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Functional Identification of an 8-Oxoguanine Specific Endonuclease from *Thermotoga maritima*

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To date, no 8-oxoguanine-specific endonuclease-coding gene has been identified in *Thermotoga maritima* of the order *Thermotogales*, although its entire genome has been deciphered. However, the hypothetical protein *Tm*1821 from *T. maritima*, has a helix-hairpin-helix motif that is considered to be important for DNA binding and catalytic activity. Here, *Tm*1821 was overexpressed in *Escherichia coli* and purified using Ni-NTA affinity chromatography, protease digestion, and gel filtration. *Tm*1821 protein was found to efficiently cleave an oligonucleotide duplex containing 8-oxoguanine, but *Tm*1821 had little effect on other substrates containing modified bases. Moreover, *Tm*1821 strongly preferred DNA duplexes containing an 8-oxoguanine:C pair among oligonucleotide duplexes containing 8-oxoguanine paired with four different bases (A, C, G, or T). Furthermore, *Tm*1821 showed AP lyase activity and Schiff base formation with 8-oxoguanine in the presence of $NaBH_4$, which suggests that it is a bifunctional DNA glycosylase. *Tm*1821 protein shares unique conserved amino acids and substrate specificity with an 8-oxoguanine DNA glycosylase from the hyperthermophilic archaeon. Thus, the DNA recognition and catalytic mechanisms of *Tm*1821 protein are likely to be similar to archaeal repair protein, although *T. maritima* is an eubacterium.

Keywords: DNA glycosylase, DNA repair, Hypothetical protein, 8-Oxoguanine, *Thermotoga maritima*

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Introduction

Enormous progress has been made in the physical mapping and genome sequencing of a large number of organisms in recent years (Bentley, 2000; Fraser *et al*., 2000; Sterky and Lundeberg, 2000). The sequences of the entire genome of the hyperthermophilic bacterium *Thermotoga maritima*, which grows at an optimum temperature of 80°C, have also been published (Nelson *et al*., 1999). However, the biochemical functions of a significant portion (46%) of the gene products from *T. maritima* cannot be assigned based on sequence homology to genes from other organisms. The ultimate goal of genome sequencing is to determine the biochemical function and biological role of each newly sequenced gene. Traditionally, the biochemical functions of these genes have been predicted on the basis of searches for well-characterized relatives in sequence databases (Brock and Bairoch, 1996). Both the sequence alignment and motif searching methods have been applied successfully to the functional identification of genes (Bork, 1992; Bork and Gibson, 1996). A combined motif search/sequence alignment approach is therefore feasible, and would allow more precise functional predictions to be made for newly appearing sequences.

Although the entire genome of *T. maritima* has been determined (Nelson *et al*., 1999), the 8-oxoguanine DNA glycosylase gene has not been identified. 8-oxoguanine DNA glycosylase is an enzyme involved in DNA repair. More specifically, it excises 8-oxoguanine formed by oxidative damage of guanine in DNA (Fortini *et al*., 2003), which can mispair with adenine, and induce a G:C to T:A transversion mutation (Sekiguchi and Tsuzuki, 2002; Tudek, 2003). Several DNA repair enzymes, including DNA glycosylase/lyase, prevent mutagenesis by 8-oxoguanine (Petit and Sancar, 1999; Lee, 2001). Genes that encode 8-oxoguanine-excising enzyme have been found in bacteria (Michaels *et al*., 1991), archaea (Gogos and Clarke, 1999), and eukaryotes (Nash *et al*., 1996; Radicella *et al*., 1997).

In this study, we identified the hypothetical protein *Tm*1821 in *T. maritima* as an 8-oxoguanine-specific endonuclease that hydrolyzes DNA duplex substrates containing an 8-oxoguanine residue. DNA glycosylase homologs in the *T. maritima* genome were searched for using BLAST (Altschul *et al*., 1997), and candidates containing a helix-hairpin-helix (HhH) motif were selected. Finally, the ORF of *Tm*1821 was compared with the amino acid sequences and the HhH motifs of other proteins. The DNA cleavage activity of *Tm*1821 was examined using various oligonucleotide duplexes containing modified bases, like 8-oxoguanine.

