Purification and amino-terminal amino acid sequence of an apurinic/apyrimidinic endonuclease from calf thymus

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Received June 8, 1987; Accepted June 22, 1987

ABSTRACT

An apurinic/apyrimidinic (AP) endonuclease (E.C.3.1.25.2) has been purified 1100 fold to apparent homogeneity from calf thymus by a series of ion exchange, gel filtration and hydrophobic interaction chromatographies. The purified AP endonuclease is a monomeric protein with an apparent molecular weight on SDS-PAGE of 37,000. On gel filtration the protein behaves as a protein of apparent molecular weight 40,000. DNA cleavage by this AP endonuclease is dependent on the presence of AP sites in the DNA. DNA cleavage requires the divalent cation Mg^{2+} and has a broad pH optimum of 7.5-9.0. Maximal rates of catalysis occur at NaCl or KCl concentrations of 25-50 mM. The amino acid composition and the amino-terminal amino acid sequence for this AP endonuclease are presented. Comparison of the properties of this AP endonuclease purified from calf thymus with the reported properties of the human AP endonuclease purified from HeLa cells or placenta indicate that the properties of such an AP endonuclease are highly conserved in these two mammalian species.

INTRODUCTION

Apurinic and apyrimidinic (AP) sites are produced in the mammalian genome by several different mechanisms. Under physiologic conditions purines are spontaneously released from the DNA at a rate of 6,000 - 12,000 per cell per day (1,2). AP sites are also produced by the action of alkylating agents (3), by the action of some antitumor antibiotics such as bleomycin and neocarzinostatin (4-9) and by the action of DNA repair glycosylases (for review see 10). AP sites are mutagenic for both bacterial and mammalian cells (11-14) and are usually "stop" sites for DNA polymerases (15). Mutation is believed to result from "bypass" of AP sites during replication resulting in the insertion of an improper base (13,15-18).

Mammalian cells contain endonucleases that recognize and cleave DNA at AP sites (19-21). Such enzymes are present in a variety of species and tissues (10). Among the most extensively purified and characterized are AP endonucleases of calf thymus (20,22), calf liver (23), rat liver (24,25), mouse plasmacytoma (26), human placenta (27,28) and HeLa cells (29). However,

prior to this report, the properties of eukaryotic AP endonuclease, purified to homogeneity, have been reported only for enzyme obtained from a single species, either human HeLa cells or human placenta (27-29). The exact extent of similarity among the AP endonucleases of human and other species can not be defined in the absence of detailed information concerning the properties and primary structure of AP endonucleases derived from non-human mammalian sources.

Multiple chromatographically distinct forms of AP endonuclease have been identified in extracts of human placenta (30), fibroblasts (31-33) and lymphoblasts (34). At least some of the chromatographically distinct forms of AP endonuclease are likely the result of artefactual proteolysis (24,27). However, the possibility that multiple AP endonucleases exist in mammalian cells, products of multiple genes and with distinct roles in DNA repair can not be excluded at present (34). Resolution of this question will require more detailed information concerning the primary structure of these proteins and their genes.

In an early report by Ljungquist and Lindahl (20) of an AP endonuclease activity in mammalian tissues, this activity was partially purified from extracts of calf thymus. Because calf thymus is a readily available and cheap tissue that is rich in enzymes of DNA replication and repair, it is a particularly suitable source for the purification of DNA repair enzymes. In preliminary experiments we identified calf thymus as a tissue with specific activity of AP endonuclease in crude extracts equivalent to the specific activity of extracts of cultured human cells such as HeLa or lymphoblasts. In this report, we describe a procedure for the purification to homogeneity of the major AP endonuclease of calf thymus, some of the properties of this enzyme and a portion of its amino acid sequence.

MATERIALS AND METHODS

Materials. DEAE-cellulose (DE-52), Phosphocellulose (P-11), and CM-cellulose (CM-23) were purchased from Whatman. Sephadex G75, Sepharose S-200, Phenyl Sepharose, the Mono S cation exchange column, and the Superose 12 HR 10/30 column were purchased from Pharmacia (Uppsala, Sweden). Protein standards of known molecular weight were purchased from Sigma (St. Louis, MO).

