteines (with the exception of endo III of *S. pombe*; see Fig. 1) that ligate the iron-sulfur cluster suggests that this structure is very similar in the endo III and MutY protein families (35).

The recombinant SpMYH protein has been shown to incise A/G-, N/G-, A/GO-, 2AP/G-, and A/2AP-containing DNA. Cleavage is achieved by both DNA glycosylase and AP lyase activities. Two lines of evidence strongly suggest that SpMYH possesses AP lyase activity. 1) The detection of the nicked product in a sequencing gel without heating the samples at 90 °C (Fig. 4A) argues that the cleavage activity observed in SpMYH reaction is not caused by heating at high pH, which may catalyze a β-elimination at the AP site. 2) SpMYH can be trapped in a stable covalent enzyme-DNA intermediate with A/G-containing DNA in the presence of sodium borohydride. Imino enzyme-DNA intermediates are characteristic of a group of glycosylase/AP lyases including T4 endonuclease V, *Micrococcus luteus* UV endonuclease, *E. coli* MutY (23–25), *E. coli* endonuclease III, and *E. coli* FPG (MutM) (43–45). These enzymes use an amino group as the nucleophile, resulting in an imino enzyme-DNA intermediate (43). In addition, SpMYH cleavage activity is not inhibited by 8 mM EDTA. Thus, SpMYH belongs to that class of DNA glycosylases that possess concomitant AP lyase activity.

The glycosylase/AP lyase activity of SpMYH on A/G-20 and A/GO-20 is comparable with that of MutY enzyme on A/G-20 but is about 3-fold lower than that of MutY on A/G-20 (Table III). Thus, the catalytic activity of SpMYH is similar to MutY, although the imino intermediate of SpMYH and A/G-containing DNA is less easily trapped than MutY and the same substrate. The weak trapping activity of SpMYH with A/G-containing DNA is partly attributable to its weakened binding affinity and catalytic activity in the presence of salt. In contrast to MutY, addition of NaCl higher than 80 mM abol-

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**Fig. 6. Cleavage of mismatch-containing oligonucleotides by SpMYH and *E. coli* MutY proteins.** Oligonucleotide substrates (3’-end-labeled 20-mer, 1.8 fmol) containing indicated mismatches were incubated with 3.6 nM SpMYH (lanes 1–10) or MutY (lanes 11–20) with enzyme to DNA ratio of 40. The cleaved DNA fragment (N) and intact DNA (I) are indicated by arrows. Oligonucleotides containing the following mismatches or homoduplexes were used: A/G (lanes 1 and 11), A/GO (A/O, lanes 2 and 12), A/C (lanes 3 and 13), N/G (lanes 4 and 14), I/G (lanes 5 and 15), 2AP/G (2/G, lanes 6 and 16), A/2AP (A/2, lanes 7 and 17), C/G (C/O, lanes 8 and 18), A/I (lanes 9 and 19), and C/G (lanes 10 and 20).
zymes. The C2-amino group of mismatched G, presented in the C6-keto group as in the I/G pair blocks catalysis of both SpMYH protein, like MutY, cannot be rationalized on the basis less stable than the A/G base pairing. Thus, the reactivity of conformations (50, 51), N/G pairing would be expected to be and C/G. The C6-amino group of a mismatched A is not critical similar order and have very weak or no cleavage on I/G, A/I, C/GO, derivatives. Both enzymes cleave A/G, N/G, and A/GO in sim-

Enzyme DNA $K_m$ $V_{max}$ $h_{cat}$ $h_{cat} \cdot K_m^{-1}$

| MutY | A/G-44 | 19.1 ± 2.5 | 1.8 ± 0.3 | 10 ± 2 |
| MutY | A/GO-44 | 0.123 ± 0.015 | 0.141 ± 0.008 | 31 ± 49 |
| MutY | C/G-44 | 87.4 ± 10.1 | 315 ± 9 |
| MutY | A/GO-20 | 168 ± 26 | 5.3 ± 0.5 |
| MutY | C/GO-20 | 0.239 ± 0.015 | 0.060 ± 0.013 |
| MutY | A/GO-20 | 715 ± 89 | 370 ± 80 |
| MutY | 2AP/G-20 | 163 ± 10 | 12 ± 3 |
| MutY | A/2AP-20 | 812 ± 98 | 94 ± 14 |

a Binding constants for MutY are derived from Lu et al. (30).

