Properties of a Purified Rat-Liver Nuclease

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1. The pH optimum, ionic requirement and heat-stability of a purified liver nuclease have been examined with RNA and denatured DNA as substrates. 2. The enzyme attacked DNA and RNA in an endonucleolytic manner, forming oligonucleotides terminated by 5'-phosphate groups. No clear specificity was found with respect to the bases at the site of cleavage. 3. Comparison of the results obtained with RNA and denatured DNA as substrates suggests that the ribonuclease and deoxyribonuclease activities are associated with the same protein.

The characteristics of the nuclease purified from rat-liver mitochondria (see Curtis, Burdon & Smellie, 1966) have been examined to determine whether it could be identified with a known nuclease in rat liver, e.g. the acid ribonuclease and deoxyribonuclease of the lysosomes (Roth, 1957), the alkaline ribonuclease (ribonuclease I) of the nuclei (Heppel, Ortiz & Ochoa, 1956) or the alkaline ribonuclease (ribonuclease II) of the mitochondria (Roth, 1957).

MATERIALS AND METHODS

Materials. Salmon-sperm DNA, adenosine, guanosine, cytidine and adenosine 2'- and 3'-monophosphate were purchased from the California Corp. for Biochemical Research, Lucerne, Switzerland, inosine from the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A., deoxyinosine and thymidine from the Sigma Chemical Co., London, S.W. 6, and deoxyadenosine, deoxyguanosine, deoxycytidine and adenosine 5'-monophosphate from Schwarz BioResearch Inc., Mount Vernon, N.Y., U.S.A.

Pancreatic deoxyribonuclease I, pancreatic ribonuclease, spleen phosphodiesterase and crude snake venom (*Crotalus adamanteus*) were purchased from the Sigma Chemical Co., and *Escherichia coli* alkaline phosphatase as an $(\text{NH}_4)_2\text{SO}_4$ suspension from the Nutritional Biochemicals Corp. Purified snake-venom phosphodiesterase was prepared from the crude venom by a combination of published methods as follows: step 1, acetone fractionation (Bjork, 1963); step 2, ethanol fractionation (Williams, Sung & Laskowski, 1961); step 3, ethanol fractionation (Williams *et al.* 1961); step 4, acetone fractionation (Sulkowski & Laskowski, 1962); step 5, CM-cellulose (Felix, Potter & Laskowski, 1960); step 6, DEAE-cellulose (Felix *et al.* 1960). The spleen and purified snake-venom phosphodiesterases showed no detectable phosphomonoesterase activity after incubation at 37° for 4 hr. with 2'- and 3'-AMP and 5'-AMP respectively when the products of the digestion were examined chromatographically.

Liver nuclease. Unless otherwise stated the pooled

fraction of the liver nuclease from Sephadex G-75 was used. The methods employed for assay of the activity of this enzyme towards native DNA, denatured DNA and RNA were as described by Curtis *et al.* (1966).

Inactivation of *E. coli* alkaline phosphatase. The solution was refluxed with an equal volume of ethanol for 1 hr. after which the ethanol was allowed to evaporate. An equal volume of chloroform was added and the solution was shaken vigorously for 2 hr., after which the two layers were separated by centrifuging.

Column chromatography of oligonucleotides. A DEAE-cellulose column (2 cm. $\times$ 40 cm.) equilibrated with 10 mM-triethylammonium hydrogen carbonate buffer, pH 8.6, was used. The column was developed by using a linear gradient with 1.4 l. of 10 mM-triethylammonium hydrogen carbonate buffer, pH 8.6, in the mixing vessel and 1.4 l. of 1.0 M-triethylammonium hydrogen carbonate buffer, pH 8.6, in the reservoir. A hydrostatic pressure of 1 m. above the top of the column was used. The gradient was measured by determining the nitrogen content of selected fractions. Samples were digested with conc. H_2SO_4 and the resulting $(\text{NH}_4)_2\text{SO}_4$ was estimated, after neutralization with alkali, with Nessler's reagent.

