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Enzymic Detection of Uracil in a Cloned and Sequenced Deoxyribonucleic Acid Segment†

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ABSTRACT: Uracil can occur in DNA either as a result of utilization of dUTP by DNA polymerases or from in situ deamination of cytosine. The latter results in transition mutations following the next round of replication. We describe a technique for the detection of uracil in DNA by a modification of the Maxam-Gilbert sequencing procedure. Reaction of end-labeled DNA with uracil-DNA glycosylase followed by 1 M piperidine results in scission products corresponding to locations of uracils. These are detected by autoradiography following electrophoresis through a sequencing gel. Comparison of these scission products with the DNA sequences elucidates the mechanism of origin of the DNA uracils. The technique was tested with a cloned human DNA sequence grown in a dut⁻,ung⁻ strain of *Escherichia coli*, which incorporates uracil in place of thymine in its DNA, and by chemical deamination of cytosines in that sequence. The technique was expanded by use of alkaline and enzymic probes to investigate possible accumulation of uracil, base losses, and other modifications in human liver and brain DNA. No damaged DNA moieties were detected. This method is applicable to the study of any recoverable reiterated sequence by any enzyme preparation that can recognize modifications in DNA.

Uracil, while not a normal component of DNA, may arise either as a result of utilization of dUTP in place of dTTP by DNA polymerases or from in situ deamination of cystosine (Lindahl, 1979). Replicative polymerases have the same K_m for dUTP as for dTTP and will utilize dUTP if it is available (Schlomai & Kornberg, 1978; Dube et al., 1979). Deamination of cytosine in DNA may occur either spontaneously (Lindahl & Nyberg, 1974) or by reaction with environmental agents such as sodium bisulfite or nitrous acid (Lindahl, 1979). If uracil derived from cytosine is not removed, transition mutations (G-C → A-T) will be observed following the next round of replication (Drake & Baltz, 1976).

Two mechanisms normally exclude uracil from DNA. Deoxyuridine-5′-triphosphate nucleotidohydrolase (dUTPase) hydrolyzes dUTP to dUMP, making dUTP unavailable as a

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0006-2960/83/0422-4501$01.50/0 © 1983 American Chemical Society

substrate for DNA polymerase. Uracil already present in DNA is removed by uracil-DNA glycosylase [dUra(DNA)-glycosylase],[1] leaving an apyrimidinic site. The DNA is subsequently cleaved by an apurinic/apyrimidinic endonuclease, and the correct sequence is restored via the excision repair pathway (Lindahl, 1979). There is direct genetic evidence for the importance of these enzymes in excluding uracil from DNA in *Escherichia coli*. Strains of *E. coli* lacking dUra(DNA)glycosylase (ung^-) are mutators that accumulate G-C → A-T mutations (Duncan & Weiss, 1978; Duncan & Miller, 1980). Bacteriophage grown in mutant strains (BD897) that lack both dUTPase and dUra(DNA)glycosylase enzymes (dut^-,ung^-) can substitute as much as 30% of DNA thymines by uracils (Warner & Duncan, 1978; Warner et al., 1979). Study of these mechanisms in eukaryotic cells is more difficult because no cell line has been isolated that is deficient in dUra(DNA)glycosylase activity (Sekiguchi et al., 1976; Kuhnlein et al., 1978). However, it has been shown in a number of mammalian cell lines that levels of both dUTPase and dUra(DNA)glycosylase are minimal in nondividing cells and are increased during cell division (Sirover, 1979; Caradonna & Cheng, 1980; Duker & Grant, 1980; Gupta & Sirover, 1981). Reduced levels of dUra(DNA)glycosylase have been reported in tissues composed of nondividing cells (Aprelikova & Tomilin, 1982). Therefore, DNA uracil might possibly accumulate in tissues composed of nondividing cells.

There is ample evidence for other forms of significant hydrolytic damage to DNA in aqueous solution. Substantial losses of purines (Lindahl & Nyberg, 1972) and pyrimidines (Lindahl & Karlstrom, 1973) result from heating of DNA at neutral pH. Extrapolations from these studies suggest that, in postmitotic cells such as human neurons, 3% of the purines and 0.15% of the pyrimidines would be lost from cellular DNA during a human lifetime should such damage remain unrepaired (Lindahl, 1977). In addition, other types of hydrolytic or oxidative degradation of DNA may occur at physiological conditions (Lindahl, 1977). The repair capacities of the different human tissues for these damages have not yet been specified.

