An Enzyme Activity from *Escherichia coli* That Attacks Single-Stranded Deoxyribopolymers and Single-Stranded Deoxyribonucleic Acid Containing Apyrimidinic Sites†

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ABSTRACT: We have isolated an enzyme activity from extracts of *Escherichia coli* that catalyzes the hydrolysis of phosphodiester bonds in the single-stranded deoxyribopolymer (dU·[^{3}H]dT)$_{(2000)}$ containing depyrimidinated sites created by enzymatic removal of uracil with uracil–DNA glycosylase. Nondepyrimidinated polymer is not degraded by the enzyme, nor is the depyrimidinated polymer degraded after reduction of apyrimidinic sites with sodium borohydride. The enzyme also degrades circular M13 DNA containing uracil or denatured phage PBS2 DNA (which naturally contains uracil) only after the removal of uracil from these substrates by uracil–DNA glycosylase. Undamaged or depurinated duplex PM2 or ColE1 DNA and undamaged M13 single-stranded DNA are not attacked by the enzyme. The activity sediments in glycerol gradients with a relative *s* value of 4.2 S and has a Stokes radius of 31 Å, yielding a calculated M_r ~56000. The fractional ratio (f/f_0) is calculated at 1.25. The enzyme has no requirement for any known cofactors and is insensitive to inhibition by *p*-(chloromercuri)benzoate. Activity is inhibited in the presence of adenosine 5′-triphosphate (ATP), tRNA, or high ionic strength and is slightly stimulated by $MnCl_2$ or $CaCl_2$.

The loss of purines or pyrimidines from DNA creates apurinic or apyrimidinic sites, respectively, collectively referred to as AP sites.[1] The spontaneous loss of bases from DNA is believed to occur at biologically significant rates in living cells (Lindahl & Nyberg, 1972; Lindahl & Karlstrom, 1973). In addition, UV[2] or ionizing irradiation of DNA, as well as treatment with certain chemicals (particularly, monofunctional alkylating agents), promotes base loss either directly or by the enzymatic excision of damaged bases. The latter is effected by a class of enzymes called DNA glycosylases that catalyze the hydrolysis of the *N*-glycosylic bonds linking the bases to the deoxyribose–phosphate backbone of DNA [see Friedberg et al. (1978) and Lindahl (1979) for recent reviews]. Several endodeoxyribonucleases that catalyze the hydrolysis of phosphodiester bonds at or near AP sites in DNA have been identified from both prokaryote and eukaryote sources (Friedberg et al., 1977, 1981; Lindahl, 1979). None of these enzymes has been shown to discriminate between sites of purine or pyrimidine loss, and hence, they are referred to simply as AP endonucleases.[1] In light of the observation that sites of base loss are potentially lethal and/or mutagenic (Drake, 1970; Shearman & Loeb, 1979), it is believed that such enzymes are involved in the repair of AP sites in DNA in vivo.

Quantitatively, the principal AP endonuclease in extracts of *Escherichia coli* is designated as the AP endonuclease function of exonuclease III (Gossard & Verly, 1978; Verly, 1978). Genetic (Milcarek & Weiss, 1972; Yajko & Weiss, 1975) and biochemical (Weiss, 1976, 1981) evidence indicates that this activity is one of multiple catalytic functions associated with exonuclease III (Richardson et al., 1964), a product of the *xth* gene (Milcarek & Weiss, 1972; Yajko & Weiss, 1975).[3] A second AP endonuclease (endonuclease IV) (Ljungquist, 1977) has been identified in extracts of *E. coli* strains carrying deletions of the *xth* gene. In addition, endonucleases III (Radman, 1976; Gates & Linn, 1977a) and V (Gates & Linn, 1977b) of *E. coli* have been reported to catalyze the hydrolysis of phosphodiester bonds in DNA containing sites of base loss, although neither enzyme shows strict specificity for such lesions.

All of the above-mentioned enzymes have been shown to degrade *duplex* DNA containing AP sites. We report here the partial purification and preliminary characterization of an AP endonuclease activity from *E. coli* that catalyzes the degradation of single-stranded DNA or single-stranded deoxyribopolymers containing depyrimidinated sites but does not degrade duplex DNA containing apurinic sites or undamaged single-stranded or duplex DNA. The physical and biochemical properties of this enzyme suggest that it may be distinct from previously described AP endonucleases of *E. coli*; however, until this activity has been more extensively investigated we have decided not to designate the enzyme in terms of the standard nomenclature employed for *E. coli* endonucleases. Thus for the purposes of this report we refer to this enzyme activity as single-strand (SS) AP endonuclease.[4]

Materials and Methods

Bacterial Strains. SS AP endonuclease was isolated from *E. coli* strain BD1118, a mutant deficient in Ura–DNA glycosylase activity (*ung*$^-$). Bacteriophage M13 was grown in *E. coli* BW313, an *Hfr* strain deficient in both dUPTase (*dut*$^-$)

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[1] At The International Conference on "DNA Repair Mechanisms" at Keystone, CO, Feb 1978, it was agreed that endodeoxyribonucleases specific for sites of base loss in DNA (apurinic and apyrimidinic sites) would be referred to by the common name of AP endonucleases and the substrate sites recognized by such enzymes as AP sites.

[2] Abbreviations: UV, ultraviolet; Ura–DNA glycosylase, uracil–DNA glycosylase; dUTPase, deoxyuridine triphosphate hydro-lyase; dUTP, deoxyuridine 5′-triphosphate; dTTP, thymidine 5′-triphosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

[3] Available evidence suggests that exonuclease III is almost certainly the same enzyme as that originally termed endonuclease II of *E. coli*. For a clarification of this nomenclatural confusion the interested reader is referred to Verly (1978) and Friedberg et al. (1981).

[4] In preliminary publications we have referred to this activity as endonuclease VII of *E. coli*.

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and Ura–DNA glycosylase (*ung*⁻), or in *E. coli* BW273 (*Hfr dut*⁺ *ung*⁺). Both strains were obtained from Dr. B. Weiss, Department of Microbiology, Johns Hopkins University. Bacteriophage PM2 was grown in *Alteromonas* BAL31, both obtained from Dr. Stuart Linn, Department of Biochemistry, University of California, Berkeley. *Bacillus subtilis* strain SB168 was used both for the purification of Ura–DNA glycosylase and for the propagation of bacteriophage PBS2.

Substrates. (1) Deoxyribopolymers with Intact Bases. A single-stranded deoxyribopolymer containing a random mixture of uracil and thymine was prepared with calf thymus terminal deoxynucleotidyltransferase. The procedure for preparing (dU·[^{3}H]dT)$_{(2000)}$ was essentially as described by Bollum et al. (1964). Reaction mixtures (0.2 mL) contained 40.0 mM KCl, 0.2 M cacodylate buffer, pH 7.2, 1.0 mM $CoCl_2$, 1.0 mM 2-mercaptoethanol, 11.3 nmol (as nucleotide) of d(pT)$_{(34)}$, 0.42 μmol each of dUTP and dTTP (Sigma), 0.5 mCi of [*methyl*-^{3}H]thymidine 5′-triphosphate (15.5 Ci/mmol, ICN), and 950 units of terminal transferase. Incubations were at 37 °C for 20 h, following which reactions were terminated by heating to 65 °C for 10 min. Polymer was isolated from the reaction mix by gel filtration through a Sephacryl S-200 column (0.5 × 15 cm) equilibrated in and eluted with 0.5 M KCl in 50.0 mM potassium phosphate buffer, pH 7.0. Fractions containing acid-precipitable material were pooled, and this material was shown to have an average size of about 2000 nucleotides by sedimentation velocity on alkaline sucrose gradients. The (dU·[^{3}H]dT)$_{(2000)}$ thus obtained had a specific radioactivity of between 200 and 400 cpm/pmol of nucleotide.

