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Isoenzymes of RNase in Senescing Morning Glory Petals

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With 3 figures

Received November 16, 1976 · Accepted November 24, 1976

Summary

Purified RNase prepared from petals of the ephemeral flowers of *Ipomoea tricolor* represents a mixture of four endonucleases. These isoenzymes can be separated by polyacrylamide gelelectrophoresis. Partial separation was achieved with isoelectric focusing. The synthesis of two isoenzymes is responsible for the dramatic increase of RNase activity in the senescing corolla.

Key words: RNase, isoenzymes, gelelectrophoresis, isoelectric focusing, senescence, Ipomoea tricolor.

Senescence in the ephemeral corolla of the morning glory *Ipomoea tricolor* is characterized by a rapid and extensive catabolism of biopolymers and by the increase of various corresponding hydrolase activities (Matile and Winkenbach, 1971; Wiemken et al., 1974). RNase (Baumgartner et al., 1975) and two glucosidases (Wiemken and Wiemken, 1975) are synthesized *de novo* in the senescent corolla when the total protein content is declining rapidly. These findings support the view that senescence in this plant organ represents an organized developmental process rather than a colapse. An attempt to characterize the enzymes responsible for the circa 50 fold increase of RNase activity in the course of flower fading has yielded further details to support the above concept of aging.

Assayed according to Ambellan and Hollander (1966) the RNase is optimally active at pH 4.5–5.0. The enzyme has been purified (Baumgartner et al., 1975). Gel filtration of digestion products obtained upon incubation of yeast RNA with the purified RNase showed that the enzyme is of the endonuclease - type. The elution patterns of breakdown products obtained after various periods of digestion resembled closely the patterns obtained by Wyen et al. (1969) with an RNase purified from *Avena* leaf tissue.

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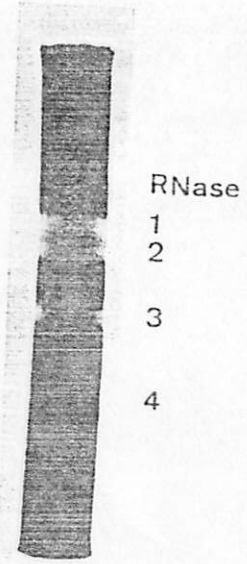

Fig. 1: Isoenzyme pattern of RNase. Polyacrylamide gelelectrophoresis according to Wilson (1971). Negative staining of RNase bands achieved by incubating the gels in the presence of 0.4 % (w/v) yeast RNA (0.1 M citrate-NaOH buffer pH 5.0) for 12 min at 37 °C; after rinsing in buffer (5 min, room temperature) the gels were stained with 0.2 % (w/v) toluidine blue (0.1 % acetic acid) for 1 min and thereafter rinsed in 0.5 % acetic acid for 10–20 h.

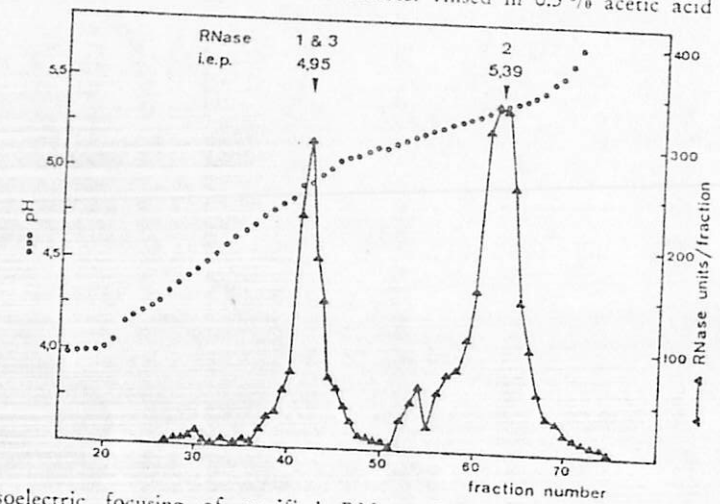

Fig. 2: Isoelectric focusing of purified RNase. Conditions: LKB isoelectric focusing column, 110 ml; 1 % ampholine pH 4–6, sucrose-gradient; anode: 1 % H_2SO_4; cathode: 1 % NaOH; 2 Watts, increasing voltage up to max. 1000 Volts; duration: 2–3 days. Reference: Haglund (1967).

