GLYCOLYTIC ENZYME PATTERN AND CONSTANT PROPORTION GROUP IN PLANT CELLS AS RELATED TO THEIR DEVELOPMENTAL AND FUNCTIONAL STATE

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1. Introduction

Glycolysis is the main route for initial degradation of respiratory substrates in higher plant tissues, and under normal conditions of tissue oxygen supply all glycolytic products are utilized in the tricarboxylic acid cycle. Only under partial or complete anaerobiosis are alcohol and lactate dehydrogenase activities revealed, and some ethanol, acetaldehyde, pyruvate, or lactate may be temporarily accumulated [1]. Limited data on glycolytic enzyme systems in plant tissues [2–4] seemed to demand further investigations of these enzyme activities in relation to developmental, functional, and taxonomic aspects. In this report we show in particular that the concept of enzyme constant proportion group developed for animal tissues and yeast cells by Bücher and his associates [5–7] is also valid with some reservations for plant tissues.

Abbreviations:
ADH: alcohol dehydrogenase (EC 1.1.1.1), GAPDH: glyceraldehydephosphate dehydrogenase (EC 1.2.1.12), GPI: glucosephosphate isomerase (EC 5.3.1.9), HK: hexokinase (EC 2.7.1.1), LDH: lactate dehydrogenase (EC 1.1.1.27), PFK: phosphofructokinase (EC 2.7.1.11), PGK: phosphoglycerate kinase (EC 2.7.2.3), PGM: phosphoglycerate mutase (EC 2.7.5.3), PK: pyruvate kinase (EC 2.7.1.40), PPG: phosphopyruvate hydratase (EC 4.2.1.11), TIM: triosephosphate isomerase (EC 5.3.1.1).

2. Methods

Maize seeds were germinated on moist absorbent paper in the dark at 27°. Root tips of 2-day seedlings were cut into 0–2, 2–4 and 10–20 mm segments consisting, respectively, of dividing, elongating, and mature cells. Coleoptiles of 2, 4 and 7-day seedlings were used to obtain the same 3 phases of cell growth. Cotyledons were isolated from dry seeds of maize, peas, and cucumber, or maize seedlings. Nine enzyme activities were measured in cell-free extracts of 12 samples of plant tissues by initial rate of NAD or NADP reduction or oxidation at 25° in direct or coupled 340 nm optical test employing the procedure of Bücher et al. [8] with some modifications described elsewhere [9] and presented as nmoles per min per g fresh tissue weight.

3. Results

When related to fresh weight all enzyme activities decreased at various rates in developing root and coleoptile cells (fig. 1A). The rate of decrease was the highest for PK and LDH and lowest for HK. Enzyme activities changed little in cotyledons of resting and germinating seeds (fig. 1B), where neither cell division nor expansion took place during germination.

Our data present some remarkable peculiarities of glycolytic enzyme pattern in plant tissues. First, all enzyme activities per g fresh weight (except PGK and PPG) are by 1 – 2 orders of magnitude lower than

