

Adenine excisional repair function of MYH protein on the adenine:8-hydroxyguanine base pair in double-stranded DNA

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ABSTRACT

Adenine paired with 8-hydroxyguanine (oh⁸G), a major component of oxidative DNA damage, is excised by MYH base excision repair protein in human cells. Since repair activity of MYH protein on an A:G mismatch has also been reported, we compared the repair activity of His₆-tagged MYH proteins, expressed in *Spodoptera frugiperda* Sf21 cells, on A:oh⁸G and A:G mismatches by DNA cleavage assay and gel mobility shift assay. We also compared the repair ability of type 1 mitochondrial protein with type 2 nuclear protein, as well as of polymorphic type 1-Q³²⁴ and 2-Q³¹⁰ proteins with type 1-H³²⁴ and 2-H³¹⁰ proteins by DNA cleavage assay and complementation assay of an *Escherichia coli mutM mutY* strain. In a reaction buffer with a low salt (0–50 mM) concentration, adenine DNA glycosylase activity of type 2 protein was detected on both A:oh⁸G and A:G substrates. However, in a reaction buffer with a 150 mM salt concentration, similar to physiological conditions, the glycosylase activity on A:G, but not on A:oh⁸G, was extremely reduced and the binding activity of type 2 protein for A:G, but not for A:oh⁸G, was proportionally reduced. The glycosylase activity on A:oh⁸G and the ability to suppress spontaneous mutagenesis were greater for type 2 than type 1 enzyme. There was apparently no difference in the repair activities between the two types of polymorphic MYH proteins. These results indicate that human MYH protein specifically catalyzes the glycosylase reaction on A:oh⁸G under physiological salt concentrations.

INTRODUCTION

8-Hydroxyguanine (oh⁸G) is a modified base of oxidative DNA damage and the formation of oh⁸G in DNA is involved in mutagenesis (1–3). The OGG1 and MYH proteins (4–16), which are functional homologs of the *Escherichia coli* MutM

and MutY proteins, respectively (17,18), have been identified as enzymes which repair oh⁸G in DNA in human cells. OGG1 possesses the ability to excise oh⁸G paired with cytosine (4–12), while MYH possesses the ability to excise adenine paired with oh⁸G (14–16). Since repair activity of MYH on an A:G mismatch had also been reported (15,16), we investigated the differences in the catalytic activities of human MYH protein towards A:oh⁸G and A:G substrates, in comparison with those of *Schizosaccharomyces pombe* MYH protein (19).

Multiple forms of human MYH proteins are expressed in human cells due to multiple transcription initiation sites and alternative splicing of MYH mRNA transcripts (14,16). Type 1 protein is localized in the mitochondria, while type 2 protein, which lacks the N-terminal 14 amino acids of type 1 protein, is localized in the nucleus (14,16). Thus, we also compared the repair activities of type 1 and 2 proteins *in vitro* and *in vivo* in this study. In a report of the first cloning and sequencing of the MYH gene, a G/C nucleotide variation in exon 12 among cDNA clones was described (13). This nucleotide variation is associated with an amino acid substitution (Gln/His) and was found to be a common single nucleotide polymorphism (SNP) in our studies (Shinmura *et al.*, unpublished data). Thus, four major types of MYH protein, type 1-Q³²⁴, type 1-H³²⁴, type 2-Q³¹⁰ and type 2-H³¹⁰, are expressed in human cells. In the human OGG1 gene we previously identified a Ser326Cys SNP as being associated with distinct oh⁸G repair activity in the complementation assay of an *E. coli mutM mutY* strain (20,21). In addition, it was shown that the catalytic efficiency (k_{cat}/K_m) of these two polymorphic proteins differed significantly by 2-fold for excision of oh⁸G from γ -irradiated DNA (22,23). With reference to this example, we also compared the repair activities of these polymorphic MYH proteins *in vitro* and *in vivo*.

