MANNITOL-ENHANCED, FLUID-PHASE ENDOCYTOSIS IN STORAGE PARENCHYMA CELLS OF CELERY (APIUM GRAVEOLENS; APIACEAE) PETIOLES

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We recently demonstrated the occurrence of a sucrose-enhanced, fluid-phase endocytic (FPE) mechanism of nutrient uptake in heterotrophic cells. In the present work, the possible enhancement/induction of FPE by photoassimilates other than sucrose was investigated by measuring the incorporation of the fluorescent endocytosis marker d-TR (dextran-Texas red; 3000 mw) into celery (Apium graveolens) petiole storage parenchyma (CSP), a tissue that transports and accumulates mannitol. Mannitol uptake in these cells is biphasic, with a hyperbolic phase at concentrations below 20 mM and a linear phase above 20 mM external solute concentration. In the absence of mannitol, or in its presence at concentrations within the hyperbolic phase, CSP cells accumulated low levels of d-TR. Conversely, d-TR accumulation by CSP cells was greatly enhanced in the presence of mannitol at concentrations within the linear phase. At high external mannitol concentration, d-TR accumulation was prevented by the endocytic inhibitors LY294002 and latrunculin B. In addition, d-TR uptake was temperature dependent under high mannitol concentration. Microscopic observations revealed that d-TR accumulated in the vacuole. These data support the occurrence of an FPE mechanism in CSP cells that participates in trapping and transport of photoassimilates to the vacuole. The FPE mechanism is enhanced by high mannitol concentrations.

Key words: apoplast; biphasic uptake curve; sugar carriers; sugar uptake; vacuole storage.

The capacity of plant heterotrophic organs to incorporate and accumulate photoassimilates (mostly in the form of sucrose) directly impacts their final size, crop productivity, and nutritional value. Photoassimilate transport within heterotrophic organs occurs either through the symplasmic continuum or through the apoplast (Offler and Patrick, 1993; Yong-Ling and Patrick, 1995; Patrick, 1997; Wood et al., 1997; Wang et al., 2003; Zhang et al., 2004). In the apoplastic route, transport of sugars into the cell is believed to be mediated by a sequence of membrane-bound transporters located at the plasmalemma and tonoplast. However, recent studies (Etxeberria et al., 2005a, b, c, 2006) revealed the existence of vesicle-mediated, fluid-phase endocytosis (FPE) that serves as a mechanism for photoassimilate uptake and traffic into the vacuole in various heterotrophic cells.

FPE in heterotrophic cells is enhanced by externally supplied sucrose, although low levels of FPE activity have been consistently observed in the presence of other photoassimilates such as hexoses and amino acids (Etxeberria et al., 2005a). In all species investigated, however, sucrose constitutes the main component of carbohydrate transport, suggesting a major role of this disaccharide in the signaling pathway leading to endocytic uptake of nutrients. Furthermore, in some species, most of the sucrose destined for starch biosynthesis is taken up by endocytosis (Baroja-Fernandez et al., 2006).

In a large number of plant species, phloem transport of sucrose is accompanied by various oligosaccharides and sugar alcohols (Turgelon, 1996). These sugars are similarly transported and stored in the vacuole of storage cells, raising the likelihood that they participate in the induction of endocytosis. In celery storage parenchyma (CSP) cells for instance, despite the fact that apoplastic transport of photoassimilates occurs primarily as sucrose and mannitol (Daie, 1987; Diettrich and Keller, 1991), vacuoles store very little sucrose but high mannitol concentrations (Keller and Matile, 1989). Kinetics studies revealed that mannitol uptake in CSP cells is biphasic, with a hyperbolic phase of carrier-mediated transport at low external concentration and a linear phase above 20 mM (Daie, 1986).

Uptake of external solutes is a highly regulated mechanism, as evidenced by the simultaneous functioning of FPE and plasmalemma-bound sugar carriers that mediate transport to different cellular compartments (Etxeberria et al., 2005c). Whether prominent sugars (or sugar alcohols) other than sucrose in plant phloem sap can also enhance FPE, and hence photoassimilate uptake, is still unresolved. To further our understanding of assimilate transport into plant storage cells, we investigated the possible involvement of mannitol in FPE enhancement in CSP cells. Our results demonstrate that mannitol is capable of inducing FPE beyond basal levels. In addition, data showing the marked uptake inhibition of the fluorescent endocytic marker dextran-Texas red (d-TR) (Ramoino et al., 2001) by the endocytic inhibitors 2-(4-morpholinyl-)-8-phenyl-4H-1 (LY294002) (Vlahos et al., 1994) and latrunculin B (LatB) (Baluska et al., 2004) in the presence of high mannitol concentrations strongly suggest that the linear phase of mannitol uptake can be ascribed to FPE.

