Acta Biochimica Polonica

Vol. 52 No. 1/2005

167–178

QUARTERLY

Inhibition of DNA repair glycosylases by base analogs and tryptophan pyrolysate, Trp-P-1✪

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Received: 17 January, 2005; accepted: 28 February, 2005

Key words: DNA repair enzymes, formamidopyrimidine DNA glycosylase, inhibitors, base analogs, Trp-P-1

DNA base analogs, 2,4,5,6-substituted pyrimidines and 2,6-substituted purines were tested as potential inhibitors of *E. coli* Fpg protein (formamidopyrimidine -DNA glycosylase). Three of the seventeen compounds tested revealed inhibitory properties. 2-Thioxanthine was the most efficient, inhibiting 50% of 2,6-diamino-4-hydroxy-5*N*-methyl-formamidopyrimidine (Fapy-7MeG) excision activity at 17.1 μM concentration. The measured K_i was 4.44 ± 0.15 μM. Inhibition was observed only when the Fpg protein was first challenged to its substrate followed by the addition of the base analog, suggesting uncompetitive (catalytic) inhibition. For two other com-

✪This work was conducted within the activity of the Center of Excellence in Molecular Biotechnology, Institute of Biochemistry and Biophysics, PAS (WP10) and was supported (Z.K.) by the Foundation for the Development of Diagnostic and Therapy, Warsaw, Poland

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Abbreviations: AP, apurinic/apyrimidinic; dRPase, deoxyribose 5-phosphatase; 7EtG, 7-ethylguanine; εA, 1,N^6-ethenoadenine; FapyA, 4,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Fapy-7MeG, 2,6-diamino-4-hydroxy-5*N*-methyl-formamidopyrimidine; 3MeA, 3-methyladenine; 7MeG, 7-methylguanine; MNU, methylnitrosourea; [^{3}H]MNU-DNA, DNA alkylated with [^{3}H]methylnitrosourea; 8-oxoG, 8-oxoguanine; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole.

pounds, 2-thio- or 2-oxo-4,5,6-substituted pyrimidines, IC_{50} was only 343.3 ± 58.6 and 350 ± 24.4 μM, respectively. No change of the Fpg glycosylase activity was detected in the presence of Fapy-7MeG, up to 5 mM.

We also investigated the effect of DNA structure modified by tryptophan pyrolysate (Trp-P-1) on the activity of base excision repair enzymes: *Escherichia coli* and human DNA glycosylases of oxidized (Fpg, Nth) and alkylated bases (TagA, AlkA, and ANPG), and for bacterial AP endonuclease (Xth protein). Trp-P-1, which changes the secondary DNA structure into non-B, non-Z most efficiently inhibited excision of alkylated bases by the AlkA glycosylase (IC_{50} = 1 μM). The ANPG, TagA, and Fpg proteins were also inhibited although to a lesser extent (IC_{50} = 76.5 μM, 96 μM, and 187.5 μM, respectively). Trp-P-1 also inhibited incision of DNA at abasic sites by the β-lyase activity of the Fpg and Nth proteins, and to a lesser extent by the Xth AP endonuclease. Thus, DNA conformation is critical for excision of damaged bases and incision of abasic sites by DNA repair enzymes.

Inhibitors of DNA repair enzymes may be a useful tool for the potentiation of cancer chemotherapy, but also may be used in mechanistic studies on DNA damage recognition. The search for the inhibitors of repair enzymes among DNA base analogs resulted in finding O^6-benzylguanine as a potent inhibitor of O^6-alkylguanine DNA-alkyltransferase, the compound which enhances the toxicity of anticancer alkylating chemotherapeutics (Dolan & Pegg, 1997), and currently is in clinical trials (Schold *et al.*, 2004). Our previous studies on the recognition of alkylated bases have shown that 3-ethyl-, 3-propyl-, 3-butyl-, and 3-benzyladenine inhibit *Escherichia coli* alkylpurine DNA glycosylase I, the TagA protein, but not the *E. coli* alkylpurine DNA glycosylase II, AlkA, nor its human homolog, ANPG. A more polar derivative, 3-hydroxyethyladenine did not affect TagA, suggesting that hydrophobic interactions are involved in the mechanism of inhibition and/or recognition and excision of alkylated purines by the TagA protein (Tudek *et al.*, 1998). This conclusion was further confirmed after resolution of the enzyme's crystal structure, which showed that the TagA glycosylase possesses a unique, deep, aromatic pocket, binding 3MeA (Drohat *et al.*, 2002), which in other repair glycosylases of alkylated bases is much wider and less restricted to hydrophobic interactions.