Paper chromatography. For the separation of deoxyribonucleosides a two-solvent system described by Sulkowski & Laskowski (1962) was used. Solvent I consisted of 95% (v/v) ethanol-m-ammonium acetate adjusted to pH 7.5 (2:1, v/v) and solvent II was propan-2-ol-water-satd. $(\text{NH}_4)_2\text{SO}_4$ (1:9:40, by vol.). Whatman no. 3MM paper was used and both solvents were used in a descending direction. The development time for the first solvent was 16 hr. and for the second 12 hr. The ribonucleosides separated in a similar manner except that cytidine and uridine overlapped. The proportion of these pyrimidines in the merging spots was estimated by comparing the spectra of the eluted spots with the spectra of known mixtures of cytidine and uridine.

Solvent III was propan-2-ol-water-aq. NH_3 (sp.gr. 0.88) (7:2:1, by vol.). Whatman no. 1 paper was used with this solvent and the development time was 16 hr.

Spots were located by viewing the chromatograms with a Hanovia Chromatolite lamp. The material in a spot was eluted by cutting out the spot and attaching it to a paper

wick, which was then immersed in 10mN-HCl. The eluted material was identified and estimated by recording the spectrum in a Cary model 11 spectrophotometer and by utilizing the data given in the publication *Properties of the Nucleic Acid Derivatives* by Calbiochem, Los Angeles, Calif., U.S.A.

Examination of the products of prolonged digestion. The digestion mixture contained 40mg. of heat-denatured DNA from Landschutz ascites-tumour cells, 1.5m-moles of imidazole-HCl buffer, pH6.8, 15 μ moles of MgCl₂ and 300 μ moles of 2-mercaptoethanol in a total volume of 20ml. The solution was incubated at 37° for 12hr. and at zero time 90 deoxyribonuclease units of liver nuclease were added followed by a further 90 units after 4hr. and 8hr. After incubation the solution was deproteinized by shaking with an equal volume of chloroform-3-methylbutan-1-ol (4:1, v/v) for 1hr. The aqueous layer was diluted to 150ml. and applied to a DEAE-cellulose column (2cm. $\times$ 40cm.), after which gradient elution was commenced. The effluent was divided into fractions according to the E_{260} values. The pooled fractions were freeze-dried until the characteristic smell of triethylamine could no longer be detected. The material was then dissolved in 4ml. of water.

The chain length of the pooled fractions was determined by comparing the total phosphorus with the terminal phosphorus of a sample of an oligonucleotide fraction. The terminal phosphorus was estimated by incubating the sample with 0.02ml. of alkaline-phosphatase suspension and 50 μ moles of tris-HCl buffer, pH8.0, in a volume of 2ml. at 37° for 1hr. The inorganic phosphorus released was estimated by the method of Allen (1940).

The digestion products of the ribonuclease activity on 40mg. of yeast RNA were examined in a similar manner.

Position of the terminal phosphate. Dephosphorylated oligodeoxyribonucleotides were prepared by the digestion of 4mg. of DNA from Landschutz ascites-tumour cells with 200 μ g. of pancreatic deoxyribonuclease in the presence of 200 μ moles of tris-HCl buffer, pH7.5, and 100 μ moles of MgCl₂ in a total volume of 5ml. at 37° for 1hr. The pH of the solution was then adjusted to 8.0 and 0.01ml. of alkaline-phosphatase suspension was added. The solution was incubated at 37° for a further 1hr. The phosphatase was then inactivated. Dephosphorylated oligoribonucleotides were prepared similarly by the digestion of 4mg. of yeast RNA with 200 μ g. of pancreatic ribonuclease.

Oligodeoxyribonucleotides were also prepared by incubating 2mg. of denatured salmon-sperm DNA with 25 μ moles of imidazole-HCl buffer, pH6.8, 0.5 μ mole of MgCl₂, 10 μ moles of 2-mercaptoethanol and 5 deoxyribonuclease units of the purified liver nuclease at 37° for 4hr. Oligoribonucleotides were similarly prepared from 2mg. of yeast RNA.

