Incision at Diaminopurine:Thymine Base Pairs but Not at Guanine:O^4-Methylthymine Base Pairs in DNA by Extracts of Human Cells†

Sibghat-Ullah,‡ Yao-Zhong Xu,§ and Rufus S. Day, III*,‡

Molecular Oncology Program, Department of Medicine, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta T6G 1Z2, Canada, and Cancer Research Campaign Nitrosamine-Induced Cancer Research Group, Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, England

Received February 22, 1995; Revised Manuscript Received April 4, 1995⊗

ABSTRACT: Cell-free extract from the A1235 human malignant glioma cell line was employed to study the possibility of incision at 2,6-diaminopurine:T (DiAP:T), 2-amino-6-methylaminopurine:T (AMAP:T), and G:O^4-methylthymine (G:^{m4}T) mismatches, each placed in a 45 bp DNA at a defined site. The incision of a 45 bp DNA containing a G:T mispair at the same site was followed to determine the relationship between base pair structure and repair activity (ies) in the extract. The cell-free extract incised DNAs containing DiAP:T, AMAP:T, and G:T pairs similarly. Reminiscent of the known pattern of incision at G:T mismatches, products from each substrate were consistent with two incisions, one immediately 5′ and one immediately 3′ to the mismatched T, and only in the strand containing the mismatched T. While DNA with an O^6-methylguanine:T (^{m6}G:T) pair was also incised, DNA containing the G:^{m4}T pair was not, but was rendered inciseable by pretreatment with O^6-methylguanine−DNA methyltransferase. Incision of DiAP:T-containing DNA by the extract was less in the presence of unlabeled DNA containing G:T mispairs than in the presence of A:T- or G:A-containing DNA or in the absence of competing DNA. We suggest that the mechanism operating on DiAP:T and/or AMAP:T pairs may be the same as the human G:T repair pathway, possibly initiated by the action of a glycosylase as described by Wiebauer and Jiricny [Wiebauer, K., & Jiricny, J. (1989) *Nature 339*, 234−236; Wiebauer, K., & Jiricny, J. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5842−5845]. That related activities recognize DiAP:T, AMAP:T, G:T, and ^{m6}G:T pairs but not G:^{m4}T pairs suggested a unifying mechanism of action.

DiAP[1] is an analog of adenine that replaces adenine in the DNA of the cyanophage S-2L (Kirnos et al., 1977; Khudyakov et al., 1978; Vorlicková et al., 1991) showing that DiAP in DNA is compatible with normal DNA function. DiAP:T pairs in DNA exist as Watson−Crick pairs stabilized by three hydrogen bonds in either A DNA (Vorlicková et al., 1991), B DNA (Chazin et al., 1991; Howard & Miles, 1984), or Z DNA (Coll et al., 1986) with structural parameters mimicking those of A:T pairs. Counteracting the thermostabilizing force produced by the three hydrogen bonds in B DNA is believed to be a destabilizing effect caused by the interference of DiAP's 2-amino group with the spine of hydration that accompanies sequential A:T pairs [see Howard and Miles (1984)]. As a consequence of these unequal opposing effects, DNAs with DiAP:T pairs are more thermostable than those with A:T pairs (Kirnos et al., 1977; Khudyakov et al., 1978; Howard & Miles, 1984; Hoheisel & Lehrach, 1990; Chazin et al., 1991; Cheong et al., 1988) except when the cooperative stabilizing effect conferred by the spine of hydration to runs of A:T pairs is reduced by the inclusion of a single run-breaking DiAP:T pair (Cheong et al., 1988).

DiAP is toxic to cultured cells, and most mutants that are resistant to DiAP have reduced APRT (Lieberman & Ove, 1960; Harris & Ruddle, 1961; Blair & Hall, 1965; Atkins & Gartler, 1968) or elevated levels of ADA (Dutton & Chovnick, 1990). This is consistent with biochemical findings that it is normally metabolized to DiAP ribonucleoside by APRT (Blair & Hall, 1965) and then deaminated to guanosine by ADA (Weckbecker & Cory, 1989) as suggested by Garber and Gots (1980) on genetic grounds. Further, DiAP is mutagenic, albeit weakly (Alderson, 1964). The picture is consistent with the view that DiAP somehow produces at least some of its cytotoxic effects by being incorporated into DNA. Its base pairing structure (Figure 1) suggested to us a possible cellular response to DiAP:T pairs. This paper describes our finding that extracts of a human glioblastoma cell line incise a 45 bp double-stranded oligodeoxynucleotide containing a single DiAP:T base pair and an initial characterization of the products of the reaction. For comparative purposes we studied whether 45 bp DNAs containing a single AMAP:T pair or a single G:^{m4}T pair are incised. The structure of AMAP:T base pairs was expected to mimic closely that of ^{m6}G:T pairs (see Figure 1) that were found to serve as substrates for incision by cell-free extracts in a previous study (Sibghat-Ullah & Day, 1992).