Preparation of DNA Containing AP Sites. Covalently closed circular (Form I) DNA of plasmid pUC18 (35) was prepared and quantitated as described by Maniatis and coworkers (36). The plasmid DNA was purified from any residual

RNA by second CsCl density gradient centrifugation in the presence of ethidium bromide. Purified DNA was ethanol-precipitated and resuspended in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Depurinated DNA was prepared immediately before use by heating Form I pUC18 DNA (670 ug/ml) in an equal volume of depurination buffer (20 mM NaCl; 20 mM sodium citrate; 20 mM NaH₂PO₄ (adjusted to pH = 5.2 with 6 N HCl)) at 70°C for 80 min. DNA treated in this fashion contained 3-4 apurinic sites per pUC18 DNA molecule.

Assay for AP Endonuclease. The assay mixture, in a final volume of 50 ul, contained 100 mM HEPES-NaOH (pH 8.25), 3 mM MgCl₂, 1 ug of depurinated Form I pUC18 DNA and 0-10 ul of an appropriate enzyme dilution. Assay mixtures were incubated at 37°C for 20 min. Reactions were stopped by the addition of 6 ul loading buffer (10% SDS, 2.5% Ficoll and 0.025% bromophenol blue) and chilling. Form I and Form II DNA in reaction mixtures were resolved by electrophoresis in 0.9% agarose and stained with ethidium bromide (36). Gels were photographed and the amount of Form I and Form II DNA quantitated from densitometer scans of negative films. The number of nicks was quantitated by Poisson analysis. One unit of enzyme is defined as the amount that produces one pmol of nicks per minute under these conditions (29).

SDS-Polyacrylamide Gel Electrophoresis and Gel Filtration. Samples of purified AP endonuclease were loaded onto SDS-15% polyacrylamide gels and subjected to electrophoresis, under reducing conditions, as described by Laemmli (37). For determination of molecular weight, samples of bovine serum albumin (66,000 daltons), glyceraldehyde-3-phosphate dehydrogenase (36,000 daltons), carbonic anhydrase (29,000 daltons), trypsinogen (24,000 daltons), lactoglobulin (18,400 daltons) and lysozyme (14,000 daltons) were loaded in parallel lanes. Protein bands were located by staining with Coomassie Brilliant Blue R in methanol-acetic acid. Determination of apparent molecular weight by gel filtration was performed using a Superose 12 HR 10/30 column (Pharmacia FPLC System). The elution buffer was 200 mM NaCl, 10 mM Tris-HCl (pH 7.6), 1 mM EDTA and the flow rate was 0.4 ml/min. The elution volume of AP endonuclease was determined by the standard assay and the apparent molecular weight calculated from the relative elution volumes of protein standards including bovine serum albumin, carbonic anhydrase, cytochrome C and apoprotinin.

Location of DNA Cleavage Sites by DNA Sequencing Gels. End-labeled DNA was prepared according to Maxam and Gilbert (38). pUC18 DNA was cleaved with restriction endonuclease Bam HI prior to labeling with ³²P. Labeling at the

3' end was performed in a reaction with Klenow fragment of DNA polymerase I and the four alpha-³²P-labeled deoxyribonucleoside triphosphates. Fragments labeled in a single strand were then obtained by cleavage with Hae II followed by preparative gel electrophoresis. 3'-end-labeled DNA was treated with 15 mM methyl methanesulfonate, 10 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA, 10% (v/v) ethanol at 37°C for 60 min. The DNA was ethanol-precipitated and resuspended in 20 mM NaCl, 20 mM sodium citrate, 20 mM NaH₂PO₄ (pH 7.4), and then held at 50°C for 5 h to produce apurinic sites (26, 39).^{2,4} Depurinated DNA was incubated with 32,000 units of AP endonuclease (Fraction VII) in 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 1 mM EDTA, 7 mM MgCl₂ at 37°C for 30 min. The same DNA fragments that had not been subjected to methyl methanesulfonate and heat were subjected to Maxam and Gilbert (38) DNA sequencing reactions and loaded in adjacent lanes on the same gel. Gel electrophoresis and autoradiography were performed as previously described (40).

