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Sulfur dioxide inhibits the sucrose carrier of the plant plasma membrane

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Plasma membrane vesicles were prepared by phase partition from a microsomal fraction of broad bean (*Vicia faba* L.) leaf. In order to study the effects of sodium sulfite on active uptake of sucrose, the vesicles were artificially energized by a transmembrane pH gradient (ΔpH) and/or a transmembrane electrical gradient ($\Delta\psi$). At 1 mM, sulfite strongly inhibited sucrose uptake but did not affect the two components of the proton motive force, ΔpH (measured by dimethyloxazolidine dione) and $\Delta\psi$ (measured by tetraphenylphosphonium). Moreover, sulfite did not inhibit the proton-pumping ATPase of the plasma membrane vesicles. These data demonstrate that sulfite may inhibit transport of photoassimilates in plant by a direct inhibition of the sucrose carrier of the plasma membrane.

Introduction

Sulfur dioxide is one of the major gaseous air pollutants that causes damage to agricultural crops and natural vegetation, thereby affecting plant productivity. Exposure of plants to high concentrations of SO_2 can cause chlorosis and necrosis of leaf tissue, which lead to reduction in growth. Reduced plant growth in the absence of any visible injury has also been observed at relatively low ambient SO_2 concentration [1]. A concentration of 0.035 ppm SO_2 in air can give rise to 35 ppm sulfite in aqueous solutions [2]. Taking into account this important solubility in water, this pollutant can be accumulated in leaf tissues and disturb major physiological activities such as photosynthesis [3,4], respiration [5–7] and translocation of assimilates [8–10]. The latter process is not related to the effect on photosynthesis itself and results from an inhibition of phloem loading [11,12]. Phloem loading is a major physiological process in plants, because it controls the osmotic gradient that allows long distance transport of assimilates via a pressureflow mechanism. Yet, the mechanism of inhibition of phloem loading by sulfite is still poorly understood. It has been suggested that this inhibition may be due to a direct effect of SO_2 on the carrier molecules involved in phloem loading [11,12], but this suggestion has not been experimentally confirmed.

In *Vicia faba*, photosynthetic sucrose is released into the leaf apoplast and sucrose is accumulated from this compartment into the complex transfert cell – phloem cell [13,14]. The mechanism of sucrose accumulation into the conducting complex is a H^+-sucrose cotransport. The energy is provided by a plasma membrane H^+-pumping ATPase which creates a pmf [15,16]. SO_2 or its aqueous derivates (sulfite and bisulfite ions) could therefore affect either the primary (ATPase) and/or the secondary (sucrose carrier) translocation. The potential targets of the pollutant are the H^+-pumping ATPase, the phospholipid bilayer (permeability to ions), the processes supplying ATP and the sucrose carrier.

At concentrations of sulfite higher than 0.1 mM, inhibition of sucrose uptake into leaf discs is due at least in part to a decrease of the ATP supply which normally fuels the proton-pumping ATPase [17]. Yet, at 50 μM sulfite also inhibits phloem loading [18], while it does not affect respiration and energy charge [17]. These data suggest that the sucrose carrier is another target of the pollutant.

Abbreviations: BSA, bovine serum albumin; BTP, Bistris-propane; DMO, dimethyloxazolidine-2,4-dione; DTT, dithiothreitol; Mops, 3-(*N*-morpholino)propanesulfonic acid; PEG, poly(ethylene glycol); pmf, protonmotive force; TPP, tetraphenylphosphonium.

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The purpose of the present work was to test further this hypothesis by studying the effects of sulfite on active uptake of sucrose by purified plasma membrane vesicles. This experimental approach avoids some problems linked to the use of leaf discs (metabolism of the substrate, diffusion through the wall). Indeed, plasma membrane vesicles energized by an artificial protonmotive force provide the simplest model for studying sucrose uptake directly at the membrane level [19–21].

Although leaves are net exporters of sucrose, this export strongly depends on the ability of the mesophyll cells to retrieve the assimilates from the leaf apoplast, until they are leaked in the vicinity of the phloem cells, and on the ability of the phloem cells to take up sucrose. Therefore, export of sucrose is tightly dependent of at least two uptake processes at the plasma membrane level [22].

