Spleen Acid Deoxyribonuclease (1,2)

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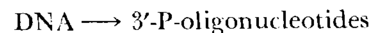
Several methods have been described for the partial purification of spleen acid deoxyribonuclease (see ref. 3 for a review). The preparation of the enzyme as a homogeneous protein was first reported by Bernardi and Griffé (1). The original purification procedure has been successively modified (2). Both procedures lend themselves, with slight modifications, to the preparation of several other enzymatic activities, such as acid phosphomonoesterase (p. 236), acid ribonuclease (p. 37), exonuclease (p. 144), and nucleoside polyphosphatase. Both methods will be described because the protein composition of the two crude nuclease preparations (*vide infra*) is different. Furthermore, for a given tissue one method may be more convenient than the other.

ASSAY

Reaction



The deoxyribonuclease activity is assayed by measuring the liberation of acid-soluble oligonucleotides from DNA.

Reagents

1. The reaction mixture (total volume 2.5 ml)† contains
   a. 2.4 μmoles of DNA-P‡
      375 μmoles of acetate buffer, pH 5.0
      25 μmoles of EDTA
   b. Enzyme—if necessary, this is diluted with 0.15 M acetate buffer + 0.01 M EDTA, pH 5.0, containing 0.05% beef heart cytochrome c (Sigma Chemical Company, type V).
2. 12% Perchloric acid

Procedure

After 10 min of incubation at 37 C the reaction is stopped by addition of 0.5 ml of 12% perchloric acid. The mixture is then chilled in an ice bath for 10 min and clarified by centrifugation at 4 C. The extent of DNA hydrolysis is determined by measuring the absorption at 260 mμ of the supernatant fluid. After subtracting a suitable blank, readings are corrected for dilution with the enzyme solution and the perchloric acid.

Assays are performed using enzyme concentrations to obtain OD_{260} readings in the range 1–4. Under these conditions a linear relationship obtains between enzyme concentration and acid-soluble oligonucleotide formation. One activity unit is defined as the amount of enzyme that under the conditions defined above catalyzes in 1 min the liberation of oligonucleotides having a (corrected) OD_{260} equal to 1. If the volumes used in the assay are halved, the activity value must be divided by 2. Since the molar extinction coefficient at 260 mμ of the oligonucleotide-phosphorus present in the final, completely acid-soluble, digest is 9100, one activity unit corresponds to the liberation of 0.22 μmoles of oligonucleotide-phosphorus. The specific activity is calculated by dividing the activity by the OD_{280} of the enzyme solution.

Assay of Other Enzymatic Activities

Ribonuclease, exonuclease, and acid phosphomonoesterase activities are assayed as described on pp. 37, 144, and 236 respectively.

Phosphodiesterase activity is assayed like phosphomonoesterase; Ca [bis(*p*-nitrophenyl)phosphate]$_2$ is used as the substrate; the solvent

† If desired, all volumes may be halved.
‡ Calf thymus DNA prepared according to Kay *et al.* (4) was used.

is 0.25 M succinate buffer, pH 6.4; the 2 N $(NH_4)OH$ solution used to stop the reaction is 0.1 M in EDTA. Under these conditions the assay detects essentially the nucleoside polyphosphatase activity (5).

PREPARATION OF CRUDE SPLEEN NUCLEASE

Two different methods, I (1) and II (2), will be described. They give rise to two different enzyme preparations, which will be called crude spleen nuclease I and II, respectively. These preparations contain among other enzymes acid deoxyribonuclease, three or more ribonucleases, and an exonuclease. The yield in acid deoxyribonuclease is slightly higher with the more laborious Method I, than with Method II, which was specifically designed for the large scale preparation of acid deoxyribonuclease and some of the accompanying enzymes.

Method I

General Directions

All operations are performed at 4 C; the precipitates are allowed to stand overnight; the centrifugations are carried out at 8000 × g for 1 hr, except where otherwise stated (a Lourdes Instruments Corp., Brooklyn, N.Y., centrifuge was used in the original work).

STEP 1. EXTRACTION. Spleens obtained at the abattoirs from freshly slaughtered pigs, are trimmed, minced, and homogenized for 3 min in a Waring Blendor with 0.15 M NaCl–0.02 M $CaCl_2$. Batches of 250 g are treated with 300 ml of solvent to which 1 ml of isooctanol has been added. The suspension is shaken for 18 hr and then centrifuged for 30 min at 3000 × g. The sediment is again homogenized with fresh solvent (1 liter/kg of spleen) and treated as before. The turbid combined supernatant fluids are centrifuged. The supernatant liquid so obtained is filtered through cheesecloth ("clarified extract").

STEP 2. FIRST AMMONIUM SULFATE FRACTIONATION. Solid $(NH_4)_2SO_4$ is added to the clarified extract (200 g/liter; this is equivalent to 0.34 saturation at 20 C), and the resulting suspension is centrifuged. The sediment is discarded. The supernatant fluid is filtered through cheesecloth and paper and saturated with $(NH_4)_2SO_4$ by adding 520 g of salt/liter. The precipitate is collected by centrifugation or filtration on paper.

STEP 3. ACID PRECIPITATION. The precipitate from the previous step is dissolved in distilled water (about 700 ml/kg of spleen) and adjusted to pH 2.5 by dropwise addition of 0.3 N HCl, under mechanical stirring. The suspension is then centrifuged and the sediment is discarded.

STEP 4. SECOND AMMONIUM SULFATE FRACTIONATION. Solid $(NH_4)_2SO_4$ is added to the supernatant fluid from the previous step (245 g/liter; 0.4 saturation at 20 C). The precipitate is removed by centrifugation and discarded. The supernatant liquid is brought to 0.8 saturation of $(NH_4)_2SO_4$ (20 C) by adding 285 g of salt/liter. The precipitate is collected by centrifugation and the supernatant fluid is discarded.

