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Purification and Properties of the Major Apurinic/Apyrimidinic Endodeoxyribonuclease of Rat-Liver Chromatin

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Two nucleases active on alkylated-depurinated DNA have been extracted from rat liver chromatin with 1 M KCl. The major enzyme was purified to near homogeneity; it has a molecular weight of 12500 (although some dimerization might occur), needs Mg^{2+} or Mn^{2+} for activity. The endonuclease activity is specific for apurinic/apyrimidinic sites in DNA; the enzyme has no associated exonuclease activity.

An apurinic/apyrimidinic (AP) endodeoxyribonuclease was first discovered in *Escherichia coli* [1,2]. The enzyme was completely purified [3] and named endonuclease VI [4]. The endonucleolytic activity of endonuclease VI is specific for apurinic/apyrimidinic (AP) sites in double-stranded DNA [3–5]; endonuclease VI is, however, the same protein as exonuclease III [4,6,7]. DNA containing AP sites has been repaired on incubation with three purified enzymes: endonuclease VI and DNA polymerase I of *E. coli* and phage T4 ligase [8]; the details of the repair have been worked out [4].

E. coli possesses a second endonuclease specific for AP sites named endonuclease IV [9]; this enzyme has no associated exonuclease activity. AP endodeoxyribonucleases have been isolated from other bacteria: *Micrococcus luteus* [10–12], *Bacillus stearothermophilus* [13], *Hemophilus influenzae* [14], *Bacillus subtilis* [15].

AP endodeoxyribonucleases have been found in fungi, algae and the roots and leaves of higher plants [16]. The *Phaseolus multiflorus* embryo enzyme has been isolated [17]; the AP endodeoxyribonuclease of barley leaves has also been purified [18]. Endonucleases specific for AP sites in DNA have been purified from rat liver [19], calf thymus [20], human fibroblasts [21], lymphoblasts [22] and placenta [23].

Abbreviations. AP, apurinic/apyrimidinic; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

Enzymes. Apurinic/apyrimidinic (AP) endodeoxyribonuclease (EC 3.1.25.2); pancreatic deoxyribonuclease (EC 3.1.21.1); *E. coli* alkaline phosphatase (EC 3.1.3.1); polynucleotide 5′-hydroxylkinase (EC 2.7.1.78).

Thibodeau and Verly [16] have studied the cellular localization of the *P. multiflorus* embryo AP endodeoxyribonuclease; it was found located mostly in the non-histone proteins of chromatin. A more recent work on rat liver [24,25] has shown the presence of AP endodeoxyribonuclease activities in different cell compartments: chromatin, nuclear sap, cytosol, cytoplasmic membranes, nuclear membranes and mitochondria. It was also shown that the membrane, nuclear sap and chromatin enzymes were different entities. It is very likely that the chromatin AP endodeoxyribonucleases are the only enzymes active on nuclear DNA and it has been hypothesized that the enzymes in membranes and nuclear sap might be precursors of the chromatin enzymes [25].

The present paper deals with the chromatin enzymes. Two species have been found; the major chromatin AP endodeoxyribonuclease has been purified to near homogeneity and its properties are described.

MATERIALS AND METHODS

The Substrates

Radioactive DNA, prepared from *Escherichia coli* B-41 grown in the presence of [^{3}H]thymidine (10 Ci/mmol, New England Nuclear; 1 μCi/ml), has a specific radioactivity of about 50000 dis. min^{-1} μg^{-1}. Alkylation with methyl methanesulfonate and partial depurination were previously described [5]. The alkylated DNA contains approximately one alkylated site per seven nucleotides and the alkylated-depurinated

DNA one AP site per 20 nucleotides. Treatment of the alkylated-depurinated DNA with 0.2 M NaOH for 15 min at 37 °C, which introduces a strand break near each AP site, yields an acid-soluble fraction of 35 %. The labeled double-stranded DNA (untreated, alkylated or alkylated-depurinated) was dissolved in 15 mM NaCl, 1.5 mM sodium citrate, 20 mM $MgCl_2$, pH 8.0.

