The AAG family of 3-methyladenine DNA glycosylases was initially thought to be limited to mammalian cells, but genome sequencing efforts have revealed the presence of homologous proteins in certain prokaryotic species as well. Here, we report the first molecular characterization of a functional prokaryotic AAG homologue, i.e. YxylJ, termed bAag, from Bacillus subtilis. The B. subtilis aag gene was expressed in Escherichia coli, and the protein was purified to homogeneity. As expected, B. subtilis Aag was found to be a DNA glycosylase, which releases 3-alkylated purines and hypoxanthine, as well as the cyclic etheno adduct 1,N6-ethenoadenosine from DNA. However, kinetic analysis showed that bAag removed hypoxanthine much faster than human AAG with a 10-fold higher value for \( k_{cat} \) compared to human AAG. Nevertheless, bAag moved hypoxanthine much faster than human AAG with a 10-fold higher value for \( k_{cat} \) compared to human AAG. However, its preference for different modified residues is significantly different from that of the human enzyme, and it recognizes none of these types of enzymes but another, structurally different protein termed AAG/ANPG/MPG (the term AAG will be used here) (12). Mammalian AAG recognizes and removes a broad range of damaged base residues, including deaminated adenine (hypoxanthine), cyclic etheno adducts, e.g. 1,N6-ethenoadenosine, and a variety of different alkylated base residues, including 3mA, 3mG, and 7-methylguanine (7mG) (8, 12–19).

Through genome sequencing, open reading frames with homology to the mammalian AAG enzymes have also been identified in prokaryotic species, for instance in Bacillus subtilis, Mycobacterium tuberculosis, and Borrelia burgdorferi. B. subtilis also possesses two AlkA homologues and a third DNA glycosylase protein with close similarity to AlkC/YbaZ, a recently identified repair gene in Bacillus cereus. The high number of 3mA DNA glycosylases in Bacillus species probably reflects the heavy exposure of earth bacteria to environmental alkylating agents like methyl chloride. E. coli possesses an inducible repair response to changes in environmental exposure, termed the adaptive response (20, 21). The Ada protein, which is a methyltransferase that becomes activated upon methylation exposure, regulates the adaptive response in E. coli and induces transcription of AlkA and other repair proteins involved in protection against alkylated damage. A similar response system is also shown to be present in B. subtilis (22). The fact that AAG enzymes have been conserved in some but not all bacteria raises questions about the possible functional significance of this type of enzyme in prokaryotic cells. To elucidate the role of AAG-like proteins in prokaryotes, we have expressed the AAG homologue of B. subtilis, YxylJ, in E. coli, and characterized its enzymatic activities. We have shown that bAag indeed is a functional DNA glycosylase, excising many of the same modified base residues as its human counterpart. However, its preference for different modified residues is significantly different from that of the human enzyme, and it

---

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† A Fellow of the Research Council of Norway.
§ Supported by grants from The Research Council of Norway, the Norwegian Cancer Society, and the Simon Fougnier Hartmanns Famielend.
¶ To whom correspondence should be addressed. Tel.: 47-23074060; Fax: 47-23074061; E-mail: magnar.bjoras@labmed.uio.no.

The abbreviations used are: 3mA, 3-methyladenine; 3mG, 3-methylguanine; 7mG, 7-methylguanine; bAag, B. subtilis Aag; MMS, methyl methanesulfonate; MES, 4-morpholinethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; HPLC, high pressure liquid chromatography.

1. Ali, T., Lindback, K. I., Kristian, L., Lillegård, O., Økstad, M., Bjørås, E., Seeberg, and A. B. Kolsto, submitted for publication.
B. subtilis Homologue of the Mammalian AAG DNA Glycosylase

seems to have a more important role in the removal of pre-mutagenic residues induced by denaturation and lipid peroxidation rather than for cytotoxic residues induced by alkylating agents.

**MATERIALS AND METHODS**

**Bacterial Strains and Plasmids**—DNA from the B. subtilis strain 186 was used for the cloning of the aag gene. E. coli strain BK2118 (tag alkA) was used in survival assays on methyl methanesulfonate (MMS)-containing plates and for expression of Bacillus aag, whereas ER2566 (New England Biolabs) and BL21 codon plus (Stratagene) were used for expression only. E. coli expression vectors included pUC18 (New England Biolabs), pUC19 (New England Biolabs), pT7SSI (USB), and pQE30 (Qiagen).