(2) Depyrimidinated Deoxyribopolymers. Depyrimidination of (dU·[^{3}H]dT)$_{(2000)}$ was effected enzymatically by preincubation of the intact polymer with purified *B. subtilis* Ura–DNA glycosylase (Cone et al., 1977; Cone & Friedberg, 1981). Preincubation mixtures (0.05 mL) contained 10.0 mM Tris-HCl, pH 7.5, 1.0 mM EDTA, 25.0 mM NaCl, 1.0 mg/mL bovine serum albumin, 0.2–0.5 nmol (as nucleotide) of polymer, and varying amounts of Ura–DNA glycosylase, depending on the extent of uracil excision required for a given experiment. Under these conditions, incubation at 37 °C for 10 min with 80 units of enzyme resulted in the excision of sufficient uracil from 0.5 nmol (as nucleotide) of (dU·[^{3}H]-dT)$_{(2000)}$ to render the polymer totally acid soluble after incubation with sodium hydroxide.

(3) Undamaged DNAs. DNA from bacteriophage PBS2 was labeled with [5-^{3}H]uracil and purified as previously described (Friedberg et al., 1975). Phage M13 was grown in *E. coli* strain BW273 (wild type) or in strain BW313 (*dut*⁻ *ung*⁻) in the presence of [*methyl*-^{3}H]thymidine at 5.0 or 10.0 μCi/mL. The latter strain carries mutations in the genes coding for dUTPase and for Ura–DNA glycosylase, thereby facilitating the incorporation and retention of uracil in M13 DNA during its replication. M13 phage were harvested and DNA was isolated and purified as described by Marvin & Schaller (1966). [^{3}H]Thymine-labeled ColE1 DNA (sp act. 7.4×10^4 cpm/μg) was a gift from P. Seawell, Department of Biological Sciences, Stanford University. [^{3}H]Thymine-labeled PM2 DNA (sp act. 5×10^4 cpm/μg) was prepared according to the procedure given by Espejo et al. (1969).

(4) Depurinated or Depyrimidinated DNAs. Duplex circular PM2 or ColE1 DNA was depurinated by dialyzing labeled DNA (5.3 nmol as nucleotide) against 0.1 M NaCl–0.01 M sodium acetate, pH 5.5, for 1 h at 4 °C, with several changes. The DNA was then heated in a water bath at 60 °C for 60 min and dialyzed at 4 °C against 30 mM potassium phosphate buffer, pH 7.0, with 5.0 mM EDTA and 0.1 mM dithiothreitol. This treatment resulted in the production of approximately one AP site per molecule of DNA. Depyrimidinated M13 DNA was prepared by enzymatic removal of incorporated uracil by incubation with Ura–DNA glycosylase. Reactions were terminated and deproteinized by the addition of 2 volumes of cold buffered (pH 7.9) phenol. The DNA was extracted with chloroform–isoamyl alcohol (20:1 v/v) and then with anhydrous ether and extensively dialyzed against 10.0 mM potassium phosphate buffer, pH 7.0. Exposure of this M13 DNA to 0.25 N NaOH at 37 °C for 60 min resulted in extensive degradation as determined by neutral agarose gel electrophoresis (see below), indicating the presence of alkali-labile depyrimidinated sites; however, we were not able to quantitate the number of such sites per M13 DNA molecule. M13 DNA not containing uracil was subjected to the same incubation and purification procedure and shown to contain no alkali-labile sites.

Single-stranded depyrimidinated PBS2 DNA was prepared as follows. [^{3}H]DNA labeled in uracil was denatured by incubation in 0.25 N NaOH for 10 min at room temperature and then quenched to pH 7.0 with HCl at 0 °C. The resultant product was shown to be 90% digested to acid-soluble product by the single-stranded DNA-specific nuclease (S_1 nuclease) of *Aspergillus oryzae*. Depyrimidination of this denatured DNA was effected by enzymatic removal of uracil as described above. Reactions were terminated by the addition of an equal volume of buffered phenol (pH 7.0). The PBS2 DNA was extracted successively with phenol, chloroform–isoamyl alcohol (20:1 v/v), and anhydrous ether and was then dialyzed against 10.0 mM Tris-HCl, pH 7.5, plus 5.0 mM EDTA.

Enzymes. Ura–DNA glycosylase (fraction V) was prepared by the procedure of Cone & Friedberg (1981). This fraction was free of detectable single- and double-strand endonuclease activity as determined by the absence of degradation of M13 and ColE1 DNA, respectively, measured by agarose gel electrophoresis (see below). Endonuclease III of *E. coli* (fraction VII) (Gates & Linn, 1977a) was generously provided by Dr. S. Linn, University of California, Berkeley. Endonuclease IV of *E. coli* (fraction V) (Ljungquist, 1977) was a gift from Dr. T. Lindahl, University of Göteborg, Sweden, terminal deoxynucleotidyl transferase was kindly supplied by Dr. R. L. Ratliff, Los Alamos Scientific Laboratory, and S_1 nuclease of *Aspergillus oryzae* was purchased from Miles Laboratories, Elkhart, IN.

Assay of SS AP Endonuclease. The standard assay of SS AP endonuclease measures the release of acid-soluble radioactivity from depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$ relative to that released from intact (nondepyrimidinated) polymer. For measurement of activity, 15.0 μL of a 10× reaction mixture containing 0.3 M potassium phosphate, pH 7.0, 50.0 mM EDTA, 1.0 mM dithiothreitol, and 2 mg/mL bovine serum albumin was added to the depyrimidinated or control substrates (0.2–0.5 nmol as nucleotide) along with a limiting amount of enzyme. The reaction volume was brought to 0.15 mL by the addition of water. After incubation at 37 °C for 90 min, 0.075 mL of bovine serum albumin (10 mg/mL) and 0.075 mL of 20% cold trichloroacetic acid were added to each reaction. The tubes were kept on ice for at least 5 min and then centrifuged at 2000 rpm for 10 min in a Sorvall GLC tabletop centrifuge. Aliquots (0.15 mL) of the supernatants were dispensed into liquid scintillation vials, to which were added 1.0 mL of water and 10.0 mL of a Triton X-100/toluene based liquid scintillation mixture. Radioactivity was measured in a Beckman LS230 or LS250 liquid scintillation spectrometer.

As a check on the extent of depyrimidination of the polymer, 5.0 μL of 5.0 N NaOH was added to the reaction mixture in lieu of enzyme and incubated at 37 °C for 90 min. The depyrimidination conditions used rendered 95–100% of the polymer acid soluble after NaOH treatment, while alkali treatment of intact polymer did not result in the release of measurable acid-soluble radioactivity. One unit of enzyme activity is defined as the amount of enzyme that renders 1.0 pmol of depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ acid soluble in 90 min under standard reaction conditions.