We therefore modified the degradative sequencing procedure of Maxam & Gilbert (1980) to locate and characterize DNA damage in the human cerebellum and liver α sequences. This segment is a 340 base pair, tandemly reiterated, DNA segment (Wu & Manuelidis, 1980). The assay for DNA uracils is based on the introduction of alkali-labile apyrimidinic sites following excision of this base by purified dUra(DNA)-glycosylase (Cone et al., 1977; Lindahl et al., 1977). The method was tested by locating uracil at specific sites in a DNA segment made by cloning the α sequence into the plasmid pBR322 and transferring the resulting recombinant plasmid into the dut^-,ung^- *E. coli* strain BD897. Uracils were detected at thymine positions in a 92 base pair fragment of this DNA, and sequence analysis distinguished such uracils from those resulting from cytosine deamination. Samples of DNA purified from human cerebellum and liver were examined for uracil and other types of DNA damage by this technique. Additional probes included alkaline hydrolysis as a probe for base-loss sites (Kochetkov & Budovskii, 1972) and an extract from *Micrococcus luteus* as a probe for a broad spectrum of DNA damages (Paterson et al., 1981). No damaged sites were detected in human DNAs by this method. Therefore, it appears that the capacity for DNA repair in these tissues remains intact throughout a human lifetime.

Experimental Procedures

Preparation of DNA. HeLa cells and cells from a continuously proliferating lymphoblastoid line transformed by Epstein–Barr virus (C91-3) were grown according to Henderson et al. (1981). Cells were lysed in 0.2% sodium dodecyl sulfate (SDS) in SSC buffer (0.15 M sodium chloride–0.015 M sodium citrate, pH 7.4), followed by digestion by RNase (60 min) and Pronase (60 min) at 37 °C. After two extractions with redistilled phenol, the DNA was extensively dialyzed into TE buffer (10 mM Tris-HCl–1 mM EDTA, pH 8.0).

Adult cerebellum and liver tissue were obtained at autopsy within 8 h of death. Tissues were obtained from an 18-year-old male who died of drowning, a 69-year-old male who died as a result of disseminated oat cell carcinoma of the lung without receiving cytotoxic chemotherapy, and a 72-year-old male who died of multiple trauma. Nuclei were obtained as described (Wu & Manuelidis, 1980) and lysed in 1% sarcosyl–1× SSC buffer. After overnight digestion by proteinase K (1 mg/mL) at 37 °C, the DNA was extracted twice with chloroform–isoamyl alcohol (19:1) and twice with phenol, dialyzed into TE buffer, precipitated with 2 volumes of 95% ethanol, and redissolved in TE buffer. Human DNA obtained both from cell cultures and from tissues was digested by *Eco*RI restriction endonuclease according to Grunberg & Haseltine (1980), and the 340 and 680 base pair α fragments were recovered from 4% polyacrylamide gels by electroelution (Smith, 1980).

The dut^-,ung^- *E. coli* strain BD897 was kindly provided by Dr. Bruce Duncan. Plasmid DNA was extracted from BD897 and from the *E. coli* strain HB101 by the SDS lysis procedure of Guerry et al. (1973), followed by isopycnic centrifugation in a cesium chloride gradient containing 500 μg/mL ethidium bromide. Two successive CsCl centrifugations were necessary to purify plasmid grown in BD897, because the SDS lysis procedure did not separate the bulk of the cellular DNA from the plasmid.

Cloning of α Sequences in pBR322. pBR322 DNA was digested with a 10-fold excess of *Eco*RI, dialyzed against 1 M sodium chloride in TE buffer followed by TE buffer alone, and then digested with a 10-fold excess of bacterial alkaline phosphatase in 40 mM Tris-HCl, 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol, pH 8.5. After phenol extraction and ethanol precipitation, 1 μg of vector DNA was incubated overnight at 12 °C with 1 μg of 340 base pair α fragment in the presence of T4 DNA ligase (Bolivar & Backman, 1979). The ligation products were used to transform HB101 to ampicillin resistance. Colonies were replica plated and screened by filter hybridization (Grunstein & Wallace, 1979) to 0.1 μg of the 340 base pair or 680 base pair α fragment labeled with ^{32}P by nick translation (Maniatis et al., 1975). Lysed human lymphoblasts were included on the filter as positive controls. Positive colonies showed a wide and reproducible range of variation in degree of hybridization. The strongest positive colony was designated pHα1 and used for subsequent plasmid preparation and analysis.

BD897 was transformed with a small amount of pHα1 obtained from the HB101 host by a rapid isolation (minilysate) procedure. DNA from 200 μL of an overnight culture of HB101 carrying the plasmid yielded approximately 50 ampicillin-resistant colonies, one of which was grown up for isolation of uracil-containing pHα1. Base content of this DNA was analyzed by acid hydrolysis followed by paper and thin-layer chromatography (Randerath & Randerath, 1967; Duker

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; TE buffer, 10 mM Tris-HCl and 1 mM EDTA, pH 8.0; dUra(DNA)glycosylase, uracil-DNA glycosylase; SDS, sodium dodecyl sulfate; SSC buffer, 0.15 M NaCl and 0.015 M sodium citrate, pH 7.4.

