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PHOSPHORYLASE ISOENZYMES: LOCALIZATION AND OCCURRENCE IN DIFFERENT PLANT ORGANS IN RELATION TO STARCH METABOLISM

SJ. J. GERBRANDY and J. D. VERLEUR
Department of Botany, Free University, Amsterdam, The Netherlands

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Abstract—Phosphorylase isoenzyme patterns obtained by the use of polyacrylamide gel electrophoresis technique were studied in relation to starch metabolism, localization, and organ differences. The following plants were investigated: *Solanum tuberosum* (L), *Vicia faba* (L), *Phaseolus vulgaris* (L) and *Allium cepa* (L). In potato tubers nine phosphorylase isoenzymes were found during the period of starch synthesis, while in a period of starch breakdown only two isoenzymes were observed. In seeds of *Vicia* and *Phaseolus*, a relation was also found between starch metabolism and the occurrence of phosphorylase isoenzymes. In extracts from potato amyloplasts only one isoenzyme was detectable. There is a correlation between the activity of this isoenzyme and the amount of amyloplasts present in different organs.

INTRODUCTION

SEVERAL authors reported experiments on the localization and function of phosphorylase isoenzymes in plant tissues. Siepmann and Stegemann[1] separated potato phosphorylase into several isoenzymes. After polyacrylamide gel electrophoresis of extracts from freshly harvested potato tubers, phosphorylase activity could be observed in 4 zones, whereas extracts from tubers after storage yielded two zones, and extracts from leaves and sprouts only one zone.

The intracellular localization of phosphorylase has been investigated mainly in leaves. de Fekete[2,3] stated that part of the phosphorylase activity of *Vicia faba* and *Zea mays* leaves was bound to isolated chloroplasts. Tsai *et al.*[4,5] found that in developing maize seeds the stage of development with fast starch synthesis was correlated with the occurrence of some additional phosphorylase isoenzymes which were supposed to have a function in starch synthesis. Recently de Fekete[6,7] suggested a function for phosphorylases in cotyledons of *Vicia*, in both the breakdown and synthesis of starch.

The present study was initiated in order to get more information about function and localization of phosphorylase isoenzymes in plants.

The Phosphorylase Isoenzyme Pattern in Solanum tuberosum

The phosphorylase isoenzyme pattern was investigated in old potatoes (1–2 months after planting), in young starch synthesizing tubers (dia. *ca.* 3 cm), and in leaves and roots. The results are presented in Fig. 1.

[1] R. SIEPMANN and H. STEGEMANN, *Z. Naturforsch.* **22**, 949 (1967).
[2] M. A. R. DE FEKETE, *Planta* **79**, 208 (1968).
[3] W. HUBER, M. A. R. DE FEKETE and H. ZIEGLER, *Planta* **87**, 360 (1969).
[4] C. Y. TSAI and Q. E. NELSON, *Plant Physiol.* **44**, 159 (1969).
[5] C. Y. TSAI and Q. E. NELSON, *Plant Physiol.* **43**, 103 (1968).
[6] M. A. R. DE FEKETE, *Planta* **87**, 311 (1969).
[7] M. A. R. DE FEKETE, *Planta* **87**, 324 (1969).

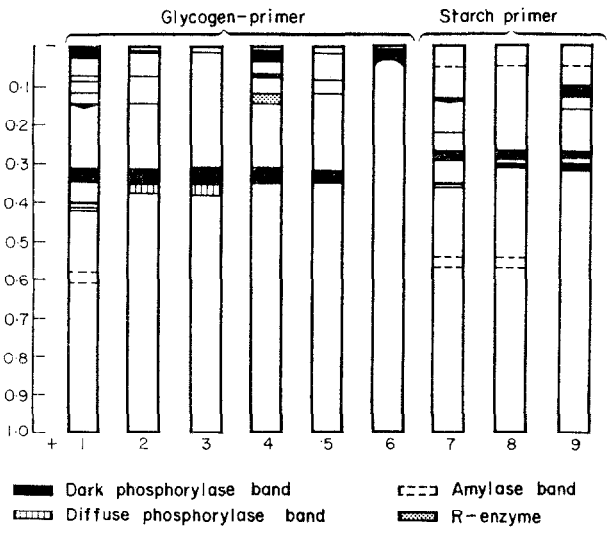

FIG. 1. ELECTROPHORETIC ENZYME PATTERNS FROM POTATO PLANTS.
(1) Young potato. (2) Old potato, 1 month after planting. (3) Old potato, 2 months after planting. (4) Leaves. (5) Roots. (6) Amyloplasts. (7) Young potatoes. (8) Old potatoes. (9) Leaves.

