

Mesophyll Resistances to SO₂ Fluxes into Leaves¹

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ABSTRACT

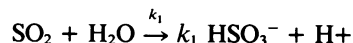
Uptake of label from solutions containing ³⁵SO₂, H³⁵SO₃⁻ and ³⁵SO₃²⁻ into mesophyll protoplasts, vacuoles, and chloroplasts isolated from young barley leaves was measured at different pH values. Uptake was fast at low pH, when the concentration of SO₂ was high, and low at high pH, when the concentration of SO₂ was low. When the resistance (*R*) of plasmalemma, tonoplast, and chloroplast envelope to the penetration of SO₂ was calculated from rates of uptake of label, comparable values were obtained for the different biomembranes at low pH values. *R* was close to 8000 seconds per meter and permeability coefficients were close to 1.25 × 10⁻⁴ meters per second. Under these conditions *R* may describe resistance to SO₂ diffusion across a lipid bilayer. At higher pH values, *R* decreased. As *R* was calculated on the assumption that SO₂ is the only penetrating molecular species, the data suggest that carrier-mediated anion transport contributes to the uptake of sulfur at physiological pH values thereby decreasing apparent *R*_{SO₂}. The contribution of anion transport appeared to be smaller for transfer across the plasmalemma than for transfer across the tonoplast. It was large for transfer across the chloroplast envelope. The phosphate translocator of the chloroplast envelope catalyzed uptake of SO₃²⁻ into chloroplasts at neutral pH. Uptake was decreased in the presence of high levels of phosphate or sulfate and by pyridoxal phosphate. SO₂ transfer into cells leads to the intracellular liberation of one or two protons, depending on pH and oxidizing conditions. When the divalent sulfite anion is exchanged across the chloroplast envelope, bisulfite formation results in proton uptake in the chloroplast stroma, whereas SO₂ uptake into chloroplasts lowers the stroma pH.

Diffusional fluxes are known to obey Fick's law. Flux is proportional to gradients in chemical potential of a gas or a solute and inversely proportional to resistance encountered along a diffusional path. A low concentration of CO₂ in air (0.03%) is sufficient to support high rates of photosynthesis in leaves even though the CO₂ concentration at the site of carboxylation in the chloroplast stroma is significant and, therefore, the CO₂ gradient is not large. Obviously, leaves are constructed so as to permit effective gas exchange as long as stomates are open and stomatal resistance to gas flow is accordingly low.

Effectiveness of gas transport cannot be restricted to a particular gas. If CO₂ can enter into leaves, so can gaseous air pollutants. They encounter the same barriers that must be overcome during CO₂ diffusion. However, different gases possess different

diffusion coefficients, different solubilities, and different reactivities in lipid and aqueous phases. These characteristics influence flow rates.

Diffusion of SO₂ in air is only about half as fast as diffusion of water vapor in air. Thus, if stomatal resistance to water loss by transpiration from the leaves has been determined, stomatal resistance to SO₂ entry can be calculated simply by multiplying the water resistance value with the factor 1.89 (25). Mesophyll resistances to SO₂ penetration are not known. Because the solubility of SO₂ in water exceeds that of CO₂ by a factor of about 40 (4, 12), and because dissolved SO₂ is readily removed from aqueous solutions according to



(rate constant $k_1 = 3.4 \times 10^6 \text{ s}^{-1}$, [19]), the intercellular SO₂ concentration is often considered to be zero (20, 25). This is at best an approximation. On its path into the cells, SO₂ meets several membrane barriers. They can be overcome only if there is a gradient in SO₂ concentration across the barriers. We were interested in estimating permeability characteristics of plasma membranes. Knowing them, and knowing intracellular pH values, we can calculate fluxes of SO₂ into different cell compartments. In the present study, we report on SO₂ transfer into leaves, across the plasmalemma of mesophyll cells, the tonoplast, and the chloroplast envelope.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* var Gerbel) was grown in a growth cabinet. The daily illumination period was 12 h (90 W m⁻²), the temperature 22° C in the light and 20° C in the dark. For the isolation of protoplasts and organelles, 10-d-old plants were used.

Isolation of Protoplasts. Mesophyll protoplasts were isolated according to Kaiser *et al.* (13). The concentration of the cellulase used for digesting cell walls was 1% (w/v), that for macerozyme 0.5%. For purification, a three step gradient was prepared with the lower phase containing 8 ml protoplast suspension (1 mM CaCl₂, 0.5 M sorbitol, 30% [w/v] Percoll, and 30 mM Mes or Hepes) adjusted to various pH values. Phase two consisted of 0.5 M sorbitol, 1 mM CaCl₂, 20% Percoll, and 30 mM Mes or Hepes. On top of phase two, a medium containing 0.5 M sorbitol, 1 mM CaCl₂, and 30 mM Mes or Hepes was layered. Mes was used for protoplast isolations at pH 5 and 6 and Hepes for isolations at pH 7. The pH was adjusted with KOH. After centrifugation for 7 min at 250g (4° C) protoplasts were collected from the upper interphase. Light-dependent oxygen evolution in a Clark type electrode served as criterion for intactness of the protoplasts and functionality of protoplasts metabolism. The medium for measuring protoplast metabolism contained 0.5 M sorbitol, 1 mM CaCl₂, and 30 mM Mes or Hepes at different pH values. Chl was determined according to Arnon (1).

