ENZYME DEGRADATION IN HIGHER PLANTS: PHOSPHOGLUCOMUTASE

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1. Introduction

Whereas enzyme induction — as increase in the rate of synthesis of a particular enzyme in response to a changing environmental parameter — has been studied extensively in higher plant systems [1], our knowledge of enzyme degradation and its control is rather scarce. Doubtless the process of specific degradation of enzymes is of outstanding importance for a cell as a means of adapting its metabolism optimally to a new environment.

Theoretically a higher plant cell could have developed various mechanisms to regulate the disappearance of enzymatic activity (inactivation by negative allosteric effectors, through a block of the active center by inhibitors, among others also proteins, dissociation into inactive subunits, physical or metabolical separation of the catalyst from its substrate or cofactors, enzyme — catalyzed chemical modification of the enzyme [2], degradation by specific or unspecific proteolytic cleavage of the polypeptide chains, which shifts the balance between synthesis and degradation in favor of degradation, etc., see [3]), but practically none of these possibilities have been verified experimentally.

In resting potato tuber tissue a series of enzymes are highly active (i.e. phosphoglucomutase, enolase, glutamate-pyruvate-transaminase), whereas slicing of the organ into thin tissue disks and incubation of these slices in a moist atmosphere induces a rapid decay of enzyme action [4, 5]. The mechanism of this rapidly occurring disappearance of enzyme activity (here described as "enzyme degradation" preliminarily) is unknown, but can be studied in this plant tissue. Some of the more general properties of this system are introduced by this paper.

2. Materials and methods

The plant material (tubers of *Solanum tuberosum* L., cv. "Hansa") and its treatment up to the freeze-drying process has been reported in detail and extraction and measurement of phosphoglucomutase (EC 2.7.5.1.) activity was essentially as described [4].

Polyacrylamide gel electrophoresis was performed according to Stegemann [6]. The extract, dialyzed overnight against 0.03 M Tris-HCl, pH 7.5; 0.0027 M EDTA; 0.01 M $MgCl_2$ and 30% glycerol, was mixed with a small amount of bromophenol blue and layered as 50–100 µl portions into the slots of polyacrylamide gel slabs of 1 or 3 mm thickness. Cyanogum-41 (Serva, Heidelberg) or a mixture of acrylamide and *N,N'*-methylene-bisacrylamide in 0.125 M Tris-borate-buffer, pH 8.9 was used for electrophoresis. Gel formation was catalyzed by *N,N,N',N'*-

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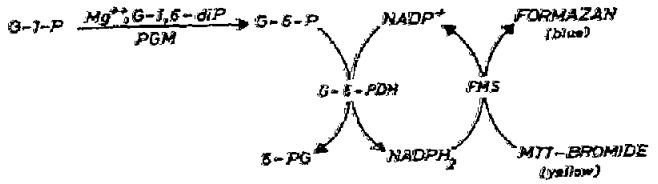

Scheme 1.

tetramethylenediamine (Serva, Heidelberg) together with $(NH_4)_2S_2O_8$ and sodium sulfite [7].

Optimal electrophoretical separation of the phosphoglucomutase isozymes from each other and from the interfering glucose-6-phosphate-dehydrogenase (G-6-PDH) and 6-phosphogluconate-dehydrogenase (6-PGDH) isozymes was achieved in 3 hr at 0° and 100 mA/slab in 5% Cyanogum gel. After the run the gels were preincubated in ice-cold 0.03 M Tris-HCl, pH 8.0 together with 0.01 M $MgCl_2$.

Phosphoglucomutase activity on the slabs was detected after incubation of the gel in a mixture of 4.6 mM glucose-1-phosphate (G-1-P), 0.05 mM glucose-1,6-diphosphate (G-1,6-diP), 10 mM $MgCl_2$, 0.12 mM $NADP^+$, 0.1 mg/ml phenacine methosulphate (PMS), 0.1 mg/ml 3 (4,5-dimethylthiazolyl-2-) 2,5-diphenyl-tetrazolium bromide (MTT-bromide, Serva, Heidelberg) and 1 μg/ml G-6-PDH (Boehringer, Mannheim) in 0.03 M Tris-buffer pH 8.0 according to the scheme:

This reaction is complete after 1–2 hr in the dark and at 30°. The formazane blue is deposited at the sites of its reduction as tiny granules.

G-6-PDH and 6-PGDH activity on the gel was detected by substituting G-1-P and G-1,6-diP by 1.5 mg/ml glucose-6-phosphate and 1 mg/ml 6-phosphogluconate, respectively.

After staining the bands of the PGM isozymes were documented immediately, since light induced colorization of the MTT-gels together with diffusion of the formazane caused disappearance of the stained zones. For conservation the gels were either fixed in 7% trichloroacetic acid or 7% acetic acid or sealed in black plastic bags and stored in the refrigerator.