Assay of Pyrimidine Dimer–DNA Glycosylase Activity. Pyrimidine dimer–DNA glycosylase activity in preparations of SS AP endonuclease was measured by the procedure of Radany et al. (1981) using *E. coli* DNA labeled with [^{3}H]-thymidine and UV irradiated to a thymine dimer content of 7% of the total thymine.

Borohydride Reduction of Sites of Base Loss in DNA or Deoxyribopolymer. Apyrimidinic sites are normally alkali labile but can be stabilized by reduction with sodium borohydride (Gossard & Verly, 1978). Reduction was carried out either prior to or subsequent to incubation with SS AP endonuclease. In both cases, samples were diluted 1:1 with 0.2 M sodium borate on ice. The final pH was approximately 9.5. Sodium borohydride (75 mg/mL in 50.0 mM NaOH) was prepared immediately before use and added to the diluted substrate to a final concentration of 7.5 mg/mL. Reactions were carried out on ice for 2 h. Controls for determining the extent of reduction were incubated with only 50 mM NaOH. One-tenth the reaction volume as 1.0 M H_3PO_4 was added to each tube to destroy excess borohydride, and tubes were kept at room temperature for an additional 2 h, or until bubbling stopped. The pH after the addition of H_3PO_4 was approximately 7.5. Samples were extensively dialyzed against 50.0 mM Tris-HCl and 1.0 mM EDTA, pH 7.5.

"s" Value Determination. The relative sedimentation coefficient of SS AP endonuclease was determined by sedimentation in 15–40% glycerol gradients (4.0 mL) in 10.0 mM potassium phosphate, pH 7.0, 0.25 M KCl, 1.0 mM EDTA, and 1.0 mM 2-mercaptoethanol. Aliquots (0.2 mL) of endonuclease were layered onto the gradients and centrifuged at 57 000 rpm for 24 h at 3 °C in a Beckman SW60 Ti rotor. Gradients were fractionated from the bottom with a peristaltic pump, and each 4-drop fraction was collected into 10.0 μL of bovine serum albumin (10.0 mg/mL). Individual fractions were assayed for SS AP endonuclease activity as well as for endonuclease activity on undamaged and depurinated ColE1 DNA. Marker proteins (bovine serum albumin, ovalbumin, and chymotrypsinogen) were sedimented under identical conditons.

Gel Filtration. Bio-Gel P100 (Bio-Rad) was swollen for 24 h in 20.0 mM Tris-HCl, pH 7.5, 0.25 M NaCl, and 1.0 mM EDTA, degassed, and then poured into a 1.5 cm (diameter) × 60 cm column to a final bed height of 55.6 cm. The column was equilibrated in the above buffer containing 10% glycerol. The column was calibrated with standard proteins [chymotrypsinogen (M_r 25 000), ovalbumin (M_r 45 000), and bovine serum albumin (M_r 67 000)], which were eluted at a flow rate of 10 mL/h. Blue dextran and phenol red were used to determine excluded and included volumes, respectively. Fraction V of the enzyme was eluted under identical conditions used for filtration of the marker proteins.

Agarose Gel Electrophoresis. Endonuclease activity on duplex supercoiled (form I) ColE1 or PM2 DNA was determined by measuring the conversion of form I to form II by electrophoresis through 0.8% agarose. Electrophoresis was in 50.0 mM Tris-HCl, 20.0 mM sodium acetate, 18.0 mM NaCl, and 2.0 mM EDTA, pH 8.0, at approximately 0.5 V/cm. Gels were stained for 30 min with 1.0 μg/mL ethidium bromide in water, and stained gels were illuminated with a short-wavelength UV source. Fluorescent bands were cut out of the gels, acidified, and melted before measuring radioactivity in 10.0 mL of a Triton X-100/toluene based liquid scintillation mixture. Enzyme activity on single-stranded M13 DNA was determined by electrophoresis through 1.4% agarose in the above-mentioned buffer. Electrophoresis of M13 DNA was for about 24 h at 1 V/cm after which the gels were stained with ethidium bromide as described above. The degradation of form I PM2 or ColE1 circular DNA to form II was quantitated and the number of breaks per molecule calculated from the first term of the Poisson equation $N/N_0 = e^{-x}$, where N = amount of DNA remaining as form I molecules, N_0 = total amount of DNA, and x = average number of breaks per molecule.

Protein Determination. Protein determinations were performed by the amido black procedure (Schaftner & Weissman, 1973) or by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Enzyme Purification. (1) Crude Extract. E. coli strain BD1118 (*ung*$^-$) is a Ura–DNA glycosylase deficient strain (kindly provided by Dr. Bruce Duncan, Fox Chase Cancer Center, Philadelphia) and was grown to mid or late log phase in an 80-L fermentor in YET medium (1% yeast extract, 1% NaCl, 0.5% tryptone) at 30 °C with forced aeration. Cells were harvested by centrifugation and stored at 20 °C until used. In a typical enzyme preparation 100 g of frozen cell paste was resuspended in 250.0 mL of 50.0 mM Tris-HCl, pH 7.9, 1.0 mM EDTA, and 10% sucrose. Lysozyme was added to a final concentration of 0.5 mg/mL, and the suspension was kept on ice for 60 min and then incubated at 37 °C for 5 min. The resulting lysate was centrifuged at 50 000 rpm for 1.5 h at 4 °C in a Beckman 60 Ti rotor. The supernatant (142 mL) was designated as fraction I.

(2) Ammonium Sulfate Precipitation. Saturated, neutralized ammonium sulfate was slowly added to fraction I at 0 °C with constant stirring to a final concentration of 35%. After gentle stirring on ice for 2 h, the suspension was centrifuged at 10 000 rpm for 20 min at 4 °C in the GSA rotor of a Sorvall RC5B centrifuge. The pellet was discarded and the supernatant brought to 50% saturation with ammonium sulfate. This suspension was maintained on ice with gentle stirring for 2 h and then centrifuged as just described. The resulting pellet was resuspended in 50.0 mM Tris-HCl, pH 7.5, to 66 mL and extensively dialyzed against this buffer at 4 °C (fraction II).

(3) DEAE-cellulose Chromatography. A 353-mL DEAE-cellulose column [5 cm (diameter) × 18 cm] was equilibrated with 50.0 mM Tris-HCl, pH 7.5. Fraction II (66 mL) was applied to the column at about 5.4 mL/min and washed with 50.0 mM Tris-HCl, pH 7.5, until the A_{280} of the effluent was at a constant minimum. A small amount of activity that degraded the depyrimidinated polymer flowed through the DEAE column in the 50.0 mM Tris-HCl wash. Further activity was eluted from the column with a 1500-mL linear NaCl gradient (0–0.25 M) in 50.0 mM Tris-HCl, pH 7.0, at a flow rate of 163 mL/h. Fractions active only on depyrimidinated and not intact $(dU\cdot[^3H]dT)_{(2000)}$ were pooled and dialyzed against 50.0 mM potassium phosphate buffer, pH 7.0 (fraction III).

