SULFITE INHIBITION OF SUCROSE TRANSPORT INTO VACUOLES OF COTTONWOOD LEAVES

GABRIELA LORENC-PLUCIŃSKA

Polish Academy of Sciences, Institute of Dendrology,
62-035 Kórnik, Poland

(Received: November 23, 1995. Accepted: December 1, 1995)

ABSTRACT

The mechanism of sucrose transport and the influence of various concentrations of sulfite on its activity was studied in the mesophyll vacuoles isolated from cottonwood (*Populus deltoides* Bartr. ex Marsh) leaves.

Kinetic analysis of [^{14}C]sucrose uptake by isolated vacuoles revealed the presence of a saturable component with K_m for sucrose uptake = 23 mM and $V_{\max} = 21.2 \text{ nmol sucrose } 10^{-6} \text{ vacuoles min}^{-1}$. The saturable component was inhibited by SH-blocker, p-chloromercuribenzenesulfonic acid (PCMBs) and was substrate specific. The transport of sucrose was not changed by ATP with or without Mg^{2+} , by the ionophore, valinomycin and by protonophores, carbonylcyanide m-chlorophenyl hydrazone (CCCP) or dinitrophenol (DNP).

Sulfite at concentrations of 0.01-2.5 mM inhibited the uptake of [^{14}C]sucrose by isolated vacuoles. Inhibition was of the noncompetitive type with $K_i = 2.48 \text{ mM}$ of sulfite (22°C).

KEY WORDS: isolated vacuoles, *Populus deltoides*, sucrose uptake mechanism.

INTRODUCTION

The regulation of photosynthate transport is central for the relationship between photosynthesis and growth. During photosynthesis, triose phosphates synthesized in the chloroplasts are transferred by the phosphate translocator into the cytosol and converted into sucrose (Stitt et al. 1987). Part of the newly synthesized sucrose is exported from the mesophyll into the phloem. Another portion can be transported from the cytosol into the vacuole (Kaiser et al. 1982, Kaiser and Heber 1984, Gerhardt et al. 1987). When sucrose accumulates in the mesophyll, the rate of sucrose synthesis decreases and more primary products of photosynthesis are diverted to starch in the chloroplasts (Stitt and Quick 1989).

Sulphur dioxide and its aqueous derivatives, sulfite and bisulfite play an important role in the regulation of photosynthate partitioning (Gould et al. 1988, Mooney and Winner 1988). Under influence of SO_2 and sulfite the rate of export of triose phosphate out of chloroplasts is stimulated (Hampp and Ziegler 1977, Marques and Anderson 1986). An increased level of triose phosphates, especially PGA, was observed in Norway spruce needles (Einig and Hampp 1990, Hampp et al. 1990). In the presence of increased levels of triose phosphates, the rate of sucrose synthesis in the cytosol should be stimulated (Stitt et al. 1987). SO_2 inhibits the loading of sucrose into the conducting complex as a result of sulfite injury to the sucrose protein carrier and electrochemical proton gradient involved in phloem loading (Maurousset and Bonnemain 1990, Maurousset et al. 1992, and literature cited therein). The effect of SO_2 and sulfite ions on the mechanism

of sucrose transport into the vacuoles has not been described so far.

We have previously determined some characteristics of the effect of sulfite on sucrose release from the symplast, accumulation in the apoplast and loading to the phloem in the cottonwood leaves (Lorenc-Plucińska 1994). The purpose of the present investigation is to elucidate the effect of various concentrations of sulfite on sucrose transport into vacuoles.

MATERIALS AND METHODS

Plant material

Hardwood cuttings (20 cm long) of cottonwood (*Populus deltoides* Bartr. ex Marsh) were collected on January 1995 from an experimental plot of the Institute of Dendrology in Kórnik (52°15'W/17°06'E). All cuttings were moistened and stored in plastic bags at 4°C until planting. Cuttings were planted on March 07 in 1.5 l plastic pots containing garden soil mixed 3:1 (v/v) with peat. One day before planting, the cuttings were soaked in water for 24 h at 22°C. Plants were grown in a greenhouse under natural light and a day/night temperature of 22/14°C and 60-80 % relative humidity. They were watered every day. On April 03 and 10 the fertilizer "Florovit" (Incoveritas, Poland) in the concentration of 1:400 was added to each pot. Fully expanded leaves, leaf plastochron index (LPI) of 6 and 7 (Dickmann et al. 1971) were harvested 2.5 h after the beginning of the light period. They were pooled to make one sample.

