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Hydroxymethyluracil DNA glycosylase in mammalian cells

(DNA repair/oxidative damage/glycosylases for thymine glycol and uracil/α-hydroxythymine)

MONICA C. HOLLSTEIN†, PETER BROOKS†, STUART LINN, AND BRUCE N. AMES

Department of Biochemistry, University of California, Berkeley, CA 94720

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ABSTRACT **An activity has been purified 350-fold from extracts of mouse plasmacytoma cells that forms 5-hydroxymethyluracil (α-hydroxythymine) and apyrimidinic sites with phage SPO1 DNA, which contains this base in place of thymine. This DNA glycosylase presumably functions to eliminate hydroxymethyluracil, a major thymine-derived DNA lesion produced by ionizing radiation and oxidative damage. The enzyme has no cofactor requirement and is active in EDTA. Neither intermediate formation nor hydrolysis of hydroxymethyldeoxyuridine or hydroxymethyldeoxyuridine monophosphate was detected. The enzyme does not cleave apyrimidinic sites in DNA. It does release uracil from the uracil-containing DNA of phage PBS2, but this activity is less than 2% of the predominant uracil DNA glycosylase activity of the cell, which is separated by phosphocellulose chromatography. The major uracil DNA glycosylase does not release hydroxymethyluracil from SPO1 DNA. The hydroxymethyluracil glycosylase is also separated upon phosphocelluose chromatography from a thymine glycol DNA glycosylase activity that is accompanied by an apyrimidinic endonuclease activity.**

There is an increasing awareness of the importance of oxidative damage to DNA (1) which may be mediated primarily by highly reactive oxygen radicals such as the hydroxyl radical produced by ionizing radiation (2). Hydrated thymine derivatives, including 5,6-dihydroxythymine (thymine glycol), are recognized to be important products of ionizing radiation and are released from bacterial and mammalian DNA *in vivo* after treatment with various oxidative agents (3). DNA from γ-irradiated HeLa cells contains some 5-hydroxymethyluracil (hmU) (α-hydroxythymine) as well as thymine glycol (3, 4), but repair of hmU has not been described.

Demple and Linn demonstrated that *Escherichia coli* endonuclease III (5) has thymine glycol DNA glycosylase activity (6). Endonuclease activities that are specific for DNA damaged by ionizing radiation, high doses of UV irradiation, or OsO_4 have also been reported from eukaryotes (7–11), and thymine glycol DNA glycosylase activity has been reported to be present in a preparation of calf thymus urea DNA glycosylase (12). We report here that the "DNA repair endonuclease" preparation from mouse MPC-11 cells described by Nes (7) contains a mixture of thymine glycol DNA glycosylase and apyrimidinic endonuclease (AP endonuclease) activities. Moreover, we describe the isolation and initial characterization of a hmU DNA glycosylase activity from these cells. The latter activity, which is separated from any AP endonuclease activity, was identified by using as substrate DNA derived from the *Bacillus subtilis* phage SPO1, which contains hmU in place of thymine (13).

MATERIALS AND METHODS

Materials. Pyrimidine bases, DNAse I, DNAse II, and bacterial alkaline phosphatase (type III-S) were from Sigma; nucleosides and nucleotides were from P-L Biochemicals; snake venom phosphodiesterase and spleen phosphodiesterase were from Calbiochem; and *Eco*RI and T4 DNA ligase were from New England Biolabs. HeLa AP endonuclease (14) and *E. coli* endonuclease III (15) were gifts of Caroline Kane and Bruce Demple, respectively. ^{3}H-labeled hydroxymethyluracil deoxynucleoside (hmdU) and its 3′- and 5′-monophosphates (3′-hmdUMP and 5′-hmdUMP) were prepared by enzymatic digestion of SPO1 [^{3}H]DNA and subsequent purification by HPLC. [*methyl*-^{3}H]Thymidine, 16 Ci/mmol (1 Ci = 37 GBq), was from Schwarz/Mann.

