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Plasmodesmal-mediated cell-to-cell transport in wheat roots is modulated by anaerobic stress

Rapid communication

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Summary. Cell-to-cell transport of small molecules and ions occurs in plants through plasmodesmata. Plant roots are frequently subjected to localized anaerobic stress, with a resultant decrease in ATP. In order to determine the effect of this stress on plasmodesmal transport, fluorescent dyes of increasing molecular weight (0.46 to 10 kDa) were injected into epidermal and cortical cells of 3-day-old wheat roots, and their movement into neighboring cells was determined by fluorescence microscopy. Anaerobiosis was generated by N_2 gas or simulated by the presence of sodium azide, both of which reduced the ATP levels in the tissue by over 80%. In the absence of such stress, the upper limit for movement, or size exclusion limit (SEL), of cortical plasmodesmata was < 1 kDa. The ATP analogue TNP-ADP (mw 681) moved across the plasmodesmata of unstressed roots, indicating that plasmodesmata may be conduits for nucleotide (ATP and ADP) exchange between cells. Upon imposition of stress, the SEL rose to between 5 and 10 kDa. This response of plasmodesmata to a decrease in the level of ATP suggests that they are constricted by an ATP-dependent process so as to maintain a restricted SEL. When roots are subjected to anaerobic stress, an increase in SEL may permit enhanced delivery of sugars to the affected cells of the root where anaerobic respiration could regenerate the needed ATP.

Keywords: Adenosine triphosphate; Anaerobiosis; Fluorescein-dextrans; Plasmodesmata; *Triticum aestivum*.

Abbreviations: F-dextran fluorescein-coupled dextran; LYCH Lucifer Yellow CH; SEL size exclusion limit; TNP-ADP 2′-O-(trinitrophenyl)adenosine-5″-diphosphate.

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Introduction

Plasmodesmata facilitate biochemical and physiological coordination between plant cells and organs. This coordination is achieved, in part, by regulation of the size exclusion limit (SEL) of the plasmodesmata (Robard and Lucas 1990). Normally the SEL of plasmodesmata is around 800 Da. However, plasmodesmata are dynamic structures whose SEL can be reduced by injection of calcium (Erwee and Goodwin 1983, Baron-Epel et al. 1988) or IP_3 (Baron-Epel et al. 1988). On the other hand, an increase in the SEL occurs in cells that have been infected by viruses (Derrick et al. 1992) or in transgenic plants expressing the viral movement proteins (Wolf et al. 1989, Ding et al. 1992).

Within the root, plasmodesmata form the conduits for movement of ions from the epidermal cells to the xylem (Lüttge and Higinbotham 1979). The movement of sugars from sieve elements to the surrounding cells of the stele and cortex is considered to occur via plasmodesmata (Giaquinta et al. 1983). Roots are subject to a variety of stresses, some of which might modify the SEL of plasmodesmata, and thus alter the symplastic flow of ions and sugars. One stress frequently encountered by roots is localized anaerobiosis, with a resultant decrease in ATP levels. Measurement of the osmotic volume of wheat root cells using the micro-pressure probe technique led to the suggestion that anaerobiosis might cause the SEL to decrease (Zhang and Tyerman

1991). This conclusion is consistent with the fact that azide decreased electrical coupling between *Avena* coleoptile cells (Drake 1979). In order to assess the possible effects of such an anaerobic stress on symplasmic transport in roots, a study was made of the effect of reduced ATP on the SEL of wheat root plasmodesmata.

Materials and methods

Plant material

Seedlings of wheat (*Triticum aestivum* cv. Yecora Rojo) were grown for 3 days on wet filter paper with occasional white light. Roots 3–8 cm long, excised or still attached, were incubated for 30 min in 0.2 M mannitol, which lowered the turgor to 0.12 to 0.20 mPa (determined by osmometry measurements, data not shown). This treatment reduced the pressure required to perform dye injections, increasing the success rate of injections, but did not alter the response to the stress (data not shown). Roots were maintained in this same solution throughout the experiment, unless otherwise stated.

