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Starch degradative enzymes in the roots of leafy spurge

S. A. MacISAAC and J. D. BEWELY

Department of Botany University of Guelph, Guelph, Ontario, Canada N1G ZW1

Introduction

The accumulation of storage reserves is an important aspect for survival of perennial species including leafy spurge. Proteins, lipids and carbohydrates are storage materials. In leafy spurge, storage carbohydrates form the bulk of reserve material in the perennial roots. Seasonal fluctuations in non-structural storage carbohydrates have been observed in roots of leafy spurge and in many other species. The yearly pattern of non-structural carbohydrates in leafy spurge roots is a rapid accumulation of starch in late summer to autumn followed by a winter phase in which starch is slowly degraded.

The distinct changes in quantities of starch suggest seasonal alterations in the activities of the enzymes involved in degradation of starch. The pathway of starch breakdown and the seasonal pattern of activity of these enzymes was investigated in leafy spurge roots to determine if there are changes which can account for the pattern of starch accumulation.

The pathway of starch breakdown in other species involves a number of enzymes. α-amylase is an endoamylase which cleaves 1,4-α-glucose linkages of native starch granule producing branched oligosaccharides, maltose, and maltodextrins. The small molecular weight dextrins are further degrade by α-amylase, β-amylase, debranching enzymes and starch phosphorylase. Alphaglucosidase and maltose phosphorylase degrade maltose to release glucose. The presence of these enzymes in leafy spurge roots and the seasonal pattern of activity were determined.

Starch degradation

The yearly pattern of total amylase activity was determined in crude extracts of leafy spurge roots. In this assay, the production of maltose by α-amylase and β-amylase from

starch is determined colorimetrically. This assay underestimates the activity of α-amylase by ignoring the other products of its action on starch but is a useful assay to determine the total amount of amylase activity in crude extracts. There are marked seasonal changes in the activity of total amylase. Total amylase activity is lowest in the summer during the growing season and highest in fall and winter.

The activity of α-amylase in crude extracts containing both α-amylase and β-amylase was done using a carbohydrate substrate specific for α-amylase. A similar pattern of α-amylase activity was observed with enzyme activity lowest in the summer during the growing season and highest in the fall growing season and winter months. However, the magnitude of the change in α-amylase activity is less than that for the total amylase activity. This suggests that the observed changes in total amylase activity were also due to an increase in β-amylase activity.

Characterization of leafy spurge root-amylase

The properties of leafy spurge root α-amylase were determined for comparison to other species. Heat stability, calcium requirement for activity and the number of multiple forms of the enzyme were determined. Leafy spurge root α-amylase is not heat stable in the presence or absence of calcium. Activity is reduced by approx. 50% in the presence of a calcium chelator EGTA, indicating a requirement for Ca^{+2}.

Separation of proteins by SDS-PAGE followed by activity gels allow the characterization of enzyme molecular weight. A protein extract from leafy spurge roots reveals the presence of a band with molecular weight of 44 kD similar to that of barley. Separation of proteins by isoelectric focusing (IEF) followed by activity gels allows the visualization of multiple forms of α-amylase which differ in their isoelectric point. Several bands of activity are visible which can be divided into two groups based on their isoelectric points. Group 1 with pI ranging from 5.0 to 5.3 and group 2 with pI from 6.0 to 6.9 are visible. The forms of leafy spurge α-amylase have similar Pi to that of barley aleurone. The gel also shows that all forms of the enzyme are coordinately regulated and that there are no seasonal changes in particular isoforms.

Leafy spurge root α-amylase has properties of both barley aleurone and spinach leaf enzyme. It is, however, antigenically, distinct and does not cross-react with antibodies to barely α-amylase.

Maltose metabolism

The cleavage of maltose by α-glucosidase and maltose phosphorylase is the final step in the complete degradation of starch. Both enzymes are detectable in leafy spurge roots, but there are no significant seasonal differences in the activity of either α-glucosidase or maltose phosphorylase.

Conclusions

Seasonal changes in starch content are correlated with changes in activity of α- and β-amylases. Alpha-amylase from leafy spurge roots has some characteristics of barley and spinach leaf enzymes but is antigenically distinct Multiple forms of α-amylase are present, and all forms appear to be coordinately regulated. α-glucosidase and maltose phosphorylase activities are present in leafy spurge roots but do not show any seasonal changes in activity.