3. Results and discussion

Slicing of resting potato tuber tissue enhances the decay of phosphoglucomutase activity, although

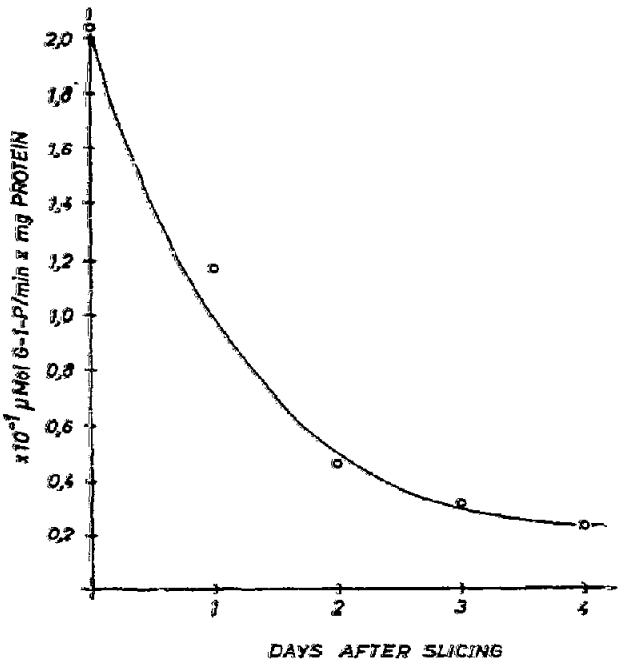

Fig. 1. Decay of phosphoglucomutase activity after slicing white potato tuber tissue.

stimulation of glycolysis occurs simultaneously [4]. After one day of incubation of the tissue disks in moist atmosphere at 25° about 50% of the initial activity is lost from the tissue and after another three days only about 20% is left (fig. 1). This decrease in activity is prevented by inhibitors of translation, added immediately after slicing the tissue. Thus amino acid analogues (100 μg/ml) and cycloheximide (20 μg/ml) more or less blocked the fall in PGM activity as did exposure of the tissue to a nitrogen atmosphere (fig. 2). As in other systems [3] protein synthesis supposedly is a prerequisite for enzyme degradation in potato tuber slices.

It is well established that the cells of a variety of higher organisms possess more than one molecular form of phosphoglucomutase. Joshi et al. [8] found two PGM-isozymes in sweet potato roots (*Ipomoea batatas*) and white potato tissue (*Solanum tuberosum* L.), differing in their elution behaviour from a DEAE-Sephadex column. In view of the results reported here, it was of interest to know if the decay of PGM concerns only one or possibly several existing isozymes. For this purpose the separation and staining techniques had to be elaborated.

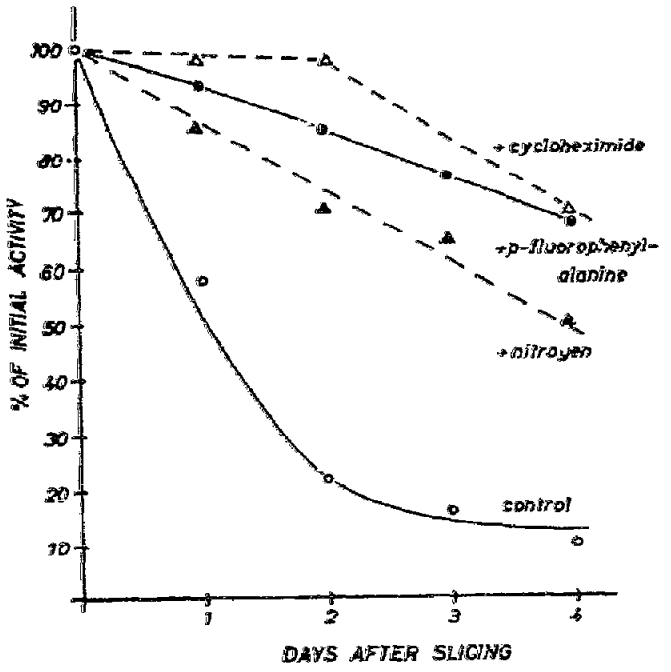

Fig. 2. Inhibition of the slicing-induced degradation of phosphoglucomutase by cycloheximide (20 μg/ml), *p*-fluorophenylalanine (100 μg/ml) and a nitrogen atmosphere.

A variety of tetrazolium salts as electron acceptors was examined for their properties in the staining reaction (table 1). Most of the salts were not reduced at all by potato PGM, but unspecifically stained the gels throughout. Best results were obtained with 3(4,5-dimethylthiazolyl-2-) 2,5-diphenyltetrazolium bromide (MTT, Serva, Heidelberg). Although the PGM isozymes could be detected on the gel with tetranitroblue-tetrazolium chloride, 2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) and *p*-nitroblue-tetrazolium chloride (Nitro BT), the intensity of reduction was only weak and the gels were coloured throughout after short exposure to light. MTT-bromide was used for staining of the potato PGM isozymes.