RESULTS

Purification of AP Endonuclease from Calf Thymus.

Calf thymus was obtained fresh from a local slaughterhouse, trimmed free of connective tissue and immediately frozen at -70°C. All procedures were performed at 4°C unless otherwise noted. Frozen tissue (100 g) was broken into small pieces and added to 400 ml of TME buffer (50 mM Tris-HCl (pH 7.4), 1 mM 2-mercaptoethanol, 1 mM EDTA) containing 0.1 M NaCl. The tissue was homogenized in a Waring blender for two periods of 30 s and 0.4 ml of 100 mM phenylmethylsulfonylfluoride was added. With gentle stirring, 5 M NaCl was added to bring the final concentration to 0.7 M NaCl. The viscous solution was stirred for 30 min and then the concentration of NaCl was brought to 0.3 M by the addition of TME buffer. The extract was gently mixed for a further 30 min and centrifuged for 20 min at 17,000 X g. The supernatant was filtered through two layers of gauze. The filtrate (Fraction I) was loaded onto a column of DEAE-cellulose (5 cm length X 5 cm diameter) pre-equilibrated with TME buffer containing 0.3 M NaCl. Filtration through DEAE-cellulose consistently resulted in an apparent increase in the amount of protein and AP endonuclease activity relative to the crude extract, probably due to removal of material such as nucleic acids that may inhibit these assays. Material not retained by the DEAE-cellulose column was collected and brought to 70% saturation with the slow addition of solid ammonium sulfate. Following 30 min of stirring, the precipitate was collected by centrifugation at 14,000 X g for 25 min. The precipitate was resuspended in 275 ml of TME buffer (Fraction

II). Four preparations of Fraction II were combined, dialyzed against 0.03 M potassium phosphate buffer (pH 6.5), and loaded onto a column of CM-cellulose (10 cm length X 7 cm diameter) that had been equilibrated in the same buffer. The column was washed with the same buffer until the OD_{280} was $< .02$ and the AP endonuclease was eluted with 2500 ml of the same buffer containing 1.0 M NaCl. Protein eluted by the NaCl step was pooled, precipitated by the addition of solid ammonium sulfate to 80% saturation and resuspended in 580 ml of TME (Fraction III).

Phosphocellulose Chromatography. Fraction III was dialyzed against 20 mM Tris-HCl (pH 8.0), 60 mM NaCl and passed through a column of DEAE-cellulose (5 cm length X 5 cm diameter) that had been equilibrated in the same buffer. Protein not retained by the column was pooled and dialyzed against TME with 50 mM NaCl and loaded onto a column of phosphocellulose (3 cm diameter X 10 cm length) equilibrated in the same buffer. The column was washed with 1200 ml of the same buffer and AP endonuclease activity was eluted with a 600 ml linear gradient from 0.05 to 0.7 M NaCl. The major peak of apurinic endonuclease activity eluted over the range 0.35 to 0.45 M NaCl (Figure 1). These fractions were pooled and ammonium sulfate-precipitated as described above (Fraction IV). In addition, a minor peak of apurinic endonuclease activity eluting between 0.25 and 0.35 M NaCl was observed in most preparations. The minor component of AP endonuclease activity eluting at 0.25 to 0.35 M NaCl was not investigated further.

Gel Filtration and Hydrophobic Interaction Chromatographies. Fraction IV was dialyzed against TME containing 0.1 M NaCl and loaded onto a gel-filtration column (3 cm diameter x 110 cm length) of Sephacryl S-200. Fractions (10 ml each) containing apurinic endonuclease activity (270-310 ml) were pooled (Fraction V), brought to 1.8 M ammonium sulfate and loaded onto a column of Phenyl-Sepharose (1 cm diameter x 24 cm length). The column was washed with 80 ml of TME containing 1.8 M ammonium sulfate and AP endonuclease was eluted with an 800 ml linear gradient decreasing from 1.8 M to 0 M ammonium sulfate (Figure 2). Fractions (8 ml each) containing apurinic endonuclease activity (1.2 M to 0.9 M ammonium sulfate) were pooled (Fraction VI) and dialyzed against TME buffer containing 75 mM NaCl.