Preparation of [^{3}H] DNAs. Phage PBS2 [^{3}H]DNA (74,000 cpm/nmol of uracil) (16) and partially depurinated phage PM2 [^{3}H]DNA (13,400 cpm/nmol of nucleotide) (17) were prepared as described. PM2 DNA was irradiated on ice with a 254-nm lamp at 2 J/m^2·sec with a total dose of 1260 J/m^2. PM2 [^{3}H]DNA was treated at 0°C with 0.2 M NaOH/0.4 M NaCl/2% (vol/vol) glycerol. After 10 min at 0°C, 1.5 ml of the solution was neutralized with 0.5 ml of 0.58 M HCl/80 mM Hepes (Na^+), pH 7.9/3.3 M NaCl/4 mM EDTA to form the denatured form I_d DNA (18); then 4 ml of 0.3% OsO_4 in 0.4 M NaCl was added. After 20 min at 25°C, the DNA was extracted three times with 3 vol of ether, renatured by adding 6 ml of 12 mM NaOH/0.16 M 3-(cyclohexylamino)-1-propanesulfonic acid (Na^+), pH 11.0/3 M NaCl (18); then, after 15 min, it was neutralized with 3 ml of 0.26 M HCl/100 mM Hepes (Na^+), pH 7.9/8% (vol/vol) glycerol. Nonrenatured DNA was removed by nitrocellulose filtration and, after dilution to 0.4 M NaCl, the DNA was concentrated by precipitation with 2 vol of ethanol. Final recovery was 56%, of which 85% was form I_d with 0.3% of the thymine oxidized; the product was not sensitive to AP endonuclease.

^{3}H-labeled phage λ DNA (10,400 cpm/nmol of nucleotide) was prepared from the lysogen *E. coli* W3110 *c*I857S7 (thymine-requiring), induced at 42°C in the presence of [*methyl*-^{3}H]thymidine. ^{3}H-labeled SPO1 DNA (59–430 cpm/pmol of hmdU) was prepared as described by Cregg and Stewart (19). Briefly, *B. subtilis* 168M was grown in CHT medium (20) and infected with bacteriophage SPO1 (provided by E. P. Geiduschek, University of California, San Diego), with a multiplicity of infection of 10 and [6-^{3}H]uridine (New England Nuclear, 25 Ci/mmol) was added to a concentration of 5 μCi/ml. After differential centrifugation, phage were purified by CsCl density gradient centrifugation and extracted with phenol; then the DNA was dialyzed against 20 mM Tris·HCl, pH 7.5/1 mM EDTA. After digestion with DNase I, venom phosphodiesterase, and bacterial alkaline phosphatase, 44% of the radioactivity was in deoxycytidine, 56% in hmU.

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Abbreviations: hmU, 5-hydroxymethyluracil (α-hydroxythymine); hmdU, 5-hydroxymethyluracil deoxynucleoside; hmdUMP, 5-hydroxymethyl deoxynucleoside monophosphate; form I_d DNA, "irreversibly" covalently closed circular duplex DNA; AP endonuclease, apyrimidinic endonuclease.

†Present address: International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon, France.

Circularization of SPO1 DNA. SPO1 [^{3}H]DNA was digested with *Eco*RI* in a reaction mixture containing 25 mM Tris·HCl at pH 8.6, 2 mM $MgCl_2$, 1 mM dithiothreitol, acetylated bovine serum albumin at 0.1 mg/ml, 67 μM DNA nucleotide, and *Eco*RI at 1 unit/μl for 6.5 hr at 37°C to yield the expected digest (21). After incubation at 70°C for 3 min, the reaction was adjusted to 3 μM DNA nucleotide, 50 mM Tris·HCl at pH 7.8, 10 mM $MgCl_2$, acetylated bovine serum albumin at 0.1 mg/ml, 1 mM ATP, and T4 DNA ligase at 0.03 unit/ml (cohesive end ligation units as defined by New England Biolabs). After 10 hr at 15°C, 5.5 hr at 0°C, and 21.5 hr at 15°C, the solution was adjusted to 2.1 M ammonium acetate and the DNA was precipitated with 2 vol of ethanol. Enrichment for covalently closed circles was by denaturation in 325 mM NaCl/30 mM NaOH, pH 12.4, for 4 min at room temperature, neutralization by adjustment to 0.5 M ammonium acetate, and filtration through nitrocellulose. The final product contained 86% covalently closed circles with an average size of 2500 base pairs.

