CONCOMITANCE OF HOG SPLEEN ACID DNASE AND PHOSPHODIESTERASE ACTIVITIES

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1. Introduction

Among the various problems encountered during the purification of hog spleen acid deoxyribonuclease (DNase II), the most puzzling was the presence of a non-specific phosphodiesterase activity so firmly bound to DNase II that all attempts of chromatographic or electrophoretic separation failed [1].

Recently, in the case of the beef spleen enzyme, a separation of these two activities on CM-cellulose after thermal treatment at 60° was reported [2], whereas the same treatment resulted in an inactivation of phosphodiesterase when applied to the sheep spleen enzyme [3,4].

In this report, we present evidence that for hog spleen DNase II, such a treatment did not allow either separation or inactivation of its phosphodiesterase activity.

A constant ratio DNase II/phosphodiesterase was observed in our homogeneous, electrophoretically pure preparations.

2. Methods

DNase II and acid RNase were assayed according to Bernardi et al. [5], by measuring the liberation of acid-soluble oligonucleotides. The specific activity of DNase II was calculated by dividing the activity by the A_{280nm} of the enzyme solution.

Non-specific phosphodiesterase was estimated by hydrolysis of Ca(bis[*p*-nitrophenyl] phosphate), according to Hodes et al. [6].

Acid phosphomonoesterase was determined according to Chersi et al. [7], using Na_2(*p*-nitrophenyl phosphate) as the substrate.

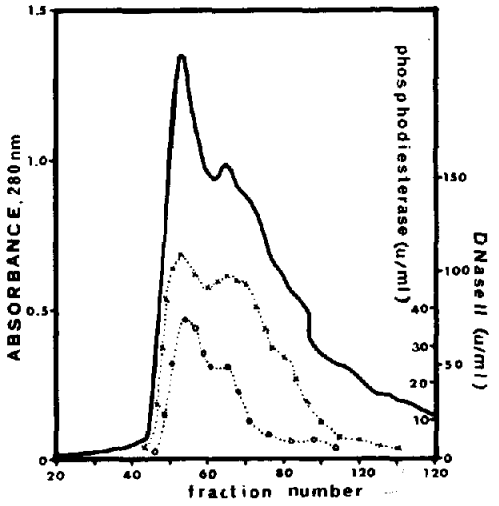

Fig. 1. First chromatography of DNase II on CM-cellulose. ——: A_{280} nm; x.....x: DNase II activity; o.....o: phosphodiesterase activity.

3. Results

Except when indicated, all purifications-operations were performed at 4°.

3.1. *Preparation of crude DNase II*

103 kg of hog spleen were processed by batches of 7–8 kg, to obtain the so-called "crude nuclease II" of the Bernardi's method [8].

3.2. *Thermal treatment*

The crude DNase II (2650 g) was dissolved in cold water. After clarification by centrifugation at 10,000 *g*, the solution was dialysed overnight against water

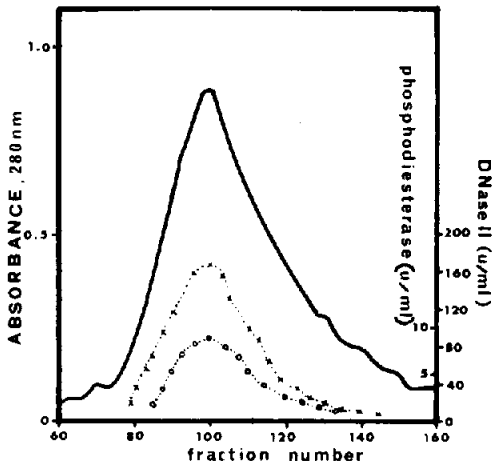

Fig. 2. Chromatography on DEAE-Sephadex A-50. ——: $A_{280\ nm}$; x.....x: DNase II activity; o.....o: phosphodiesterase activity.

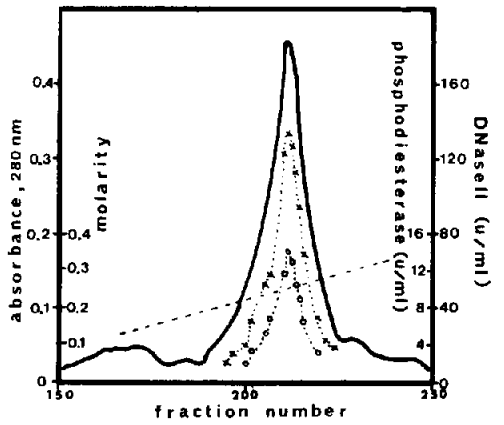

Fig. 4. Chromatography of the 2nd DNase II component on CM-Sephadex C-50. ——: $A_{280\ nm}$; - - - - - -: molarity of PPB; x.....x: DNase II activity; o.....o: phosphodiesterase activity.

and centrifuged again at 10,000 *g*. The supernatant (5600 ml) was adjusted at pH 4.50; aliquots of 250 ml were heated for 60 min at 60°, then they were pooled and dialysed overnight against 0.05 M ammonium acetate buffer, pH 6.00 (AAB).

3.3. *First CM-cellulose chromatography*

The dialysed heated solution (6250 ml) was loaded on a 9 × 97 cm CM-cellulose column equilibrated with 0.05 M AAB. Most of the DNase II was eluted by increasing the molarity of AAB to 0.40; 50 ml fractions were collected. The elution peak presented dissymmetry; in addition to DNase II it contained the three other acid hydrolases (fig. 1).