EXPERIMENTAL PROCEDURES

Oligodeoxynucleotides and Preparation of Double-Stranded DNAs. Forty-five nt single-stranded DNA oligos with the four naturally occurring bases were prepared as described

† This work was supported by NIH NCI Grant CA49936 to R.S.D.

* To whom correspondence should be addressed. Telephone: 403-492-8048. Fax: 403-492-0884.

‡ Cross Cancer Institute.

§ University College London.

⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1995.

[1] Abbreviations: m5C, 5-methylcytosine; AP, abasic site; ^{m6}G, O^6-methylguanine; DiAP, 2,6-diaminopurine; 2AP, 2-aminopurine; oligo, oligodeoxynucleotide; AMAP, 2-amino-6-methylaminopurine; ^{m4}T, O^4-methylthymine; TDT, terminal deoxynucleotidyl transferase; DTT, dithiothreitol; TE, 0.01 M Tris, 0.001 M EDTA, pH 8.0; BSA, bovine serum albumin; ADA, adenosine deaminase; APRT, adenine phosphoribosyl transferase.

0006-2960/95/0434-7438$09.00/0

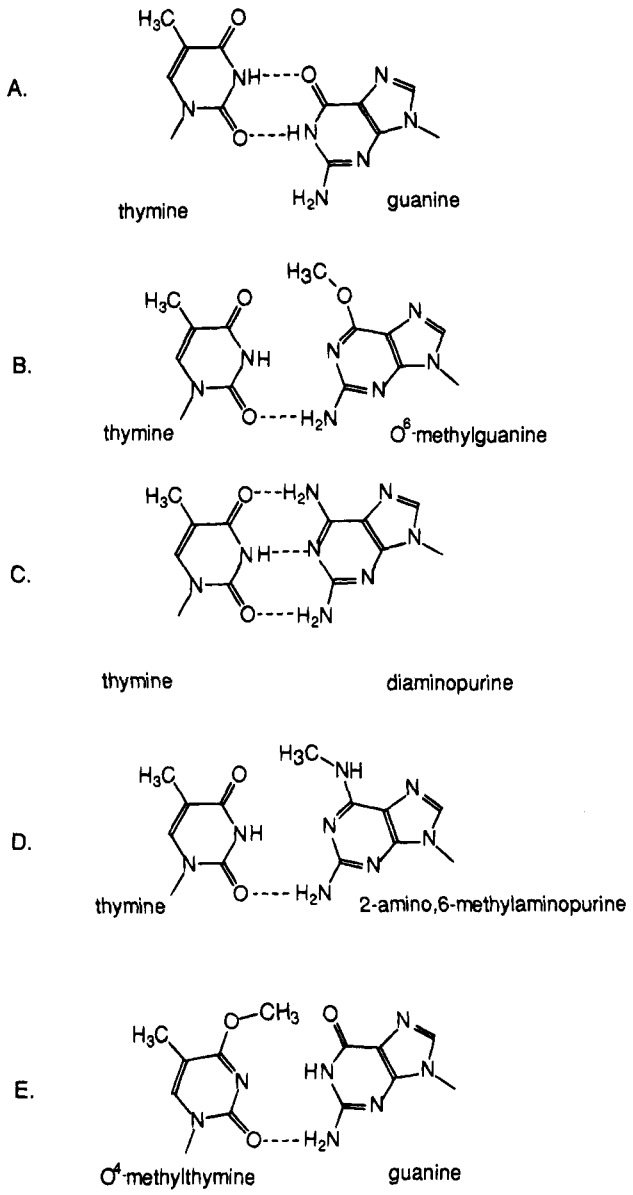

FIGURE 1: Structures of some of the base pairs incorporated into the 45-bp substrates used in this study. G:^{m4}T and ^{m6}G:T are weak Watson–Crick pairs (Kalnick et al., 1990; Swann, 1990), G:T is a wobble pair (Kennard, 1985; Swann, 1990), and DiAP:T is a strong Watson–Crick pair (Coll et al., 1986; Chazin et al., 1991).

previously by the DNA Synthesis Laboratory, Department of Microbiology, University of Alberta by conventional phosphoramidite technology (Sibghat-Ullah & Day, 1992).