MATERIALS AND METHODS

Preparation of DNA substrates

The sequences of oligonucleotides used as substrates or as a size marker are listed in Table 1. Three sets of double-stranded oligonucleotides, A:G (Sequences 1 and 2), A:T (Sequences 1 and 3) and A:oh⁸G (Sequences 1 and 4), were prepared and 3'-end-labeled with Exo⁻ Klenow (Stratagene) and [α -³²P]dCTP (Amersham), as previously described (11).

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Table 1. Oligonucleotides used in this study

Name	Sequence
Sequence 1	5'-TTGGGGAATGAGTCAGGCCAC-3'
Sequence 2	5'-GGTGGCCTGACGCATTCCCCAA-3'
Sequence 3	5'-GGTGGCCTGACTCATTCCCCAA-3'
Sequence 4	5'-GGTGGCCTGACG*CATTCCCCAA-3'
Sequence 5	5'-GTCAGGCCACC-3'

G*, 8-hydroxydeoxyguanosine.

Preparation of human and *S.pombe* MYH expression vectors

PCR products with the coding sequences for types 1-Q³²⁴ and 1-H³²⁴ and types 2-Q³¹⁰ and 2-H³¹⁰ human MYH proteins were inserted into the *Eco*RI site of the pGEX1 λ T (Pharmacia) and pFASTBAC HTa (Gibco BRL) vectors. DNA prepared from an *S.pombe* cDNA library was used as a template in a PCR amplification of the coding sequence for the *S.pombe* MYH gene. The PCR product was inserted into the *Bam*HI site of the pGEX1 λ T and pFASTBAC HTb (Gibco BRL) vectors. pGEX1 λ T-human OGG1 type 1a-Ser³²⁶ was prepared as previously (11).

Preparation of MYH proteins

Type 1-Q³²⁴/1-H³²⁴ and type 2-Q³¹⁰/2-H³¹⁰ human MYH proteins and *S.pombe* MYH proteins, tagged with His₆ at their N-terminal ends, were prepared using the BAC-TO-BAC Baculovirus Expression System (Gibco BRL) according to the manufacturer's protocol. Briefly, plasmid vectors pFASTBAC HTa/human MYH and pFASTBAC HTb/*S.pombe* MYH were transfected into DH10BAC competent cells which contain the bacmid with a mini-attTn7 target site and the helper plasmid. After selection of colonies containing recombinant bacmids, recombinant bacmid DNAs were isolated and transfected into *Spodoptera frugiperda* Sf21 cells. Recombinant MYH proteins were expressed in Sf21 cells by infection of the viral stocks and purified with TALON metal affinity resins (Clontech). The proteins were then dialyzed against buffer containing 10 mM Tris-HCl pH 7.6, 0.5 mM dithiothreitol, 0.5 μ g/ml leupeptin, 0.5 μ g/ml pepstatin, 0.5 μ g/ml chymostatin, 0.5 μ g/ml antipain, 1 mM phenylmethylsulfonyl fluoride and 10% glycerol. MYH protein concentrations were determined by western blot analysis using an anti-Tetra His antibody (Qiagen) with recombinant His₆-tagged chloramphenicol acetyltransferase protein as a quantitative control. The concentration of chloramphenicol acetyltransferase was measured using the method of Bradford (24). Western blot analyses were performed as described previously (25).

DNA cleavage assay

The reaction mixture contained 12 nM MYH protein, 10 mM Tris-HCl pH 7.6, 0.5 mM dithiothreitol, 0.5 mM EDTA, 1.45% glycerol, 50 μ g/ml bovine serum albumin, 2 nM labeled DNA and various concentrations of NaCl. The standard reaction was performed with 20 μ l of the mixture at 37°C for 60 min. After the reaction, two separate 9 μ l aliquots were taken and one aliquot was exposed to 0.1 M NaOH and heated to 95°C for 4 min. Then, 10 μ l of denaturing formamide dye was added to both aliquots. The aliquots were then heat denatured and subjected to 7 M urea-10% polyacrylamide gel

electrophoresis. Sequence 5 in Table 1 was 5'-end-labeled, as described previously (11), and used as the size marker of cleaved products. The radioactivities of intact and cleaved oligonucleotides were quantified with a bioimaging analyzer (BAS2000; Fuji Photo Film, Tokyo). For a kinetic study of DNA cleavage, MYH proteins were reacted with various amounts (0.5, 1, 2, 4 and 8 nM) of the A:oh⁸G substrate in a buffer containing 150 mM NaCl at 37°C for 15 min. Lineweaver-Burk plots representing the reciprocal of initial rates of adenine excision versus the reciprocal of substrate concentrations were utilized to determine the Michaelis constant (K_m) and the catalytic constant (k_{cat}).

