THE INFLUENCE OF LOW TEMPERATURES ON ACTIVITIES OF STARCH DEGRADATIVE ENZYMES IN A COLD-REQUIRING PLANT

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Summary: An increase in the activities of α-amylase, β-amylase and glucan phosphorylase was observed in roots of Verbascum thapsus when plants were subjected to low temperatures. Both amylases showed their greatest activities at 15°C, whereas the phosphorylase enzyme had its greatest activity at 10°C. α-Amylase and phosphorylase showed another increase in activities when plants commenced growing at higher temperatures after a long exposure to 4°C. β-Amylase in the root does not appear to be directly related to the growth of the shoot system following vernalization.

Low temperatures have been reported to stimulate the activities of glycogenolytic enzymes in the killifish (1). There is reason to believe that activities of analogous degradative enzymes of certain plants may also be stimulated by low temperatures. Significant amounts of starch degradation have been reported for some tree species when they were exposed to low temperatures (2). Starch breakdown associated with low temperatures was also observed in the roots of Verbascum thapsus, a cold-requiring biennial weed (3). The present study is an examination of the effects of low temperatures on the activities of three starch degradative enzymes in this biennial species.

MATERIALS AND METHODS

Verbascum thapsus L. overwinters as a leafy rosette. Such exposure to low temperatures is necessary for growth and flowering which occur during the following summer. Rosettes were grown from seed in a greenhouse which had average temperatures of 24° - 29°C. Pots with six-month old rosette plants with an average diameter of

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50 cm were placed for 3 days at 20°C in a room with controlled temperatures. The temperature was lowered in 5° steps at 3-day intervals for a period of 6 days. Following this 9-day period of cold acclimation, the rosettes were subjected to 4°C for 12 weeks, which is referred to as the vernalization period. Several vernalized rosette plants were returned to the greenhouse in order to study the same enzyme activities in growing plants.

Root extracts were obtained at different times from greenhouse grown plants, both before and after treatment with low temperatures, and also from plants which underwent the cold acclimation and vernalization processes. Prior to preparing the extracts, soil was gently washed from the roots with running water, and shoot material as well as all root material less than 3 mm in diameter were discarded. Two samples of extract from the remaining roots were prepared for each temperature treatment, and two replicates were made from each sample at the time of the enzyme assays. Roots were cut into small segments on an ice-cold glass plate, and enzyme preparations were made by homogenizing for 5 minutes 5-8 g of tissue in 10-16 ml of TRIS-HCl buffer, pH 7.3. The resulting brei was centrifuged at 48,200 xg for 30 minutes, at 2.4° C. Polyvinylpyrollidone powder (PVP) was added to the supernatant at 0.1 g per 1.0 g of roots, fresh weight, following the method of Loomis and Battaile (4). The supernatant was saved for later use. The pellet was resuspended in the same buffer and at the same proportions as described above, and was homogenized for 5 minutes. Following another centrifugation, PVP was added to the collected supernatant as in the first extraction. The supernatant was allowed to stand for 45 minutes, and a final centrifugation at 48,200 xg yielded a supernatant which was added to the supernatant collected earlier.

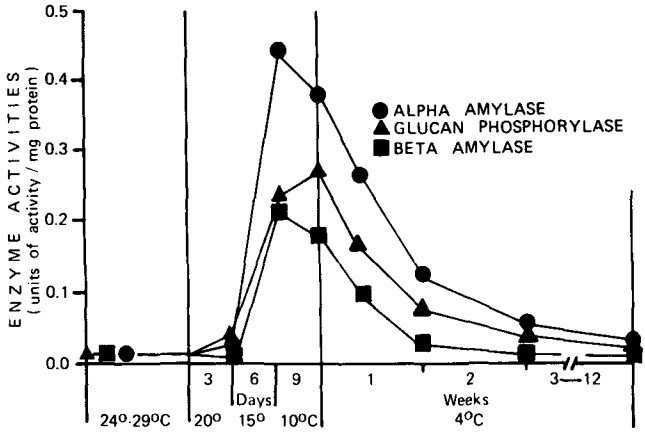

Figure 1 Activities of starch degradative enzymes in root extracts of Verbascum thapsus. Plants averaging 50 cm in diameter were grown in the greenhouse (24-29°C) and were then subjected to decreasing temperatures.