Fraction VI consisted largely (60-90% in various preparations) of a single protein of apparent molecular weight 37,000 on SDS-PAGE. However, the preparation was still contaminated with small amounts of other proteins at this stage. To remove these impurities, cation-exchange chromatography on a "Mono S" column (Pharmacia FPLC system) was employed. A portion (21 ml) of

Fig. 1 Phosphocellulose Chromatography of AP Endonuclease Fraction III (2 g protein, 3.6×10^6 U of AP endonuclease) was passed through a DEAE-cellulose column and subjected to phosphocellulose chromatography as described in Experimental Procedures. Fractions of 10 ml were collected during the application of a linear gradient of NaCl. Concentrations of NaCl (.....) were determined by conductivity. AP endonuclease activity (----) was determined as described in Methods.

dialyzed Fraction VI was applied to the column at a flow rate of 1.0 ml/min. Retained proteins were eluted by a 40 ml linear gradient of 0.075 to 0.5 M NaCl. Fractions (0.5 ml) containing apurinic endonuclease eluted at .27 to .34 M NaCl (Figure 3a) coincident with the elution of the 37,000 dalton protein (Figure 3b), and these fractions were pooled to yield Fraction VII. The AP endonuclease activity in Fraction VII is stable for greater than two months when stored at 4°C and for greater than one year when brought to 50% glycerol and stored at -70°C. A typical purification is summarized in Table I. When 5 ug of Fraction VII protein was run on SDS-PAGE only one band was detected by staining with Coomassie Brilliant Blue R (Figure 3b). Fraction VII was used in all the studies described below.

Characterization of the Enzyme: Catalytic Properties of Homogeneous Calf Thymus AP Endonuclease.

Purified AP endonuclease has a requirement for DNA containing AP sites as a substrate (Table 2) and also required Mg^{++} as a cofactor. The divalent

Fig. 2 Hydrophobic Interaction Chromatography of AP Endonuclease Fraction V (88 mg protein, 7.4×10^5 units) of the AP endonuclease purification sequence was subjected to chromatography on Phenyl-Sepharose as described in Methods. Symbols are as in Figure 1. Protein concentrations were determined by the method of Bradford (43).

cations Co^{++} or Zn^{++} can not replace Mg^{++} as a cofactor and Mn^{++} can only partially replace Mg^{++} . Maximal activity occurs during incubation at 37°C . This enzyme has a broad pH optimum over the range pH 7.5-9.0 (data not shown). Stimulation of enzyme activity occurs at low concentrations (25-50 mM) of either KCl or NaCl and activity is inhibited by concentrations of > 100 mM KCl or NaCl (Table 2). Under standard assay conditions (Experimental Procedures) the turnover number for cleavage is 7.4 apurinic sites min^{-1} and the reaction rate is linear for at least 20 minutes.

Inhibition by Adenine. It has been reported (29) that purified human AP endonuclease is inhibited by adenine and other low molecular weight adenine-containing compounds. It was of interest to determine whether calf thymus AP endonuclease is also inhibited by adenine. Calf thymus AP endonuclease is inhibited at adenine concentrations ≥ 50 μM and is inhibited by 50% at approximately 0.2 mM adenine (data not shown).

Location of Cleavage Sites of AP Endonuclease. We wished to ascertain that the bovine AP endonuclease cleaves apurinic DNA specifically at the AP sites along a DNA sequence and is not simply activated by apurinic sites for cleavage at some other location along the DNA. For this purpose we have

Fig. 3 FPLC Cation-Exchange Chromatography of AP Endonuclease Yields a Homogeneous Enzyme (Panel a) A portion of Fraction VI (0.85 mg) was subjected to chromatography on a Pharmacia "Mono S" column as described in Methods. Samples (0.5 ml) were assayed for protein and AP endonuclease content. (Panel b) A portion (16 μ l each) of samples 46-52 were resolved under denaturing conditions by SDS-polyacrylamide gel electrophoresis. The lane on the far right contains 5 μ g of Fraction VII.