(4) Phosphocellulose Chromatography. Fraction III (413 mL) was applied to a Whatman P11 phosphocellulose column

Table I: Purification of SS AP Endonuclease of *E. coli*

fraction	volume (mL)	protein (mg)	depyrimidinated polymer: sp act. (units/mg of protein) × 10^{-3}	depyrimidinated polymer: units	intact polymer: sp act. (units/mg of protein) × 10^{-3}	intact polymer: units
I, high-speed supernatant	142	5023	154	7.73×10^8	14	7.08×10^7
II, ammonium sulfate (50% sat.)	66	2323	30	7.15×10^7	14	3.28×10^7
III, DEAE-cellulose	413	529	9	4.9×10^6	0	
IV, phosphocellulose I	151	21	166	3.5×10^6	0	
V, phosphocellulose II	25	11	250	2.75×10^6		

(2.5 × 13.5 cm) equilibrated in 50.0 mM potassium phosphate, pH 7.0, plus 10% glycerol. The column was eluted with 500 mL of a 0.05–0.9 M potassium phosphate gradient, pH 7.0, in 10% glycerol. SS AP endonuclease activity eluted at approximately 0.53 M potassium phosphate. Active fractions were pooled and dialyzed against 50 mM potassium phosphate, pH 7.0, with 10% glycerol. The dialyzed fraction (fraction IV) was concentrated by rechromatography on a Whatman P11 phosphocellulose column (5.0-mL bed volume). This column was eluted with 0.9 M potassium phosphate at pH 7.0 with 10% glycerol, and essentially all SS AP endonuclease activity was recovered in a volume of about 25.0 mL (fraction V).

Results

Partial Purification of SS AP Endonuclease. The standard assay of SS AP endonuclease measures the degradation to acid-soluble products of $(dU\cdot[^3H]dT)_{(2000)}$ containing apyrimidinic sites created by enzymatic removal of uracil. Extracts of *E. coli* containing Ura–DNA glycosylase catalyze the excision of uracil from the intact (control) polymer, thus precluding the detection of selective degradation of the depyrimidinated substate. We therefore purified SS AP endonuclease from a strain of *E. coli* (BD1118, *ung*$^-$) defective in Ura–DNA glycosylase (Duncan et al., 1978). The partial purification is shown in Table I.

When either fraction I or fraction II was incubated under standard assay conditions with intact $(dU\cdot[^3H]dT)_{(2000)}$, considerable degradation of this polymer was observed. Analysis of the degradation products by thin-layer chromatography showed that most of the acid-soluble radioactivity was in the form of nucleotides and/or oligonucleotides. These results suggested that the degradation of the nondepyrimidinated polymer was due to contaminating exo- and/or endodeoxyribonuclease activity.

A previously characterized enzyme activity in extracts of *E. coli* known to degrade single-stranded DNA in the presence of EDTA is called exonuclease VII (Chase & Richardson, 1974). Comparative studies with extracts of wild-type *E. coli* and of a *xse*$^-$ mutant strain defective in exonuclease VII activity (Chase & Richardson, 1977) demonstrated that this activity indeed accounted for most, if not all, of the degradation of the intact polymer observed with fractions I and II. The SS AP endonuclease activity eluted from DEAE-cellulose ahead of exonuclease VII; however, there was some overlap between the two. Complete resolution of these two activities was achieved by phosphocellulose chromatography of a DEAE-cellulose fraction pooled to exclude contaminating exonuclease VII. Fraction III and all subsequent fractions in the purification were free of detectable activity that catalyzed degradation of intact $(dU\cdot[^3H]dT)_{(2000)}$ to acid-soluble product.

The activity that flowed through DEAE-cellulose has not been further characterized. This activity selectively attacked only the depyrimidinated polymer and may reflect the presence of polyamines in fraction II. The latter are highly basic low molecular weight compounds that do not bind to DEAE-cellulose and are known to promote β elimination reactions at sites of base loss in DNA (Lindahl, 1979). The presence of the DEAE-cellulose flow-through material and of exonuclease VII complicated the accurate measurement of units of SS AP endonuclease activity during the early steps of purification.

Characterization of the SS AP endonuclease was performed mainly with fraction V, which was stable to freezing and thawing and to storage at −20 °C for at least 8 months. For some experiments, fraction V was concentrated further by ultrafiltration using an Amicon concentrator operated at 25 psi of nitrogen gas with a PM10 membrane. This procedure afforded about a 5-fold concentration without detectable loss of enzyme activity. Fraction V was shown by agarose gel electrophoresis to be free of detectable endonuclease activity on form I undamaged duplex DNA and on single-stranded circular M13 DNA under the standard assay conditions described.

Fraction V is, however, contaminated by endonuclease activity that selectively attacks duplex DNA containing apurinic sites. Gel filtration through Bio-Gel P100 resolved a fraction of SS AP endonuclease that was free of any detectable contaminating AP endonuclease (Figure 1), and for certain experiments this fraction of SS AP endonuclease was used. However, this purification step was not used routinely and is not included in the purification table because gel filtration resulted in a significant loss of endonuclease activity and the resulting preparation was very unstable. The two activities were also partially resolved by velocity sedimentation in glycerol gradients (see later and Figure 6), but this procedure did not provide a fraction of SS AP endonuclease totally free of the endonuclease active on duplex AP DNA. The two activities could be distinguished but not adequately resolved by chromatography on double-stranded DNA–cellulose or by chromatography on agarose Cn-NH_2 series or agarose Cn series hydrophobic matrices.

Characterization of SS AP Endonuclease. Figure 2 shows the kinetics of degradation of depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ and its dependence on enzyme concentration. The control (nondepyrimidinated) substrate was not degraded to measurable acid-soluble product (Figure 2B). The kinetics of formation of acid-soluble labeled product were distinctly nonlinear at very early times of incubation (Figure 2A), suggesting that the release of acid-soluble product did not occur by a "single-hit" mechanism, a result expected from an endonucleolytic rather than an exonucleolytic mode of action. This reaction shows a linear dependence on the concentration of substrate and reaches a plateau (Figure 3), suggesting that the activity displays typical Michaelis–Menten kinetics.

More definitive demonstrations of endonucleolytic activity were provided by the use of single-stranded DNA containing apyrimidinic sites. In one series of experiments we grew phage

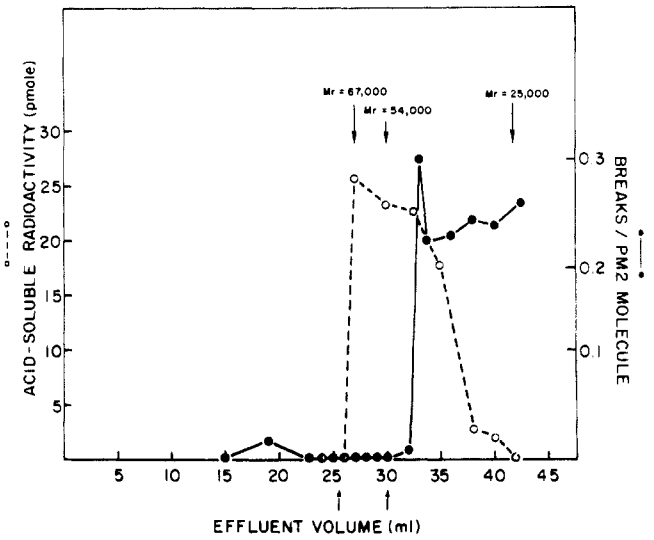

FIGURE 1: Separation of SS AP endonuclease from duplex AP endonuclease activity by gel filtration. A Bio-Gel P100 column was prepared as described under Materials and Methods. Fraction V (1.5 mL containing 1.65×10^5 units of activity) was applied to the column, which was eluted with 10.0 mM Tris-HCl, pH 7.5, 0.25 M NaCl, 1.0 mM EDTA, and 10% glycerol. Fractions (0.75 mL) were collected and assayed for SS AP endonuclease on depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$ as described under Materials and Methods and for AP endonuclease activity on duplex depurinated PM2 DNA containing a total of 0.1 pmol of AP sites/reaction with an average of one AP site per DNA molecule. Fractions active only on the polymer (indicated by the arrows on the abscissa) were pooled. No nicking of the PM2 DNA by the pooled fraction was detected by agarose gel electrophoresis as described under Materials and Methods. In this particular experiment (which was for preparative purposes) the assay of duplex AP endonuclease activity was not continued beyond fraction 43.