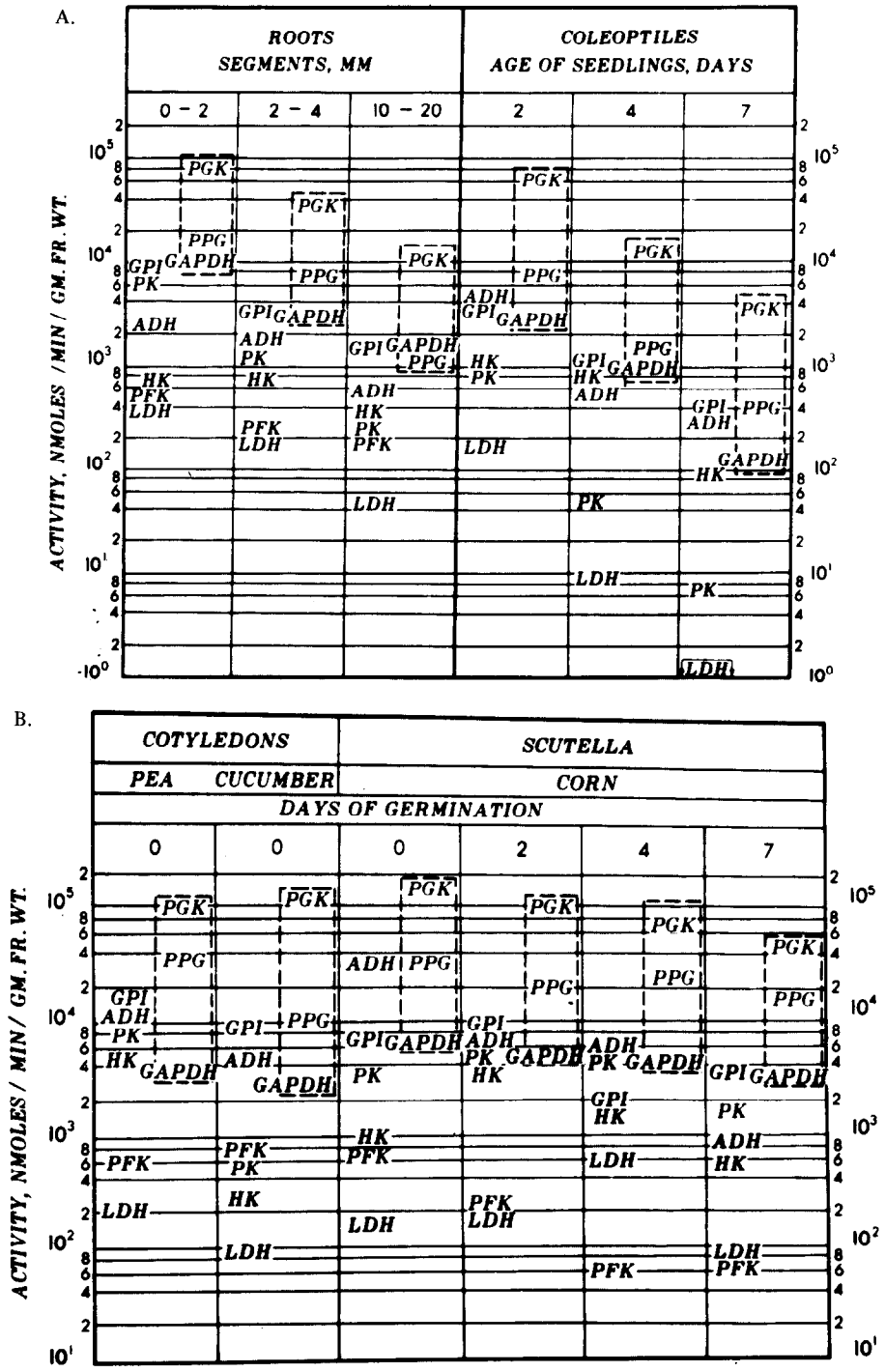

Fig. 1. Logarithmic plot of glycolytic enzyme activities in plant tissues. A) Developing maize roots and coleoptiles; B) cotyledons of dry and germinating seeds. Abbreviations as in text.

in animal tissues or yeast [7]. Second, in contrast to animal tissues enzyme levels in plants varied little when compared in 3 taxonomically distant species (maize, pea, and cucumber), in different organs of the same maize seedlings, and in root or coleoptile cells at successive developmental stages. Third, the investigated tissues appeared to differ in the ratio of key glycolytic kinases. PFK had the lowest activity and seemed to be the rate-limiting enzyme of glycolysis in maize seedlings and pea cotyledons. However, 2 other kinase ratios were different in growing maize root cells, maize scutellum, and pea cotyledon where PK was more active than HK or in mature maize root cells and coleoptiles where HK was much more active especially after the end of cell divisions. Cucumber cotyledons were quite an exception: here PFK had the highest and HK the lowest activity resembling some animal tissues [7]. The fourth peculiarity of investigated plant tissues was very low activity of LDH, even under thick seed coat in peas. ADH to LDH ratio was about 5 in partly anaerobic root meristem, 10–15 in elongating root cells, and 50–200 in other tissues. LDH development was insignificant and ADH activity increased only by 50% even when peas were germinated under partly anaerobic conditions.