**Expression of B. subtilis DNA**—10 ml of overnight culture was washed twice in sterile water, and the cells were resuspended in 0.2 ml of 2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.5, 1 mM EDTA, 0.2 ml of phenol/chloroform/isoamyl alcohol (24:24:1), and 0.3 g of glass beads (Sigma). The suspension was vortexed three times for 1 min and cooled on ice in between to break the cell wall. The DNA was precipitated with 96% ethanol, incubated for 5 min at 37 °C with RNase A and precipitated with ethanol.

**Cloning of B. subtilis aag**—Genomic B. subtilis DNA was used as a template in a PCR reaction to amplify a DNA fragment containing the B. subtilis aag gene, termed bAag. The forward primer 5′-CTCGATATCGTCTCCG-3′ (PstI restriction site underlined) was located 45 nucleotides upstream of the putative GCC start codon, and the reverse primer 5′-GGATCCTAACAGGGCGCG-3′ (BamHI restriction site underlined) was located 55 nucleotides downstream of the putative stop codon. The PCR product was cloned into the PstI and BamHI restriction sites of pUC18, pUC19, pQE30, and pT7SSI.

**Alkylation Survival of BK2118 (tag alkA) Transformed with B. subtilis aag**—Exponentially growing BK2118 transformed with pUC19tagAag and pUC19 (control), were harvested, washed in PBS, and incubated for 30 min on ice, and appropriate dilutions were spread on plates containing 100 μg/ml ampicillin and 6, 0.1, 0.5, and 1 mM MMS. Plates were incubated at 37 °C for 2 days, and surviving colonies were counted.

**Expression and Purification of B. subtilis and Human AAG Protein**—Various B. subtilis aag constructs were used for expression analysis in BK2118 (pUC19, pT7SSI, and pQE30), ER2566 (pT7SSI), and BL21-RIL codon plus (pT7) at different temperatures (37, 22, and 16 °C), and the highest expression levels were obtained using BK2118/pUC19 growing at 37 °C. 2 liters of cell culture from freshly transformed colonies were grown in LB medium with 100 μg/ml ampicillin to A600 of 1 and induced by 0.5 μM isopropyl-1-thio-β-D-galactopyranoside for 2 h. Cells were resuspended in 50 mM Tris, pH 7, 50 mM NaCl, and 10 mM β-mercaptoethanol and sonicated three times for 30 s. (Vibra Cell Sonicator, Sonics and Materials Inc.) Extracts were diluted with volumes of 50 mM Tris, pH 7, and 10 mM mercaptoethanol and loaded onto a phosphocellulose column (10 ml) equilibrated with buffer A (50 mM Tris, pH 7, 50 mM NaCl, and 10 mM mercaptoethanol). Active fractions were step-eluted by buffer B (50 mM Tris, pH 7, 1 mM NaCl, and 10 mM mercaptoethanol), and alkyl base DNA glycosylase activity was used to monitor bAag purification. Because of the BK2118 tag alkA phenotype, the extracts have no background 3mA DNA glycosylase activity. Active fractions were pooled, dialyzed against buffer C (50 mM MES, pH 6, 50 mM NaCl, and 10 mM mercaptoethanol) and applied to a MonoS column (HR 5/5, Amersham Biosciences). The column was eluted with a linear gradient of 50–500 mM NaCl in buffer C, and fractions were tested for glycosylase activity and analyzed by denaturing SDS-PAGE. Human AAG was purified as described previously (14).

**Alkylbase DNA Glycosylase Assay**—10,000–40,000 dpm (glycosylase assay and HPLC, respectively) of calf thymus DNA alkylated with 3H-6-ethenoG (20 Ci/mmol; New England Nuclear) and 3H-6-hydroxyG (20 Ci/mmol; New England Nuclear) were incubated with different amounts of cell extracts or purified protein for 30 min at 37 °C, as described previously (37). The DNA was precipitated with ethanol, and radioactivity in the supernatant was measured in a liquid scintillation analyzer (Tri-Carb 2900TR, Packard).

**HPLC Analyses of Alkylated Bases**—Reverse phase HPLC of methylated bases released by the purified glycosylases (B. subtilis and human AAG) was performed as follows (5). The supernatant was centrifuged through Millipore Ultrafuge MC filters (10,000 NMWC filter unit) and made up to 1 ml with alkylated bases and TBE markers. Separations were performed by HPLC on a Bio-Bac Bio-Tang (5 μm, 250 × 4.6 mm) column using a linear gradient of 0–100% (v/v) of 0.1 m triethyl ammonium acetate buffer, pH 7.3 or pH 5.4, in methanol for elution (1 ml/min.). Fractions of 0.5 ml were collected, and the radioactivity was measured in a liquid scintillation analyzer (Tri-Carb 2900TR, Packard).

