

# **De novo Synthesis of Glucose-6-Phosphate- (E.C. 1.1.1.49) and 6-Phosphogluconate Dehydrogenase (E.C. 1.1.1.44) in Plant Storage Tissue Slices**

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Resting potato tuber tissue possesses only faint activity of the two dehydrogenases of the oxidative pentose phosphate cycle, glucose-6-phosphate- and 6-phosphogluconate dehydrogenase. Slicing of the tissue, however, greatly enhances the action of both enzymes. The slicing-induced increase in activity is a consequence of intensified action of at least 5 glucose-6-phosphate dehydrogenase isozymes and a more differentiated activation/inactivation of seven 6-phosphogluconate dehydrogenase isozymes.

Using density labelling and isopycnic equilibrium centrifugation it could be demonstrated, that the bulk of both enzymes appearing after slicing the tissue is the result of *de novo* synthesis rather than activation of pre-existing proenzymes.

Slicing of dormant plant storage organs such as potato tubers initiates large-scale alterations in genetic and metabolic activities of the tissue. These activities are quantitatively as well as qualitatively different from those of the dormant cells (for review see<sup>1,2</sup>). During the slicing-induced activation of cellular metabolism a great number of enzymes enhances activity, but some enzymes are inactivated or degraded simultaneously<sup>3</sup>.

If this phenomenon is interpreted correctly, then the harmonious activation of some and degradation of other enzymes is essential for a balanced cellular metabolism. Both processes then are of fundamental importance for our understanding of metabolic regulation. Accordingly, the exact mechanism of activation and inactivation/degradation of enzymes in the plant storage tissue slice system and other plants systems as well should be evaluated.

The enhancement of activity of glucose-6-phosphate- and 6-phosphogluconate dehydrogenase in white potato tuber slices as such can be prevented by inhibitors of protein synthesis<sup>4</sup>, which is already a vague indication that the enzymes are synthesized as a consequence of slicing. Unequivocal proof for such a *de novo* synthesis through density labelling is presented in this paper.

## **Material and Methods**

### *Material*

Tubers of *Solanum tuberosum* L., cv. "Saskia" of equal size were coarsely peeled and washed thoroughly. The pith tissue was chopped, washed with sterile water and incubated in moistened desiccators for different periods of time. Fixation of the tissue in liquid nitrogen and its lyophilization has been described<sup>4</sup>.

### *Methods*

Extraction and measurement of glucose-6-phosphate- and 6-phosphogluconate dehydrogenase are essentially as reported in detail<sup>4</sup>.

### *Gel electrophoresis*

For electrophoresis the crude extracts were centrifuged at  $24\,000 \times g$  for 30 min and then dialyzed overnight against a 1000 fold volume of 0.12 M Tris-HCl, pH = 8.0, containing  $3 \cdot 10^{-4}$  M EDTA,  $2 \cdot 10^{-3}$  M cysteine and 30% glycerol. Aliquots of this enzyme solution, adjusted to a protein content of 50  $\mu$ g (G-6-PDH) or 80  $\mu$ g (6-PGDH) were layered on 7.3% polyacrylamide gels (85  $\times$  5 mm) in a Shandon analytical column electrophoresis unit, Mk. I (SAE 2710) equipped with a Vokam power supply (300 V/80 mA). The gel concentration used

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*Abbreviations:* G-6-PDH (E.C. 1.1.1.49), Glucose-6-phosphate dehydrogenase; 6-PGDH (E.C. 1.1.1.44), gluconate-6-phosphate dehydrogenase; LDH (E.C. 1.1.1.28), lactate dehydrogenase.

proved to be most suitable for the separation of the dehydrogenase isozymes, which was achieved in  $4.9 \cdot 10^{-3}$  M Tris-glycine-buffer, pH = 8.3 according to Maurer<sup>5</sup> and complete after 2 hours at 3 mA per tube and at 4 °C.

After the run the gels were incubated in a staining solution containing 5 mM glucose-6-phosphate or 3 mM 6-phosphogluconate respectively, 10 mM MgCl<sub>2</sub>, 0.12 mM NADP<sup>+</sup>, 0.1 mg/ml phenacine methosulfate (PMS) and 0.1 mg/ml 3(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT-bromide, Serva, Heidelberg) in 0.12 M Tris-EDTA-cysteine-buffer, pH = 8.0<sup>3</sup>. After 30–60 min staining of the isozyme bands was complete and documented immediately. The two dehydrogenases do not interfere with each other in the staining reaction. Even prolonged staining of the G-6-PDH gels, which should result in accumulation of 6-phosphogluconate within the gels did not give rise to bands characteristic for 6-PGDH.

