BBA 65929

THE PARTIAL PURIFICATION AND SOME PROPERTIES OF A DEOXYRIBONUCLEASE FROM REGENERATING RAT LIVER

P. J. O'CONNOR
Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester (Great Britain)

(Received February 24th, 1969)

SUMMARY

An endonuclease (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.5) which preferentially hydrolyzes native DNA has been purified from whole cell homogenates of regenerating rat liver. A procedure is described which employs fractionation at pH 5, $(NH_4)_2SO_4$ precipitation and chromatography on hydroxylapatite to give a purification of at least 60-fold. The enzyme shows maximum activity in the presence of Mg^{2+} at pH 8 and is shown by gel filtration and DEAE-cellulose chromatography to act as an endonuclease which produces oligodeoxynucleotides bearing 5'-phosphomonoester end groups. This enzyme differs from the alkaline deoxyribonuclease from rat liver mitochondria in its specificity for DNA, its preference for native DNA and its activity in the presence of bivalent cations.

INTRODUCTION

Alkaline deoxyribonuclease (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.5) activity has been demonstrated in calf kidney[1], in the mitochondria of guinea pig liver[2] and in mouse tissues[3]. In rat liver this activity was shown to be principally contained in the mitochondria[4] and the preferred substrate proved to be heat denatured DNA[5]. The enzyme from rat liver has been purified extensively[6] and was found to be a nonspecific nuclease which hydrolyzed both denatured DNA and RNA[7].

While studying the purification procedure for soluble DNA polymerase from regenerating rat liver[8], two contaminating nucleases were observed in partly purified polymerase preparations. The principal contaminant was the nuclease which hydrolyzed denatured DNA preferentially. The other proved to be an alkaline deoxyribonuclease which hydrolyzed native DNA preferentially[9]. In the present studies the latter enzyme has been partially purified, and some of its properties are described.

MATERIALS AND METHODS

Materials

The following materials were obtained from the Sigma (London) Chemical Co.: the 2-deoxynucleosides, adenosine, cytidine, guanosine and thymidine and the four corresponding 5'-monophosphates; adenosine 3'- and 5'-monophosphate; calf thymus DNA type I, yeast RNA Type XI and crude snake venom *(Crotalus adamanteus)*.

Purified snake venom exonuclease was obtained from the crude venom by the following procedures: Step 1, acetone fractionation[10]; Steps 2 and 3, ethanol fractionations[11]; Step 4, acetone fractionation[12]; Step 5, CM-cellulose chromatography[13] and Step 6, DEAE-cellulose chromatography[13]. The purified material was incubated for 4 h with adenosine 3'- and 5'-monophosphate. No phosphomonoesterase activity could be detected when the products were examined by paper chromatography and for free phosphate by the procedure of FISKE AND SUBBAROW[14]. Purified spleen exonuclease[15] free of phosphomonoesterase activity was kindly provided by Dr. G. Bernardi. Acid prostatic phosphatase was purified from carefully dissected human prostate tissue as far as the acid supernatant stage[16] and was freeze-dried. Crude Russell's Viper venom was the gift of Dr. G. R. Barker.

Chemical estimations

Protein, DNA and RNA were estimated using the procedure of LOWRY *et al.*[17], BURTON[18] and CERIOTTI[19], respectively.

Preparation of solutions of polynucleotide substrates

1 mg/ml native calf thymus DNA was dissolved in 10 mM potassium phosphate buffer (pH 7.7) and was stored at 2°. Heat denatured DNA was prepared by heating 1-ml portions for 10 min in boiling water and by quenching in ice-cold water. RNA was dissolved in the same buffer at the same concentration.