Table I Purification of Calf Thymus AP Endonuclease

	Fraction	Vol(ml)	Protein(mg)	Units ₃ (X10 ⁻³)	Unit/mg (X10 ⁻³)	Purif.	Yield(%)
Crude Extract	I	3900	25802	5471	0.21	1	N.D. ^b
DEAE-cellulose	II	1100	37620	12991	0.34	1.6	100
CM-cellulose	III	580	2001	3578	1.8	8.4	27
Phosphocellulose	IV	16	266	696	2.6	12	5
Sephacryl S-200	V	50	88.5	1505	17	80	9
Phenyl Sepharose	VI	84	3.4	737	219	1033	5
FPLC Mono S	VII ^a	4	.56	129	230	1090	1.0

^aThe results for Fraction VII are the combined yield of four FPLC runs. The apparent increase in the activity of AP endonuclease activity on passage over DEAE-cellulose precludes an accurate estimate of the yield of enzyme from the crude extract.

utilized ³²P end-labeled DNA fragments of defined sequence and DNA sequencing techniques to locate the site of DNA cleavage. For these experiments, treatment of the DNA with methyl methanesulfonate and heat was chosen for the production of apurinic sites in the DNA. This method can be used to produce a large number of apurinic sites in DNA, largely at sites of guanines (26). DNA containing apurinic sites and control DNA, without apurinic sites, was incubated in either the presence or absence of purified AP endonuclease. The resulting DNA fragments were resolved by polyacrylamide gel electrophoresis under denaturing conditions (38) (Figure 4). Control DNA fragments lacking apurinic sites (i.e., not treated with methyl methanesulfonate) remained full length when incubated in either the presence or absence of calf thymus AP endonuclease. DNA treated with methyl methanesulfonate and heat has undergone some fragmentation predominantly at guanines, the major site of depurination. When incubated with AP endonuclease this treated DNA underwent 4.7 fold greater fragmentation, again predominantly at guanine sites. From these results, we conclude that DNA cleavage by calf thymus AP endonuclease occurs directly at the guanine sites. Detailed studies of the mechanism of action of this enzyme, including the chemical nature of the DNA termini produced, will be the subject of a later report.

Structural Properties of Purified Calf Thymus AP Endonuclease.

Comparison of the migration of purified calf thymus AP endonuclease with the migration of proteins of known molecular weight on SDS-PAGE indicated a molecular weight of approximately 37,000. On gel filtration chromatography,

Table 2 Optimal Requirements for AP Endonuclease Activity

Assay Conditions	Activity (%)
Standard ^a	100
-depurination	<1
-Mg ⁺⁺ , +EDTA	<5
-Mg ⁺⁺ , +Mn ⁺⁺	25
-Mg ⁺⁺ , +Zn ⁺⁺	<5
-Mg ⁺⁺ , +Co ⁺⁺	<5
15° C Incubation	31
25° C Incubation	62
37° C Incubation	100
50° C Incubation	74
+ 25 mM NaCl	120
+ 50 mM NaCl	118
+100 mM NaCl	99
+200 mM NaCl	45
+400 mM NaCl	31
+ 25 mM KCl	101
+ 50 mM KCl	105
+100 mM KCl	70
+200 mM KCl	10
+400 mM KCl	2

^a Assays were performed under standard conditions as described in experimental procedures or with the indicated changes in divalent cation or incubation temperature. For each set of reaction conditions the ability of AP endonuclease (Fraction VII) to cleave control DNA without AP sites was determined and found to be < 1% of its activity against DNA containing AP sites. Where indicated Mn⁺⁺, Zn⁺⁺, Co⁺⁺, or EDTA were present at 5 mM.

the bovine AP endonuclease eluted as a protein of M_r 40,000 suggesting that the calf thymus AP endonuclease is a single monomeric protein. Purified calf thymus AP endonuclease was subjected to amino acid analysis. The results of this analysis are shown in Table 3.