Endonuclease Assays. Reaction mixtures at 37°C contained for "UV endonuclease": 40 mM Tris·HCl at pH 8.0, 100 mM KCl, 3 mM EDTA, 10 mM 2-mercaptoethanol, acetylated bovine serum albumin at 0.05 mg/ml, and 40 μM UV-irradiated PM2 DNA. For AP endonuclease they contained 40 mM Tris·HCl at pH 7.5, 50 mM KCl, 3 mM EDTA, 0.5 mM dithiothreitol, acetylated bovine serum albumin at 0.05 mg/ml, 40 μM depurinated PM2 [^{3}H]DNA, and 10 mM $MgCl_2$ where indicated. Strand breaks were quantified by nitrocellulose filtration (17). One unit of endonuclease produces 1 fmol of strand breaks per min.

DNA Glycosylase Assays. To monitor hmU base release, reaction mixtures contained 40 mM Tris·HCl at pH 8.0, 2 mM EDTA, enzyme, and 40 μM SPO1 [^{3}H]DNA unless otherwise noted. After incubation at 37°C for 30–90 min, unlabeled markers were added and, to remove DNA and protein, reactions were immediately applied to Amicon MPS-1 filter holders containing Amicon YMT membranes (no. 40402) and centrifuged for 20 min at 1000 × *g* at 4°C. The filtrate (25–75 μl) was injected with a U6K injector onto a 4.6 × 250 mm, 5-μm particle C_{18} column (Supelco) for HPLC separation using Waters Associates 6000A pumps, solvent programmer, and absorbance detector and a Nelson analytical model 4416 data system for recording UV absorption. Elution was with solvent A (25 mM potassium phosphate, pH 7.0) at 0.7 ml/min for 12 min and then 1 ml/min at minute 13. This was followed by a linear gradient at 1 ml/min of 100% solvent A to 20% solvent B [solvent B is methanol/solvent A, 50:50 (vol/vol)] at minute 20, 40% solvent B at minute 25, and 50% solvent B at minute 30. The eluate was monitored at 254 nm and fractions were collected directly into scintillation vials and their radioactivities were measured. One unit of glycosylase releases 1 pmol of hmU per min at 37°C.

To monitor thymine glycol base release, 100-μl reaction mixtures contained 10 mM Tris·HCl at pH 8.0, 100 mM KCl, 3 mM EDTA, 10 mM 2-mercaptoethanol, acetylated bovine serum albumin at 0.05 mg/ml, 560 μM OsO_4-treated PM2