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Fig. 1. Uptake of the endocytic marker dextran-Texas red (d-TR) by celery parenchyma cells incubated at two concentrations of mannitol in the presence or absence of 5 μM LY294002 or 10 μM latrunculin B. Accumulation of d-TR was based on alcohol dehydrogenase (ADH) activity to standardize the number of cells.

MATERIALS AND METHODS

Plant material—Celery stalks (Apium graveolens L.) were purchased at a local grocery store and refrigerated until use. Stalks were washed, and cylinders of CSP cells were obtained by cutting perpendicular to the surface using a #3 cork borer. Epidermal cells and vascular tissues were removed from the edges of the cylinders, and the remaining storage cells were cut in discs of approximately 0.5 mm. Tissue discs were rinsed with double-distilled water before experimentation.

Incubation experiments—Tissue samples (1 g) were incubated in a 10-mL flask each with 3 mL of 25 mM Tris/MES (pH 5.6), 0.5 mM CaCl₂, 0.5 mM MgCl₂, 2 mM dithiothreitol, and 1 mg mL⁻¹ of the endocytic marker d-TR (dextran-Texas Red, 3000 mw, neutral; Invitrogen Corp, Carlsbad, California, USA) or latrunculin B (L5288; Sigma, St. Louis, Missouri, USA) with an excitation filter of 590/10 and emission filter of 620/10. Fluorescence was analyzed using a BioRad Versafluor fluorometer (Bio-Rad Laboratories, Hercules, California, USA) with an excitation filter of 590/10 and emission filter of 620/10. Conversion of fluorescence to micrograms of d-TR was based on a predetermined standard curve, Values for d-TR are presented based on the amount of live cells. ADH activity was measured at 340 nm essentially as described by Etxeberria et al. (2005a). One unit (U) is the amount of activity that consumes 1 μmol ethanol per min. Data from temperature dependent experiments were based on volume of protoplast preparation. All experiments were carried out in triplicates, and data are presented as the average ± SD.

Microscopy—Microscopy of protoplasts was performed with a Leica TCS SL confocal microscope (Leica, Heidelberg, Germany).

RESULTS AND DISCUSSION

FPE enhancement by mannitol occurs at concentrations within the linear phase of uptake—Mannitol uptake in CSP cells is biphasic, with a hyperbolic phase of carrier-mediated transport at concentrations below 20 mM and a linear phase above 20 mM (Daie, 1987; Keller, 1991). To investigate the possible involvement of FPE in the transport of mannitol, we analyzed d-TR incorporation into CSP cells incubated with mannitol concentrations within both phases of the uptake curve.

CSP cells incubated with d-TR either in the absence or the presence of mannitol at concentrations within the hyperbolic phase of the uptake curve accumulated low levels of d-TR (6.5 μg d-TR/U ADH) (Fig. 1). Notably, in the presence of 200 mM mannitol, d-TR accumulation sharply increased to 18.2 μg d-TR/U ADH, suggesting that mannitol is capable of inducing or stimulating FPE in CSP cells. Sugars such as sucrose and glucose were less enhancing than mannitol, because CSP cells incubated with either 200 mM glucose or 200 mM sucrose accumulated only 11.9 and 8.7 μg d-TR/U ADH, respectively (Fig. 2). Mannitol-induced FPE was substantially reduced in the presence of glucose and sucrose (Fig. 2), suggesting that CSP cells possess a single sensor recognizing different sugars and with different degrees of sugar-sensing capacity. Sugar specificity was also observed in sucrose-grown, cultured sycamore cells, where mannitol was not able to induce FPE over control levels (unpublished results), as was the case for glucose and fructose (Etxeberria et al., 2005a).

Similar to cultured sycamore cells (Etxeberria et al., 2005a; Barroja-Fernández et al., 2006) and in agreement with the occurrence of a manitol-inducible endocytic transport of d-TR, both LY294002 and LatB substantially reduced FPE accumulation of d-TR in 200 mM mannitol (Fig. 1). These structurally different compounds inhibit the endocytic process in a multitude of plant (Baluska et al., 2004) and animal (Vlahos et al., 1994) species by interfering with phosphatidylinositol 3-kinase and by depolymerizing F-actin filaments, respectively. In addition, and consistent with the occurrence of basal FPE activity in heterotrophic cells (Etxeberria et al., 2005a), d-TR incorporation in the absence of mannitol was also suppressed by the endocytic inhibitor LY294002.

Uptake of d-TR was temperature dependent as illustrated in Fig. 3. At 4°C, endocytic uptake of d-TR was minimal, in agreement with previously reported data on temperature effect.
on the endocytic process (Weigel and Oka, 1980). Increasing temperature to 15°C sharply enhanced d-TR accumulation, with the highest levels of endocytic d-TR uptake at 30°C.