Isolation of vacuoles

Vacuoles were prepared by a slight modification to the method of Blom-Zandstra et al. (1990). The leaves without the mid-rib were sliced into segments of 0.5 to 1.0 mm width, and macerated by incubation for 1.5 h in the dark at 30°C in 1% (w/v) Cellulase R-10 (Serva), 0.5% (w/v) Macerozyme R-10 (Serva), 0.1% (w/v) Pectinase (Serva), 0.6 mM sucrose, 0.5% bovine serum albumin (BSA), 0.1% polyvinylpyrrolidone-40 (PVP), 25 mM 2-(N-morpholino)ethanesulfonic acid – tris(hydroxymethyl)aminomethane (MES-Tris) pH 5.6 (30 g fresh weight of leaves per 150 ml). The next steps of the isolation procedure were performed essentially as described by Blom-Zandstra et al. (1990). Osmolarity of solutions were measured with Osmometer 800 cl (Trident Med., Poland). The yield of purified vacuoles was determined by counting and measuring marker enzymes (Martinoia et al. 1981).

Transport studies

Sugar uptake was measured by a modification of the methods described by Kaiser and Heber (1984) and Martinoia et al. (1987). We added 30 µl of vacuole suspension to a 70 µl incubation medium consisting, if not indicated otherwise, of 0.4 M sorbitol, 40% (v/v) Percoll, 0.2% (w/v) BSA, 30 mM 2-(N-morpholino)ethanesulfonic acid (MES) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)-KOH, 2 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM dithiothreitol (DTT), 1 mM CaCl₂, 1 mM MgCl₂, 30 mM K-gluconate, different concentrations of (U-¹⁴C)-sucrose (spec. act. 2 GBq/mmol) with or without sulfite and various chemicals in centrifugation tubes. Details are given in the legends of tables and figures. Total osmotic concentration was maintained constant in all assays by reducing the sorbitol in the assay by an amount appropriate to compensate for the added sugar. Sulfite was prepared separately, each time directly before use, by dissolving Na₂SO₃ in the MES buffer and bringing it to the pH of the incubation medium. Stock solutions of inhibitors, ionophores and uncouplers were made with absolute ethanol. Ethanol concentrations in experimental solutions were less than 0.1 % (v/v) and an equivalent concentration of ethanol was added to the control solutions without reagents. The suspension was overlaid with 200 µl of silicon oil (AR 200, Fluka) and 60 µl H₂O and incubated for different times at 22°C. Sugar uptake was terminated by centrifugation for 30 s at 10 000 g. The rate of ¹⁴C-sucrose uptake was determined by scintillation counting.

Calculations

Uptake rates were calculated using a linear regression between ¹⁴C-sugar uptake and time, as in Kaiser and Heber (1984).

RESULTS AND DISCUSSION

Characteristics of sucrose uptake mechanism

From the work of Kaiser et al. (1982) and Gerhardt et al. (1987) it appears that portion of the products synthesized during photosynthesis is sequestered from the cytosol through the tonoplast into the vacuole of the mesophyll cells.

Isolated mesophyll vacuoles are a proper material for the study of the transport processes through the tonoplast (Boller and Wiemken, 1986). They are capable of taking up different metabolites from the suspending medium with or without a specific carrier system and energization (Guy et al. 1979, Martinoia et al. 1981, 1985, 1986, Homeyer and Schultz

Fig. 1. Time course of [¹⁴C]sucrose uptake by isolated vacuoles at different sucrose concentrations (1, 10 and 100 mM) in the medium (pH 5.8, 22°C). Uptake units (V) were nmol sucrose 10⁻⁶ vacuoles. Sucrose uptake data were extrapolated to zero incubation time. This served to determine the amount of contamination of vacuoles with sucrose of the incubation medium that occurred after separation from the medium. Data are from three independent experiments, each with four parallel samples.