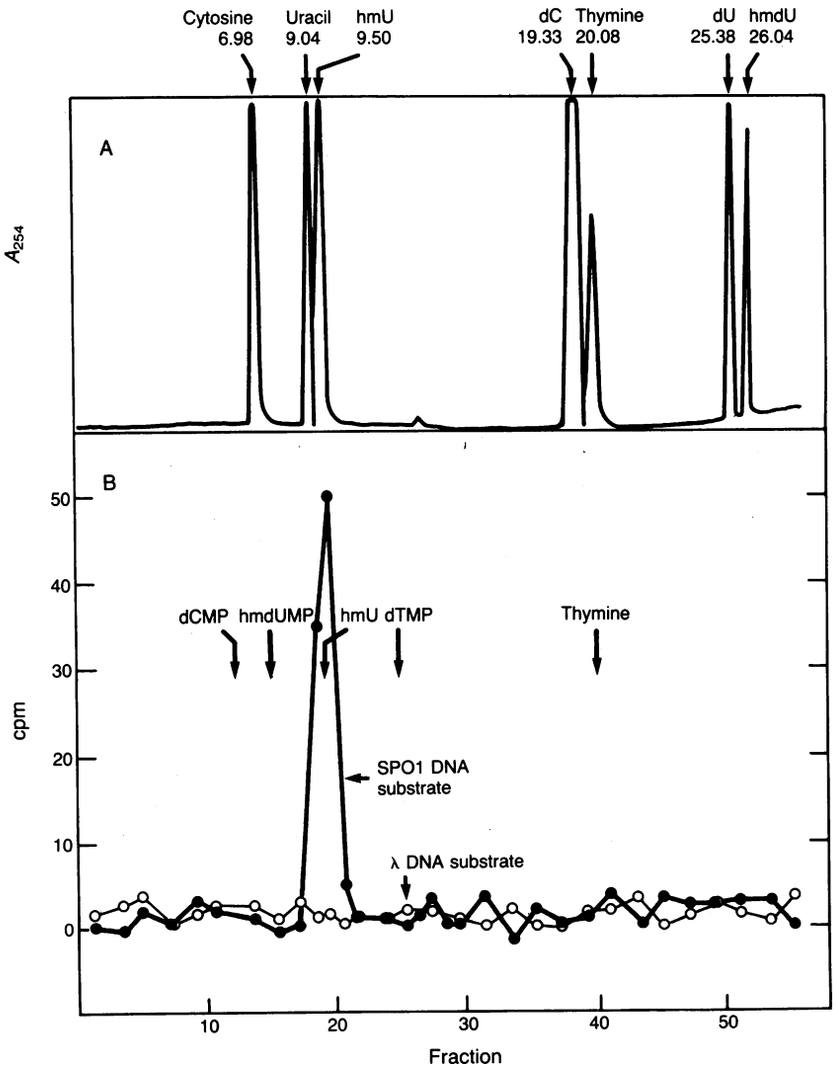

FIG. 1. (*A*) HPLC reverse-phase separation of pyrimidine bases and deoxynucleosides. Separation with a phosphate buffer/methanol gradient is described in *Materials and Methods*. A full-scale deflection equals an A_{254} of 0.01. The numbers under the compounds are retention times in min. (*B*) Enzymatic release of hmU base. Radioactive product released from SPO1 [^{3}H]DNA by fraction III was run on a C_{18} column as described in *Materials and Methods* (●). Sixty 0.5-min fractions were collected during the 30-min gradient. hmU and thymine eluted in fractions 19 and 41, respectively, as identified by monitoring of UV absorption of added unlabeled bases during each separation. No significant radioactivity coeluted with thymine when [^{3}H]thymine-containing λ DNA was substituted for SPO1 DNA (○).

[^{3}H]DNA, and 21 units of endonuclease III or 18 units of the MPC-11 mouse cell UV endonuclease eluting at 0.35 M KCl from phosphocellulose. Strand breaks were determined by the nicked circle assay, using 2 μl of the reaction mixture. To the remainder were added 12.5 μg of thymine glycol and 40 μg of salmon sperm DNA; after adjustment to 0.5 M NaCl, DNA was precipitated with ethanol. Supernatants were evaporated and the residues were resuspended in water and injected onto the HPLC column. The samples were eluted with water at 1 ml/min, absorbance at 254 nm was monitored, and 0.5-ml fractions were collected for scintillation counting. Thymine glycol and thymine eluted with 4.5 and 16 ml of water, respectively.

To monitor uracil base release, 100-μl reaction mixtures contained 40 mM Tris·HCl at pH 8.0, 2 mM EDTA, 1.3 μM PBS2 [*uracil*-^{3}H]DNA, and 20 μl of enzyme. After 45 min at 37°C, reaction mixtures were filtered through YMT membranes and 40 μl of filtrate was removed for scintillation counting. Where filtrates contained significant ^{3}H, unlabeled uracil was added to the remaining filtrate, and the identity of the ^{3}H with uracil base was verified by HPLC.

Reaction mixtures to monitor apyrimidinic site formation contained 40 mM Tris·HCl at pH 8.0, 50 mM KCl, 3 mM EDTA, 0.5 mM dithiothreitol, acetylated bovine serum albumin at 0.05 mg/ml, 0.2 μM circularized SPO1 [^{3}H]DNA, and enzyme in 50 μl. After 10–30 min at 37°C, the DNA was probed for apyrimidinic sites by either (*i*) incubation for 3 min at 70°C, adjustment to 12 mM $MgCl_2$, addition of 0.2 unit of HeLa cell AP endonuclease, and, after 30 min at 37°C, quantitation of strand breaks by the nicked circle assay; or (*ii*) addition of 150 μl of 0.01% sodium dodecyl sulfate/20 mM EDTA and 200 μl of 0.3 M potassium phosphate at pH 12.35 and incubation at room temperature for 3–12 hr before neutralization and quantitation of strand breaks as above.