5′-GGCCAGCTAGTGGTGGTGGGCGCCRGCGGTGTGGGCATTCGTAAT-3′
3′-CCGGTCGATCACCACCACCCGCGGYCGCCACACCCGTAAGCATTA-5′

Oligos, containing either DiAP or AMAP at the purine position (designated R), were prepared as described (Xu et al., 1992a,b) as 26-mers having sequences representing the 3′-most 26 nt of the top strands. To prepare the 45 bp DNAs, these 26-mers were 5′-phosphorylated with T4 kinase and unlabeled ATP, mixed with equimolar amounts (~7 pmol) of both the 19 nt 5′-most segments of the top strand and entire 45 nt bottom strands containing T at the pyrimidine position Y (3′ labeled as above). In each case (DiAP:T or AMAP:T DNAs), the three oligos were hybridized by heating to 65 °C and cooling to room temperature and incubated with 2 units of T4 DNA ligase for 4 h at room temperature. Another 2 units of ligase was added and allowed to react at 4 °C for 4 h. After electrophoresis through DNA sequencing gels, the 45-mer region was excised, and the DNAs were electroeluted, rehybridized, and repurified by phenol, chloroform, and ethanol precipitation. Substrates were repurified either on denaturing gels (in which case they were rehybridized later) or on native gels. G:T and G:A mismatched DNAs, as well as A:T and G:C matched DNAs, were prepared from 45 nt oligos as described previously (Sibghat-Ullah & Day, 1992).

The ^{m4}T-containing bottom strand was prepared (Xu et al., 1992b) in two segments, the 3′-most 30 nt containing ^{m4}T at the position designated Y and the 5′-most 15 nt. The 30-mer was 5′-phosphorylated with unlabeled ATP, hybridized along with the 15-mer (5′-phosphorylated with ATP 5′ γ-^{32}P), to the top strand (R = G), ligated and purified as described in the preceding.

The DiAP:T, AMAP:T, G:T, and G:^{m4}T base pairs comprise the base at position 25 from the 5′ terminus of the top strand and the base at position 21 from the 5′ terminus of the bottom strand. For convenience this base pair is referred to in the text as "the base pair at position 25".

Cultured Cells, Cell Extracts, and DNA Incision Assay. The A1235 human malignant glioma cell line was cultured in Dulbecco's Modified Eagle Medium plus 10% fetal calf serum and antibiotics (Sibghat-Ullah & Day, 1992). Cell-free extract was prepared according to the procedure of Manley et al. (1980) as described previously (Sibghat-Ullah et al., 1989). Incision reactions were performed as described (Sibghat-Ullah & Day, 1992, 1993) generally following the procedures of Wiebauer and Jiricny (1989, 1990).

For the incision assay, substrates (2 ng) were prepared either ^{32}P-labeled on the 5′-termini of their bottom strands using T4-kinase and 5′-[γ-^{32}P]ATP or on the 3′-termini of their bottom strands using TDT and cordycepin-5′-[α-^{32}P]-triphosphate. Substrates were incubated with 10 μg of A1235 extract in buffer (25 mM Hepes, pH 7.9, 0.5 mM EDTA, 0.01 M $ZnCl_2$, 0.5 mM DTT, 100 μg/mL BSA) and 8–10 ng of unlabeled nonmismatched double-stranded 45 bp DNA in 50 μL reaction volumes at 30 °C for 16 h (Sibghat-Ullah & Day, 1992). The treated DNAs were extracted with phenol and then chloroform, precipitated with ethanol, vacuum-dried, and dissolved in 20 μL of formamide/dyes. After electrophoresis through 12% acrylamide sequencing gels, gels were exposed to X-ray film at −80 °C for 1–3 days to obtain autoradiograms.