Enzyme Assays

To 20 µl of labeled DNA solution (0.4 µg DNA) were added 20 µl of the enzyme preparation. After a 15-min incubation at 37 °C, the tubes were cooled on ice, then 100 µl 0.15 M NaCl, 15 mM sodium citrate, pH 7.0, containing 200 µg calf thymus DNA and 900 µl 5.78 % perchloric acid were added successively. The tubes were left for 15 min at 0 °C, then centrifuged at 12000 × *g* for 15 min. The supernatant was used to measure the acid-soluble radioactivity. Results were corrected for controls without enzyme which always gave a very low acid-soluble fraction.

With the alkylated-depurinated DNA, the acid-soluble fraction was nearly proportional to the AP endodeoxyribonuclease activity when this was between 1 % and 12 %. One unit of AP endodeoxyribonuclease is the amount which releases 10 % of the total alkylated-depurinated DNA radioactivity into the acid-soluble fraction in the conditions described above.

Assays for Protein and DNA

Protein concentration was measured by the method of Lowry et al. [26] using bovine serum albumin as standard, or by absorbance at 230 nm and 260 nm using the relation given by Kalb et al. [27]. DNA concentration was estimated by the diphenylamine method [28].

EXPERIMENTS AND RESULTS

Preparation of Chromatin and Extraction of the Enzyme

The preparation and purification of rat liver nuclei, in a medium of low ionic strength containing high-molecular-weight polymers which enabled us to retain most of the AP endodeoxyribonuclease activity in the nuclei, have been previously described [25].

Purified nuclei (260 mg DNA) were incubated at 0 °C during 30 min in 60 ml water containing 0.5 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$) adjusted to pH 8.0. The swollen nuclei were disrupted in a Potter-Elvehjem apparatus of low clearance (10–30 µm) and the suspension centrifuged 10 min at 3000 × *g*. The sediment was resuspended in 60 ml 0.5 mM $PhMeSO_2F$, pH 8.0, and submitted to a second homogenization followed by a centrifugation; after an identical treatment of the second sediment, a chromatin gel was obtained which was collected by centrifugation.

The chromatin was suspended in 60 ml 1 M KCl, 5 mM Tris · HCl, pH 8.0. After 30 min at 0 °C, the suspension was centrifuged at 40000 × *g* during 30 min. The supernatant, which contained 55 mg protein, was dialyzed five times against 1 l 50 mM Tris · HCl, pH 8.0. A small precipitate which formed in the dialysis bag was discarded by centrifugation; the 72-ml supernatant contained 51 mg protein and 287000 units AP endodeoxyribonuclease activity.

Chromatography of the 1 M KCl Extract of Chromatin

The 72 ml of protein extract were poured onto a column (2.6 × 23 cm) of DEAE-cellulose equilibrated with 50 mM Tris · HCl, 0.1 mM EDTA, 0.1 mM 2-mercaptoethanol, pH 8.0, at a rate of 12 ml/h. The column was washed with 160 ml of the same buffer, then eluted, at a rate of 15 ml/h, with 1 l of a 0–0.5 M NaCl linear gradient always in the same buffer; 10-ml fractions were collected. Absorbance at 230 nm and activity on alkylated-depurinated DNA were measured on each fraction. Fig. 1 shows three enzyme peaks: the first is eluted with the washings, the second is eluted at 30 mM NaCl, and the third at 80 mM NaCl.

Fractions 34–42 of the second enzyme peak, which is the major one, were pooled and dialyzed five times against 1 l 5 mM potassium phosphate, pH 6.8. The dialyzed solution (88 ml) containing 4.4 mg protein and 89000 enzyme units, was poured at a rate of 10 ml/h onto a column (1.6 × 34 cm) of hydroxyapatite equilibrated with 5 mM potassium phosphate, pH 6.8. The column was eluted by steps with 120 ml of 0.05 M, 80 ml of 0.1 M, 120 ml of 0.2 M, 100 ml of 0.3 M and 150 ml of 0.5 M potassium phosphate, pH 6.8, at a rate of 12 ml/h. Absorbance at 230 nm and activity on alkylated-depurinated DNA were measured on each 10-ml fraction. Fig. 2 shows a peak of protein eluted with 0.2 M potassium phosphate followed immediately by a sharp peak containing most of the enzyme activity.