In extracts from young potatoes (Fig. 1.1) 9 bands with phosphorylase activity were detected. The two main components have R_f values of 0·04 and 0·33. In extracts from planted tubers, in a period of starch breakdown, the minor components with phosphorylase activity tend to disappear (Fig. 1.2 and 1.3). Old potatoes (1 month after planting), in which still several amyloplasts per cell could be seen by microscopic examination, produced a low but (still) reasonable active zone at R_f 0·04 (Fig. 1.2), whereas older tubers (2 months after planting), in which hardly any starch was left, showed only a trace of activity at the same place (Fig. 1.3). The young, starch synthesizing potatoes (Fig. 1.1) showed less activity in zone R_f 0·33 than the old ones (Fig. 1.2 and 1.3).

In gel systems containing starch as a primer the fast moving band (R_f 0·33) from old potatoes proved to be separated into two bands (Fig. 1.8). In these gels the band at R_f 0·04 did not appear, probably because of the amylase activity in the same region (Figs. 1.7–1.9).

Potato leaf extracts (Fig. 1.4) showed high phosphorylase activity at R_f 0·07 in addition to the main components at R_f 0·33 and R_f 0·04. When starch was used, the fast moving band separated into two distinct zones (Fig. 1.9).

Similar to the tuber extracts, a band at R_f 0·04 could not be demonstrated. Apart from the fast moving band potato roots contained little phosphorylase activity (Fig. 1.5).

Amylase and Debranching Enzyme

The electrophoretic pattern in glycogen-gels of the extracts from all leaves investigated included a light brown zone at R_f 0·14 (Figs 1.4, 2.3, 3.3, 4.3). This zone was also found after incubation of the gels in a medium without Glucose-1-phosphate (G-1-P) or inorganic phosphate. In starch gels a dark blue zone appeared at the same place (Fig. 1.9). These phenomena may be caused by hydrolysis of the α-1:6-branch linkages of the glycogen by R enzyme action, resulting in a greater capacity of the unit chains to adopt the helical

configuration for iodine complex formation.[8] The zones that were colourless after incubation with or without G-1-P and staining with iodine-potassium iodide were assumed to indicate the presence of amylase activity. They appeared both in glycogen gels and in starch gels, suggesting that these amylases do not have a high substrate specificity.

The Phosphorylase Isoenzyme Pattern of Vicia faba

In order to compare isoenzyme patterns of seeds in a starch synthesizing period and in a period of starch breakdown, extracts from developing seeds and from cotyledons of seedlings were investigated in addition to extracts from roots and leaves.

The pattern from cotyledons showed the greatest number of bands with one dominating zone at R_f 0·29 (Fig. 2.1). The developing seeds (Fig. 2.2) contained the same slow moving isoenzyme (at R_f 0·06) as found in the cotyledons but with higher activity. In addition, a

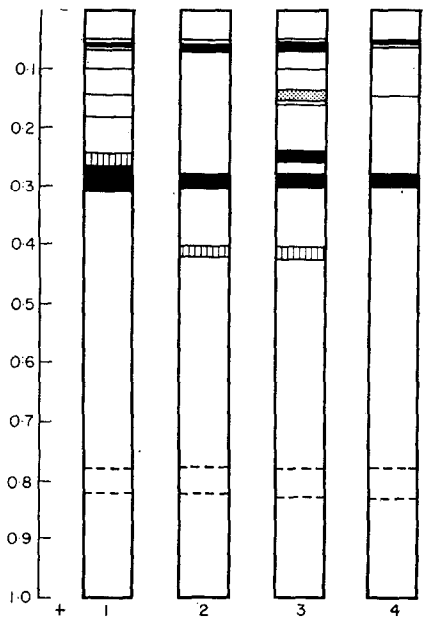

FIG. 2. ELECTROPHORETIC ENZYME PATTERNS FROM *Vicia* PLANTS.
(1) Cotyledons. (2) Young seeds. (3) Leaves. (4) Roots. For legends see Fig. 1.

zone with moderate activity was found at R_f 0·42. The latter could also be demonstrated in leaf extracts (Fig. 2.3), in which two other components separated at R_f 0·25 and 0·29. Band R_f 0·06 was also present in addition to some minor components. In the pattern of *Vicia* root extract, the zone at R_f 0·29 was most active, while the other components showed rather low activities (Fig. 2.4).

