Ribonucleases in the microsomes from pea seedlings

Previous communications[1,2] from this laboratory reported that the microsomes from young wheat roots contained RNase which was inactivated by EDTA. A similar enzyme was sought in the microsomes from pea seedlings. Contrary to our expectation the RNase activity of the microsomal suspension was not inhibited by the addition of EDTA; it was rather somewhat activated. When the microsomes were fractionated into endoplasmic reticulum and ribonucleoprotein particles different RNases were found in each fractions.

3-day-old pea seedlings were detached from their cotyledons, cut into small pieces, cooled to 5° and ground in a mechanical mortar with 2 vol. 0.5 *M* sucrose. The mixture was filtered through cotton cloth and the filtrate was fractionated by differential centrifugation. The filtrate was centrifuged at 16,000 × *g* for 15 min and the mitochondrial sediment removed. The supernatant was centrifuged at 50,000 × *g* for 30 min in a Spinco preparative centrifuge (with No. 30 rotor) to give a sediment of microsomes (endoplasmic reticulum and a part of ribonucleoprotein particles). The supernatant was further centrifuged at 100,000 × *g* for 120 min to give a sediment of ribonucleoprotein particles.

RNase in the endoplasmic reticulum

Washed microsomes were suspended in water and 0.2 vol. butanol was added to the suspension. After sonic oscillation for 10 min, NaCl was added to 0.15 *M* and the sonic oscillation repeated for 10 min. The suspension was centrifuged at 5,000 × *g* for 20 min, and the clear water phase separated from the butanol phase.

Some of the particles precipitated and others adsorbed on the butanol layer. The clear water phase was removed and dialyzed against water for 24 h in a cold room. The RNase in this solution was inactivated in 0.1 *M* acetate buffer, pH 6.0, with the addition of $2.5 \cdot 10^{-3}$ *M* EDTA, suggesting that this RNase requires a metal ion for its activity. For this reason, EDTA or deoxycholate which are generally used for the solubilization of the enzymes in the microsomes cannot be used for detecting this enzyme. The pH optimum was at 6.0 (see Fig. 1).

RNase in the ribonucleoprotein particles

The RNase in the ribonucleoprotein particles could be easily solubilized by metal-binding compounds, such as EDTA, deoxycholate, citrate or phosphate (see Table I). However, since the enzyme could not be completely solubilized by acetate buffer, it is clear that the enzyme was bound to particles. Apparently it does not require metal ions for its activity since it was not inhibited by EDTA. The enzyme solution which was solubilized from the ribonucleoprotein particles by citrate–phosphate, pH 6.0, was dialyzed against water for 24 h in a cold room. The pH optimum of this RNase was 5.6 (see Fig. 1).

From these results, it appears that the endoplasmic reticulum and ribonucleoprotein particles of the microsomes from pea seedlings contain different RNases which differ in pH optimum and in the effect of EDTA.

The RNase in the endoplasmic reticulum corresponds to the RNase which was previously found in the microsomes from young wheat roots[2].

Abbreviations: RNase, ribonuclease; EDTA, ethylenediaminetetraacetic acid.

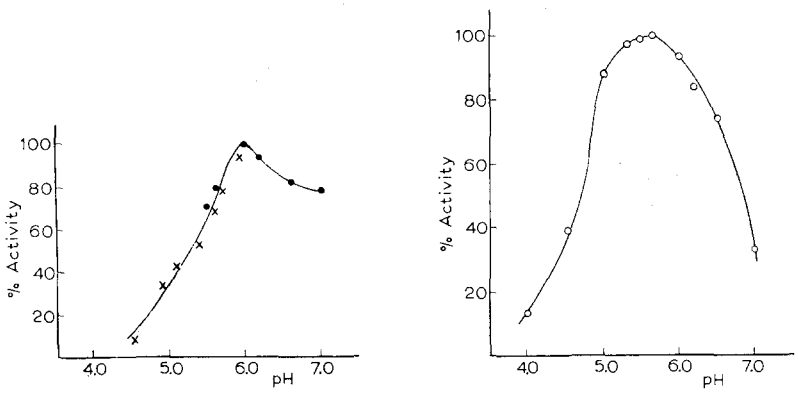

Fig. 1. pH-activity curve of endoplasmic reticulum RNase; ×—×, in 0.1 *M* acetate buffer; ●—●, in 0.1 *M* cacodylate buffer. ○—○, pH-activity curve of ribonucleoprotein particle RNase in 0.1 *M* citrate-phosphate buffer.

TABLE I

SOLUBILIZATION OF THE RIBONUCLEASE FROM THE RIBONUCLEOPROTEIN PARTICLES

Ribonucleoprotein particles from the pea seedlings were suspended in the indicated solutions. The suspensions were centrifuged at 10,000 × *g* for 30 min in order to sediment the particles. Both suspensions and supernatants were assayed for RNase activity. The RNase activity was determined spectrophotometrically in acetate buffer, pH 6.0. Activity is reported as absorbancy at 260 mμ.

Treatment	*Ribonuclease activity*		*Solubilization* %
	Suspension	*Supernatant*	
Acetate buffer, 0.1 *M*, pH 6	1.328 (100)	0.133	10
EDTA, 0.1 *M*; acetate buffer, 0.1 *M*, pH 6	1.472 (111)	1.480	100
EDTA, 0.01 *M*; acetate buffer, 0.1 *M*, pH 6	1.470 (111)	0.701	48
EDTA, 0.001 *M*; acetate buffer, 0.1 *M*, pH 6	1.338 (101)	0.271	20
Citrate-phosphate buffer, 0.1 *M*, pH 6	1.461 (110)	1.416	97
Phosphate buffer, 0.1 *M*, pH 6	1.272 (96)	1.220	96
0.2% Deoxycholate	1.073 (81)	0.652	61

The RNase in the ribonucleoprotein particles may correspond to the RNases which were found in rat-liver microsomes by TASHIRO[3], in the mouse-pancreas microsomes by DICKMAN[4] and in the ribonucleoprotein of *Escherichia coli* by ELSON[5]. But these RNases were all latent and did not reveal their activity until the microsomes or ribonucleoprotein were treated with EDTA or urea. In the plant, on the other hand, the RNase in the ribonucleoprotein particles could also be solubilized by the addition of EDTA, but the suspension of the washed ribonucleoprotein particles themselves contained more of the RNase activity. Therefore, it is concluded that the RNase is bound on the surface of the particles.

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