Purification and Characterization of Bovine Aorta Ribonucleases

By R. A. LEWIS AND W. GAMBLE

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oreg. 97331, U.S.A.

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1. Two ribonucleases (aorta ribonuclease I and aorta ribonuclease II) from bovine aorta were purified 4611-fold and 667-fold respectively. Ethanol precipitation, acid extraction, isoionic precipitation at pH 3.5 and Bio-Rex 70 column chromatography were the methods employed. 2. Aorta ribonuclease I exhibited no deoxyribonuclease or alkaline phosphatase activity. 3. Aorta ribonuclease I appeared to be homogeneous when subjected to discontinuous gel electrophoresis. 4. Aorta ribonuclease II exhibited the same properties as aorta ribonuclease previously isolated. 5. The activities of the aorta ribonucleases and pancreatic ribonuclease on homopolymers and dinucleoside phosphates were compared. 6. Aorta ribonuclease I exhibited optimum pH 7.5 and, under the assay conditions used, optimum temperature 60°.

The presence of a ribonuclease in bovine aorta has been demonstrated by Gamble, Nayar & Kiersky (1967). To our knowledge no other studies on arterial ribonuclease have been presented. A recent report by Henry (1968) indicated a correlation between the RNA content of arteries and the development of atherosclerosis. It occurred to us that a detailed study of the properties of arterial ribonucleases might prove useful in understanding arterial nucleic acid metabolism and the relationship of this to the development of atherosclerosis. The present paper describes the purification and characterization of two bovine aorta ribonucleases (aorta ribonuclease I and aorta ribonuclease II). Aorta ribonuclease I was purified 4611-fold and aorta ribonuclease II 667-fold. Aorta ribonuclease I rapidly depolymerized poly U* whereas poly C was hydrolysed slowly, one-twentyfourth the rate exhibited with poly U. Poly G, poly A and poly I were not hydrolysed by the enzyme. Aorta ribonuclease II depolymerized poly C 18 times the rate and poly U one-seventh the rate exhibited by aorta ribonuclease I with the respective homopolymers. CpC and UpC were converted into C>p and cytidine and into U>p and cytidine respectively by

aorta ribonuclease I. Aorta ribonuclease I did not hydrolyse ApC and GpC. It was concluded that aorta ribonuclease I was an endonuclease with a specificity for the formation of pyrimidine cyclic phosphates during the hydrolysis of polynucleotides and a preference for uridylic acid residues. Aorta ribonuclease II exhibited the same properties as aorta ribonuclease isolated by Gamble *et al.* (1967).

MATERIALS AND METHODS

Materials

Bio-Rex 70 (200–400 mesh; Na⁺ form), Cellex-E (ECTEOLA-cellulose) and Bio-Gel P-2 were obtained from Bio-Rad Laboratories (Richmond, Calif., U.S.A.). Mann Research Laboratories Inc. (New York, N.Y., U.S.A.) was the source of Gp, Ap, Cp, Up and *p*-nitrophenyl phosphate. Reagent grade (NH₄)₂SO₄, H₂SO₄, propan-2-ol, aq. NH₃, acetic acid and NaCl were acquired from the Baker and Adamson Co. (Allied Chemicals, Morristown, N.J., U.S.A.). Poly U, poly C, poly A, poly G, poly I and GpC were obtained from Miles Chemicals Co. (Clifton, N.J., U.S.A.). K₂HPO₄, KH₂PO₄, trichloroacetic acid and KOH were purchased from Mallinckrodt Chemical Works (St Louis, Mo., U.S.A.). Schwarz Bioresearch Inc. (Orangeburg, N.Y., U.S.A.) was the source of C>p. Reagent-grade isobutyric acid was purchased from the J. T. Baker Co. (Phillipsburg, N.J., U.S.A.). Sodium RNA was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.).

Tris, bovine pancreatic ribonuclease type IIIA (90–95% homogeneous), DEAE-cellulose, U>p and G>p were products of Sigma Chemical Co. (St Louis, Mo., U.S.A.). β-Alanine, UpC, CpC and ApC were purchased from Calbiochem (Los Angeles, Calif., U.S.A.). Glycine was obtained from Matheson, Coleman and Bell Inc. (East Rutherford, N.J., U.S.A.). Cytidine and pC were purchased from P-L

* Abbreviations: C>p, 2',3'-cyclic cytidylic acid; U>p, 2',3'-cyclic uridylic acid; G>p, 2',3'-cyclic guanylic acid; A>p, 2',3'-cyclic adenylic acid; Cp, 2',3'-cytidylic acid; pC, 5'-cytidylic acid; Up, 2',3'-uridylic acid; CpC, cytidyl-(3'-5')-cytidine; UpC, uridylyl-(3'-5')-cytidine; ApC, adenylyl-(3'-5')-cytidine; GpC, guanylyl-(3'-5')-cytidine; poly C, polycytidylic acid; poly U, polyuridylic acid; poly A, polyadenylic acid; poly I, polyinosinic acid; poly G, polyguanylic acid.