Gel mobility shift assay

Reactions were performed under the same conditions as for the DNA cleavage assay, except for the incubation time of 30 min. Protein-DNA complexes were analyzed as described previously (11).

Complementation assay

pGEX1 λ T-human MYH, pGEX1 λ T-*S.pombe* MYH and pGEX1 λ T-human OGG1 were used for the complementation assay. The detailed protocol has been described previously (5). Briefly, overnight cultures of YG5132 cells transformed with MYH- or OGG1-overproducing plasmids and of control cells were analyzed for rifampicin-resistant (Rif^R) mutation events. In each group, four to six plates were prepared and the average numbers of Rif^R colonies/10⁸ cells were recorded.

RESULTS

Preparation of human and *S.pombe* MYH proteins

For functional analysis of human MYH proteins we first tried to express GST-MYH fusion proteins in *E.coli*. However, we could not obtain any bacterial clones with expression of the fusion proteins. Therefore, we next constructed baculovirus expression vectors for human and *S.pombe* MYH proteins. Vectors for polymorphic MYH proteins of both mitochondrial type (1-Q³²⁴ and 1-H³²⁴) and nuclear type (2-Q³¹⁰ and 2-H³¹⁰), as well as *S.pombe* MYH protein, were prepared and expressed in Sf21 insect cells. Since the pFASTBAC HT vector with a His₆ tag was used for expression of these proteins, human and *S.pombe* proteins were N-terminally fused with polypeptides with a predicted molecular size of ~3.7 and 3.3 kDa, respectively. The levels of MYH protein expression in Sf21 cells were low, as in the case of MYH protein expression in bacteria (14-16), however, purified MYH proteins were detected by western blot analysis using an anti-Tetra His antibody. Human MYH type 1 and type 2 and *S.pombe* MYH proteins with molecular sizes of 64, 62 and 57 kDa, respectively, were detected (Fig. 1). The sizes determined by western blot analysis corresponded to those calculated from the His₆-MYH cDNA sequences. Therefore, we used these proteins for further analyses.

Adenine DNA glycosylase and apurinic/aprimidinic (AP) lyase activities of MYH protein

Type 2-Q³¹⁰ protein was first prepared as a representative nuclear form of MYH proteins and used for analysis of adenine DNA glycosylase activity and AP lyase activity on the A:oh⁸G mismatch. These activities were evaluated initially with NaCl-free

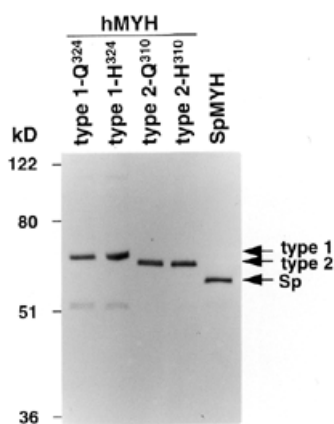


Figure 1. Detection of MYH proteins by western blot analysis. Human MYH (hMYH) types 1-Q³²⁴, 1-H³²⁴, 2-Q³¹⁰ and 2-H³¹⁰ and *S.pombe* MYH (SpMYH) proteins were expressed in Sf21 cells and purified with TALON metal affinity resins. Twenty nanograms of the purified MYH proteins were applied to each lane. MYH proteins N-terminally fused with polypeptides containing a His₆ tag were detected by western blot analysis using an anti-Tetra His antibody. Arrows indicate the positions of the proteins.