Materials and Methods

Preparation of plasma membrane vesicles

Growth of broad bean (*Vicia faba* L. cv Aguadulce) has been described by M'Batchi et al. [23]. Plasma membrane vesicles were prepared by phase partition from a microsomal fraction of leaf tissues according to Gallet et al. [24] with minor changes. The final composition of the two phase systems was 6.5% (w/w) Dextran T-500, 6.5% (w/w) PEG 3350, 300 mM sorbitol, 3 mM KCl 0.5 mM DTT, 5 mM potassium phosphate (pH 7.8). The vesicles were equilibrated for 30 min in medium K (50 mM potassium phosphate (pH 7.5), 300 mM sorbitol, 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 0.5 mM DTT). After pelleting for 45 min at $100000 \times g$, the vesicles were resuspended as a concentrated solution (about 10 mg protein-ml^{-1}) in medium K without DTT, aliquoted, frozen in liquid nitrogen and stored at −80°C until further use. Characterization of these preparations by using marker enzymes has shown that they are highly enriched in plasma membranes [25].

Determination of the internal volume of the vesicles

The internal volume of the vesicles was determined by the equilibration method using ^3H_2O (marker for the water space) and [^{14}C]dextran (marker for the extracellular space) [26,27]. Vesicles (1 mg ml^{-1}) were incubated in 150 μl buffer K containing ^3H_2O (69 kBq ml^{-1}) and [^{14}C]dextran (42 kBq ml^{-1}) for 10 min. Vesicles were then pelleted by a 5-min centrifugation at $165000 \times g$ in an Airfuge (Beckman) [27]. Two 50-μl aliquots of the supernatant added with 100 μl of 0.1% Triton X-100 were taken for the determination of the volumic activities for both ^{3}H and ^{14}C. The tip of the airfuge tube containing the vesicle pellet was cut off and placed in a scintillation vial with 150 μl of 0.1% Triton X-100. The supernatant aliquots and the pellet were counted for determinations of ^{3}H and ^{14}C radioactivity. The volume corresponding to the water space and to the dextran space in the pellet were calculated from the corresponding volumic activity and the internal volume of vesicles was obtained by substracting the dextran space from the water space.

Sucrose uptake experiments

Uptake experiments were run according to Lemoine and Delrot [21] except that DTT was omitted in the incubation media. Briefly, a 2-μl aliquot of vesicles (10 mg protein ml^{-1}) equilibrated for 30 min with medium K containing 5 μM valinomycin was diluted in 400 μl of incubation medium whose composition depended on the gradient to be created. At the end of the incubation, vesicles were collected by filtration and rinsed [21]. For experiments in energized conditions, vesicles were diluted either in medium K buffered at pH 5.5 to generate ΔpH, or in medium Na pH 7.5 (same as medium K except that it was buffered at pH 7.5 with 50 mM sodium phosphate) to create $\Delta\psi$, or in medium Na pH 5.5 (50 mM sodium phosphate buffer, pH 5.5) to combine the electrical and the pH gradients ($\Delta\psi$ + ΔpH). Resuspension of the vesicles in medium Na resulted in the generation of $\Delta\psi$, due to rapid diffusion of internal potassium towards the extravesicular space, along its concentration gradient. In all cases, the incubation medium also contained 1 mM [^{3}H]sucrose (radioactivity: 25.9 kBq, final specific activity: 64.8 GBq mol^{-1}).

To study the effect of sodium sulfite on sucrose uptake, salts of Na_2SO_3 were dissolved into the experimental buffers and the pH of the solution were readjusted if necessary. The K_m value for sucrose uptake in plasma membrane vesicles from broad bean leaves under energized conditions (ΔpH + $\Delta\psi$) is about 0.6 mM (Maurousset et al., Ref. 38). Altough this does not allow to reach the V_{max}, the concentration of 1 mM sucrose was used in the present study, in order to avoid too rapid dissipation of the protonmotive force imposed by high sucrose concentrations, and to allow longer times for sampling, thus reducing the experimental error.