The solutions of the dephosphorylated oligodeoxyribonucleotides prepared from the deoxyribonuclease I digest and the oligodeoxyribonucleotide products of the liver nuclease were each divided into three fractions. The first fraction gave a measure of mononucleotides released in the preliminary digestion. The second fraction was incubated at pH6.5 and 37° for 1hr. after adding 200 μ g. of spleen phosphodiesterase. The reaction was stopped by heating at 100° for 10min. The pH of the third fraction was adjusted to pH8.8 and 0.1ml. of the purified snake-venom phosphodiesterase was added. Incubation was at 37° for 1hr. and then the reaction was stopped by heating at 100°

for 10min. The dephosphorylated oligoribonucleotides from the pancreatic-ribonuclease digest and the oligoribonucleotide products of the liver nuclease were treated similarly. To facilitate the chromatographic separation of the mononucleotides produced by the action of the phosphodiesterase from the other products each fraction was dephosphorylated. The pH of each fraction was adjusted to 8.0, 0.01ml. of alkaline-phosphatase suspension was added and the solutions were incubated at 37° for 30min. The fractions were concentrated by freeze-drying and the material was redissolved in 0.05ml. of 10mN-HCl. Then 0.03ml. of each fraction was spotted on Whatman no. 1 paper and the chromatogram was developed in a descending direction in solvent III for 16hr.

Determination of the terminal bases of the oligonucleotides. The oligodeoxyribonucleotides and oligoribonucleotides were prepared by incubating 20mg. of salmon-sperm DNA or 20mg. of yeast RNA, 250 μ moles of imidazole-HCl buffer, pH6.8, 5 μ moles of MgCl₂, 100 μ moles of 2-mercaptoethanol and 50 units of the liver nuclease in a total volume of 10ml. at 37° for 4hr. The two solutions of oligonucleotides were dephosphorylated by adjusting the pH of each solution to 8.0 and adding 0.02ml. of alkaline-phosphatase suspension. The solutions were incubated at 37° for 1hr., the alkaline phosphatase was inactivated and the two solutions were divided into two. One half was adjusted to pH8.8, 0.5ml. of purified snake-venom phosphodiesterase was added and the solution was incubated at 37° for 4hr. In this time the digestion had proceeded to completion. The other half was adjusted to pH6.5, 1.0mg. of spleen phosphodiesterase was added and the solution was incubated at 37° for 8hr., the extent of the digestion being approx. 90%. The four digests were freeze-dried and redissolved in 0.1ml. of 10mN-HCl. The nucleoside and nucleotide products were separated by the two-dimensional paper-chromatographic system of Sulkowski & Laskowski (1962).

Fig. 1. Time-course of the hydrolysis of RNA and denatured DNA by the purified liver nuclease. The assay medium contained 200 μ g. of RNA or denatured DNA, 25 μ moles of imidazole-HCl buffer, pH6.8, 0.25 μ mole of MgCl₂, 5 μ moles of 2-mercaptoethanol and 6.7 μ g. of protein from the DEAE-cellulose fraction for deoxyribonuclease assays or 2.7 μ g. for ribonuclease assays in a total volume of 0.5ml. Incubation was at 37° for the indicated time-intervals. The results are expressed as percentages of the amount of the substrate converted into acid-soluble products. ○, Deoxyribonuclease activity; Δ, ribonuclease activity.

RESULTS

Since the rate of hydrolysis of RNA was greater than that of DNA, less enzyme was employed in experiments with RNA. The time-course of the hydrolysis of denatured DNA and RNA showed that the rate was linear for approx. 1 hr., when 50% of the substrate had become acid-soluble (Fig. 1). With RNA as a substrate there was direct proportionality between the rate of hydrolysis and the concentration of the enzyme over the range 0–10 μg . of protein/ml. (Fig. 2). The rate of hydrolysis of DNA showed lack of proportionality at concentrations below 12 μg . of protein/ml. (Fig. 2). Above this concentration there was direct proportionality.

The effect of pH was studied in a number of different buffer systems. It was found that phos-

phate and glycine-sodium hydroxide buffers gave relatively low activities in comparison with imidazole-hydrochloric acid and tris-hydrochloric acid buffers. Both activities showed sharp pH optima, deoxyribonuclease at pH 6.6–6.8 and ribonuclease at pH 6.8–7.0 (Fig. 3).