Alkaline Sucrose Gradient Centrifugation. Twenty-five microliters of [^{3}H]DNA was mixed with 25 μl of 0.4 M NaOH, incubated 3.5 hr at room temperature, then layered onto a 5–20% gradient of sucrose in 0.2 M NaOH. After centrifugation at 4°C for 2.5 hr at 45,000 rpm in a Beckman SW 50.1 rotor, 0.25-ml fractions were collected from the tube bottom and neutralized, and radioactivity was determined.

RESULTS

Assays of hmU DNA Glycosylase. Phage SPO1 DNA, which contains hmU in place of thymine, was an extremely convenient substrate for studying this activity. After incubation of this DNA with extracts, samples were put through YMT filters to remove DNA and protein, and then the products were separated by HPLC under conditions that resolve hmU and cytosine and their respective deoxynucleosides and deoxynucleotides (Fig. 1*A*). When SPO1 DNA labeled in both hmU and cytosine by growth in [6-^{3}H]uridine was incubated with extracts from mouse plasmacytoma cells, radioactivity was released in the form of free hmU. With crude fractions approximately 50% of the radioactivity in YMT filtrates was in hmU, but with pure fractions at least 80% of the radioactivity in the filtrates migrated as hmU (Fig. 1*B*).

Enzyme activity could also be quantitated by measuring the apyrimidinic sites produced in covalently closed circular SPO1 DNA produced by ligation of fragments generated by *Eco*RI*. After incubation of these circles with enzyme, they were treated with either alkali or AP endonuclease to cleave apyrimidinic sites that had been produced; then strand breaks were quantitated by nitrocellulose filtration (see Fig. 2*B*).

Enzyme Fractionation. hmU DNA glycosylase activity could be purified from cells extracted by nitrogen cavitation as described by Nes (7), but sonication in high salt yielded approximately 3 times the activity. When the sonic extract was fractionated by Sephacryl S-200 chromatography and ammonium sulfate precipitation and then chromatographed upon phosphocellulose (Table 1), the endonuclease activity that recognizes thymine glycol residues that Nes has described (7) eluted at 0.35 M KCl, the major uracil DNA glycosylase activity eluted at 0.27 M KCl, while hmU DNA glycosylase eluted at 0.06 M KCl (Fig. 2*A*). The major Mg^{2+}-dependent AP endonuclease eluted at 0.19 M KCl and a Mg^{2+}-independent AP endonuclease cochromatographed with the endonuclease described by Nes.

An activity generating sites in SPO1 DNA circles sensitive to phosphodiester bond cleavage by alkali or AP endonuclease coeluted with activity releasing hmU (Fig. 2*B*) as expected for a DNA glycosylase. On the other hand, this fraction contained no detectable endonuclease when assayed with untreated or depurinated SPO1 or PM2 DNA circles in the absence of subsequent alkali or AP endonuclease, and baseless sites were not generated in PM2 DNA (see Fig. 2*B*). Fraction IV was stable in 50% glycerol at −20°C.

Substrate Specificity of the hmU DNA Glycosylase. Since SPO1 DNA contains approximately equal amounts of [^{3}H]hmU and [^{3}H]cytosine, it provides an internal control for nonspecific base release. As shown in Fig. 1, fraction IV released only hmU from SPO1 DNA: no radioactivity eluted at positions corresponding to cytosine, deoxycytidine, hmdU, dCMP, or hmdUMP, and no [^{3}H]thymine release was detected when [^{3}H]thymidine-labeled phage λ DNA replaced SPO1 DNA. Also, fraction IV did not form hmU from 3′-hmdUMP, 5′-hmdUMP, or hmdU, nor did it generate baseless sites from [^{3}H]thymidine-labeled unirradiated or UV-irradiated DNA.

