Chromatographic purification of acid ribonuclease from calf thymus

The presence of an acid RNAase activity has been shown both in calf-thymus extracts[1] and in preparations of calf-thymus acid DNAase[2]. However, no isolation of this enzyme has yet been reported. In the present communication a method will be described for the purification of calf-thymus acid RNAase; at the same time acid DNAase is also obtained in a purified form.

A crude acid DNAase preparation is first obtained according to the following three steps, essentially derived from LASKOWSKI *et al.*[2] and FRÉDÉRICQ AND OTH[3].

(1) 230 g calf thymus (stored 4 months at -30°) is minced and homogenized in small portions for a few minutes with 1 l 0.15 *M* NaCl–0.02 *M* $CaCl_2$ (pH 5), containing 3 ml isooctanol. The mixture is stirred for 20 h at 4°, then centrifuged for 1 h at 20000 rev./min in a Spinco model L centrifuge. The enzyme is precipitated from the supernatant between 0.3–0.9 satd. ammonium sulfate.

(2) The precipitate is dissolved in 400 ml water, the pH is adjusted to 2.5. The enzyme is precipitated between 0.4–0.8 satd. ammonium sulfate.

(3) The precipitate is dissolved in 60 ml distilled water, the resulting solution is exhaustively dialyzed against distilled water, then freeze-dried. 50 mg of the product, which essentially represents the albumin fraction of calf thymus, is dissolved in 2 ml of 1 m*M* potassium phosphate buffer (pH 6.8) (the absorbancy at 280 mμ of the solution is 10.0) and loaded onto a 1.3 × 14 cm column of hydroxyapatite prepared according to TISELIUS *et al.*[4]. Elution is carried out stepwise at room temperature with potassium phosphate buffer (pH 6.8) of increasing molarities, and fractions of approx. 3.1 ml are collected at a flow rate of approx. 80 ml/h (Fig. 1).

Pooled fractions from each peak were dialyzed against 0.15 *M* NaCl (pH 5.0) and tested for both DNAase and RNAase activity. 1 ml of each dialyzed fraction was added to 4-ml samples of Ehrlich ascites-tumor cell RNA (weight average mol. wt., $1.4 \cdot 10^6$, $A_{260m\mu} = 10.3$) or chicken-erythrocytes DNA (preparation B5, weight average mol. wt., $6 \cdot 10^6$; $A_{260m\mu} = 10.6$) solution in 0.15 *M* NaCl (pH 5.0). Digestion was allowed to proceed for 2 h at 37°. Acid-soluble nucleotides were determined by

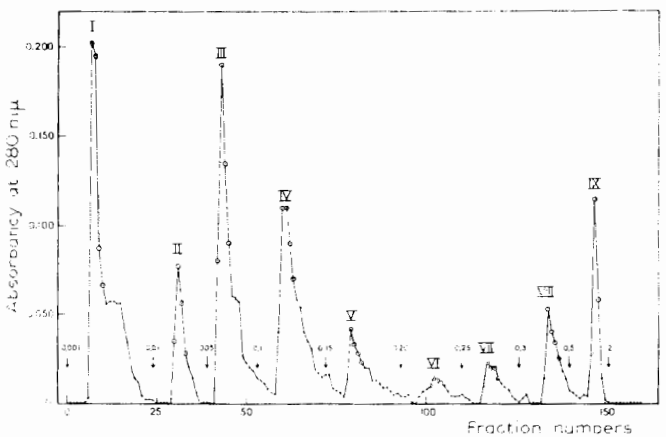

Fig. 1. Chromatography of a crude calf-thymus acid DNAase preparation on hydroxyapatite. Arrows indicate changes of eluent. Circled points represent fractions which were pooled together.

the method of SCHNEIDER AND HOGEBOOM[5]. Larger samples (30 ml) of DNA were also incubated with 1-ml fractions as above; at the end of digestion these were centrifuged 1 h at 30000 rev./min at 0°; supernatants were made up to 50 ml with 2 *M* NaCl, clarified by centrifugation and used for light-scattering measurements. Results are given in Table I.

TABLE I

Chromatographic fractions			*RNAase activity*		*DNAase activity*		
Fraction No.	*Phosphate buffer (M)*	$A_{260m\mu}$	*Activity**	*Specific** activity*	*Activity**	*Specific** activity*	*Mol. wt. × 10⁻⁶*
I	0.001	0.088	0.15	1.65	—	—	6
II	0.01	0.086	0.60	7	—	—	6
III	0.05	0.162	5.9	37	—	—	
IV	0.10	0.132	8.9	68	0.022	—	4
V	0.15	0.074	6.4	86	0.103	2.6	2
VI	0.20	0.110	3.7	34	0.078	0.8	2.5
VII	0.25	0.040	1.9	47	2.3	58	0.2
VIII	0.30	0.058	0.53	10	—	—	6
IX	0.50	0.090			—	—	6
Starting product (diluted)		0.250	8.4	33	7.3	28	0.2

* $A_{260m\mu}$ of acid-soluble RNA or DNA after correction for the blank.
** Activity divided by the $A_{260m\mu}$ of enzymic preparation used.

It appears that by just one chromatographic run, acid RNAase is substantially freed from inactive proteins (fractions I, II and IX) and from acid DNAase (fraction VII). On the basis of the rather wide range of buffer molarities eluting acid RNAase activity it might be suspected that more than one enzyme with acid RNAase activity is present in calf thymus, whereas acid DNAase activity essentially appears in one peak only. Similar suggestions have been already put forward by MAVER *et al.*[6] for the acid RNAase and DNAase activities of calf spleen.

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