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The effect of SO_2 on sucrose efflux from source leaves of poplar

Abstract: The present study was aimed at the determining the influence of various concentrations of sulphite (0.5–2.5 mol m^{-3}) on the regulation of sucrose efflux from leaf discs of poplar (*Populus deltoides*). Sucrose efflux was enhanced by sulphite at a concentration of 1.0 mol m^{-3} for the first 60 min of treatment. However, after longer period under sulphite treatment both an attenuation of the stimulation of sucrose release and a reduction of efflux were observed.

Additional key words: *Populus deltoides*, metabolic uncouplers, sulfhydryl reagents, sulphite solution

Abbreviations: CCCP – carbonyl cyanide m-chlorophenylhydrazone, FC – fusicoccin, HEPES – N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid, PCMBS – p-chloromercuribenzenesulfonic acid, MES – morpholinoethanesulfonate, TRIS – tris(hydroxymethyl)amino metane, VAN – sodium orthovanadate

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Introduction

There is considerable evidence that SO_2 plays an important role in the regulation of photoassimilate distribution over the plant. Under the influence of SO_2 the rate of translocation of assimilates is inhibited (Gould et al. 1988). The inhibition of assimilate export from leaves to roots is due to an increased retention of carbohydrates in the leaves. The cause of this may be SO_2 injury to the sucrose protein carrier and electrochemical proton gradient involved in the phloem loading mechanism (Maurousset et al. 1992) rather than a direct effect on long-distance transport (Lorenc-Plucińska and Ziegler 1987).

In poplar leaves phloem loading of sucrose is an active proton-sugar cotransport process (Lorenc-Plucińska and Bojarczuk 1995). SO_2 inhibits the phloem loading of sucrose into leaf discs, leading to an increase of the sucrose content in the apoplast. The increase in apoplastic sucrose creates an unfavourable gradient which would block sucrose efflux from the mesophyll cells. The exact mechanism of the influence of SO_2 and its aqueous derivates (sulphite and bisulphite) on assimilate release is not known exactly (Lorenc-Plucińska 1994). Therefore the objective of this work was to investigate the action of various concentrations of sulphite on the regulation of sugar release from poplar source leaves.

Materials and methods

Plant material

Populus deltoides Bartr. ex Marsh plants were grown from hardwood cuttings in a greenhouse as described previously (Lorenc-Plucińska 1996). Fully expanded leaves, leaf plastochron index (LPI) of 6 and 7 (Dickmann 1971) were harvested 5.5 h after the beginning of the light period. They were pooled to make one sample. The leaves were rinsed twice in tap water and leaf discs (6 mm in diameter) were punched with a cork borer avoiding major veins.

Efflux experiments

Immediately after isolation, leaf discs were placed in a medium consisting of 0.5 mol m^{-3} $MgCl_2$, 0.5 mol m^{-3} $CaCl_2$ and 25 mol m^{-3} HEPES adjusted with NaOH to pH 7. They were vacuum infiltrated until most discs no longer floated on the medium. Then 10 discs were incubated in 2.5 ml of medium containing 25 mol m^{-3} HEPES adjusted with 25 mol m^{-3} MES or TRIS to different pH values, 0.5 mol m^{-3} $MgCl_2$, 0.5 mol m^{-3} $CaCl_2$, with or without various compounds (CCCP, FC, mannitol, PCMBS, VAN and sulphite (details are given in the legends to tables). Stock solutions of inhibitors, ionophores and uncouplers were made in absolute ethanol. Ethanol concentrations in experimental solutions were up to 0.1% (v/v) and an equivalent concentration of ethanol was added to the control solutions without reagents. Sulphite was prepared each time before use by dissolving Na_2SO_3 in the buffered solution and adjusted to the pH of the incubation medium. The use of sulphite simulates the effect of gaseous sulfur dioxide. The leaf discs were incubated in the light (200 mol $m^{-2}s^{-1}$) at 20°C. Samples of 0.1 ml were taken from the medium at specific time intervals and boiled for 2 min. After cooling the samples, sucrose content was analyzed by enzymatic methods adapted from Jones et al. (1977).

Statistical analysis

All experiments were repeated at least two times with a total of 8–12 replications. Statistical data analyses were performed using analysis of variance with the statistical package *STATISTICA for Windows*. Statistical significance of differences between the sulphite, effectors-treated leaf discs and the controls (without sulphite and effectors) were accepted when $P < 0.05$.

Results and discussion

The influence of sulphite on the rate of sucrose efflux depended on its concentration (0.5–2.5 mol m^{-3}), the length of exposure and the pH (5.5 and 7.5) of the incubation medium (Table 1 and 2). The greatest changes in sucrose efflux from leaf discs occurred immediately after the beginning of sulphite treatment. The decrease of sucrose efflux during the course of experiment could result from a decline in sulphite concentration in the incubation medium. We did not correct the sulphite concentration during the experiments, thus level of sulphite in the medium could have decreased. Miszalski and Ziegler (1989) have observed that the highest rates of sulphite uptake by plant samples occurred over a very short time (i.e. from 30 s to 5 min) after sulphite was supplied to the incubation medium and then an equilibrium between the experimental plant material and the medium was established.

Sucrose unloading from the mesophyll was stimulated under the influence of 1.0 and 2.5 mol m^{-3} of sulphite in the first 60 min of the experiments (Table 1). However, in the following 60 min and especially after 180 min, this stimulation declined and a reduction in efflux began (Table 1). It seems that the one of the reasons for the enhancement of efflux may be the dependence of sucrose release on the availability of protons. It is well known that the action of sulphite can lead to acidification of the cytosol coupled with an increase in alkalization of the cell exterior (Pfanz et al. 1987; Lorenc-Plucińska and Ziegler 1989). As a consequence, sucrose efflux can be promoted because the concentration of protons inside the cell increases and the proton gradient is toward the apoplast.