The rationale of this study was to search for base analogs inhibiting the Fpg protein (formamidopyrimidine-DNA glycosylase) from *E. coli* because a number of DNA lesions induced by the drugs used in cancer chemotherapy (e.g. thiotepa) are substrates for the Fpg protein (Cussac & Laval, 1996; Gros *et al.*, 2002), and structural homologues of the Fpg protein have recently been found in mammalian cells (Hazra *et al.*, 2002a; 2002b; Das *et al.*, 2004). We investigated the inhibition of the Fpg protein by 2,4,5,6-substituted pyrimidines and 2,6-substituted purines. We also investigated the effect of secondary DNA structure on the effectiveness of DNA processing by the Fpg and other DNA glycosylases of oxidized pyrimidines (Nth) and alkylated bases (bacterial TagA, AlkA, and human ANPG proteins) (reviewed in Gros *et al.*, 2002) as well as bacterial AP endonuclease (Xth). For this purpose we used tryptophan pyrolysate, Trp-P-1, which was shown to change the secondary DNA structure into non-B, non-Z (Inohara *et al.*, 1995).

The Fpg protein is an *E. coli* DNA glycosylase/AP lyase, which removes from DNA a broad spectrum of oxidized and alkylated bases: 8-oxoguanine, unsubstituted and substituted imidazole ring-opened purines introduced into DNA by hydroxyl radicals (e.g. FapyG, FapyA; Boiteux *et al.*, 1992; Jurado *et al.*, 1998) as well as by chemical carcinogens, including anticancer drugs (e.g. Fapy-7MeG, Fapy-7EtG, Fapy-7aminoethylG; Tudek *et al.*, 1998; Cussac & Laval, 1996). Fpg has two additional activities: (i) it cleaves DNA at abasic (AP) sites by β-δ-elimination (Bailly *et al.*, 1989); and (ii) displays a dRPase activity (Graves *et al.*, 1992) which removes the 5′ ter-

minal deoxyribose phosphate from DNA incised by an AP endonuclease. The Fpg protein contacts six bases in the repaired strand and one base opposite the lesion in the complementary strand. The enzyme does not recognize lesions paired with adenine (Tchou *et al.*, 1991; Speina *et al.*, 2001) or lesions in Z-DNA (Lagravère *et al.*, 1984). In mammalian cells the Fpg protein has two structural/functional homologues – NEIL1 and NEIL2, which similarly to the Fpg protein use the N-terminal Pro as the active site, and utilize two DNA binding motifs, a helix-two-turn-helix and a zinc finger (Das *et al.*, 2004). Both enzymes excise oxidized pyrimidines from DNA, but NEIL1 additionally removes FapyG and FapyA (Hazra *et al.*, 2002b).

In *E. coli* the major glycosylase/AP lyase excising oxidized pyrimidines from DNA and, to a lesser extent, some Fapy lesions is the Nth protein (Dizdaroglu *et al.*, 1993; 2000). The enzyme has an iron-sulfur cluster, a helix-turn-helix motif for DNA binding, and a deep cleft between two domains for substrate binding (Kuo *et al.*, 1992a, 1992b). The enzyme cleaves AP-sites by β-elimination mechanism (reviewed by Gros *et al.*, 2002).

DNA glycosylases of alkylated bases are monofunctional enzymes which remove alkylated bases, but do not cleave DNA at AP-sites. *E. coli* has two such enzymes: constitutive TagA protein, which specifically excises 3MeA and 3MeG from DNA (Thomas *et al.*, 1982), and inducible AlkA glycosylase, which also recognizes 3MeG, 7MeG (Laval *et al.*, 1981), 7EtG (Tudek *et al.*, 1998) as well as hypoxanthine and 1,N^6-ethenoadenine (Saparbaev & Laval, 1994; Saparbaev *et al.*, 1995). In eukaryotic cells, only one alkylpurine-DNA glycosylase (in humans – ANPG protein) has been found with the substrate specificity, but not structure, resembling that of the *E. coli* AlkA protein (O'Connor & Laval, 1991; Saparbaev & Laval, 1994; Saparbaev *et al.*, 1995).

