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Purification of *Escherichia coli* Endonuclease Specific for Apurinic Sites in DNA*

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The endonuclease specific for apurinic sites in DNA has been isolated from *Escherichia coli* B41 as a pure monomeric protein of 32,000 daltons. The enzyme hydrolyzes a phosphodiester bond near the apurinic sites in double-stranded DNA; it does not hydrolyze untreated DNA and its action on alkylated DNA is restricted to the apurinic sites always present. This enzyme is not endonuclease II which is most probably a mixture of two enzymes, one a glycosidase (Kirtikar, D. M., and Goldthwait, D. A. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 2022-2026), the other an endonuclease for apurinic sites which is the enzyme isolated in this work.

The observation by Verly and Paquette (1, 2) that intact apurinic sites could not be found in *Escherichia coli* DNA after treatment with an alkylating agent led to the demonstration and partial purification of an endonuclease which hydrolyzes a phosphoester bond near each apurinic site (3). The enzyme is specific for apurinic sites and does not hydrolyze intact DNA strands or strands with alkylated sites (3).

DNA containing apurinic sites is repaired when incubated with the *E. coli* endonuclease for apurinic sites, DNA polymerase I and the four deoxyribonucleoside triphosphates, and T4 ligase and its cofactor (4). DNA spontaneously loses purines (5) so that an endonuclease specific for apurinic sites may participate not only in the repair of DNA after treatment with an alkylating agent, but also in the general maintenance of DNA in normal cells to keep the genetic information intact (6). Endonucleases specific for apurinic sites have been found in thermophilic bacteria,[1] rat liver (7), calf thymus (8), and plants (6).

In this work, the *E. coli* endonuclease for apurinic sites has been isolated as a pure protein.

MATERIALS AND METHODS

Preparation of Substrates

^{3}H-labeled DNA—Escherichia coli B41 was obtained from Dr. Hoffman-Berling of the Max-Planck Institut für Medezinische Forschung at Heidelberg. The bacteria were grown overnight in the minimal medium of Thomas and Abelson (9) containing 2 μCi/ml of [*methyl*-^{3}H]thymidine (6.0 Ci/mmol) and 250 μg/ml of 2′-deoxyadenosine (10). The DNA was extracted according to the method of Marmur (11), dissolved in 0.05 M Tris·HCl/0.015 M sodium citrate, pH 7.0 (saline-citrate), at a concentration of 60 μg/ml and stored at −20°. The specific radioactivity was 50,000 dpm/μg of DNA.

^{3}H-labeled Alkylated DNA—To the ^{3}H-labeled DNA dissolved in saline-citrate (60 μg/ml) were added 2 volumes of 1 M sodium phosphate buffer, pH 7.0/methyl methanesulfonate (Eastman Organic Chemicals) to a final concentration of 0.3 M and the solution was incubated at 37° for 1 hour. The mixture was cooled on ice and dialyzed at 4° against three changes of saline-citrate. The final alkylated DNA concentration was 20 μg/ml. Under these conditions, the DNA contains about 550 methyl groups/10^6 daltons (3).

^{3}H-labeled Depurinated DNA—The ^{3}H-labeled alkylated DNA in saline citrate was heated at 50° for 6 hours and dialyzed at 4° against three changes of saline-citrate containing 0.02 M $MgCl_2$. This treatment leaves the DNA in the native form and is associated with the loss of about 160 alkylated purines/10^6 daltons of DNA (3). The final depurinated DNA solution of 15 μg/ml was stored at −20°.

Enzyme Assay

Paquette and Verly[2] have shown that the *E. coli* endonuclease for apurinic sites has a maximum activity in Tris buffer at pH 8.5. On the other hand, depurinated DNA cannot be kept for a long time in Tris buffer at pH 8.5 because of a slow hydrolysis of phosphoester bonds near apurinic sites (4). The following compromise was worked out for the enzyme assay.

To 20 μl of depurinated DNA (0.3 μg; 15,000 dpm) in saline-citrate containing 0.02 M $MgCl_2$, were added 10 μl of enzyme in Buffer A (0.5 M Tris·HCl/0.1 mM EDTA/0.1 mM mercaptoethanol, pH 8.0); the pH of the mixture is 8.0. After a 10-min incubation at 37°, the tube was cooled in crushed ice, 0.1 ml of saline-citrate containing 200 μg of calf thymus DNA and 0.9 ml of 10% perchloric acid were added successively. The tubes were gently shaken, centrifuged for 15 min at 12,000 × *g*, and the supernatant solution was assayed for radioactivity. Enzyme controls, which were always below 1% of the substrate radioactivity, were subtracted from the experimental values.