1988, Dietz et al. 1990, Rautenkranz et al. 1994). Sucrose is transferred into isolated vacuoles of mesophyll cell *via* a carrier-mediated passive transport (Kaiser and Huber 1984, Martinoia et al. 1987).

Uptake of externally supplied ¹⁴C-sucrose at different concentration (1, 10 and 100 mM) into isolated vacuoles of cottonwood mesophyll cells was linear for at least 20 min (Fig. 1). The uptake rate depended on the pH of the incubation medium and showed an optimum at pH 5.8 (Fig. 2). This is in agreement with the results presented by Guy et al. (1979), Thom et al. (1982) and Kaiser and Huber (1984) for sugar uptake into vacuoles isolated from tissues of various plants.

Fig. 2. Uptake of [¹⁴C]sucrose by isolated vacuoles at various pH values. The vacuoles were incubated for 8 min at 22°C. Uptake units were nmol sucrose per 10⁻⁶ vacuoles. The data are means ± SD of at least two different experiments with three parallel samples.

Fig. 3. Concentration dependence of $[^{14}\text{C}]$ sucrose uptake by isolated vacuoles. Vacuoles were incubated at pH 5.8 and 22°C for 10 min. Uptake units (V) were $\text{nmol sucrose } 10^{-6} \text{ vacuoles min}^{-1}$. Each value is the average of three independent experiments with three parallel samples.

The transport of sucrose was a combination of the operation of two components (Fig. 3). One component, which showed saturation kinetics was superimposed on a second nonsaturable, diffusion-like component (Fig. 4). The saturable component had an apparent K_m for sucrose of 23 mM and a $V_{\max}$ of 21.2 $\text{nmol sugar transported}/10^6 \text{ vacuoles/min}$. The similar transport kinetic constants were observed by Kaiser and Huber (1984).

Several sugars were examined for their ability to compete with sucrose transport (Table 1). Uptake of 1 mM ^{14}C -sucrose was inhibited by 100 mM sucrose whereas the influence of glucose and fructose was negligible. Sucrose transfer was significantly inhibited by a non-penetrating inhibitor of sulfhydryl groups, p-chloromercuribenzenesulfonic acid (PCMBS) (Table 2). These results may indicate that the sucrose transport system does not interact with hexoses and that the uptake of sucrose is specific and consists of a protein carrier-mediated system.

Uptake of sucrose was not affected by ATP addition into the incubation medium with or without Mg^{2+} , by an iono-

TABLE 1. Competition of several sugars with sucrose uptake by isolated vacuoles. Competition for sites on sucrose carrier was measured by providing 100 mM of potential competitors and 1 mM $[^{14}\text{C}]$ sucrose. Vacuoles were incubated at pH 5.8 and at 22°C for 10 min. Uptake units were $\text{nmol sucrose } 10^{-6} \text{ vacuoles min}^{-1}$. The data are means $\pm$ SD of at least two different experiments with two parallel samples. ** – values were significantly different from the control at the 0.01 confidence level.

Competing substances	Uptake ($\pm$ SD)	% of control
1 mM $[^{14}\text{C}]$ sucrose	0.720 ± 0.079	100
+ 100 mM sucrose	$0.303 \pm 0.045^{**}$	42
+ 100 mM glucose	0.626 ± 0.109	87
+ 100 mM fructose	0.576 ± 0.115	80
+ 100 mM raffinose	0.540 ± 0.124	75
+ 100 mM galactose	0.742 ± 0.178	103

Fig. 4. Eadie-Hofstee transformation of sucrose uptake data presented in Fig. 3.

phore, valinomycin (with K^+ present) and by metabolic uncouplers, carbonylcyanide m-chlorophenyl hydrazone (CCCP) or dinitrophenol (DNP) (Table 2). The data may be interpreted as indicating that sucrose transport into vacuoles of cottonwood mesophyll cells is not energy-dependent.