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   3' (TTAA)GGGTCA TTGAAGGAAC AAAACCAACA CAAGTTGAGT GTCTCAACTT GAAAGTAAAT GTGTCTCGTC
             A                 C   AC    T                      CT  G

        TAAACTTTGT GAGAAAAACA CCTTAAACGT TTACATCTAA AGTTCGCGAA ACTCCGGTTT CCGTCTTTTC
         G                 G              C  C         CG           A   A   A    A

        CTTTATAGAA GCAAAGTTTT GATCTGTCTT AGTAAGAGTC TTTGACGAGA CGCTACACAC GCAAGTTGAG
                    G T TC                C              G  A   A

        AGTGTCAAAT TGAAAAGAAA AGTAAGTCGT CAAACCTTTG TGAGACAAAC ATTTCAGACG TGCACCTATT
        T  C         G            TC        T                              T       A

        GAACTGGTGA ATCTCCGGAA GCAACCTTTG CCCATAAAAA GTACATTCGG ATCTGTCTTC TTAA       5'
        AGT    A    A                       TA  G      T A AC
```

FIGURE 1: Sequence of insert in pHα1 compared with consensus sequence of human α DNA. The sequence of the α DNA fragment in pHα1 is shown in its entirety. The consensus sequence derived from the 340 base pair human *Eco*RI satellite band (Wu & Manuelidis, 1980) is shown where it differs from the sequence in pHα1. The sequence presented here is the complementary strand to that published by Wu & Manuelidis (1980), but in the same orientation. The *Eco*RI* site used to generate the 92 base pair indicator fragment is underlined.

et al., 1981). All handling of organisms containing recombinant DNA was carried out under P1 containment conditions.

Labeling and Sequencing of DNA. The 340 base pair α fragment, obtained by *Eco*RI digestion of either human cellular DNA or pHα1 plasmid, was labeled at the 3′-end with [α-^{32}P]dATP for 30 min at 15 °C by the Klenow fragment of *E. coli* DNA polymerase I (Grunberg & Haseltine, 1980). The reaction mixture contained 20 mM Tris-HCl (pH 7.6), 6 mM $MgCl_2$, 6 mM NaCl, 6 mM 2-mercaptoethanol, 0.1 mg/mL gelatin, 100 μCi of [α-^{32}P]dATP, 100 μM dTTP, 1–5 μg of DNA, and 1 unit of enzyme in 50 μL. After passage over Sephadex G-50 and ethanol precipitation, labeled DNA was once again digested with *Eco*RI, this time under *Eco*RI* conditions (25 mM Tris-HCl, pH 8.6, 2 mM $MgCl_2$, 20% glycerol, and 100 units of *Eco*RI, incubated at 37 °C for 2 h) (Polisky et al., 1975). The 92 base pair fragment was isolated on an 8% polyacrylamide gel, located by autoradiography, and recovered from the gel by electroelution. Preparative gels contained 40 mM Tris–acetate, 20 mM sodium acetate, and 5 mM EDTA, pH 7.9; electroelution was carried out in 0.1× gel buffer.

Standard DNA sequencing reactions were performed according to Maxam & Gilbert (1980) except that half the recommended amount of dimethyl sulfate (0.5 μL) was used in the guanine reaction.

Enzymic Sequencing Procedure. Two enzymic preparations were used for sequencing reactions to locate uracil. dUra(DNA)glycosylase from *Bacillus subtilis* was purified according to Cone et al. (1977). Fraction VI was concentrated by ultrafiltration in TE buffer containing 0.5 mM dithiothreitol. A protein extract was made from *M. luteus* and precipitated with ammonium sulfate according to Paterson et al. (1981) and dialyzed into 5 mM potassium phosphate, pH 7.5. One microliter of either purified dUra(DNA)glycosylase or *M. luteus* extract was added to 10 μL of labeled 92 base pair fragment in TE buffer and incubated for 30 min at 37 °C. In most cases, the sample was then divided in two. Half was lyophilized and redissolved directly in loading buffer (90% formamide, 10 mM NaOH, 1 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). The other half was lyophilized, redissolved in 1 M piperidine and incubated at 90 °C for 30 min, and then repeatedly lyophilized according to the protocol of Maxam & Gilbert (1980) and taken up in loading buffer. Except as noted, samples were heated at 90 °C for 1 min in loading buffer before electrophoresis on sequencing gels (8% polyacrylamide–50% urea). Gels were subject to autoradiography overnight with a Kodak Lightning Plus intensifying screen.