M13 in a host defective in both dUTPase and Ura–DNA glycosylase activities. Under these conditions dUMP was incorporated into M13 DNA, as evidenced by the detection of alkaline-labile sites following the incubation of this DNA with purified Ura–DNA glycosylase (Figure 4A,B). Alkaline lability was not observed in uracil-containing DNA that was not preincubated with Ura–DNA glycosylase (Figure 4E). When enzymatically depyrimidinated M13 DNA was incubated with SS AP endonuclease, a significant fraction of the circular DNA was degraded (Figure 4C,D). In control incubations, M13 DNA containing no uracil (not shown), or containing uracil that was not enzymatically removed, was not degraded by the AP endonuclease (Figure 4F,G).

Another experiment to demonstrate endonuclease activity involved the use of phage PBS2 DNA, which naturally contains uracil instead of thymine (Takahashi & Marmur, 1963). This DNA was denatured and incubated with or without Ura–DNA glycosylase, such that under the former conditions ~0.5% of the total uracil was excised. This substrate was incubated in the presence of either SS AP endonuclease or 0.25 N NaOH. Following incubation, the DNA was reduced with sodium borohydride to protect any remaining alkali-labile sites and then sedimented in alkaline sucrose density gradients. Sedimentation profiles (Figure 5A) showed that the endonuclease did not attack PBS2 DNA that had not been preincubated with Ura–DNA glycosylase, nor were alkali-labile sites detected in this DNA. However, incubation of depyrimidinated PBS2 DNA with SS AP endonuclease or alkali resulted in a significant reduction in sedimentation velocity (Figure 5B). Number-average molecular weights were calculated from each of the profiles shown in Figure 5. These calculations showed that preincubating single-stranded PBS2

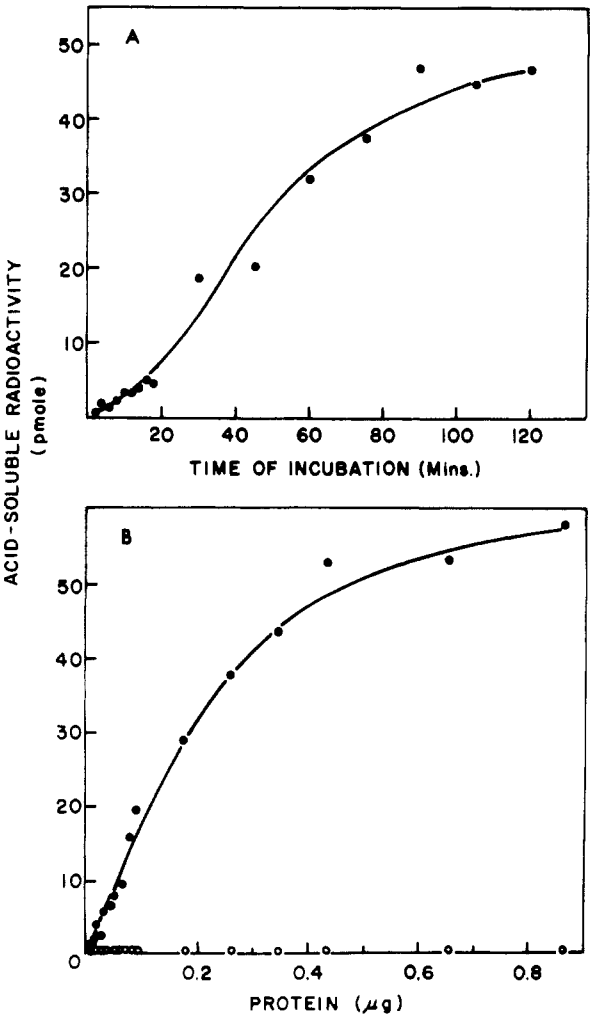

FIGURE 2: Release of acid-soluble radioactivity from depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$ as a function of time of incubation at 37 °C with 34 units of SS AP endonuclease (fraction V) (A) and as a function of the amount of enzyme added (160 units/μg of protein) (B). Reaction conditions were described under Materials and Methods. Reactions in (B) were terminated after 90 min at 37 °C. The open circles show the activity on nonpyrimidinated polymer. Each data point represents the average of two experimental determinations.

DNA with Ura–DNA glycosylase resulted in ~1 alkali-sensitive site/340 nucleotides, or about 8.1 such sites/10^6 daltons, of which 2.3 sites/10^8 daltons were nicked by SS AP endonuclease. There was no detectable release of acid-soluble radioactivity dependent on incubation with the AP endonuclease in any of the samples. These data indicate an endonucleolytic mode of degradation of the depyrimidinated PBS2 DNA also. Qualitatively identical results were obtained by using lightly depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$ instead of denatured PBS2 DNA.

The requirements for SS AP endonuclease activity are shown in Table II. The enzyme was active in the presence of 5.0 mM EDTA. The absence of EDTA did not result in increased activity, and the addition of $MgCl_2$ up to 10.0 mM did not stimulate activity. In the presence of either $MnCl_2$ or $CaCl_2$ up to 2.0 mM, about 50% stimulation of activity was observed; however, concentrations greater than 5.0 mM resulted in significant inhibition. ATP at concentrations of 2.0 mM or higher was a potent inhibitor of activity, and inhibition was also observed in the presence of tRNA. High concentrations of sulfhydryl binding reagents resulted in a very modest inhibition of activity. SS AP endonuclease activity was also inhibited about 50% in the presence of 0.15 M NaCl.

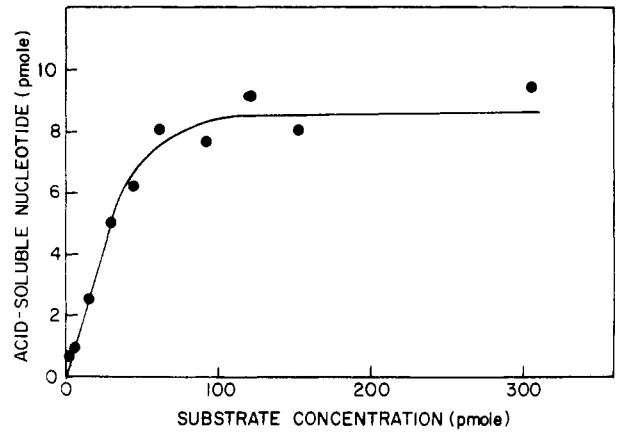

FIGURE 3: Degradation of depyrimidinated single-stranded polymer as a function of substrate concentration. (dU·[^{3}H]dT)$_{(2000)}$ was depyrimidinated by incubating 6140 pmol of polymer with 38 units of Ura–DNA glycosylase for 20 min at 37 °C as described. This reaction rendered the substrate about 50% acid soluble after treatment with 0.17 N NaOH. The amounts of substrate shown were incubated with 55 units (fraction V) of SS AP endonuclease for 30 min at 37 °C. Acid-soluble radioactivity was measured as described.