ATPase activity (P_i release) and proton pumping

For these experiments, vesicles were equilibrated in a medium containing 300 mM sucrose, 100 mM KCl, 0.5 mM DTT and 10 mM Mops-BTP at pH 7.0. Plasma membrane vesicles were frozen in liquid N_2 and thawed in water at 25°C a total of four times, in order to produce a mixture of inside-out and right-side-out vesicles [28]. H^+ uptake into the vesicles was monitored as the absorbance decrease at 495 nm of the pH probe acridine orange [29]. The assay medium consisted of 300 mM sucrose, 100 mM KCl, 0.1% BSA, 20 μM acridine orange, 3 mM ATP-BTP, 2.5 μM valinomycin,

10 mM Mops-BTP (pH 7.0) and 150 μg membrane proteins in a total volume of 1.5 ml. After a 5-min preincubation in the dark at ambiant temperature, the reaction was initiated by addition of 3 mM $MgCl_2$. The rate of H^+ accumulation was estimated from the initial slope of absorbance quenching (ΔA_{495}) of acridine orange. At the end of the recording (25 min) the proton gradient was dissipated with 5 μM nigericin and the absorbance returned routinely to the initial value before the addition of $MgCl_2$. Aliquots of 100 μl were withdrawn after 5, 10, 15 and 20 min for P_i determination according to Ames [30].

Determination of ΔpH and $\Delta\psi$

The two components of the pmf were measured simultaneously according to Ref. 27 using DMO to monitor ΔpH and TPP to monitor $\Delta\psi$. The two components of the pmf were determined simultaneously by incubating the vesicles as described above, except that sucrose in the incubation media was replaced with 4 μM [^{3}H]tetraphenylphosphonium ([^{3}H]TPP, 4.8 TBq mol^{-1}) to evaluate $\Delta\psi$ and 10 μM [^{14}C]dimethyloxazolidinedione ([^{14}C]DMO, 2.01 TBq mol^{-1}) to evaluate ΔpH. For those experiments, cellulose acetate filters (Sartorius, ref. 1110625N) were used to collect the vesicles at the end of incubation because they retained less TPP than the regular filters (mixed esters of cellulose, Millipore ref. HAWP) routinely used for sugar uptake experiments. The retention of the vesicles on both types of filters was the same.

Four sets of experiments were run in parallel: one in the presence of $\Delta\psi + \Delta$pH (external buffer sodium phosphate, pH 5.5), one in the presence of ΔpH alone (external buffer potassium phosphate, pH 5.5), one in the presence of $\Delta\psi$ alone (external buffer Na-phosphate, pH 7.5), and the last one in the absence of any gradient (external buffer potassium phosphate, pH 7.5). For the calculations, the uptake values in the absence of gradients were considered as a measurement of the unspecific binding of the probes to the vesicles. The electrical gradient was calculated from the equation:

$$\Delta\psi = Z \log([TPP]_{in}/[TPP]_{out})$$

where $[TPP]_{in}$ is the difference (TPP uptake in the presence of the gradient(s) studied minus TPP uptake in the absence of gradient) divided by the internal volume of the vesicles (2.3 μl (mg protein)$^{-1}$). The dissociation equilibrium of DMO ($pK_a = 6.3$) is different at pH 5.5 (conditions used for $\Delta\psi + \Delta$pH and ΔpH) and at pH 7.5 (conditions used for $\Delta\psi$ and for $\Delta pH = \Delta\psi = 0$). Therefore, in the former case the values obtained for DMO uptake were converted into ΔpH according to the equation:

$$\Delta pH = \log([DMO]_{in}/[DMO]_{out})$$

whereas in the latter case $\Delta pH = pH_{in} - pH_{out}$ with:

$$pH_{in} = \log\{([DMO]_{in}/[DMO]_{out})(10^{pK} + 10^{pH}_{out}) - 10^{pK}\}$$

These calculations were made according to Rottenberg [26].

The protein content was measured according to Ref. 31 using BSA as a standard.