After the altered base is removed by DNA glycosylase, AP endonuclease cleaves the phosphodiester backbone 5′ to the AP site. The major AP endonuclease in *E. coli* is the Xth protein. This enzyme possesses multiple catalytic activities: (1) a nucleotidyl hydrolase activity cutting 5′ to apurinic/apyrimidinic sites and urea residues in DNA; (2) a 3′ to 5′ exonuclease activity specific for double-stranded DNA; (3) an RNase H activity preferentially degrading the RNA strand of a DNA:RNA hybrid, and (4) an activity that can remove a number of 3′ termini from duplex DNA including 3′-phosphates, 3′-phosphoglycolate, 3′-phosphoglycolaldehyde, and 3′-*trans*-4-hydroxy-2-pentenal-5-phosphate residues (Kuo *et al.*, 1993).

Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole), together with other heterocyclic amines, like harman (1-methyl-9*H*-pyrido[3,4-*b*]indole) and norharman (9*H*-pyrido[3,4-*b*]indole) have been isolated from the charred parts of broiled fish and meat and found to be bacterial and mammalian mutagens as well as rodent carcinogens in concentrations highly exceeding the dietary intake of these substances (Mori *et al.*, 1993). However, in low, nontoxic and nonmutagenic concentrations Trp-P-1 and other heterocyclic amines, namely Trp-P-2 (3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole) and harman have been shown to increase UV induced mutation frequencies in *E. coli* (Shimoi *et al.*, 1992) or chromosomal aberrations in cultured mammalian cells (Sasaki *et al.*, 1992) by inhibiting DNA repair, as later shown the nucleotide excision repair (Mori *et al.*, 1993).

Here we show that the Fpg protein is inhibited by 2-thioxanthine (Fig. 1, compound **2**) and to a lesser extent by 5,6-diamino-4-oxo-2-thioxopyrimidine (compound **3**) and 4,6-diamino-5-nitroso-2-oxopyrimidine (compound **4**). We have also found that Trp-P-1 (Fig. 1, compound **1**) differentially inhibits excision of alkylated bases by DNA glycosylases and incision activities of the Fpg and Nth DNA glycosylases as well as *E. coli* Xth AP endonuclease.