Fractions 34 and 35 were pooled and dialyzed five times against 1 l 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10 % glycerol. The dialyzed solution (24 ml), containing 120 µg protein and 35000 enzyme units, was poured at a rate of 10 ml/h onto a column (1.6 × 15 cm) of DNA-cellulose prepared according to the method of Bautz and Dunn [29] and equilibrated with the buffer used in the preceding dialysis. The column was eluted by steps with 180 ml of 0.2 M, 84 ml of 0.3 M and 100 ml of 0.6 M KCl in the same buffer, at a rate of 12 ml/h;

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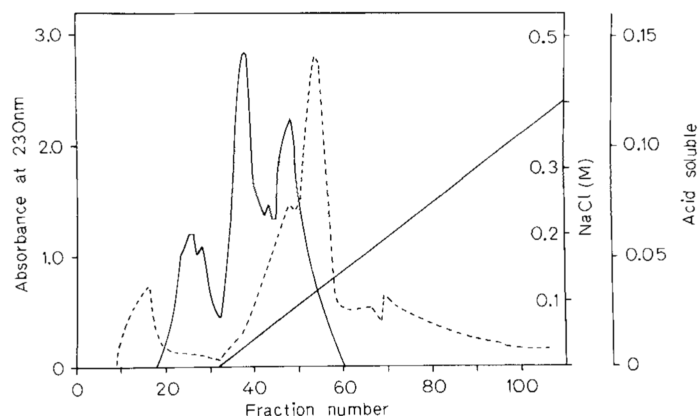

Fig. 1. *Chromatography on DEAE-cellulose of the proteins extracted with 1 M KCl from rat liver chromatin.* After dialysis against 50 mM Tris · HCl, 0.1 mM EDTA, 0.1 mM 2-mercaptoethanol, pH 8.0, the protein solution was poured on a column (2.6 × 23 cm) of DEAE-cellulose. After washing with 160 ml of the same buffer, the column was eluted with 1 l of a 0–0.5 M NaCl linear gradient in the same buffer; 10-ml fractions were collected. The straight line represents the gradient as it emerges from the column. The absorbance at 230 nm was measured on each fraction (dashed line); after a 15-fold dilution, 20-µl aliquots were taken from the fractions and tested on alkylated-depurinated [^{3}H]DNA (acid-soluble fraction; continuous line)