Materials and Methods

Materials The pET28a expression vector and *E. coli* BL21 (DE3) strain were obtained from Novagen (Madison, USA). DNA restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, USA), and *Taq* DNA polymerase and dNTP solutions were from Takara (Shiga, Japan). The genomic DNA of *T. maritima* MSB8 was obtained from ATCC (Manassas, USA), and all oligonucleotides for cloning and sequencing were synthesized by Bioneer (Daejeon, Korea). The FPLC system and columns were supplied by Amersham Biosciences (Uppsala, Sweden). Oligonucleotide sizing markers and [γ-^{32}P]ATP were also purchased from Amersham Biosciences. All other reagents used in this study were of analytical grade.

Selection and cloning of *Tm*1821 To search for the presumed 8-oxoguanine endonuclease in the *T. maritima* genome, a sequence search of ORFs of unknown function (hypothetical proteins) was undertaken using local similarity BLAST at NCBI (Altschul *et al*., 1997). The amino acid sequences of the hypothetical genes of *T. maritima* ORFs (of amino acid residue number <400) were compared with known proteins in the BLAST database. Selected candidates were scrutinized in order to determine if these proteins possessed a helix-hairpin-helix (HhH) motif. The complete coding sequence of *Tm*1821 was amplified by polymerase chain reaction (PCR) using GeneAmp PCR system 2400 (Perkin Elmer, Norwalk, USA) using a pair of DNA primers, namely, 5'-GCCTCACGT CATATGGAAGAACTGCTGAAA-3' (where the underlining indicates the *Nde*I site), and 5'-GCTAGGCATGGATCCCTATTTATCCACC TTCCC-3', (where the underlining indicates the *Bam*HI site). PCR was performed in 100 µl reaction mixtures containing 600 ng of genomic DNA as a template, 200 mM dNTPs, 2 units of *Taq* DNA polymerase, 10 µl of 10X reaction buffer, and 1 pmol of each primer. PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), and digested with *Nde*I and *Bam*HI. The fragments produced were subcloned into a pET28a expression vector (Novagen), and pET-*Tm*1821 plasmid was used to transform *E. coli* DH5a for propagation. The DNA sequence of the *Tm*1821 gene in pET28a vector was confirmed using an ABI 373 DNA automatic sequencer (Applied Biosystems, Foster City, USA).

Protein expression and purification of *Tm*1821 The recombinant plasmid (pET-*Tm*1821) was introduced into *E. coli* BL21 (DE3). To purify recombinant *Tm*1821 protein, *E. coli* BL21 (DE3) harboring pET-*Tm*1821 was grown in LB broth (1% tryptone, 0.5% yeast extract, 1% NaCl) with 35 µg/ml of kanamycin at 37°C. The recombinant protein was induced with 0.5 mM IPTG when the culture achieved an absorbance of 0.6 at 600 nm. After another 4h of growth, cells were harvested and resuspended in 50 ml of buffer A (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 1 mM PMSF). The cells were then lysed by ultrasonication and centrifuged at 16,000 rpm for 30 min at 4°C to pelletize insoluble materials. The supernatant was then loaded onto a Ni-NTA affinity column (Qiagen) and washed with buffer A containing 20 mM imidazole. His-tagged *Tm*1821 protein was eluted using a linear gradient of imidazole (from 20 to 500 mM) in buffer A. Additional amino acids that contained hexahistidine in His-tagged *Tm*1821 N-terminus were removed by thrombin digestion at 4°C. *Tm*1821 protein was purified by gel filtration chromatography using a Superdex-75 FPLC column (Amersham Biosciences, Uppsala, Sweden) pre-equilibrated with buffer A. Proteins were separated in a 15% SDS polyacrylamide gel and visualized using Coomassie blue staining in order to estimate yield and purity. Protein concentrations were determined using the Bradford method (Bradford, 1976) with a bovine serum albumin standard.