#### Density labelling experiments<sup>19</sup>

For these experiments the chopped tuber tissue was divided into two portions. One portion was incubated in a certain volume of H<sub>2</sub>O, the other one in 99.8% D<sub>2</sub>O (Serva, Heidelberg) according to the procedure of Sacher *et al.*<sup>6</sup>. Bathing the tissue fragments for 30 min in both solutions was followed by blotting and a 28-hours-incubation in petri dishes and water respectively 99.8% D<sub>2</sub>O. Sacher and co-workers reported that this method yields an endogenous concentration of 75% D<sub>2</sub>O, which in our experiments inhibited the development of enzyme activity up to 30–40% of the water control. After this period the enzymes were extracted with Tris-EDTA-cysteine-buffer, pH = 8.0, the extract centrifuged at 48 000 × *g* and 4 °C and aliquots layered on top of a CsCl gradient.

#### Density gradient ultracentrifugation

The procedure of Brunk and Leick<sup>7</sup> and Schopfer and Hock<sup>8</sup> was followed with minor modifications. The gradients consisted of a lower layer with 2 ml of CsCl solution (0.825 g/ml) and an upper part with 2 ml of CsCl solution containing 0.22 g/ml. On top of this step gradient 0.3–0.5 ml of enzyme solution was layered and sealed with another 0.5 ml of liquid paraffin. As a buoyant density marker enzyme 20 µg of lactate dehydrogenase (Sigma) was used, the activity profiles for the LDH marker from labelled and unlabelled gradients were superimposed and drawn as one.

The tubes were centrifuged at 160 000 × *g* in a Spinco SW 50.1 rotor for 55–60 hours at 4 °C.

The gradient was fractionated into one-drop-fractions (20 µl). To each of these fractions 2 ml of Tris-EDTA-cysteine-buffer (pH = 8.0) were added and enzyme activity recorded immediately.

Preliminary experiments revealed that CsCl even in maximal concentrations, which might be present in the assay of gradient fractions (200 mM) did not inhibit glucose-6-phosphate dehydrogenase more than 20% and 6-phosphogluconate dehydrogenase activity more than 35% of the control (Table I).

Table I. Inhibition of glucose-6-phosphate- and 6-phosphogluconate dehydrogenase activity by CsCl.

CsCl [mM]	Enzyme activity [ΔOD <sub>340 nm</sub> · min <sup>-1</sup> · ml <sup>-1</sup> ]	
	G-6-PDH	6-PGDH
0	4.2	1.5
10	3.9	1.2
100	4.0	1.1
200	3.5	1.0

After dilution of the density gradient fractions with 2 ml buffer the concentration of CsCl in the test was too low to inhibit the dehydrogenases at all.

Storage of extracts in 0.1 M CsCl for 3 days in the cold resulted in the loss of about 11% of G-6-PDH and 6% of 6-PGDH activity as compared with the initial activity. These conditions were identical with those employed during equilibrium sedimentation of both enzymes. CsCl-free controls had the same residual activity.

The refractive index of every tenth fraction was determined on a Bausch and Lomb Abbe-32 refractometer.

## Results

Slicing of resting storage tissues enhances the activity of glucose-6-phosphate- and 6-phosphogluconate dehydrogenase, both prominent enzymes of the oxidative pentose phosphate shunt (Fig. 1). This has been shown to be true for white potato tuber<sup>4, 6</sup>, carrot, chicory and swede<sup>6, 11</sup>, sweet potato<sup>12</sup> and red beet tissue<sup>10, 13</sup> and can be taken as indication of the induction phenomenon in plant storage tissue slices generally.

The time-course of enzyme activity does not mean too much, if not based on data of the activity pattern of the corresponding isozymes. Separation of the bulk of enzyme activity by means of polyacrylamide gel electrophoresis revealed the presence of at least 5 isozymes of glucose-6-phosphate dehydrogenase and at least 7 isozymes of 6-phosphogluco-

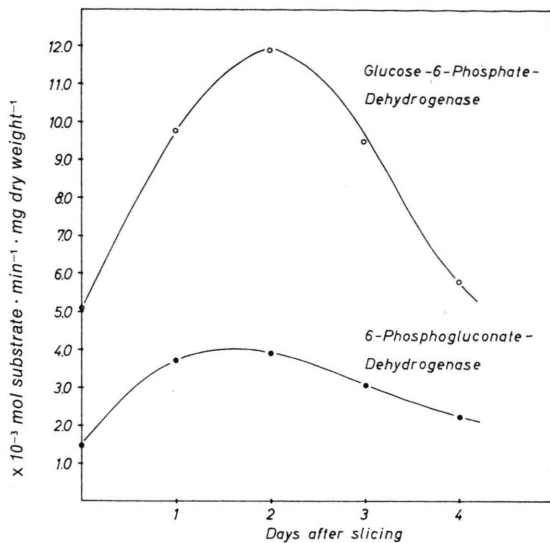


Fig. 1. Glucose-6-phosphate- and 6-phosphogluconate dehydrogenase activity in slices of white potato tuber tissue.