Fig. 1 gives the acid-soluble radioactivity formed as a function of the amount of enzyme; the acid-soluble radioactivity increased rapidly first and approached a maximum value of 50%. This is the acid-soluble radioactivity obtained after an incubation without enzyme of the same duration followed by a treatment with NaOH which introduces a strand break near each apurinic site. Fig. 1 shows that, as long as the acid-soluble radioactivity remains under 30%, it is approximately proportional to the amount of enzyme.[3] In all assays used to measure

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[1] V. Bibor and W. G. Verly, unpublished observation.

[2] Y. Paquette and W. G. Verly, unpublished observation.

[3] A careful study (J. P. Hardy, unpublished results) has indicated that the proportionality is no longer found for very low amounts of enzyme (the curve representing the acid-soluble radioactivity as a function of the amount of enzyme is sigmoid), but the deviation is not enough to alter greatly the calculated amount of enzyme.

the amount of endonuclease for apurinic sites, the enzyme was diluted in Buffer A so that the acid-soluble radioactivity liberated from labeled depurinated DNA fell below 30%. One unit of enzyme activity was defined as the amount of enzyme necessary to release 10% acid-soluble radioactivity.

Molecular Weight Determination on Sephadex G-75

A column (1.6 × 60 cm) of Sephadex G-75 (Pharmacia) has been used with two different eluents: 0.05 M ammonium acetate, pH 8.0, and 0.04 M sodium phosphate, pH 7.5. The Sephadex was first equilibrated with the chosen buffer, and then the sample (about 100 μg of protein) dissolved in 1 ml of the same buffer was applied to the column and eluted at a rate of 6 ml/hour; 3-ml fractions were collected. The void volume (V_0) was measured with blue dextran (Pharmacia), while the total volume (V_t) was determined with either riboflavin or *N*-glycylglycine. Four different standard proteins were used for calibration, the absorption at 280 nm was read on each fraction, and the elution volume (V_e) was determined; the elution constant (K_{av}) then was calculated ($K_{av} = (V_e - V_0)/(V_t - V_0)$).

Polyacrylamide Gel Electrophoresis

Gel Electrophoresis at pH 8.3 of Native Enzyme—The method described by Davis (12) was followed. The sample containing 20 to 50 μg of protein was applied on the stacking gel and the electrophoresis was carried out in 0.025 M Tris/0.2 M glycine, pH 8.3, buffer, utilizing a 3 ma current/tube. The gels were stained with Coomassie blue for protein detection or sliced for enzyme assay. The stained gel was scanned at 550 nm and the per cent transmission was recorded. For enzyme assay, 3- or 1.5-mm slices were placed in 0.2 ml of Buffer A, for one night at 4°, before testing for the nuclease activity.

Gel Electrophoresis in 8 M Urea at pH 4.5—The method of Reisfeld *et al.* (13, 14) was used. The sample containing 40 μg of protein in Buffer A saturated with urea was applied on the stacking gel. The electrophoresis was carried out in 0.13 M acetic acid/0.35 M β-alanine/8 M urea, pH 4.5 buffer; the other conditions were as above. The gels were stained for protein detection and scanned at 550 nm.

Gel Electrophoresis at pH 7.1 in Presence of Sodium Dodecyl Sulfate—We followed the method of Shapiro *et al.* (15) and Weber and Osborn (16). Three volumes of protein solution were incubated with 1 volume of 0.08 M sodium phosphate (pH 7.1), 0.4% sodium dodecyl sulfate, 4% mercaptoethanol, 20% sucrose, and some bromphenol blue for 30 min at 37°. An aliquot containing 25 μg of protein was used for the electrophoresis which was carried out in 0.1 M sodium phosphate/0.1% sodium dodecyl sulfate, pH 7.1, at 8 ma/tube. The gel was stained with Coomassie blue and scanned at 550 nm. The total length (*La*) of the extracted gel and the distance migrated by the bromphenol (*Db*) were measured. After coloration, the total length of the gel (*Lb*) was again measured and also the distance migrated by the protein (*Dp*). A migration coefficient (*mc*) then was calculated: $mc = (Dp \times La)/(Db \times Lb)$. The migration coefficient was determined not only for the denatured enzyme, but also for bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease A.

Amino Acid Composition—The pure enzyme (250 μg) was hydrolyzed in HCl and analyzed on an automatic JEOL, JLC-SAH apparatus. The molar per cent for each amino acid was calculated from the specific absorption determined experimentally with pure amino acids.