Results and Discussion

Determination of the internal volume of plasma membrane vesicles

The knowledge of the internal volume of the vesicles is necessary to evaluate the internal sucrose concentration of the vesicles and the size of the gradients (ΔpH and $\Delta\psi$) actually imposed. A sucrose concentration inside the vesicles higher than the external one is a necessary condition to demonstrate an active uptake of sucrose occurring against a concentration gradient. We have used the method described in [27] which allows a precise determination of the osmotic volume of the vesicles by comparing the volume correspondig to a permeant compound (^3H_2O) with the volume corresponding to a compound which does not enter the vesicles ([^{14}C]sorbitol or [^{14}C]dextran). Although the use of sorbitol allowed measurements of the extravesicular volume with plasma membrane vesicles from sugar beet leaves [27], preliminary experiments showed that sorbitol entered to some extent into the plasma membrane vesicles from broad bean. Therefore, [^{14}C]dextran was used instead of [^{14}C]sorbitol to measure the extravesicular volume in the present study. The difference between the dextran space and the water space was studied at different osmolalities (Fig. 1). When the lines are extrapolated to infinite osmolality, the two lines should merge because the internal volume of the vesicles should be zero. This is not observed in Fig. 1. According to Ref. 26, this difference between the two volumes at infinite osmolality (9.0 μl (mg protein)$^{-1}$ in Fig. 1) could correspond to non-osmotically active water (i.e. water that cannot move freely into and out of the vesicles in response to osmotic changes). Some explanations for this phenomenon have been proposed (binding of water to lipids, presence of unstirred layers, see Ref. 27). It is possible to calculate the total non-osmotic water space by adding the value found at infinite osmolality (9.0 μl (mg protein)$^{-1}$) to the dextran space as represented in Fig. 1 (open circles). The internal volume corresponding to osmotically active water is 2.3 μl (mg protein)$^{-1}$ under the osmotic conditions used for uptake experiments (300 mM sorbitol). This value is very close to the one determined with the same method on plasma membrane vesicles isolated from sugar beet leaves (2.2 μl (mg protein)$^{-1}$, [27]). The osmotic volume value determined has been

used for the subsequent calculations of sucrose concentrations, ΔpH and $\Delta\psi$.

Sucrose uptake

Most of the plasma membrane vesicles prepared by phase partition are right-side-out (80%, [27]). This makes it impossible to energize proton-sucrose cotransport via the supply of ATP to the proton-pumping ATPase, whose active site is inaccessible by external ATP. However, uptake of sucrose in these vesicles can be studied after imposing artificial gradients [19–21].

In the absence of any gradient (same buffer inside and outside the vesicles, non-energized conditions), the vesicles took up very little sucrose (Fig. 2, closed circles). Even after 10 min, the amount of sucrose taken up (1.8 nmol (mg protein)$^{-1}$) was smaller than the amount that could be expected from the passive (or diffusive) equilibration of sucrose (2.34 nmol for a 1 mM external sucrose concentration). It is therefore likely that sucrose slowly entered the vesicles by diffusion under these conditions.

In the presence of ΔpH alone, a rapid accumulation of sucrose into the vesicles occurred during the first minute of incubation (Fig. 2, closed squares). Then, the sucrose content of the vesicles slowly decreased up to 10 min. Given the internal volume estimated previously, 2.3 μl (mg protein)$^{-1}$, the maximal accumulation ratio (internal sucrose concentration/external sucrose concentration) after 1 min incubation was about 3, confirming that sucrose uptake occurred against a concentration gradient. Energization by $\Delta\psi$ led to a slightly lower uptake of sucrose by vesicles (about 7 nmol (mg protein)$^{-1}$, Fig. 2, open squares), but statistically the difference was not significant. Thus, $\Delta\psi$ and ΔpH are apparently equivalent in energizing sucrose transport across the plasma membrane. This result has also been reported by Kalinin and Opritov [32], Lemoine and Delrot [21]. In the presence of $\Delta\psi$ + ΔpH (Fig. 2, open circles), the maximum uptake of sucrose (14.6 nmol (mg protein)$^{-1}$) was reached after 2 min of incubation, giving an accumulation ratio of about 6.2. The value of sucrose uptake measured in the presence of ΔpH + $\Delta\psi$ corresponded to the sum of the values obtained in the presence of ΔpH or $\Delta\psi$ alone. When the duration of incubation was longer than 2 min, sucrose uptake decreased and was stable around 10 nmol (mg protein)$^{-1}$. The absolute values of sucrose uptake, as well as the maximum accumulation ratio were about 2-fold lower in broad bean plasma membranes (Fig. 2) than in sugar beet plasma membranes [27]. The curve obtained is typical for an overshoot phenomenon which was due to a rapid dissipation of the protonmotive force [33]. This phenomenon was also reported in the studies of sucrose uptake conducted with plasma membranes from other plant materials: sugar beet [27], *Ricinus* cotyledons [34].