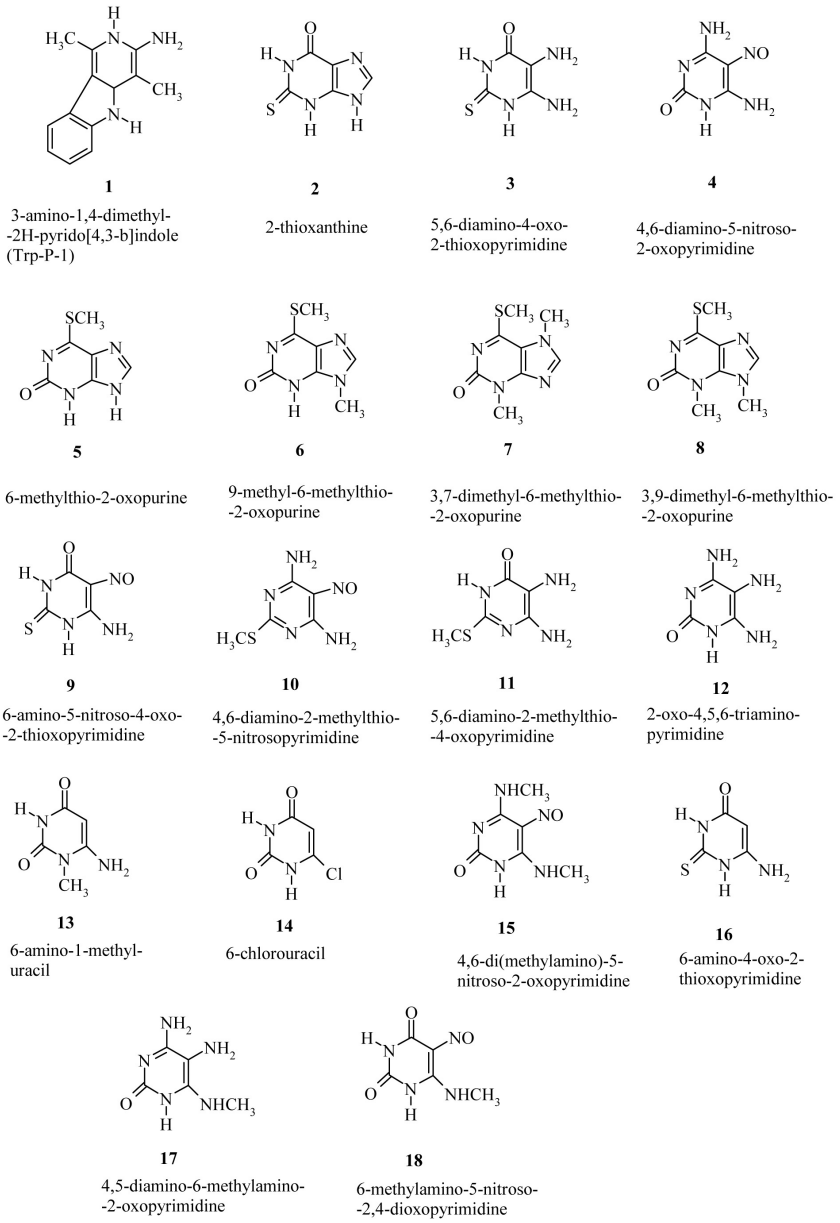

Figure 1. Chemical structures of compounds used in the present study.

MATERIALS AND METHODS

Reagents. Radiolabeled methylnitrosourea, [^{3}H]MNU (18 Ci/mmol) was obtained from Amersham. Trp-P-1 was a kind gift of Dr. K. Wakabayashi (National Cancer Center Research Institute, Tokyo, Japan). Base analogs (see Fig. 1 compounds: **2**–**18**) were obtained according to previously described procedures: compounds **2**, **3**, **9** (Beaman, 1954); **4**, **12** (Bendich *et al.*, 1948); **5** (Yamazaki *et al.*, 1968); **6** (Lichtenberg *et al.*, 1972); **7** (Hayashi *et al.*, 1980); **8** (Kazimierczuk & Shugar, 1974); **10**, **11** (Taylor & Chain, 1952); **13** (Papesch & Schroeder, 1951); **14, 15** (Kazimierczuk *et al.*, 1972); **16** (Traube, 1904); **17** (Pfleiderer & Fink, 1962); **18** (Huebsch & Pfleiderer, 1988). The purity of the compounds was ascertained by TLC, UV, and mass spectra analysis (not shown). pBR322 plasmid DNA was isolated from DH5α *E. coli* strain according to the standard alkaline lysis procedure (Sambrook *et al.*, 1989).

Enzymes. *E. coli* formamidopyrimidine DNA glycosylase (the Fpg protein), alkylpurine DNA glycosylases (the TagA, AlkA, and ANPG proteins), and thymine glycol DNA glycosylase (the Nth protein) were purified according to standard procedures with minor modifications (Boiteux *et al.*, 1987; Tudek *et al.*, 1998; O'Connor & Laval, 1990; Dizdaroglu *et al.*, 1993). Bacterial exonuclease III (the Xth protein) was obtained from Promega (Maddison, WI, U.S.A.). Protein content was quantified as described by Bradford (1976).

Modification of DNA. [^{3}H]MNU-DNA and [^{3}H]MNU-poly[d(G-C)] were prepared by alkylation of respective polynucleotides with [^{3}H]MNU as described (Boiteux *et al.*, 1984). [^{3}H]FapyMe-poly[d(G-C)] was obtained by imidazole ring opening of [^{3}H]MNU-poly[d(G-C)] as described (Laval, 1977; Boiteux *et al.*, 1984).

DNA of pBR322 plasmid was oxidized by an 8-oxoG inducing agent as follows: to 20 μg of plasmid DNA in 10 mM Tris/HCl, pH 8 (50 μl), methylene blue was added to a final concentration of 3.12 μM and the mixture was irradiated with visible light (bulb 200 W) for 10 min in an open tube on ice. For DNA depurination, 100 μg of plasmid DNA in 100 mM sodium acetate buffer, pH 4.8 (120 μl) was incubated at 70°C for 30 min, followed by cooling below 30°C for 1 h. Oxidized or depurinated DNA was pelleted with ice-cold ethanol and suspended in water.