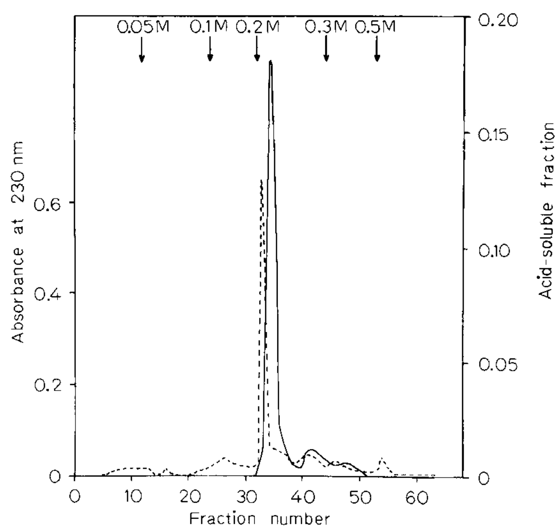

Fig. 2. *Chromatography on hydroxyapatite of the major AP endodeoxyribonuclease activity of rat liver chromatin separated on DEAE-cellulose.* The protein of the second AP endodeoxyribonuclease activity peak from the DEAE-cellulose chromatography (fractions 34–42; Fig. 1) was dialyzed against 5 mM potassium phosphate, pH 6.8. The solution was poured onto a column (1.6 × 34 cm) of hydroxyapatite and the protein eluted by steps with 120 ml of 0.05 M, 80 ml of 0.1 M, 120 ml of 0.2 M, 100 ml of 0.3 M and 150 ml of 0.5 M potassium phosphate, pH 6.8; 10-ml fractions were collected. The arrows indicate when the solutions of increased ionic strength emerge from the column. The absorbance at 230 nm was measured on each fraction (dashed line); after a 15-fold dilution, 20-µl aliquots were taken from the fractions and tested on alkylated-depurinated [^{3}H]DNA (acid-soluble fraction; continuous line)

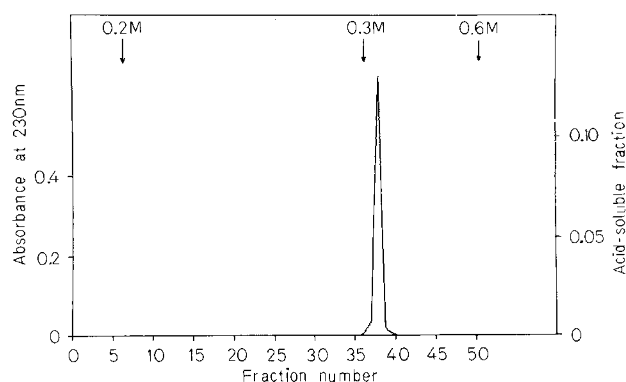

Fig. 3. *Chromatography on DNA-cellulose of the major AP endodeoxyribonuclease of rat liver chromatin.* Fractions 34–35 from the hydroxyapatite chromatography were pooled and dialyzed against 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10% glycerol. The solution was poured onto a column (1.6 × 15 cm) of DNA-cellulose. After washing with the buffer, the protein was eluted by steps with 180 ml of 0.2 M, 84 ml of 0.3 M, and 100 ml of 0.6 M KCl always in the same buffer; 6-ml fractions were collected. No absorbance could be measured at 230 nm; after a 100-fold dilution, 20-µl aliquots taken from the fractions were tested on alkylated-depurinated [^{3}H]DNA (acid-soluble fraction; continuous line)

6-ml fractions were collected. The proteins were undetectable at 230 nm; the activity on alkylated-depurinated DNA was measured on each fraction. Fig. 3 shows that the enzyme was eluted almost in a single fraction (fraction 38) which contained 43000 units of enzyme activity.

The results of the four purification steps are summarized in Table 1.

PROPERTIES OF THE PURIFIED ENZYME

The preparation used in these experiments comes from the second peak of the DEAE-cellulose chromatography and has been further purified on hydroxyapatite and DNA-cellulose (fraction 38 of Fig. 3).

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Table 1. *Purification of the major AP endodeoxyribonuclease of rat liver chromatin*
Preparation I is the protein extracted from chromatin with 1 M KCl; II is the major peak from DEAE-cellulose after dialysis; III is the active fractions from the hydroxyapatite chromatography after dialysis; IV is the fraction 38 from the DNA-cellulose chromatography

Preparation	Enzyme activity	Protein	Specific activity	Purification
	units	mg	units/mg protein	-fold
I	287000	51	5600	1
II	89000	4.4	20200	4
III	35000	0.12	292000	52
IV	43000	< 0.012	> 3580000	> 640

Table 2. *Specificity of the chromatin major AP endodeoxyribonuclease of rat liver*
[^{3}H]DNA (0.4 µg) was incubated for 15 min at 37 °C with or without 15 units enzyme in 40 µl solution. The acid-soluble fraction was measured either directly or after a treatment with NaOH. The results obtained with the enzyme are corrected for controls without enzyme