Deamination of Cytosines in DNA. DNA cytosines were deaminated according to the procedure of Ullman & McCarthy (1973). Labeled 92 base pair fragment was suspended in freshly dissolved 1 N NaOH and heated at 65 °C for 3 h and then cooled and neutralized with 1 M acetic acid, diluted 3-fold with TE buffer, ethanol precipitated, and redissolved in TE buffer for reaction with purified dUra(DNA)glycosylase.

Results

Cloning of α Fragments and Characterization of pHα1. We have cloned the human α sequence, a 340 base pair tandem repeat constituting about 1% of the human genome, into the *Eco*RI site of pBR322. One of the clones from the resulting library, pHα1, has been put into *E. coli* strains HB101 and BD897 (dut^-,ung^-) to facilitate study of uracil incorporation into α sequences. The insert in pHα1 has been sequenced (Figure 1). This sequence differs by 15% from the consensus sequence for the 340 base pair α satellite band obtained from *Eco*RI-digested human DNA (Wu & Manuelidis, 1980). The variations are mostly the result of randomly distributed point mutations, except for one apparent short inversion (AAGT → TGAA). The uracil sequencing studies presented here utilized the 92 base pair *Eco*RI–*Eco*RI* fragment of the insert. The pHα1 preparation used in the experiments shown here was found to have a thymine to uracil ratio of 6.9:1, representing 13% substitution of uracil for thymine, assuming all uracil to be in the thymine position.

Detection of Uracil with Uracil-DNA Glycosylase. End-labeled 92 base pair α fragment for sequencing was obtained from pHα1 grown in either BD897 or HB101. Treatment of the 92 base pair fragment from BD897 with dUra(DNA)-glycosylase, followed by piperidine hydrolysis and electrophoresis on a sequencing gel, resulted in prominent bands at positions corresponding to all thymine residues in the sequence but not at other positions (Figure 2A). This was determined by comparing the scission products, observed after enzyme treatment, with the sequence obtained by the chemical cleavage reactions (Maxam & Gilbert, 1980). Identical treatment of DNA from HB101 showed no increase in band intensity over the untreated control sample, even on an overexposed autoradiogram (Figure 2B), confirming the specificity of the procedure for the detection of uracil in DNA. A 3-fold increase either in the amount of dUra(DNA)glycosylase added

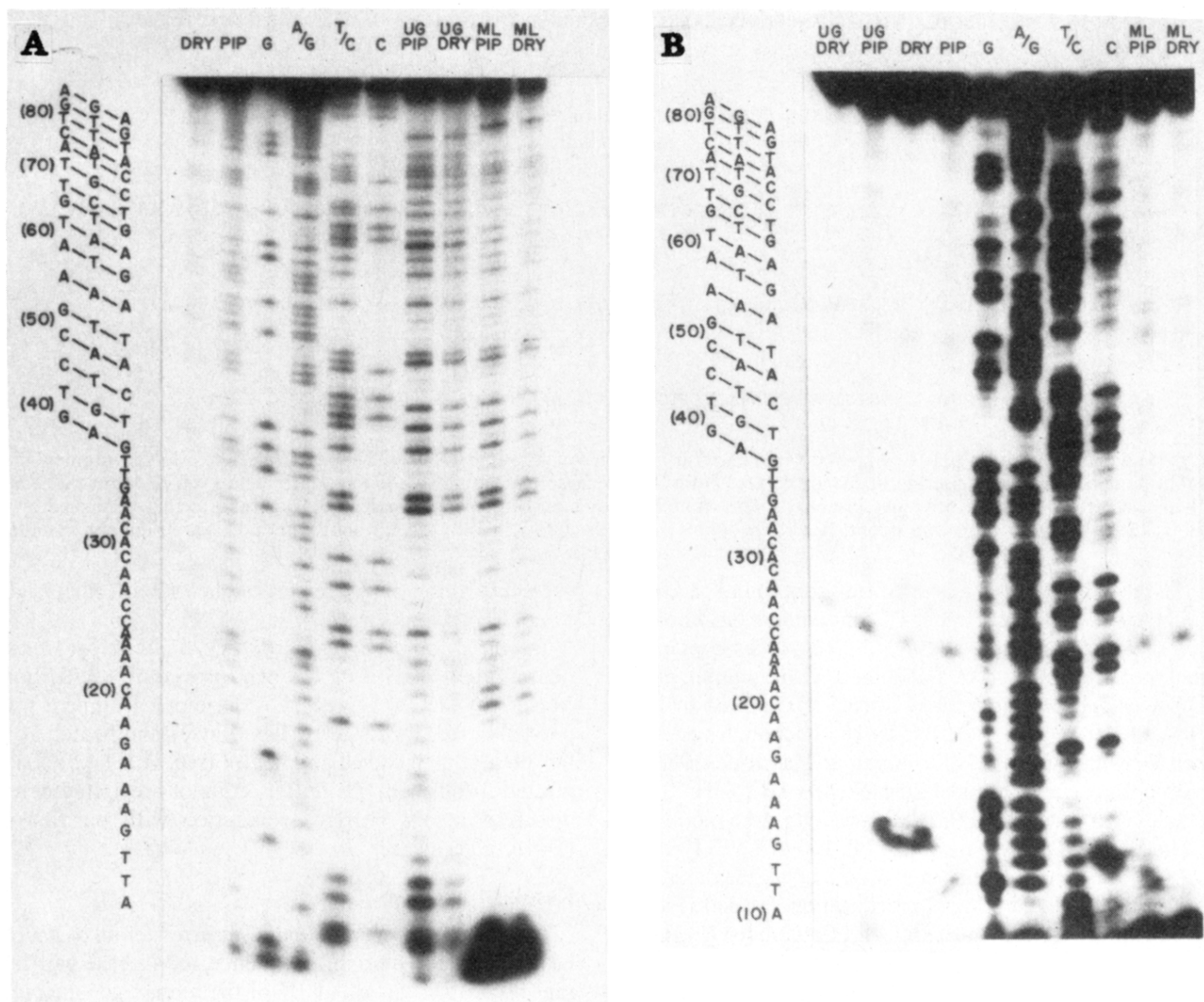

FIGURE 2: (A) Autoradiogram of an 8% sequencing gel of the 92 base pair fragment of pHα1 extracted from BD897. Aliquots of the 3′-end-labeled DNA were lyophilized (lane 1), heated in piperidine (lane 2), subjected to base-specific chemical cleavage (lanes 3–6), and treated with purified dUra(DNA)glycosylase followed by heating in 1 M piperidine (lane 7) or lyophilization (lane 8) or treated with the extract from *M. luteus* followed by heating in piperidine (lane 9) or lyophilization (lane 10). (B) Autoradiogram of an 8% sequencing gel of the 92 base pair fragment of pHα1 extracted from HB101. Aliquots of 3′-end-labeled DNA were treated with purified dUra(DNA)glycosylase followed by lyophilization (lane 1) or heating in piperidine (lane 2), simply lyophilized (lane 3), heated in piperidine (lane 4), subjected to base-specific chemical cleavage (lanes 5–8), or treated with the extract from *M. luteus* followed by heating in piperidine (lane 9) or lyophilization (lane 10).