The two activities showed an absolute requirement for Mg^{2+} or Mn^{2+} (Fig. 4). The requirement was about the same for both cations and for both activities, i.e. the optimum concentration for deoxyribonuclease and ribonuclease was about 0.1 mM with Mg^{2+} and with Mn^{2+} . In the absence of Mg^{2+} , Ca^{2+} showed no stimulatory effect (Fig. 5), and inhibited both activities in the presence of Mg^{2+} . There was no indication of synergism in the presence of both cations. Univalent cations inhibited both activities markedly, though at a higher concentration than Ca^{2+} (Fig. 6).

The effect of heat was examined at pH 5.1 and pH 7.2 (Fig. 7). Both deoxyribonuclease and ribonuclease were rapidly inactivated at pH 5.1, but at pH 7.2 both activities survived heating at higher temperatures than at pH 5.1.

Fig. 2. Effect of the protein concentration on the rate of hydrolysis of RNA and denatured DNA. The assay medium contained 300 μg . of RNA or denatured DNA, 25 μmoles of imidazole-HCl buffer, pH 6.8, 0.25 μmole of MgCl_2 , 5 μmoles of 2-mercaptoethanol and various amounts of protein of the DEAE-cellulose fraction in a total volume of 0.5 ml. Incubation was at 37° for 30 min. (a) ○, Deoxyribonuclease activity; (b) Δ, ribonuclease activity.

Fig. 3. Effect of pH on the activity of the purified liver nucleases. The assay medium contained 300 μg . of RNA or denatured DNA, 0.25 μmole of MgCl_2 , 5 μmoles of 2-mercaptoethanol, 25 μmoles of buffer and 1.1 μg . of purified liver nuclease protein for deoxyribonuclease assays or 0.6 μg . for ribonuclease assays in a total volume of 0.5 ml. The buffers used were: (i) acetate at pH 5.1 and 5.6; (ii) imidazole-HCl at pH 6.0–7.6; (iii) tris-HCl at pH 7.5–8.8. Incubation was at 37° for 30 min. ○, Deoxyribonuclease activity; Δ, ribonuclease activity.

Fig. 4. Effect of Mg^{2+} and Mn^{2+} on the activity of the purified liver nuclease. The assay medium contained 300 μg. of RNA or denatured DNA, 25 μmoles of imidazole-HCl buffer, pH 6.8, 5 μmoles of 2-mercaptoethanol and 0.9 μg. of purified liver nuclease protein for deoxyribonuclease assays or 0.45 μg. for ribonuclease assays in a total volume of 0.5 ml. MgCl_2 (a) and MnCl_2 (b) were added to give the indicated molarities. Incubation was at 37° for 30 min. O, Deoxyribonuclease activity; Δ, ribonuclease activity.

Specificity of the liver nuclease

Products of prolonged digestion. Exhaustive digestion of the substrate was excluded since it was considered that under conditions necessary to achieve complete hydrolysis of the substrate the contaminating activities, i.e. phosphomonoesterase and phosphodiesterase, would contribute appreciably to the result. A 12 hr. incubation was therefore used since after such a time exo- and endonucleolytic activity would be clearly distinguished. The elution profiles of the DNA and RNA digests on DEAE-cellulose are given in Figs. 8 and 9: 98% of the ultraviolet-absorbing material of the DNA digest and 100% of that in the RNA digest were recovered. The chain lengths of the fractions indicated in Figs. 8 and 9 were determined as described in the Materials and Methods section (Table 1): for both DNA and RNA about 95% of the recovered nucleotide was in the form of oligonucleotides. To enable these results to be

Fig. 5. Effect of CaCl_2 in the presence and absence of the optimum concentration of MgCl_2 on the activity of the purified liver nuclease. The assay medium contained 300 μg. of RNA or denatured DNA, 25 μmoles of 10 mM-imidazole-HCl buffer, pH 6.8, 5 μmoles of 2-mercaptoethanol and 0.9 μg. of purified liver nuclease protein for deoxyribonuclease assays or 0.45 μg. for ribonuclease assays in a total volume of 0.5 ml. Incubation was at 37° for 30 min. (a) Deoxyribonuclease activity in the absence (●) and presence (○) of 0.25 μmole of MgCl_2 ; (b) ribonuclease activity in the absence (▲) and presence (Δ) of 0.25 μmole of MgCl_2 .

evaluated the phosphomonoesterase and phosphodiesterase activities in the enzyme solutions used in both digests were assayed and the values obtained were calculated so that the extent of hydrolysis due to the contaminating activities under the conditions of the digestion could be estimated. It was found that for the DNA digest phosphomonoesterase would account for 0.3% and phosphodiesterase II for 0.25% of the total digest, and phosphodiesterase I showed no activity. For the RNA digest phosphomonoesterase would account for 0.18% and phosphodiesterases I and II for 0.02% each of the total digest.