Estimation of enzymes

Alkaline deoxyribonuclease

The activity of the liver deoxyribonuclease with native and denatured DNA was estimated by following the release of acid soluble products[20]. The assay medium (Medium A) contained 30 μmoles glycine–NaOH (pH 8.0), 3 μmoles $MgCl_2$ and 0.5 μmole 2-mercaptoethanol, substrate and enzyme in a final 0.5-ml volume. The substrate was 25 μg native or denatured DNA, and the mixtures were incubated for 1 h at 37° in the presence of 4–34 μg of Fraction 4 *(see Preparation of enzyme)*. The reaction was terminated by the addition of 0.1 ml of denatured carrier DNA (2 mg/ml) and 0.4 ml of 1.6 M $HClO_4$. After standing for 15 min in ice, the tubes were centrifuged at 2000 $\times$ *g* for 30 min. The absorbance at 260 nm of the supernatant was determined in microcuvettes of 1-cm light path. The amount of DNA rendered acid soluble was estimated using a molar absorbance coefficient (ε_M) of 10 200 (ref. 21). One unit of activity was defined as the amount of enzyme which released 0.1 μmole acid-soluble nucleotide per h.

Other enzymes

Ribonuclease activity was measured in the same way by replacing DNA with 25 μg RNA and using the corresponding ε_M of 10 600 (ref. 21). Deoxyribonuclease II

activity[22] was measured as above, but the assay medium contained 25 μg native DNA, 30 μmoles sodium acetate (pH 4.6), 0.5 μmole $MgCl_2$ and 0.5 μmole 2-mercaptoethanol in a final 0.5-ml volume. Determinations of phosphodiesterase[23] and alkaline phosphatase[24] activity were made using 0.4 μmole of either sodium bis-(*p*-nitrophenyl) phosphate or sodium phenylphosphate as substrate with 24 μg of liver deoxyribonuclease in 0.5 ml of Medium A.

DEAE-cellulose chromatography of the products of prolonged digestion

Native DNA was digested for 26 h at 37°. The initial reaction medium contained 20 mg native DNA, 2.4 mmoles glycine–NaOH (pH 8), 240 μmoles $MgCl_2$, 40 μmoles 2-mercaptoethanol and 1.54 mg liver deoxyribonuclease in a total 40-ml volume. Further additions of enzyme were made; 1.54 mg at 4 h, 1.97 mg at 8 h and 2.2 mg at 12 h. Appropriate amounts of reagents were included with each addition in order to maintain the concentration of the reaction medium. The product was rendered 100% acid soluble by 21 h, and digestion was continued for an additional 5 h. After cooling, the mixture was deproteinized by shaking with an equal volume of chloroform–3-methylbutan-1-ol (4:1, v/v). The aqueous phase was diluted to 400 ml and was applied to a 2 cm × 30 cm column of DEAE-cellulose (Whatman DE 52) which had been prepared in the chloride form. Tritium-labeled 5'-TMP was added as a marker. The column was developed with a linear gradient according to the procedure of TOMLINSON AND TENER[25] using 1 l of 7 M urea containing 2.5 mM sodium acetate buffer (pH 4.7) in the mixing vessel and 1 l of the same solution made 0.3 M with respect to NaCl in the reservoir. The effluent was monitored at 260 nm, and 10.5-ml fractions were collected. Inorganic phosphate from the buffer solutions used to prepare enzyme and substrate and the added [^{3}H]TMP was used to indicate the mononucleotide region of the column effluent. The dimers, trimers and oligomers were eluted in order of increasing chain length[25]. Separation was obtained with oligomers up to 7 deoxynucleotides long. The chain lengths of the larger oligodeoxynucleotides were determined by estimating terminal and total phosphate[26] after recovering the products on small DEAE-cellulose columns eluted with 0.8 M $(NH_4)_2CO_3$ (ref. 25).

Identification of terminal phosphate

A digestion mixture containing 4 mg native DNA and 1.08 mg liver deoxyribonuclease in 27 ml of Medium A was incubated for 7 h at 37° to reach 70% acid solubility. The digest was then heated to 100° for 10 min, centrifuged and divided into five 5-ml portions. Four of these were treated as described under Table III. The fifth portion was diluted, and the deoxyribonuclease products were collected on a 2 cm × 1 cm DEAE-cellulose column, were eluted and were freeze-dried to remove salt[25]. The product was digested by the snake venom enzyme, was heated, was freeze-dried and taken up in 10 mM HCl. A portion was applied to Whatman 3MM paper and separated by high voltage electrophoresis[27] using sodium acetate buffer (pH 3.5). The mononucleotides were eluted from the paper with 10 mM HCl. After measuring the $A_{260\ nm}$ value, the solution was adjusted to pH 8.9 and incubated at 37° for 30 min with 50 μl of a solution of Russell's Viper venom. The products were freeze-dried and separated by paper chromatography.