DNA	Enzyme	NaOH	Acid-soluble	
			measured	corrected
			%	
Untreated	–	–	0.1	
	+	–	0.1	0
	–	+	0.1	
	+	+	0.1	0
Alkylated	–	–	0.2	
	+	–	1.2	1.0
	–	+	1.3	
	+	+	1.2	0

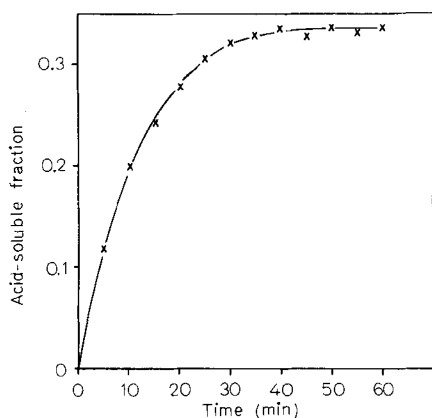

Fig. 4. *Action of the purified major AP endodeoxyribonuclease of rat liver chromatin on alkylated-depurinated DNA.* The purified enzyme (50 units) and 10 µg alkylated-depurinated [^{3}H]DNA in 1 ml buffer containing 10 mM $MgCl_2$ were incubated at 37 °C; 40-µl aliquots were taken at intervals

Specificity of AP Sites

Aliquots of fraction 38 were diluted 75-fold or 10-fold with 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10% glycerol. 0.5 ml of 75-fold diluted enzyme solution or buffer without enzyme was mixed with 0.5 ml of solution containing alkylated-depurinated [^{3}H]-DNA and incubated at 37 °C; 40-µl aliquots (0.4 µg DNA; 2 units when enzyme is present) were taken at various times to determine the acid-soluble radioactivity. Fig. 4 shows that the acid-soluble radioactivity reached a plateau; this maximum corresponds to the acid-soluble radioactivity given by the same amount of substrate after a 15-min treatment at 37 °C in 0.2 M NaOH.

20 µl of 10-fold diluted enzyme solution (15 units) or buffer were mixed with 20 µl of solution containing 0.4 µg of untreated or alkylated [^{3}H]DNA and incubated at 37 °C for 15 min before measuring the acid-soluble radioactivity. In some tubes, the incubation with or without enzyme was followed by the addition of an equal volume of 0.4 M NaOH and a further 15-min incubation at 37 °C before measuring the acid-soluble radioactivity. Table 2 shows that the enzyme has no action on untreated DNA; its action on alkylated DNA is restricted to the alkali-labile sites, i.e. the AP sites, since a preliminary incubation with a large amount of enzyme does not increase the acid-soluble radioactivity given by an exposure to NaOH.

Polyacrylamide Gel Electrophoresis

1 ml of fraction 38 was placed in a dialysis bag and the solution concentrated by burying the bag in Sephadex G-25 powder. The concentrated solution (100 µl in 5 µM Tris · HCl, pH 8.0) was then submitted to electrophoresis on polyacrylamide following the method of Davis [30]. The sample, mixed with some bromophenol blue, in 40% sucrose, was applied on the stacking gel. The electrophoresis was carried out in 25 mM Tris, 0.2 M glycine, pH 8.0 buffer; the 3-mA current was stopped when the bromophenol blue reached the end of the gel. After staining with Coomassie blue, the gel was scanned at 550 nm and the absorbance recorded versus the migrated distance. Fig. 5 shows only one major protein band. When an unstained gel prepared identically was sliced, no significant activity on alkylated-depurinated DNA was found. Assuming that the detected band is the enzyme, the chemical purity of the protein appears to be above 90%.

