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RIBONUCLEASE ACTIVITIES IN BEAN ROOTS

J. P. TREBAL, N. BEOPOULOS and R. ESNAULT*

Physiologie Cellulaire Végétale, Université de Paris VII, 2 Place Jussieu, 75005 Paris, France

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Abstract—*Vicia faba* meristematic and elongating root cells (zones 0–4 and 10–20 mm) contained one nuclease (A_1) and four ribonucleases (A_2, A_3, C_1, C_2). When the overall activity of each enzyme was expressed per cell, the elongating cells contained 4-, 4-, 4-, 10- and 17-fold more activity than meristematic cells for A_1, C_1, C_2, A_2 and A_3, respectively.

INTRODUCTION

Little is known about the control mechanisms in higher plants that prevent nucleases from degrading cellular nucleic acids. The relative proportion of higher plant endonucleases, grouped into RNases I, II and nuclease I, varies in different organs and cell fraction, or after a period of growth, senescence and stress [1, 2]. As pointed out by Dove [1], most studies have been done with crude or partially purified material. For a better understanding, it would be necessary to assay the different enzymes separately and establish their individual functions in RNA metabolism. This paper is devoted to an analysis of the distribution of several RNases [3, 4] extracted from *Vicia faba* meristematic (zone A: 0–4 mm) and elongating (zone B: 10–20 mm) root cells.

RESULTS AND DISCUSSION

The enzyme extracts (cf. Experimental) from both meristematic and elongating cells were separated into anionic and cationic species by chromatography on a CM-cellulose column equilibrated at pH 5.5 [3]. At this pH most of the proteins are negatively-charged and are not retained, while the positive species were eluted by M NaCl. Both fractions contain ribonuclease (RNase), phosphomonoesterase (PME) and phosphodiesterase (PDE) activities. The RNase activities were freed from PME and PDE by filtration on Biogel columns.

The anionic and cationic RNase activities from meristematic and elongating cells were then subjected to chromatography on DEAE- and CM-cellulose columns, respectively. Separation of the anionic fractions led to the isolation of two peaks (A_β and A_γ), eluted with 0.1 and 0.25 M NaCl, respectively (Fig. 1). After concentration, each fraction was independently chromatographed using NaCl gradients from 0 to 0.2 M (A_β) or 0 to 0.5 M (A_3) eluting at 0.25 M NaCl (Fig. 1). Successive chroma- 0.1 M (A_2), while from A_γ only one species was obtained (A_3) eluting at 0.25 M NaCl (Fig. 1). Successive chromatography of each fraction did not result in any further fractionation.

The cationic species were chromatographed on CM-cellulose, equilibrated with 10 mM phosphate pH 6, and eluted with a KCl gradient from 0 to 1 M. Two species were isolated, eluting at 0.2 (C_1) and 0.5 M KCl (C_2), respectively (Fig. 1). Further chromatography using shallower gradients gave only one peak from each active fraction.

Both meristematic and elongating root cells of *Vicia faba* contain the same spectrum of enzymatic activities, i.e. 3 anionic species (A_1, A_2, A_3) and two cationic species (C_1, C_2). This agrees very well with previous results obtained with whole roots [4]. Furthermore it was confirmed that A_1 is an unspecific endonuclease, degrading both DNA and RNA, and that A_2, A_3, C_1 and C_2 are endoribonucleases.

The elongating cells contain much more activity than the meristematic ones. When activity is expressed per cell or per mg of protein in one cell (Table 1), large differences appear between these different enzymes. On a per cell basis, the zone B/zone A ratios are 17 (for A_3), 10 (for A_2) and 4 (for A_1, C_1 and C_2 each).

Previous studies with different plant parts such as leaves [5–8] or roots [9–15] have led to the conclusion that RNase activity, often expressed per cell or per unit

Table 1. Comparison of the activities of five isolated enzymes extracted from zones A (0.4 mm) and B (10–20 mm) of *Vicia faba* roots

Activity in enzyme units per	Enzyme	Zone A*	Zone B*	Ratio A/B
mg protein/cell ($\times 10^{-10}$)	A_1	19.4	62.5	3.2
	A_2	2.7	20.3	7.5
	A_3	29.3	382.0	13.0
	C_1	13.7	44.4	3.2
	C_2	12.6	37.5	2.9
cell ($\times 10^{-8}$)	A_1	27.1	113.0	4.1
	A_2	3.8	36.6	9.6
	A_3	41.0	688.0	16.8
	C_1	19.2	80.0	4.1
	C_2	17.6	67.5	3.8

* Zone A contained 0.4 mg protein/segment and 3.9×10^5 cells/segment, while zone B contained 0.18 mg protein and 1.2×10^{15} cells.

* To whom reprint requests should be addressed.