For chromatography, the freeze-dried exonuclease and 5'-nucleotidase products were taken up in 75 μl of 10 mM HCl. 25-μl samples were applied to Whatman No. 1

paper which was developed for 18 h in a descending direction to obtain a clear separation of nucleosides from other digestion products[7]. The solvent used was propan-2-ol–water–NH_4OH (7:2:1, v/v). The areas on the papers corresponding to the nucleoside markers and adjacent control areas were eluted in order to determine the $A_{260\ nm}$ values.

Preparation of enzyme

Regenerating liver from Wistar rats was used as the enzyme source. Partial hepatectomy[28] was performed on 8 males (230–250 g body weight), and after 36 h the regenerating liver was removed. The tissue was cut into small pieces and frozen on dry ice. All subsequent operations were carried out at 3°. The four stages in the purification procedure are described below and summarized in Table I.

TABLE I

PURIFICATION OF THE LIVER DEOXYRIBONUCLEASE

Fraction	*Total protein (mg)*	*Total units*	*Specific activity (units/mg)*	*Yield (%)*	*Activity with native DNA / Activity with denatured DNA*
1. Crude enzyme	2050	1230	0.60	100.0	0.8
2. pH 5 supernatant	1170	210	0.18	17.1	1.2
3. $(NH_4)_2SO_4$ precipitate	259	132	0.51	10.8	1.8
4. Hydroxylapatite	*1.44	*14.8	10.30	5.5	3.0

* These values are the yield from 56.5 mg of Fraction 3.

Fraction 1: crude enzyme

The liver tissue was homogenized (40% w/v) in 0.05 M potassium phosphate (pH 8.0) containing 1 mM EDTA and 2 mM 2-mercaptoethanol using an Eppendorf glass homogenizer. Triton X-100 (5% v/v in the same buffer) was added to give a final concentration of 0.25%. The homogenate was then frozen in acetone-dry ice and thawed by immersion in an iced water bath. The process of freezing and thawing was repeated once more, and the homogenate was centrifuged at 105 000 × *g* for 90 min. The supernatant was termed Fraction 1.

Fraction 2: pH 5 supernatant

Fraction 1 was adjusted to pH 5.0 by the slow addition of 1 M acetic acid, was stirred 20 min and was centrifuged at 10 000 × *g* for 20 min to remove insoluble material. The supernatant was adjusted to pH 7.4 by the addition of 2 M Tris–HCl (pH 9.4), was dialyzed overnight against 60 vol. of Medium B (1 mM potassium phosphate (pH 8.0) containing 1 mM EDTA and 2 mM 2-mercaptoethanol), with one change after 4 h. The dialyzed supernatant was termed Fraction 2.

Fraction 3: $(NH_4)_2SO_4$ precipitate

The protein concentration of Fraction 2 was adjusted to approx. 10 mg/ml by the addition of Medium B. Pulverized $(NH_4)_2SO_4$ was added slowly with constant stirring to Fraction 2 to give a final concentration of 0.225 g/ml. The solution was adjusted to pH 7.4 by the addition of 2 M NH_4OH. After stirring for 30 min, the precipitate was removed by centrifugation at 10 000 × *g* for 30 min. The process was repeated and 0.110 g salt for each ml of the supernatant was added to give a

final concentration of 0.335 g/ml. The precipitate was collected and dissolved in 0.1 M potassium phosphate buffer (pH 8.0) containing 1 mM EDTA and 2 mM 2-mercaptoethanol. The solution was dialyzed against 100 vol. of Medium B with one change after 4 h and was termed Fraction 3. This could be stored at −15° without loss of activity for at least 2 weeks.

Fraction 4: hydroxylapatite eluate

Hydroxylapatite (Biogel HTP) was packed as a 4 cm × 1 cm column under a hydrostatic pressure of 25 cm and equilibrated with Medium B. Approx. 50 mg of Fraction 3 in a total volume of 2.0 ml were applied to the column, and washing was continued with Medium B at the same pressure. The enzyme was eluted between 2 and 6 column volumes (Fig. 1). The column retained 96% of the protein under these conditions. Further washing of the column with Medium B containing 10 mM potassium phosphate eluted more enzyme activity, but this had the same specific activity as Fraction 3.