reaction buffer, which was previously used for analysis of *S.pombe* MYH protein (19). Since NaOH treatment is known to cleave any AP sites generated by DNA glycosylases (26,27), this treatment was adapted to distinguish adenine DNA glycosylase activity from AP lyase activity. In other words, AP lyase activity was assessed without NaOH treatment of DNA substrates after reaction with MYH proteins, whereas DNA glycosylase activity was assessed after NaOH treatment. Oligonucleotides containing A:T, A:oh⁸G or A:G reacted with type 2-Q³¹⁰ protein were treated or not with NaOH before loading onto the gel and the percentage of cleaved products per total oligonucleotides was calculated (Fig. 2). The protein had an activity cleaving oligonucleotides with an A:oh⁸G or A:G mismatch but did not have an activity cleaving oligonucleotides with A:T (Fig. 2A). Such an activity was more prominent with NaOH treatment than without NaOH treatment. This result indicated that human MYH protein possesses adenine DNA glycosylase activity and AP lyase activity against both A:oh⁸G and A:G mismatches. AP lyase activity was also shown by production of a stable covalent MYH protein–DNA complex with DNA containing an A:oh⁸G or A:G mismatch in the presence of borohydride (data not shown).

The time-course assay on A:oh⁸G repair activity of 2-Q³¹⁰ protein indicated that the amount of cleaved products plateaued within 60 min and was steady in the period of 60–180 min (Fig. 2B). The percentage of AP lyase activity relative to DNA glycosylase activity was ~15% throughout the course of the experiment. Thus, AP lyase activity of MYH protein almost correlates with adenine DNA glycosylase activity of MYH protein and the reaction time of 60 min was considered to be enough to evaluate these activities. Based on these results, DNA glycosylase activity with NaOH treatment was assessed with a reaction time of 60 min in further experiments.

Salt requirement of the adenine DNA glycosylase activity

We next investigated the effect of NaCl concentration on the DNA glycosylase activity of MYH protein. Type 2-Q³¹⁰

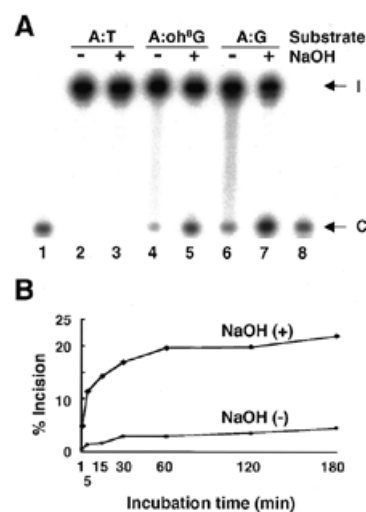


Figure 2. Detection of the glycosylase and AP lyase activities of MYH type 2-Q³¹⁰ protein. (A) Substrate specificity of the DNA cleavage reaction. The reactivity of the 2-Q³¹⁰ protein on 3'-end-labeled 22mer oligonucleotides containing either an A:T (lanes 2 and 3), A:oh⁸G (lanes 4 and 5) or A:G (lanes 6 and 7) base pair at position 12 from the labeled end were examined with an incubation time of 60 min. Samples with (+) or without (-) NaOH treatment were loaded onto the denaturing polyacrylamide gel. The positions of intact (I) and cleaved (C) oligonucleotides are indicated by arrows (lanes 2–7). A 5'-labeled 11mer marker was co-electrophoresed for confirmation of the size of cleaved products (lanes 1 and 8). (B) Time dependency of the DNA cleavage reaction. 3'-End-labeled oligonucleotides containing an A:oh⁸G mismatch were reacted with 2-Q³¹⁰ protein for the period indicated. Samples with (+) or without (-) NaOH treatment were loaded onto the gel and the radioactivities of the intact and cleaved oligonucleotides were quantified. The percentages of cleaved products per total oligonucleotides were calculated.

protein was reacted with either an A:oh⁸G or A:G substrate in a buffer containing 0–200 mM NaCl (Fig. 3A and B). At low NaCl concentrations the activity against the A:G substrate was higher than that against the A:oh⁸G substrate. However, the activity of the protein on the A:G substrate was extremely reduced in buffers with >100 mM NaCl, whereas that on the A:oh⁸G substrate was retained in buffers containing 0–150 mM NaCl. In a report on the repair activity of *S.pombe* MYH protein, no activity on the A:G substrate at 80 mM NaCl was detected and no experiment on A:oh⁸G was performed under other than NaCl-free conditions (19). Thus, the activity of human MYH protein on A:G was similar to that of *S.pombe* MYH protein. On the other hand, it was revealed in this study that the glycosylase activity of the human protein on A:oh⁸G was retained up to 150 mM NaCl.