Preparation of oligonucleotide duplex substrates The 32-mer oligonucleotide duplexes containing a single modified base or a mismatched base (8-oxoguanine, 3-methyladenine, 7-methylguanine, thymine glycol, hypoxanthine, 8-bromoguanine, apurinic/apyrimidinic site, uracil, or an A:G mismatch) at position 16 were used as substrates to assess the DNA cleavage activity of *Tm*1821 protein (Table 1). Oligonucleotides containing a single modified base and their complementary strands were obtained from Bio-Synthesis, Inc. (Lewisville, USA). These were labeled on their 5'-ends with [γ-^{32}P]ATP using T4 polynucleotide kinase (Takara, Shiga, Japan) at 37°C. Unincorporated [γ-^{32}P]ATP was removed following purification of the oligonucleotides using a Microspin G-50 column (Amersham Biosciences, Uppsala, Sweden). Duplexes were prepared by annealing with an unlabeled complementary strand at a 1.5-fold molar excess in buffer (20 mM Tris-Cl, pH 7.4, 100 mM NaCl, 1 mM DTT, 1 mM EDTA, and 3% glycerol). To generate duplex molecules, annealing was performed at 75°C for approximately 5 min and this was followed by gentle cooling to room temperature. The annealed DNA so obtained was eluted by ethanol precipitation, dried, and resuspended in doubly-distilled water.

DNA cleavage assay DNA cleavage reactions of *Tm*1821 protein were performed in 40 µl reaction mixtures using 10 ng of *Tm*1821 at 50°C. Reactions were carried out with 1 pmol of radiolabeled 32-mer oligonucleotide duplexes in a reaction buffer containing 20mM Tris-Cl (pH 8.5), 100 mM NaCl, 1 mM DTT, and 1 mM EDTA. After reaction termination with phenol/chloroform and ethanol precipitation, oligonucleotides were resuspended in 20 µl of formamide loading buffer (containing 0.05% bromophenol blue and 0.05% xylene cyanol). These suspensions were then heated for 5 min at 90°C, and subjected to electrophoresis through a denaturing 15% polyacrylamide gel containing 7 M urea in 1X TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA). Gels were then dried and placed on an imaging plate. DNA cleavage products were quantified using a BAS2000 image analyzer (Fuji, Tokyo).