Radioactivity Determinations

The solution to be analyzed was diluted with water to a volume of 1 ml and prepared for counting by addition of 10 ml of a scintillation mixture made of 1 part Triton X-100 and 2 parts toluene containing 0.4% (w/v) 2,5-diphenyloxazole (PPO) and 0.01% (w/v) of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl-POPOP). An internal ^{3}H-labeled hexadecane standard was used; radioactivity was measured in a Packard Tri-Carb scintillation spectrometer and expressed in disintegrations per min.

Assay of Proteins

Protein concentration was measured by Lowry's method (17) using bovine serum albumin as standard or by absorption at 280 nm.

EXPERIMENTS AND RESULTS

Purification of Enzyme—Escherichia coli B41, a mutant lacking endonuclease I (18), was grown in a 10-liter fermentor in an enriched Trypticase soy broth (17 g of Trypticase peptone, 3 g of Phytone peptone, 5 g of NaCl, 2.5 g of dipotassium phosphate, 5 g of glucose, 5 g of yeast extract, and 1 liter of water, pH 7.2). The bacterial cells (100 g), collected at the end of the exponential phase of growth, were ground in a mortar with 250 g of alumina for 30 min at 4°. An electron microscope examination showed that more than 90% of the bacterial cell walls were disrupted by this treatment. To the thick paste were added 500 ml of Buffer A, and the mixture was suspended with a Dounce homogenizer. Alumina was removed by centrifugation for 15 min at 2500 × *g*; the supernatant then was centrifuged at 7000 × *g* for 20 min to remove the bacterial debris.

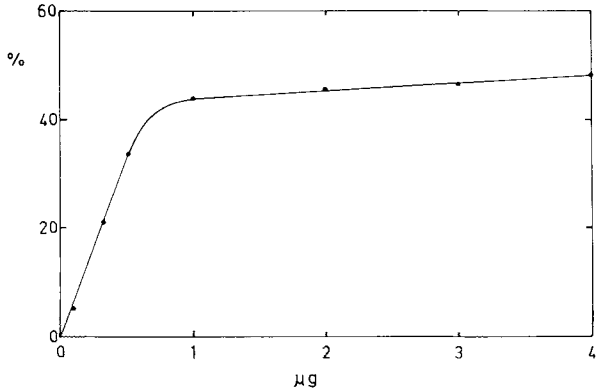

FIG. 1. Action of the endonuclease for apurinic sites on depurinated DNA. To 20 μl of depurinated DNA (0.3 μg; 15,000 dpm) in saline-citrate, 0.02 M $MgCl_2$, were added 10 μl of Buffer A containing increasing amounts of the purified enzyme prepared by Paquette *et al.* (3). After 10 min at 37°, the acid-soluble radioactivity was measured; all data were corrected for a control without enzyme. The acid-soluble radioactivity (in per cent of the substrate radioactivity) is given as a function of the amount of enzyme (expressed in micrograms of protein).

To the 600 ml of final supernatant, 200 ml of 3.2% streptomycin sulfate in Buffer A were added slowly at 4° to a final concentration of 0.8%. The suspension was centrifuged for 15 min at 10,000 × *g*. The supernatant, called Preparation I, contained about 5,000,000 enzyme units with a specific activity of 340 units/mg of protein.

A pilot fractional precipitation with ammonium sulfate was done before committing the entire preparation. In a typical large scale operation, 780 ml of Preparation I were treated with solid ammonium sulfate at 0° to reach 50% saturation; after centrifugation, the supernatant was brought to 70% saturation. After centrifugation, the sediment was redissolved in 150 ml of Buffer A, then dialyzed against 4 × 2 liters of Buffer A containing 0.1 M NaCl. The dialyzed material (Preparation II, 175 ml) contained 3,400,000 enzyme units with a specific activity of 650 units/mg of protein.

A column (5 × 40 cm) of DEAE-cellulose (Sigma Chemical Co.) was equilibrated at 4° with Buffer A containing 0.1 M NaCl. Preparation II was applied and the column was washed with 100 ml of the same buffer. The elution rate was 100 ml/hour, with 4 liters of a 0.1 to 0.3 M NaCl linear gradient in Buffer A; fractions of 25 ml were collected. Fig. 2*a* shows that the endonuclease for apurinic sites was eluted between 0.15 and 0.17 M NaCl. Fractions 50 to 76 were pooled; they contained 2,900,000 enzyme units with a specific activity of 3,600 units/mg of protein. The pooled fractions were dialyzed against Buffer B (0.04 M sodium phosphate/0.1 mM EDTA/0.1