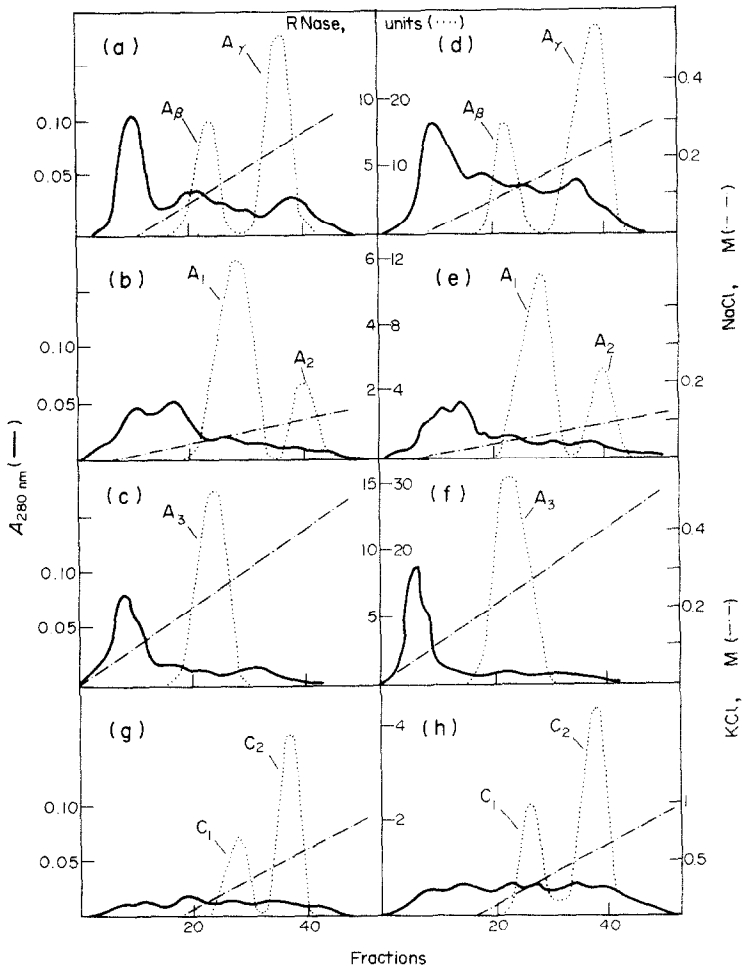

Fig. 1. Separation of anionic enzymes from zone A_β (a, b, c) and A_γ (d, e, f) on DEAE-cellulose and cationic enzymes (g, zones A and h, zone B) on CM-cellulose. The anionic fractions from gel filtration were chromatographed on DE52 columns and eluted with linear gradients of 0–0.5 M NaCl (a, d). The A_β and A_γ fractions were then independently concentrated, dialysed and applied to DE52 and eluted with NaCl gradients of 0–0.2 M for A_β (b, e) and 0–0.5 M NaCl for A_γ (c, b). The cationic fractions from gel filtration were chromatographed on CM52 columns and eluted with linear gradients of 0–1 ; KCl (g, h).

protein, is usually low in young or meristematic cells and increases with age or elongation and maturation. With the exception of work by Sahulka [13] or Hirai *et al.* [15], where the electrophoretic distribution of the RNases present in a partially-purified extract [13] or the subcellular localization of the enzymes [15] were analysed, all other studies have been carried out with crude homogenates. The overall determination of the RNase activity shows the same trend when activity is expressed per cell or per unit protein: that is, an increase of activity in elongating and maturing root cells. However, the increase of activity is not of the same order for all the RNases in *Vicia*. We did not observe that the fundamental enzymatic spectrum differs between meristematic and elongating root cells. This is in agreement with the results of Sahulka [13] but not with those of Wilson [10] who showed a nuclease specific for corn root tip cells.

EXPERIMENTAL

Plant material. Pre-soaked beans (*Vicia faba* cv Aguadulce), placed in moist vermiculite, were incubated at 25° in darkness for 48 hr. Following careful washing in sterile H_2O, roots from 2.5 to 2.8 cm were harvested and segments from 0 to 4 mm (zone A) or 10 to 20 mm (zone B) excised.

Preparation of enzyme extracts. All procedures were carried out at 4°. 1000 segments (corresponding to 10 and 63 g for zones A and B, respectively) were homogenized in 50 mM Na citrate, pH 5.5 and the homogenates brought to 0.5 M KCl in order to solubilize particulate RNases [16]. After 15 min the extracts were centrifuged at 2000 *g*, and the supernatants brought to 30% satn with satd $(NH_4)_2SO_4$ adjusted to pH 5.5. The solns were stirred for 1 hr and the ppts. removed and discarded after centrifugation at 18000 *g* for 1 hr. The resulting supernatants were brought to 80% satn. After stirring for 1 hr, the ppts. were collected by centrifugation at 18000 *g* for 1 hr and dissolved in 20 mM Na citrate, pH 5.5. The resulting solns were dialysed against 50 vol. of 20 mM Na citrate, pH 5.5 with constant stirring for 30 hr using 3 changes of buffer. After dialysis, the solns were centrifuged, if necessary, at 3000 *g* for 10 min and taken as the 'enzyme extract'.

The enzyme extract was chromatographed on CM-cellulose (CM52) column (2.5 × 12 cm) equilibrated with 20 mM Na

citrate, pH 5.5; while anionic proteins were not retained, those of cationic nature were eluted with M NaCl. The anionic and cationic groups of proteins were applied to Biogel P30 (equilibrating buffer: 20 mM Na citrate, pH 5.5) and P60 (equilibration buffer: 80 mM Na Pi, pH 7.5) columns (2.5 × 80 cm), respectively. Proteins were eluted with the corresponding buffer with a flow rate of 6 ml/cm^2/hr and 5 ml fractions were collected. Protein elution was monitored by *A* at 280 nm and enzyme activities measured on 200 µl samples.

The anionic and cationic RNases were subjected to several successive chromatographic steps: on DEAE-cellulose (DE52) columns (2.5 × 4 cm) equilibrated with 10 mM Na citrate, pH 5.5 for the anionic species and on CM52 columns (2.5 × 4 cm) equilibrated with 10 mM NaPi, pH 6 for the cationic ones. Elutions were achieved by using NaCl and KCl gradients, respectively, with a flow rate of 12 ml/cm^2/hr and 5 ml fractions were collected. Protein elution was monitored at 280 nm and samples of 200 µl were assayed.

Protein determination and enzyme assays. These have been described previously [4].

Cell counts. Thinly sliced segments were macerated in 10% chromic acid for 24 hr. After homogenization, the dissociated cells were counted in 100 µl aliquots using a haemocytometer (Nageotte) with at least 10 determinations from 5 independent prepns.

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