To clarify whether the effect of NaCl concentration on glycosylase activity is associated with binding activity of the protein to the substrates, the effect of salt concentration on the affinity of MYH protein for both substrates was examined by gel mobility shift assay. The specific binding complexes of 2-Q³¹⁰ protein and oligonucleotides containing either an A:oh⁸G or A:G mismatch were compared to each other under various NaCl concentrations (Fig. 3C). Oligonucleotides containing the normal A:T base pair instead of a mismatch base pair were used as a control. The binding activity of type 2-Q³¹⁰ protein on the A:oh⁸G substrate was similar at 0–150 mM NaCl, while that on the A:G substrate was extremely reduced at >100 mM

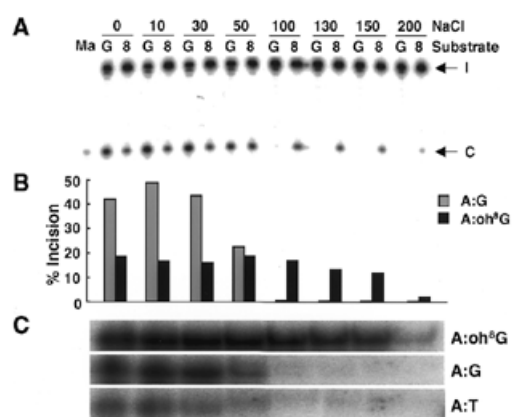


Figure 3. Effect of NaCl concentration on the DNA repair properties of MYH proteins. (A) Effect of NaCl concentration on the glycosylase activity of MYH type 2-Q³¹⁰ protein was examined by DNA cleavage assay. Oligonucleotides containing an A:G (G) or A:oh⁸G (8) mismatch were reacted with the protein for 60 min under various NaCl concentrations (mM) and subjected to gel electrophoresis after NaOH treatment. The positions of intact (I) and cleaved (C) oligonucleotides are indicated by arrows. The 5'-labeled 11mer marker was co-electrophoresed on the left for confirmation of the size of the cleaved product. (B) Percentages of oligonucleotides cleaved by MYH protein in the experiment in (A) were calculated and are shown as bars. (C) The effect of NaCl concentration on the binding activity of type 2-Q³¹⁰ protein to oligonucleotides with an A:oh⁸G (upper), A:G (middle) or A:T (lower) mismatch was examined by gel mobility shift assay. Reaction mixtures were electrophoresed on a 6% non-denaturing polyacrylamide gel. The specific protein-DNA binding complex under the condition of NaCl concentrations corresponding to experiment (A) is shown.

NaCl. Thus, binding activity correlated well with glycosylase activity of this protein. These results indicate that MYH glycosylase activity is dependent on NaCl concentration and is regulated by the affinity of the protein for the mismatched DNA.