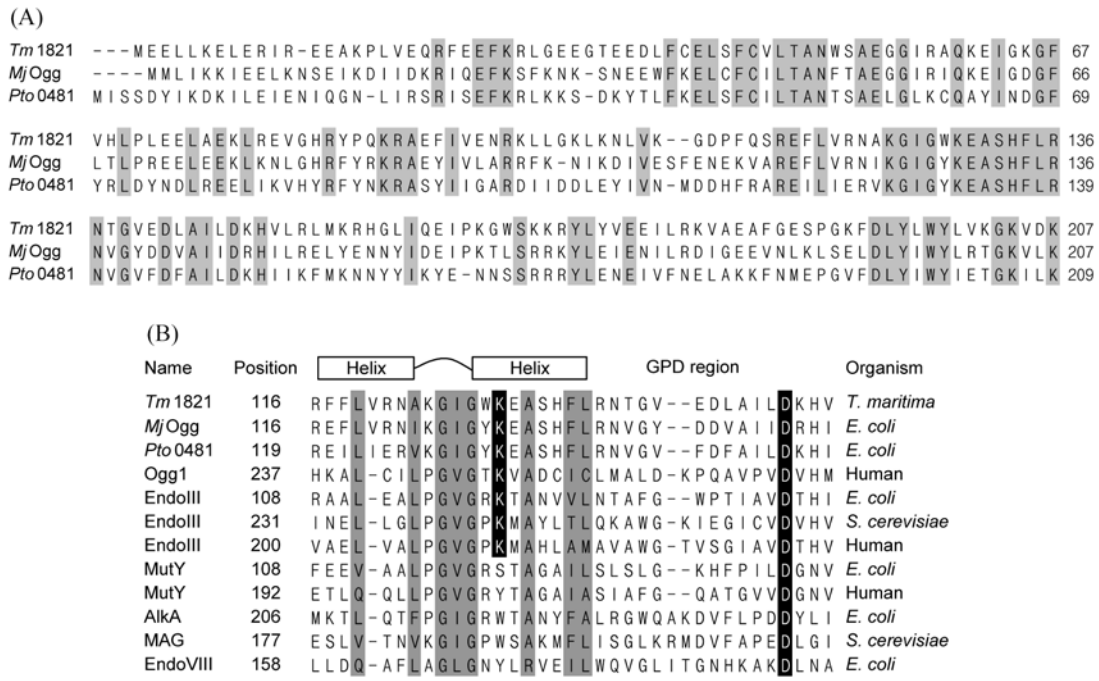

Fig. 1. Sequence alignment of *Tm*1821 and other proteins. (A) Amino acid sequence alignment of *Tm*1821 and its homologous proteins. The shadowed boxes represent conserved amino acid residues. Multiple alignments of amino acid sequences were performed using ClustalX (Heringa, 1999). (B) Alignment of the helix-hairpin-helix (HhH) motif of *Tm*1821 with those of other proteins. Hydrophobic residues important for achieving the HhH structure are shaded in gray. Black boxes represent charged residues involved in catalytic reactions. Endo III, DNA endonuclease III; Ogg, 8-oxoguanine DNA glycosylase; MutY, mutY-DNA glycosylase; AlkA, alkyl-DNA glycosylase; MAG, 3-mA-DNA glycosylase; Endo VIII, DNA endonuclease VIII.

DNA trapping assay *Tm*1821 protein was incubated with 1 pmol of radiolabeled 32-mer oligonucleotide duplexes in the presence of 20 mM Tris-Cl (pH 8.5), 1 mM EDTA, 1 mM DTT, and 50 mM $NaBH_4$ in a total volume of 10 ml. The reaction mixture was incubated at 50°C for 30 min and mixed with 2.5 μl of 5X SDS loading buffer (100 mM Tris-Cl (pH 6.8), 10% SDS, 20% glycerol, 5% 2-mercaptoethanol, and 0.2% bromophenol blue). Samples were boiled at 90°C for 5 min, and electrophoresed in 10% SDS-polyacrylamide gel. Trapping efficiencies were quantified using a BAS2000 image analyzer.

Results and Discussion

Identification and purification of *Tm*1821 ORFs of unknown function in *T. maritima* genome were searched for using BLAST at NCBI (Altschul *et al*., 1997). The amino acid sequences of hundreds of hypothetical genes of *T. maritima* were compared with known proteins. However, only those with an amino acid residue number <400 were searched for because most DNA glycosylases (including 8-oxoguanine endonuclease) have amino acid residue numbers between 200 and 400. Some of the hypothetical genes, including *Tm*1821, showed a high hit score for the endonuclease family. Among these, two ORFs (*Tm*0366, 213 amino acids and *Tm*0382, 232 amino acids) have been already annotated as endonuclease III and putative endonuclease protein, respectively. A search of the GenBank database, several *Tm*1821 homologs in other organisms, e. g., *Methanococcus jannashcii* (8-oxoguanine DNA glycosylase, *Mj*Ogg) (Gogos and Clarke, 1999) and *Picrophilus torridus* (*Pto*0481, hypothetical endonuclease III-related protein) (Futterer *et al*., 2004) (Fig. 1A). The amino acid sequence of *Tm*1821 showed 45-52% identity with these homologous proteins, and interestingly, they also possess a highly conserved helix-hairpin-helix (HhH) motif and amino acid residues (Fig. 1B), which are critical for catalysis frequently associated with the DNA glycosylase family. (Doherty *et al*., 1996; Rosenquist *et al*., 1997). Sequence-specific interaction between protein and DNA is fundamental to DNA synthesis and repair. Many of these DNA-binding proteins contain small structural motifs that utilize either α-helices or β-strands to bind to the phosphate backbone or the grooves of DNA (Mullen and Wilson, 1997; Feng *et al*., 1998). The importance of the HhH motif for DNA-binding and recognition was proposed for the endonuclease III family, which includes the DNA glycosylases (Krokan et al. 1997; Mullen and Wilson 1997). Therefore, we investigated the activities of *Tm*1821 protein in order to determine its biochemical function.