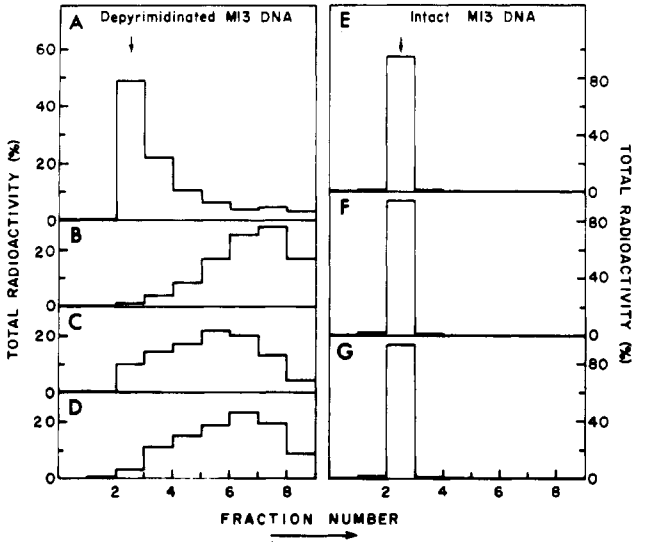

FIGURE 4: Incubation of SS AP endonuclease with depyrimidinated or intact circular M13 DNA. [^{3}H]Thymine-labeled M13 DNA (25.0 μg, sp act. 4.1 × 10^4 cpm/μg) containing deoxyuridine residues (see Materials and Methods) was depyrimidinated by incubation with 2100 units of Ura–DNA glycosylase for 10 min at 37 °C. A separate aliquot of DNA was incubated without the addition of Ura–DNA glycosylase. DNA was deproteinized as described under Materials and Methods, and 2 μg of depyrimidinated (left panels) or intact DNA (right panels) was incubated without addition of SS AP endonuclease (A and E), with 0.2 N NaOH (B and F), with 400 units of SS AP endonuclease (fraction V) (C), or with 800 units of SS AP endonuclease (fraction V) (D and G) for 60 min at 37 °C. Incubation mixtures (0.075 mL) also contained 5.0 mM EDTA and 10.0 mM Tris-HCl, pH 7.5. Reactions were terminated by the addition of 2 volumes of chloroform–isoamyl alcohol (20:1 v/v). Electrophoresis of DNA fractions (25.0 μL) was carried out in a 1.4% agarose gel as described under Materials and Methods. Following electrophoresis the gel was sliced into fractions and the radioactivity in each fraction measured as described under Materials and Methods. The arrows mark the electrophoretic position of intact M13 DNA. Identical results with those shown in the right-hand panels were obtained with M13 DNA not containing deoxyuridine.

Both single-stranded nondepyrimidinated DNA and polymer were inhibitors of activity on depyrimidinated substrates. The pH optimum of the activity was about 7.0. About one-third the optimal activity was detected at pH 6.0 and about two-thirds optimal activity at pH 8.0.

Glycerol gradient sedimentation of SS AP endonuclease

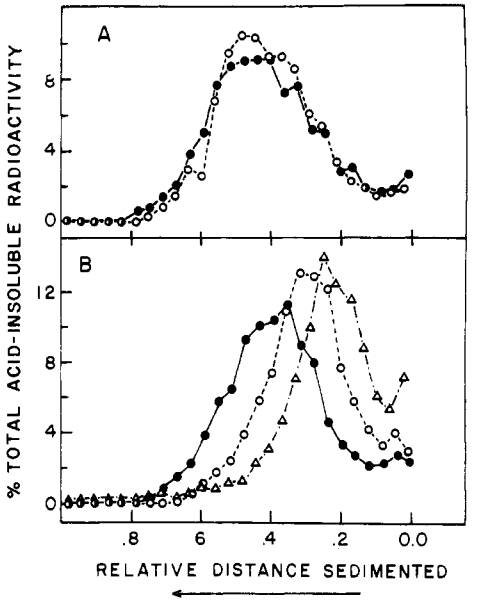

FIGURE 5: Sedimentation profiles of nondepyrimidinated (A) or lightly depyrimidinated (B) denatured PBS2 DNA. [5-^{3}H]Uracil-labeled PBS2 DNA (79.5 nmol as nucleotide; 1.75 × 10^4 cpm/nmol) was denatured by incubating in 0.25 N NaOH at 30 °C for 15 min. The DNA was then neutralized by adding 5.0 N HCl to 0.25 N (final concentration). Incubations (1.0 mL) contained 48 nmol (as nucleotide) of denatured PBS2 DNA, 10.0 mM Tris-HCl, pH 7.5, 1.0 mM EDTA, 25.0 mM NaCl, 0.2% bovine serum albumin, and 840 units of Ura–DNA glycosylase. The reaction was at 25 °C for 8 min. This treatment resulted in the removal of approximately 0.5% of the uracil from the PBS2 DNA. The reaction was stopped and deproteinized with phenol, which was removed from the aqueous phase by ether extraction, followed by extensive dialysis against 10.0 mM potassium phosphate buffer, pH 7.0, with 1.0 mM EDTA. Control PBS2 DNA was treated in exactly the same way except that no Ura–DNA glycosylase was added. Both depyrimidinated (2 nmol as nucleotide containing 10 pmol of AP sites) and control DNA (2 nmol as nucleotide) were incubated with either NaOH (0.25 N final concentration), 120 units of SS AP endonuclease (fraction V), or no further additions, for 90 min at 37 °C under the standard assay conditions for SS AP endonuclease described under Materials and Methods. Following incubation the DNA was reduced with sodium borohydride as described under Materials and Methods. Samples were brought to pH 12.5 by addition of NaOH and were sedimented through alkaline sucrose density gradients (5–20% sucrose in 0.5 M NaCl, 0.2 M NaOH, 10.0 mM EDTA) in a Beckman SW56 rotor at 20 °C at 21 000 rpm for 14.5 h. Gradients were fractionated, radioactivity profiles measured, and weight-average molecular weights calculated as described by Reynolds (1978). (A) Sedimentation profiles of control ^{3}H-labeled PBS2 DNA. (●) No enzyme; (○) incubated with SS AP endonuclease. (B) Sedimentation profiles of depyrimidinated ^{3}H-labeled PBS2 DNA. (●) No enzyme; (○) incubated with SS AP endonuclease; (△) incubated with NaOH.

revealed a peak of activity at a relative *s* value of 4.2 S (Figure 6). This result was repeatedly observed both in the presence and in the absence of salt at 0.25 M. The Stokes radius was calculated at 31 Å according to the procedure of Ackers (1964). The parameter "*r*" in the equation was determined from gel filtration measurements on the basis of the known Stokes radii of the protein markers used. By using the Stokes radius and *s* value, a M_r ~56 000 was computed by the procedure of Siegel & Monty (1966). The frictional ratio was calculated at 1.25.