STEP 5. DIALYSIS AND LYOPHILIZATION. The precipitate obtained between 0.4 and 0.8 $(NH_4)_2SO_4$ saturation is dissolved in distilled water (about 100 ml/kg of spleen) and dialyzed against distilled water with several changes. The precipitate formed is removed by centrifugation and the supernatant fluid is freeze-dried.

Properties of Crude Spleen Nuclease I

The crude spleen nuclease I is obtained in a yield of about 1 g/kg of trimmed tissue. Whereas no loss of enzymatic activity is found upon freeze-drying, storage at −15 C of the brownish, freeze-dried preparation for several months is accompanied by some loss of activity.

The crude spleen nuclease has a specific activity equal to 3–4; its total activity is about 3,000 units/kg of trimmed tissue. The specific acid deoxyribonuclease activity of the crude spleen nuclease so obtained is about the same as that of commercial preparations of spleen acid deoxyribonuclease (Worthington, Freehold, N.J.) obtained according to the procedure of Shimomura and Laskowski (6) through dialysis against acetate buffer (Step 5).

The crude spleen nuclease contains acid phosphomonoesterase, exonuclease, nucleoside polyphosphatase activities, and basic and acid ribonuclease activities.

Method II

General directions as in Method I. All operations however, are carried out at room temperature, except where otherwise stated.

STEP 1. Hog spleens are trimmed, ground with an electrical meat grinder, and homogenized with 0.1 N H_2SO_4 (1 liter/kg of ground spleen; 1 ml of isooctanol is added to each batch). 4.2 is the final pH. 0.2 N H_2SO_4 is added to the suspension dropwise under mechanical

stirring until a pH of 2.5 is obtained; about 1 liter/kg of spleen is needed. Solid $(NH_4)_2SO_4$ (243 g/liter; 0.4 saturation at 20 C) and EDTA (2.43 g/liter) are added to the suspension. This is then allowed to stand overnight at 4 C. The suspension is then centrifuged for 1 hr at 8000 × *g* at 4 C and the sediment is discarded.

STEP 2. Solid $(NH_4)_2SO_4$ (285 g/liter; 0.8 saturation at 20 C) and EDTA (2.85 g/liter) are added to the supernatant liquid from the previous step and the suspension is stored for several days or weeks at 4 C.

The suspension is then filtered. In the original work this was done using a k_0 00 Seitz filter† with a Seitz press-filter; over 60 liters of suspension may be easily filtered through the same filter, under atmospheric to +0.5 kg/cm² pressure.

STEP 3. The filtered precipitate is dissolved in a small volume of distilled water (about 100 ml per 10 kg of spleen) and dialyzed against several changes of distilled water at 4 C for 48 hr. The precipitate that forms upon dialysis is centrifuged off.

STEP 4. The supernatant fluid is concentrated by freeze-drying and dialyzed against 0.05 M phosphate buffer, pH 6.8, and used for the chromatographic purification.

Properties of the Crude Spleen Nuclease II

The crude spleen nuclease II is obtained in a yield of about 0.2 g (dry weight)/kg of trimmed spleen.

TABLE 1. *Crude Spleen Nuclease II (Preparation HS 11)*[a]

	Total Activity	Specific Activity
Acid deoxyribonuclease	160,000	12.8
Acid ribonuclease	153,000	12.5
Acid phosphomonoesterase	600	0.048
Phosphodiesterase	185	0.015

[a] This preparation was obtained from 60 kg of hog spleen.

Its specific activity is about 10. The total activity is about 2700 units/kg of trimmed tissue. The OD_{280}/OD_{260} ratio is equal to 1.3–1.5. The properties of crude spleen nuclease II are summarized in Table 1.

† Seitz-Asbest-Werke, Bad Kreuznach, Germany.

CHROMATOGRAPHIC PURIFICATION

Three different chromatographic procedures, A, B and C, have been developed to purify the crude spleen nuclease preparation. Procedures A and B (1) have been only used on crude spleen nuclease I preparations. Procedure C has been developed more recently (2) to purify large quantities of spleen nuclease II preparations.

Column Chromatography

Hydroxyapatite was prepared according to Tiselius *et al.* (7). DEAE-cellulose was purchased from Serva, Heidelberg, Germany, and Calbiochem (Biorad Cellex D); the exchange capacities were 0.63 meq/g and 0.6 meq/g, respectively. CM-cellulose was a product of Serva; its exchange capacity was 0.65 meq/g. Sephadex, DEAE-Sephadex, and CM-Sephadex were obtained from Pharmacia AB, Stockholm, Sweden. Amberlite IRC-50 was purchased from Fisher, Philadelphia, Pa. (Rexyn CG-51) or Serva (C 950/II, 200–400 mesh); the latter product was treated according to Hirs (8) before use.

Chromatographic experiments were performed in a cold room (4 C) using a Gilson Medical Electronics fraction collector. The transmission of the effluent at 280 mμ was continuously recorded; 50–100 cm hydrostatic heads were used in most experiments. Molarity gradients were checked by refractometry using a Zeiss refractometer or by phosphorus analysis.

Procedure A

The results obtained for preparation HS I are summarized in Table 2.

STEP 1. DEAE-CELLULOSE. Crude spleen nuclease I was dissolved in water, adjusted to 0.005 M in phosphate buffer, pH 8.0, and adsorbed on DEAE-cellulose columns equilibrated with the same buffer. Under these conditions acid deoxyribonuclease is not retained. The total deoxyribonuclease activity eluted was about 10% higher than that of the starting material because of the adsorption of an inhibitor on the column. This inhibitor was later identified as sulfate ion. The adsorbed proteins could be eluted with 0.15 M acetate buffer, pH 5.0; this buffer also eluted sulfate. A brown substance, unidentified as yet, was removed from the column with 0.1 N HCl.