b Data for MutY are derived from Lu et al. (30).

ished both SpMYH glycosylase and binding activities. We expect sodium borohydride will have the same effect on Sp-

The MutY and endonuclease III families are highly homolo-
gous (Fig. 1) (31, 46, 47). Lysine 120 of E. coli endonuclease III at the HhH motif is conserved in the endo III family and has been suggested to be necessary for the formation of the enzyme-substrate intermediate (35). Lys-249 of human OGG1 located at the same position as Lys-120 of endo III has been shown to be the active amine for Schiiff’s base formation (48). However, MutY and SpMYH have a serine and tyrosine residues at this position, respectively. In fact, MutY was grouped initially as one of the monofunctional glycosylases based on its lack of this conserved lysine (43, 49). It will be interesting to see which amino acid of SpMYH is involved in the nucleophilic attack upon the C-1’ carbon of the sugar of adenine.

The role of functional groups in catalysis by SpMYH and MutY proteins were elucidated and compared by cleavage as-

hanced in GO(anti) pair (53) but is not protone-

TABLE II

| DNA duplex | SpMYH $K_m$ | MutY $K_m$ |
| --- | --- | --- |
| A/G-44 | 19.1 ± 2.5 | 1.8 ± 0.3 |
| A/GO-44 | 0.123 ± 0.015 | 0.141 ± 0.008 |
| C/G-44 | 87.4 ± 10.1 | 315 ± 9 |
| A/GO-20 | 168 ± 26 | 5.3 ± 0.5 |
| C/GO-20 | 0.239 ± 0.015 | 0.060 ± 0.013 |
| A/GO-20 | 715 ± 89 | 370 ± 80 |
| 2AP/G-20 | 163 ± 10 | 12 ± 3 |
| A/2AP-20 | 812 ± 98 | 94 ± 14 |

a Binding constants for MutY are derived from Lu et al. (30).

b Data for MutY are derived from Lu et al. (30).

| Enzyme | DNA | $K_m$ | $V_{max}$ | $h_{cat}$ | $h_{cat} \cdot K_m^{-1}$ |
| --- | --- | --- | --- | --- | --- |
| MutY | A/G20 | 2.6 ± 0.3 | 21 ± 2 | 0.026 ± 0.002 | 10 ± 2 |
| MutY | A/GO20 | 6.5 ± 1.3 | 17 ± 2 | 0.021 ± 0.002 | 3.3 ± 1.0 |
| SpMYH | A/G20 | 4.2 ± 1.0 | 4.9 ± 0.6 | 0.012 ± 0.002 | 29 ± 0.7 |
| SpMYH | A/GO20 | 8.7 ± 1.2 | 8.3 ± 0.6 | 0.021 ± 0.002 | 24 ± 0.2 |
| SpMYH | A/G44 | 6.2 ± 1.5 | 21 ± 3 | 0.053 ± 0.003 | 86 ± 2.0 |
| SpMYH | A/GO44 | 14 ± 1.1 | 5.5 ± 0.1 | 0.014 ± 0.001 | 99 ± 0.7 |

a Binding constants for MutY are derived from Lu et al. (30).

b Data for MutY are derived from Lu et al. (30).










References

1. Ames, B. N., and Gold, L. S. (1991) Mutat. Res. 250, 3–16
2. Kasai, H., and Nishimura, S. (1991) in Oxidative Stress: Oxidants and Antioxidants.
Adenine Glycosylase (SpMYH) of S. pombe

25105

Antioxidants (Sies, H., ed) pp. 99–116, Academic Press, London

3. Halliwell, B., and Gutteridge, J. M. C. (1989) Free Radicals in Biology and Medicine, Academic Press, New York.

4. Fraga, C. G., Shigenaga, M. K., Johnson, J.-W., Degen, P., and Ames, B. N. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4533–4537.

5. Mori, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1122–1126.

6. Moriya, M., Ou, C., Bodepudi, V., Johnson, F., Takeshita, M., and Grollman, A. P. (1991) Mutat. Res. 254, 281–288.

7. Wood, M. L., Dzlharouglu, M., Gajewski, E., and Essigmann, J. M. (1990) Biochemistry 29, 7024–7032.

8. Cheng, K. C., Cahlil, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1991) J. Biol. Chem. 267, 166–172.

9. Michaels, M. L., and Miller, J. H. (1992) J. Biological Chem. 174, 6321–6325.

10. Tchou, J., and Grollman, A. P. (1993) Mutat. Res. 309, 277–287.

11. Tajiri, T., Maki, H., and Sekiguchi, M. (1995) Mutat. Res. 336, 257–267.

12. Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Grollman, A. P., and Nishimura, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4690–4694.