or in the duration of piperidine treatment did not increase the intensity of the uracil bands (not shown), indicating that under the conditions of the standard procedure, cleavage at the site of uracils is quantitative.

The decrease in band intensity as a function of fragment length on these gels is consistent with a finding of about 10% substitution with uracil and with essentially complete cleavage at all uracil positions. Under these conditions, we would expect each band to be about 10% less intense than the one below it, and this is qualitatively in agreement with our results. The uniformly decreasing intensity of these bands in the glycosylase-treated channel also indicates that uracil is substituted uniformly at all thymines, regardless of surrounding sequences.

The protein extract from *M. luteus* was used as a probe for a wide variety of DNA lesions. The following activities were found to be present in our preparation: (1) pyrimidine dimer-DNA glycosylase, assayed according to Friedberg et al. (1980) with UV-irradiated PM2 DNA; (2) dUra(DNA)-glycosylase, assayed according to Friedberg et al. (1975); (3) apurinic/apyrimidinic site endonuclease, assayed according to Teebor & Duker (1975); (4) endonuclease activity for photoalkylated DNA, assayed according to Livneh et al. (1979). Another activity reported in a similarly prepared extract is an endonuclease directed against γ-irradiated DNA (Paterson et al., 1981). Reaction with this extract generated strand breaks at thymine positions in DNA grown in BD897 but not in HB101 (Figure 2), confirming the presence of dUra(DNA)glycosylase in the extract. In addition, several bands of variable intensity corresponding to adenines are in all channels containing DNA reacted with *M. luteus* extract; none of these is present in DNA treated with *B. subtilis* dUra(DNA)glycosylase. A particularly prominent band is present in some plasmid preparations in the A_5 region near the 5′-end of the 92 base pair fragment. The nature of these sites is uncertain.

Detection of Uracil from in Vitro Deamination of Cytosine. In order to confirm that we can distinguish uracil substituted for cytosine from that in place of thymine, a sample of labeled 92 base pair α fragment from BD897 was subjected to cytosine deamination (Ullman & McCarthy, 1973). When this DNA was treated with purified dUra(DNA)glycosylase and piperidine and run on a sequencing gel, prominent bands were seen corresponding to positions of cytosine when these scission products are compared to the original sequence (Figure 3). Uniformity of band intensity indicates that deamination of cytosine by this procedure is not sequence dependent. A

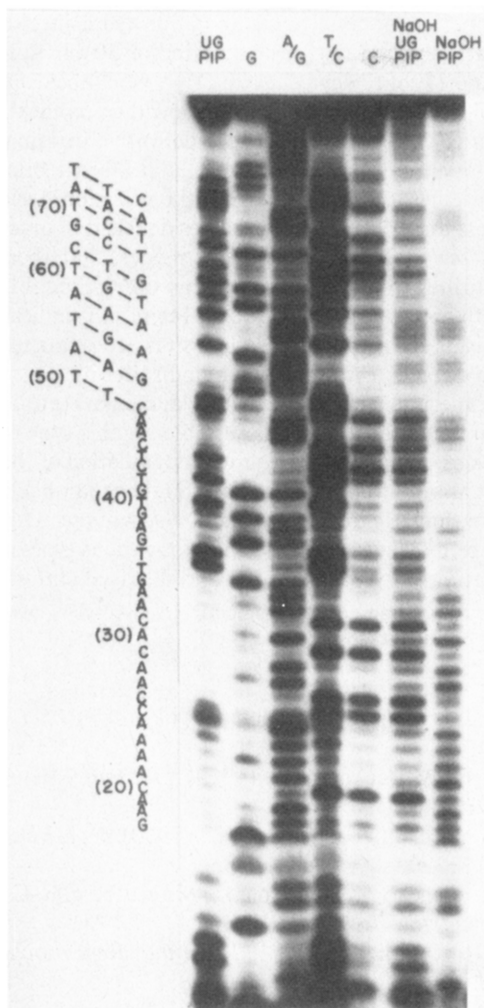

FIGURE 3: Effect of deamination by NaOH and treatment with dUra(DNA)glycosylase on the 92 base pair fragment of pHα1 extracted from BD897. Aliquots of 3′-end-labeled DNA were treated with purified dUra(DNA)glycosylase followed by heating in piperidine (lane 1), subjected to base-specific chemical cleavage (lanes 2–5), or deaminated by heating in 1 N NaOH (lanes 6 and 7) followed either by incubation with dUra(DNA)glycosylase and then heating in piperidine (lane 6) or by simply heating in piperidine (lane 7).

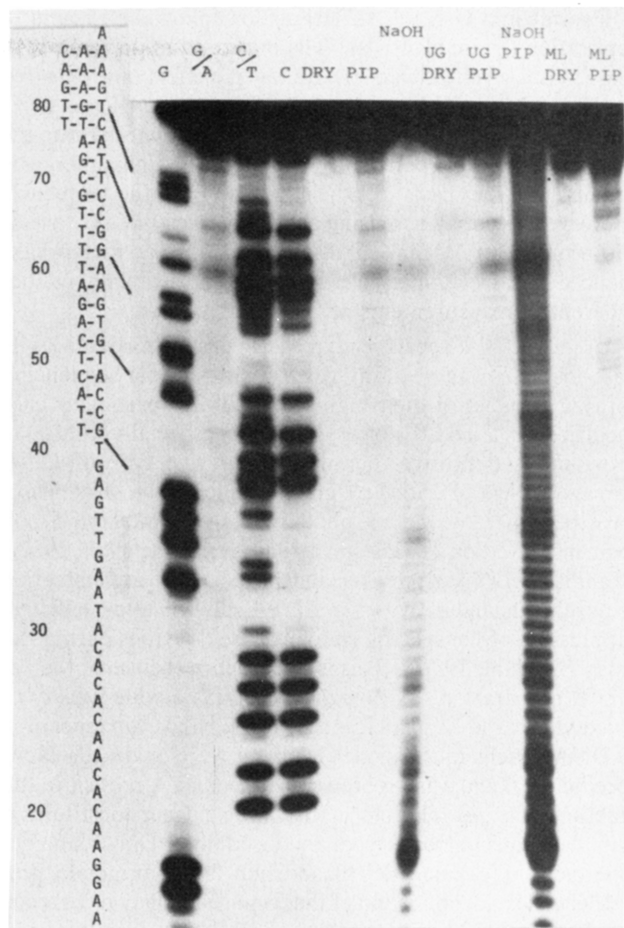