TABLE 2. *Chromatographic Purification of Spleen Acid Deoxyribonuclease (Procedure A)*[a]

	Volume (ml)	Total OD_{280}	Total Activity	Specific Activity
Crude spleen nuclease I	200	12,200	47,500	3.9
Step 1. DEAE-cellulose	814	7,090	51,200[b]	7.2
Step 2. Hydroxyapatite	289	493	36,600	74.1
Step 3. DEAE-cellulose[c]				
Step 4. Hydroxyapatite	285	157	19,500	124.2
Step 5. Hydroxyapatite	50	60.4	10,680	176.8
Step 6. IRC-50 Amberlite[d]	34	5.5	1,650	302

[a] Only the figures concerning the fractions that were processed further or the final product are given. A complete Table is given in ref. 1. The reported data refer to preparation HS 1.

[b] See text for the explanation of this increase.

[c] No data are available, since the fraction eluted at pH 8.0, $\mu = 0.005$ was immediately made 0.02 M with potassium phosphate buffer, pH 6.8, and loaded on hydroxyapatite.

[d] This chromatography was performed on a fraction (18%) of the enzyme obtained from Step 5.

STEP 2. HYDROXYAPATITE. The activity-rich fraction from Step 1 was made 0.02 M with phosphate buffer, pH 6.8, and adsorbed on hydroxyapatite. Elution was carried out stepwise, using 0.02, 0.1, 0.3, and 0.5 M phosphate buffer, pH 6.8.

STEP 3. DEAE-CELLULOSE. The 0.3 M fraction from the previous step was freeze-dried, dissolved in water, and dialyzed against 0.005 M phosphate buffer, pH 8.0, and then adsorbed on DEAE-cellulose. The fraction that was not retained was immediately made 0.02 M with phosphate buffer, pH 6.8, and absorbed on hydroxyapatite.

STEP 4. HYDROXYAPATITE. Elution was carried out in four steps: 0.02, 0.2, 0.3, and 0.5 M phosphate buffer, pH 6.8. Activity was found in the 0.2 and 0.3 M fractions. Active fractions were pooled together and adsorbed on another hydroxyapatite column.

STEP 5. HYDROXYAPATITE. Elution was carried out with a linear molarity gradient of phosphate buffer, pH 6.8, from 0.05 to 0.5 M. A single peak was eluted but the activity curve did not coincide with the optical density curve. The central fractions of the peak were pooled, concentrated by being rolled in visking cellulose tubes over dry Sephadex, frozen at an optical density at 280 mμ of about 3.0, and stored at

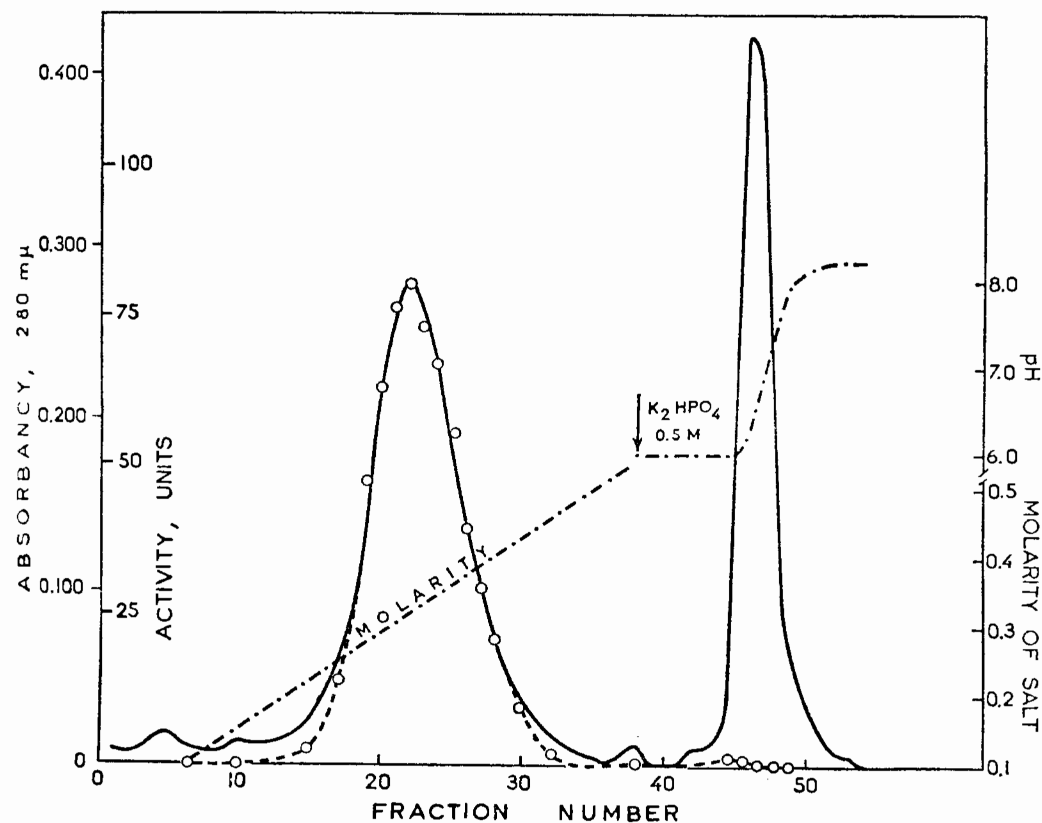


Fig. 1. Chromatography of acid deoxyribonuclease on Amberlite IRC-50 (Procedure A, Step 6; see Table 2). A molarity gradient of phosphate buffer, pH 6.0 (right-hand ordinate, lower scale) was followed by 0.5 M K_2HPO_4 (the pH of effluent is given in the right-hand ordinate upper scale). The continuous line indicates the absorption at 280 mμ. Circles indicate the deoxyribonuclease activity (left-hand, inner scale). (Reproduced from Biochemistry. 1964. *3:* 1419. With the permission of the American Chemical Society, publisher and copyright owner.)