The results indicate that both activities, deoxyribonuclease and ribonuclease, are almost exclu-

Fig. 6. Effect of NaCl on the activity of the purified liver nuclease. The assay medium contained 300 μ g. of RNA or denatured DNA, 25 μ moles of imidazole-HCl buffer, pH 6.8, 0.25 μ mole of $MgCl_2$, 5 μ moles of 2-mercaptoethanol and 3 μ g. of purified liver nuclease protein for deoxyribonuclease assays or 1.5 μ g. for ribonuclease assays in a total volume of 0.5 ml. NaCl was added to give the indicated molarities. Incubation was at 37° for 30 min. O, Deoxyribonuclease activity; Δ , ribonuclease activity.

sively endonucleolytic in their mode of action, though the amount of mononucleotide product cannot be accounted for by phosphodiesterase activity.

Determination of the position of the terminal phosphate group. The position of the terminal phosphate group was determined by comparing the hydrolysis by snake-venom and spleen phosphodiesterases of dephosphorylated oligodeoxyribonucleotides and oligoribonucleotides with the hydrolysis of oligonucleotides produced by the liver nuclease. The extent of hydrolysis by the phosphodiesterases was examined by paper chromatography. The mononucleotides were hydrolysed to nucleosides to facilitate their complete separation from all other products. The material corresponding in R_F to the four known nucleosides was eluted and the E_{280} value measured (Table 2). The results show that, by comparison with the dephosphorylated oligonucleotides, the oligodeoxyribonucleotides and oligoribonucleotides were both relatively resistant to spleen phosphodiesterase but both were readily hydrolysed by snake-venom phosphodiesterase. Since snake-venom phosphodiesterase

Fig. 7. Effect of heat on the activity of the purified liver nuclease. Samples containing 11 μ g. of purified liver nuclease protein and 50 μ moles of acetate buffer, pH 5.1, or imidazole-HCl buffer, pH 7.2, in a volume of 1.2 ml. were heated at 40°, 45°, 50°, 60° or 70° for 5 min. and then were immediately cooled in ice until assayed. The control was kept in ice until assayed. The assay medium contained 300 μ g. of RNA or denatured DNA, 25 μ moles of imidazole-HCl buffer, pH 6.8, 0.25 μ mole of $MgCl_2$, 5 μ moles of 2-mercaptoethanol and 0.1 ml. of the samples in a total volume of 0.5 ml. Incubation was at 37° for 30 min. (a) Heated at pH 5.1; (b) heated at pH 7.2. O, Deoxyribonuclease activity; Δ , ribonuclease activity.

attacks oligonucleotides bearing terminal 5'-phosphate but not terminal 3'-phosphate groups, and spleen phosphodiesterase shows the reverse specificity, it was concluded that the deoxyribonuclease and ribonuclease activities of the purified liver nuclease produce oligonucleotides terminated by 5'-phosphate groups.

Determination of the terminal bases of the oligonucleotides. Dephosphorylated oligonucleotides were produced by hydrolysis of DNA and RNA by the purified liver nuclease followed by incubation with alkaline phosphatase. After inactivation of the alkaline phosphatase, hydrolysis of the

Fig. 8. Fractionation of the products of digestion of denatured DNA by the purified liver nuclease. The digestion medium contained 40mg. of denatured Land-schutz ascites-carcinoma DNA, 1.5m-moles of imidazole-HCl buffer, pH6.8, 15 μ moles of $MgCl_2$ and 300 μ moles of 2-mercaptoethanol in a total volume of 20ml. and was incubated at 37° for 12hr. At zero time 90 deoxyribonuclease units of the purified liver nuclease were added and again after 4hr. and 8hr. The gradient, consisting of 1.4l. of 10mM-triethylammonium hydrogen carbonate buffer, pH8.6, and 1.4l. of 1.0M-trimethylammonium hydrogen carbonate buffer, pH8.6, was applied to a column (2cm. $\times$ 40cm.) of DEAE-cellulose. The flow rate was 4ml./min. and 16.5ml. fractions were collected. —, E_{260} ; ----, concn. of buffer.