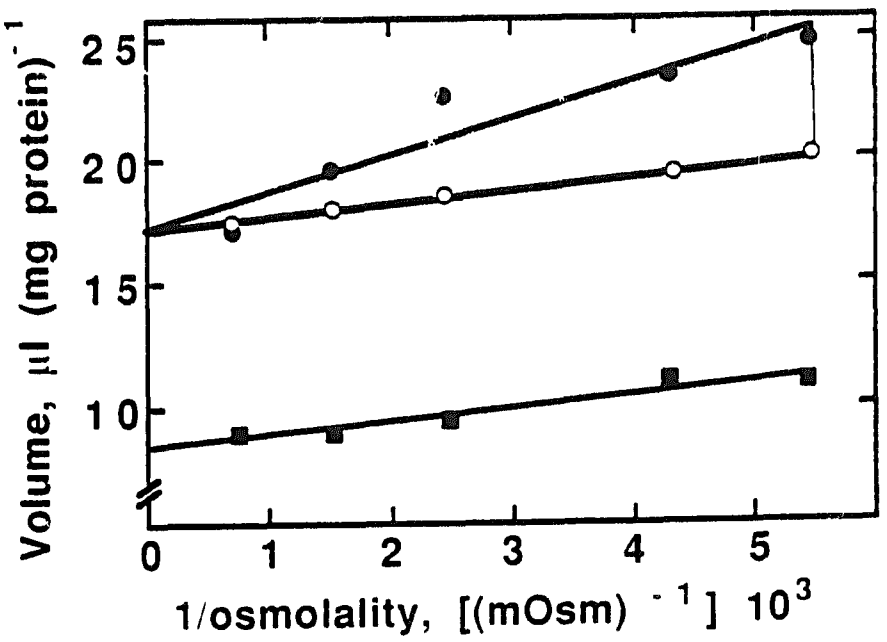

Fig. 1. Measurement of the internal volume of the vesicles at different osmolalities. The sorbitol concentration ranged from 50 to 1000 mM. ■, volume accessible to [^{14}C]dextran. ●, volume accessible to ^3H_2O. ○, volume accessible to non-osmotically active water. The lines were drawn by linear regression. The shaded area represents the variation of the osmotic space. Results are the mean of four replicates in one typical experiment. The standard errors were smaller than the symbols used. The arrow indicates the osmolality of the standard media used for uptake experiments.