DNA glycosylase assay. The AlkA and TagA protein standard reaction mixture (50 μl) contained [^{3}H]MNU-DNA (5 pmols of alkylated bases), 70 mM Hepes/KOH, pH 7.8, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol. For ANPG this buffer was supplemented with 100 mM KCl. The Fpg protein incubation mixture contained [^{3}H]FapyMe-poly-[d(G-C)] (5 pmols of alkylated bases), 70 mM Hepes/ KOH, pH 7.6, 100 mM KCl, 2 mM EDTA. The substrates were incubated at 37°C with an amount of enzyme which ensured that after reaction no more than 50% of substrate was used, then 50 μl of DNA/BSA (0.5 mg/ml calf thymus DNA, 0.26 mg/ml bovine serum albumin, 250 mM NaCl) was added to stop the reaction. The DNA was precipitated with 300 μl of cold ethanol and the enzyme activity was calculated on the basis of the amount of liberated modified bases assayed in supernatants by liquid scintillation.

DNA glycosylase and AP endonuclease assay. Fpg, Nth, and Xth standard reaction mixtures (50 μl) contained oxidized or depurinated pBR322 DNA (50 μg) in an appropriate buffer (70 mM Hepes/KOH, pH 7.6, 100 mM KCl, 2 mM EDTA for the Fpg protein. The Nth protein reaction buffer comprised 50 mM Tris/HCl, pH 7.8, 50 mM KCl, 2 mM EDTA. The Xth protein buffer was supplied by the enzyme's manufacturer). The substrates were incubated at 37°C with an amount of enzyme which ensured that after reaction no more than 50% of substrate was digested and aliquots of each reaction were separated on 0.8% agarose gels containing ethidium bromide. Images of the gels were recorded using the UVP Gel Documentation System (UVP Inc., Upland, CA, U.S.A.).

Inhibition of DNA glycosylase and AP endonuclease activity by base analogs and Trp-P-1. DNA glycosylase reaction mixtures containing [^{3}H]MNU-DNA or [^{3}H]-FapyMe-poly[d(G-C)] were supplemented with different concentrations of Trp-P-1 or base analogs (1 μM to 5 mM). Following 10 min incubation at 37°C, the DNA was ethanol precipitated in the presence of DNA/BSA and liberated bases assayed as described above. Concentration of compounds, inhibiting 50% of enzymes' activities (IC_{50}) were calculated. DNA glycosylase and/or AP endonuclease reaction mixtures containing depurinated or oxidized pBR322 DNA were supplemented with 1 mM Trp-P-1 and incubated at 37°C for 10 min. The enzyme activity was judged on the basis of the relative band intensity of the *ccc* and *oc* DNA forms after agarose gel electrophoresis.

Estimation of K_i for Fpg glycosylase activity inhibition by 2-thioxanthine. Reactions were conducted in 10 μl sample volume which contained the substrate (2.5–12 nM), 70 mM Hepes/KOH, pH 7.6, 100 mM KCl, 2 mM EDTA, 1% glycerol, BSA (20 μg/ml), 2-thioxanthine (4 or 5 μM) in Me_2SO (final concentration 16%), and 0.4 ng of the Fpg protein added last. The samples were incubated at 37°C for 10 min and reactions were stopped by the addition of 90 μl DNA/BSA. DNA was precipitated and liberated bases measured as above. Kinetic constants were calculated using a program based on the Eisenthall-Cornish-Bowden nonparametric algorithm (Kamiński & Domino, 1987). The K_i value was calculated from replots of ordinate and abscissa intercepts.