Biochemicals Inc. (Milwaukee, Wis., U.S.A.). Polyacrylamide gel and ammonium persulphate were acquired from the E-C Apparatus Corp. (Philadelphia, Pa., U.S.A.). Bovine aortas were a gift from D. E. Nebergall Meat Co. (Albany, Oreg., U.S.A.).

Methods

Ribonuclease assay. To a 3 ml. test tube were added 0.1 ml. of 0.1 M-potassium phosphate buffer, pH 7.0, 0.1 ml. of enzyme solution and 0.2 ml. of 1.0% sodium RNA (purified as described by Gamble *et al.* 1967). As controls two other reaction solutions were prepared by substituting water for sodium RNA and enzyme solution respectively. The reaction solutions were incubated for 5 min. at 37° in a shaking water bath. The reaction was stopped by the addition of 0.33 ml. of 10% (w/v) trichloroacetic acid solution. This mixture was kept on ice for 5 min., then centrifuged at 12 100g for 15 min. at 3° in a Sorvall RC2-B centrifuge. The supernatant was transferred quantitatively to graduated centrifuge tubes and diluted to 4.0 ml. with water. Acid-soluble nucleotides were measured at 260 nm. on a Beckman DB spectrophotometer. The reference sample was a 0.83% trichloroacetic acid solution. The results are reported as the difference between E_{260} of the acid-soluble nucleotides of the reaction solution and that of the controls.

A unit of activity is defined as the amount of protein required to cause a net E_{260} value of 1.00 under the assay conditions. Specific activity is expressed as units/mg. of protein.

The effluents of all columns used in the purification of the enzyme were assayed by this procedure except that the reaction solutions were incubated for 15 min.

When poly C, poly U, poly G, poly A and poly I were used as substrates, 0.1–0.327% solutions were substituted for the 1% sodium RNA. Incubation periods of 5 and 15 min. for poly U, 5, 15 and 60 min. for poly C and 195 min. for poly G, poly A and poly I were employed. The reactions were stopped as described above except when poly U was the substrate, when 1.5 ml. of saturated $(\text{NH}_4)_2\text{SO}_4$ solution was used.

Dinucleoside phosphate activity of aorta ribonuclease I was determined by using 0.1% solutions of UpC, CpC, GpC and ApC each substituted for 1% sodium RNA. Incubations were for 24 hr. with 50 μg . of enzyme/ml. The reaction was stopped by placing the solution on ice and separating the protein from the nucleotides and nucleosides on a Bio-Gel P-2 column (Gamble *et al.* 1967). Substrate and enzyme blanks were performed as controls. Pancreatic ribonuclease A was assayed similarly. The nucleotides and nucleosides were isolated by Cellex-E column chromatography (Gamble *et al.* 1967) and the identities confirmed by paper chromatography.

Protein determination. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Estimations of protein eluted from columns were made by direct spectrophotometric readings E_{280} or by the E_{280}/E_{260} -ratio method of Warburg & Christian (1941).

Paper chromatography. Both ascending and descending chromatography were employed. A solution of 40 g. of $(\text{NH}_4)_2\text{SO}_4$ in 100 ml. of 0.1 M-potassium phosphate buffer, pH 7.0, was used as the solvent in ascending chromatography. Isobutyric acid–1 M- NH_3 –0.2 M-EDTA (125:75:1, by vol.) and propan-2-ol–water–aq. NH_3 (sp.gr. 0.88)

(1400:600:1, by vol.) with NH_3 in the vapour phase were the two solvents employed in ascending chromatography. Whatman no. 1 and Whatman no. 3 chromatography paper were used in ascending and descending chromatography respectively. The compounds were detected by viewing under u.v. light (254 nm.). Identifications of nucleotides were confirmed by eluting the spots with water and comparing their u.v. spectra with standards and with the data of Stanley & Bock (1965).