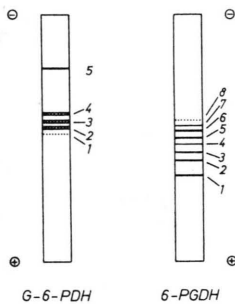


Fig. 2. Glucose-6-phosphate- and 6-phosphogluconate dehydrogenase isozymes in white potato tubers.

nate dehydrogenase within the intact storage tissue (Fig. 2). This result is a consequence of improved methods, since separation of the isozymes in tris-borate-buffer, pH = 8.9 and 5% polyacrylamide gels, as used previously, has been shown to be inadequate, yielding one G-6-PDH enzyme band and three

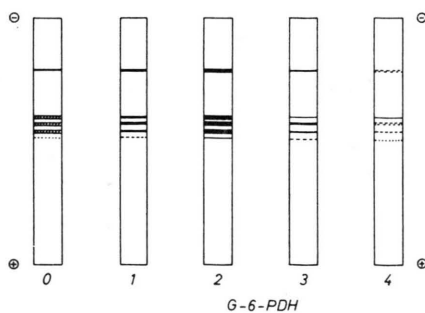


Fig. 3. Isozyme pattern of glucose-6-phosphate dehydrogenase in slices of white potato tuber tissue. 0, intact tuber; 1–4, 1–4 days after slicing.

6-PGDH isozyme bands only<sup>3</sup>. Slicing of the tissue does not alter the pattern, but increases the intensity of all 5 isozyme activity bands of G-6-PDH (Fig. 3). As a consequence of activation of all molecular variants of this enzyme, the overall activity enhances simultaneously (Fig. 1).

The pattern of 6-PGDH isozymes does not respond as uniformly to slicing. Isozyme I, III, V, VI, VII and VIII considerably increase in activity after slicing the tissue and begin to decrease their activity after 3–4 days. These isozymes determine the extent of overall activation of this enzyme (Fig. 1). Isozymes II and IV, however, lose activity and cannot be detected with certainty 24–48 hours after slicing of the tissue (Fig. 4). Generally the isozyme

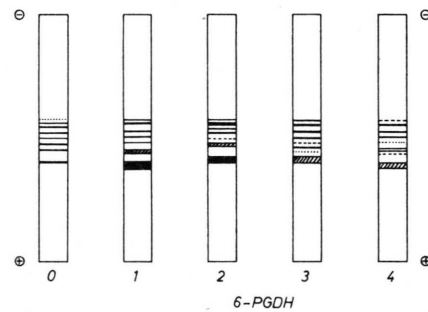


Fig. 4. Isozyme pattern of 6-phosphogluconate dehydrogenase in slices of white potato tuber tissue. 0, intact tuber; 1–4, 1–4 days after slicing.

pattern of 6-PGDH is more differentiated than that of G-6-PDH.

Inhibitors of transcription and protein synthesis prevent the increases of both dehydrogenases after slicing the storage tissues<sup>4</sup>. This is a rather vague indication, but no proof for *de novo* synthesis of these enzymes. However, density labelling experiments clearly indicate, that the bulk of G-6-PDH and 6-PGDH is synthesized *de novo* after slicing the tissue (Figs 5 and 6). The buoyant density of both enzymes increases as a result of the treatment with D<sub>2</sub>O and there is no reason to believe that this density shift is the result of deuteration of a possible carbohydrate moiety of the enzymes<sup>14, 15</sup>.

In most experiments the shift in buoyant density was less clear-cut in case of the 6-phosphogluconate dehydrogenase as compared with the deuterated glucose-6-phosphate dehydrogenase. Mixing homogenates derived from water and D<sub>2</sub>O-incubated tissues — a procedure which eliminates any variation between tubes of a single centrifuge run — resulted

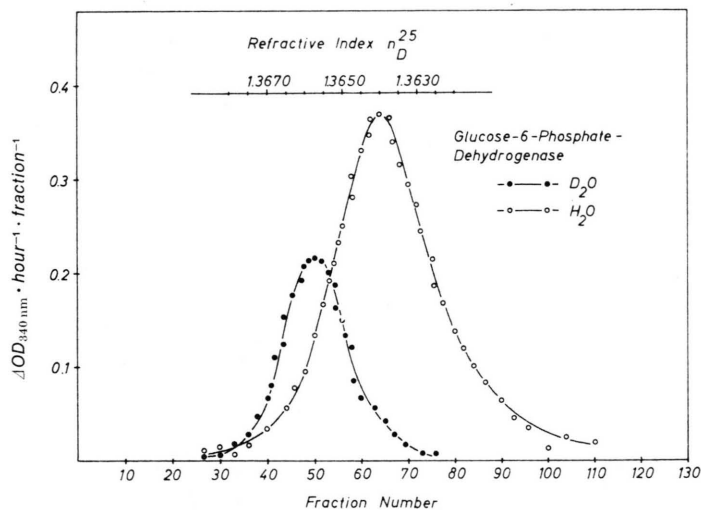


Fig. 5. Equilibrium distribution in CsCl gradients of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) of white potato tuber slices incubated in  $D_2O$  respectively  $H_2O$  for 28 hours.