Figure 6 also demonstrates that the endonuclease did not degrade depurinated ColE1 *duplex* DNA and was partly resolved by glycerol gradient sedimentation from contaminating AP endonuclease that did not degrade the depyrimidinated polymer but did degrade depurinated form I ColE1 DNA. The contaminating AP endonuclease demonstrated in Figures 1 and 6 could represent endonucleases III and/or IV of *E. coli*,

Table II: Requirements for SS AP Endonuclease[a]

reaction conditions	acid-soluble radioactivity (pmol)	% act.
complete	72.0	100.0
no EDTA	68.2	94.7
no EDTA, 2.0 mM $MgCl_2$	71.3	99.0
no EDTA, 10.0 mM $MgCl_2$	58.7	81.6
no EDTA, 2.0 mM $MnCl_2$	111.0	154.0
no EDTA, 10.0 mM $MnCl_2$	36.0	50.0
no EDTA, 2.0 mM $CaCl_2$	101.0	140.0
no EDTA, 10.0 mM $CaCl_2$	48.9	67.9
no EDTA, 2.0 mM ATP	16.4	22.7
no EDTA, 10.0 mM ATP	16.4	22.8
plus 10.0 mM *N*-ethylmaleimide	57.5	79.9
plus 20.0 mM *N*-ethylmaleimide	49.3	68.4
plus 0.3 nmol of tRNA (as nucleotide)	36.0	50.0

[a] The standard reaction (see Materials and Methods) contained either intact or depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ (0.23 nmol as nucleotide) and 70 units of SS AP endonuclease (fraction V). Incubations with divalent cations were carried out in 20.0 mM Hepes-KOH buffer, pH 7.0, instead of potassium phosphate, and EDTA was omitted. All reactions were carried out in the absence of dithiothreitol. No degradation of intact polymer was observed under any of the reaction conditions. All reactions were carried out in the linear range of both enzyme concentration and time of incubation.

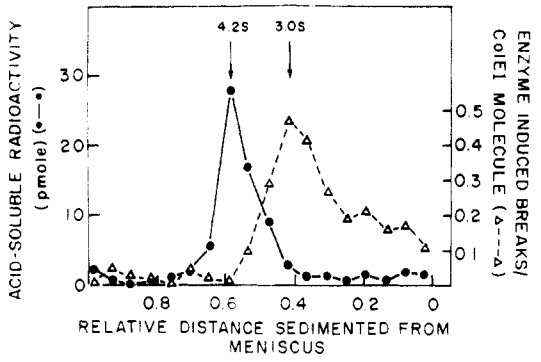

FIGURE 6: Sedimentation of SS AP endonuclease through glycerol density gradients. A 0.2-mL aliquot of SS AP endonuclease (1.93×10^4 units of fraction V) was layered on a 15–40% glycerol gradient containing 10.0 mM potassium phosphate buffer, pH 7.0, 0.25 M KCl, 1.0 mM EDTA, and 1.0 mM 2-mercaptoethanol and centrifuged for 24 h at 3 °C in a Beckman SW60Ti rotor at 57 000 rpm. Gradients were fractionated into tubes containing 10.0 μL of bovine serum albumin (10.0 mg/mL). Aliquots of each fraction (25 μL) were assayed for activity on depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ (0.4 nmol as nucleotide/assay) under standard reaction conditions. Additionally, 10.0-μL aliquots were incubated with heat/acid depurinated ColE1 DNA (containing a total of 0.05 pmol of AP sites/reaction with an average of one AP site per DNA molecule) or undamaged ColE1 DNA 0.28 nmol (nucleotide) in the standard SS AP endonuclease reaction buffer (volume = 20 μL) for 90 min at 37 °C. Reactions were stopped by adding sodium dodecyl sulfate to 0.1% (final concentration). Nicks in the ColE1 substrate were quantitated by electrophoresis in 0.8% gels as described under Materials and Methods. Activity on the depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ is shown with closed circles. Activity on depurinated ColE1 DNA (corrected for any nicking on the native ColE1 DNA) is represented by the open triangles.

both of which degrade duplex DNA containing sites of base loss in the presence of EDTA (Ljungquist, 1977; Gates & Linn, 1977a).

Both endonucleases III (*s* value = 2.7 S) and IV (*s* value = 3.4 S) are considerably smaller proteins than the SS AP endonuclease. However, in order to further distinguish the latter activity from endonucleases III and IV, we directly compared the activity of each enzyme on depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ and on heat–acid depurinated duplex PM2 DNA. The results shown in Table III demonstrate that only the SS AP endonuclease preparation catalyzed the degradation of the depyrimidinated polymer to acid-soluble nucleotide. Both endonucleases III and IV were active preparations, as indicated by the degradation of the depurinated duplex PM2 DNA (Table III).

Table III: Activity of Endonucleases III and IV and SS AP Endonuclease of *E. coli* on Depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ and on Depurinated Duplex PM2 DNA[a]

enzyme	depyrimidinated polymer (acid-soluble product) (pmol)	depurinated PM2 DNA [total nicks (fmol)]
endonuclease III	0.13	70
endonuclease IV	0.0	60
SS AP endonuclease	17.0	39

[a] Incubation of endonuclease III (25 units; Gates & Linn, 1977a) or SS AP endonuclease (fraction V, 30 units) with the depyrimidinated polymer was carried out under the standard conditions described for the assay of SS AP endonuclease under Materials and Methods. Endonuclease IV (530 units; Ljungquist, 1977) was incubated in a reaction mixture containing 5.0 mM Hepes-KOH, pH 8.3, 0.02 M NaCl, 1.0 mM EDTA, 0.1 mM dithiothreitol, 100 mg/mL bovine serum albumin, and 0.6 nmol (as nucleotide, containing 72 000 cpm) of depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$. Incubations were for 30 min at 37 °C, and the extent of degradation of the polymer (acid-soluble radioactivity) was measured as described under Materials and Methods. PM2 DNA was depurinated by heat-acid treatment as described under Materials and Methods to yield ~2 AP sites/molecule (0.1 pmol of AP sites/reaction) and incubated with endonuclease III or IV under the conditions described above. Incubations were at 37 °C for 1 h, and reactions were terminated by adding sodium dodecyl sulfate to 0.2% (final concentration). The extent of nicking of form I PM2 DNA was determined by agarose gel electrophoresis as described under Materials and Methods.

The degradation of depurinated duplex PM2 DNA by the preparation of SS AP endonuclease (Table III) reflects the presence of the contaminating AP endonuclease. It is instructive to note that despite the presence in all three enzyme preparations shown in Table III of approximately equal levels of AP endonuclease active on duplex AP DNA, only the preparation containing SS AP endonuclease activity significantly degraded the depyrimidinated single-stranded polymer. This result is particularly relevant in light of the fact that the concentration of AP sites in the SS depyrimidinated polymer is much greater than in the duplex DNA substrate. Thus it is unlikely that the degradation of the depyrimidinated polymer is a spurious activity of duplex AP endonuclease dependent on substrate concentration.

Enzymatically depyrimidinated $(dU\cdot[^3H]dT)_{(2000)}$ that was reduced with sodium borohydride *prior* to incubation with SS AP endonuclease was refractory to degradation (Table IV). It was for this reason that the reduction of depyrimidinated sites in PBS2 DNA shown in Figure 5 was carried out *after* enzyme treatment of the DNA.

Fraction V of the SS AP endonuclease did not contain detectable pyrimidine dimer–DNA glycosylase activity (see Materials and Methods) when tested with ^3H-labeled *E. coli* DNA containing 7.0% thymine-containing pyrimidine dimers. In these experiments the pyrimidine dimer–DNA glycosylase activity of the phage T4 UV endonuclease (Radany & Friedberg, 1980) was used as a positive control.