Amino-Terminal Amino Acid Sequence of Calf Thymus AP Endonuclease. A sample of purified AP endonuclease was subjected to Edman degradation and gas-phase amino acid sequencing. The twenty-two amino-terminal amino acids of this protein have been identified (Table 4). In another experiment, the amino-terminal sequence of a second, independently isolated preparation of AP endonuclease was obtained to position sixteen, confirming this portion of the

Fig. 4 Sequence Specificity of AP Endonuclease Cleavage for DNA containing Apurinic Sites. ^{32} P-3'-end-labeled DNA was depurinated by methyl methanesulfonate and heat treatment as described in Methods. This DNA was then either left untreated (lane 3; 17,000 cpm loaded) or incubated with AP endonuclease (lane 4; 8,000 cpm loaded) and run on a denaturing polyacrylamide gel. Sites of cleavage were compared with DNA cleaved by Maxam and Gilbert (38) pyrimidine (lane 1; 6,000 cpm loaded) and purine (lane 2; 7,000 cpm loaded) sequencing reactions. Control DNA lacking apurinic sites was either left untreated (lane 5; 9,000 cpm loaded) or incubated with enzyme (lane 6; 7,000 cpm loaded) to determine the specificity of the enzyme for depurinated DNA. Quantitation of cleavage was determined by densitometer scanning of autoradiograms.

sequence. Comparison of this sequence with the amino acid sequences on file in the National Biomedical Research Foundation protein data base (Version 6.0) did not identify any known proteins containing this sequence.

Table 3 Amino Acid Composition of Calf Thymus AP Endonuclease^a

<u>Amino Acid</u>	<u>Calculated Residues^b</u>
Ala	18.1
Arg	23.3
Asp + Asn	28.9
Cys	6.5
Glu + Gln	43.4
Gly	16.2
Ile	7.3
Leu	38.1
Lys	36.1
Met	3.4
Phe	16.0
Pro	15.6
Ser	17.3
Thr	10.0
Tyr	20.4
Val	16.3

^aPurified AP endonuclease (10 ug Fraction VII) was desalted using an Amicon Centrifree filter. Amino acid composition was obtained using a Beckman Analyzer Model 6300. ^bResidues per enzyme were calculated on the basis of a molecular weight of 37,000. No correction was made for the amounts of the amino acids his and trp that are not determined by this method.

DISCUSSION

Although AP endonuclease activity has been described in many species, its purification to homogeneity from mammalian sources has only been previously reported from human HeLa cells and placenta (27,29). This procedure uses ion exchange chromatography on DEAE-cellulose, CM-cellulose and phosphocellulose as the initial steps. Phosphocellulose chromatography appears to resolve a minor form of AP endonuclease activity and for this reason this step was retained in the procedure despite its minor contribution to the overall purification. The availability of hydrophobic interaction chromatography on Phenyl-Sepharose and FPLC cation-exchange chromatography proved particularly useful as final purification steps to obtain homogenous enzyme. Sufficient pure protein can be obtained by this procedure to allow studies of catalysis and structural studies, such as determination of amino acid composition and the amino terminal amino acid sequence.

In our studies, the properties of AP endonuclease of calf thymus appear to be quite similar to those reported for the AP endonucleases of human placenta and HeLa cells and to those of the partially purified enzyme described by Ljungquist and Lindahl (20). A comparison of the catalytic and

Table 4 Amino-Terminal Amino Acid Sequence of the Calf Thymus AP
Endonuclease

<u>Position</u>	<u>Amino Acid</u> ^a
1	Pro (.65) ^b
2	Lys (1.7)
3	Arg (n.d)
4	Gly (.52)
5	Lys (1.0)
6	Lys (1.0)
7	Gly (.42)
8	Ala (.50)
9	Val (.46)
10	Val (.38)
11	Glu (.42)
12	Asp (.60)
13	Ala (.48)
14	Glu (.28)
15	Glu (.45)
16	Pro (.31)
17	Lys (.44)
18	Thr (.32)
19	Glu (.18)
20	Leu (.15)
21	Pro (.15)
22	Ala (.16)