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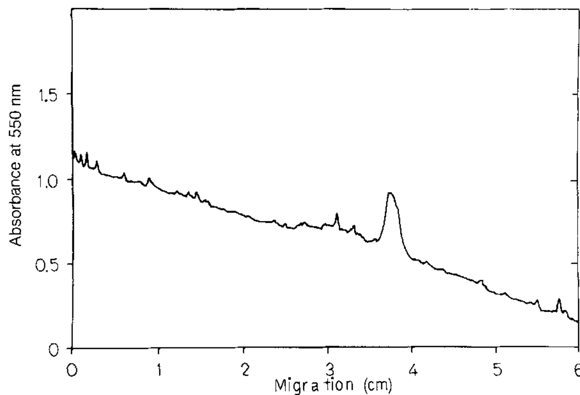

Fig. 5. *Gel electrophoresis of the purified major AP endodeoxyribonuclease of rat liver chromatin.* A concentrated solution of the purified enzyme was submitted to electrophoresis on polyacrylamide gel following the method of Davis [30]. After staining with Coomassie blue, the gel was scanned at 550 nm and the absorbance recorded versus the migrated distance

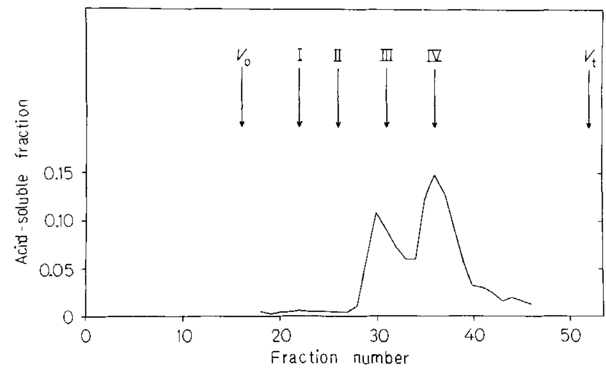

Fig. 6. *Sephadex G-100 chromatography of the purified major AP endodeoxyribonuclease of rat liver chromatin.* The enzyme in 2 ml 0.1 M NaCl, 50 mM Tris · HCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, pH 8.0, was poured on a column (2.6 × 95 cm) of Sephadex G-100. The elution was carried out with the same buffer; 10-ml fractions were collected. Aliquots of 20 µl taken from the fractions were tested on alkylated-depurinated [^{3}H]DNA (acid-soluble fraction; continuous line). Void volume (V_0), total volume (V_t) and elution volumes of bovine serum albumin (I), ovalbumin (II), chymotrypsinogen (III) and cytochrome *c* (IV) are indicated by arrows

Chromatography on Sephadex G-100

A column (2.6 × 95 cm) of Sephadex G-100 was equilibrated with 0.1 M NaCl, 50 mM Tris · HCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, pH 8.0. Void volume (V_0) and total volume (V_t) were measured with blue dextran and riboflavin respectively. The column was also calibrated with four proteins of known molecular weights: cytochrome *c* (12500), chymotrypsinogen (25000), ovalbumin (45000) and bovine serum albumin (67000); each protein was dissolved in 2 ml buffer and applied separately onto the column. The elution was carried out with the same buffer at a rate of 12 ml/h, 10-ml fractions were collected and the absorbance at 230 nm measured to determine the elution volume (V_e). The elution constants $K_{av} = (V_e - V_0)/(V_t - V_0)$ were a linear function of the logarithm of the molecular weights.

An aliquot of fraction 38, dialyzed against the buffer used to equilibrate the Sephadex G-100 column and brought to 2 ml, was placed onto the column and the elution carried out with the same buffer; the activity on alkylated-depurinated [^{3}H]DNA of each 10-ml fraction was measured. Fig. 6 shows two peaks: the major one corresponds to a molecular weight of 12500 while the other corresponds to a molecular weight approximately twice as great.

Effect of Heat, pH, Ionic Strength, and Divalent Cations

A part of fraction 38 was diluted 150-fold with water containing 0.1 % bovine serum albumin adjusted to pH 8.0. The enzyme solution (1.6 ml) was placed in a glass tube, previously treated with silicone grease, and warmed at 45 °C. Aliquots of 20 µl were taken at various times, mixed with 20 µl alkylated-depurinated [^{3}H]DNA and incubated at 30 °C for 10 min before measuring the acid-soluble radioactivity. The logarithm of the acid-soluble radioactivity plotted against time of warming at 45 °C yielded, in two different experiments, a straight line, the slope of which indicates a half-life of 9 min.