*Tm*1821 was expressed in *E. coli* BL21 (DE3) as a hexahistidine-tagged recombinant protein. The *Tm*1821 gene was amplified from genomic DNA by PCR using two oligonucleotide primers coding for the amino and carboxy

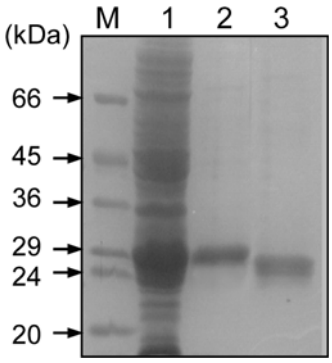

Fig. 2. Purification of *Tm*1821 protein. Lane M, molecular weight makers; lane 1, crude extract of cells harboring pET-*Tm*1821 after 4 h induction with IPTG; lane 2, protein fraction eluted from a Ni-NTA affinity column after heat-denaturation; lane 3, *Tm*1821 protein purified by gel filtration after thrombin treatment.

terminal ends of the ORF, and the PCR product obtained was cloned into an expression vector (pET28a vector), which places a hexahistidine tag at the 5'-end of the gene. The recombinant plasmid pET28a-*Tm*1821 was then introduced into *E. coli* BL21 (DE3), and cells harboring the recombinant plasmid were induced to express proteins by adding IPTG to growth medium. The recombinant protein was expressed at a high level in the soluble fraction, proteins in the soluble fraction were denatured by heating at 80°C for 30 min and removed by centrifugation, whereas the recombinant protein remained soluble and was recovered from the supernatant. The histidine-tagged *Tm*1821 recombinant protein was further purified using a Ni-NTA affinity column and eluted with imidazole. After cleavage of the N-terminus of the histidine-tagged *Tm*1821 protein with thrombin, the protein was purified by Superdex-75 gel filtration FPLC. The final product had a single detectable band at 24 kDa, which is compatible with its molecular mass as predicted by SDS-PAGE, and was >95% pure (Fig. 2).

The 8-oxoguanine endonuclease activity of *Tm*1821 protein Several oligonucleotide duplexes were used to determine the enzymatic activity of *Tm*1821 protein. Initially, we examined the DNA cleavage activity of *Tm*1821 protein using defined double-stranded oligonucleotides containing a single modified base or a mismatched base, i.e., thymine glycol, 3-methyladenine, 7-methylguanine, hypoxanthine, uracil, a reduced abasic residue, and 8-oxoguanine or A:G mismatch (Table 1). Each substrate was incubated with *Tm*1821 protein, and products were analyzed by PAGE. Although *T. maritima* has an optimum growth temperature of 80°C, these reactions were performed at 50°C to prevent thermal denaturation of the oligonucleotide duplex substrates. Since the oligonucleotide containing 8-oxoguanine was labeled on its 5'-end with [γ-^{32}P]ATP, cleaved products could be observed in the gel. Of the various substrates, *Tm*1821 protein specifically incised the

Table 1. Sequences of oligonucleotide duplexes used for DNA cleavage activity assays. The modified bases are represented by the following italic letters:- *T*, thymine glycol; *A*, 3-methyladenine; *G*, 7-methylguanine; *H*, hypoxanthine; *U*, uracil; *R*, reduced abasic residue; *O*, 8-oxoguanine