TABLE 2. Influence of various effectors on $[^{14}\text{C}]$ sucrose uptake by isolated vacuoles. The effectors were added to the suspension medium together with 1 mM $[^{14}\text{C}]$ sucrose. Valinomycin (Val) was tested in the presence of 50 mM K^+ . Vacuoles were incubated at pH 5.8 and at 22°C for 10 min. Uptake units were $\text{nmol sucrose } 10^{-6} \text{ vacuoles min}^{-1}$. The data are means $\pm$ SD of two different experiments with four parallel samples. ** – values were significantly different from the control at the 0.01 confidence level.

Effectors	Uptake ($\pm$ SD)	% of control
1 mM $[^{14}\text{C}]$ sucrose	0.670 ± 0.1	100
+ 1 mM PCMBS	$0.074 \pm 0.102^{**}$	11
+ 2 mM ATP	0.697 ± 0.084	104
+ 2 mM MgATP	0.678 ± 0.122	101
+ 1 μM Val	0.717 ± 0.115	107
+ 10 μM CCCP	0.750 ± 0.15	112
+ 100 μM DNP	0.610 ± 0.13	91

The presence of a saturable component, sensitivity to PCMBS and to competitive substrates and energy independence lead to the postulation that the uptake of sucrose in the isolated mesophyll cell vacuoles of cottonwood is passive and takes place via a facilitated diffusion.

Sulfite action

The influence of sulfite on the rate of sucrose transport into the vacuole depended on its concentration (0.01–2.5 mM). Sulfite at a concentration ≥ 1.0 mM significantly inhibited sucrose uptake (Fig. 5). This inhibition was of the noncompetitive type. This may suggest that sulfite does not affect the

Fig. 5 A and B. The effect of various sulfite concentrations on $[^{14}\text{C}]$ sucrose uptake by isolated vacuoles. Sucrose at different concentrations (1, 2.5, 20, 50 and 100 mM) was applied in the incubation medium together with the sulfite. Vacuoles were incubated at pH 5.8 and 22°C for 10 min. Uptake units (V) were nmol sucrose 10^{-6} vacuoles min^{-1} . Mean values ($\pm$ SD) from three independent experiments with three parallel samples.

active site of the protein carrier-mediated system directly, but it may be a result of the influence of sulfite on sulfhydryl groups and bisulfite bonds of proteins and structural changes of the membrane (Ziegler 1975).

The inhibitor constant K_i of this noncompetitive inhibition was to be 2.48 mM of sulfite. It was higher than K_i calculated previously for mesophyll cell protoplasts of broadbean (1.69) or oat (1.40) (Lorenc-Plucińska and Ziegler 1989, Lorenc-Plucińska et al. 1989). In the mesophyll cell protoplast, the transport of sugars takes place *via* a proton co-transport system and the inhibitive effect of sulfite on the active uptake of sugar was the result of disturbances in the activity of the protein carrier and proton sugar symport mechanism. In contrast, the transfer of sucrose into vacuoles isolated from mesophyll cells of cottonwood was passive, and differences in the degree of sulfite inhibition of sucrose uptake may be a consequence of the different character of the transport mechanisms. On the other hand, it is rather doubtful, that the lower reduction of sucrose uptake into vacuoles than into protoplasts observed after sulfite action is due to different resistances of the tonoplast and plasmalemma to sulfite. At low pH values resistance of these biomembranes to the penetration of dissolved SO_2 is comparable (Pfan et al. 1987 a and b).

ACKNOWLEDGEMENTS

This work was supported by Grant no. 6 P204 101 04 from the State Committee for Scientific Research, Poland. Thanks are due to M. Sc. A. Konwińska and R. Żytkowiak and to Mrs. D. Szymańska and A. Niemir for their help in performing the isolation of vacuoles.