Next, to elucidate whether glycosylase activity is also affected by KCl, instead of NaCl, we examined activity in a buffer with KCl concentrations of 0–170 mM (Fig. 4A). KCl had the same effect as NaCl on glycosylase activity of the protein. Physiologically, intracellular concentrations of Na⁺ and K⁺ ions are maintained at 5–15 and 140 mM, respectively (28), and the nuclear envelop is non-selective for Na⁺ and K⁺ ions (29). With reference to these reports, glycosylase activity of the type 2 nuclear protein was further examined under conditions of 10 mM NaCl with various concentrations of KCl (Fig. 4B). The activity on the A:G substrate almost disappeared at 10 mM NaCl + 90 mM KCl, whereas activity on the A:oh⁸G substrate was retained at up to 10 mM NaCl + 160 mM KCl. Thus it was concluded that glycosylase activity of MYH protein was different in its salt (NaCl and/or KCl) requirements between A:oh⁸G and A:G substrates. It was also concluded that an A:G mismatch was poorly repaired by MYH protein at a 150 mM salt concentration, which is similar to physiological conditions. Glycosylase activity of *S.pombe* MYH protein was also examined under various NaCl concentrations (Fig. 4C). The trend of activity of the *S.pombe* protein against the two substrates was similar to that of the human protein. Thus, the distinct salt requirement of glycosylase activity against A:oh⁸G and A:G is conserved between *S.pombe* and human proteins.

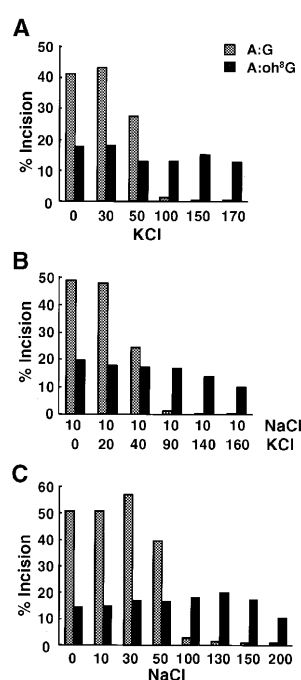


Figure 4. Effect of salt concentrations on the glycosylase activity of MYH proteins. Effect of KCl (A) or NaCl + KCl (B) concentration on the glycosylase activity of type 2-Q³¹⁰ protein examined by DNA cleavage assay. (C) Effect of NaCl concentration on the glycosylase activity of *S.pombe* MYH protein examined by DNA cleavage assay. Oligonucleotides containing an A:G or A:oh⁸G mismatch were reacted under various salt concentrations (mM) and subjected to gel electrophoresis after NaOH treatment. The percentages of oligonucleotides cleaved by MYH proteins were calculated and are shown as bars.

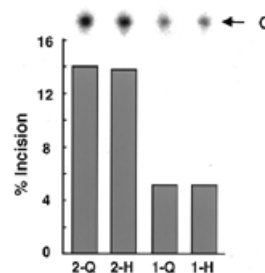


Figure 5. Comparison of glycosylase activity between type 2 and type 1 as well as between polymorphic MYH proteins. Oligonucleotides containing an A:oh⁸G mismatch were reacted with 2-Q³¹⁰ (2-Q), 2-H³¹⁰ (2-H), 1-Q³²⁴ (1-Q) or 1-H³²⁴ (1-H) protein in the 150 mM NaCl reaction buffer for 60 min. Products treated with NaOH were subjected to gel electrophoresis after heating. The percentages of oligonucleotides cleaved by each protein are presented as bars. A photograph of each cleaved product (C) is shown at the top.

Comparison of the glycosylase activity of the type 1 and type 2 proteins

Both mitochondrial type 1 and nuclear type 2 proteins are expressed in human cells. Thus, glycosylase activity on an A:oh⁸G mismatch at a 150 mM salt concentration was compared between the 2-Q³¹⁰ and 1-Q³²⁴ proteins (Fig. 5). The activity of type 2-Q³¹⁰ was 2.7 times higher than that of type 1-Q³²⁴

after 60 min reaction. To further confirm the difference in the activities of the type 1 and type 2 proteins, the Michaelis constant (K_m) and the catalytic constant (k_{cat}) of the glycosylase reaction of these proteins for an A:oh⁸G mismatch were determined in a reaction buffer with a 150 mM salt concentration (Table 2). The k_{cat}/K_m value, representing catalytic efficiency, of type 2-Q³¹⁰ protein was 2 times higher than that of type 1-Q³²⁴ protein. Thus, it was indicated that glycosylase activity of the type 2 protein is greater than that of the type 1 protein.