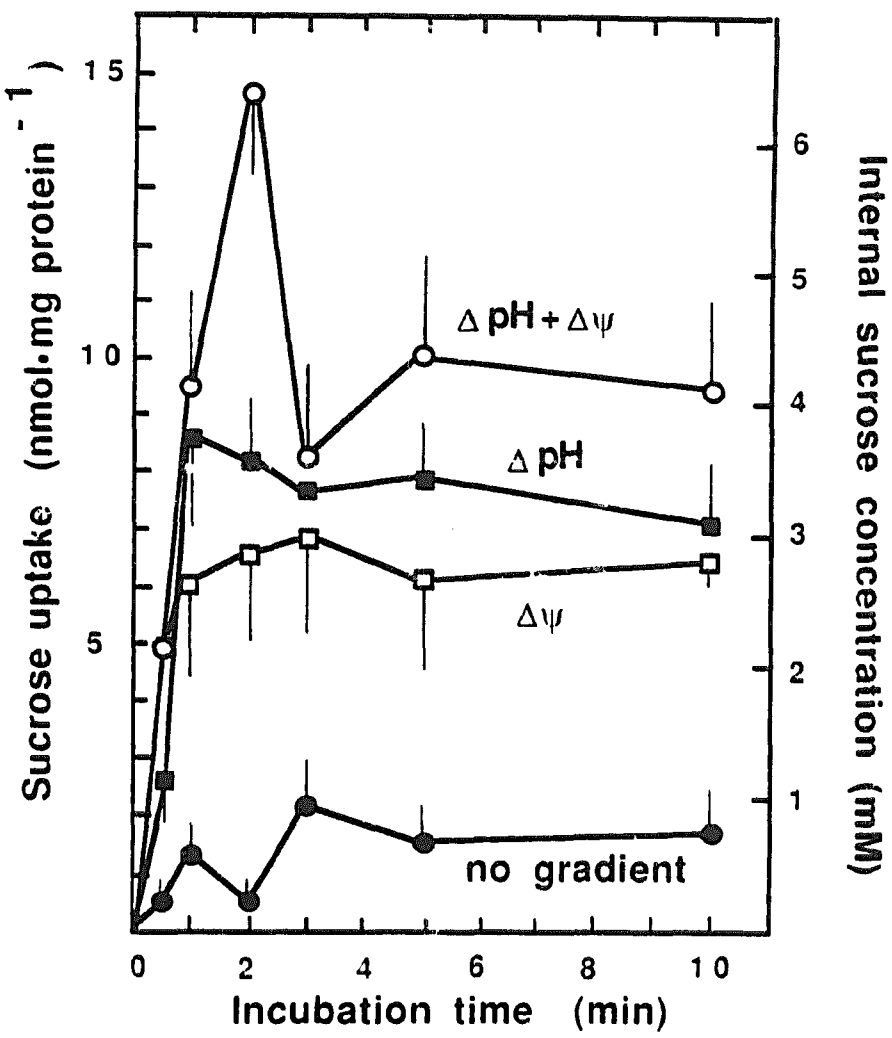

Fig. 2. Time dependent uptake of 1 mM sucrose. Experiments were run in the presence of ΔpH (■), $\Delta\psi$ (□) and ΔpH + $\Delta\psi$ (○). The uptake values under non-energized conditions (absence of gradient) are also shown (●). Each point is the mean ± S.E. of 18 replicates (five independent experiments). The left ordinate corresponds to uptake values whereas the right ordinate corresponds to the sucrose internal concentration computed from the uptake values and the internal volume of the vesicles (2.3 μl (mg protein)$^{-1}$).

The lower accumulation of sucrose in broad bean may be due to a lower efficiency of the artificial energization provided, to a lower density of the sucrose carriers, or to a weaker coupling between the imposed pmf and the sucrose carrier. Although measurements of the gradients actually imposed to the plasma membranes show that they are somewhat smaller in broad bean than in sugar beet, this explanation is not sufficient to account for the difference of sucrose uptake between both species (see below).

No trace of sucrose metabolism during uptake into plasma membranes from broad bean leaf was found [38], confirming earlier data obtained with sugar beet [21].

It is also interesting to compare the rate of sucrose measured into purified plasma membrane vesicles from broad bean with the rate of sucrose uptake measured in vivo with leaf tissues of the same species. The rate of uptake of sucrose into peeled broad bean leaf tissues (from a 1 mM sucrose solution) is about 0.2 nmol cm^{-2} min^{-1} [16]. The amount of plasma membrane recovered after phase partitioning from 1 g FW (= 56.5 cm^2) leaf tissue is about 14.2 μg [35]. If all sucrose uptake activity was recovered, the uptake measured in plasma membrane vesicles (9.5 nmol (mg protein)$^{-1}$) after the first min of incubation (Fig. 2) would convert to an uptake rate of 2.4 pmol cm^{-2} min^{-1}. This figure is about 80 times lower than that measured in vivo, which is not surprising, since substantial loss of activity may be expected from the fractionation process and the phase partitioning. Moreover, the rates measured in vivo are measured under steady states conditions, with a constant energization of the membrane via the proton-pumping ATPases, while the rates measured with the vesicles are obtained after imposition of a transient and artificial protonmotive force.

Effect of Na_2SO_3 on sucrose uptake

The effect of sulfite was studied at the time when the uptake of sucrose was maximum, i.e. after 2 min of incubation. First, some experiments were conducted to verify whether a pretreatment was necessary to observe any inhibition by sulfite as was the case for leaf discs [18]. Sulfite was present during the incubation with sucrose, at the same concentration as during the pretreatment. Inhibition of sucrose uptake into vesicles energized by $\Delta\psi + \Delta$pH increased with the pretreatment duration and reached 50% after 1 h pretreatment. It did not increase significantly for longer pretreatments (data not shown). Thus, the effect of different concentrations of sulfite was studied on sucrose uptake into vesicles treated by sulfite for 1 to 2 h. The inhibition of sucrose uptake by sulfite (25%) was not statistically significant when uptake was energized by $\Delta\psi$ alone, while this inhibition reached 55% and was statistically significant when uptake was energized by

TABLE I

Effect of the sulfite concentration on the uptake of 1 mM sucrose energized by ΔpH $\Delta\psi$ or both

The uptake values indicated are for a 2-min incubation. The results are the mean ± S.E. of 16 replicates (four experiments). The numbers in parentheses indicate the percentage of inhibition relative to the corresponding control. Probability: *, uptake values significantly different (0.05 level) from the corresponding control.

