StCDPK1 is expressed in potato stolon tips and is induced by high sucrose concentration

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Abstract

StCDPK1 encodes a calcium-dependent protein kinase (CDPK) from Solanum tuberosum, which is transiently induced upon tuberization in swelling stolons. In situ hybridization determined that StCDPK1 mRNA is localized in the apical dome of tuberizing stolon tips, close to the region where sucrose was reported to accumulate. The expression of StCDPK1, and other tuber-specific genes was enhanced when in vitro-cultured potato plants were transferred to high sucrose or high sorbitol containing media. Glucose, fructose or a mixture of both showed no effect on CDPK expression. Okadaic acid blocked sucrose-inducible gene expression, suggesting that phosphatases from the PP1/PP2A family could also participate in the regulation of StCDPK1 and other tuberization-related genes.

Key words: Gene expression, okadaic acid, potato stolons, StCDPK1, sucrose.

Sugars are not only important energy sources and structural components, they are also central regulatory molecules controlling metabolism, the cell cycle, development, and gene expression (Sheen et al., 1999). It was suggested that sugars could act as morphogens providing positional information to the cell cycle machinery and different developmental programmes (Rolland et al., 2002).

Potato tuberization is an interesting system in which to study the sucrose regulation of gene expression. This process involves a switch in assimilate phloem unloading in the subapical region of the developing stolon. Sucrose is the most abundant sugar in swelling stolons, while glucose and fructose concentration remain lower and show a similar pattern at all developmental stages (Viola et al., 2001). In vitro tuber formation is dependent on sucrose concentration and microtuber production can be obtained without any addition of other growth regulators in the culture medium (Garner and Blake, 1989). Sucrose seems to be a specific signal since neither glucose nor fructose is as effective as sucrose in meeting the sugar requirement for tuber development (Ewing, 1987).

High sucrose concentrations induce the transcription of several genes involved in tuber storage metabolism (Salanoubat and...
Previously, it was suggested that a calcium-dependent protein kinase (CDPK) could be involved in the events leading to tuber formation (MacIntosh et al., 1996). Furthermore, StCDPK1, an active CDPK that is differentially expressed in swelling stolons was isolated (Raõces et al., 2001). It was then interesting to study if StCDPK1 could be a potential target of sucrose regulation.

The spatial distribution of StCDPK1 transcripts was analysed in thin stolons (Fig. 1A), induced stolons (Fig. 1B, C) and mature tubers, using in situ hybridization as described by Crespi et al. (1994). Purple staining, indicating a positive hybridization signal, was found in the shoot apical dome of swelling stolons (Fig. 1E) whereas no hybridization signal was observed in early stolons (Fig. 1D), larger induced stolons (Fig. 1F), mature tubers (data not shown) or in sections incubated with the sense StCDPK1 probe (negative control, data not shown). These results correlate with previous Northern analysis (Raõces et al., 2001) except for the larger induced stolons. The weak signal detected at this stage could suggest that the kinase transcripts are dispersed or that mRNA expression was already down-regulated, since StCDPK1 is completely absent in mature tubers.

The strong signal detected in induced stolons (Fig. 1E) was observed in the cells from the apical region (Fig. 1G, H) and faded towards the proximal portion of the developing tuber, which is adjacent to the attached stolon. A large amount of starch granules was observed in the parenchyma of the region that lacks StCDPK1 expression (Fig. 1I), suggesting that StCDPK1 transcript levels decline in the differentiated storage tissue.

StCDPK1 localization, close to the site where sucrose concentration accumulates upon tuber differentiation, prompted the authors to analyse whether sucrose was involved in the induction of StCDPK1 expression. In vitro-cultured adult plants were transferred to liquid media containing 2% sucrose (control), 8% sucrose or osmotically equal concentrations of sorbitol, glucose, fructose or glucose plus fructose for 16 h. Northern blots indicated that sucrose and sorbitol treatments induced a 3.5-fold accumulation of StCDPK1 transcripts (Fig. 2A) that correlated with a 2.5±4-fold increase in CDPK activity (data not shown). Although a slight increase in transcript accumulation could be observed with glucose, fructose or a combination of both, none mimicked the induction obtained with sucrose or sorbitol (Fig. 2A). When the tuber-specific genes Patatin and Pin2, which are known to be up-regulated by sugars (Roitsch, 1999), were analysed, sucrose, sorbitol and all the monosaccharides tested were able to increase mRNA accumulation (Fig. 2A).

The fact that StCDPK1 expression is specifically up-regulated by sucrose, but not by hexoses, could be of physiological relevance since sucrose is the most abundant sugar in swelling stolons tips and acts as a specific signal during in vitro tuber induction (Ewing, 1987). The effect of sorbitol on StCDPK1 expression suggests that
osmotic stress could be a component of the sugar signal. It is possible that osmotic-stress activation of sucrose-phosphate synthase, which leads to an increase in sucrose concentration (Toroser and Huber, 1997; Geigenberger et al., 1999), could be involved.

Both kinase and phosphatase activities are induced at the onset of tuberization (MacIntosh et al., 1996) and have been implicated in the signal transduction pathways triggered by sugars (Smeekens, 2000). Kinase and phosphatase inhibitors were added to sucrose-treated plants to analyse their effect on sugar-inducible StCDPK1 expression. The protein kinase inhibitor, staurosporine, and the calmodulin antagonist, chlorpromazine, had no effect on StCDPK1 expression or any of the sugar-regulated genes analysed. By contrast, the addition of okadaic acid, a potent inhibitor of protein phosphatases from the PP1 and PP2A family, blocked the induction of StCDPK1, Pin2 and patatin (Fig. 2B). These results suggest that, in the transduction of carbohydrate signals, protein dephosphorylation is required for the transcriptional activation of some tuberization-related genes. It can be proposed that StCDPK1 could be a key mediator in the signal transduction pathways triggered by sucrose during tuber development.

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