Table 2. Kinetic constants of the glycosylase reaction of MYH proteins on an A:oh⁸G substrate

Type of protein	K_m (nM)	k_{cat} (min ⁻¹)	k_{cat}/K_m (min ⁻¹ · μ M ⁻¹)
MYH type 1-Q ³²⁴	1.0	5.7×10^{-4}	0.55
MYH type 1-H ³²⁴	1.2	5.8×10^{-4}	0.50
MYH type 2-Q ³¹⁰	2.1	2.6×10^{-3}	1.22
MYH type 2-H ³¹⁰	2.2	2.1×10^{-3}	0.99

Comparison of the glycosylase activity among polymorphic proteins

A G/C SNP in exon 12 in the *MYH* gene results in production of type 1-Q³²⁴/1-H³²⁴ and type 2-Q³¹⁰/2-H³¹⁰ proteins. To elucidate whether or not there are interindividual variations in MYH repair ability due to this SNP, glycosylase activity on the A:oh⁸G mismatch at 150 mM NaCl was compared among these polymorphic proteins. The percentage incision value (Fig. 5) and the k_{cat}/K_m value (Table 2) were similar between the type 1-Q³²⁴ and 1-H³²⁴ proteins as well as between the type 2-Q³¹⁰ and 2-H³¹⁰ proteins. Thus, it was indicated that both of the polymorphic MYH proteins efficiently catalyze the glycosylase reaction at similar levels.

Comparison of the ability to suppress spontaneous mutagenesis between type 1 and type 2 proteins as well as polymorphic proteins

The ability to suppress spontaneous mutagenesis was also compared between the type 1 and type 2 as well as polymorphic proteins. Since strain YG5132 is a *mutM mutY* mutant of *E.coli* strain CC104, the numbers of Rif^R mutants in YG5132 transformed with plasmids containing each of the *GST-MYH* cDNA fragments were compared (Table 3). Plasmids expressing *GST-S.pombe* MYH and *GST-human* OGG1 proteins were used as positive controls. The number of Rif^R YG5132 colonies was significantly higher than that of Rif^R CC104 colonies. All of the *GST-human* MYH fusion proteins had activities suppressing spontaneous mutagenesis of the YG5132 strain, as in the cases of the *GST-S.pombe* MYH and *GST-human* OGG1 fusion proteins. The activity of type 2 was greater than that of type 1 protein, correlating with the difference in glycosylase activity between them. The activity was similar between polymorphic proteins, also correlating with the similarity in glycosylase activity between them. Based on the analyses of glycosylase activity *in vitro* and suppression activity *in vivo*, we concluded that type 2 protein possesses greater repair ability than type 1 protein and that polymorphic

proteins of the Gln and His types catalyze the DNA repair reaction at similar levels.

Table 3. Complementation of an *E.coli mutM mutY* mutant with *MYH*-overproducing plasmids

<i>E.coli</i> strain	Plasmid	No. of Rif ^R cells/10 ⁸ cells ^a
CC104	–	1.7 ± 0.91
YG5132 ^b	–	516.1 ± 94.6
YG5132	pGEX-1 λ T	402.1 ± 87.1
YG5132	GST–human MYH type 1-Q ³²⁴	111.1 ± 27.6
YG5132	GST–human MYH type 1-H ³²⁴	90.9 ± 35.1
YG5132	GST–human MYH type 2-Q ³¹⁰	14.4 ± 6.3
YG5132	GST–human MYH type 2-H ³¹⁰	11.3 ± 2.8
YG5132	GST– <i>S.pombe</i> MYH	1.4 ± 0.76
YG5132	GST–human OGG1 type 1a-Ser ³²⁶	4.6 ± 1.67

^aMean ± SE is shown.

^bStrain YG5132 is a *mutM mutY* double mutant of *E.coli* CC104.