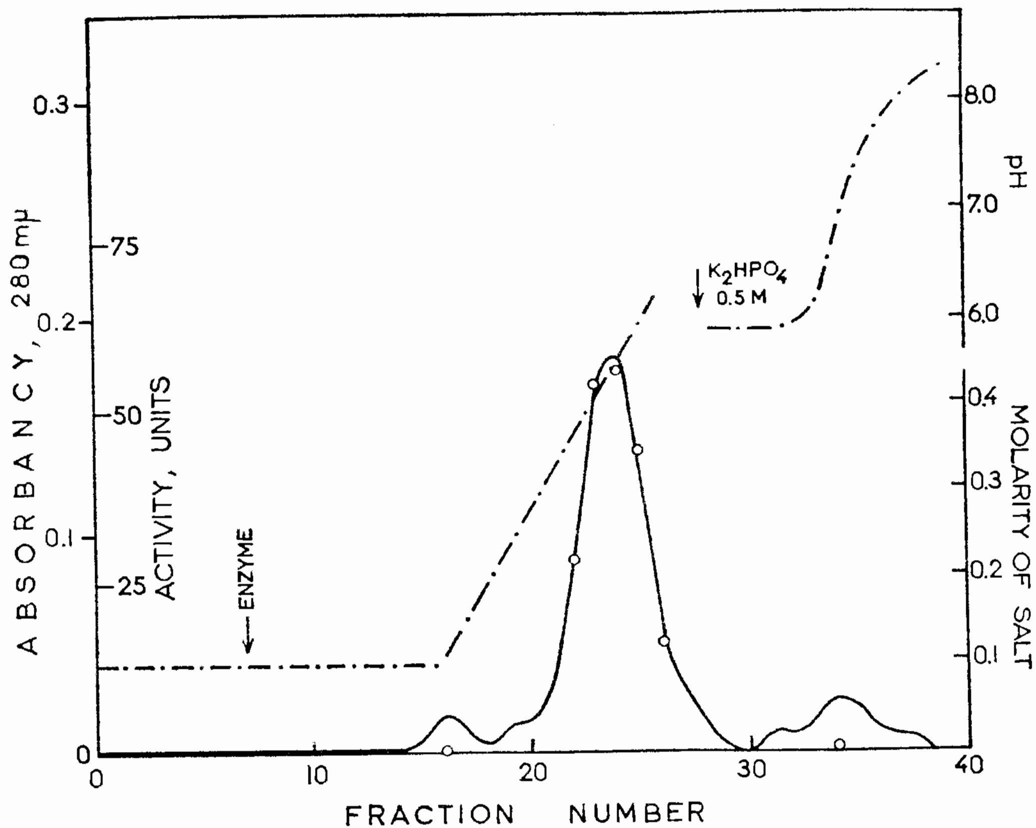


Fig. 2. Rechromatography of acid deoxyribonuclease on Amberlite IRC-50. Elution was carried out as in Fig. 1. All indications as in Fig. 1.

—60 C. A portion of the frozen enzyme was thawed and rerun on hydroxyapatite; no improvement whatsoever over Step 5 was obtained.

STEP 6. AMBERLITE IRC-50. Other samples of enzyme from the previous step were dialyzed against 0.1 M phosphate buffer, pH 6.0, and adsorbed on Amberlite IRC-50 equilibrated with the same buffer (Fig. 1). Some inactive material was not retained. A molarity gradient of phosphate buffer, pH 6.0, from 0.1 to 0.5 eluted the enzyme at a constant specific activity of 300,† the eluting molarity being extremely dependent upon the pH of the buffer. Additional inactive material was eluted with 0.5 M K_2HPO_4. Upon rechromatography the first fraction was again eluted (Fig. 2) at a constant specific activity of 300 with the molarity gradient, whereas the inactive fraction still required the pH gradient for elution.

Procedure B

STEP 1. DEAE-CELLULOSE. This was used as in Procedure A. The fraction not retained by the column contained deoxyribonuclease, phosphodiesterase, and acid phosphomonoesterase in that order; basic ribonuclease and some acid ribonuclease were also present. Adsorbed proteins were eluted with a gradient of molarity and/or pH, with 0.15 M acetate buffer, pH 5.0, as the final eluent: this resulted in a partial separation of a second acid phosphomonoesterase and the bulk of acid ribonuclease.

STEP 2. HYDROXYAPATITE. Elution was carried out as in Step 5 of Procedure A. Deoxyribonuclease was eluted immediately after a bright-red fraction showing absorption bands centered at 280, 414, 520, and 550 mμ (tentatively identified with cytochrome c). Phosphodiesterase and acid phosphomonoesterase were eluted in that order before deoxyribonuclease.

STEP 3. HYDROXYAPATITE. Elution was performed as before, the enzyme being desorbed at about 0.2 M phosphate buffer, pH 6.8. The active fractions were concentrated and frozen.

STEP 4. AMBERLITE IRC-50. Elution was performed as in Step 6 of Procedure A, with the same results upon rechromatography. The central fractions showed a constant specific activity and were pooled, dialyzed against 0.01 M phosphate buffer, pH 6.0, 0.02 M NaCl, con-

† See ref. 1 for an explanation of this lower value, compared to those obtained from Procedures B and C.

centrated to an OD_{280} = 2.5, and frozen. Deoxyribonuclease activity was recovered in a final yield of 25%, taking as 100% the activity measured after Step 1. The specific activity was 350.