Fluorescent dyes, microinjection, and fluorescence microscopy

Lucifer Yellow CH (LYCH, 453 Da), 2'-O-(trinitrophenyl)-adenosine-5"-diphosphate (TPN-ADP, 681 Da), and fluorescein-dextrans (F-dextrans), were purchased from Molecular Probes, Inc. (Eugene, OR). The polydisperse 4 kDa F-dextran was fractionated over a Biogel P-10 column (BioRad, Richmond, CA) as described previously by Ding et al. (1993). From this procedure, 1–2, 2–3, 3–5, 5–7, and 7–10 kDa F-dextran probes were obtained and used in this study. The dyes were pressure-injected into epidermal cells or into cortical cells 1 or 2 cell layers below the epidermis, in the root-hair zone. Each root was subjected sequentially to a number of injections over a period of up to 90 min. Movement of dye was monitored with a Leitz Orthoplan epifluorescence microscope fitted with a blue (BP 390 to 490) excitation filter, and quantitation of dye movement was monitored with a Hamamatsu image analysis system (model C 1966-20). Since dye movement was rapid (usually less than 10 s), injections were scored as positive if the dye moved to neighboring cells within the first minute. When observations were continued for 10 min, there was no movement between cells where movement had failed to occur in the first minute.

ATP reduction and measurement

Two procedures were used to reduce the intracellular ATP concentration; addition of azide or anaerobic conditions produced by N_2 gas. After control microinjections, the solution around the root was changed to one containing 1 or 10 mM Na-azide, in addition to 0.2 M mannitol. Injections were resumed immediately. Anaerobiosis was achieved by sealing the wheat seedling and injection-pipette inside a 12 × 12 inch ZipLock bag through which N_2 was circulated. In this situation, dye injections were not started until 30 min after the beginning of the N_2 treatment.

ATP and ADP concentrations were measured by the procedure of Wilson et al. (1985). Roots were frozen in liquid N_2, extracted with $HClO_4$ and ATP and ADP were converted to fluorescent analogues by reaction with chloroacetaldehyde, and then separated by HPLC (Beckman Series 344 Gradient Liquid Chromatograph) equipped with an Ultrasphere IP column. The eluting peaks were detected by using a fluorescence detector (Beckman Model 157).

Results

Both azide treatment and anaerobiosis caused a rapid and extensive decline in the ATP concentration. 10 min after addition of azide the ATP was reduced to 22% of control by 1 mM azide and 10% of control by 10 mM azide (Fig. 1). The decline in ATP with N_2 was less rapid, but reached the same low levels after 30 min (Fig. 1).

In the absence of metabolic inhibitors, wheat roots showed excellent dye coupling with LYCH. For most cells, F-dextrans of > 1 kDa failed to move out of the target cell (Fig. 2 a), but on occasion, limited dye movement was seen with the 1–2 kDa F-dextran and an occasional coupling was seen with 2–3 kDa F-dextran (Table 1).

Upon addition of 1 or 10 mM azide there was an appreciable change in the SEL (Table 1). The SEL increased to above 5 kDa in most cases (Fig. 2 b), and some movement of 7–10 kDa F-dextran was often observed. The change in the plasmodesmal SEL did not begin until at least 5 min after addition of 10 mM azide, while it appeared that at least 30 min in 1 mM azide was required to increase the SEL above 5 kDa (data not shown).

Measurement of dye-coupling was begun 30 min after the start of the N_2-induced anaerobiosis, in order to allow time for the ATP level to decrease to a low level (cf. Fig. 1). Dye-coupling was consistently recorded with 2–3 kDa and 3–5 kDa F-dextrans (Fig. 2 c), and occasionally with 5–7 kDa F-dextran. These data indicate that anaerobic stress, with the attendant reduction in ATP, caused the plasmodesmal SEL to increase, and thus potentiated greater cell-to-cell symplastic transport in wheat roots.

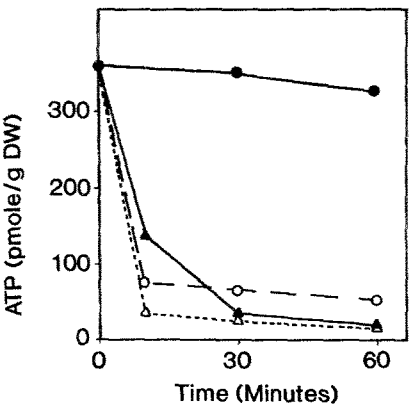

Fig. 1. Time-course of reduction in ATP in wheat root cells in response to azide or N_2-induced anaerobiosis. Roots pretreated 30 min in 0.2 M mannitol, then with addition of 1 mM sodium azide (○), 10 mM sodium azide (△), N_2 gas (▲) or without additions (●)