FIGURE 4: Autoradiogram of an 8% sequencing gel of the 92 base pair fragment of human α DNA isolated from the cerebellum of a 72-year-old male who died of multiple trauma. Aliquots of 3′-end-labeled DNA were subjected to base-specific chemical cleavage (lanes 1–4), simply lyophilized (lane 5), heated in piperidine (lane 6), treated with dUra(DNA)glycosylase followed by lyophilization (lane 7) or heating in piperidine (lane 8), incubated in 0.1 N NaOH at 37 °C for 30 min followed by heating in piperidine (lane 9), or incubated with *M. luteus* extract (lanes 10 and 11) followed by lyophilization (lane 10) or heating in piperidine (lane 11).

background of nicks or piperidine-labile sites, predominantly at adenine positions, is present; however, subsequent incubation with dUra(DNA)glycosylase produced an increase in band intensity only at cytosine and thymine positions, corresponding to uracils that arose by in vitro deamination and in vivo incorporation, respectively. Comparison of channels 1 and 6 in Figure 4, in which dUra(DNA)glycosylase generated bands in T and C positions, respectively, illustrates the distinction between uracil incorporated during DNA synthesis and uracil arising from deamination of cytosine on the basis of position in the sequence. These results directly verify that dUra(DNA)glycosylase reacts with uracils opposite either adenine or guanine (Lindahl et al., 1977).

Sequence from DNA Extracted from Human Tissues. Because dUra(DNA)glycosylase levels are low in nondividing cells (Sirover, 1979; Caradonna & Cheng, 1980; Duker & Grant, 1980; Gupta & Sirover, 1981), the possible presence of uracil in liver and brain DNA was investigated. DNA extracted from autopsy material was found to be suitable for sequencing. Using the dUra(DNA)glycosylase procedure, we were unable to locate uracils in the DNA of these tissues, of which one is shown in Figure 4. No base losses were exposed by the piperidine treatment, indicating that these lesions must be repaired in nondividing cells. Breakage at some adenine positions when the DNA was incubated with the *M. luteus* extract including a band in the 5′-A_5 region was observed; but these are not related to age, and their significance is uncertain.

Piperidine treatment of human DNA samples (as well as plasmid DNA samples) exposed to ultraviolet light during the isolation procedure gave rise to bands at C in the sequence TTCAA (Figure 4), which occurs twice in the 92 base pair indicator fragment. A similar UV-induced alkali-labile base alteration has been reported by Lippke et al. (1981) at T*C* sites in cloned copies of the *E. coli lac* promoter.

Discussion

This sequencing assay both detects uracil in DNA and indicates the mode of its generation. Assessment of the significance of uracil in a given DNA sample depends on determination of the mechanism by which it arose. Uracil inserted in place of thymine appears to have little effect on cell function in *E. coli*, since the heavily substituted dut⁻,ung⁻ strain (BD897) displays unimpaired viability. The hydrogen-bonding properties of uracil in DNA are expected to be very similar to those of thymine, so that uracil arising from substitution of uracil for thymine during synthesis may be classified as nonmutagenic. On the other hand, replacement of cytosine by uracil, as a result of deamination by water (spontaneously) or by various chemicals such as nitrous acid or sodium bisulfite,