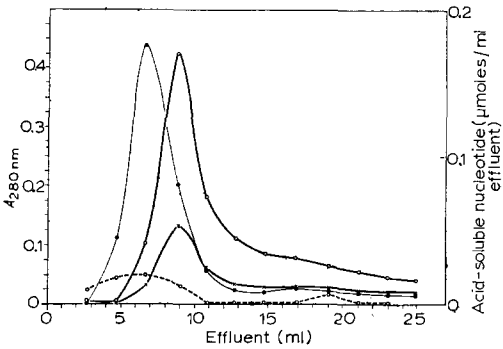
RESULTS

Purification

A summary of the purification procedure is given in Table I which shows a 57-fold purification relative to the pH 5 supernatant. The separation of the nuclease which preferentially hydrolyzes denatured DNA from the deoxyribonuclease which preferentially hydrolyzes native DNA is indicated under the activity ratios in Table I. A substantial separation of these two activities was achieved by acid precipitation. The activity ratio for the pH 5 supernatant was 1.2 indicating a slight preference for native DNA, whereas the ratio for the pH 5 precipitate was 0.4 (not given in Table I) showing that this fraction was twice as active with denatured DNA. Although the activity with denatured DNA was progressively reduced by the purification procedure, Fraction 4 retained some activity towards the denatured substrate. When Fraction 3 was chromatographed on a column of hydroxylapatite, the elution profiles for the activities with native and denatured DNA were perfectly superimposed and were in a ratio of approx. 3 (Fig. 1), indicating that chromatographically similar proteins were responsible for the two activities. The low level of ribonuclease activity eluted from the column was associated with the protein peak and not with the deoxyribonuclease activity. Deoxyribonuclease II was also removed in the purification. The activity of this enzyme relative to the activity of the alkaline enzyme which preferentially utilizes native DNA was 10, 50, 15 and 1% respectively for Fractions 1–4. Tests for alkaline phosphatase activity with sodium phenylphosphate and phosphodiesterase activity with sodium bis-(*p*-nitrophenyl) phosphate were negative.

General properties

Fraction 4 exhibited an absorbance ratio at 280 nm and 260 nm of approx. unity and a protein concentration of approx. 110 μg/ml. Analyses showed the preparation to contain 3.8 μg DNA and 18.0 μg RNA per mg protein. It could be stored without loss of activity for at least 2 weeks at 2°, but the inactivation produced by freezing and thawing could be as high as 70%. Under standard reaction conditions the activity of the enzyme was linear with respect to time for at least 90 min with



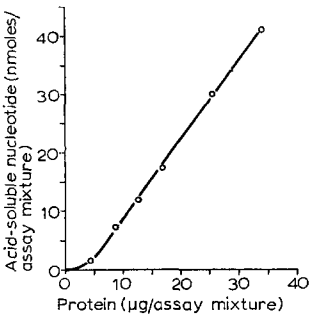


Fig. 1. Elution profile of liver deoxyribonuclease activity after column chromatography on hydroxylapatite. The eluant was Medium B; ○—○, activity with native DNA; ×—×, activity with denatured DNA; ○····○, activity with RNA; ●—●, absorbance at 280 nm.

Fig. 2. The effect of protein concentration on the rate of hydrolysis of native DNA. The reaction mixture is described under MATERIALS AND METHODS.

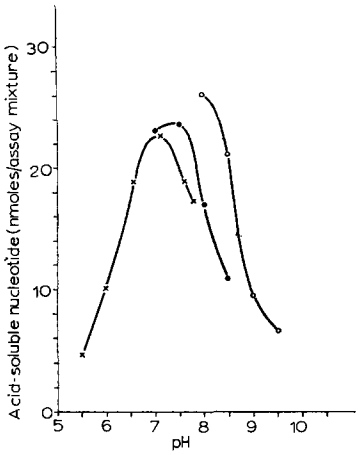
15.6 μg protein per assay mixture and with respect to protein concentrations from 6–34 μg protein per assay mixture (Fig. 2). There was an initial lag with low protein concentrations possibly due to the formation of large acid-insoluble polynucleotides.