Table 1. Chain length of the products of digestion of RNA and denatured DNA by liver nuclease

Experimental details are given in the text.

Fraction	Chain length (no. of nucleotides)	% of total digest
RNA		
I (18-31)	0	5.7
II (36-60)	1*	2.9
III (63-72)	1.9	3.4
IV (73-96)	2.5	13.1
V (97-114)	2.8	21.9
VI (115-123)	2.9	13.1
VII (124-134)	3.4	16.0
VIII (135-170)	5.3	24.0
DNA		
I (15-21)	0	3.3
II (37-66)	1*	1.8
III (70-83)	2.2	4.3
IV (84-91)	2.7	7.2
V (92-107)	4.7	18.9
VI (108-130)	6.1	43.8
VII (131-160)	10.1	20.7

* The position of this fraction in the DEAE-cellulose effluent and the presence of phosphorus in the H_2SO_4 digest indicate that these fractions contain mononucleotides, though no phosphorus was released by alkaline phosphatase.

Fig. 9. Fractionation of the products of digestion of RNA by the purified liver nuclease. The digestion medium contained 40mg. of yeast RNA, 1.5m-moles of imidazole-HCl buffer, pH6.8, 15 μ moles of $MgCl_2$ and 300 μ moles of 2-mercaptoethanol in a total volume of 20ml. and was incubated at 37° for 12hr. At zero time 90 ribonuclease units of the purified liver nuclease were added and again after 4hr. and 8hr. The gradient, consisting of 1.4l. of 10mM-triethylammonium hydrogen carbonate buffer, pH8.6, and 1.4l. of 1.0M-trimethylammonium hydrogen carbonate buffer, pH8.6, was applied to a column (2cm. $\times$ 40cm.) of DEAE-cellulose. The flow rate was 4ml./min. and 16.5ml. fractions were collected. —, E_{260} ; ----, concn. of buffer.

Similarly, hydrolysis with spleen phosphodiesterase released the terminal bases at the 3'-hydroxy end as nucleosides.

The nucleosides were resolved by two-dimensional paper chromatography with the solvent systems of Sulkowski & Laskowski (1962). The digests gave one ultraviolet-absorbing spot common to all four that ran with the front of solvent I. This spot was identified as 2-mercaptoethanol. Apart from this spot, snake-venom phosphodiesterase gave four spots corresponding in their positions to adenosine, cytosine, guanosine and uridine and to the corresponding deoxy compounds. Digestion with spleen phosphodiesterase produced five spots corresponding in their positions to adenosine, cytosine, guanosine, uridine and inosine and the five corresponding deoxy compounds. The additional spots corresponding to inosine and deoxyinosine were expected, since Winter & Bernheimer (1964) had shown that the commercial preparation of spleen phosphodiesterase contained a deaminase acting on adenosine and deoxyadenosine. Incubation of adenosine and deoxyadenosine with the preparation of spleen phosphodiesterase used in these experiments produced inosine and deoxyinosine.

The nucleosides were eluted and estimated by recording their spectra and utilizing the molar extinction coefficients quoted in the publication

dephosphorylated oligonucleotides with snake-venom phosphodiesterase released the terminal bases at the 5'-hydroxy end as nucleosides.

Table 2. *Digestion of the oligonucleotide products of the liver nuclease with spleen and snake-venom phosphodiesterases*

Experimental details are given in the text.

Fraction	<i>E</i> ₂₆₀ of nucleotides released	
	Spleen phosphodiesterase	Snake-venom phosphodiesterase
DNA products		
(1) Dephosphorylated oligonucleotides	0.44	2.16
(2) Oligonucleotide products of liver nuclease	0.01	1.01
RNA products		
(1) Dephosphorylated oligonucleotides	0.34	0.49
(2) Oligonucleotide products of liver nuclease	0.08	0.64

Table 3. *Terminal nucleosides of the oligonucleotide products of the liver nuclease*

Experimental details are given in the text. The oligonucleotide products are expressed as percentages of the total amount of nucleosides released. The values for adenosine and deoxyadenosine are the sums of adenosine plus inosine and of deoxyadenosine plus deoxyinosine respectively. Values for yeast RNA were taken from Magasanik (1955), and those for salmon-sperm DNA from Chargaff (1955).