RESULTS AND DISCUSSION

The Fpg, TagA, AlkA, and Nth proteins were purified to homogeneity according to standard procedures (Boiteux *et al.*, 1987; Tudek *et al.*, 1998; Dizdaroglu *et al.*, 1993). This yielded proteins of high purity: a single band, as judged by polyacrylamide gel electrophoresis (not shown), and the proteins were further used for inhibition studies. We found that the classical excision product of the Fpg protein Fapy-7MeG very weakly inhibited the Fpg activity; at 1 mM concentration the excision of Fapy-7MeG from DNA was decreased only by 16% and at 5 mM by 32%. In order to look for more efficient inhibitors of this activity, we tested seventeen substituted purine and pyrimidine intermediates for purine synthesis (Fig. 1, compounds **2**–**18**). The aim of this search was to find a possible "leading structure" for further studies on the inhibitors of the Fpg glycosylase. Three of them (compounds **2**, **3**, and **4**) revealed inhibitory properties. The other compounds, tested in up to 1 mM concentration, had no effect on the enzyme activity. The most efficient inhibitor was a purine analog, 2-thioxanthine (2-thio-6-oxopurine, compound **2**). It inhibited 50% of the enzyme activity (IC_{50}) at 17.1 μM (Table 1) and 80% at 50 μM. Because of the high substrate usage in kinetics experiments (20–60%), for the calculation of the Fpg protein kinetics, mean substrate concentrations (arithmetic mean of initial and final concentration) and mean velocity (fmol substrate used/min) were used. The measured K_m value of the Fpg protein activity was 2.43 ± 0.68 nM, whereas V_{max} was 3.19 ± 0.41 fmol/min. The obtained K_m values for Fapy-7MeG excision are similar to the values obtained in other studies, 7 nM (Tudek *et al.*, 1998), and 10 nM (Jurado *et al.*, 1998). For other Fpg glycosylase substrates the K_m values are also low, e.g. for 8-oxoG excision from the 8-oxoG:C pair K_m values varying between 4 nM and 16 nM have been reported (Jurado *et al.*, 1998; Duarte *et al.*, 2000; Tchou *et al.*, 1994), and for excision of pyrimidine ring-opened 1,N^6-ethenoadenine derivative the K_m is 6 nM (Speina *et al.*, 2001).

Table 1. Inhibition of Fpg glycosylase activity by various base analogs*

Base analog	IC_{50} [μM]
2-Thioxanthine	17.1 ± 0.6
5,6-Diamino-4-oxo-2-thioxo-pyrimidine	343.3 ± 58.6
4,6-Diamino-5-nitroso-2-oxopyrimidine	350 ± 24.4

*Data from three assays are presented as mean ±S.D.

K_i of Fpg inhibition by 2-thioxanthine (Fig. 1, compound **2**) was 4.44 ± 0.15 μM. The character of inhibition was found to be uncompetitive (catalytic, Fig. 2), which means that 2-thioxanthine is able to bind only the Fpg protein–DNA complex, but not the free enzyme. This suggests that binding of the Fpg protein to DNA significantly changes the protein conformation, creating the recognition site for the inhibitor. A comparative study of crystal models of Fpg glycosylases obtained

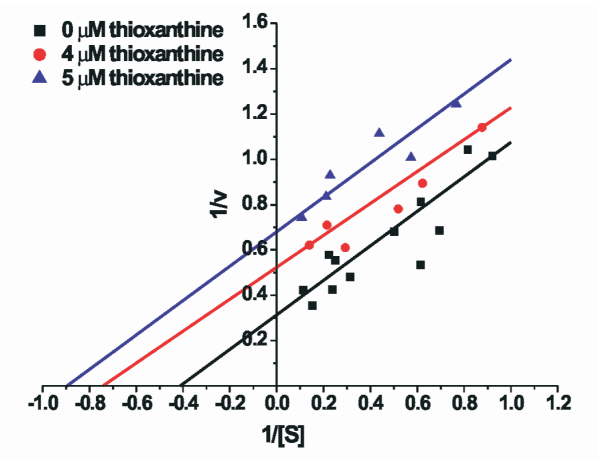

Figure 2. Lineweaver-Burk plot for the uncompetitive (catalytic) inhibition of Fpg glycosylase activity by 2-thioxanthine

The Fpg protein standard reaction mixtures containing [^{3}H]FapyMe-poly[d(G-C)] were supplemented with 4 or 5 μM thioxanthine and incubated at 37°C for 10 min. Following DNA precipitation liberated bases were measured and the enzyme activity assayed. Plots for non-inhibited and inhibited enzyme were calculated by the use of the Eisenthall-Cornish-Bowden nonparametric algorithm. K_i value was calculated from ordinate and abscissa intercepts.

from different bacteria species, either as free enzymes or in complex with an abasic site-containing DNA, shows that the Fpg protein reveals high dynamics upon binding to damaged DNA (Amara *et al.*, 2004). Our results suggest that the enzyme–substrate complex binds to the inhibitor leading to a ternary complex which is enzymatically inactive. It is possible that the damaged base influences the dynamics of the whole enzyme. The site of 2-thioxanthine binding is unknown, however it is possible that once the inhibitor is bound to the enzyme–substrate complex, another conformational change takes place causing enzyme inhibition.