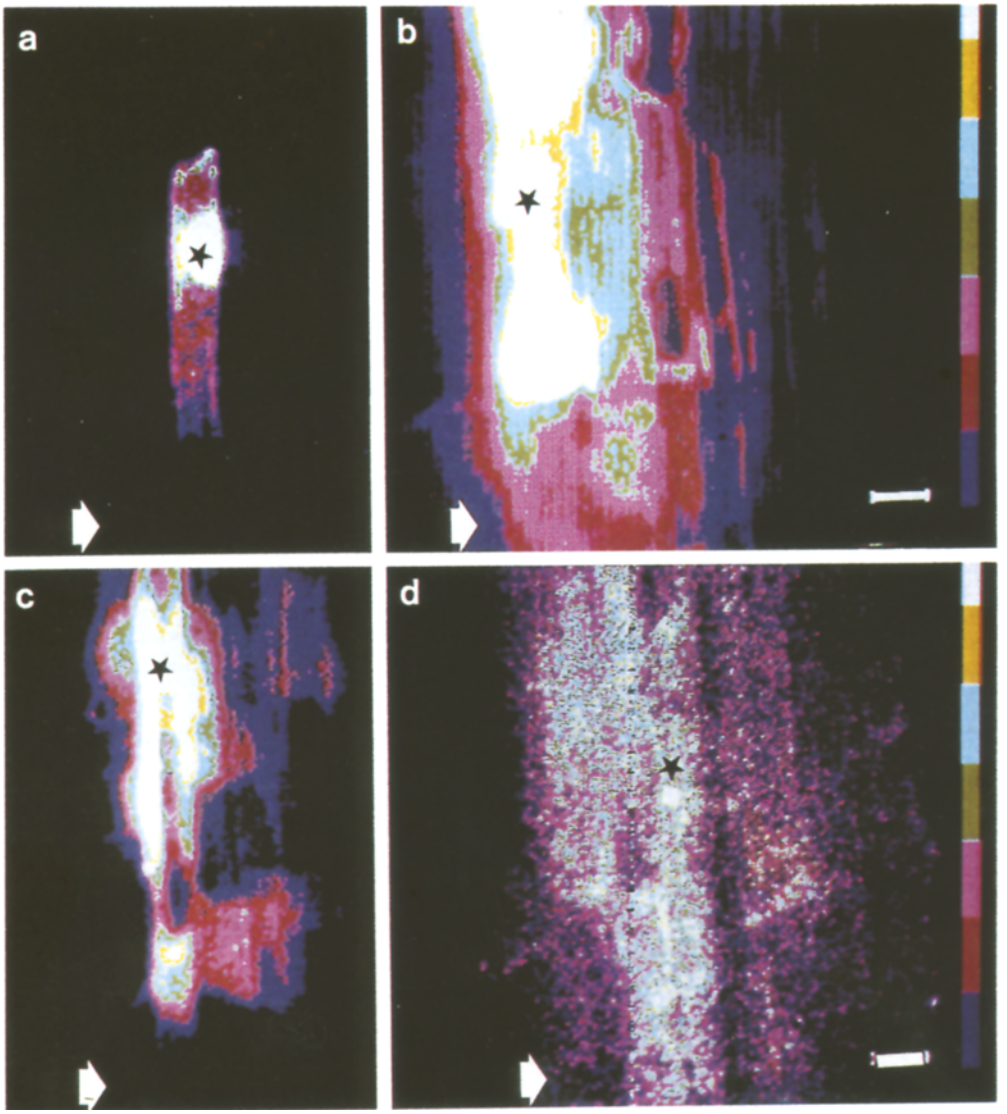

Fig. 2. Dye-coupling of F-dextrans and TNP-ADP as influenced by azide and anaerobic conditions. Experimental conditions same as in Table 1. The intensities of fluorescence are presented as false-color images as shown by the bars in **b** and **d**; white represents the highest intensity while black is background. All images are at the same magnification (bars: in b and d, 50 μm). Injected cells are indicated by stars and the horizontal arrows indicate the extremity (edge) of the wheat root. **a** Lack of dye-coupling with 1–2 kDa F-dextran in the absence of azide. **b** Dye-coupling of 3–5 kDa F-dextran 30 min after addition of 1 mM azide. **c** Dye-coupling of 3–5 kDa F-dextran 30 min after imposition of a N_2 treatment. **d** Cell-to-cell movement of TNP-ADP (10 mM) following its injection into a wheat root cortical cell

Since ATP has a molecular weight of 509, it should pass through plasmodesmata, even without a stress-induced increase in the SEL. To test this prediction, cells were injected with TNP-ADP, an ATP analogue (681 Da). This compound is very weakly fluorescent when free in solution, but becomes strongly fluorescent when bound to ATP receptors (Grubmayer and Penefsky 1981). By use of the Hamamatsu C 1966-20 image intensifier system it was possible to visualize the fluorescence of TNP-ADP after injection and as it moved from cell to cell. As shown in Fig. 2 d, TNP-ADP showed good dye-coupling, indicating that ATP and ADP should be able to move between cells through plasmodesmata. These results show that the SEL of wheat root cells is >681 Da, in contrast to the roots of *Egeria densa* where it is between 453 and 536 Da (Erwee and Goodwin 1985).