Name	Duplex oligonucleotide sequences
Duplex A:T	5'-GGATCCTCTAGAGTC**A**ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**T**TGGACGTCCGTACGTT
Duplex G:C	5'-GGATCCTCTAGAGTC**G**ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**C**TGGACGTCCGTACGTT
Duplex TG:A	5'-GGATCCTCTAGAGTC***T***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**A**TGGACGTCCGTACGTT
Duplex 3-mA:T	5'-GGATCCTCTAGAGTC***A***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**T**TGGACGTCCGTACGTT
Duplex 7-mG:C	5'-GGATCCTCTAGAGTC***G***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**C**TGGACGTCCGTACGTT
Duplex HX:T	5'-GGATCCTCTAGAGTC***H***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**T**TGGACGTCCGTACGTT
Duplex U:A	5'-GGATCCTCTAGAGTC***U***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**A**TGGACGTCCGTACGTT
Duplex A:G	5'-GGATCCTCTAGAGTC**A**ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**G**TGGACGTCCGTACGTT
Duplex rAB:C	5'-GGATCCTCTAGAGTC***R***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**C**TGGACGTCCGTACGTT
Duplex 8-oxoG:C	5'-GGATCCTCTAGAGTC***O***ACCTGCAGGCATGCAA 3'-CCTAGGAGATCTCAG**C**TGGACGTCCGTACGTT

oligonucleotide duplex containing an 8-oxoguanine (°G) (Fig. 3). Oligonucleotide duplex substrates of other DNA glycosylases, and which contained a modified base or a mismatched base, were examined as substrates for *Tm*1821 protein, but were not hydrolyzed (Fig. 3). In order to eliminate the possibility that the observed 8-oxoguanine endonuclease activity was due to contamination by endogenous *E. coli* protein, a purified sample was heated at 80°C for 30 min. Since *Tm*1821 protein is a thermostable protein, no significant loss of *Tm*1821 8-oxoguanine endonuclease activity was observed after heating (data not shown). *Mj*Ogg from *M. jannashcii*, which is a hyperthermophilic archaeon, also shows high activity at high temperature (Gogos and Clarke, 1999). *T. maritima*, which grows optimally at 80°C, belongs to the hyperthermophilic bacterium and was originally isolated from geothermal heated marine sediment (Nelson *et al*., 1999).

Substrate specificity of *Tm*1821 To investigate the specificity of its 8-oxoguanine excision, *Tm*1821 was examined using DNA duplexes containing an 8-oxoguanine residue opposite each of the four common bases (A, C, G, or T). DNA cleavage reactions by *Tm*1821 using 1 pmol of substrate were performed at 50°C. *Tm*1821 recognized 8-oxoguanine paired with C most efficiently followed by T, A, and G in descending order (Fig.

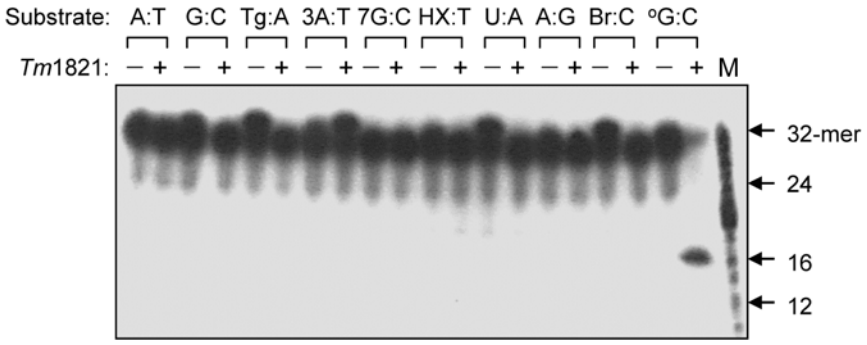

Fig. 3. The 8-oxoguanine endonuclease activity of *Tm*1821 protein. DNA cleavage activity assays of *Tm*1821 were performed using various oligonucleotide duplexes containing modified or mismatched bases. Reactions were performed in 40ml mixtures containing 10ng of *Tm*1821 protein at 50°C. Reaction samples were separated on 15% polyacrylamide gel. Tg, thymine glycol; 3A, 3-methyladenine; 7G, 7-methylguanine; HX, hypoxanthine; U, uracil; Br, 8-bromoguanine; °G, 8-oxoguanine; M, oligonucleotide size markers.