Three of 5 enzymes forming Bücher's constant glycolytic group: TIM, PGK, GAPDH, PGM, and PPG, were measured in our experiments, and these data are specially marked in fig. 1. GAPDH activities are rather low in plant tissues compared to animals [cf. 2, 10, 11], so the ratios of PGK and PPG to this enzyme were much higher than in animal tissues. The ratios of 3 enzyme activities seemed to be rather constant in 12 investigated samples of plant tissues: less variable in developing cells of the same organ, more variable in 3 organs of the same seedling, and most variable when distant species were compared.

When protein synthesis and development of glycolytic enzymes in elongating maize root cells were inhibited by low concentrations of puromycin and cycloheximide [12] the ratios of 3 constant group enzyme activities were not changed significantly (table 1).

In addition to functional considerations some statistical criteria are evidently in need for enzyme selection into constant proportion group. When correlation coefficients were computed the values for

Table 1
Constant group enzyme ratios in maize root cells elongating in the presence of protein synthesis inhibitors.

Treatment	PGK / PPG	PGK / GAPDH	PPG / GAPDH
Initial control	4.2	9.6	2.6
After 6 hr:			
Water control	5.8	11.1	2.3
100 μg/ml puromycin	4.0	11.8	2.9
0.4 μg/ml cycloheximide	6.2	–	–

PGK–PPG, PGK–GAPDH or PPG–GAPDH pairs were not significantly higher than those for example for PFK–PGK, GPI–PGK, ADH–PGK, and even for HK–PK. Another approach was to compare the upper and lower limits of enzyme activity ratios. The values for PGK, PPG, and GAPDH in plant samples were a little higher than those in animal tissues (table 2). Although such a criterion seemed to be efficient enough to put a definite borderline, however, it could not be used for unambiguous discrimination of constant group components among 9 enzymes of glycolysis.

Thus, the glycolytic constant group may be found in any already investigated animal and plant tissues. It is most important that constant group pattern appears to be present at the successive developmental stages in plant cells and muscle [15, 16]. However, we think that in quantitative studies of enzyme synthesis this concept should be used with some reservations.

4. Discussion

In developmental studies of higher plant tissues enzyme activities per g fresh weight appear not to be a reliable representation, as plant cell growth involves a rapid increase in water content and accumulation of non-nitrogenous cell wall material [17]. Per protein unit seems to be more representative except in cotyledons with a high content of reserve proteins, however, protein content itself changes significantly during cell growth and development. Per cell level appears to be the most easily interpreted physiological unit. Both in root and coleoptile all activities increased several-fold during elongation, reached maximal

Table 2
Upper/lower limits of enzyme activity ratios in plant and animal tissues.

	PGK/PPG	PGK/GAPDH	PPG/GAPDH	PK/PGK	HK/PGK	PFK/PGK	GPI/PGK	HK/PK	HK/PFK	PK/PFK
12 plant samples described in fig. 1	5	6	10	45	32	18	8	210	56	100
16 samples of animal tissues [6, 7, 13, 14]	4	2	3	4	750	5	7	233	415	12

values in mature root cells, but dropped below the level of division phase after coleoptile cells ceased to grow (for details see [9, 12]).

Our data on enzyme activities per g fresh weight and mg protein are in good agreement with those for pea cotyledons [18] and parenchyma tissues of red beet roots (except PFK, PGK, and PK) [2] and potato tubers [3, 19]; Per cell levels of PFK and PK in root tip of pea seedlings (calculated on the basis of data [4]) and in suspension of *Acer pseudoplatanus* cells [10] correspond to those in maize root tip, however, GAPDH level in pea roots and GAPDH and PGK levels in Acer were much lower than in maize.

Although the constant group concept was not applied previously to plant tissues some already published data fit well enough into constant group pattern, e.g. all 5 enzymes in beet [2], PGK/GAPDH in developing wheat seedlings [11] and dividing Acer cells [10], TIM/GAPDH in germinating pine seeds [20], and more variable TIM, PGK, PGM, and PPG ratios in derepressed potato tuber tissue [3, 19].

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