Treatment of ^{m4}T-Containing DNA with Ada Protein. Five nanograms of the G:^{m4}T double-stranded DNA (~0.2 pmol), ^{32}P-labeled at the 5′-termini of the bottom strands, was incubated for 2 h at 37 °C in the presence (or absence) of 1 μg (~50 pmol) of the 19 kDa MGMT portion of the *Escherichia coli* Ada protein (McCarthy et al., 1984; a kind gift of Dr. Daniel Yarosh, Applied Genetics, Inc., Freeport, NY) in 50 μL volumes in 50 mM Tris-HCl, pH 7.6, 50 mM NaCl, 1 mM EDTA, and 5 mM DTT. Ada-treated and mock-treated control DNAs were repurified with phenol and chloroform, ethanol precipitated, dried, and resuspended in 10 μL of TE. They were then incubated with A1235 extract and analyzed for mismatch incision as described in the preceding.

RESULTS

Incision of DNAs Containing Modified Bases. Forty-five bp DNAs containing either G:T, DiAP:T, or AMAP:T pairs

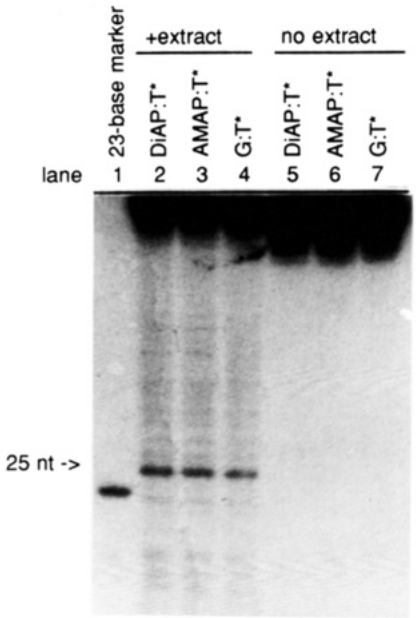

FIGURE 2: Incision of 3′-terminally labeled 45 bp DNAs containing DiAP:T or AMAP:T pairs by cell free extract of A1235 human malignant glioma cells. The DNAs indicated were prepared with label at the 3′-terminus of their bottoms strands (see structure in Experimental Procedures). They were incubated with cell-free extract (lanes 2−4) or without cell-free extract (lanes 5−7) from A1235 cells. The DNAs were repurified, subjected to electrophoresis along with a 23 base marker, and autoradiographed.

FIGURE 3: Incision of 5′-terminally labeled 45 bp DNAs containing DiAP:T or AMAP:T pairs by cell free extract of A1235 human malignant glioma cells. The DNAs indicated were prepared with label at the 5′-terminus of their bottom strands. They were incubated with cell-free extract from A1235 cells, repurified, and subjected to electrophoresis along with 20 and 21 base markers, and autoradiographed. The markers were the 5′-labeled 20 and 21 nt oligos 5′-ATTACGAATGCCCACACCGC-3′ and 5′-ATTACGAATGCCCACACCGCT-3′ having sequences of the 5′ terminus of the bottom strand of the DNA shown in Experimental Procedures.

were prepared with ^{32}P label in the 3′ termini of their bottom strands using cordycepin-5′-[α-^{32}P]triphosphate and TDT. (The bottom strand is designated, as shown in Experimental Procedures, as the strand containing the T that pairs with the G, DiAP, or AMAP residue. That particular T will be designated the T at base pair 25.) These DNAs were incubated for 16 h with or without cell-free extract from the A1235 human glioblastoma cell line at 30 °C. The DNAs were freed from protein and electrophoresed along with a 23 nt marker that was prepared by *Hpa*II digestion of the G:C counterpart of the substrate labeled in the 3′ terminus of the top strand. Figure 2 shows the results of the experiment. Each of the three DNAs served as substrate for a process that produced a 3′-labeled 25 nt fragment (lanes 2, 3, and 4) in an extract-requiring reaction (compare lanes 2−4, obtained with extract, with lanes 5−7, without extract). Because the 3′-labeling process added 1 nt to the 3′ terminus, the 25 nt lengths of the reaction products indicate that incision was immediately 3′ to the T at base pair 25. This is a site at which human extracts incise G:T mismatches in DNA (Wiebauer & Jiricny, 1989; Sibghat-Ullah & Day, 1992). A similar experiment, in which the 5′ termini of the bottom strands were labeled with T4 polynucleotide kinase and 5′[γ-^{32}P]ATP, showed that the 5′ products of reactions of extract with DNAs having DiAP:T, AMAP:T, or G:T pairs were 20 nt long in all cases (Figure 3, lanes 3−5), indicating that an incision had been made immediately 5′ to the T at position 25. A DNA with an A:T pair was not incised (Figure 3, lane 2). When DNAs were prepared with either 3′ or 5′ label in their top strands, no product was observed (data not shown). Thus, the DNAs containing DiAP:T, AMAP:T, and G:T base pairs were incised only in their bottom strands and both immediately 3′ and immediately 5′ to the T belonging to the base pair at position 25. DNAs having the same overall sequences, but containing A:T pairs or G:C pairs in the **R** and **Y** positions, were not incised [Figure 3 and Sibghat-Ullah and Day (1992)]. The reaction rates were low, as observed previously for the incision of DNAs with either G:T or ^{m6}G:T mispairs (Wiebauer & Jiricny, 1989; Sibghat-Ullah & Day, 1992). Ten micrograms of extract protein usually converted about 3−5% of the substrate (~2−3 fmol) to product in 16 h.