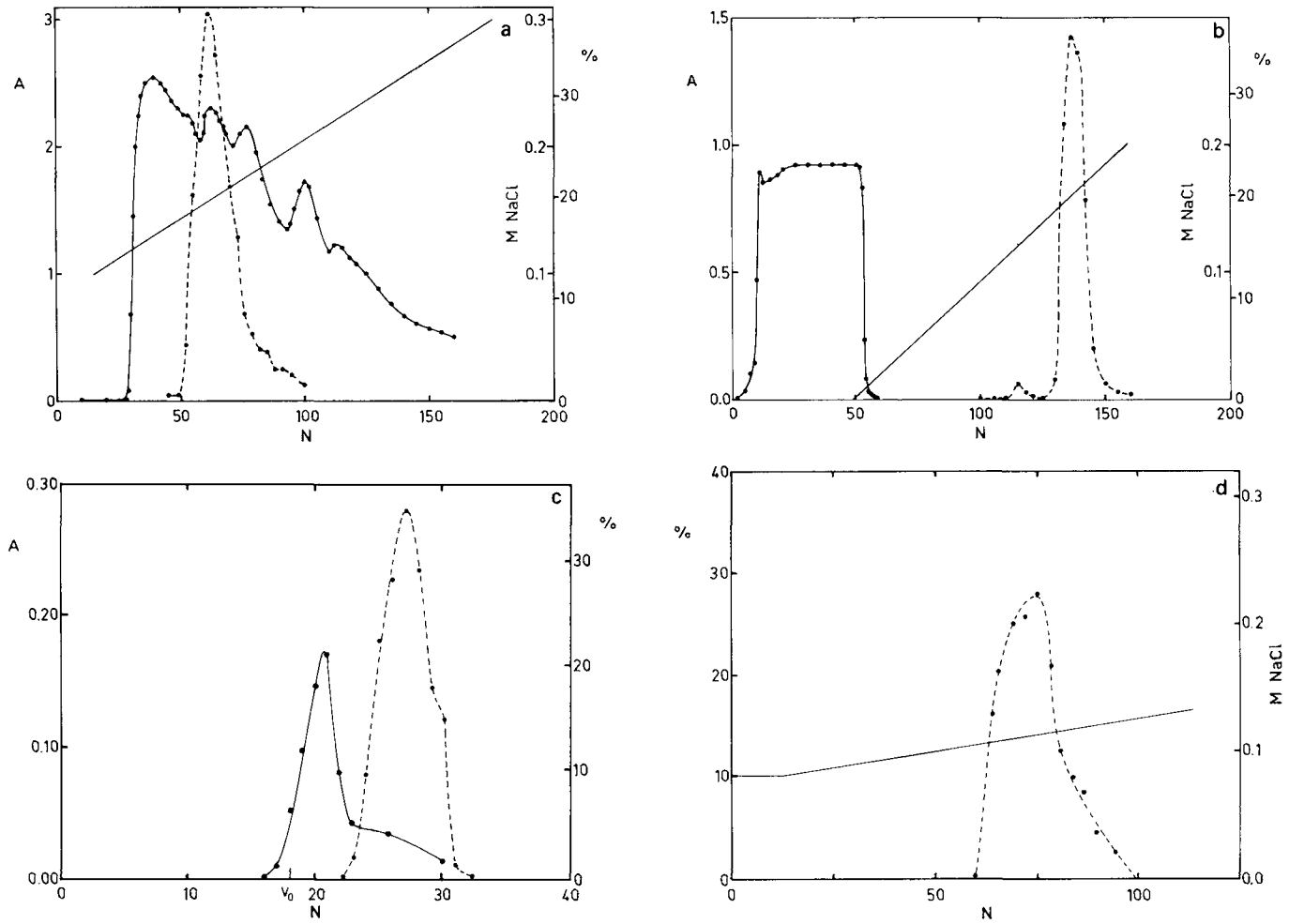

FIG. 2. Chromatographic purification of the endonuclease for apurinic sites. Absorbance at 280 nm (*A*, *continuous line*) and enzyme activity on depurinated DNA (acid-soluble radioactivity in per cent of substrate radioactivity; *dotted line*) are given for each fraction (*N* = fraction number). *a*, DEAE-cellulose, Preparation II in Buffer A containing 0.1 M NaCl (175 ml) was placed on a DEAE-cellulose column (5 × 40 cm) equilibrated with the same solvent at 4°. After a 100-ml washing with this solvent, the elution was carried out, at a rate of 100 ml/hour, with 4 liters of 0.1 to 0.3 M NaCl linear gradient (straight line) in Buffer A; 25-ml fractions were collected. *b*, phosphocellulose, Preparation III (650 ml in Buffer B) was applied at a rate of 50 ml/hour, to a phosphocellulose column (2.6 × 32 cm) equilibrated with Buffer B at 4°, the elution was carried out, at the same rate, with 2 liters of 0 to 0.25 M NaCl linear gradient (*straight line*) in Buffer B; 15-ml fractions were collected. *c*, Sephadex G-75, Preparation IV, in 10 ml of Buffer A containing 0.5 M NaCl, was placed on a Sephadex G-75 column (2.6 × 92 cm) equilibrated with Buffer A at 4°. Elution was carried out with the same buffer at a rate of 30 ml/hour; 10-ml fractions were collected. *d*, DEAE-Sephadex, Preparation V in Buffer A, containing 0.08 M NaCl (50 ml) was placed on a DEAE-Sephadex column (0.9 × 12 cm) equilibrated with the same solvent at 4°. Elution was carried out, at a rate of 8 ml/hour, with 500 ml of 0.08 to 0.13 M NaCl linear gradient (*straight line*) in Buffer A; 5-ml fractions were collected. The absorbance at 280 nm was negligible and is not recorded.

mM mercaptoethanol, pH 6.5), yielding Preparation III (650 ml).

A column (2.6 × 32 cm) of phosphocellulose (Sigma) was equilibrated at 4° with Buffer B. Preparation III was applied to the column at a flow rate of 50 ml/hour. Elution was performed, at the same flow rate, with 2 liters of a 0 to 0.25 M NaCl linear gradient in Buffer B; absorbance at 280 nm and enzyme activity were measured on the collected 15-ml fractions. Fig. 2*b* indicates that the enzyme was eluted between 0.15 and 0.17 M NaCl, far from the bulk of proteins. Fractions 130 to 145 were pooled and dialyzed against Buffer A to give Preparation IV (250 ml) which contained 2,400,000 enzyme units with a specific activity of 97,000 units/mg of protein. Gel electrophoresis indicated that Preparation IV was extremely heterogeneous (Fig. 3).