Previously described methods were used in assaying the following starch degradative enzymes: α-amylase (5), β-amylase (5) and glucan phosphorylase (6). Ammonium molybdate, an inhibitor of phosphatases (7), and mercuric chloride, an inhibitor of amylases (8), were found to be effective in the present study at 10^{-3}M and 10^{-5} M, respectively. The earlier report that phosphorylase tolerated these inhibitors (9) was confirmed in this study. One unit of activity of phosphorylase was defined as the amount which liberated 0.1 mg of phosphorous at 35°C.

For both α- and β-amylases, one unit of activity was defined as that amount which liberated one μmole of maltose at 25° C. Citrate buffer at 5 x 10^{-2} M was used in the assay for both amylases, however, the optimum pH for amylase was found to be 6.0, while that of β-amylase was 5.5. α-Amylase inhibitor was purchased from Nutritional Biochemical Corporation, and it was effectively used in the assay for -amylase at 1.0 mg per 1.0 ml of starch solution.

Protein determinations were made according to Lowry et al.

(10) and specific activities of enzymes are expressed in terms of units of activity per mg of protein.

RESULTS AND DISCUSSION

Declining temperatures resulted in an increase in the activities of all three starch degradative enzymes (Figure 1). Maximum variation in the phosphorylase assay was 0.01 units of activity per mg protein; maximum variation for α- and β-amylase was 0.02 and 0.012 units of activity per mg protein, respectively. Of the three enzymes, α-amylase showed the greatest increase in activity, and this was observed during the 3-day period of 15°C. A lesser activity is seen in α-amylase at the end of the 3-day period of 10°C, and further reduction in activity occurs at 4°C. α-Amylase nevertheless shows greater activity in roots of Verbascum plants subjected to 4° C than in roots of greenhouse grown specimens. Activities of glucan phosphorylase and β-amylase follow this general pattern in relation to treatment with low temperatures, although the phosphorylase enzyme differed from both amylases in that it showed a peak of activity at 10°C. Both -amylase and phosphorylase show a second increase in their activities during the postvernalization period (Figure 2). It is during this period of higher temperatures that the shoot system of vernalized specimens of Verbascum begins the rapid growth process known as bolting. One can assume from the low level of β-amylase activity during this period that β-amylase of the root contributes very little directly to the bolting process.

The correlation between higher amylolytic enzyme activities and decreasing temperatures agrees with the earlier observations of starch reduction in this species (3). The phenomenon of cold-induced starch reduction is certainly not universal in plants, as shown for example by Digitaria decumbens, a tropical plant in

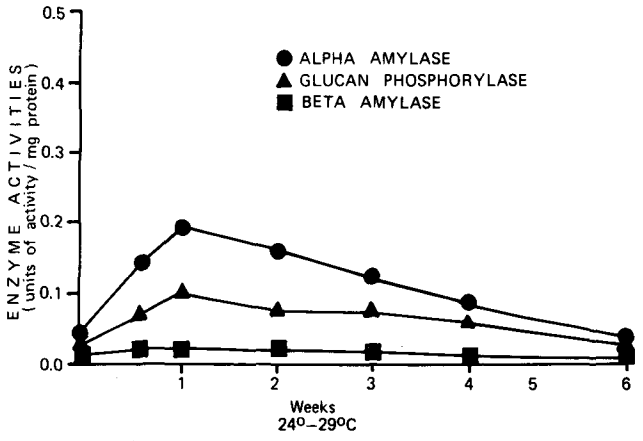

Figure 2 Activities of starch degradative enzymes in root extracts of vernalized Verbascum rosettes. α-Amyalse and glucan phosphorylase show increases which become maximum at the end of the first week of postvernalization period.

which starch accumulates during low night temperatures (11). Yet the higher amylolytic enzyme activities in Verbascum, a hardy weed of northern latitudes, might result in an increase in soluble carbohydrates which have a cryoprotective role (12, 13). That such a survival mechanism exists remains to be determined for this species.

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