Free uracil was released from PBS2 DNA by fraction IV. The apparent K_m values are approximately 0.05 μM and

Table 1. Purification of hmU DNA glycosylase from MPC-11 mouse cells

Fraction	Protein, mg	Total activity, units	Specific activity, units/mg
I. Crude extract	104	21.2	0.20
II. Sephacryl S-200	4.3	7.4	1.7
III. Ammonium sulfate	2.4	5.6	2.3
IV. Phosphocellulose (peak fraction 23)	0.016	1.2	72

The growth and harvesting of cells and enzyme purification procedures were adapted from Nes (7). Cells (6.5×10^9) were thawed after storage over liquid nitrogen, pelleted, and resuspended in 33 ml of 10 mM Tris·HCl, pH 7.5/0.1 M KCl/1 mM EDTA/7.5% sucrose/10 mM 2-mercaptoethanol. The suspension was adjusted to 0.7 M KCl with 3 M KCl/10 mM Tris·HCl, pH 8.2/1 mM EDTA/10 mM 2-mercaptoethanol and sonicated for 20 s with a Branson large probe. After adjustment to 0.3 M KCl with 20 mM Tris·HCl, pH 8.2/0.5 mM EDTA/10 mM 2-mercaptoethanol, the extract was sonicated for 30 s longer, gently stirred for 2 hr at 0°C, and centrifuged at 4°C for 20 min at 27,000 × *g*. The supernatant (fraction I) was concentrated to 11 ml in an Amicon Diaflow apparatus with a PM-10 membrane and then applied to a 2.5 × 80 cm Sephacryl S-200 column equilibrated with 0.3 M KCl/20 mM Tris·HCl, pH 8.0/0.1 mM EDTA/10 mM 2-mercaptoethanol and eluted with the same buffer into 6-ml fractions. Fractions 39–44 were pooled (fraction II). To 25 ml of fraction II was slowly added 58 ml of neutralized 3.9 M ammonium sulfate with stirring at 0°C; after stirring for 30 min, the suspension was centrifuged at 4°C for 30 min at 27,000 × *g*. The pellet was resuspended in 1.8 ml of 20 mM Tris·HCl, pH 8.0/0.1 mM EDTA/10 mM 2-mercaptoethanol (buffer A) and dialyzed against buffer A (fraction III). A 0.5-ml aliquot of fraction III was applied to a 0.8 × 35 cm phosphocellulose column equilibrated with buffer A. After washing with buffer A the column was eluted with a 20-ml gradient from 0 to 0.45 M KCl in buffer A and elution was continued with 0.45 M KCl. Fractions (0.54 ml) were mixed with 0.5 ml of glycerol and stored at −20°C (fraction IV). Protein was assayed according to Bradford (22).