Na_2SO_3 (mM)	Sucrose uptake (nmol (mg protein)$^{-1}$) in the presence of		
	ΔpH	$\Delta\psi$	$\Delta\psi + \Delta$pH
0	8.7 ± 1.4	7.4 ± 1.4	15.4 ± 2.3
0.1	4.8 ± 1.6 * (45)	5.7 ± 1.2 (23)	12.4 ± 4.6 (20)
1	3.7 ± 1.1 * (57)	5.5 ± 1.1 (26)	7.6 ± 1.9 * (50)
1.0			4.6 ± 2.2 * (70)

ΔpH alone (Table I). Equivalent degree of inhibition was achieved with 0.1 and 1 mM sulfite. Of interest, inhibition showed a sulfite concentration dependence when the two driving forces were combined (Table I). These percentages were the same as those obtained with leaf discs after a 2-h preincubation followed by 30 min incubation ([18], Fig. 1). Therefore, a good agreement between the biochemical (use of plasma membrane vesicles) and the physiological (use of leaf discs) approaches was obtained. Inhibition of sucrose uptake not only in leaf tissues, but also in vesicles energized artifically, suggests that the effect of sulfite may not be restricted to a single mechanism, ATP supply [17].

Determination of $\Delta\psi$ and ΔpH

The two components of the pmf (ΔpH, $\Delta\psi$) were measured according to Ref. 27 to investigate a possible effect of sulfite on the ionic permeability of the plasma membrane vesicles. The radiolabelled probes used ([^{3}H]TPP and [^{14}C]DMO) allow a reliable estimation of the gradients as long as the problem of binding and passive entry of the probes are taken into consideration [36]. Therefore, the data presented here were corrected by substracting the values measured in the absence of gradient (non-energized conditions) from the values measured in the presence of an imposed gradient [27]. For TPP, the values measured under non-energized conditions represented 5% of the values measured with vesicles energized by $\Delta\psi$, and for DMO, they represented 40% of the values measured in the presence of ΔpH. Sulfite did not affect the passive binding of the probes.

In agreement with data obtained with plasma membranes from sugar beet leaf [27], ΔpH and $\Delta\psi$ showed a tendency to decrease during a 20-min incubation (data not shown), reflecting the passive dissipation of the ionic gradients. The different gradients measured and the effect of 1 mM sulfite are presented in Table II. Table II shows that a 1 h preincubation with 1 mM

TABLE II

Effect of sodium sulfite on ΔpH and Δψ imposed to the plasma membrane vesicles for the energization of sucrose uptake

Vesicles were pretreated with sulfite for 1 h. The results are the mean of four replicates (one experiment).

Na_2SO_3 (mM)	ΔpH (unit)		Δψ (mV)	
	2 min	10 min	2 min	10 min
0	0.83	0.70	−122	−101
1	0.87	0.93	−126	−100

sulfite did not affect the ΔpH measured after 2 min incubation and increased the ΔpH after 10 min incubation. In control vesicles, Δψ decreased from a value of −122 mV after 2 min to a value of −101 mV after 10 min. These values are only slightly lower than the one measured on sugar beet leaves plasma membrane vesicles (respectively, −155 mV and −110 mV after 2 and 10 min incubation, 27]). Preincubation with 1 mM sulfite did not affect Δψ (Table II). It is therefore possible to conclude that sulfite did not alter the ΔpH and Δψ imposed to energize the uptake of sucrose. The lack of effect of sodium sulfite on ΔpH and Δψ at a concentration which markedly inhibited active uptake of sucrose by the vesicles implies a direct inhibition of the sucrose carrier by the pollutant, confirming the hypothesis made by several authors [9–12]. In preliminary experiments (data not shown), sulfite inhibition of sucrose uptake was not detected if vesicles had been stored in the presence of 1 mM DTT, suggesting a possible reaction of sulfite with the thiol groups present in (or close to) the binding site of the sucrose carrier [23].