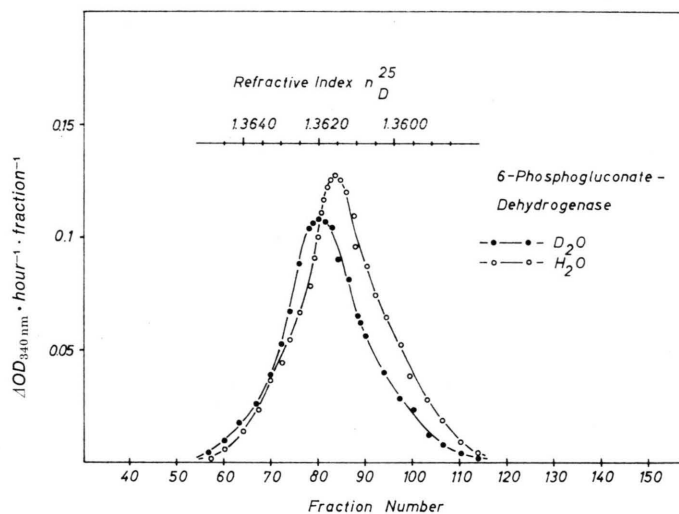


Fig. 6. Equilibrium distribution in CsCl gradients of 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) of white potato tuber slices incubated in  $D_2O$  or  $H_2O$  respectively for 28 hours.

in a broadened activity distribution in the gradient. This is taken as indication of inhomogeneity of the enzyme population. The actual reason for the weak density labelling of 6-PGDH is unknown. Possibly in this case the small change in buoyant density does not represent an exact index for the actual increase in enzyme mass<sup>16</sup>.

### Discussion

Slicing of resting plant storage tissues, especially white potato tubers lead to the transition of the differentiated storage cell to a cell with mitotic capabilities (for review see<sup>1,2</sup>). Concomitant with this cellular differentiation and subsequent histological

development a molecular differentiation takes place. The differentiated cell alters its isoenzyme complement and consequently its enzyme activities in a highly sophisticated manner. On one hand the different molecular variants of an enzyme may be activated throughout or activated differentially, on the other they may be inactivated throughout or inactivated differentially. An example of the first type is glucose-6-phosphate dehydrogenase, the 5 isozymes of which respond to slicing of quiescent tissue with a rapid increase in activity (Fig. 3). The second type is represented by phosphoglucoisomerase (E.C. 5.3.1.9) of potato tuber slices (Kahl, unpublished data), inactivation occurs with all three phosphoglucomutase isozymes (E.C. 2.7.5.1) in the

same tissue<sup>3</sup>. 6-phosphogluconate dehydrogenase is representative for the last type: Slicing induces the activation of 5 and temporal disappearance of 2 of seven isozymes (Fig. 4). However, it is difficult to interpret changes in isozyme patterns, especially if very complicate in detail (see Fig. 4), since aggregation-disaggregation processes, limited proteolytic attack of single polypeptide chains, conjugation with other proteins and various chemical modifications of the different isozymes (*i. e.* simple binding of substrate, coenzyme or other effectors of low molecular weight) during the extraction procedure may well be responsible for observed changes<sup>20</sup>.

It now seems that all variations at the molecular level following slicing are subject to transcriptional and translational control. Whereas inhibitor studies already stressed the importance of both these fundamental processes after slicing, unequivocal evidence

for, say, enzyme induction as *de novo* synthesis came from incorporation studies and density labelling experiments only. Thus it can be taken as proved that the activity rise of peroxidase in sweet potato tissue slices is a result of *de novo* synthesis of the enzyme<sup>17, 18</sup>. The enhancement of activity of phenylalanine ammonia-lyase (E.C. 4.3.1.5)<sup>6</sup> and both dehydrogenases of the pentose shunt in potato tuber slices clearly is a consequence of the appearance of newly built enzymes as judged from density labelling (Figs 5 and 6).

Induction then is well established as a consequence of slicing quiescent plant storage tissue, the mechanism of disappearance of isozymes, however, remaining as a process still scarcely understood.

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