

Endoribonuclease from roots of the *Vicia faba* L. ssp. *minor* seedlings

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Ribonuclease (RNase; EC 3.1.4.23) from roots of broad bean seedlings (*Vicia faba* L. ssp. *minor* var. 'Nadwiślański') was isolated and partially purified. The seeds were placed in 10 cm Petri dishes on 2 discs of Whatman No. 2 filter paper wetted with 10 cm³ of distilled water, and germinated for 3 days in darkness at 25°C. RNase from the roots of 3-day-old seedlings was extracted and partially purified (Table 1); the enzyme activity was determined according to [1].

The enzyme should be regarded as an endoribonuclease [2] as shown by the Sephadex G-50 chromatography of the digestion products in the course of hydrolysis of the high molecular yeast rRNA (Fig. 1). The undigested substrate was eluted in the void volume of the column (fraction no. 3); oligonucleotides of approximate molecular mass below 30 kDa appeared after 10 min (fraction no. 4), followed by oligonucleotides, and finally by mononucleotides (fraction no. 7).

The molecular mass of plant ribonucleases varies from 17.5 kDa in *Pisum sativum* L. [3], to

155 kDa in *Beta vulgaris* [4]. Chromatography on Sephadex G-100 [5] of the extract from roots

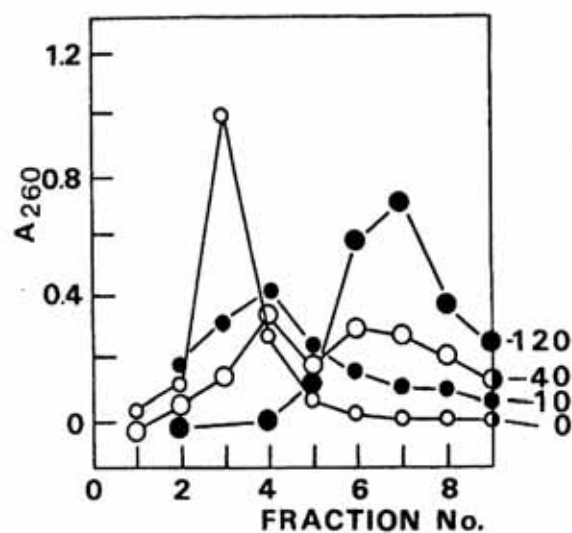


Fig. 1. Endoribonucleolytic cleavage of yeast rRNA by RNase from *Vicia faba* L. ssp. *minor* roots. The successive hydrolysis products were collected at the indicated time intervals (0 - 120 min) and chromatographed on Sephadex G-50 [2]

Table 1

Partial purification of RNase from roots of *Vicia faba* L. ssp. *minor* var. 'Nadwiślański'

Purification steps	Activity (units)		Protein		Specific activity
	U	%	mg	%	U/mg protein
1. Crude extract	46	100	20	100	2.3
2. Sephadex G-25	35	76	18	93	2.0
3. Sephadex G-100	33	71	2	10	16.5
4. DEAE-Sephadex A-25	27	59	0.24	1.2	112.5
5. ConA Sepharose	25	54	0.22	1.1	113.0

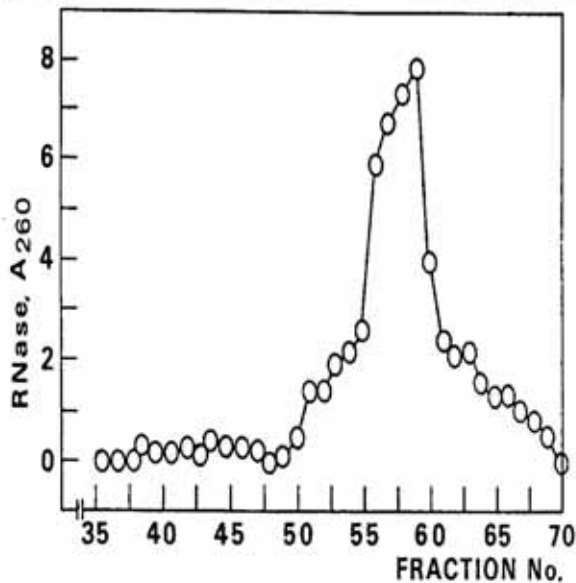


Fig. 2. Sephadex G-100 chromatography of RNase of *Vicia faba L. ssp. minor*.

Roots from 3-day-old seedlings were extracted with 0.05 M Tris/HCl buffer (pH 7.45). The column (2.6 × 89 cm) was loaded with 5 cm³ of the extract and eluted with the same buffer. Fractions of 5 cm³ were collected. The enzyme activity was assayed in 0.1 M citrate buffer (pH 6.0) and expressed in units. One unit of activity is defined as that amount of enzyme which causes an increase of A₂₆₀ of 1.0 per g fresh tissue mass per min

of broad bean seedlings revealed the presence of one molecular form of the enzyme, of apparent molecular mass of 35 kDa (Fig. 2). The enzyme obtained by ion exchange chromatography on DEAE Sephadex A-25, and eluted with 0.07 M NaCl was homogeneous (not shown).

RNase of higher plants are purine-specific [6], base unspecific, or pyrimidine-specific [7]. The enzyme from broad bean hydrolysed synthetic ribopolymers (60 min of hydrolysis) in the following order: poly(U) [100%] > poly(I) [50%] > rRNA = poly(A) = poly(A-C) [46%] > poly(C) [4%]. Digestion products of poly(A) and poly(A-C) were chromatographed on PEI-cellulose. In the latter hydrolysate only adenosine-2'-P and adenosine-3'-P were identified. Cytidylic acid did not appear since poly(C) was practically not hydrolysed.

The RNase binds to Con A Sepharose and is washed out of the column with 0.05 M α -methyl-D-mannoside supplemented with 0.2 M NaCl. This points to glycoprotein structure of the enzyme.

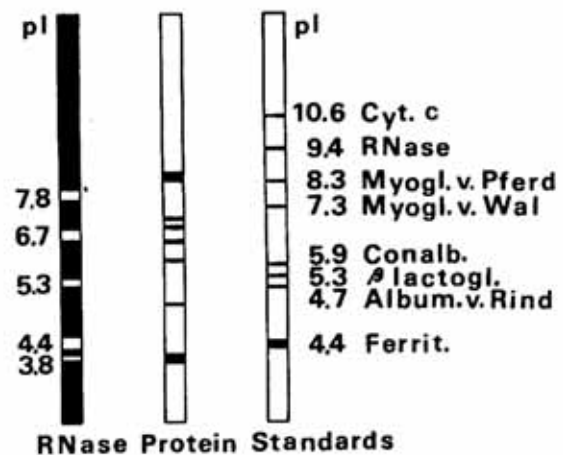


Fig. 3. Isoelectrofocusing of the RNase from *Vicia faba L.*

Protein (100 μ g) was loaded onto 7.5% gel containing urea (8%) and ampholine (pI = 3.5 - 10)

Polyacrylamide gel electrophoresis [8] of the crude extract revealed two active bands. Isoelectrofocusing with urea (8%) and ampholine (2.2%, pH 3.5 - 10) in 7.5% gel [8] revealed five bands of RNase activity at: pI 7.7, 6.7, 5.3, 4.4, 3.8 (Fig. 3). Staining for RNase and protein was performed according to [1].

Optimal temperature for the enzyme was 45°C; with the maximum activity at pH 6.0 in 0.05 M Tris/acetate buffer.

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