Purification of the enzyme

Preparation of crude homogenate. The method was as described by Gamble *et al.* (1967). Aortas were obtained immediately after the cattle had been killed. They were washed with cold distilled water. Surgical gloves were worn whenever it was necessary to handle the aortas, to avoid contamination of the preparation. Excess of fat and adventitia of the aortas were removed with scissors. The aortas were then cut into small pieces, and the preparation was homogenized in a Waring Blendor with an equal weight of ice-cold distilled water for about 1 min. The mixture was centrifuged in a Sorvall RC2-B centrifuge at 12 100g for 30 min. The supernatant (homogenate) was used immediately or could be frozen and stored without loss of activity. The homogenate was subsequently subjected to ethanol fractionation before the isolation was continued by the standard column-chromatographic procedure. In this way the protein containing the ribonuclease activity was separated from most of the lipid, which would have interfered with the purification if it had been allowed to remain in the mixture.

Precipitation with ethanol. The procedure is the same as that of Gamble *et al.* (1967). The crude homogenate was fractionated by adjusting the ethanol concentration to 50% (v/v).

Acid extraction. The 50%-ethanolic precipitate was extracted with 0.25 M- H_2SO_4 (10 ml./200 mg. of precipitate) by homogenization with a Potter–Elvehjem Teflon homogenizer. Insoluble material was removed by centrifugation at 12 100g for 30 min. at 3°. The supernatant solution was adjusted to pH 3.5 with 10 M-KOH and the resulting precipitate removed by centrifugation. The precipitate was inactive. The soluble fraction was dialysed against cold (4°) water overnight. Insoluble material, subsequently shown to be inactive, was removed by centrifugation. The dialysed supernatant was freeze-dried and stored dry at 0° until used.

Bio-Rex 70 column chromatography. Bio-Rex 70 (200–400 mesh), an IRC resin, in the Na^+ form was washed with excess of 0.1 M-potassium phosphate buffer, pH 7.0. The fines were decanted after the mixture had stood for 15 min. This step was repeated. A column (2.5 cm. $\times$ 41 cm.) was prepared and washed with 200 ml. and 1000 ml. of 2 M-NaCl and 2 M-NaCl in 0.1 M-potassium phosphate buffer, pH 7.0. The column was then washed free of NaCl by eluting with 0.1 M-potassium phosphate buffer, pH 7.0, until the effluent was free of Cl^- ions. The freeze-dried acid extract was dissolved in 0.1 M-potassium phosphate buffer, pH 7.0, and placed on the column. The sample was washed on to the column with an equal volume of the same buffer. Elution of the column was at the rate of 1.2 ml./min. with 40 ml. of 0.1 M-potassium phosphate buffer, pH 7.0, followed by a linear gradient of 0.2 M-NaCl in 0.1 M-potassium phosphate

Table 1. *Purification of aorta ribonuclease I and aorta ribonuclease II*

Purification step	Volume (ml.)	Units	Total units	Yield (%)	Specific activity	Fold purification
1 Crude homogenate	2250	0.5	11 474		0.45	1
2 Acid extraction; neutralization	1072	3.7	39 661	100	92	202
3 Dialysed acid extract	1262	2.7	34 062	86	133	295
4 Bio-Rex 70						
Peak I	13.6	32.0	4352	11	46	104
Aorta ribonuclease II	27.0	48.0	12 960	33	300	667
Aorta ribonuclease I	14.0	41.5	5810	15	2075	4611

buffer, pH 7.0. Fractions (2.5 ml.) were collected by means of a Technicon fraction collector. A total of 110–209 E_{280} units of protein was placed on the column.

Discontinuous vertical gel electrophoresis. Discontinuous vertical gel electrophoresis was performed at alkaline pH conditions (stacking at pH 8.9) by the method outlined in the *E-C Bulletin* [vol. 2, no. 1 (1966); distributed by E-C Apparatus Inc., Philadelphia, Pa., U.S.A.] with a 7.0% running gel and 4.0% spacer gel. Electrophoresis at acidic pH (stacks at pH 4.5) was by the method of Reisfeld, Lewis & Williams (1962), modified for use in the E-C 470 vertical gel cell. The small-pore gel was 8% polyacrylamide and the large-pore gel 4% polyacrylamide; 0.1 g. of ammonium persulphate/100 ml. of gel solution and 0.14 g. of ammonium persulphate + 0.21 g. of sodium sulphite/100 ml. of gel solution were added for the large-pore and small-pore gels respectively. Protein samples were mixed with a 20% sucrose–Sephadex G-200 solution and then applied to the sample slots. A current of 180 ma was employed for electrophoresis. The gels were then stained for a 3 hr. period. An E-C 489 destainer was used to destain the samples.