Discussion

The data presented here indicate that the plasmodesmata of wheat roots are maintained in a constricted

Table 1. Size exclusion limit of wheat root cortical and epidermal plasmodesmata in the absence and presence of azide

Root	Dye coupling (no. coupled/total no. injections)							
	Minus azide			Plus azide				
	F-dextrans (kDa)							
	1–2	2–3	5–7	1–2	2–3	3–5	5–7	7–10
Excised roots								
1	0/6			4/5	1/2	1/1	3/5	0/5
2	0/4			4/5	2/3	1/1	3/3	1/3
3	2/6	0/3		2/3	3/4	1/4	3/5	
Intact roots								
4	0/5			3/3	2/4	2/3	2/4	
5		1/6		2/2	2/2	3/3	2/16	
6		1/5				3/3	0/18	
7		1/6	0/6				5/9	2/5

Excised or intact roots of 3-day-old wheat seedlings were preincubated 30 min in 0.2 M mannitol, then pressure-injected with 2 mM LYCH or 3–5 kDa F-dextran or 1 mM 1–2, 2–3, 5–7, or 7–10 kDa F-dextran (Ding et al. 1992). Injections were into epidermal or subepidermal cortical cells in root hair zone. Fluorescence observed at × 160 with a Leitz Orthoplan epifluorescence microscope and Hamamatsu C 1966 image intensifier. Injections were done sequentially, starting with the lowest molecular weight probe. Data indicate the number of injections in which the fluorescent probe moved relative to the total number of injections performed with that particular probe. Blanks mean that that particular size F-dextran was not tried on that root

state by an ATP-dependent process. This may be a general phenomenon, since Tucker (1993) has found that azide caused some increase in the rate of cell-to-cell diffusion in staminal hairs of *Setcreasea purpurea*. The mechanism by which ATP modulates the SEL remains to be established. ATP might be required to phosphorylate a plasmodesmal protein (see, e.g., Citovsky et al. 1993) involved in the constriction phenomenon. A putative connexin-like protein (Meiners et al. 1991) thought to be localized to the plasmodesmata (Yahalom et al. 1991) has strong homology to a protein kinase (Mushegian and Koonin 1993). Spiral filaments, which are seen in some plasmodesmata, may contain actin which may well be involved in constricting the cytoplasmic annulus (White et al. 1992). Alternatively, the lack of ATP may perturb the dynamic balance between callose deposition and degredation at the annulus of the plasmodesma so as to bring about a reduction in callose and thus an increase in SEL (Wolf et al. 1991).

The data also indicate that cells may form a symplasm in terms of their energy-charge; i.e., the availability of ATP would be more-or-less uniform among cells. The mobility of ATP through the plasmodesmata between companion cells and sieve-elements has long been assumed (see, e.g., Eschrich and Heyser 1975) but not demonstrated; however, the possibility of ATP movement between other cells does not seem to have been considered. Cells which are involved in extensive transport at the plasma membrane, and are thus major users of ATP, may not have to generate all the ATP in their own mitochondria, but may be able to import ATP from neighboring cells.

There are distinct advantages in having an increased SEL in root cells during times of reduced ATP, such as under localized anaerobic stress. For example, this should permit an increased influx of soluble sugars into these cells, enhancing ATP synthesis by anaerobic respiration. Such an increase in soluble sugars upon anaerobiosis has been detected with wheat roots (Wiedenroth 1993). In addition, ATP may move into the affected cells from their more aerobic neighbors above or below, in exchange for ADP. Likewise, NADH may move out in exchange for NAD^+, further facilitating ATP production by glycolysis. Furthermore, ATP may move transversely from cortical cells to the more metabolically-active stele. This process, enabling anaerobic respiration to occur within the root periphery, may permit the continued production of ATP, while ethanol, a potentially damaging end-product, may be released into the soil solution.

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