Optimal reaction conditions

Fig. 3 shows the pH activity curves for the hydrolysis of native DNA with three different buffers. The region of highest activity was pH 7.0–8.0, and although the precise optimum varied with the buffer employed, maximum activity was obtained with glycine buffer at pH 8.0. At the optimum pH for a given buffer, changes in concentration from 10–60 mM did not affect activity in the case of glycine and glycylglycine, but the activity with 60 mM potassium phosphate was only 9% of that exhibited with the same buffer at a concentration of 10 mM.

In the presence of $MgCl_2$ enzyme activity was maximal between 4 and 7 mM but was progressively inhibited at higher concentrations. At a concentration of 24 mM, activity was reduced to 43% of the maximum. When $MgCl_2$ was excluded from the reaction mixtures, there was a residual activity of 20% which was abolished in the presence of 4 mM EDTA. The maximum activity obtained in the presence of $ZnCl_2$ or $MnCl_2$ was with concentrations from 0.2–0.4 mM or in the presence of $CaCl_2$ from 0.5–1.0 mM. The activation produced by these cations was in each case approx. 50% of the activity shown with Mg^{2+} alone. In the presence of 6 mM $MgCl_2$, the addition of 0.4 mM $CaCl_2$ did not alter the activity; increasing the concentration of $CaCl_2$ to 4 mM progressively inhibited the enzyme to 85% of the activity with Mg^{2+} alone. The univalent cations K^+ and Na^+ both inhibited the liver nuclease by approx. 50% at a concentration of 50 mM KCl or NaCl in the presence of 6 mM $MgCl_2$.

For maximum activity the enzyme required a substrate with a high degree of secondary structure. Table I shows that the activity of the nuclease was 3 times greater when the substrate was native DNA. This was confirmed when native DNA was denatured by heat to varying degrees and used as substrate (Fig. 4). The enzyme was apparently sensitive to the presence of partially denatured DNA. Once the



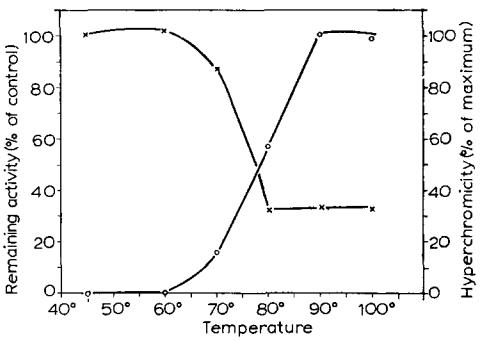


Fig. 3. The effect of pH on the activity of the liver deoxyribonuclease with native DNA. The reaction mixture is described under MATERIALS AND METHODS except for the buffers used, which were ×—×, 10 mM potassium phosphate (pH 5.4–7.8); ●—●, 60 mM glycylglycine–NaOH (pH 7.0–8.5); ○—○, 60 mM glycine–NaOH (pH 8.0–9.5).

Fig. 4. The effect of heat denaturation on the utilization of DNA as substrate by the liver deoxyribonuclease. The reaction mixture is described in MATERIALS AND METHODS and contained samples of DNA which had been heated at a concentration of 250 μg/ml in 10 mM potassium phosphate buffer (pH 7.7) for 6 min at the temperatures shown in the figure. An increase in hyperchromicity to a maximum of 37% was attained. Hyperchromicity (○—○) is expressed as a percentage of the maximum and enzyme activity (×—×) as a percentage of the activity with control native DNA.

secondary structure was substantially disrupted, as at the mid-point of the thermal transition curve, the DNA was hydrolyzed at the rate for fully denatured DNA.

Mode of action of the liver deoxyribonuclease

A gel filtration procedure[29] has been used to show that the enzyme acts predominantly as an endonuclease. Limited digests of native DNA by the liver enzyme and of denatured DNA by the snake venom enzyme were compared. The profiles obtained for digests by the liver deoxyribonuclease showed a wide spectrum of polynucleotides, whereas profiles obtained for digests with the snake venom exonuclease showed only undigested DNA and mononucleotide material.