Preparation IV was applied to a column (1.7 × 2 cm) of DEAE-cellulose equilibrated with Buffer A at 4°; the endonuclease which was completely retained was eluted in 10 ml of 0.5 M NaCl in Buffer A. This eluate was placed on top of a column (2.6 × 92 cm) of Sephadex G-75 (Pharmacia) equilibrated with Buffer A at 4°. The elution was performed with Buffer A at a rate of 30 ml/hour; absorbance at 280 nm and enzyme activity were measured on the collected 10-ml fractions (Fig. 2*c*). Fractions 25 to 29 were pooled to form Preparation V (50 ml) which contained 2,400,000 enzyme units with a specific activity of 925,000 units/mg of protein. Gel electrophoresis revealed that Preparation V was still heterogeneous (Fig. 3).

A column (0.9 × 12 cm) of DEAE-Sephadex (Pharmacia) was equilibrated with 0.08 M NaCl in Buffer A at 4°. Preparation V was dialyzed against Buffer A/0.08 M NaCl and applied to the column. The elution was carried out, at 8 ml/hour, with 500 ml of a 0.08 to 0.13 M NaCl linear gradient in Buffer A; enzyme activity was measured on the collected 5-ml fractions, and the endonuclease for apurinic sites was shown to be eluted between 0.106 and 0.114 M NaCl (Fig. 2*d*). Fractions 69 to 77 were pooled and dialyzed against Buffer A, giving Preparation

VI (45 ml) which contained 2,200,000 enzyme units with a specific activity of 2,750,000 units/mg of protein. Gel electrophoresis revealed a single protein band which corresponded to the enzyme activity (Fig. 3).

Two other enzyme purifications were carried out. The data summarizing the three preparations are in Table I.

Properties of Enzyme

Chemical Properties—A Sephadex G-75 column (1.6 × 60 cm) was calibrated with four proteins of known molecular weight using two different solvents. The K_{av} values were calculated in each case (Table II) and, for each set, plotted against the logarithm of the molecular weight. The enzyme taken after the phosphocellulose chromatography (Preparation IV) was passed through the same column with the same solvents and the K_{av} was determined. Using the appropriate curve, a molecular weight of 32,000 was found with each of the two solvents.

The four reference proteins were submitted to gel electrophoresis at pH 7.1 in the presence of sodium dodecyl sulfate; the migration constants were calculated and plotted against the logarithm of the molecular weight. The pure endonuclease for apurinic sites (Preparation VI) was submitted to the same treatment. Staining with Coomassie blue revealed a single protein band (Fig. 4*a*) whose migration constant corresponded to a molecular weight of 33,000. Gel electrophoresis at pH 4.5 in the presence of 8 M urea also gave a single protein band (Fig. 4*b*).