In all organs amylase activity occurred at R_f 0·80.

The isoenzyme patterns obtained by using starch as primer were essentially similar to those with glycogen as primer.

[8] W. J. WHELAN, *Handbuch der Pflanzen Physiologie*, VI, p. 219 Springer-Verlag, Berlin (1958).

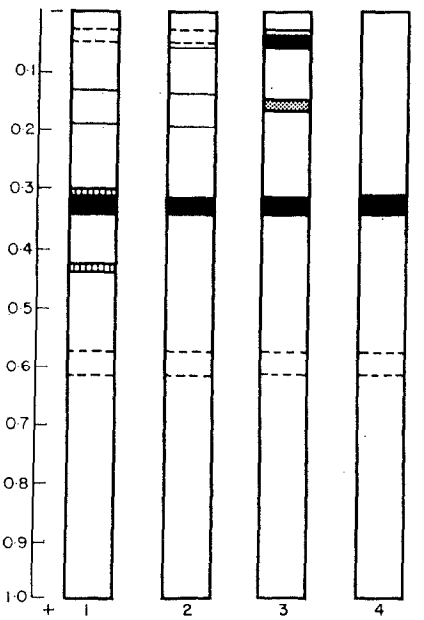

FIG. 3. ELECTROPHORETIC ENZYME PATTERNS FROM *Phaseolus* PLANTS.
(1) Cotyledons. (2) Young seeds. (3) Leaves. (4) Roots. For legends see Fig. 1.

The Phosphorylase Isoenzyme Pattern from Phaseolus vulgaris

Isoenzyme distributions were studied in the same organs as in the experiments with *Vicia faba*. The developing seeds (Fig. 3.2) showed an active component at R_f 0·33 and some minor components.

The band with R_f 0·06 could not be demonstrated in extracts from roots and cotyledons (Figs. 3.1 and 3.4) but showed a marked activity in leaf extracts (Fig. 3.3). The fast moving zone with R_f 0·33 was more active in cotyledons than in developing seeds. All extracts had amylase activity at R_f 0·59 while an additional amylase activity at R_f 0·05 could only be demonstrated in the extracts from cotyledons and seeds.

Phosphorylase Isoenzymes in Allium cepa

Allium cepa was selected for investigation because this plant in normal conditions contains hardly any starch.[9] Extracts from resting bulbs, leaves and young growing bulbs were studied. In general phosphorylase activity was very low. The resting bulbs produced one band at R_f 0·33 (Fig. 4.1). Extracts from young bulbs (Fig. 4.2) contained additional components with low activity at R_f 0·04, 0·06 and 0·15. Green leaves showed only one band at R_f 0·04 (Fig. 4.3).

Localization of Phosphorylase

Extracts from isolated potato tuber amyloplasts showed one single moving active phosphorylase band at R_f 0·04 (Fig. 1.6). Extracts from *Vicia* amyloplasts isolated from seeds showed several active phosphorylase bands, from which the isoenzyme at R_f 0·06 was more active when compared with extracts from whole seeds.

[9] J. W. GATES and G. M. SIMPSON, *Can. J. Botany* **46**, 1459 (1968).

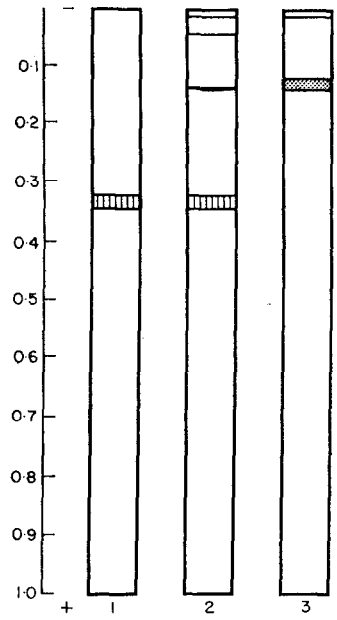

FIG. 4. ELECTROPHORETIC ENZYME PATTERNS FROM *Allium* PLANTS.
(1) Resting bulbs. (2) Young bulbs. (3) Leaves. For legends see Fig. 1.

DISCUSSION

The phosphorylase isoenzyme patterns of the storage organs in the plants investigated in comparing periods of starch synthesis and of starch breakdown showed differences. During the breakdown period the fast moving bands possessed a relatively high activity in both potato tubers (Fig. 1) and in cotyledons of *Vicia faba* and *Phaseolus vulgaris* (Figs. 2.1 and 3.1). This suggests that these enzymes might be mainly concerned with the digestion of starch. During a period of starch synthesis the electrophoretically slow moving components are more active than during a period of starch breakdown. This phenomenon is most pronounced in extracts from potato tubers (Fig. 1). This indicates that these isoenzymes are perhaps mainly concerned with starch synthesis.