Samples of a solution containing 25 mM Tris, 25 mM Hepes, 10 mM $MgCl_2$ were adjusted by addition of HCl or NaOH to different pH values from 6.0 to 9.5. Aliquots of the alkylated-depurinated [^{3}H]-DNA solution were dialyzed against these buffers whereas the enzyme from fraction 38 was diluted 100-fold with the same buffers. 20 µl substrate and 20 µl enzyme in buffers of the same pH were mixed and incubated for 15 min at 37 °C before measuring the acid-soluble radioactivity. Values from controls without enzyme were subtracted. Fig. 7 shows that the optimum pH for the major chromatin AP endodeoxyribonuclease activity is 8.0.

To investigate the role of ionic strength, a part of fraction 38 was diluted 100-fold with 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10 % glycerol. 20 µl of the diluted enzyme solution were mixed with 20 µl alkylated-depurinated [^{3}H]DNA solution and 10 µl water containing NaCl or KCl to have a final concentration varying from 0 to 500 mM; the mixtures were incubated at 37 °C for 15 min before measuring the acid-soluble radioactivity. The enzyme activity did not change much between 0 and 100 mM NaCl or KCl; there seemed, however, to be a slight stimulation between 10 and 30 mM. The activity was inhibited above 100 mM, the inhibition reaching a factor of about 5 at 500 mM.

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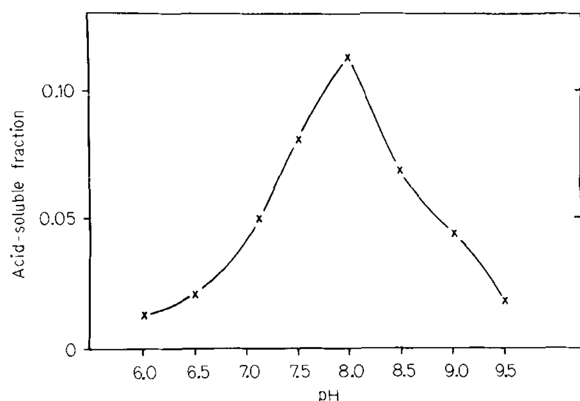

Fig. 7. *Effect of pH on the activity of the major AP endodeoxyribonuclease of rat liver chromatin.* Enzyme and alkylated-depurinated [^{3}H]DNA were incubated in 25 mM Tris, 25 mM Hepes, 10 mM $MgCl_2$, adjusted to different pH with HCl or NaOH. After 15 min at 37 °C, the fraction of the substrate which had become acid-soluble was measured

The magnesium of the alkylated-depurinated [^{3}H]-DNA solution was removed by dialysis against 10 mM EDTA; to remove the EDTA, a second dialysis was performed against 15 mM NaCl, 20 mM Tris · HCl, pH 8.0. 20 µl of this substrate solution were mixed with 20 µl of the diluted fraction 38 and 10 µl water containing $MgCl_2$ to reach a final concentration varying between 0 and 100 mM; the mixtures were incubated at 37 °C for 15 min before measuring the acid-soluble radioactivity. The enzyme is inactive in the absence of Mg^{2+}; the optimum concentration for this cofactor is between 10 and 20 mM. Mn^{2+}, but not Ca^{2+}, can substitute for Mg^{2+}.

Absence of Exonuclease Activity

[^{3}H]DNA (4 µg) in 200 µl 15 mM NaCl, 1.5 mM sodium citrate, 10 mM $MgCl_2$, pH 8.0, was mixed with 20 µl water containing. 0.008 unit pancreatic deoxyribonuclease and incubated for 15 min at 37 °C. The nuclease was then inactivated by heating 10 min at 77 °C or 100 °C to obtain either double-stranded or single-stranded activated [^{3}H]DNA. At this point, an acid-soluble radioactivity of 1% was measured, indicating a mean frequency of about one break/200 nucleotides. The activated [^{3}H]DNA solution (200 µl) was mixed with an equal volume of 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10% glycerol and 20 units chromatin AP endodeoxyribonuclease (fraction 38), and incubated at 37 °C; aliquots were taken at various times up to 120 min to measure the acid-soluble radioactivity. The results were identical for double-stranded or single-stranded activated [^{3}H]DNA: the acid-soluble radioactivity remained the same as for the unincubated substrates.