LITERATURE CITED

BLOM-ZANDSTRA M., KOOT H.T.M., VAN HATTUM J., 1990. Direct isolation of vacuoles from leaf tissue of lettuce (*Lactuca sativa*) retaining protoplasts within the leaves. *Physiol. Plant* 79: 693-699.

- BOLLER T., WIEMKEN A., 1986. Dynamics of vacuolar compartmentation. *Annu. Rev. Plant Physiol.* 37: 137-164.
- DICKMANN D.I., 1971. Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* Bartr.). *Bot. Gaz.* 132, 253-259.
- DIETZ K.J., JÄGER R., KAISER G., MARTINOIA E., 1990. Amino acid transport across the tonoplast of vacuoles isolated from barley mesophyll protoplasts. *Plant Physiol.* 92: 123-129.
- EINIG W., HAMPP R., 1990. Carbon partitioning in Norway spruce: amounts of fructose 2,6-bisphosphate and intermediates of starch/sucrose synthesis in relation to needle age and degree of needle loss. *Trees* 4: 9-15.
- GERHARDT, R., STITT M., HELDT H.W., 1987. Subcellular metabolite levels in spinach leaves: regulation of sucrose synthesis during diurnal alterations in photosynthetic partitioning. *Plant Physiol.* 83: 399-407.
- GOULD R.P., MINCHIN P.E.H., YOUNG P.C., 1988. The effects of sulphur dioxide on phloem transport in two cereals. *J. Exp. Bot.* 39: 997-1007.
- GUY M., REINHOLD L., MICHAELI D., 1979. Direct evidence for a sugar transport mechanism in isolated vacuoles. *Plant Physiol.* 64: 61-64.
- HAMPP R., ZIEGLER I., 1977. Sulfate and sulfite translocation via the phosphate translocator of the inner envelope membrane of chloroplasts. *Planta* 137: 309-312.
- HAMPP R., EINIG W., EGGER B., 1990. Energy and redox status, and carbon allocation in one- to three-year-old spruce needles. *Environ. Poll.* 68: 305-318.
- HOMMEYER U., SCHULTZ G., 1988. Transport of phenylalanine into vacuoles isolated from barley mesophyll protoplasts. *Planta* 176: 378-382.
- KAISER G., HEBER U., 1984. Sucrose transport into vacuoles isolated from barley mesophyll protoplasts. *Planta* 161: 562-568.
- KAISER G., MARTINOIA E., WIEMKEN A., 1982. Rapid appearance of photosynthetic products in the vacuoles isolated from barley mesophyll protoplasts by a new fast method. *Z. Pflanzenphysiol.* 107: 103-113.
- LORENC-PLUCIŃSKA G., 1994. Sucrose distribution in *Populus deltoides* leaves exposed to sulfite. *Arbor. Kórn.* 39: 125-133.
- LORENC-PLUCIŃSKA G., ZIEGLER H., 1989. Mechanisms of sugar uptake inhibition by sulfite in leaf discs and protoplasts of *Vicia faba* L. *Biochem. Physiol. Pflanzen* 184: 107-126.