The experiments demonstrated, at least in potatoes, the presence of only one phosphorylase isoenzyme (R_f 0·04) in the amyloplast fraction. It seems reasonable to suppose that *in vivo* this slow moving enzyme is exclusively associated with amyloplasts. The extracts from the old potatoes (Figs. 1.2 and 1.3) containing little activity of this enzyme were prepared from tubers containing a reduced amount of amyloplasts. Furthermore, in comparing the different organs the low activity of this enzyme is correlated with the presence of few amyloplasts as, for instance, in the investigated roots, and in leaves and bulbs of *Allium*.

All investigated leaves show a pronounced phosphorylase activity at R_f 0·06, suggesting a special function in the metabolism of transitory starch. More detailed information on the structure of the investigated isoenzymes is desirable to clarify their physiological functions.

EXPERIMENTAL

Plant Materials

Potato tubers (*Solanum tuberosum* L. var Bintje) were stored in the dark at 8° and planted at intervals. After growing for 1 or 2 months in the greenhouse, the 'old tubers', the newly formed 'young tubers', the leaves and the roots were separately collected.

Plants of *Phaseolus vulgaris* L. and *Vicia faba* L. were grown in the greenhouse and harvested 14 days after sowing. Onions (*Allium cepa* L.) were purchased at the local market.

Preparation of Tissue Extracts

Tissue samples were cut into small pieces and homogenized in a blender for 1 min, with twice the volume (w/v) of homogenizing medium for all types of tissue except for the developing seeds where 5 times the volume of medium was used. The medium contained 0·1 M Na-citrate buffer pH 6·5, 0·4 M sucrose, 0·002 M EDTA and in order to prevent browning of the extracts,[1,10,11] 1 ml per 100 ml medium of 5 g Na_2SO_3 plus 3·75 g $Na_2S_2O_5$ in 100 ml water.

The homogenate was pressed through perlon gauze and centrifuged at 10,000 *g* for 15 min. The supernatant fluid was used as the extract for electrophoresis.

Preparation of Extracts from Potato Tuber Amyloplasts

Tissue samples of 60 g were homogenized in 250 ml medium, filtered through perlon gauze and centrifuged for 3 min at 200 *g*. The sediment was washed 5× by resuspension in 3 × the vol. of medium, centrifugation, and ultimately resuspended in 2× the volume of medium. Microscopic examination of the resulting amyloplast suspension revealed that it was not visibly contaminated with other cell fragments. To solubilize the particle bound proteins the amyloplast suspension was sonified for 5 min by a 'Branson sonifier' at maximal power. After centrifugation for 5 min at 1000 *g* the starchy sediment was discarded and the supernatant was used as the amyloplast extract. All procedures were carried out at about 2°.

Gel Electrophoresis

Discontinuous polyacrylamide gel electrophoresis was performed according to Maurer[12] using gel system No. 2 with 7% acrylamide. Tris-glycine buffer pH 8·3 served as the electrode buffer. Both buffer and gels contained 0·002 M EDTA. As a primer for phosphorylase action 0·1% glycogen or starch (Merck) was polymerized with the gel.[1] Electrophoresis was conducted at 4° with 3 mA per tube. Bromphenol blue served as the front indicator.

Enzyme Assay

Phosphorylase activity was demonstrated in the gels usually by the synthesizing reaction using glycogen or starch as primer and incubating for 5 hr in 3 ml of a medium consisting of 0·1 M Na-Citrate pH 5·0 and 0·025 M glucose-1- Phosphate. After incubation the gel was stained with a solution of 0·01 M I and 0·014 M KI. The gels stained light brown due to the glycogen; the zones with phosphorylase activity appeared as dark brown bands.

When incubation took place in a medium containing 0·1 M Na-Citrate and 0·1 M Na_2HPO_4. pH 6·0, the zones with phosphorylase activity were colourless due to the breakdown of the primer.

[10] J. W. ANDERSON, *Phytochem.* **7**, 1973 (1968).
[11] D. M. STOKES, J. W. ANDERSON and K. S. ROWAN, *Phytochem.* **7**, 1509 (1968).
[12] H. RAINER MAURER, *Disk-elektrophorese*, Walter de Gruyter, Berlin (1968).