Incision of Modified DNAs in the Presence of Competitor DNAs. To test whether the activity that acts on DNAs with DiAP:T or AMAP:T pairs binds to DNAs having G:A pairs (Yeh et al., 1991) or G:T pairs (Wiebauer & Jiricny, 1989), competition experiments were performed. DNA with DiAP:T pairs (5′-terminally labeled in the bottom strands) was incubated with extract from A1235 cells in the presence of 5, 10, and 20 ng of unlabeled DNAs containing either G:C, A:T, G:A, or G:T pairs (as **R** and **Y**, respectively) as the base pair at position 25. Figure 4 shows that incision of the labeled DiAP:T-containing substrate was strongly inhibited by unlabeled DNA having the G:T mismatch (lanes 12−14), somewhat by 20 ng of the DNA with the A:T match (lane 8), but not by unlabeled nonmismatched G:C or mismatched G:A DNAs (lanes 3−5 and 9−11). Very similar results were obtained in another such experiment and in two experiments using labeled DNA with an AMAP:T pair as substrate (data not shown). Insufficient amounts of unlabeled DiAP:T- or AMAP:T-containing DNAs remained for the reverse competion experiments. We conclude that the 45 bp DNA having a G:T mismatch interferes with the activity (ies) that incises DNAs having DiAP:T pairs or AMAP:T pairs in a way that DNAs containing G:C, A:T or G:A pairs do not.

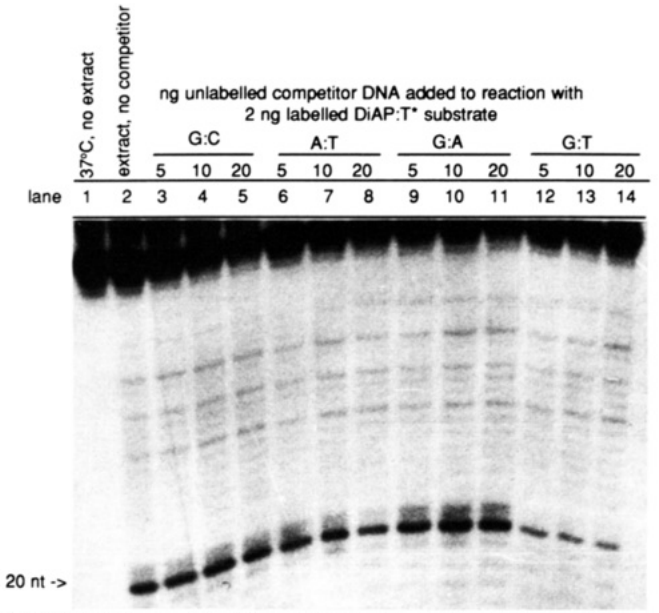

FIGURE 4: Effect on incision of labeled DiAP:T substrate of added unlabeled DNAs. Aliquots of DiAP:T-containing DNA, prepared with label at the 5′-terminus of the bottom strand, were incubated with cell-free extract from A1235 cells in the presence and absence of 5, 10, and 20 ng of the DNAs having the base pair at position 25 as designated.