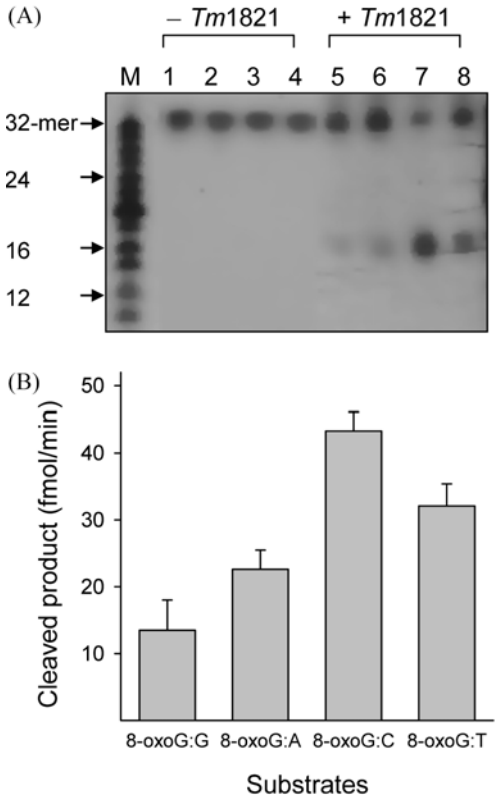

Fig. 4. Substrate specificity of *Tm*1821 for oligonucleotide duplexes containing the 8-oxoguanine residue. Reactions were performed in 40 ml mixtures with 10 ng of *Tm*1821 protein at 50°C. Post-reaction samples were separated on 15% polyacrylamide gel. The figure is representative of at least three independent experiments (A). Results were obtained using a BAS2000 image analyzer and the graph shows activity levels (B). Lane M, oligonucleotide size markers; lane 1 and 5, 8-oxoguanine:G pair; lane 2 and 6, 8-oxoguanine:A pair; lane 3 and 7, 8-oxoguanine:C pair; lane 4 and 8, 8-oxoguanine:T pair.

4). This opposite base-dependent efficacy of 8-oxoguanine excision by *Tm*1821 resembles that of the thermophilic 8-oxoguanine DNA glycosylase from *M. jannaschii* UDG (*Mj*Ogg) (Gogos and Clarke, 1999). Human 8-oxoguanine DNA glycosylase also strongly prefers substrate DNA duplexes containing an 8-oxoguanine:C pair (Hazra *et al*., 1998). Moreover, 8-oxoguanines paired with T, G, or C were found to be good substrates for *E. coli* Fpg, whereas 8-oxoguanine:A was poorly recognized (Boiteux *et al*., 1990). The amino acid sequence of *Tm*1821 showed only 19.3% homology with that of *E. coli* Fpg (data not shown); moreover, *Tm*1821 lacks the N-terminal proline residue (Pro2) that is responsible for the glycosylase/AP lyase activity of Fpg (Zharkov *et al*., 1997). This N-terminal Pro2 participates in the enzymatic activities of Fpg protein, and Fpg mutants containing Pro2 substitutions barely possess 8-oxoguanine DNA glycosylase activity, nor did they cleave DNA-containing a preformed AP site, though they readily produced Schiff base complex from a 8-oxoguanine-containing substrate (Sidorkina and Laval, 2000). Thus, we believe that the reaction mechanism of *Tm*1821 differs from that of *E. coli* Fpg.