**Assays for Enzyme Cleavage of Hypoxanthine, 1,N4-Ethenoadenine, 8-Oxoguanine, and Tetrahydrofuran (THF)**—Assays were performed with duplex DNA fragments containing hypoxanthine at position 14 (5′-GCTCATGCGCAG[hypoxanthine]CGCCGCTACTG-3′), 1,N4-ethenoA at position 15 (5′-GGATACGGCGG[1,N4-ethenoA]GGCCTA-CTGC-3′), 8-oxoguanine (8oxoG) at position 13 (5′-GGCGGATGACC-C[8-oxoG][GAGGCCCATC-C]-3′) and tetrahydrofuran (THF) at position 20 (5′-GCTGGTAGATCCGTGCG[THF]TTAAGCCACCTGCTAC-3′). Different amounts of B. subtilis Aag or human AAG and 80 fmol of 3P-15Fabeled duplex nucleotides in reaction buffer (70 mM MOPS, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, and 5% glycerol) were incubated for 30 min at 37 °C. The reaction was stopped by boiling. Products were analyzed by HPLC (Waters 600 pump, Waters 990 photodiode array detector) and Signalizer software (PerkinElmer) or on an Agilent 1100 capillary electrophoresis instrument (Agilent Technologies) using a 36-cm-long polyacrylamide gel (3% acrylamide; 4.5% bisacrylamide; Agilent Technologies). A mixture of calf thymus DNA (1.5 Ci/mmol; American Radiolabeled Chemicals) and 3H-methyl-1,N4-etheno-A was used to monitor bAag purification. Because of the BK2118 tag alkA phenotype, the extracts have no background 3mA DNA glycosylase activity. The cleavage products were visualized with a FluorImager (Amerham Biosciences model 4455)

The kinetic constants, kcat and km, were determined by incubating the respective proteins with increasing concentrations of oligo-nucleotides containing a single hypoxanthine or 1,N4-ethenoadenine. The initial velocities were calculated by LineweaverBurk plots.

**DNA Sequencing and Sequence Analysis**—Sequence analysis was performed using the GeneTing/Tang program (GeneTing, Lillestrøm, Norway). Homology searches were performed by the blast algorithm (30). Protein domains were searched on a 20% sequence similarity level (NCBI), and alignment analysis was done with the ClustalW program from Pole Bio-Infotmatique, Lorraine, France.

GenBank™ identification (gi) numbers for AAG homologues are as follows: B. subtilis, gi 3912954; Staphylococcus aureus, gi15925334; Borrelia burgdorferi, gi 15594767; Chlamydia pneumoniae, gi15818416; Mycobacterium tuberculosis, gi15698828; Arabidopsis thaliana, gi15229928, Mus musculus, gi 7106361; and Homo sapiens, gi12084553.

**RESULTS**

An Open Reading Frame in Bacillus subtilis That Encodes a Protein with Homology to the Mammalian 3-Methyladenine DNA Glycosylase—Mammalian 3mA DNA glycosylases have been characterized from mouse, man, and rat (13–15, 18, 23, 24) and are different in structure from those found in E. coli and most other bacteria. However certain bacterial species appear to possess protein homologues of the mammalian enzymes as judged from searches in the completed genome sequences. An alignment of putative AAG sequences from B. subtilis, M. tuberculosis, and B. burgdorferi shows 33, 39, and 37% identity to human AAG, respectively (Fig. 1). The yxJ gene of B. subtilis, here renamed aag, translates into a polypeptide of 196 amino acids (GenBank™ identification number gi 3912954). Compared with the human AAG, this putative B. subtilis protein contains all of the key residues involved in substrate recognition and catalysis, except for Tyr-162 and Tyr-165 which are replaced by His.