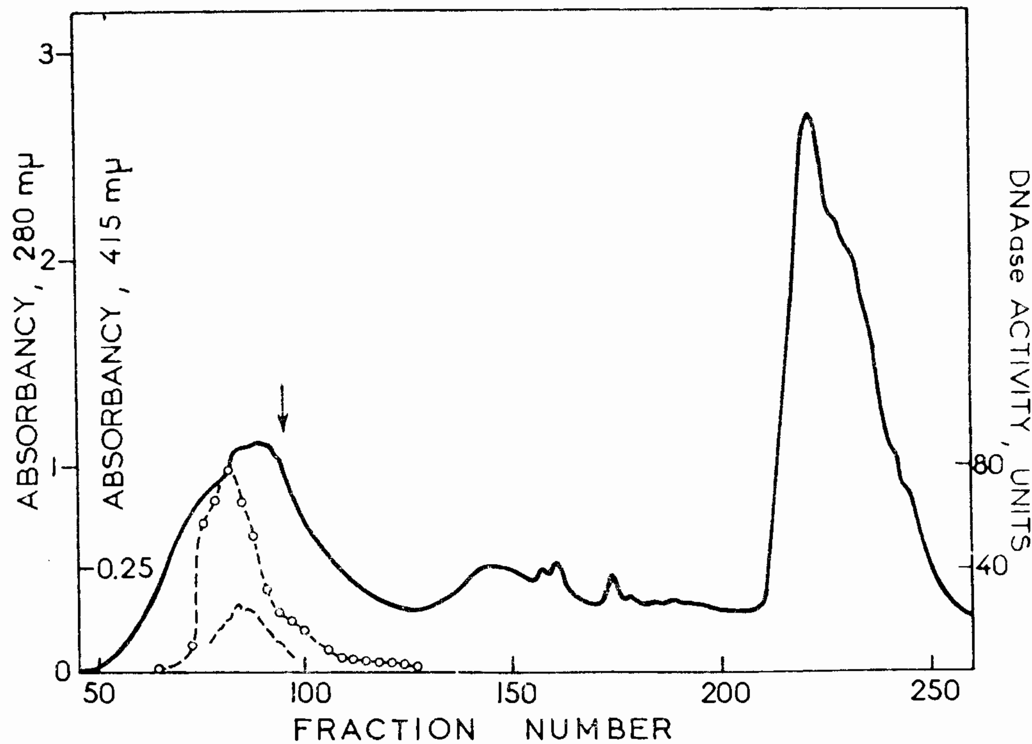


Fig. 3. Chromatography on DEAE-Sephadex A-50 of crude spleen nuclease II (Procedure C, Step 1). 330 ml of preparation HS 9 (OD_{280} = 10.3; OD_{260} = 6.9) were loaded on a 8- × 80-cm column of DEAE-Sephadex A-50 equilibrated with 0.05 M phosphate buffer, pH 6.8. This buffer was also used to elute the first protein peak. 0.5 M phosphate buffer, pH 6.8, was loaded at the fraction indicated by the arrow. 24-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand scale). The broken line indicates the absorption at 415 mμ of cytochrome c (left-hand inner scale). Fractions 50–65 were processed further. Acid and basic ribonuclease, acid phosphomonoesterase, and phosphodiesterase were also assayed; the results are shown on pp. 39 and 238.

Procedure C

STEP 1. DEAE-SEPHADEX A-50 (FIG. 3). 10–20 g of crude spleen nuclease II in 300–500 ml of 0.05 M phosphate buffer, pH 6.8, were loaded onto a 8- × 80-cm DEAE-Sephadex A-50 column equilibrated with the same buffer. The column is then washed with the same buffer, and several enzymatic activities were not retained under these conditions: ribonuclease, nucleoside polyphosphatase, acid phosphomonoesterase, acid deoxyribonuclease, and cytochrome c.

The column is then washed with 0.5 M phosphate buffer, pH 6.8. A second large fraction, which contains acid ribonuclease activity and some acid phosphomonoesterase, is eluted. These activities are different from those present in the first peak.

STEP 2. HYDROXYAPATITE (FIG. 4). The fractions rich in deoxyribonuclease activity from the first peak of the previous step were loaded onto a 2- × 40-cm hydroxyapatite column equilibrated with 0.05 M phosphate buffer. No enzymatic activity was found in the fraction that was not retained.

A molarity gradient (0.05–0.5) of phosphate buffer, pH 6.8 (1000 + 1000 ml), eluted three main peaks of optical density. The first peak contained nucleoside polyphosphatase and a first ribonuclease, the second peak contained acid phosphomonoesterase, cytochrome c, and a second ribonuclease, and the third peak acid deoxyribonuclease.

STEP 3. CM-SEPHADEX C-50 (FIG. 5). The deoxyribonuclease-rich fraction from the previous step is dialyzed against 0.075 M phosphate buffer, pH 6.8, loaded on a 2- × 100-cm CM-Sephadex C-50 column equilibrated with 0.05 M phosphate buffer, pH 6.8. Elution is then carried out with a 0.1–0.4 M molarity gradient of phosphate buffer, pH 6.8. Acid deoxyribonuclease activity is eluted at a fairly constant specific activity in two peaks (Fig. 5). The first, minor, peak will be called deoxyribonuclease A, the second major one deoxyribonuclease B. The central parts of the two peaks are rechromatographed separately on CM-Sephadex after having reduced the phosphate molarity of the solvent to 0.075 by dialysis or Sephadex G-25 filtration. Upon rechromatography each fraction gives a single peak (Fig. 6) with a constant specific activity of about 350.