In order to obtain a clear demonstration of the nucleolytic action of this enzyme a prolonged digest with 20 mg native DNA was carried out. An exhaustive digest was excluded since an extensive purification of the enzyme has not yet been achieved. The products of this digest were examined by DEAE-cellulose chromatography (see MATERIALS AND METHODS). The products were completely eluted from the column, and the proportion of the product contained in each peak was calculated from the ultraviolet absorbing material eluted from the column. The percentage distribution and chain length of the oligonucleotide products are shown in Table II. The almost exclusively endonucleolytic action is shown by the small proportion of mononucleotides present in the digest.

The differing modes of action of snake venom and spleen exonucleases have been used to determine the position of the terminal phosphate. Native DNA was

TABLE II

CHAIN LENGTH OF THE PRODUCTS OF A PROLONGED DIGESTION OF NATIVE DNA BY THE LIVER DEOXYRIBONUCLEASE

Chain length (number of nucleotides)	1	2	3	4	5	6	7	9.7	16.6	19.3
% of digest	1.9	2.1	3.1	5.2	8.1	6.4	6.2	17.8	29.3	19.9

digested by the liver deoxyribonuclease, and portions of the product were exposed to the action of snake venom and spleen exonuclease. The untreated product was readily digested by the snake venom enzyme (Table III). Another portion of the product (see MATERIALS AND METHODS) was digested by the snake venom enzyme in order to isolate the mononucleotides and show that they were readily degraded to nucleosides by the 5'-nucleotidase contained in Russell's Viper venom. The spleen exonuclease showed little activity with the untreated liver deoxyribonuclease product (Table III), but the dephosphorylated product was digested more readily. Since oligonucleotides bearing a 5'-phosphate terminus are degraded by the snake venom enzyme to yield 5'-mononucleotides and are not attacked by the spleen enzyme[30,31], it was concluded that the liver deoxyribonuclease produced oligonucleotides with a 5'-phosphomonoester end group.

DISCUSSION

These studies indicate that the alkaline deoxyribonuclease purified from regenerating rat liver is specific for DNA and utilizes native DNA 3 times more rapidly than denatured DNA. In these respects it resembles the pancreatic deoxyribonuclease[32] but differs from the other alkaline nuclease from rat liver which utilizes RNA and denatured DNA[6] and from the lamb brain endonuclease which has almost an obligatory requirement for denatured DNA[33]. Like the pancreatic enzyme and the

TABLE III

IDENTIFICATION OF THE TERMINAL PHOSPHATE OF THE OLIGODEOXYNUCLEOTIDE PRODUCTS OF THE LIVER DEOXYRIBONUCLEASE

The oligodeoxynucleotide products were obtained from a digest of native DNA (see MATERIALS AND METHODS), and portions were exposed to the action of snake venom and spleen exonucleases as described below: Portions 1–3 were adjusted to pH 5.6; 50 μg of prostatic phosphatase were added to Sample 3, and all three were incubated for 1 h at 37°. The reaction was stopped by heating to 100° for 10 min. Portions 2 and 3 were then incubated at 37° for 2 h with 20 μl of the spleen exonuclease ($(NH_4)_2SO_4$ suspension); Portion 1 served as the control. The reactions were again terminated by heating, and Portions 1–3 were dephosphorylated as described above. Portion 4 was adjusted to pH 8.9 and incubated for 1 h at 37° with 0.4 ml of the purified snake venom exonuclease. The reaction was terminated by heating and was dephosphorylated as above. After dephosphorylation, each portion was freeze-dried, and the products were chromatographed as described under MATERIALS AND METHODS. The values given in the table refer to the samples applied to the chromatogram. Under the same conditions these enzymes were active with the appropriate substrates for phosphatase[16], for venom exonuclease[13] and for spleen exonuclease[15].

Treatment	*$A_{260\ nm}$ of nucleosides eluted*
1. Control	0.02
2. Spleen exonuclease	0.03
3. Spleen exonuclease (after dephosphorylation)	0.31
4. Snake venom exonuclease	2.17

nonspecific liver nuclease, this enzyme hydrolyzes DNA endonucleolytically to produce oligonucleotides with a 5'-phosphate terminus.