- LORENC-PLUCIŃSKA G., MISZALSKI Z., ZIEGLER H., 1989. Sugar transport and sulfite action in etioprotoplasts, semi-etio-protoplasts and green protoplasts of *Avena sativa* L. *Botanica Acta* 102: 37-45.
- MARQUES I.A., ANDERSON L.E., 1986. Effect of arsenite, sulfite, and sulfate on the photosynthetic carbon metabolism in isolated pea (*Pisum sativum* L., cv Little Marvel) chloroplasts. *Plant Physiol.* 82: 488-493.
- MARTINOIA E., HECK U., WIEMKEN A., 1981. Vacuoles as storage compartments for nitrate in barley leaves. *Nature* 289: 292-294.
- MARTINOIA E., FLÜGGE U.I., KAISER G., HEBER U., HELDT H.W., 1985. Energy dependent uptake of malate into vacuoles isolated from barley mesophyll protoplasts. *Biochem. Biophys. Acta* 806: 311-319.
- MARTINOIA E., SCHRAMM M.J., KAISER G., KAISER W.M., HEBER U., 1986. Transport of anions in isolated barley vacuoles. I. Permeability to anions and evidence for a Cl⁻-uptake system. *Plant Physiol.* 80: 895-901.
- MARTINOIA E., KAISER G., SCHRAMM M.J., HEBER U., 1987. Sugar transport across the plasmalemma and the tonoplast of barley mesophyll protoplasts. Evidence for different transport systems. *J. Plant Physiol.* 131: 467-478.
- MAUROUSSET L., BONNEMAIN J.-L., 1990. Mechanism of the inhibition of phloem loading by sodium sulfite: effect of the pollutant on the transmembrane potential difference. *Physiol. Plant.* 80: 233-237.
- MAUROUSSET L., LEMOINE R., GALLET O., DELROT S., BONNEMAIN J.-L., 1992. Sulfur dioxide inhibits the sucrose carrier of the plant plasma membrane. *Biochim. Biophys. Acta* 1105: 230-236.
- MOONEY H.A., WINNER W.E., 1988. Carbon gain, allocation, and growth as affected by atmospheric pollutants. In: Schulte-Hostede S., Darral N.M., Wellburn A.R. (eds). *Air pollution and plant metabolism*. Elsevier Applied Sciences, London and New York, 272-288.
- PFANZ H., MARTINOIA E., LANGE O.-L., HEBER U., 1987a. Mesophyll resistance to SO₂ fluxes into leaves. *Plant Physiol.* 85: 922-927.
- PFANZ H., MARTINOIA E., LANGE O.-L., HEBER U., 1987b. Flux of SO₂ into leaf cells and cellular acidification by SO₂. *Plant Physiol.* 85: 928-933.
- RAUTENKRANZ A.A.F., LI L., MÄCHLER F., MARTINOIA E., OERTLI J.J., 1994. Transport of ascorbic and dehydroascorbic acids across protoplast and vacuole membranes isolated from barley (*Hordeum vulgare* L. cv Gerbel) leaves. *Plant Physiol.* 106: 187-193.
- STITT M., QUICK W.P., 1989. Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. *Physiol. Plant.* 77: 633-641.
- STITT M., HUBER S., KERR P., 1987. Control of photosynthetic sucrose formation. *The biochemistry of plants* 10: 327-409.
- THOM M., KOMOR E., MARETZKI A., 1982. Vacuoles from sugar-cane suspension cultures. II. Characterization of sugar uptake. *Plant Physiol.* 69: 1320-1325.
- ZIEGLER I., 1975. The effect of SO₂ pollution on plant metabolism. *Res. Rev.* 56: 79-105.

WPLYW SIARCZYNU NA TRANSPORT SACHAROZY DO WAKUOLI IZOLOWANYCH Z LIŚCI TOPOLI

STRESZCZENIE

Badano mechanizm transportu sacharozy do wakuoli izolowanych z mezofilu liści topoli *Populus deltoides* Bartr. ex Marsh i wpływ różnych stężeń jonów siarczynowych na jego aktywność.

W mechanizmie transportu sacharozy stwierdzono obecność kinetyki typu dwufazowego. Komponent wysycony był wrażliwy na działanie kwasu p-chlorortęciobenzenosulfonowego (PCMBs) i pH środowiska inkubacyjnego oraz kompetycyjny względem substratu. Aktywność pobierania sacharozy przez izolowane wakuole nie ulegała istotnym zmianom w obecności ATP, lub MgATP, antybiotyku jonoforowego, walinomycyny oraz rozpręgaczy, karbonylocyjanku m-chlorofenylohydrazonu (CCCP) i dwunitrofenolu (DNP).

Siarczyn w stężeniu 0.01-2.5 mM hamował pobieranie sacharozy przez izolowane wakuole. Hamowanie to było typu niewspółzawodniczego, a stałą inhibicji K_i skalkulowano dla 2.48 mM siarczynu.

SŁOWA KLUCZOWE: *Populus deltoides*, izolowane wakuole, transport sacharozy, działanie siarczynu.