FIGURE 5: DNA containing G:^{m4}T pairs is a poor substrate for G:T-type mismatch incision. A:T, G:^{m4}T, and G:T DNAs were prepared labeled on the 5′-terminus of their bottom strands and were either mock treated or treated with the 19 kDa MGMT portion of the *E. coli* Ada protein. The DNAs from all samples were repurified, incubated with extract from A1235 (which lacks detectible MGMT activity; Sibghat-Ullah & Day, 1992), again repurified, and subjected to electrophoretic analysis.

Incision of DNA Containing G:^{m4}T Pairs. We had previously found that DNA containing a single ^{m6}G:T base pair at position 25 was a substrate for incision (Sibghat-Ullah & Day, 1992). The thermodynamic stability of the DiAP:T pair should be much greater than that of the ^{m6}G:T pair (cf. Howard & Miles, 1984; Swann, 1990), yet DNAs containing either pair served as substrate for a G:T mismatch-like incision process. Thus we tested whether DNA containing a G:^{m4}T base pair (see Figure 1E), which has a binding energy which approximates that of the ^{m6}G:T pair (see Figure 1B; Swann, 1990), was incised. We incubated the 45 bp DNA containing a single G:^{m4}T base pair at position 25 with extract from A1235 cells and processed the products as before. We found very little, but detectable, incision of the G:^{m4}T substrate compared to a G:T substrate (data not shown) that may have arisen from a small amount of substrate that had suffered demethylation of ^{m4}T during preparation. To show that the reaction would have taken place to a greater extent if the same DNA had contained G:T pairs, and to demonstrate that ^{m4}T was indeed present in the substrate, we treated the G:^{m4}T DNA as well as control A:T and G:T DNAs with and without *E. coli* Ada protein to convert G:^{m4}T pairs to G:T pairs (McCarthy et al., 1984) prior to assaying for mismatch incision. The demethylation of ^{m4}T to T was accompanied by a substantially increased sensitivity to the mismatch incision activity (compare lanes 3 and 4, Figure 5). In the same experiment, the lack of incision of the control nonmismatched A:T DNA (lane 1) was unaltered by MGMT treatment (lane 2), and the incision of control mismatched G:T DNA (lane 5) was not enhanced by the treatment (lane 6). Thus, even though the binding energies of the ^{m6}G:T and G:^{m4}T base pairs may be approximately equal, only the former is incised by extracts of human cells.

DISCUSSION

We have presented evidence that 45 bp DNAs containing either a single DiAP:T pair or a single AMAP:T pair are incised by extract from human cells, but that a similar DNA containing G:^{m4}T pairs is not. The pattern of incision of the DiAP:T- and AMAP:T-containing DNAs is the same as that for incision by human extracts of G:T pairs (Wiebauer & Jirincy, 1989) and ^{m6}G:T pairs (Sibghat-Ullah & Day, 1992): all of these DNAs are incised both immediately 3′ and immediately 5′ to the T of the respective base pair and only in the strand that contains that T. The result is somewhat surprising given that the stability of DiAP:T base pairs is reported to be greater than that of A:T base pairs, except in regions of runs of As. The fact that a DNA having a single ^{m6}G:T base pair is incised, while DNA having a single G:^{m4}T pair is not, even though the two share similar stability (Swann, 1990), strengthens the argument that base pairing energy may be a relatively minor factor in determining proclivity toward such incision.

The competition experiment showed that DNA that contains G:T base pairs interferes with incision of DNA at DiAP:T or AMAP:T pairs, while DNA containing A:T pairs shows very weak interference, and DNAs with G:C and G:A pairs show none. The competition we observe reflects binding to G:T mispairs by at least one protein involved in incision of DiAP:T or AMAP:T pairs. Such a protein could be a G:T-binding protein involved in mismatch recognition and/or a glycosylase specific for G:T mismatches (Neddermann & Jiricny, 1993). Thus, the G:T mismatch repair system described by Wiebauer and Jiricny (1989, 1990) may also incise DNAs containing DiAP:T, AMAP:T and ^{m6}G:T base pairs. This G:T mismatch repair system, which possibly arose to correct base mismatches caused by the spontaneous hydrolytic deamination of C to U or ^{m5}C to T, was found to involve a 55 kDa G:T- or G:U- specific thymine (or uracil) glycosylase, now purified to apparent homogeneity (Neddermann & Jiricny, 1993). The glycosylase initiates the removal of the mismatched thymine as the free base (Wiebauer & Jiricny, 1990; Nedderman & Jiricny, 1993) without breaking the DNA backbone (Nedderman & Jiricny, 1993). In the extract, other proteins, presumably an AP-endonuclease or AP-lyase, produce a one-nucleotide gap by incising the DNA backbone just 3′ and just 5′ to the

thymineless site (Wiebauer & Jiricny, 1989). DNA polymerase β, presumably followed by one of the DNA ligases, restores the G:C pair (Wiebauer & Jiricny, 1990).