Bio-Gel P-2 column chromatography. The method used was as described by Gamble *et al.* (1967).

Cellex E column chromatography. Cellex E column chromatography was performed as described by Gamble *et al.* (1967).

Deoxyribonuclease activity. Deoxyribonuclease activity was determined at pH 5.0 and 7.0 in the presence of Mg^{2+} by the method of Kunitz (1950). The reaction solutions were incubated for 30 min.

Alkaline phosphate activity. Alkaline phosphatase was measured by the method of Garen & Levinthal (1960), with *p*-nitrophenyl phosphate and 30 min. incubation periods.

RESULTS

Purification of the enzyme. The results are summarized in Table 1. Fractionation of 2250 ml. of crude homogenate by ethanol (50%, v/v) yielded 29 g. of dried product. Storage for up to 6 months at 0° caused no loss of activity. Acid extraction resulted in a 202-fold purification of the enzyme. Fig. 1 shows the results of Bio-Rex 70 column chromatography of the freeze-dried acid-extracted enzyme preparation. Three purified protein fractions were obtained. The first of the three fractions was in tubes 15–19 (peak I), the second in tubes 20–28 (aorta ribonuclease II) and the third in

Fig. 1. Bio-Rex 70 column chromatography of dialysed acid extract of bovine aorta. The column (2.5 cm. $\times$ 41 cm.) was eluted at a rate of 1.2 ml./min. with 40 ml. of 0.1 M-phosphate buffer, pH 7.0, followed by a linear gradient of 0–2 M-NaCl in 0.1 M-phosphate buffer, pH 7.0. The fraction volume was 2.5 ml. ●, Protein concentration expressed as E_{280} units; ○, ribonuclease activity expressed as E_{260} units.

tubes 60–73 (peak II, aorta ribonuclease I). The purification results are summarized in Table 1. When the pooled fractions were dialysed aorta ribonuclease I was precipitated out of solution. The enzyme was redissolved in 0.5 M-sodium chloride. Aorta ribonuclease I was 4611-fold purified and aorta ribonuclease II 667-fold purified. Peak I was not characterized further.

Discontinuous vertical gel electrophoresis. Fig. 2 shows the results of electrophoresis under acidic conditions of aorta ribonuclease I, pancreatic ribonuclease and chymotrypsinogen. Aorta ribonuclease I migrated slightly faster than pancreatic ribonuclease, which, in turn, ran faster than chymotrypsinogen. Aorta ribonuclease I exhibited only one band whereas aorta ribonuclease II (not

shown) under similar conditions exhibited several bands. Aorta ribonuclease I did not migrate under alkaline conditions.

Deoxyribonuclease activity. Aorta ribonuclease I did not exhibit deoxyribonuclease activity when assayed at pH 5.0 and 7.0 for 30 min.; moreover, no deoxyribonuclease activity was observed in any fractions exhibiting ribonuclease activity.

Alkaline phosphatase activity. No alkaline phosphatase activity was observed with *p*-nitrophenyl phosphate as substrate.

Effect of pH on activity. Fig. 3 shows that aorta ribonuclease I has an apparent optimum pH 7.5.

Effect of temperature. Fig. 4 shows that, under the assay conditions, aorta ribonuclease I exhibited optimum activity at approx. 60°. At 95° enzyme activity was 2% of that measured at 60°.

Fig. 2. Acid gel discontinuous electrophoresis. (a) From left to right: (1) and (2) 12 μ g. of chymotrypsinogen; (3) 15 μ g. of pancreatic ribonuclease in 0.5 M-NaCl; (4) 15 μ g. of aorta ribonuclease I; (5) 15 μ g. of pancreatic ribonuclease; (6) and (7) 15 μ g. of aorta ribonuclease I; (8) 23 μ g. of chymotrypsinogen. (b) From left to right: (1) 20 μ g. of aorta ribonuclease I; (2) 20 μ g. of pancreatic ribonuclease. Electrophoresis was carried out at pH 5.0, with small-pore gel 8% polyacrylamide and large-pore gel 4% polyacrylamide. Other conditions are described under 'Methods'.

