Changes in Ribonuclease and Glucose-6-Phosphate Dehydrogenase Activities during PVY-RNA Biosynthesis in Potato Leaf Discs

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Abstract. Changes in fresh matter content, protein content, chlorophyll content, ribonuclease activity, and glucose-6-phosphate dehydrogenase activity, associated with potato Y-virus multiplication (common strain, PVY ordinary) were studied in discs cut from potato leaves. The results obtained showed that marked decreases in disc fresh matter, in protein content, and in chlorophyll content occurred during a 5-day-long cultivation period. The activity of glucose-6-phosphate dehydrogenase, that is of the rate limiting enzyme of the pentose phosphate pathway, and the activity of ribonucleases which characterize the rate and intensity of host RNA degradation were markedly enhanced in this period. The fact that activity curves of both these enzymes were in linear relationship with the PVY reproduction curve indicates that not only nucleotides produced in the reactions of the oxidative pentose phosphate pathway but also nucleotides released in the process of host RNA degradation were the main source of nucleotides necessary for PVY-RNA biosynthesis, in spite of a high photosynthetic rate.

Virus RNA can be synthesized in an infected host cell both from intermediates of the reductive pentose phosphate pathway during photosynthesis and from intermediates of the oxidative pentose phosphate pathway (mainly in the dark), and also from nucleotides released from degraded host RNA. All these three metabolic pathways are involved in virus RNA biosynthesis, but some of them are usually preferred in dependence on the virus, host, and environmental conditions.

The biosynthesis of virus RNA via the reductive pentose phosphate pathway is limited by the photosynthetic rate. A virus disease causes a marked reduction in photosynthetic activity in leaf tissues as soon as by day two following the inoculation which is associated with reduced size and number of chloroplasts, reduced chlorophyll content, and low efficiency of CO₂ fixation in the chloroplasts (for example HAMPTON et al. 1966, JENSEN 1968, TÜ et al. 1968). Thus it seems that the involvement of this metabolic pathway in virus RNA biosynthesis is low even immediately after the inoculation.

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The oxidative pentose phosphate pathway is more advantageous for virus RNA biosynthesis from the physiological viewpoint, because both free and storage carbohydrates can be used for the synthesis of ribose-5-phosphate necessary for the biosynthesis of purine and pyrimidine nucleotides serving for the biosynthesis of virus RNA. Data indicating permanent utilization of this metabolic pathway were reported by a number of authors, who also found lower sugar contents (e.g. ORLOB and ARNY 1961, ŠINDELÁŘ 1975, MAKOVCOVÁ et al. 1980), and lower starch content (DOKE andHIRAI 1969, ŠINDELÁŘ et al. 1980) in infected tissues. Increased activity of glucose-6-phosphate dehydrogenase, a rate-limiting enzyme of this metabolic pathway, was reported by many authors (e.g. TtEN and TANG 1963, MERRETT and SUnderLAND 1967, HUTH 1973), and the results obtained in our earlier studies also confirmed such increased activity (e.g. ŠINDELÁŘ 1975, MAKOVCOVÁ and ŠINDELÁŘ 1981, ŠINDELÁŘ 1986). However, a final response to this question can be obtained only after complex studies of the compartmentation and regulation of enzymes of the oxidative pentose phosphate pathway.

The pathway of the degradation of host RNA by ribonucleases, in which nucleotides necessary for virus RNA biosynthesis are released, is a universal pathway. However, it seems that this pathway is triggered only when the sources of nucleotides formed de novo by the reductive and oxidative pentose phosphate pathways are insufficient for the synthesis of a high virus content (as in the case of the tobacco mosaic virus), or for a very fast virus RNA synthesis. Such a situation was reported by REDDI (1963), CHEO (1971), and ŠINDELÁROVÁ et al. (1988), who found that TMV was synthesized mainly from nucleotides released from host RNA.

The biosynthesis of virus RNA is a complex process in which obviously differentiated utilization not only of the above three metabolic pathways occurs but in which the utilization of the pool of free nucleotides and other intermediates is also involved (ŠINDELÁŘ 1984). For this reason, using the model of discs cut from potato leaves infected with potato virus Y, we tried to study the origin of the sources of intermediates utilized for the biosynthesis of virus RNA as a complex of the oxidative pentose phosphate pathway and the host RNA degradation pathway.

**MATERIAL AND METHODS**

Experimental *Solanum tuberosum* cv. Resy plants were grown in soil in a glasshouse. Three detached leaves of medium insertion and of equal size (plant age 40 days) were mechanically inoculated with sap prepared by grinding leaf tissues of *Nicotiana tabacum* L. cv. Samsun plants infected with potato virus Y (common strain, PVY ordinary, received from Dr. Nohejl from the Potato Research and Breeding Institute at Havířkov Brod), diluted 1 : 2 with water. Discs, 1 cm in diameter, were cut from these inoculated leaves, rinsed with water, and cultivated on the surface of a half-strength Knop’s nutrient solution. Corresponding discs of control healthy plants were prepared similarly, but infectious leaf sap was replaced.