In another experiment, [^{3}H]DNA was also treated with pancreatic deoxyribonuclease; sedimentation analysis of the denatured DNA in the ultracentrifuge indicated one break/2060 nucleotides. The double-stranded activated [^{3}H]DNA was successively treated with alkaline phosphatase and polynucleotide 5′-hydroxyl-kinase together with [γ-^{32}P]ATP following the method of Weiss et al. [31]. After phenol deproteinization and precipitation with ethanol, a doubly-labeled DNA was obtained which had 30000 dis. ^{3}H min^{-1} μg^{-1} and 10700 dis. ^{32}P min^{-1} μg^{-1}; it was dissolved in 50 mM Hepes/KOH, 2 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 0.05% bovine serum albumin, pH 8.5. 20 µl of the solution (0.2 µg DNA) were mixed with 20 µl 20 mM Tris · HCl, 1 mM EDTA, 0.2 mM dithiothreitol, pH 8.1, containing 10% glycerol and 2 units chromatin AP endodeoxyribonuclease (fraction 38); the mixture was incubated at 37 °C for up to 120 min: no ^{3}H or ^{32}P was released in the acid-soluble fraction. Deoxyribonuclease IV isolated from rat liver following the method of Lindahl et al. [32] quickly set free ^{32}P in a mixture of mononucleotides and dinucleotides from this substrate.

DISCUSSION

Chromatography on DEAE-cellulose of the proteins eluted with 1 M KCl from rat liver chromatin showed three peaks of activity on alkylated-depurinated DNA. When the first one was rechromatographed on the same column, the activity appeared in the position of the other peaks. It would thus seem that rat liver chromatin contains only two different enzymes active on alkylated-depurinated DNA. We do not know whether these two enzymes are both endonucleases specific for AP sites in DNA. So far, we have purified only the enzyme corresponding to the major peak. After additional chromatographies on hydroxyapatite and DNA-cellulose, polyacrylamide gel electrophoresis showed that the preparation contained a protein responsible for more than 90% of the material revealed by Coomassie blue; it cannot be confirmed that this protein is the AP endodeoxyribonuclease since the enzyme is inactivated during the electrophoresis. Chromatography of the purified enzyme on Sephadex G-100 yielded two peaks of activity: one corresponded to a molecular weight of 12500 which is the same as for the AP endodeoxyribonuclease activity found after dissociation of chromatin in NaCl/urea [25]; a second peak of less importance corresponded to a higher molecular weight. We do not know yet if this results from the dimerization of the low-molecular-weight enzyme.

The endonucleolytic activity of the purified enzyme specific for AP sites in DNA; the enzyme has no action on intact DNA strands or on alkylated

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sites. Although chromatin contains exonucleases [25], the purified major AP endodeoxyribonuclease of rat liver chromatin has no associated exonuclease activity. In particular, it has no 5'-3' exonuclease working from breaks in double-stranded DNA comparable to that of deoxyribonuclease IV, although this latter enzyme might have an associated AP endodeoxyribonuclease activity (unpublished results).

The major chromatin AP endodeoxyribonuclease of rat liver needs Mg^{2+} or Mn^{2+} to be active; it is inhibited by NaCl or KCl above 0.1 M and has an optimum pH of 8.0.

The minor chromatin species active on alkylated-depurinated DNA will be purified and studied as will also the enzymes from nuclear sap, cytosol and membrane. The aim of the research is to assess how many independent families of AP endodeoxyribonucleases exist in the rat liver cell.

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