will result in a G-C → A-T transition following a round of replication (Lindahl, 1979). The increased mutation rate in ung⁻ cells is a reflection of the mutagenic effect of unrepaired uracil arising by this mechanism (Duncan & Weiss, 1978; Duncan & Miller, 1980). This assay for uracil permits the distinction between potentially mutagenic and nonmutagenic uracils, on the basis of their location in the sequence. Moreover, use of a sequencing assay for uracil in DNA avoids the problem of RNA contamination by measuring breaks induced by a DNA-specific enzyme, in contrast to ambiguities inherent in measurements of the released base.

Use of DNA repair enzymes to generate alkali-labile base-loss sites is a versatile general strategy for sequencing assays. Because of their high specificity for particular base modifications, DNA glycosylases are especially useful in providing a definitive identification of the type of DNA damage present. Purified pyrimidine dimer-DNA glycosylases have been used to detect photodimers in sequenced DNA segments (Gordon & Haseltine, 1980; Haseltine et al., 1980). In addition, DNA glycosylase independence of divalent cation requirements and activities in EDTA solution allow effective suppression of nonspecific endonuclease activities during the assay (Lindahl, 1979). This is apparent in the use of the *M. luteus* preparation. Although it contains a wide variety of glycosylases and endonucleases, specific bands corresponding to DNA uracils in the cloned segment were obtained. Nonspecific breakage was suppressed by the EDTA present in the reaction mixture. Therefore, this crude extract constitutes a suitable means to locate types and locations of DNA damages in sequenced fragments. This location can, in principle, precede chemical identification of the damaged moiety or rigorous purification and characterization of all DNA repair enzymes present in *M. luteus*.

This assay provides a means of detecting accumulation of uracils, base losses, or other types of damaged sites in human DNA. Our choice of the cloned segment of the α sequence to test this assay was prompted by our interest in its application to human tissue DNAs. Because of its high copy number and ease of separation from the bulk of high molecular weight DNA, cellular α DNA can be sequenced directly. It has been used in studies of DNA damage in cultured human cells (Grunberg & Haseltine, 1980). It is possible that such sequences may accumulate base modifications at higher rates than bulk DNA, as does the α sequence in African green monkey cells (Zolan et al., 1982). The tissues examined here, brain and liver, consist predominantly of nondividing cells. Neurons are permanent cells no longer capable of division; hepatocytes are stable cells that, though normally nondividing, are capable of rapid division following partial hepatectomy (Robbins & Cotran, 1979). Close correlations have been noted between proliferative activity in in vivo rat tissues and specific activities of dUra(DNA)glycosylases (Gombar et al., 1981; Aprelikova & Tomilin, 1982). Therefore, the capacity to exclude uracil from DNA might be diminished in reiterated sequences of such cells, allowing the possible accumulation of uracil in such DNA. These were investigated in aging tissues, where progressive loss of cell function might reflect accumulation of damaged moieties in DNA.

We have observed neither uracil nor alkali-labile base-loss sites within the limit of detection of our assay in any of the normal human tissue we have examined to date. This could be either because these tissues retain sufficient dUra(DNA)glycosylase activity to keep pace with the rate of cytosine deamination or because deamination is too slow to result in detectable accumulation even without repair over a substantial period of time. Extrapolation from in vitro measurements suggests that over a period of 30 years, between 0.01% and 1% of the