Two other base analogs, 5,6-diamino-4-oxo-2-thioxopyrimidine and 4,6-diamino-5-nitroso-2-oxopyrimidine (Fig. 1, compound **3** and **4**, respectively) were weaker inhibitors. Their IC_{50} values were 343.3 ± 58.6 and 350 ± 24.4 μM, respectively (Table 1). The character of the inhibition was not studied for these compounds.

We also investigated the effect of the modification of the secondary DNA structure on the activity of different base excision repair enzymes. 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (tryptophan pyrolysate, Trp-P-1; Fig. 1, compound **1**) belongs to a class of heterocyclic amines that have been isolated from cooked food and found to be mutagenic and carcinogenic (Watanabe & Ohta, 1993; Ohgaki *et al.*, 1986). Heterocyclic amines intercalate into DNA and change the structure of the double helix into a non-B, non-Z form (Inohara *et al.*, 1995). We studied the release of alkylated bases from radiolabeled polynucleotides or DNA by the Fpg, TagA, AlkA, and ANPG proteins in the presence of Trp-P-1 (Table 2). Trp-P-1 inhibited the glycosylase activity of the Fpg protein by 50% at 187.5 μM, and almost completely at 500 μM. The values of IC_{50} for the TagA and ANPG proteins were 96 ± 15.6 μM and 76.5 ± 19.1 μM, respectively. Trp-P-1 most efficiently inhibited the activity of the AlkA glycosylase (IC_{50} = 1 ± 0.1 μM). All of the above DNA glycosylases, except for the Fpg protein, require double stranded DNA as a substrate, and thus may be more sensitive to DNA structure change than the Fpg protein, which can excise damaged bases also from a single stranded substrate, al-

Table 2. Inhibitory effect of Trp-P-1 on DNA glycosylase activity*

DNA glycosylase	IC_{50} [μM]
Fpg	187.5 ± 46
TagA	96 ± 15.6
AlkA	1 ± 0.1
ANPG	76.5 ± 19.1

*Data from three assays are presented as mean ±S.D. Fpg, *E. coli* formamidopyrimidine DNA glycosylase; TagA and AlkA, *E. coli* alkylpurine DNA glycosylase I and II, respectively; ANPG, human alkylpurine DNA glycosylase.

though with about 100-fold lower efficiency (Boiteux *et al.*, 1990).

We also used a plasmid system in which we measured conversion of the supercoiled (*ccc*) to the relaxed (*oc*) form upon nicking by AP lyases or AP endonuclease at AP sites caused by the release of the damaged base by DNA glycosylase. When the plasmid DNA was modified by methylene blue in the presence of visible light (a system generating almost exclusively 8-oxoguanine; Tudek *et al.*, 1993), it was found that the activity of the Fpg and Nth proteins against the oxidized DNA was abolished in the presence of 1 mM Trp-P-1 (Fig. 3A). This suggests that repair of 8-oxoG and other oxidized bases recognized by the studied glycosylases/AP lyases is inhibited by Trp-P-1.

We also investigated if Trp-P-1 would influence incision of DNA at abasic sites by the Xth protein (the major *E. coli* AP endonuclease) and bifunctional DNA glycosylases (Fpg and Nth proteins). Using depurinated DNA as a substrate, it was found that the incision activity of the Fpg, Nth and Xth proteins was completely inhibited by 1 mM Trp-P-1 (Fig. 3B). Judging from the ratio of the *ccc* to *oc* plasmid forms, this inhibition was less efficient for AP endonuclease than for the AP lyase activities of the Fpg and Nth proteins.