Cloning and Expression of B. subtilis aag in E. coli—To characterize bAag, the coding region, including its promoter sequence, was cloned in the E. coli expression vector pUC19 (Fig. 2B). There are two putative start codons (ATG and GTG) located within the open reading frame, but upstream Shine-Dalgarno and promoter-like sequences makes the second putative start site, GTG, the most probable initiation codon (Fig. 2B). With this vector, bAag was expressed and purified to apparent physical homogeneity by a two-step procedure including phosphocellulose and MonoS chromatography (see “Materials and Methods”). The double mutant strain BK2118 (tag alkA) was used for the expression to avoid interference by possible contamination of endogenous 3mA DNA glycosylase activities during purification and characterization. The purification was monitored by standard assays for 3mA DNA glycosylase activity, and the final preparation was analyzed by SDS-PAGE (data not shown).
Inefficient Excision of Methylated Bases by B. subtilis Aag—Mammalian AAG has been shown to remove various methylated bases, including 3mA, 3mG, and 7mG (13, 15, 24). We examined the ability of the purified bAag to remove alkylated bases from calf thymus DNA treated with [3H]methyl-N-nitrosourea (Fig. 3). The amounts of methylpurines present in such DNA were determined by HPLC to be 65% 7mG, 10% 3mA, and 0.7% 3mG (5). The relative activities for release of the different alkylated base residues were analyzed by treating methylated DNA with different amounts of enzyme and quantifying the radiolabeled excision products after separation by HPLC. Enzyme activity measurements were performed with purified human AAG as well as with the B. subtilis enzyme to compare efficiency and specificity of base excision by the two enzymes (Fig. 3). It appears that the rate of removal of 3mA and 3mG by bAag for the same amount of enzyme proceeds /H110111020 times more slowly than for human AAG, whereas the activity toward 7mG is /H110211021% . The difference between human and Bacillus Aag in the efficiency of repair of alkylated bases indicates that bAag may not be the major activity in B. subtilis for removal of alkylated base residues.

Efficient Removal of Deaminated Adenine and 1,N6-ethenoadenine—Mammalian AAG has been reported to be active against a wide variety of premutagenic lesions, such as hypoxanthine (25), cyclic etheno adducts, e.g. 1,N6-ethenoadenine and N2-ethenoguanine (26), and 8-oxoguanine (27). The activity of the bAag to remove these lesions was analyzed on oligonucleotides with a single lesion. bAag cleaved 1,N6-ethenoadenine with an efficiency similar to that of the human AAG, whereas hypoxanthine excision was much more efficient with bAag than with the human enzyme (Fig. 4). Further experiments were performed to compare the kinetics of hypoxanthine or 1,N6-ethenoadenine removal by B. subtilis and human AAG. There was no difference in the Km and kcat values observed between

Fig. 1. Multiple alignment of bacterial and eukaryotic AAG proteins. Bacterial sequences are from A. thaliana (gi 15229928), M. musculus (gi 7106361) and man (H. sapiens; gi 12984553). Identical residues in all members of the group are highlighted in black, strongly similar in dark gray, weakly similar in light gray. The residues of human AAG essential in catalytic activity and “flipping” (12), are indicated with bold or bold with underlining, respectively. The ClustalW sequence analysis program from Pole Bio-Informatique was used to align the sequences.
AAG and bAag for excision of 1,6-ethenoadenine (Table I). In contrast, bAag showed a 10-fold higher $k_{\text{cat}}$ value for hypoxanthine removal than did human AAG, whereas the $K_m$ values were found to be similar. This indicates that the difference in repair of hypoxanthine could be attributed to an increased turnover capacity of bAag compared with human AAG. No activity toward δ-oxoG, formamidopyrimidine (faPy), or abasic sites was found to be associated with bAag (data not shown).

**B. subtilis Aag Can Only Partially Complement the Alkyla-**

**DISCUSSION**

We have characterized the product of the *yxlJ* gene in *B. subtilis*, a homologue of the 3-mA DNA glycosylases found in mammalian cells. Like the mammalian enzymes, bAag is a DNA glycosylase removing several different types of damaged base residues from DNA. To our knowledge, this appears to be the first characterization of a prokaryotic enzyme of this type. AAG homologues are also found in several other (among them pathogenic) bacterial species. Surprisingly, HPLC analysis revealed that removal of the cytotoxic lesions 3mA and 3mG is 10–15 times less efficient for bAag than for human AAG. In contrast, bAag is 10-fold more efficient than human AAG in the excision of the premutagenic adenine deamination product, hypoxanthine. The two enzymes are similar in their affinity toward the cyclic etheno adduct 1,6-ethenoadenine. It thus appears that the primary function of the *B. subtilis* AAG enzyme would be to avoid mutagenesis and repair DNA containing deaminated adenine and cyclic etheno adducts rather than to prevent the cytotoxic effects of methylated base residues. This agrees with the presence in *B. subtilis* of several other 3mA DNA glycosylases like, for example, two AlkA homologues...