The possiblity that one glycosylase initiates the incision of DiAP:T, AMAP:T, ^{m6}G:T, and G:T pairs is consistent with the competition data as well as with observation that the incision products of DNAs containing these base pairs are the same, i.e., incision both immediately 3′ and immediately 5′ to the T of the respective base pair. If the same glycosylase is responsible, a possible implication with regard to the mechanism of recognition of base mismatches by the repair protein may be drawn. Recognition of target base pairs could be achieved by the glycosylase scanning base pairs simultaneously for purine 2-amino groups and for pyrimidine 4-keto oxygens (or N3 protons). The G:T mismatch glycosylase, at ~55 kDa (Nedderman & Jiricny, 1993), is larger than other glycosylases: the protein sequence may include purine 2-amino/pyrimidine 4-keto (and/or N3-proton) recognition motifs.

It will be interesting to determine whether the effective antitumor DiAP analogue 2-chlorodeoxyadenosine (Saven et al., 1992), which is resistant to adenosine deaminase (unlike DiAP), owes a portion of its considerable toxicity to such a mechanism.

An analogue of DiAP, 2AP, produces C→T transitions predominantly at m5C sites in three C^{m5}C(A/T)GG locations (hot spots) when generating amber mutations in the *lacI* gene of *E. coli* K strains (Coulondre et al., 1978; Glickman, 1985). A high level of spontaneous C→T transitions occurs at these same sites because the very short patch (VSP) repair of G:T mispairs (Lieb, 1991; Hennecke et al., 1991) that are produced when m5C spontaneously deaminates to T [see Duncan and Miller (1980)] is not totally effective (Claverys & Lacks, 1986; Modrich, 1991; Radman, 1988). Were the *E. coli* VSP system to have a recognition mechanism similar to that described in the preceding, the DNA sequence specificity of 2AP in *E. coli* could arise when VSP repair becomes saturated by attempting to restore VSP-restoration sites [CC(A/T)G, C(A/T)GG; see Hennecke et al. (1991)] that contain 2AP:T pairs. The repair of G:T mismatches that arise via 5mC deamination would then be less effective, and the observed specificity would result. This expection is strengthened by the finding that *dcm* mutants, lacking m5C in their DNA, show 5−6-fold reduced levels of 2AP-induced mutants at these hot spots (Glickman, 1985).

^{m4}T is 15−22-fold more mutagenic than ^{m6}G when incorporated into single-stranded *lac* Zα-containing plasmids and analyzed in repair-deficient (Ada$^-$, MNNG-treated) *E. coli* (Dosanjh et al., 1991). This disparity may be due, in part, to mismatch repair recognition of ^{m6}G:T pairs, but not of G:^{m4}T pairs.

ACKNOWLEDGMENT

S.-U. and R.S.D. are indebted to Dr. Peter F. Swann of the Cancer Research Campaign Nitrosamine-Induced Cancer Research Group, Department of Biochemistry and Molecular Biology, University College London, for his enthusiasm, encouragment, and discussions during the collaboration that allowed the study of the 2,6 DiAP- and AMAP-containing oligonucleotides. We thank Drs. M. C. Paterson and R. Godbout for critically reading the manuscript.