Microscopic observations of vacuolar incorporation of d-TR—Solute taken up by the sucrose-inducible FPE process are transported into the vacuole (Etxeberria et al., 2005a, b). If mannitol-induced d-TR uptake by CSP cells is transported to the vacuole by FPE, fluorescence should be observed emanating from the vacuole. High definition confocal micrographs of CSP protoplasts after 12 h incubation in 200 mM mannitol demonstrated the vacuolar origin (Fig. 4). Furthermore, the higher fluorescence intensity at 200 mM mannitol confirmed the enhancement of FPE by high mannitol concentrations (Fig. 1).

Additional remarks and conclusions—Discovery of FPE as a mechanism of nutrient uptake and traffic has altered our view of the processes driving sink strength and productivity in plants. Better understanding of the mechanisms involved in endocytic uptake of nutrients will require additional molecular, biochemical, and cell biological investigations. Only then we will be in a position to exploit the full potential of this mechanism in the design of more efficient crops.

From the data presented in this communication, several conclusions can be drawn with high degree of certainty. First, FPE can be induced by phloem sap components other than sucrose, most likely a species-specific characteristic. In celery for instance, mannitol present in the phloem sap appears to be the most efficient FPE enhancer. Second, aside from nutrient-enhanced, vesicle-mediated endocytic uptake, a basal level of endocytosis in plant cells that mediates the dynamic turnover of intracellular components (Robinson et al., 1998) inevitably traps extracellular fluids, some of which are transported to the vacuole. Third, the lack of differences between d-TR accumulation in CSP cells incubated in the absence and presence of low mannitol concentrations (Fig. 1) implies that mannitol is not taken up by FPE within the hyperbolic phase of the uptake curve, but is mainly transported by a membrane-transported, carrier-mediated system as previously established (Daie, 1986; Keller, 1991; Noiraud et al., 2000). Fourth, the linear component of sugar uptake in plant cells is likely mediated by FPE and not by facilitated diffusion as generally suggested (Niemietz and Jenner, 1993; Daie, 1986; Wright and Oparka, 1989). The increased d-TR fluorescence within the cells at high external mannitol concentration, the strong inhibition by LY294002 and latrunculin B (Fig. 1), and FPE’s dependence on temperature (Fig. 3) supports this contention. In yeast (Saccharomyces cerevisiae), for example, researchers have recognized that the low-affinity linear component of glucose transport is not due to passive diffusion (Gamo et al., 1995). It is noteworthy that endocytic uptake of metabolites is also characterized by linear uptake kinetics (Kyle et al., 1988).

Fig. 2. Uptake of the endocytic marker dextran-Texas red (d-TR) by celery parenchyma cells incubated in the presence of the indicated concentrations of mannitol, sucrose, or glucose. The d-TR content was based on alcohol dehydrogenase (ADH) activity to standardize the number of cells.

Fig. 3. Uptake of the endocytic marker dextran-Texas red (d-TR) by celery parenchyma cells incubated in 1 mg/mL d-TR and 200 mM mannitol for 12 h at various temperatures. Data were based on milliliter of protoplasts due to variations in alcohol dehydrogenase activity among temperature treatments.
believed to be responsible for the continuous turnover of membrane components (Robinson et al., 1998), the retrieval and desensitization of receptors for signal dispersal (Sorkin and von Zastrow, 2002; Piddini and Vincent, 2003), and the dynamic redistribution of cell wall pectins (Baluska et al., 2002).

Like many other plant storage organs, the celery petiole constitutes a reversible sink tissue, undergoing a rapid transformation from sink to source during its biennial existence (Keller and Matile, 1989). During petiole growth and solute accumulation, in vivo mannitol concentration in phloem cells has been estimated at approximately 50 mM (Daie, 1987). This concentration, well above the 20 mM upper limit of the hyperbolic phase, is sufficiently high to induce endocytic uptake and eventual transport of mannitol (and other photo-assimilates) into the vacuole as previously demonstrated for cultured sycamore cells (Etxeberria et al., 2005a). Whether endocytic vesicles are transported directly into the vacuole or whether they are routed through a series of intermediary structures (Bolte et al., 2004) is still unresolved. However, to generate a mannitol concentration gradient across the vacuole in storage parenchyma cells (Daie, 1987; Keller and Matile, 1989), the participation of an energy-dependent mechanism is guaranteed. At this point, we can only speculate on such mechanism(s) by analogy to rat kidney cells (Traub et al., 1989), where a retrograde system of ~100 nm clathrin-coated vesicles from the lysosome has been observed. Based on calculations of surface area to volume ratio, retrograde vesicles originating from the vacuole must be considerably smaller than incoming endocytic vesicles to retrieve comparable amount of membrane without subtracting significant amounts of vacuole solutes (Swanson, 1989). As tonoplast is energetically retrieved, the remaining volume must be compensated by water flux across the tonoplast into the cytosol. We are currently investigating all these aspects in plant systems.

LITERATURE CITED


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