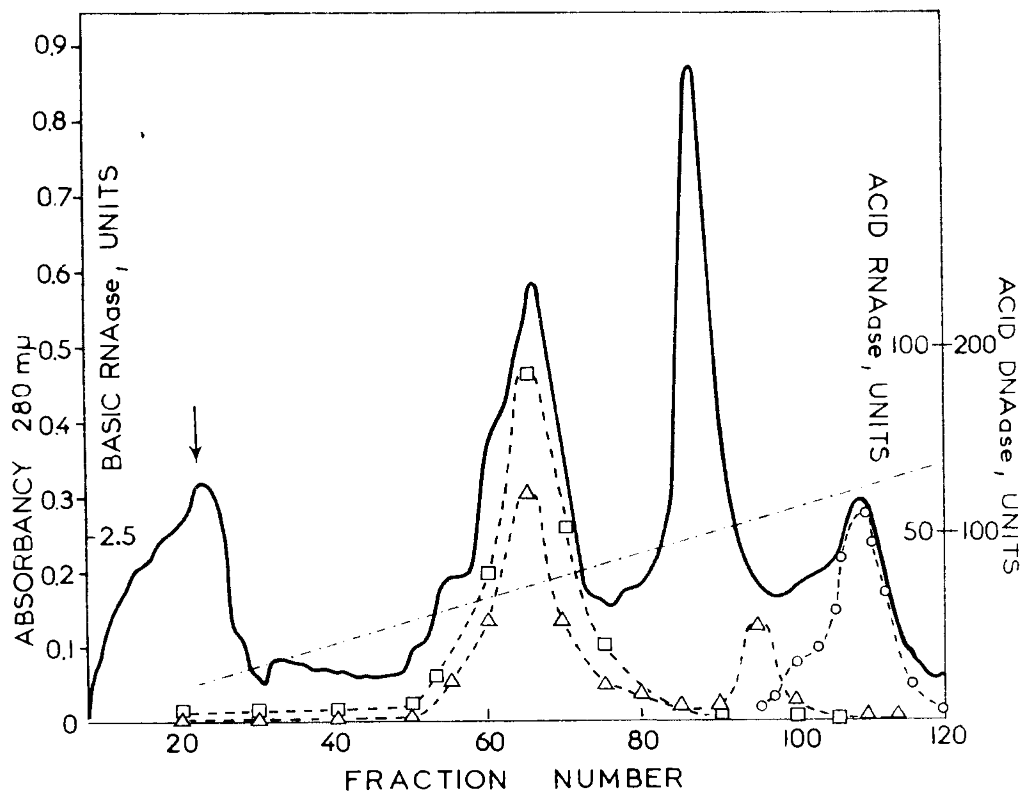


Fig. 4. Chromatography on hydroxyapatite of fractions 50–65 from Step 2 (Procedure C; Step 2). 370 ml (OD_{280} = 1.48) were loaded on a 2- × 40-cm column of hydroxyapatite equilibrated with 0.05 M phosphate buffer, pH 6.8. A molarity gradient (0.05–0.5) was started at the fraction indicated by the arrow; at fraction 120 the molarity of the effluent was 0.35. 24-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand scale). Cytochrome c was eluted as a sharp peak centered on fraction 86 (OD_{415} = 0.82; not shown on the figure). Acid ribonuclease (squares; right-hand scale) and basic ribonuclease (triangles; left-hand inner scale) are also shown. [Phosphomonoesterase and diesterase were also assayed (p. 239).] Fractions 100–115 were concentrated by freeze-drying to about 70 ml, filtered through Sephadex G-25 equilibrated with 0.075 M phosphate buffer, pH 6.8, and processed further.

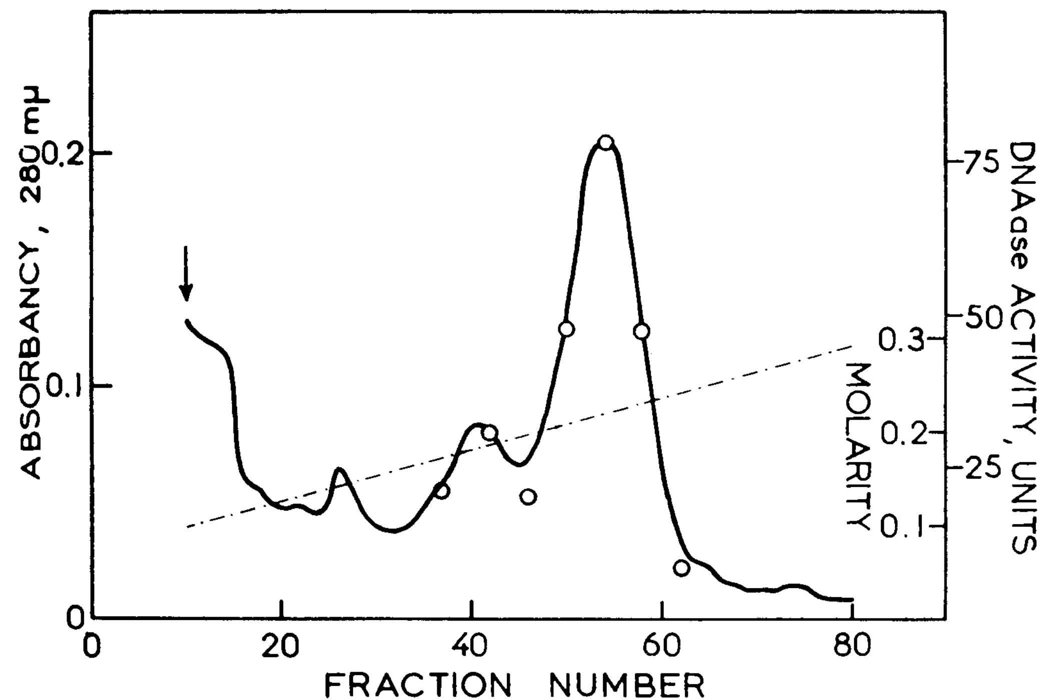


Fig. 5. Chromatography on CM-Sephadex C-50 of fractions 100–115 from Step 2 (Procedure C; Step 3). 115 ml (OD_{280} = 0.490) were loaded on a 2- × 100-cm column of CM-Sephadex C-50 equilibrated with 0.05 M phosphate buffer, pH 6.8. A molarity gradient (0.1–0.4) of phosphate buffer, pH 6.8, was started at the fraction indicated with an arrow (right-hand inner scale). 11-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand outer scale). Fractions 37–48 and 49–61 were processed further.