The stimulation of sucrose efflux by sulphite was stronger at pH 5.5 (Table 2) than at 7.5 (Table 1), which could be a result of faster and greater accumulation of sulphite in the alkaline cytoplasm at lower external pH than at higher (Pfanz et al. 1987).

Addition of CCCP, osmoticum and VAN into the medium resulted in attenuation of the stimulation of sucrose release by sulphite and PCMBS cancelled it out completely (Table 2). This could be due to disturbances in the energy state of the cells, in the permeability and integrity of membranes, in protein activity, especially through the blocking of SH – groups and the breaking of the disulphite bonds by sulphite (Lüttge et al. 1982; Lorenc-Plucińska and Ziegler 1989).

On the other hand, the stimulation of sucrose efflux by sulphite in the presence of FC (Table 2) could be a consequence of the FC stimulation of sulphite uptake (Lorenc-Plucińska and Bojarczuk 1995) through FC stimulation of transmembrane movement of sulphite into the cell across the plasmalemma (Olszyk and Tingey, 1984).

As reported previously (Lorenc-Plucińska 1994) the retention of sucrose in the apoplast on account of phloem loading inhibition by sulphite can block sucrose efflux from mesophyll cells. The rise in the accumulation of starch confirmed our supposition.

However, the results of the present investigation indicate that sulphite, depending on the duration of treatments, enhanced or only slightly reduced the efflux of sucrose. The unexpected lack of an unequivocal blockage of sucrose release by sulphite for the duration of the treatments might be a consequence of the fact that sucrose unloading from the mesophyll of poplar leaves is not passive down a gradient from a high symplastic to a low apoplastic concentration. It is a regulated process which sulphite can disturb in a variable manner.

Table 1. Effect of sulphite on sucrose efflux (nmol cm^{-2}) in the light. Leaf discs were incubated in 0.5 mol m^{-3} $MgCl_2$, 0.5 mol m^{-3} $CaCl_2$, 25 mol m^{-3} HEPES, pH 7.5 in absence (control, 0.0) or with sulphite at concentrations of 0.5, 1.0 and 2.5 mol m^{-3}. Data are means from 4–5 independent experiments ±SE. *, ** – values were significantly different from the control at a 0.05 and 0.01 confidence level, respectively

Time (min)	Sulphite concentration (mol m^{-3})			
	0.0	0.5	1.0	2.5
15	16.4±0.6	18.2±1.1	27.8±1.5*	35.3±1.4**
30	27.3±1.5	25.9±1.5	44.2±2.4*	53.8±3.1*
60	38.8±1.9	46.6±3.3	59.8±4*	66.8±2.9*
120	58.1±2.6	73.8±3.7	72.6±5.1	80.8±6.1
180	73.4±4.6	82±5.3	69.7±4.2	55.1±2.7

Table 2. Effect of various effectors on sucrose efflux (nmol cm^{-2} h^{-1}) in the light. Leaf discs were incubated for 10 min in 0.5 mol m^{-3} $MgCl_2$, 0.5 mol m^{-3} $CaCl_2$, 25 mol m^{-3} HEPES, pH 5.5 with or without 250 mol m^{-3} mannitol, 1 mol m^{-3} PCMBS, 50 mmol m^{-3} CCCP, 100 mmol m^{-3} VAN, 20 mmol m^{-3} FC, and sulphite at concentrations of 1.0 and 2.5 mol m^{-3}. Data are means from 2 experiments ± SE replicated 4–6 times. *, ** – values were significantly different from the control (nil and without sulphite) at a 0.05 or 0.01 confidence level, respectively

	Sulphite concentration (mol m^{-3})		
	0.0	1.0	2.5
nil	19.4±0.6	34.1±1.4**	42±1.5**
+ mannitol	13.6±0.5**	14.7±0.9*	19±1
+ PCMBS	8.7±0.5**	7.3±0.4**	6±0.3**
+ CCCP	27.3±2.3*	32.4±2.5*	33.8±1.8*
+ VAN	24.2±0.9*	25.2±1.5	27.7±2*

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Wypływ sacharozy z liści topoli traktowanych SO_2

Streszczenie

Badano wpływ jonów siarczynowych na natężenie wypływu wewnątrzkomórkowej sacharozy. Doświadczenia wykonano na krążkach izolowanych z całkowicie rozwiniętych liści topoli czarnej amerykańskiej (*Populus deltoides* Bartr. ex Marsh). Krążki liściowe traktowano roztworami siarczynu sodu o stężeniach 0,5, 1,0 i 2,5 mol m^{-3} (pH 5,5 i 7,5) przez 15–180 min w obecności lub bez karbonylocyjanku m-chlorofenylohydrazonu (CCCP), kwasu p-chlororтęciobenzenosulfonowego (PCMBS), ortowanadanu sodu (VAN), mannitolu i fuzikokcyny (FC). Doświadczenia prowadzono na świetle (200 mol $m^{-2}s^{-1}$) w temperaturze 20°C. Jony siarczynowe stosowano w celu symulacji wpływu gazowego dwutlenku siarki.

Pod wpływem działania jonów siarczynowych o stężeniach od 0,5 do 2,5 mol m^{-3} przez okres od 15 do 60 min notowano stymulację wypływu endogennej sacharozy z krążków liściowych. Działanie jonów siarczynowych przez 120 i 180 min nie zmieniało natężenia transportu cukru. Obecność PCMBS prowadziła do zahamowania wypływu endogennej sacharozy przez siarczyn. W przeciwieństwie do powyższego, CCCP, mannitol i VAN obniżały wzrost natężenia analizowanego procesu przez jony siarczynowe.