DISCUSSION

In the analysis of the DNA repair reaction by DNA cleavage assay and gel mobility shift assay type 2 nuclear protein possessed glycosylase activity as well as binding activity to both A:oh⁸G and A:G mismatches in a reaction buffer with a low salt concentration. However, in a reaction buffer with a 150 mM salt concentration, which is similar to the physiological condition in the cells, glycosylase activity and affinity of the type 2 protein for A:G, but not A:oh⁸G, were extremely reduced. Thus, it was strongly indicated that an adenine paired with oh⁸G is physiologically more preferable as a substrate of MYH protein than an A:G mismatch. The glycosylase activity of MYH protein under the physiological salt concentration is likely to reflect the repair activity *in vivo*.

The salt concentration-dependent differences in glycosylase activity of the MYH proteins between each mismatch was regulated, at least in part, by the affinity of the MYH proteins for these substrates. Thus, the factor influencing affinity between the protein and DNA substrates may affect the differences in glycosylase activity. In relation to DNA substrates, the presence or absence of a C8-keto group in guanine causes the distinct nature of hydrogen bonding between A:oh⁸G and A:G (30,31). This difference may be related to the distinct level of dissociation of the protein from substrate DNA when the salt concentration is elevated, although the detailed mechanism is unclear at present. In general, *in vitro* studies on the efficiency of DNA repair activity are not always done under physiological salt concentrations. However, for instance, G:T mismatch repair activity *in vitro* was reported to be remarkably different between the physiological and other salt concentrations (32). A difference in DNA cleavage activity of APEX protein was also detected between these two conditions (33). Thus, the experiment at physiological salt concentration should be important in understanding DNA repair activity *in vivo*.

In both the DNA cleavage and complementation assays the activity of type 2 protein was greater than that of type 1 protein. This result may imply a higher repair ability of the nuclear MYH form compared with the mitochondrial MYH form. Such a difference may be associated with the greater number of nucleotides in chromosomal DNA, compared with those in mitochondrial DNA. However, the type 1 protein is suggested to be processed during mitochondrial transport in cells and the mature form of the protein is unclear (14). Therefore, repair activity of the full-length mitochondrial form may not always reflect the activity *in vivo*.

Both the type 1-Q³²⁴ and 1-H³²⁴, as well as the type 2-Q³¹⁰ and 2-H³¹⁰, polymorphic proteins showed glycosylase activity and an ability to suppress spontaneous mutagenesis at similar levels. Thus, this SNP is likely to be neutral with respect to repair activity. However, it is possible that addition of GST or His₆ polypeptides to native proteins has made it difficult to detect a difference in repair capacities of these polymorphic proteins. Since this SNP exists outside the catalytic domain for DNA glycosylase activity, it is also possible that other properties may differ between these polymorphic proteins.

In this study it was indicated that human MYH protein possesses both adenine DNA glycosylase activity and AP lyase activity. The percentage of AP lyase activity relative to DNA glycosylase activity of MYH protein was ~15% in the time-course experiment (Fig. 2B). Such a low AP lyase activity of MYH protein in comparison with its DNA glycosylase activity has also been reported by others (15,16,34). In contrast, there is controversy whether *E.coli* MutY protein possesses AP lyase activity in addition to DNA glycosylase activity (35). Low AP lyase activity relative to DNA glycosylase activity of MutY protein was reported, while a similar level of AP lyase activity to glycosylase activity of the protein was also reported (35). It has been thought that formation of a covalent DNA-protein complex by borohydride reduction of the Schiff base intermediate is a characteristic of DNA glycosylases with AP lyase activity. However, Williams and David demonstrated that MutY protein possesses only glycosylase activity and not AP lyase activity, in spite of forming a covalent complex (35). Thus, although the covalent complex of human MYH protein and substrate DNA was detected in our preliminary experiment, such a finding would not be enough to support the presence of AP lyase activity in MYH protein. Since the AP lyase activity of MYH protein was much lower than its glycosylase activity so far reported, as well as examined in this study, further studies will be needed to elucidate the catalytic mechanism of eukaryotic and prokaryotic MYH proteins.

In summary, it is indicated here that MYH protein specifically catalyzes the glycosylase reaction on A:oh⁸G under physiological salt concentrations. This approach will provide new insights into the DNA repair machinery surrounding MYH on oxidatively damaged and mismatched DNA *in vivo*.

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