^a A sample of purified calf thymus AP endonuclease (Fraction VII, 55 ug) was desalted using an Amicon Centrifree filter and subjected to gas phase amino acid sequencing (42). ^b The recovery of each identified amino acid in nmol for the analysis is indicated in parentheses.

structural properties of these enzymes is shown in Table 5. Each of the enzymes is strongly Mg^{2+} -dependent and the homogeneous preparations have similar specific activities. Kane and Linn (29) have described the inhibition of HeLa cell endonuclease by low molecular weight adenine containing molecules. The mechanism of such inhibition is unknown but it provides a further test of the similarity between the calf thymus AP endonuclease and the HeLa cell AP endonuclease. The calf thymus and HeLa cell AP endonucleases are inhibited to a similar extent by adenine.

On SDS-PAGE we obtained the same estimate of molecular weight as that reported by Shaper and coworkers (27) for the human placental AP endonuclease -- 37,000 daltons. This is slightly smaller than the estimate of 41,000 daltons for the HeLa cell AP endonuclease reported by Kane and Linn (29). However, a recent report by Kuhnlein (41) describes the molecular weight of HeLa cell AP endonuclease as 37,600 daltons. Therefore, all the Mg^{+2} -

Table 5 Comparison of Mammalian AP Endonucleases.

Spec Act ^a	pH Opt	Mg ⁺² Required	Inhibition by Adenine	Mol.Wt.	Reference
<u>HeLa cells</u>					
200	6.7-9.0	Yes	ND	41,000 ^b	(29)
		Yes	ND	37,600 ^b	(41)
<u>Human Placenta</u>					
300	7.4-7.6	Yes	Yes	37,000 ^b	(27)
<u>Calf Thymus</u>					
ND	8.5	Yes	ND	32,000 ^c	(20)
230	7.5-9.0	Yes	Yes	37,000 ^b	This report

^aSpecific activity of homogeneous AP endonuclease expressed as units/mg X 10⁻³. ^bMolecular weight estimates by SDS-PAGE. ^cMolecular weight estimated from sedimentation velocity. ND means not determined.

dependent mammalian AP endonucleases appear to have similar molecular weights as assessed by SDS-PAGE. Further evidence consistent with a substantial conservation of this enzyme among species is that antisera raised against the purified bovine AP endonuclease cross reacts with a protein of the same molecular weight in extracts of human lymphoblasts (J. Toney, unpublished data). The exact extent of the similarity between the bovine and human AP endonucleases must be established by future studies that directly compare such features as tryptic digestion patterns, amino acid sequences and immunologic cross-reactivity.

There is now a considerable amount of information available concerning the prokaryotic genes involved in DNA repair, their regulation, and their protein products. In contrast, relatively little is known of the genes involved in mammalian DNA repair or the primary structure of the proteins encoded by DNA repair genes. A better understanding of the primary structure of eukaryotic DNA repair enzymes is likely to provide insights into mechanisms of mutagenesis and carcinogenesis and may provide clues about the nature of the heritable human DNA repair deficiencies.

This report provides some information concerning the primary structure of the major AP endonuclease of calf thymus; its size, amino acid composition and amino terminal amino acid sequence. There are no previous reports of the amino acid composition or amino acid sequence data for mammalian AP

endonucleases. The availability of the amino terminal amino acid sequence should allow the design of oligonucleotide probes for the selection of the cDNA or gene coding for this enzyme. Alternatively, the sequence should be useful in confirming the identity of putative AP endonuclease clones selected by other strategies, such as immunoscreening or through the complementation of repair deficient mutant cell lines.

ACKNOWLEDGEMENTS

This work was supported by grant CA35767 from the National Institutes of Health, a Scholar Award from the Leukemia Society of America to Dr. Henner, a National Research Service Award from the Public Health Service (CA07707) to Dr. Jorgensen, and a Fellowship Award from L'Association pour la Recherche contre le Cancer to Dr. Munck. We thank Dr. William Lane of the Protein Microchemistry Facility at Harvard University and Dr. Lawrence Duffy of Brigham and Women's Hospital for performing the amino acid analyses and amino acid sequencing, R. Patarca for assistance in the search of the protein sequence bank and J. Jacobs for secretarial assistance.

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