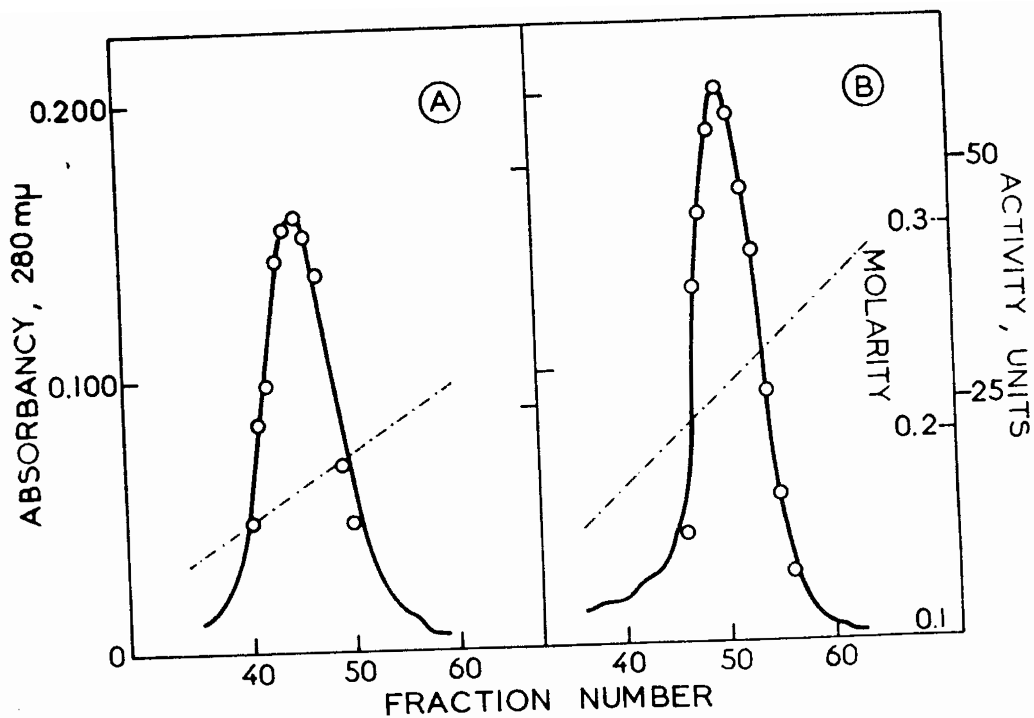


Fig. 6. Rechromatography of acid deoxyribonuclease fractions A and B on CM-Sephadex G-50. 8 OD_{280} units of each acid deoxyribonuclease (preparation HS 10) fractions A and B were loaded on two 1- × 100-cm CM-Sephadex C-50 columns. A molarity gradient (0.1–0.4) of phosphate buffer, pH 6.8, was started at fraction no. 1 (right-hand inner scale). 3-ml fractions were collected. The continuous line shows the absorption at 280 mμ. Circles indicate the deoxyribonuclease activity (right-hand outer scale).

Table 3. *Chromatographic Purification of Spleen Acid Deoxyribonuclease (Procedure C)*[a]

	Weight (g)	Volume	Total Units	Total OD_{280}	Specific Activity OD_{280}	Specific Activity Weight[b]
Crude spleen nuclease II	10	400	160,000	125,000	12.8	16
Step 1. DEAE-Sephadex	0.935	725	105,000	1,625	64.5	112
Step 2. Hydroxyapatite	0.193	425	61,500	225	273	318
Step 3. CM-Sephadex A[c]	0.0185	20	7,850	22.4	350	425
CM-Sephadex B[c]	0.073	30	31,000	88.5	350	425

[a] The reported data refer to preparation HS 11. All values quoted refer to the fractions that were processed further or to the final product. Fractions from the sides of the activity peaks were processed separately.

[b] Specific activity-data given in this column were obtained by dividing the activity (total units) by the dry weight (in mg) of the enzyme preparation.

[c] Values reported refer to fractions A and B, respectively (see text).

The central parts of the rechromatographed fractions are loaded on Sephadex G-25 equilibrated with 0.001 M acetate buffer, pH 5.0. The enzyme fractions are then concentrated by freeze-drying to an OD_{280} = 3.0 or higher, frozen, and stored at −60 C. These fractions are referred to as the final product in Table 3.

Acid deoxyribonuclease fractions A and B do not show any significant differences in any of the following properties: sedimentation constant (as measured both in the analytical ultracentrifuge and in sucrose density gradient centrifugation), ultraviolet spectra, elution volume from Sephadex G-100 columns, deoxyribonuclease and phosphodiesterase (*vide infra*) specific activities, color reaction with orcinol. The peptide map of the two fractions is identical, except that one particular peptide spot of fraction A is replaced by two well-resolved spots in fraction B. This is the only difference between the two fractions found so far.

No significant difference in physical properties, enzymatic activities and amino acid composition has been found between acid deoxyribonuclease B and the preparations obtained from crude spleen nuclease I (**1,9**).

PROPERTIES OF SPLEEN ACID DEOXYRIBONUCLEASE

Physical Properties and Amino Acid Composition (10)

These are shown in Tables 4 and 5, respectively. Recently it has been shown that the enzyme is a dimeric protein with two probably identical subunits (**11**).