It is worth noting that the ΔpH measured decreased from a value of 0.83 after 2 min to a value of 0.70 after 10 min of incubation. These values were lower than the imposed gradient of 2.0 pH units. This phenomenon was already noticed on plasma membrane vesicles of sugar beet using the same method of measurement [27] and on Zucchini vesicles using electron spin resonance spectroscopy [37]. The latter authors could not measure a gradient higher than 1.4 unit even with an imposed gradient of 2.4 units and they suggested the existence of a control mechanism dissipating the H^+ gradient above a certain threshold value. Nevertheless, the pH gradient measured here (0.83) is smaller than the one measured on sugar beet leaves vesicles (1.2, [27]).

Effect of Na_2SO_3 on the H^+-ATPase activity

Although the data presented above suggests that sulfite directly affects the sucrose carrier, previous results obtained with leaf discs or leaf fragments [18] demonstrated also a depolarizing effect of sulfite on the transmembrane potential difference. Part of the depolarizing effect of sulfite may be ascribed to the decrease of the energy charge induced by this pollutant in the tissues [17]. Yet, this does not exclude that sulfite may also affect directly the proton-pumping ATPase of the plasma membrane. Therefore, we studied the effects of sulfite on the two activities of this enzyme (cleavage of ATP and H^+-pumping).

TABLE III

Effect of sodium sulfite on the H^+-pumping and ATPase activities

The two activities have been evaluated simultaneously by measuring the release of P_i and the decrease of absorbance of acridine orange, with vesicles that have been subjected to four freeze-thaw cycles in order to get a mixture of right-side-out and inside-out vesicles. The results are the mean ± S.E. of six different experiments. Probability: *, value significantly different (0.05 level) from the control.

Na_2SO_3 (mM)	ATPase activity (nmol P_i (mg protein)$^{-1}$ min^{-1})	Proton flux ($-\Delta A$ (mg protein)$^{-1}$ min^{-1})
0	40.9 ± 2.3	0.15 ± 0.03
1	68.0 ± 6.3 *	0.20 ± 0.05

When assayed in the presence of 0.02% Triton X-100 to unmask the latent activity of the ATPase, a 1-h pretreatment with 1 mM Na_2SO_3 did not affect the ATPase activity of right-side out vesicles (data not shown). So the anions present in the sulfite solution at pH 7.0, HSO_3^- and SO_3^{2-}, had no significant effect on the ATPase activity.

For the following experiments, ATPase activity and proton pumping were studied simultaneously on a mixture of right-side-out and inside-out vesicles. According to the freeze-thaw procedure used [28], approximately 50% of the vesicles have the catalytic sites of the ATPase facing the outer medium. After a 1-h pretreatment, 1 mM Na_2SO_3 led to a 66% stimulation of the ATPase activity (Table III). An increase was noticed in H^+ flux but the difference was not statistically significant (Table III). The proton gradient could be dissipated by nigericin. After the change of orientation of the vesicles, sulfite strongly stimulated the ATPase activity. This effect of sulfite on the H^+-pumping ATPase activity might be due to the fact that the enzyme, whose catalytic site is located on the cytoplasmic side of the membrane, became more accessible to sulfite. However, the reasons of the sulfite-induced stimulation of ATPase activity are still unknown. Nevertheless, these results confirm that the inhibition of sucrose uptake by sulfite in leaf discs was not due to a direct inhibition of the plasma membrane H^+-ATPase.

Conclusion

Up till now, the effects of SO_2 or its derivatives on the uptake of photoassimilates were not studied at the membrane level. The effect of sulfite on sucrose up-

take into energized vesicles suggest that plasma membrane vesicles are a good model which reflects the in vivo conditions, because inhibition percentages were the same on leaf discs [18] and on plasma membrane vesicles. In plasma membrane vesicles from broad bean leaves, sucrose uptake occurs against a concentration gradient and is maximum in the presence of $\Delta pH + \Delta\psi$. This active uptake occurs at a lower rate than in plasma membrane vesicles from sugar beet leaf. Sulfite markedly decreased sucrose uptake, without inhibiting ATPase activity, proton pumping, or the two components of the pmf. These results confirm that the H^+-ATPase is not directly affected by the pollutant. They show that besides its effect on energy charge [17], sulfite may affect directly the transport of photoassimilates in the plant via its effect on the sucrose carrier of the plasma membrane.

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