DNA conformation is a general factor affecting the activity of DNA repair enzymes, however, the same factor may have divergent effects on different repair systems. The Fpg protein and O^6-methylguanine DNA methyltransferase are inactive towards the substrate DNA in Z-form (Lagravère *et al.*, 1984; Boiteux *et al.*, 1985). Excision of pyrimidine dimers and 6-4 photoproducts from UV irradiated DNA by UvrABC nuclease and T4 endonuclease V was decreased in the presence of Trp-P-1 (Mori *et al.*, 1993; Shimoi *et al.*, 1996).

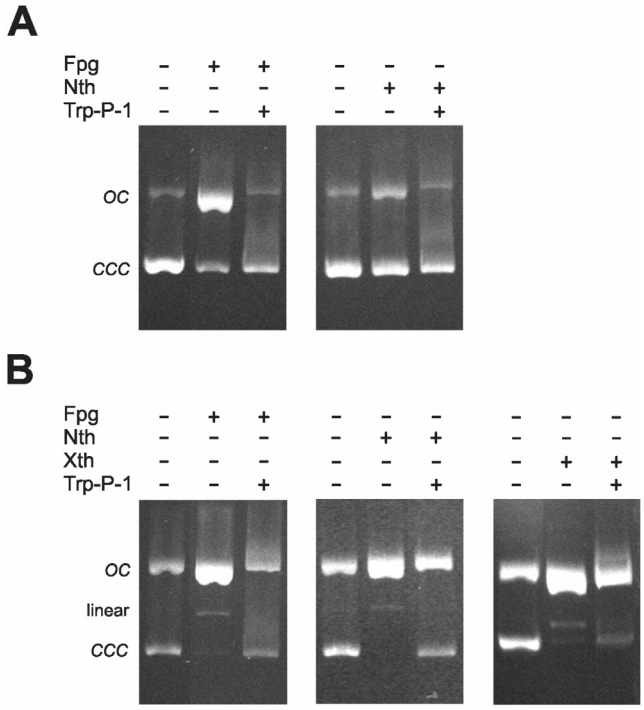

Figure 3. Inhibitory effect of Trp-P-1 on DNA glycosylase activity and strand scission by *E. coli* Fpg and Nth proteins, and on strand scission by Xth protein.

Oxidized (A) or depurinated (B) pBR322 plasmid DNA was treated with 1 mM Trp-P-1 at 37°C for 10 min and incubated with enzyme for 10 min. The reacted DNA was analyzed by agarose gel electrophoresis. Each experiment was performed in duplicate and results from one experiment are shown. *oc*, relaxed (open circular) DNA; *ccc*, supercoiled (covalently closed circular) DNA.

Other intercalating agents, like ethidium bromide or ellipticine increased the activity of uracil-DNA glycosylase about 2-fold (Leblanc *et al.*, 1982), probably by promoting formation of ssDNA fragments, for which the enzyme has a higher affinity than for dsDNA. Similarly, ethidium bromide stimulates release of 3MeA from nucleosomes by mammalian cell extracts and partially purified alkylpurine-DNA glycosylase (Price *et al.*, 1983), probably by relaxing the nucleosome structure and abolishing the steric hindrance of histones for repair enzymes. In contrast, correction of the G:T mismatch by hMutSα was inhibited by low concentration of ethidium bromide (IC_{50} – 30 μM) and intercalating anticancer drugs like adriamycine, probably by preventing mismatch binding (Larson & Drummond, 2001).

Taken together the results show that 2-thioxanthine inhibits excision of Fapy-7MeG by the Fpg protein by an uncompetitive mode, which suggests substantial dynamics of the Fpg protein upon substrate binding. We have also shown that change(s) of DNA secondary structure by Trp-P-1 may significantly influence two classes of DNA repair events: the excision of damaged DNA bases by several glycosylases, as well as the incision of DNA at AP sites by AP lyases and AP endonucleases. Thus, similarly to the inhibition of UV-induced DNA damage repair, Trp-P-1 might exert an inhibitory effect also on base excision repair, and its consumption in the human diet may also increase mutation rate caused by alkylation and oxidative DNA damage.

We thank Dr. Keiji Wakabayashi (Carcinogenesis Division, National Cancer Center Research Institute, Tokyo, Japan) for a kind gift of Trp-P-1. The authors would like to express the gratitude to Professor Jarosław T. Kuśmierek (Institute of Biochemistry and Biophysics, PAS) for many helpful discussions.

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