The activity of the nonspecific liver nuclease has an optimum at pH 6.8 with RNA and denatured DNA[7], whereas the enzyme described here is most active at pH 8.0. However, both enzymes are active over the range pH 6.5–8.0 and are in this respect similar to the pancreatic enzyme[34]. There are differences also in the bivalent metal requirements. The liver deoxyribonuclease which hydrolyzes native DNA exhibits maximum activity with Mg^{2+}, and at the optimal concentration, Mn^{2+}, Zn^{2+} and Ca^{2+} produce only 50% of the activation obtained with Mg^{2+} alone. Furthermore, there is no evidence of a synergistic effect by Ca^{2+} in the presence of Mg^{2+}. The nonspecific liver nuclease is activated to the same extent by Mg^{2+} and Mn^{2+} and is inhibited by Ca^{2+} (ref. 7). The pancreatic deoxyribonuclease, on the other hand, is activated by Mn^{2+} 3.5 times more efficiently than by Mg^{2+}. Alone, Ca^{2+} produces a weak stimulation, but in the presence of Mg^{2+} a powerful synergistic effect is observed[35]. Univalent cations inhibit the activity of the liver deoxyribonuclease, the pancreatic enzyme[34] and the nonspecific liver nuclease[7].

The total extent of purification cannot be ascertained easily. Although there is a reasonable recovery of enzyme activity in the last two stages of purification, there is a considerable loss of activity during fractionation at pH 5. Part of this loss may be due to inactivation or to precipitation of the enzyme. Part also is due to removal of the enzyme which is principally active with denatured DNA but shows some activity with native DNA[6]. The presence of activators in the crude homogenate is possibly significant here. In an attempt to reduce this loss, proteins were precipitated over a range of acid pH, but it proved necessary to adjust the preparation to pH 5 in order to effect a substantial removal of the enzyme active with denatured DNA. The extent of purification has therefore been expressed relative to the pH 5 supernatant and is shown to be 57-fold (Table I). Attempts have been made to purify the enzyme further, using DEAE-cellulose chromatography, gel filtration and isoelectric focusing, but in each case the activity was lost.

The question of whether the residual activity shown by Fraction 4 towards denatured DNA is due to the protein responsible for the hydrolysis of native DNA has not yet been resolved, although the elution profiles (Fig. 1) for the two activities from a column of hydroxylapatite suggest that they are chromatographically similar proteins.

Results of preliminary studies to examine the intracellular distribution of the enzyme have indicated that the deoxyribonuclease which is active with native DNA can be demonstrated in nuclear fractions and is located principally in the chromatin acidic protein fraction. Other reports also indicate the presence of a deoxyribonuclease which is associated with the chromatin of mammalian cells[36] and the nuclei of sea urchin embryos[37,38]. Although the biological function of alkaline deoxyribonuclease is at present unknown[36], the localization of enzyme activity is of interest in relation to the release and inhibition of deoxyribonuclease activity at different stages of the cell cycle. The significance of the release of low-level deoxyribonuclease activity during chromosome replication is of special interest in the light of preliminary experiments with the DNA polymerase from the regenerating rat liver. These have indicated that the addition of low levels of this deoxyribonuclease to polymerase reaction mixtures considerably enhanced the priming efficiency of native DNA, while

the priming efficiency of denatured DNA remained unaltered. An enhancement of priming activity due to nuclease action has been observed by others[39,40]. Further studies of the purification and properties of this enzyme are in progress.

ACKNOWLEDGMENTS

The author gratefully acknowledges the helpful advice of Dr. G. R. Barker and Dr. E. S. Canellakis and discussion with Dr. E. D. Whittle.