Discussion

We have partially purified an endonuclease activity from *E. coli* that catalyzes the degradation of single-stranded de-

Table IV: SS AP Endonuclease Activity on Reduced and Unreduced Depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$[a]

incubation conditions	acid-soluble radioact (pmol) unreduced polymer	reduced polymer
0.17 N NaOH	36.06	2.47
SS AP endonuclease	7.69	0.60

[a] (dU·[^{3}H]dT)$_{(2000)}$ (0.48 nmol as nucleotide) was depyrimidinated as described under Materials and Methods, extracted with phenol, chloroform–isoamyl alcohol (10:1 v/v), and ether, and then dialyzed into 10.0 mM Tris-HCl, pH 7.4, with 0.5 mM EDTA. The depyrimidinated polymer was divided into two equal aliquots (0.25 mL), one of which was treated with sodium borohydride as described under Materials and Methods. Both aliquots were extensively dialyzed against 2000 volumes of 10.0 mM Tris-HCl (pH 7.5)–1.0 mM EDTA and then incubated with SS AP endonuclease (34 units of fraction V) under the standard conditions described under Materials and Methods.

oxyribopolymers and single-stranded DNA containing depyrimidinated sites. In recent studies Behmoaros et al. (1981) demonstrated the hydrolysis of phosphodiester bonds at apurinic sites in DNA incubated with the tripeptide Lys-Trp-Lys. In addition, as mentioned earlier in this paper, highly basic proteins are known to promote hydrolysis of phosphodiester bonds via β elimination at sites of base loss in DNA. These reactions are nonenzymatic in nature. However, the following observations suggest the SS AP endonuclease of *E. coli* is a true enzyme. First, we have demonstrated a dependence of the AP endonuclease catalyzed reaction on the concentration of substrate AP sites, a result typical of most enzymes (Figure 4). A nonenzymatic degradation reaction would be expected to demonstrate a velocity that is *not* limited by substrate concentration, reflecting a very low binding affinity of the degradative function for the AP substrate. Additionally, our studies demonstrate that the enzyme differentiates between duplex and single-stranded polydeoxyribonucleotides containing sites of base loss, a distinction not likely to be made by a protein effecting spurious degradation at the AP sites. Thus, neither duplex PM2 nor ColE1 DNA containing apurinic sites is attacked by the SS AP endonuclease. On the other hand, (dU·[^{3}H]dT)$_{(2000)}$ and M13 DNA or denatured PBS2 DNA containing depyrimidinated sites are all effective substrates. An interesting observation is that reduction of depyrimidinated sites in (dU·[^{3}H]dT)$_{(2000)}$ with sodium borohydride results in almost total loss of substrate for SS AP endonuclease. This suggests that the enzyme does not simply recognize a missing base in a polynucleotide chain. The enzymatic mechanism may involve β elimination at sites of base loss in DNA, a reaction for which the aldehyde moiety of the deoxyribose is required (Jones et al., 1968).

In view of the fact that the purification and characterization of this enzyme are not complete, we have refrained from assigning an official nomenclatural designation to this enzyme at the present time. However, on the basis of currently available information, the SS AP endonuclease appears to be clearly distinguishable from all previously reported AP endonucleases of *E. coli*: (1) The enzyme can be distinguished from exonuclease III (the quantitatively major AP endonuclease of *E. coli*) by a number of parameters. Exonuclease III is inhibited in the presence of EDTA and has a M_r = 33000. In addition, mutants (*xth*$^-$) (including deletions) defective in AP endonuclease activity (Milcarek & Weiss, 1972; Yajko & Weiss, 1975; Weiss, 1976) have normal levels of SS AP endonuclease activity. (2) Endonuclease IV is inhibited rather than stimulated by Ca^{2+} at concentrations greater than 1.0 mM, is highly stable to both ionic strength and heat, and has a reported sedimentation coefficient of 3.4 S (Ljungquist, 1977). In addition, we have shown directly that preparations of purified endonuclease IV do not degrade depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$. (3) Endonuclease V of *E. coli* (Gates & Linn, 1977b) has an absolute requirement for Mg^{2+} and is a small protein (2.3 S). It also has an alkaline pH optimum (9.5) and attacks a variety of substrates including osmium tetraoxide treated duplex DNA, DNA irradiated with UV radiation, and native PBS2 DNA. (4) Endonuclease III was first described by Radman (1976) as an endonuclease that catalyzes the nicking of duplex UV-irradiated DNA. The enzyme, like SS AP endonuclease, is inhibited by high ionic strength and by tRNA and is active in the presence of EDTA (Gates & Linn, 1977a). However, it has a relative *s* value (2.7 S) distinctly smaller than that of SS AP endonuclease and, like endonuclease IV, has been shown in the present studies not to degrade depyrimidinated (dU·[^{3}H]dT)$_{(2000)}$.

At present we have no data that address the biological function(s) of the SS AP endonuclease of *E. coli*. Obviously such an enzyme would be lethal to single-stranded genomes containing sites of base loss. However, it seems reasonable to speculate that the enzyme is involved in the repair of sites of base loss that occur by spontaneous hydrolysis of *N*-glycosylic bonds in locally denaturated regions of *duplex* DNA in living cells. Such sites could exist in DNA with varying degrees of supercoiling, in or near replication forks, or at sites of recombination, transcription, or DNA damage.

With respect to DNA damage, pyrimidine dimers are known to result in local denaturation of the DNA duplex (Kahn, 1974). In addition apyrimidinic sites can be generated during the excision repair of pyrimidine dimers. Recent evidence accumulated from a number of laboratories indicates that the incision of UV-irradiated DNA by the so-called "UV endonuclease" from *Micrococcus luteus* (Haseltine et al., 1980) or from phage T4 infected *E. coli* (Demple & Linn, 1980; Radany & Friedberg, 1980; Seawell et al., 1980; Warner et al., 1980) is effected by a two-step mechanism, the first of which is the hydrolysis of the 5′-glycosyl bonds of the dimerized pyrimidines, thus creating apyrimidinic sites. Phosphodiester bonds at these sites are then cleaved by a 3′ AP endonuclease activity, leaving 3′-terminal apyrimidinic sites. Recent studies by Warner et al. (1980) and by Gordon & Haseltine (1981) have shown that in vitro repair synthesis (by DNA polymerase I of *E. coli*) of UV-irradiated DNA incised by the *M. luteus* UV endonuclease requires prior excision of the 3′-terminal apyrimidinic sites by a 5′ AP endonuclease. In addition, Sharma et al. (1981) have demonstrated that when ϕX174 RF 1 DNA is UV irradiated and cleaved at pyrimidine dimers by the *M. luteus* UV endonuclease, the resulting product DNA does not serve as a primer-template for DNA synthesis catalyzed by purified DNA polymerase I unless first exposed to a cell extract containing *uvr*C protein. These results suggest that *uvr*C protein may act as a 5′ AP endonuclease in vitro. Since the *uvr*C protein has a molecular weight between 64500 and 70000 (Sancar et al., 1981; Sharma et al., 1981; Yaokum & Grossman, 1981) and also binds to single-stranded DNA (Sancar et al., 1981), it is intriguing to speculate that in fact the *E. coli* SS AP endonuclease may be the *uvr*C gene product. Studies are currently in progress to examine this hypothesis.

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