![Fig. 4. Cleavage of hypoxanthine and 1,6-ethenoadenine-containing DNA by B. subtilis and human AAG. 40 fmol of duplex 25-mer oligodeoxyribonucleotide containing a single hypoxanthine (HX:T) or 1,6-ethenoadenine (eA) at position 12 was incubated for 30 min at 37 °C with 3 and 33 fmol of *B. subtilis* Aag or human AAG. The reaction mixture was heat-inactivated for 30 min at 50 °C before 10 ng of *E. coli* N6 enzyme was added to perform cleavage after removal of the damaged base. Cleavage products were separated by 20% denaturing PAGE and visualized by phosphorimaging.](http://www.jbc.org/Downloaded from...)

**TABLE I**

| Enzyme  | Ethenoadenine | Inosine   |
|---------|---------------|-----------|
|         | $K_m$ (nM) | $k_{\text{cat}}$ (min$^{-1}$) | $K_m$ (nM) | $k_{\text{cat}}$ (min$^{-1}$) |
| bAag    | 21 ± 3 | 0.10 ± 0.06 | 5.0 ± 1 | 0.053 ± 0.040 |
| Aag     | 23 ± 3 | 0.13 ± 0.06 | 5.3 ± 0.6 | 0.005 ± 0.002 |

**Fig. 4.** Cleavage of hypoxanthine and 1,6-ethenoadenine-containing DNA by *B. subtilis* and human AAG. 40 fmol of duplex 25-mer oligodeoxyribonucleotide containing a single hypoxanthine (HX:T) or 1,6-ethenoadenine (eA) at position 12 was incubated for 30 min at 37 °C with 3 and 33 fmol of *B. subtilis* Aag or human AAG. The reaction mixture was heat-inactivated for 30 min at 50 °C before 10 ng of *E. coli* N6 enzyme was added to perform cleavage after removal of the damaged base. Cleavage products were separated by 20% denaturing PAGE and visualized by phosphorimaging.
Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.

Etheno bridged exocyclic DNA adducts, e.g. 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytidine, can arise spontaneously in the cell and will also be induced by mutagens that induce lipid peroxidation or directly by other mutagens such as vinyl chloride and urethane (31). Because AAG appears to be the major enzyme in the removal of hypoxanthine than the mammalian AAG and, consequently, the structurally unrelated bAag family of proteins also. Interestingly, the genome sequence of B. subtilis also reveals the presence of a gene that translates into a protein with homology to Nfi. Studies of this gene function are required to elucidate whether this represents an alternative mechanism for repair of hypoxanthine in B. subtilis.
28. Yao, M., Hatahet, Z., Melamede, R. J., and Kow, Y. W. (1994) J. Biol. Chem. 269, 16260–16268
29. Gao, G. M., and Weiss, B. (1998) J. Bacteriol. 180, 46–51
30. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
31. Nair, J., Barbin, A., Guichard, Y., and Bartsch, H. (1995) Carcinogenesis 16, 613–617
32. Lau, A. Y., Scharer, O. D., Samson, L., Verdine, G. L., and Ellenberger, T. (1998) Cell 95, 249–258
33. Winterling, K. W., Levine, A. S., Yashin, R. E., and Woodgate, R. (1997) J. Bacteriol. 179, 1698–1703
34. Winterling, K. W., Chafin, D., Hayes, J. J., Sun, J., Levine, A. S., Yashin, R. E., and Woodgate, R. (1998) J. Bacteriol. 180, 2201–2211
35. Falnes, P. O., Johansen, R. F., and Seeberg, E. (2002) Nature 419, 178–182
36. Trewick, S. C., Henschaw, T. F., Hausinger, R. P., Lindahl, T., and Sedgwick, B. (2002) Nature 419, 174–178
37. Bjelland, S., and Seeberg, E. (1987) Nucleic Acids Res. 15, 2787–2801
The *Bacillus subtilis* Counterpart of the Mammalian 3-Methyladenine DNA Glycosylase Has Hypoxanthine and 1,N\(^6\)-Ethenoadenine as Preferred Substrates
Randi M. Aamodt, Pål Ø. Falnes, Rune F. Johansen, Erling Seeberg and Magnar Bjørås

*J. Biol. Chem.* 2004, 279:13601-13606.
doi: 10.1074/jbc.M314277200 originally published online January 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M314277200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 13 of which can be accessed free at http://www.jbc.org/content/279/14/13601.full.html#ref-list-1