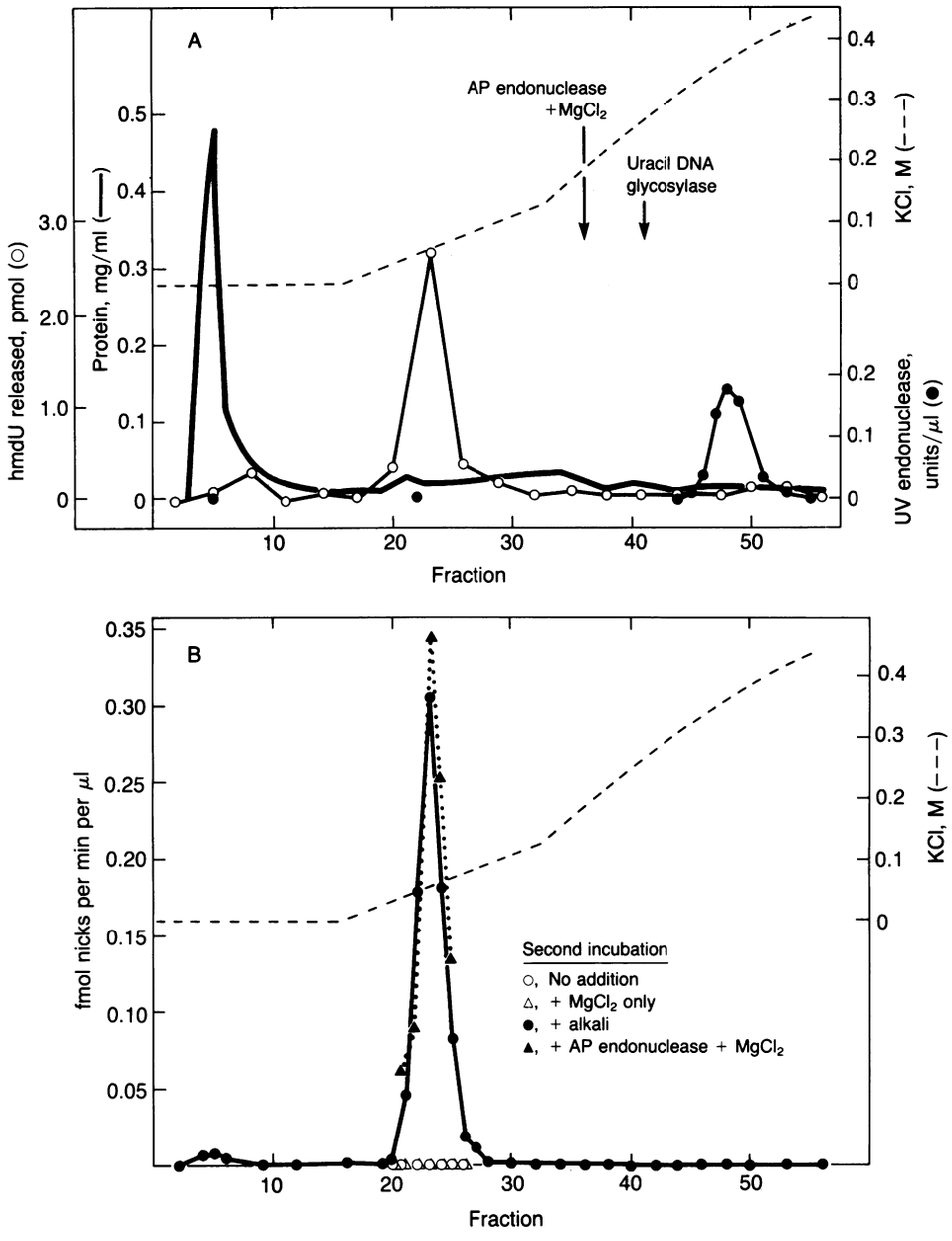

FIG. 2. Phosphocellulose chromatography of fraction III. (*A*) Elution and assays with 40 μl of eluate were described in Table 1 and *Materials and Methods*, respectively. ——, Protein; ●—●, UV endonuclease activity; ○—○, hmU DNA glycosylase activity. Arrows show peak positions of the major uracil glycosylase and AP endonuclease activities, each of which separated from the UV endonuclease and hmU glycosylase. (*B*) AP sites generated in circularized SPO1 DNA after incubation with column fractions as indicated were monitored after a second incubation with no addition (○), alkali (●), $MgCl_2$ plus AP endonuclease (▲), or $MgCl_2$ without AP endonuclease (△). Fractions 20–26 catalyzed no detectable nicking of PM2 DNA substrate with or without a second incubation with alkali or AP endonuclease (data not plotted for the sake of clarity).

0.012 μM for DNA uracil and DNA hmU, respectively. The amount of uracil DNA glycosylase activity detected in fraction IV was less than 2% of that of the major uracil DNA glycosylase, which cleanly separated from the hmU glycosylase on phosphocellulose (it elutes at 0.27 M vs. 0.06 M KCl) and which had a K_m of 0.3 ± 0.1 μM DNA uracil, a value in agreement with that reported by Kuhnlein *et al.* (23) for the major human uracil DNA glycosylase. The small amount of uracil DNA glycosylase of fraction IV could be a property of the hmU glycosylase or it could be a contaminating enzyme. It is not the major uracil glycosylase activity of the cell but could be a minor form, as has been described in human lymphocytes (24) and KB cell mitochondria (25).