13. Chetsanga, C. J., and Lindahl, T. (1979) Nucleic Acids Res. 6, 3673–3683.

14. Boiteux, S., Belleney, J., Roques, B. P., and Laval, J. (1984) Nucleic Acids Res. 12, 549–553.

15. Hatahet, Z., Kow, Y., Purmal, A. A., Cunningham, R. P., and Wallace, S. S. (1984) J. Biol. Chem. 269, 18814–18820.

16. Au, K. G., Cabrera, M., Miller, J. H., and Modrich, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9163–9166.

17. Lu, A.-L., and Chang, D.-Y. (1988) J. Biological Chem. 263, 6829–6835.

18. Lu, A.-L., and Chang, D.-Y. (1988) Cell 54, 685–691.

19. Michaels, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7022–7025.

20. Bessho, T., Tano, K., Kasai, H., Ohtsuka, E., and Nishimura, S. (1993) Cell 73, 16665–16671.

21. Radicella, J. P., Clark, E. A., and Fox, M. S. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7022–7025.

22. Su, S.-S., Lahue, R. S., Au, K. G., and Modrich, P. (1988) J. Biological Chem. 263, 6829–6835.

23. Su, S.-S., Lahue, R. S., Au, K. G., and Modrich, P. (1988) J. Biological Chem. 263, 6829–6835.

24. Lu, A.-L., Yen, D. S., and Cillo, J. (1990) J. Biological Chem. 265, 24438–24443.

25. Bologa, C. G., and Lloyd, R. S. (1997) Biochemistry 36, 11140–11152.

26. Xing, D., Cunningham, R. P., and Tainer, J. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8779–8783.

27. L. C., Chang, Y.-C., Masin, J., and Lu, A.-L. (1991) J. Biological Chem. 266, 6480–6484.

28. Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Grollman, A. P., and Nishimura, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9156–9160.

29. McGoldrick, J. P., Yeh, Y.-C., Solomon, M., Essigmann, J. M., and Lu, A.-L. (1995) Mol. Cell. Biol. 15, 969–986.

30. Slupska, M. M., Baikalov, C., Luther, W. M., Chang, J.-H., Wei, Y.-F., and Miller, J. H. (1990) J. Biological Chem. 265, 3885–3892.

31. Brown, T., Hunter, W. N., Kneale, G., and Kennard, O. (1986) J. Biological Chem. 261, 4378–4384.

32. Carbonnaux, C., van der Marel, G. A., van Boom, J. H., Guschlbauer, W., and Fazakerley, G. V. (1991) Biochemistry 30, 5449–5458.

33. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Chem. Biol. 4, 693–702.

34. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 32709–32712.

35. Nash, H. M., Pham, L., Nghiem, Y., Cruz, C., and Miller, J. H. (1990) Nucleic Acids Res. 18, 3841–3845.

36. Thayer, M. M., Ahern, H., Xing, D., Cunningham, R. P., and Tainer, J. A. (1995) EMBO J. 14, 4108–4120.

37. Nash, H. M., Bruner, S. D., Scharer, O. D., Kawate, T., Addona, T. A., Spooner, E., Lane, W. S., and Verdine, G. L. (1996) Curr. Biol. 6, 968–980.

38. Lu, R., Nash, H. M., and Verdine, G. L. (1997) Curr. Biol. 7, 397–407.

39. Aburatani, H., Hikyo, Y., Nishida, T., Takashima, R., Matsuha, C., Kodama, T., Takao, M., Yasui, A., Yamamoto, K., Asano, M., Fukasawa, K., Yoshiri, T., Inoue, H., Ohtsuka, E., and Nishimura, S. (1997) Cancer Res. 57, 2151–2156.

40. Radicella, J. P., Dherin, C., Desmazie, C., Fox, M. S., and Boiteux, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8010–8015.

41. Roldan-Arjona, T., Wei, Y.-F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R.-P., Augustus, M., and Lindahl, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8016–8020.

42. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Chem. Biol. 4, 693–702.

43. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

44. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

45. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

46. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

47. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

48. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

49. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

50. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.

51. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) Biochemistry 36, 8284–8290.