To further examine the mode of action of *Tm*1821 protein, we performed DNA trapping assays using $NaBH_4$. DNA glycosylases with associated AP lyase activity, such as, human 8-oxoguanine DNA glycosylase and *E. coli* Fpg, form a transient Schiff base (imine intermediates) during reactions with substrates containing 8-oxoguanine, and these can be trapped by $NaBH_4$ to generate covalent protein-DNA complexes (Nash *et al*., 1996) that can be detected by SDS-PAGE. Thus, DNA substrates containing 8-oxoguanine were incubated with *Tm*1821 protein in the presence of $NaBH_4$, and the complexes were analyzed by SDS-PAGE. These DNA trapping assays performed with *Tm*1821 protein showed its distinct preference for C opposite 8-oxoguanine followed by; T > A > G (Fig. 5A). These results confirm the DNA cleavage assay data mentioned above.

DNA glycosylases are classified as mono- or bi-functional based on their *N*-glycosylic cleavage abilities and their abilities to cleave apurinic/apyrimidinic (AP) sites (Dizdaroglu, 2003). *Tm*1821 protein also cleaved an oligonucleotide duplex with an AP site:C pair (Fig. 5B). The action mechanism of bifunctional DNA glycosylases may involve nucleophile

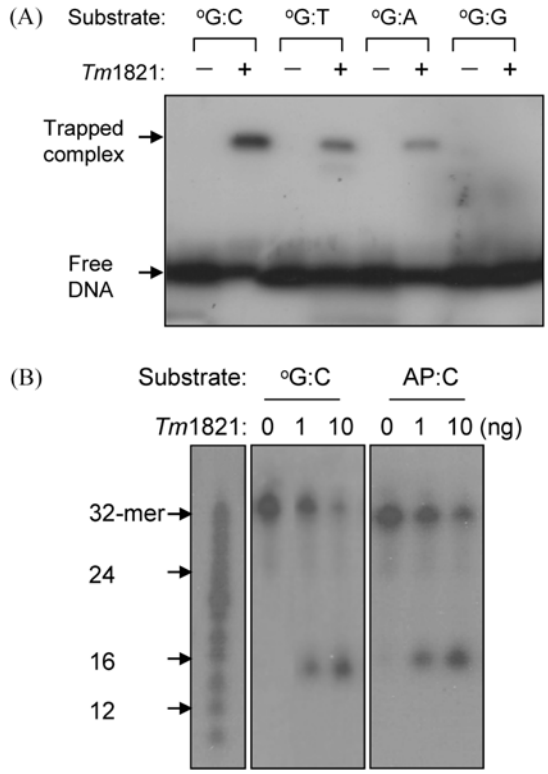

Fig. 5. DNA trapping and AP lyase activity assays. (A) *Tm*1821 protein was incubated with 1 pmol of 32-mer oligonucleotide duplexes containing 8-oxoguanine in the presence 50 mM $NaBH_4$. Reaction mixtures were incubated at 50°C for 30 min. Protein-DNA complexes were analyzed by using 10% SDS-PAGE and a BAS2000 image analyzer. (B) Reactions were performed at 50°C in 40 ml mixtures containing 1 or 10 ng of *Tm*1821 protein. Reaction samples were separated in 15% polyacrylamide gel. °G:C, 32-mer oligonucleotide duplex containing 8-oxoguanine:C pair; AP:C, 32-mer oligonucleotide duplex containing AP site:C pair.

attack and deprotonation of a lysine amino group by the conserved amino acids, lysine and aspartate, respectively (Nash *et al*., 1996). Because *Tm*1821 possesses two conserved amino acid residues (lysine and aspartate) its HhH-GPD motif (Fig. 1B), it may be a bifunctional DNA glycosylase.

The *T. maritima* genome shows a degree of similarity with the archaea in terms of its gene content and genome organization (Nelson *et al*., 1999). Actually, as we have shown, the amino acid sequence of *Tm*1821 exhibits high sequence identity with that of *Mj*Ogg from *M. jannaschii*, a hyperthermophic archaeon. Their substrate specificities are also similar. Thus, the catalytic mechanism of DNA repair proteins (including *Tm*1821) in *T. maritima* resembles that of the archaeal proteins. The structure determination of this repair protein with an oligonucleotide duplex substrate should help to clarify the unique recognition mechanism used by these thermophilic eubacteria and archaea to repair DNA.

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