Iso-Enzymes of Acid Ribonuclease in Cotyledons of *Pisum sativum* L.

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Summary. Acid ribonuclease from cotyledons of *Pisum sativum* is very stable, with a temperature optimum of 65°C. It has a molecular weight of 17,500 and there is evidence that a fragment, with a molecular weight of 3,100, retains enzyme activity. Acid ribonuclease from the cotyledons of five-day old seedlings may be fractionated into two iso-enzymes, I and II, by CM-cellulose chromatography. The increase in activity of iso-enzyme I is not inhibited by cycloheximide, whereas the increase in iso-enzyme II activity is strongly inhibited by cycloheximide. Cotyledons of 9-day old seedlings contain only iso-enzyme I, whilst cotyledons of 15-day old seedlings contain three iso-enzymes, I, IIa and III.

Introduction

In the preceding paper (Bryant et al., 1976), we demonstrated that the development of acid ribonuclease activity in the cotyledons of germinating peas exhibits a biphasic pattern. Preliminary evidence was also presented to show that the early increase in acid ribonuclease is partly independent of protein synthesis. In this paper we report a partial characterisation of acid ribonuclease, including an estimation of its molecular weight. The existence of at least three iso-enzymes is demonstrated, and data are presented which suggest that increases in the activity of one iso-enzyme are not mediated by protein synthesis.

Materials and Methods

*Growth of Plants* was as described in the previous paper (Bryant et al., 1976).

*Ribonuclease Assays* were carried out essentially according to Sri-vastava (1968).

Results and Discussion

**Stability.** Stability of the enzyme was investigated by storage of crude extracts at 1°C (Fig. 1). At 1°C the enzyme is stable for 4 days. It is also stable for 24 h at 37°C and for at least 24 h at 22°C. The temperature optimum, as determined in crude extracts, is 65°C (Fig. 2). The increased activity of the enzyme at high temperature is not caused by changes in the secondary structure of the RNA used as a substrate, since preheating the RNA, followed by rapid cooling to 37°C for assay, does not lead to enhanced activity (Fig. 2). The temperature optimum is higher than that reported for soluble acid ribonuclease from wheat (Torti et al., 1973), but is similar to that reported for the ribonuclease from bovine pancreas (Davidson, 1972). In fact the general stability of the acid ribonuclease in pea cotyledons closely resembles that of pan-
creatic ribonuclease. The acid ribonucleases from pea leaves (Frisch-Niggemeyer and Reddi, 1957) and from cucumber seedlings (Kado, 1968) also show a high level of stability.

Molecular Weight. The enzyme elutes from columns of Sephadex G-50 (fine) as two peaks, A and B (Fig. 3). The molecular weight of the early-eluting peak (A), computed by comparison with the markers, is 17,500 ± 1,560. This is within the range shown by acid ribonucleases in other plants: corn, 23,000 (Wilson, 1968), garlic, 20,000 (Carlson and Frick, 1964), cucumber 12,000 (Kado, 1968), potato, 10,000 (Pitt, 1975) and wheat, 9,000 (Torti et al., 1973). The alkaline ribonuclease from pea cotyledons is slightly larger, with a molecular weight of ca. 21,000 (J.A. Bryant and G.A. West, unpublished data). The molecular weight of the late-eluting peak (B) is 3,100 ± 530.

This molecular weight is lower than that of any other known enzyme, and it is thus unlikely that peak B represents a native enzyme. It is more probable that this low-molecular-weight ribonuclease is a fragment derived from the native enzyme by proteolysis during extraction. Support for this suggestion comes from the finding that peak B is variable in amount, making up between five and 50% of the total activity. Further confirmation of the existence of a low-molecular-weight form of acid ribonuclease comes from results of dialysis experiments. When crude extracts of acid ribonuclease are dialysed at 1°C, activity is lost (Fig. 1). This contrasts with the maintenance of activity during normal storage at 1°C (Fig. 1). The amount of activity lost during dialysis is again variable, the upper limit being 50% of the total activity. It is suggested that this loss of activity is best explained as movement of a low-molecular-weight fragment through the dialysis membrane. A small acid ribonuclease (molecular weight 5,000) has been detected by Pitt (1975) in leaves of potato (Solanum tuberosum). Pitt regards this form of acid ribonuclease as a native enzyme of exceptionally low molecular weight, although the possibility that it is a fragment of a larger protein is not excluded. If these low-molecular-weight forms are indeed fragments of the native enzyme, it suggests that acid ribonuclease from certain plants retains enzyme activity even after loss of a large number of its amino acid residues. This suggestion is supported by the finding that acid ribonuclease from pea cotyledons is not inactivated by photodynamic modification of a number of its amino acids (J.A. Bryant and P.S. Phillips, manuscript in preparation).