Nucleoside	% of total nucleoside at 5'-position	% of total nucleoside at 3'-position	Composition of substrate
Ribonuclease			Yeast RNA
Adenosine	36	46.5	25.0
Cytidine	9.5	8.5	20.1
Guanosine	33.5	30.5	30.2
Uridine	22	15	24.6
Deoxyribonuclease			Salmon-sperm DNA
Deoxyadenosine	30	42	29
Deoxycytidine	17	27	20.8
Deoxyguanosine	25	14	20.4
Thymidine	28	15	29.1

Properties of the Nucleic Acid Derivatives by Calbiochem. The results (Table 3) show that the two activities, deoxyribonuclease and ribonuclease, have no marked preference towards the bases, though when compared with the values for the percentage composition of salmon-sperm DNA and yeast RNA there is a slight predominance of adenine at the 3'-hydroxy end. In the oligoribonucleotides, cytidine occupied the 3'- and 5'-terminal positions less frequently than might have been expected.

DISCUSSION

From the evidence presented in this and the preceding paper (Curtis *et al.* 1966) the enzyme, purified from rat-liver mitochondria, appears to hydrolyse both DNA and RNA. The two activities of the enzyme are very similar in every respect except in their relative stability on storage (see Curtis *et al.* 1966). Thus the enzyme hydrolyses

DNA and RNA endonucleolytically producing oligonucleotides terminated by 5'-phosphate groups. It has a preference towards single-stranded rather than double-stranded DNA or RNA, but shows no marked preference towards any of the four bases. The pH optimum for both activities is about 6.8, and the enzyme shows an absolute requirement for Mg²⁺ or Mn²⁺; Ca²⁺ inhibits the enzyme, as also does Na⁺. The enzyme is readily denatured by heating, the two activities being lost at equivalent rates.

The deoxyribonuclease activity of the liver nuclease is similar in many respects to pancreatic deoxyribonuclease. The pH optima are similar and the requirement for Mg²⁺ is of the same order; Na⁺ inhibits at approx. 50mM and the products of hydrolysis are similar. However, there are a few important differences. Thus Ca²⁺ alone stimulates pancreatic deoxyribonuclease weakly, but in the presence of Mg²⁺ there is a powerful synergistic

effect (Wiberg, 1958). In contrast, the liver nuclease is inhibited by Ca^{2+} in the presence and absence of Mg^{2+} . The second important difference is that pancreatic deoxyribonuclease hydrolyses native DNA several times more rapidly than denatured DNA, whereas the liver nuclease shows a preference for denatured DNA. In this respect there is a closer similarity to the endonuclease from lamb brain (Healy, Stollar, Simon & Levine, 1963), though this enzyme shows an absolute requirement for denatured DNA. However, the liver nuclease and the lamb-brain enzyme respond to heat, freezing and freeze-drying similarly, and 2-mercaptoethanol and glycerol stabilize both enzymes when they are stored at 0° . In addition, the purified lamb-brain endonuclease contains ribonuclease activity (M. G. Burdon, personal communication), though whether this activity belongs to the same protein as the deoxyribonuclease activity has yet to be determined.

The ribonuclease activity of the liver nuclease is distinct from the enzyme purified from rat-liver mitochondria by Roth (1957) and Beard & Razzell (1964). This ribonuclease has a pH optimum about 7.0, but no requirement for Mg^{2+} ; for optimum activity it requires an ionic strength of 0.1 M. It is stable to heat and produces on hydrolysis of RNA a resistant 'core' and oligonucleotides terminated by pyrimidine 2',3'-(cyclic)-phosphates. Heppel *et al.* (1956) have described an enzyme from guinea-pig nuclei that hydrolyses polyadenylic acid and polyuridylic acid to oligonucleotides terminated by a 5'-phosphate group.

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