cytosines in DNA would be deaminated (Lindahl, 1979), an amount at or below the present threshold of detection by this assay. More definitive information on accumulation of uracil in human cellular DNA requires both an improvement in the sensitivity of the assay for in vivo uracil and access to human mutant cell lines deficient in uracil-DNA glycosylase. Similar extrapolations suggest that about 3% of DNA purines would be lost over this time (Lindahl, 1977). Failure to detect this level of alkali-labile sites indicates that mechanisms for repair of base-loss sites are retained even in nondividing cells throughout a human lifetime.

The experiments presented here demonstrate that DNA from human autopsy tissue is suitable for this type of assay. The background due to postmortem degradation of this DNA does not appear to be substantially higher than background nicking in similarly treated plasmid DNA samples. DNA from this source may be useful in looking for various types of DNA modifications in vivo in normal and diseased states.

Registry No. Uracil, 66-22-8; uracil-DNA glycosylase, 59088-21-0; thymine, 65-71-4; cytosine, 71-30-7.

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Properties of a Human Lymphoblast AP-Endonuclease Associated with Activity for DNA Damaged by Ultraviolet Light, γ-Rays, or Osmium Tetroxide†

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ABSTRACT: An endonuclease activity for UV-irradiated DNA, γ-irradiated DNA, and OsO_4-treated DNA that was partially purified from human lymphoblasts was found to have associated with it an endonuclease activity for partially depurinated DNA. When this apurinic endonuclease (Endo A) was characterized and compared with the cells' major apurinic endonuclease (Endo B), several notable differences were observed. (1) Endo A bound to oxidized DNA–Sepharose under conditions where Endo B did not. (2) Endo A did not require Mg^{2+}, retaining full activity in 10 mM ethylenediaminetetraacetic acid, while Endo B showed an absolute requirement for Mg^{2+}. (3) Whereas the nicks made in depurinated DNA by Endo B were efficient priming sites for *Escherichia coli* polymerase I, those made by Endo A were not. Further characterization of the nicks indicated that Endo A incises depurinated DNA 3′ to apurinic sites, leaving 3′-terminal deoxyribose, a poor priming site for DNA synthesis. Endo A action on UV-irradiated DNA produced nicks that resembled those it made in depurinated DNA, suggesting that the UV endonuclease activity acts through an apurinic/apyrimidinic site intermediate.

Biological systems possess several different mechanisms for coping with damage to DNA that either is caused by environmental chemicals and radiation or is spontaneously generated within the cell. Whereas some DNA lesions are repaired by direct reversal of the damage-inducing reaction, such as UV-dimer photoreactivation (Sutherland, 1978) or O^6-alkylguanine alkyl transfer (Olsson & Lindahl, 1980), the more frequent pathway for repair is by base or nucleotide excision that involves limited degradation and resynthesis of DNA. Such excision repair is initiated by lesion-specific DNA glycosylases or endonucleases. Several DNA glycosylases that remove demaged or abnormal bases from DNA without DNA strand scission have been described (Caradonna & Cheng, 1982), and endonuclease that results in release of intact nucleotides from UV-irradiated DNA has recently been demonstrated unequivocally for the uvr ABC system in *Escherichia coli* (Rupp et al., 1983). A third class of enzymes has been

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