Two aliquots of Preparation VI, containing 50 and 250 μg of protein, respectively, were dialyzed against 0.05 M ammonium acetate, then lyophilized and submitted to amino acid analysis. Table III gives the molar per cent of the amino acids.

Specificity of Pure Endonuclease for Apurinic Sites—The pure enzyme was incubated with labeled normal, alkylated, or depurinated DNA in the conditions given in Table IV. With normal DNA, there was no significant release of radioactivity whether the enzyme was present or not. With the alkylated DNA, the incubated sample was treated with NaOH before determination of the acid-soluble radioactivity; whatever the duration of the incubation, the acid-soluble radioactivity was the same whether or not the enzyme was present. With the

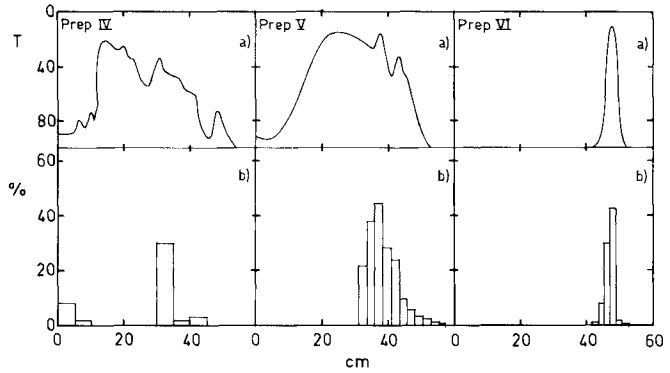

FIG. 3. Gel electrophoresis of the native enzyme. The sample (0.15 ml) containing 20 to 50 μg of protein, mixed with a drop of bromphenol blue in 40% sucrose, was layered on the stacking gel (0.5 cm) placed on top of the separating gel (6.5 cm) in 0.5-cm diameter glass tubes, the electrophoresis was carried out in Tris-glycine buffer, pH 8.3. Two gels were carried out in parallel: one was colored with Coomassie blue and scanned at 550 nm, and the per cent transmittance (T) was recorded (*a*); the other was sliced in 3- or 1.5-mm thick pieces and the enzyme activity (per cent acid-soluble radioactivity) determined after elution with Buffer A (*b*). Three samples were examined: Preparations IV, V, and VI (*Prep IV*, *Prep V*, and *Prep VI*, respectively).

TABLE II

Molecular weight determination of endonuclease for apurinic sites on Sephadex G-75

The experiment was carried out with two different solvents successively. The protein (0.1 mg), dissolved in 1 ml of the chosen solvent, was put on a Sephadex G-75 column (1.6 × 60 cm) equilibrated with the same solvent; elution was carried out at a rate of 6 ml/hour and 3-ml fractions were collected. The proteins used as standards were detected by their absorbance at 280 nm, and the enzyme by its activity on labeled depurinated DNA. In each case, the K_{av} value was calculated.

Sample	Molecular weight	K_{av}	
		0.05 M ammonium acetate, pH 8.0	0.04 M sodium phosphate, pH 7.5
Bovine serum albumin	68,000	0.058	0.060
Ovalbumin	43,000	0.119	0.122
Chymotrypsinogen	25,700	0.275	0.284
Ribonuclease A	13,700	0.433	0.435
Preparation IV		0.206	0.216

TABLE I

Three purifications of Escherichia coli endonuclease for apurinic sites

The purification factor is calculated relative to Preparation I and the specific activity is expressed in enzyme units per mg of protein. Preparation I is the supernatant after the streptomycin sulfate precipitation; Preparation II is the fraction that precipitates between 50 and 70% saturation in ammonium sulfate; the pool of the active fractions from DEAE-cellulose chromatography is Preparation III; from the phosphocellulose chromatography, Preparation IV; from the Sephadex G-75 chromatography, Preparation V; and Preparation VI is the pure endonuclease for apurinic sites obtained from the DEAE-Sephadex chromatography. Preparation VI total volumes were 45 and 40 ml for Purifications I and III, respectively.