Hydrolysis of homopolymers. The activities of aorta ribonuclease I, aorta ribonuclease II and pancreatic ribonuclease with homopolymers as substrates are compared in Table 2. The hydrolysis of poly U by aorta ribonuclease I was 24 times as fast as the hydrolysis of poly C. Aorta ribonuclease I and pancreatic ribonuclease hydrolysed poly U at equivalent rates. No activity was exhibited by aorta ribonuclease I with poly I, poly A or poly G as substrate after 190 min. incubation. Under the same conditions pancreatic ribonuclease hydrolysed poly A, but not poly I or poly G. The hydrolysis rate of poly C by aorta ribonuclease II was seven times that of poly U.

Fig. 3. pH-activity curve of aorta ribonuclease I. The activities were obtained in 0.1 M-sodium acetate (pH 4.0–5.1), 0.1 M-potassium phosphate (pH 6.2–7.5), 0.1 M-tris-HCl (pH 8.0–8.8) and 0.1 M-glycine-NaOH (pH 9.8) buffers. Ribonuclease activity is expressed as E_{260} units.

Fig. 4. Temperature-activity curve for aorta ribonuclease I in 0.1 M-phosphate buffer, pH 7.0. Ribonuclease activity is expressed as E_{260} units.

Table 2. *Homopolymers as substrates*

Concentrations of homopolymers were: poly C, 0.1%; poly U, 0.1%; poly I, 0.33%; poly G, 0.33%; poly A, 0.3%. Sodium RNA (0.1%) is shown as a comparison.

Enzyme and substrate	Concn. of enzyme ($\mu\text{g./ml.}$)	Incubation time (min.)	E_{260}
Aorta ribonuclease			
Poly C	1	5	0.042
		15	0.116
		60	0.51
Poly C	7	5	0.442
		15	0.83
Poly U	1	5	1.01
Poly I	50	195	0.004
Poly G	50	195	0.004
Poly A	50	195	0.000
Sodium RNA	1	5	0.280
Aorta ribonuclease II			
Poly C	1	5	0.79
Poly U	1	5	0.12
		15	0.68
Pancreatic ribonuclease			
Poly C	1	5	0.87
Poly U	1	5	0.99
Poly I	50	195	0.000
Poly G	50	195	0.002
Poly A	50	195	0.79

Hydrolysis of dinucleoside phosphates. Aorta ribonuclease I did not catalyse the hydrolysis of ApC and GpC in a 24 hr. incubation period at a concentration of 50 $\mu\text{g./ml.}$ CpC was converted into C > p and cytidine by aorta ribonuclease I under the same conditions. The products were isolated by desalting on Bio-Gel P-2 followed by Cellex E column chromatography. Their identities were confirmed by descending paper chromatography in the propan-2-ol-water-aq. ammonia solvent system. Similar results were obtained when the products were subjected to paper chromatography without previous column chromatography. Parallel experiments were performed with bovine pancreatic ribonuclease as a positive control. The results are shown in Figs. 5 and 6, which illustrate the fractions isolated by Cellex E column chromatography. Fig. 7 shows the results of paper chromatography of the isolated products.

DISCUSSION

A highly purified ribonuclease (aorta ribonuclease I) was isolated from bovine aorta. One other ribonuclease (aorta ribonuclease II) was isolated and purified 667-fold. Another fraction, which may have been identical with aorta ribonuclease II, was partially purified. Aorta ribonuclease II has the

same properties as the aorta ribonuclease isolated and partially purified by Gamble *et al.* (1967). It hydrolysed poly C and poly U but not poly A and poly I. The products were the cyclic mononucleotides. Aorta ribonuclease II had electrophoretic properties similar to the previously isolated aorta ribonuclease and similar temperature optimum (about 50°) and pH optimum (about 7.0). Aorta ribonuclease I exhibits a preference for poly U over poly C, hydrolysing the former 18 times as fast. Aorta ribonuclease II catalyses the hydrolysis of poly C seven times as fast as poly U. Aorta ribonuclease I catalysed the hydrolysis of poly U at a rate equivalent to that of pancreatic ribonuclease; however, pancreatic ribonuclease acts 21 times as fast on poly C. Aorta ribonuclease I did not hydrolyse poly A, poly G and poly I when tested at an enzyme concentration 50 times that necessary for degradation of poly C or poly U. Pancreatic ribonuclease hydrolysed poly A, but not poly I and poly G. However, it is known that poly I can be depolymerized in the presence of pancreatic ribonuclease at concentrations higher than those used here (Gamble *et al.* 1967). Aorta ribonuclease I catalyses the hydrolysis of poly U four times as fast as that of sodium RNA, whereas the hydrolysis of sodium RNA is seven times as fast as that of poly C. Aorta ribonuclease I hydrolysed only 13% of the

Fig. 5. Cellex E column chromatography of standard nucleoside, nucleotides and nucleoside diphosphate. The column (1 cm. $\times$ 20 cm.) was eluted with a linear LiCl gradient as described by Gamble *et al.* (1967). (a) C > p; (b) from left to right, cytidine and pC; (c) Cp; (d) CpC.