3.4. *DEAE-Sephadex A-50 chromatography*

Fractions of the preceding step, with DNase specific activity higher than 70, were pooled (3870 ml),

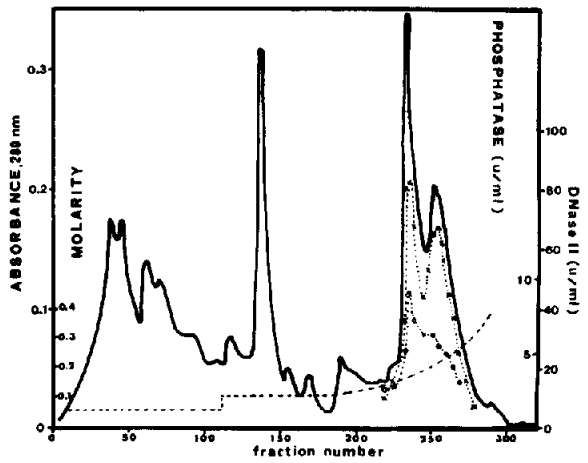

Fig. 3. Chromatography on hydroxyapatite. ——: $A_{280\ nm}$; - - - - - -: molarity of PPB; x.....x: DNase II activity; o.....o: phosphodiesterase activity.

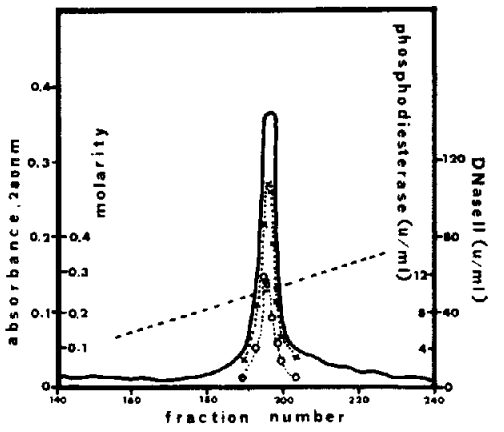

Fig. 5. Chromatography of the 2nd DNase II component on CM-cellulose. ——: $A_{280\ nm}$; - - - - - -: molarity of AAR; x.....x: DNase II activity; o.....o: phosphodiesterase activity.

Table 1
Chromatographic purification of hog spleen acid deoxyribonuclease.

Purification step	DNase II		Phosphodiesterase	Acid RNase	Acid phosphomonoesterase
	Total act.	Specific act.	Total act.	Total act.	Total act.
Crude DNase II:					
– before thermal treatment	534,780	3	250,200	64,960	2,837
– after thermal treatment	452,500	6.7	212,400	63,200	2,645
Cm-cellulose (1st chromatog)	255,486	110	26,535	8,720	63
DEAE-Sephadex A-50	131,200	118	12,050	4,735	50
Hydroxyapatite:					
– fr. 202–218 (1st component)	52,992	307	5,935	0	0
– fr. 246–265 (2nd component)	52,898	331	5,087	0	0
CM-Sephadex C-50	31,200	339	3,060	0	0

dialysed against water, lyophilized and dissolved in 0.05 M potassium phosphate buffer pH 6.80 (PPB). The resulting solution was chromatographed on a 9.2 × 97.5 cm DEAE-Sephadex A-50 column equilibrated with 0.05 M PPB; 50 ml fractions were collected. Elution of all acid hydrolases occurred without changing the PPB molarity. The elution peak was not symmetric (fig. 2).

3.5. *Hydroxyapatite chromatography*

Fractions with a specific activity higher than 110 were pooled (1730 ml) and loaded on a 4.6 × 44 cm hydroxyapatite column equilibrated with 0.05 M PPB. Inactive proteins were eluted with 0.10 M PPB. DNase II elution was carried out with a concave PPB gradient (0.10–0.50 M) devised according to Wren [9]; 50 ml fractions were collected. DNase II activity was distributed between two components, each of which showed phosphodiesterase activity, but was free of acid RNase and phosphomonoesterase (fig. 3).

3.6. *CM-Sephadex C-50 chromatography*

Fractions 246–265 of the preceding step (2nd component) were dialysed against 0.05 M PPB and run on a 3 × 80 cm CM-Sephadex C-50 column equilibrated with 0.05 M PPB. Elution with a linear PPB gradient resulted in a symmetrical peak of DNase II, with a rather constant ratio DNase II/phosphodiesterase (fig. 4).

A rechromatography on CM-cellulose of an aliquot of the central part of this peak, with elution by a linear AAB gradient (0.12–0.50 M), confirmed the simultaneous presence of both activities in a constant ratio (fig. 5).

4. Discussion

The main results of the whole purification have been gathered in table 1. The specific activity of 339 obtained after CM-Sephadex is in good agreement with the value reported by Bernardi for its purest preparations (350). An identical value was obtained after rechromatography of fractions 202–218 from hydroxyapatite (1st component), first on hydroxyapatite, then on CM-Sephadex C-50. Again a constant ratio DNase II/phosphodiesterase was obtained (ca. 10).

For both components, the sedimentation coefficient determined in a sucrose gradient was 3.4 ± 0.1. No differences were observed for the sedimentation rates of DNase II phosphodiesterase activities. No separation of these activities could be observed when both components were submitted to polyacrylamide gel electrophoresis. It is thus confirmed that, even if the two activities belong to different proteins, they cannot be separated by conventional techniques.

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