TABLE 4. *Physical Properties of Hog Spleen Acid Deoxyribonuclease*[a]

$s^{\circ}_{20,w}$ (Svedbergs)	3.4
$D_{20,w}$ (10^{-7} cm^2/sec)[b]	7.8
v(ml/g)[c]	0.72
MW	3.8×10^4
f/f_o	1.34
$E^{1\%, 1cm}_{280 m\mu}$	12.1

[a] Reproduced from Biochemistry. 1965. *4*:1725. With the permission of the American Chemical Society, publisher and copyright owner.
[b] This value was obtained at concentrations of 0.5% and about 0.1%.
[c] Calculated value.

TABLE 5. *Amino Acid Analysis of Hog Spleen Deoxyribonuclease*[a]

	Grams of Amino Acid Residues/100 g of Protein[b]				Moles of Amino Acid/Mole of Protein M/W 38,000	Nearest Integral Number of Residues/Mole of Protein[d]
	Hydrolysis time (hr) 22	48	72	Corrected Values[c]		
Lys	6.94	6.80	6.95	6.89	20.44	20
His	2.13	2.17	2.31	2.20	6.08	6
(NH_3)	(23.05)	(23.65)	(24.06)	(22.5)	(49.4)	(49)
Arg	5.04	5.55	5.37	5.46	13.30	13
Asp	10.19	10.14	9.36	9.89	32.68	33
Thr	5.44	5.20	4.80	5.63	21.16	21
Ser	8.30	8.10	7.18	8.46	36.93	37
Glu	11.03	11.09	10.66	10.93	32.19	32
Pro	7.09	7.14	6.51	6.91	27.05	27
Gly	3.88	3.97	3.87	3.90	25.99	26
Ala	4.84	4.72	4.33	4.63	24.77	25
½ Cys	1.77	1.79	1.79	1.79	6.65[e]	8
Val	2.62	3.33	3.35	3.35	12.84	13
Met	1.63	1.65	1.30	1.53	4.45	4
Ileu	2.08	2.49	2.52	2.52	8.47	8
Leu	10.47	11.13	10.49	10.67	35.87	36
Tyr	5.23	5.17	4.83	5.28	12.31	12
Phe	6.30	6.56	6.07	6.31	16.30	16
Try					6.3[f]	6
Glucosamine	3.07	3.32	3.06	3.15	7.45	(8)
Total	98.05	100.32	94.75	(99.50)		343
N recovery %	98.9	98.7	97.4			

[a] Reproduced from Biochemistry. 1965. *4*:1725. With the permission of the American Chemical Society, publisher and copyright owner.
[b] Total N is 17.2%; total S is 1.0%.
[c] In calculating the corrected values, the criteria given by Tristram and Smith (**14**) have been followed.
[d] The selection of the integral numbers of residues has been done also taking into account results from other analyses.
[e] After performic acid oxidation: cysteic acid 8.2 residues.
[f] From N-bromosuccinimide titration.

Purity

Phosphomonoesterase activity at pH 5.0 and 6.7, nucleoside polyphosphatase (5), and exonuclease activities were absent. Ribonuclease activity was present at a trace level in acid deoxyribonuclease preparations obtained according to Procedure B (1). No ribonuclease activity could be detected in the preparations obtained according to Procedure C (2).

Enzymological Properties

Spleen acid deoxyribonuclease is active on both DNA and a series of *p*-nitrophenyl phosphodiesters (1).

DEOXYRIBONUCLEASE ACTIVITY. The enzyme is able to hydrolyze both native and heat-denatured DNA; the acid-soluble oligonucleotide liberation is slower in the latter case. The initial kinetics of degradation of native DNA indicates that acid deoxyribonuclease can cause scissions of opposite bonds at the same level ("single hit" degradation) in addition to introducing single breaks on one or the other strand ("double hit" degradation) (12,13). At $\mu = 0.15$ the pH optimum appears to be close to 4.8. Mg^{2+} is slightly inhibitory above pH 4.5 and shifts the pH optimum to 4.4. EDTA enhances the enzymatic activity without shifting the pH optimum. Phosphate ion seems to be slightly inhibitory only above pH 5.0. Sulfate ion is a very strong inhibitor, the inhibition being less pronounced below pH 4.5. In succinate buffer, $\mu = 0.15$, pH = 6.7, the activity is less than 3% of that in acetate buffer, $\mu = 0.15$, pH = 5.0. At a lower ionic strength ($\mu = 0.01$) the pH optimum is shifted to a higher pH (5.5 instead of 4.8) and the enzyme shows an activity also in the 6.5–7.5 pH range.

PHOSPHODIESTERASE ACTIVITY. The enzyme shows an activity on the Ca^{2+} and Na^{+} salts of bis(*p*-nitrophenyl)phosphate and the *p*-nitrophenyl esters of thymidine, deoxyguanosine, and deoxycytidine-3′-phosphates (the deoxyadenosine derivative was not assayed). The *p*-nitrophenyl ester of thymidine-5′-phosphate is resistant. With Ca [bis(*p*-nitrophenyl)phosphate]$_2$ as a substrate, the pH optimum was found to be between 5.6 and 5.9. In the 5.1–5.6 pH range the activity in acetate is about twice as large as in the succinate buffer. In the 4.0–5.6 pH range no significant changes occur upon addition of Mg^{2+} or EDTA; in the 4.0–7.0 pH range sulfate and phosphate give a very strong inhibition at a 0.01 M level. In contrast to the deoxyribonuclease activity, the phosphodiesterase activity exhibits a sigmoid type of velocity versus substrate concentration curve (11).

Soluble RNA, ribosomal RNA, and some synthetic polyribonucleotides competitively inhibit acid deoxyribonuclease in both its activities (15). Keeping the enzyme for 20 min at 60 C destroys 50% of both activities (1).

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