The reduction of the dye has been proven to be due to PGM-action (table 2). If the substrate or coenzyme is omitted from the incubation mixture, no reaction occurred. Exclusion of G-1,6-diP resulted in appearance of only one isozyme, faint reaction was possible without PMS. If the gels were developed without added G-6-PDH, all three isozyme bands

Table 1

Various tetrazolium salts as electron acceptors in the phosphoglucomutase reaction.

Tetrazolium salt	Staining reaction
Tetranitroblue-tetrazolium chloride	Reduced by all three isozymes to black bands on the gel
"Nitro BT"; *p*-nitroblue tetrazolium chloride	Faint blue reaction with all three isozymes
Neotetrazolium chloride	No reaction
"Tetrazolium violet"; 2,5-diphenyl-3-naphthyl-2,4-tetrazolium chloride	No reaction
"TTC"; 2,3,5-triphenyltetrazolium chloride	No reaction
"Tetrazolblue"; 3,3'-dianisyl-bis-4,4'-(3,4-diphenyl)-tetrazolium chloride	No reaction
"Tetrazolium red"; 2,5-diphenyl-3-*o*-tolyl-2,4-tetrazolium chloride	No reaction
"Tetrazolpurpur ; 4,4'-bis (3,5-diphenyl-2-tetrazolium) biphenyldichloride.	No reaction
"INT"; 2 (*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride	Reduced by all three isozymes to red bands on the gel
"MTT" 3 (4,5-dimethylthiazolyl-2-) 2,5-diphenyltetrazolium bromide	Reduced by all three isozymes to blue bands on the gel. Fast and strong reaction.
"BTC"; 3,3'-dianisol-4,4'-bis-(2,5-diphenyl)-tetrazolium chloride	No reaction

Reaction conditions: concentrations of the tetrazolium salts were 2.5 mg/ml incubation mixture. Incubation of the gels in normal reaction medium at 30° for 3 hr. Phosphoglucomutase was extracted from intact tubers.

appeared due to action of endogenous G-6-PDH. Since interference with this enzyme and 6-PGDH would give misleading results, the three enzymes were separated from each other electrophoretically in a 5% polyacrylamide gel (fig. 3).

Optimal test conditions allowed the separation of three PGM-isozymes (I, II, III). In the intact potato tuber all three isozymes are extremely active. Slicing,

Table 2
Properties of the phosphoglucomutase-catalyzed staining reaction.

Reaction system	Staining reaction
Complete	Appearance of PGM I, II, III.
Without added G-6-PDH	Faint bands of PGM I, II, III.
Without G-1,6-diP	Only faint band of PGM I.
Without G-1-P	No reaction
Without $NADP^+$	No reaction
Without MTT-bromide	No reaction
Without PMS	Only faint bands of PGM I, II, III.
Boiled extract, otherwise complete reaction medium	No reaction

however, induces the gradual disappearance of all isozymes, so that only faint bands of PGM I and PGM II are visible 4 days after slicing the organ (fig. 4). PGM III disappears from the tissue or its activity (concentration) drops to very low levels, so that it cannot be detected with the methods used.

The slicing-induced degradation thus is concerning all three phosphoglucomutase isozymes to a comparable degree.

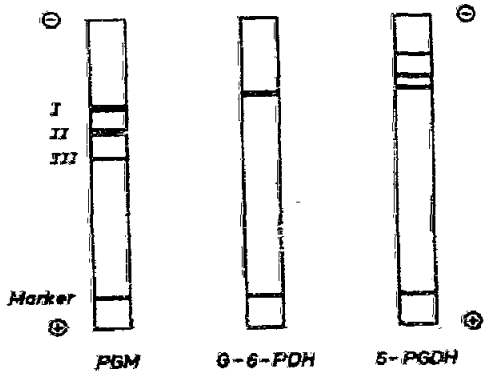

Fig. 3. Separation of phosphoglucomutase isozymes I, II and III from glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and the three 6-phosphogluconate dehydrogenases (EC 1.1.1.44) of potato tuber tissue.

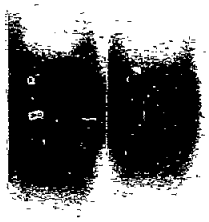

Fig. 4. Phosphoglucomutase isozyme pattern in white potato tuber tissue after slicing. Left to right: Intact tuber, tissue slices after 1, 2, 3 and 4 days of incubation in a moist atmosphere. Experimental conditions as in Materials and methods.

Acknowledgements

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