Preparation	Purification								
	I			II			III		
	Enzyme units	Specific activity	Purification factor	Enzyme units	Specific activity	Purification factor	Enzyme units	Specific activity	Purification factor
I	5,000,000	340	1	4,500,000	340	1	4,100,000	300	1
II	3,370,000	690	2	3,000,000	690	2	2,740,000	600	2
III	2,920,000	3,560	10	2,770,000	3,690	10	2,600,000	3,610	12
IV	2,400,000	97,000	280	2,260,000	90,400	260	2,140,000	95,000	320
V	2,400,000	925,000	2,650	2,010,000	2,510,000	7,200	2,000,000	975,000	3,240
VI	2,200,000	2,750,000	7,900				1,570,000	2,850,000	9,450

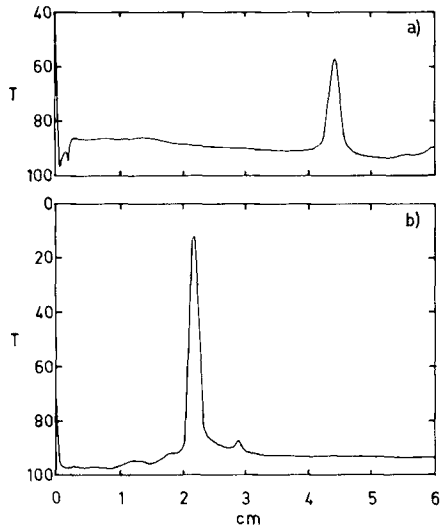

FIG. 4. Gel electrophoresis of the denatured enzyme. *a*, an aliquot of Preparation VI (25 μg of protein), after denaturation in the presence of sodium dodecyl sulfate, was placed with some bromphenol blue on a 7-cm separating gel in a 0.5-cm diameter glass tube. The electrophoresis was carried out in phosphate-sodium dodecyl sulfate buffer, pH 7.1. *b*, an aliquot of Preparation VI (40 μg of protein), after denaturation in 8 M urea, was layered, with a trace of pyronine, on the stacking gel (0.5 cm) placed on top of the separating gel (6.5 cm) in 0.5-cm diameter glass tubes. The electrophoresis was carried out in β-analine/acetic acid, pH 4.5, buffer containing 8 M urea. After coloration with Coomassie blue, the gels were scanned at 550 nm and the per cent transmittance (*T*) was recorded.

depurinated DNA, the acid-soluble radioactivity in the presence of the endonuclease, corrected for the control value without enzyme, increased to reach a plateau; this maximum corresponds to the acid-soluble radioactivity given by a NaOH treatment of the labeled depurinated DNA incubated without enzyme for the same time.

DISCUSSION

The endonuclease for apurinic sites was purified approximately 10,000-fold with a yield of 40%. The final product appears as a pure protein; gel electrophoresis at pH 8.3 gave a single protein band corresponding to the position of the enzyme activity; gel electrophoresis at pH 7.1 in the presence of sodium dodecyl sulfate or at pH 4.5 in 8 M urea also gave a single protein band. Using Sephadex G-75, the enzyme was found to have a molecular weight of 32,000; gel electrophoresis in the presence of sodium dodecyl sulfate gave a value of 33,000, suggesting that the endonuclease for apurinic sites is a monomer.

The pure enzyme was active on depurinated DNA: it liberated acid-soluble fragments up to a maximum corresponding to the acid solubility given by an alkaline treatment consecutive to an incubation, without enzyme, of the same duration. It is known that alkaline treatment hydrolyzes a phosphodiester bond near each apurinic site (19, 20). A much larger amount of the pure enzyme (25 times more) was without effect on normal double-stranded DNA, but had an action on alkylated DNA; this action however was restricted to the apurinic sites present in this substrate: the amount of acid-soluble radioactivity liberated from the radioactive alkylated DNA was the same whether it was incubated with the enzyme and then treated with NaOH, or incubated for the same time without enzyme and also treated with NaOH. Pure enzyme

TABLE III

Amino acid composition of endonuclease for apurinic sites

Preparation VI (the pure enzyme; 50 and 250 μg) was submitted to hydrolysis in 5.7 N HCl at 110° for 2 hours *in vacuo*. After evaporation, an amino acid analysis was performed on the residue in an automatic JEOL apparatus. Molar per cents were calculated from the recorded chromatogram taking into account the specific absorption of each amino acid. Because no data were available for cysteine, methionine, and tryptophan, the molar per cents do not take account of the possible presence of these amino acids in the protein. The table gives the results of two different analyses.