Baseless DNA Sites Are Also Reaction Products. Fraction IV was observed to render duplex circles of SPO1 DNA sensitive to cleavage by either alkali or AP endonuclease (Fig. 2*B*); without alkali or AP endonuclease, insignificant nicking was observed. To determine the stoichiometry between base release and nicks subsequently generated by alkali, a reaction mixture containing fraction IV and linear SPO1 DNA was incubated until 7.2 pmol of hmU was released; then the DNA was exposed to alkali and analyzed by sedimentation through alkaline sucrose. The observed conversion from an average sedimentation coefficient of 23.7 S to 4.7 S corresponded to 7.6 pmol of nicks having been formed by the alkali (calculated from the distribution of fragments, not the mean sedimentation value). As expected, the enzyme did not generate alkali-labile sites in λ DNA. In summary, after incubation of SPO1 DNA with the enzyme, alkali or AP endonuclease is required to nick the DNA, and for each hmU released by the enzyme, one nick is introduced.

Thymine Glycol DNA Glycosylase. Since *E. coli* endonuclease III is known to recognize and remove thymine glycol from DNA and then nick the resulting AP sites, the MPC-11 mouse cell UV endonuclease, which eluted at 0.35 M KCl from phosphocellulose (Fig. 2*A*), was tested for thymine glycol DNA glycosylase activity. A supercoiled substrate was chosen because the lability of thymine glycol in alkali (26) obviated the use of alkaline sucrose sedimentation for detecting strand breakage; moreover, the UV endonuclease is reported to require a supercoiled substrate (27). Therefore, PM2 DNA treated with OsO_4 was incubated with the 0.35 M KCl eluate, and then base release was measured by HPLC analysis and endonuclease by the nicked circle assay (Table

Table 2. UV endonuclease has thymine glycol glycosylase and AP endonuclease activity

Enzyme	Nicks generated per PM2 DNA molecule	Thymine glycol molecules released per PM2 DNA molecule	Nicks per thymine glycol released
MPC-11 UV endonuclease	0.33	0.18	1.8
E. coli endonuclease III	2.23	1.17	1.9

Assays of thymine glycol released and nicks put into PM2 DNA treated with OsO_4 by the 0.35 M KCl phosphocellulose eluate (Fig. 2*A*) or *E. coli* endonuclease III are described in *Materials and Methods*.

2). Thymine glycol base was released both by the mouse UV endonuclease and by *E. coli* endonuclease III. Moreover, strand cleavage was effected by AP endonuclease activities, which are present in both the bacterial and mammalian preparations. In both cases, approximately two strand breaks were detected for each thymine glycol released. It is likely that the alkali renaturation following OsO_4 treatment of the form I_d PM2 DNA resulted in hydrolysis of some thymine glycol residues to other products, including urea, which are also recognized by *E. coli* endonuclease III (28, 29) and possibly by the mouse enzyme, since a preparation from calf thymus contains both urea and thymine glycol DNA glycosylase activities (12). Removal of these secondary lesions would not have been monitored by HPLC but would generate apyrimidinic sites. In conclusion, the mouse UV endonuclease, like *E. coli* endonuclease III, appears to be a combination of thymine glycol DNA glycosylase and AP endonuclease that is separable from the hmU DNA glycosylase.

DISCUSSION

Several DNA glycosylases have been isolated that are specific for repair of oxidatively damaged bases: thymine glycol and urea glycosylase from calf thymus and human cells (12) and from *E. coli* (16, 29, 30) and formamidopyrimidine glycosylase from rodents (31) and from *E. coli* (32), which are thought to remove ring-opened purines formed by hydroxyl radicals. This report adds a mouse hmU DNA glycosylase to the list. While it is not clear whether the mammalian urea and thymine glycol DNA glycosylase activities and the accompanying AP endonuclease all reside in the same protein, the mouse hmU glycosylase is completely resolved from the thymine glycol glycosylase and from AP endonuclease. The demonstration of another DNA glycosylase whose substrate is an oxidized DNA base provides a probe for measuring oxidative damage in DNA and gives further evidence for the significance of this type of damage. That such damaged bases are routinely excised *in vivo* is evidenced by the appearance and subsequent disappearance of hydrated thymines from the DNA of cells treated with UV or ionizing radiation (3) and the presence of thymine glycol in human and rat urine (33).

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