REFERENCES

1 L. CUNNINGHAM AND M. LASKOWSKI, *Biochim. Biophys. Acta*, 11 (1953) 590.
2 M. GOUTIER-PIROTTE AND A. OTH, *Biochim. Biophys. Acta*, 22 (1956) 394.
3 J. SHACK, *J. Biol. Chem.*, 226 (1957) 573.
4 H. BEAUFAY, D. S. BENDALL, P. BAUDHUIN AND C. DE DUVE, *Biochem. J.*, 73 (1959) 623.
5 M. G. BURDON, R. M. S. SMELLIE AND J. N. DAVIDSON, *Biochim. Biophys. Acta*, 91 (1964) 46.
6 P. J. CURTIS, M. G. BURDON AND R. M. S. SMELLIE, *Biochem. J.*, 98 (1966) 813.
7 P. J. CURTIS AND R. M. S. SMELLIE, *Biochem. J.*, 98 (1966) 818.
8 R. MANTSAVINOS, *J. Biol. Chem.*, 239 (1964) 3431.
9 P. J. O'CONNOR, *Biochem. J.*, in the press.
10 W. BJORK, *J. Biol. Chem.*, 238 (1963) 2487.
11 E. J. WILLIAMS, S.-C. SUNG AND M. LASKOWSKI, *J. Biol. Chem.*, 236 (1961) 1130.
12 E. SULKOWSKI AND M. LASKOWSKI, *J. Biol. Chem.*, 237 (1962) 2620.
13 F. FELIX, J. L. POTTER AND M. LASKOWSKI, *J. Biol. Chem.*, 235 (1960) 1150.
14 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
15 A. BERNARDI AND G. BERNARDI, *Biochim. Biophys. Acta*, 155 (1968) 360.
16 G. SCHMIDT, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 2, Academic Press, New York, 1955, p. 523.
17 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
18 K. BURTON, *Biochem. J.*, 62 (1956) 315.
19 G. CERIOTTI, *J. Biol. Chem.*, 214 (1955) 59.
20 I. R. LEHMAN, M. J. BESSMAN, E. S. SIMMS AND A. KORNBERG, *J. Biol. Chem.*, 233 (1958) 163.
21 G. H. BEAVEN, E. R. HOLIDAY AND E. A. JOHNSON, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. 1, Academic Press, New York, 1955, p. 493.
22 M. LASKOWSKI, SR., in P. D. BOYER, H. LARDY AND K. MYRBÄCK, *The Enzymes*, Vol. 5, Academic Press, New York, 2nd ed., 1961, p. 123.
23 W. E. RAZZELL AND H. G. KHORANA, *J. Biol. Chem.*, 234 (1959) 2105.
24 E. J. KING AND I. D. P. WOOTTON, *Micro-Analysis in Medical Biochemistry*, Churchill, London, 1956, p. 83.
25 R. V. TOMLINSON AND G. M. TENER, *Biochemistry*, 2 (1963) 697.
26 N. B. FURLONG, in L. GROSSMAN AND K. MOLDAVE, *Methods in Enzymology*, Vol. 12, Part A, Academic Press, New York, 1967, p. 318.
27 J. D. SMITH, in L. GROSSMAN AND K. MOLDAVE, *Methods in Enzymology*, Vol. 12, Part A, Academic Press, New York, 1967, p. 350.
28 G. M. HIGGINS AND R. M. ANDERSON, *Arch. Pathol.*, 12 (1931) 186.
29 H. BIRNBOIM, *Biochim. Biophys. Acta*, 119 (1966) 198.
30 W. E. RAZZELL, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, New York, 1963, p. 240.
31 W. E. RAZZELL, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, New York, 1963, p. 247.
32 N. B. KURNICK, *J. Am. Chem. Soc.*, 76 (1954) 417.
33 J. W. HEALY, D. STOLLAR, M. I. SIMON AND L. LEVINE, *Arch. Biochem. Biophys.*, 103 (1963) 461.
34 M. KUNITZ, *J. Gen. Physiol.*, 33 (1950) 363.
35 J. S. WIBERG, *Arch. Biochim. Biophys.*, 73 (1958) 337.
36 K. F. SWINGLE, L. J. COLE AND J. S. BAILEY, *Biochim. Biophys. Acta*, 149 (1967) 467.
37 A. J. SOLARI, *J. Ultrastruct. Res.*, 17 (1967) 421.
38 L. A. LOEB, D. MAZIA AND A. D. RUBY, *Proc. Natl. Acad. Sci. U.S.*, 57 (1967) 841.
39 C. C. RICHARDSON, C. L. SCHILDKRAUT AND A. KORNBERG, *Cold Spring Harbor Symp. Quant. Biol.*, 28 (1963) 9.
40 R. MANTSAVINOS AND B. MUNSON, *J. Biol. Chem.*, 241 (1966) 2840.