REFERENCES

Alderson, T. (1964) *Mutat. Res. 1*, 205−208.
Atkins, J. H., & Gartler, S. M. (1968) *Genetics 60*, 781−792.
Blair, D. G. R., & Hall, A. D. (1965) *Can. J. Biochem. 43*, 1857−1878.
Chazin, W. J., Rance, M., Chollet, A., & Leupin, W. (1991) *Nucleic Acids Res. 19*, 5507−5513.
Cheong, C., Tinoco, I., & Chollet, A. (1988) *Nucleic Acids Res. 16*, 5115−5122.
Claverys, J.-P., & Lacks, S. A. (1986) *Microbiol. Rev. 50*, 133−165
Coll, M., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H., & Rich, A. (1986) *J. Biomol. Struct. Dyn. 4*, 157−172.
Coulondre, C., Miller, J. H., Farabaugh, P. J., & Gilbert, W. (1978) *Nature 274*, 775−780.
Dosanjh, M. K., Singer, B., & Essigmann, J. M. (1991) *Biochemistry 30*, 7027−7033
Duncan, B. K., & Miller, J. H. (1980) *Nature 287*, 560−561.
Dutton, F. L, Jr., & Chovnick, A. (1990) *Mol. Gen. Genet. 220*, 172−176.
Garber, B. B., & Gots, J. S. (1980) *J. Bacteriol. 143*, 864−871.
Glickman, B. W. (1985) *Basic Life Sci. 31*, 353−379.
Harris, M., & Ruddle, F. H. (1961) *J. Natl. Cancer Inst. 25*, 1405−1411.
Hennecke, F., Kolmar, H., Bründl, K., & Fritz H.-J. (1991) *Nature 353*, 776−778.
Hoheisel, J. D., & Lehrach, H. (1990) *FEBS Lett. 274*, 103−106.
Howard, F. B., & Miles, H. T. (1984) *Biochemistry 23*, 6723−6732.
Kalnick, J. W., Li. B. F. L., Swann, P. F., & Patel, D. J. (1990) *Biochemistry 28*, 6170−6181, 6182−6192.
Kennard, O. (1985) *J. Biomol. Struct. Dyn. 3*, 205−225.
Khudyakov, I. Y., Kirnos, M. D., Alexandrushkina, N. I., & Vanyushin, B. F. (1978) *Virology 88*, 8−18.
Kirnos, M. D., Khudyakov, I. Y., Alexandrushkina, N. I., & Vanyushin, B. F. (1977) *Nature 270*, 369−370.
Lieb, M. (1991) *Genetics 128*, 23−27.
Lieberman, I., & Ove, P. (1960) *J. Biol. Chem. 235*, 1765−1768.
Manley, J. L., Fire, A., Cano, A., Sharp, P. A., & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3855−3859.
McCarthy, T. V., Karran, P., & Lindahl, T. (1984) *EMBO J. 3*, 545−550.
Modrich, P. (1991) *Annu. Rev. Genet. 25*, 229−253.
Neddermann, P., & Jiricny, J. (1993) *J. Biol. Chem. 268*, 21218−21224.
Radman, M. (1988) in *Genetic Recombination* (Kucherlapati, R., & Smith, G. R., Eds.) pp 169−192, American Society of Microbiology, Washington, D.C.
Rappaport, H., & DeMars, R. (1973) *Genetics 75*, 335−345.
Saven, A., Carrera, C. J., Carson, D. A., Beutler, E., & Piro, L. D. (1992) *Blood 80*, 587−592.
Sibghat-Ullah & Day, R. S., III (1992) *Biochemistry 31*, 7998−8008.
Sibghat-Ullah & Day, R. S., III (1993) *Nucleic Acids Res. 21*, 1281−1287.
Sibghat-Ullah, Husain, I., Carlton, W., & Sancar, A. (1989) *Nucleic Acids Res. 17*, 4471−4484.
Swann, P. F. (1990) *Mutat. Res. 233*, 81−94.
Vorlicková, M., Khudyakov, I., Hejtmánková, Y., & Kypr, J. (1991) *J. Biomol. Struct. Dyn. 9*, 81−85.
Weckbecker, G., & Cory, J. G. (1989) *Adv. Enzyme Regul. 28*, 125−144.
Wiebauer, K., & Jiricny, J. (1989) *Nature 339*, 234−236.
Wiebauer, K., & Jiricny, J. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5842−5845.
Xu, Y.-Z., Zheng, Q., & Swann, P. F. (1992a) *Tetrahedron 48*,-1729−1740.
Xu, Y.-Z., Zheng, Q., & Swann, P. F. (1992b) *J. Org. Chem. 57*, 3839−3845.
Yeh, Y.-C., Chang, D.-Y., Masin, J., & Lu, A.-L. (1991) *J. Biol. Chem. 266*, 6480−6484.

BI950404H