MECHANISMS AND REGULATION OF MONOSACCHARIDE TRANSPORT IN SUSPENSION CULTURED CELLS OF VITIS VINIFERA

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INTRODUCTION

The grape (Vitis vinifera L.) is a productive plant considered as the world’s premier fruit, with nearly 9 million hectares of culture land in 1995. The ripening process is characterized by massive sugar import into the berries. Up to now, studies on sugar transport in V. vinifera have been carried out mainly using molecular biology approaches, and VvHT1 and VvHT2 genes associated with sugar accumulation were identified (Fitzot et al., 1995; Agasse et al., 2004).

Besides their role as carbon and energy sources, sugars can act as regulatory signals that affect expression levels of several genes controlling key processes and hence plant development. The ability to sense altered sugar concentrations is extremely important in the context of resource allocation, allowing the plant to tailor its metabolism in source tissues to meet the demands in sinks. In this work, V. vinifera heterotrophic suspension cultured cells were used as a model system to study VvHT1 expression and sugar transport into sink cells.

RESULTS

Growth in batch cultures with sucrose

Cell suspensions were cultivated in liquid minimal medium with sucrose as carbon and energy source (Fig. 1). Sucrose is hydrolysed extracellularly within 4 days and growth occurs along with glucose and fructose consumption.

Monosaccharide transport

Kinetics

Initial uptake rates of 0.02-50 mM D-[14C]glucose and L-[14C]glucose measured as described by Oliveira et al. (2002) are depicted in Figure 2. The application of a computer-assisted non-linear regression analysis (GraphPad software) to the data suggested the involvement of a saturable transport system associated with non-saturable ‘diffusion-like’ transport. The kinetic parameters were: V_{max} 0.05 ± 0.15 mM glucose and V_{min} 1.45 ± 0.44 nmol mg^{-1} min^{-1} d.w.; and for the diffusion-like component, A_{0} 0.09 ± 0.03 mg^{-1} min^{-1} d.w.

Specificity

D-glucose and D-fructose share the same monosaccharide carrier, which exhibits higher affinity for D-glucose (Fig. 3A and 3B), explaining why glucose is the first substrate to be consumed after sucrose hydrolysis (see Fig. 1). D-galactose and D-xylose are also substrates for the monosaccharide carrier. Sucrose, D-mannitol, and D-arabinose are not recognized (Fig. 3C). Glucose carrier is also able to accept 2-deoxy-D-glucose and 3-O-Me-D-glucose but not L-glucose (Fig. 3D).

Energetics

Data from energetic studies on glucose transport in V. vinifera are presented in Figure 4. Results are consistent with the involvement of a glucose/H+ symporter with a stoichiometry of 1 glucose:1 H+.

Sugar regulation of monosaccharide transport and VvHT1 expression

Monosaccharide transport activity and expression analysis of VvHT1 along sugar depletion in the medium are presented in Figure 5A and 5B. The parallel between VvHT1 transcripts and VvHT1 activity transport suggests that expression of the carrier is controlled at the transcriptional level, although other levels of regulation cannot be ruled out. VvHT2 transcripts were not detected, suggesting that it is not expressed in this cell-line model.

SUGAR TRANSPORT IN SUSPENSION CULTURED CELLS OF VITIS VINIFERA

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Glucose positivly regulates expression of VvHT1

The removal of glucose from 3 days old V. vinifera culture promoted VvHT1 transcription within 12 hours. However, the prolonged absence of glucose resulted in the decrease of VvHT1 transcription to levels lower than those detected in glucose repressing conditions (Fig. 5C). The addition of glucose to 48 h starved cells promoted the induction of VvHT1 transcription to levels observed in growing conditions. In cells treated either with glucose plus mannose plus hexokinase or 3-O-Me-D-glucose stronger accumulation of VvHT1 transcripts was observed, possibly as a result of HXK impairment in metabolizing the repression signal (Fig. 5D). This expression profile is in agreement with previous reported data from Almeida et al (2003).

Concluding remarks

- V. vinifera cultured cells are able to grow in mineral media with sucrose after the disaccharide had been hydrolysed by an extracellular invertase.
- Cells displayed activity for a monosaccharide symporter with a stoichiometry of 1 glucose:1 H+ and K_{m} 0.05 ± 0.03 mM, encoding VvHT1.
- Glucose repression activity and VvHT1 transcription mediated by a hexokinase signalling pathway.
- In addition to repression at high concentrations, glucose is required for induction of VvHT1 expression.

References