Fig. 6. Cellex E column chromatography of the products of hydrolysis of CpC by aorta ribonuclease I (a and b) and pancreatic ribonuclease (c and d), previously desalted on Bio-Gel P-2. The method used was the same as that described in Fig. 5. Identities of the compounds were confirmed by paper chromatography (see under 'Methods'). (a) C > p; (b) from left to right, cytidine and a trace of C > p; (c) from left to right, a trace of C > p and Cp; (d) cytidine.

sodium RNA. Most (84%) of the hydrolysis products of sodium RNA were at least four nucleotide units in length (determined as described by Gamble *et al.* 1967). The results suggest that aorta ribonuclease I is an endonuclease. Aorta ribonuclease I has optimum pH (7.5) slightly higher than that of the previously isolated aorta ribonuclease (optimum pH 7.0). The optimum temperature (60°) of aorta ribonuclease I was higher than that of aorta ribonuclease (50°). These differences may only be apparent, since aorta ribonuclease is less pure than aorta ribonuclease I. Aorta ribonuclease activity was linear for 8 min. with enzyme concentrations in the range 0.25–1.5 μ g. of protein/ml. The products of hydrolysis of the homopolymers by bovine aorta ribonucleases are 2',3'-cyclic nucleotides (Gamble *et al.* 1967). In agreement with the previous findings CpC and UpC were converted into the corresponding 2',3'-cyclic phosphate and nucleoside, i.e. the mono-

nucleotide product was always the cyclic derivative. The rate of hydrolysis of the nucleoside diphosphates was slow and required higher concentrations of enzyme than were necessary for hydrolysis of homopolymers. When UpC was incubated with aorta ribonuclease I (50 μ g./ml.) for 1 hr. only 12% of the UpC was hydrolysed. No conclusion is drawn from this difference, since the structure of the nucleoside diphosphate is obviously different from the homopolymers or sodium RNA.

The presence of ribonuclease activity in aorta was first noted by Gamble *et al.* (1967). There was no known relationship between nucleic acid metabolism and arterial lipid metabolism. Henry (1968) has since shown that the RNA content of arteries decreased with the development of atherosclerosis whereas DNA content remained constant. There

Fig. 7. Paper chromatography of the products of hydrolysis of CpC by aorta ribonuclease I on Whatman no. 3 paper with propan-2-ol-water-aq. NH_3 (sp.gr. 0.88) (1400:600:1, by vol.). From bottom to top: (1) standards Cp and cytidine; (2) standards Cp and C>p (Cp formed as a result of hydrolysis by solvent); (3) standard CpC; (4) standard Cp, CpC and mixture of cytidine and C>p; (5) hydrolysis products of CpC plus standard CpC: trace of Cp [shown to be due to solvent hydrolysis, confirmed by chromatography in $(\text{NH}_4)_2\text{SO}_4$ solvent (Gamble *et al.* 1967)], CpC and a mixture of cytidine and C>p; (6) hydrolysis products of CpC: Cp (due to solvent hydrolysis as explained above) and a mixture of cytidine and C>p. The results show that the products of hydrolysis of CpC are cytidine and C>p. The findings were confirmed by chromatography in $(\text{NH}_4)_2\text{SO}_4$ solvent and isobutyric acid-1 M- NH_3 -0.1 M-EDTA (125:75:1, by vol.) (see Gamble *et al.* 1967). The products of hydrolysis of UpC were identified in a similar manner.

appear to be interrelationships between nucleic acid and lipid and protein metabolism beyond the structural aspects: thus it is possible that the ribonucleases described in the present paper play significant roles in arterial metabolism.

In conclusion, two ribonucleases were isolated from bovine aorta, aorta ribonuclease I and aorta ribonuclease II. Aorta ribonuclease II is apparently identical with aorta ribonuclease isolated previously. Aorta ribonuclease I is highly purified and, like aorta ribonuclease, distinct from pancreatic ribonuclease. The enzyme appears to be electrophoretically homogeneous. It should be noted that the isolation of ribonucleases from arterial tissue requires modifications of the classical methods usually employed in purification of ribonucleases, primarily because of the high lipid content of arteries.

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