Amino acid	Molar %		
	I	II	Average
Lysine	5.9	5.3	5.6
Histidine		1.8	1.8
Arginine	3.6	3.4	3.5
Aspartic acid	11.3	11.4	11.3
Threonine	4.7	4.5	4.6
Serine	12.8	13.2	13.0
Glutamic acid	14.8	15.3	15.1
Proline		3.5	3.5
Glycine	16.8	17.0	16.9
Alanine	7.2	8.1	7.6
Cysteine			
Valine	4.2	4.7	4.4
Methionine			
Isoleucine	2.8	2.9	2.8
Leucine	5.9	5.0	5.4
Tyrosine		0.9	0.9
Phenylalanine		3.9	3.9
Tryptophan			
Total			100.3

TABLE IV

Enzyme activities of pure endonuclease for apurinic sites

Pure endonuclease for apurinic sites (Preparation VI; indicated number of units) in Buffer A (5 μl) was incubated for various times at 37° with 20 μl of saline-citrate containing labeled normal, alkylated, or depurinated DNA (radioactivity and amount indicated). After addition of 0.1 ml of saline-citrate containing 200 μg of salmon sperm DNA (Sigma chemicals) and 0.9 ml of 10% perchloric acid, the suspension was centrifuged and the acid-soluble radioactivity was determined. In the case of the alkylated DNA, at the end of the incubation, 25 μl of 0.4 N NaOH were added and the mixture kept for 15 min at 37° before addition of carrier DNA and precipitation with perchloric acid. The depurinated DNA incubated for 240 min at 37° with Buffer A containing no enzyme, after a treatment with NaOH, gave an acid-soluble radioactivity of 4650 dpm.

Substrate DNA	Enzyme units	Acid-soluble radioactivity at:				
		15 min	30 min	60 min	120 min	240 min
				dpm		
Normal,	25				205	
92,100 dpm	0				118	
0.5 μg						
Alkylated,	25	1181	1299	1512	1481	1831
17,800 dpm,	0	1196	1304	1710	1519	1764
0.3 μg						
Depurinated,	1	958	2430	3260	4600	4660
6,430 dpm,	0	178	280	330	360	480
0.6 μg	1 (corrected)	780	2150	2930	4240	4180

and NaOH thus acted on the same sites. If NaOH is known to introduce breaks near apurinic sites, it is without effect or rather stabilizes (21) the glycosidic bond of the alkylated bases. The conclusion is that the enzyme acted only on the apurinic sites present in the alkylated DNA and not at all on the far more numerous alkylated sites. The increase with time of the acid-soluble radioactivity (Table IV), whether the enzyme was present or not, was the result of progressive depurination of the alkylated DNA when incubated at 37°.

These properties are at variance with those of endonuclease II, an enzyme acting on alkylated DNA described by Friedberg and Goldthwait (22); although Hadi and Goldthwait (23) showed that endonuclease II hydrolyzed depurinated DNA, they also observed an action on alkylated sites. More recently, Kirtikar and Goldthwait (24) have found that endonuclease II contains a glycosidase which releases *N*-3-methyladenine and *O*-6-methylguanine from DNA treated with *N*-methyl-*N*-nitrosourea, possibly resulting in apurinic sites; *N*-7-methylguanine is not released by the enzyme. Endonuclease II thus appears to be a mixture of two enzymes: a glycosidase and an endonuclease for apurinic sites. The formation of a strand break near an alkylated base is likely a two-step process resulting from the successive action of these two enzymes; with *Escherichia coli* crude extract, the rate-limiting step is catalyzed by the glycosidase since the alkylated DNA becomes a much better substrate when alkylated bases are replaced by apurinic sites (3). The discovery of the glycosidase provides an explanation of the results of Papirmeister *et al.* (25) who found that a crude *E. coli* extract introduced breaks in alkylated DNA near *N*-3-alkyladenine, but not near *N*-7-alkylguanine, and also of the finding of Lawley and Orr (26) of a specific excision of *N*-3-methyladenine and *O*-6-methylguanine, but not of *N*-7-methylguanine, from DNA of *E. coli* treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. All these observations correlate with an older work by Strauss and Robbins (27) on a crude extract of *Micrococcus lysodeikticus* which contained a nuclease that formed breaks near methylated bases in DNA treated with methyl methanesulfonate. Heating the DNA at 50° to replace the methylated purines by apurinic sites did not alter the ability of the DNA to serve as a substrate for the nuclease, and the number of single strand breaks introduced was the same before and after heat treatment.

If endonuclease II is a mixture of two enzymes, one of which is a glycosidase, the nomenclature is confusing. Specific names should be given to each of the constitutive enzymes and the term endonuclease restricted to the enzyme acting on internal phosphodiester bonds, in this case the endonuclease specific for apurinic sites. We propose either that the name "endonuclease II" be dropped completely or restricted to the enzyme we have described as the *E. coli* endonuclease specific for apurinic sites.

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