Disc gelelectrophoresis of the purified *Ipomoea* RNase revealed the presence of four isoenzymes (Fig. 1). In the negatively stained gels, two bands are prominent (RNase 1 and 2), one is rather faint (RNase 4) whilst RNase 3 is intermediate.

Using isoelectrofocusing in combination with disc electrophoresis, it appeared that RNases 1 and 2 are distinct proteins with isoelectric points of 4.95 (RNase 1) and 5.39 (RNase 2); as shown in Fig. 2 the RNases 1 and 3 cannot be separated by isoelectric focusing. However, the distinct development of the isoenzymes 1 and 3 in the senescing corolla suggests that they represent distinct proteins.

Isoenzyme patterns of RNase were analyzed quantitatively at four stages of flower development. It appears from Fig. 3 that the dramatic increase of RNase activity in the fading corolla is due to the synthesis of RNases 1 and 2 exclusively. The isoenzyme 3 is unchanged throughout the development whilst isoenzyme 4 has

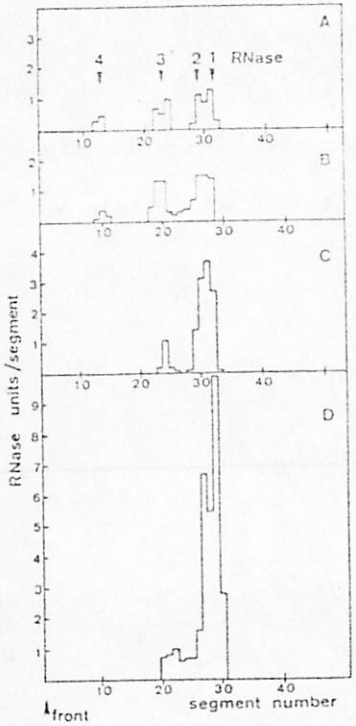

Fig. 3: Isoenzyme pattern of RNase at four stages of flower development. The gels (Wilson, 1971) were sliced into 1 mm segments which were incubated in the presence of substrate (1 % purified yeast RNA in 0.1 M Na-acetate buffer pH 5.0). Assay according to Ambellan and Hollander (1966).
A: flower buds, day before anthesis, noon. – B: day of anthesis, noon, before onset of fading and increase of RNase activity. – C: day of anthesis, 6 p.m. – D: day after anthesis, 8 a.m.

disappeared completely in the senescent organ. These changes of isoenzyme patterns reflect a specific biochemical differentiation associated with the ageing of the ephemeral corolla. The two endonucleases which are synthesized during senescence presumably have a specific function in catabolism of RNA which takes place in this phase of flower development. The two isoenzymes which are not synthesized or even disappear completely as catabolic RNA metabolism is intensified upon flower fading, are likely to have functions in the developing flower bud. A conclusive functional interpretation of RNase isoenzymes would certainly require an anlysis of their intracellular localization. An attempt to localize the RNases 1 and 2 by means of immunocytochemistry has yielded evidence for the presence of these isoenzymes in the central vacuoles of those cells in the corolla ribs that are responsible for the curling of the ageing organ (Baumgartner and Matile, 1976). As there is no strict correlation between RNase activity and decline of RNA, such a compartmentation of hydrolases and their potential cytoplasmic substrate molecules is not unexpected. The breakdown of RNA in the lytic compartment of living cells appears to represent a prerequisite of an organized recuperation and export of nitrogen in the senescent corolla.

Acknowledgements

The present work was supported by the Swiss National Science Foundation and by the Zentenarfonds ETHZ. The authors are indepted to Dr. E. Häusermann for her help with the manuscript and to Mr. Paul Frick who skillfully cultured the plants.

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