Glucose-6-phosphate dehydrogenase/6-phosphogluconate dehydrogenase ratio and the glucose-6-phosphate, 6-phosphogluconate and fructose-6-phosphate contents in tobacco plants infected with potato virus Y

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Abstract

The ratio of activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (G6P DH/6PG DH), and the contents of glucose-6-phosphate (G6P), 6-phosphogluconate (6PG) and fructose-6-phosphate (F6P) were studied at various stages of potato virus Y (PVY) multiplication in Nicotiana tabacum cv. Samsun. G6P DH/6PG DH increased through the experiment from 0.42 to 0.53 in leaves of healthy tobacco, and up to 0.59 in PVY systemically infected leaves. However, these ratios in the ruptured protoplast preparations, and the chloroplast and cytosol fractions of healthy protoplasts were similar to that from infected ones. The ratio lower than 1, found in the healthy and/or PVY-infected leaf tissues and in the infected protoplasts as well, confirms the assumption that G6P DH is the control enzyme of oxidative pentosephosphate pathway not only in the healthy but also in the infected plants. The contents of G6P, 6PG and F6P in the period of the highest PVY multiplication were strongly decreased (to 30 - 50 % when compared with control healthy leaves) and were negatively correlated with the G6P DH and 6PG DH activities.

Additional key words: protoplast, chloroplast, cytosol, regulation, oxidative pentosephosphate pathway, Nicotiana tabacum.

Introduction

It has long been recognized that the plant has the capacity to metabolize free, storage and transport saccharides through the pentose phosphate cycle, the pathway representing a functionally significant alternative route for saccharides utilization. The oxidative pentose phosphate pathway metabolizes glucose-6-phosphate to ribose-5-phosphate necessary for the de novo biosynthesis of purine and pyrimidine nucleotides of viral RNA. Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP$^+$ oxidoreductase, EC 1.1.1.49; G6P DH) and 6-phosphogluconate dehydrogenase (6-phospho-D-glucurate: NADP$^+$ oxidoreductase, EC 1.1.1.44; 6PG DH) play an important role in the regulation of the flux through this pathway at the level of its first enzyme G6P DH. This together with 6-phosphogluconolactonase hydrolysing 6-phospho-D-glucurate lactone to 6-phospho-D-glucurate forms a suitable irreversible enzyme system (Turner and Turner 1980). The physiological significance of this enzymatic regulation during virus-RNA replication is given by the variable requirements of NADPH necessary for pentoses essential for nucleotide synthesis. G6P DH and 6PG DH catalyse the two-stage, NADP$^+$ dependent, oxidation of glucose-6-phosphate, thereby constituting the direct oxidative pathway of saccharide metabolism.

G6P DH and 6PG DH are present in plant tissues in the form of two main isozyme complexes, one of which is located in chloroplasts and the other in cytosol (Eichhorn and Corbus 1988, Wendt et al. 2000). Both isozymes show similar properties: nearly the same pH optima, relative molecular mass, kinetic constants $K_M$ and $V_{max}$, etc. (Schnarrenberger et al. 1973). Mechanisms of their coarse and fine regulation by some intermediates (NADP$^+$, NADPH, AMP, ADP, erythrose-4-phosphate, phosphoenolpyruvate) and the effect of light (inhibition) or darkness (activation) are partially known (Heber et al.

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Abbreviations: F6P - fructose-6-phosphate; G6P - glucose-6-phosphate; G6P DH - glucose-6-phosphate dehydrogenase; 6PG - 6-phosphogluconate; 6PG DH - 6-phosphogluconate dehydrogenase; p.i. - post inoculation; PVY - potato virus Y.
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Materials and methods

Plant cultivation and virus inoculation: Two-month-old tobacco (Nicotiana tabacum L. cv. Samsun) plants grown under constant conditions in Hoagland No. 3 nutrient solution, at an irradiance of 100 μmol m⁻² s⁻¹ (16-h photoperiod) and average temperature of 25 °C, were used in experiments. The lower leaf, approximately 5 cm in length, was mechanically inoculated with purified potato virus Y (necrotic strain of PVY, Leiser and Richter 1978) at a concentration of 100 μg cm⁻²; the corresponding leaf of control plant was mock-inoculated with distilled water. The day of inoculation was day zero (0 d.p.i.).

Preparation of homogenate: Crude homogenate was prepared from the systemically infected and/or corresponding control leaves by grinding in a mortar with fine silica sand, 10 % (m/m) insoluble polyvinyl-pyrrolidone and TEMM buffer (20 mM Tris-HCl buffer, 1 mM EDTA, 2.5 mM MgCl₂, 30 mM 2-mercapto-ethanol, pH 7.0) in a ratio of 1:5 (m/v). The resulting homogenate was squeezed through Miracloth and nylon sieve 100 mesh and centrifuged for 10 min at 20,000 g.

Preparation of protoplasts and the fractionation of cell organelles: Protoplasts were prepared 7 d.p.i. according to Šindelářová and Šindelář (1994). Their disintegration and subcellular fractionation was performed according the methods described in Šindelář et al. (1999). Ruptured protoplasts were named the crude homogenate S₀. The pellet (P₁) containing the substantial part of chloroplasts was obtained by 5 min centrifugation at 1,000 g and was resuspended in TEMM medium. Supernatant was centrifuged for 15 min at 15,000 g and the supernatant (S₁₅) was used in experiments. The fractions were used for estimation of total protein content, chlorophyll content and NAD⁺-triosephosphate dehydrogenase (markers of chloroplasts), phosphoenolpyruvate carboxylase (marker of cytosol), G6P DH, and 6PG DH activities. All steps of these procedures were carried out at a temperature from 0 to 4 °C. Under these conditions the activities of the enzymes did not change for more than 5 h.

The number of protoplasts was determined in a haemocytometer and the number of viable protoplasts by staining with methylene blue according to Hooley and McCarthy (1980). The percentage of infected protoplasts was determined by an immunoenzymatic method according to Šindelář and Šindelářová (1994).

Determination of PVY content: PVY content was determined by the quantitative DAS-ELISA method (Clark and Adams 1977) using rabbit anti-PVY antibodies and alkaline phosphatase labeled antibodies prepared from our isolates of PVY (necrotic strain).

Determination of chlorophyll, G6P, F6P and 6PG content and enzyme activities: Chlorophyll content was determined according to Arnon (1949).

D-glucose-6-phosphate and D-fructose-6-phosphate were determined by using glucose-6-phosphate dehydrogenase and phosphoglucoisomerase (Pohorst 1963a) and D-6-phosphogluconate by using 6-phosphogluconate dehydrogenase (Pohorst 1963b) after extraction of these metabolites by 0.6 M HClO₄.

G6P DH (EC 1.1.1.49) and 6PG DH (EC 1.1.1.44) activities were determined spectrophotometrically, NADPH generation was monitored at 340 nm (Šindelář 1986, Šindelář and Šindelářová 1987a). Phosphoenolpyruvate carboxylase activity (EC 4.1.1.31) was determined according to Downton and Slatyer (1971), NAD⁺-triosephosphate dehydrogenase activity (EC 1.2.1.9) according to Heber et al. (1963).

Statistical treatment: The results are presented as arithmetical means ± standard deviation of the mean of 3 to 7 determinations in 3 independent experiments. The t-test was employed to characterise the differences.

Chemicals: Protoplasts releasing enzymes were obtained from Serva (Heidelberg, Germany), alkaline phosphatase from Boehringer (Heisenhofen, Germany) and all other fine biochemicals were purchased from Sigma Chemical Company (St. Louis, USA).