Isolation of Chloroplasts. Purified protoplasts were resuspended in a medium containing 0.37 M sorbitol, 1 mM MgCl₂, 1

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mm MnCl₂, 2 mM EDTA, 20 mM KCl, 0.5 mM KH₂PO₄, and 50 mM Mes (pH 5.0–6.5) or Hepes (pH 7.5). Protoplasts were lysed by the method of Martinoia *et al.* (15) and layered on top of a step gradient. The lower phase consisted of the medium mentioned above with 25% Percoll added; the upper chloroplast containing phase was without Percoll. Chloroplasts were pelleted by centrifugation for 4 min at 2100g (4° C) and resuspended in a solution without Percoll. Intactness was measured by phase contrast microscopy and by the ferricyanide method of Heber and Santarius (7).

Isolation of Vacuoles. Vacuoles were isolated as described by Martinoia *et al.* (16, 17) in media adjusted to pH 6.5 and 7.5.

Preparation of Tracer. Na₂³⁵SO₃ (stored at -70° C) was dissolved in incubation medium adjusted to pH 7.5 five min before the experiment (radioactive stock solution). To reduce oxidation of sulfite to sulfate, 1 mM ascorbate was added to the stock solution. Aliquots from this stock solution were added to the organelle containing solutions.

Separation of Intact Organelles from the Incubation Medium by the Silicone Oil Centrifugation Technique. For the rapid separation of intact organelles from the incubation medium, a modification of the silicone oil technique of Heldt and Sauer (10) was used. Briefly, for chloroplasts and protoplast sedimentation in narrow 400 μl polypropylene microcentrifugation tubes, a three-step gradient was prepared. The lower phase (100 μl) consisted of 20% Percoll, 0.5 M sorbitol, 1 mM CaCl₂, and 20 mM Hepes (pH 8). The alkaline pH was chosen to avoid loss of radioactivity due to the formation of ³⁵SO₂. In order to avoid contamination by the pipetted volume adhering to the wall of the tube, the tubes were centrifuged for 2 s at 10,000g in a microfuge before adding 150 μl silicone oil AR-200. The upper phase consisting of 100 μl incubation medium including protoplasts or chloroplasts (with ³H₂O and Na₂³⁵SO₃) was added during the experiment. Incubation was stopped at different time intervals (as indicated in the figures) by rapid centrifugation at 10,000g for 10 s. The final Chl concentration was 15 to 20 μg Chl/100 μl incubation medium. Uptake of radioactive solutes by vacuoles was measured in the three-step gradient described by Martinoia *et al.* (16) which was prepared during the experiment. In this gradient, vacuoles float into the upper phase, which consisted of 10 mM KOH to trap ³⁵SO₂. The samples were centrifuged in a Beckman centrifuge for 15 s at 10,000g.

Organelle volumes were measured in double-labeling experiments by adding ³H₂O to the test solutions containing labeled sulfur (16). Contamination of the organelles with adhering incubation medium was measured in similar double-labeling experiments with ³H₂O and [¹⁴C]sorbitol (10). For liquid scintillation counting the frozen tubes (-20° C) were cut with a razor blade. In the case of protoplasts or chloroplasts the sediment was used, whereas for vacuoles (vacuoles float according to their density to the top of the gradient), the upper phase of the gradient was used.

Calculations. Calculations of SO₂ fluxes (ϕ), resistances to SO₂ fluxes (R) and permeability coefficients (P) were based on the volume/chlorophyll/surface area relationships shown in Table I. The equations are specified in the text. For calculating the concentrations of the different sulfur species at a given pH, the Henderson Hasselbalch equation ($\text{pH} = \text{pK} + \log A^-/\text{HA}$) was used. The pK values of bisulfite and sulfite were from the literature ($\text{pK}_1 = 1.78$; $\text{pK}_2 = 6.99$). No attempt was made to correct the pK for the ionic strength in protoplasts or organelles.

Chemicals and Enzymes. BSA, pyridoxal-P and Percoll were obtained from Sigma; macerozyme R-10 and cellulase ONO-ZUKA R-10 from Serva (Heidelberg, Germany) and silicone oil AR-200 from Wacker Chemie (Nürnberg, Germany). Na₂³⁵SO₃ (1.1 mCi) was purchased as a solid salt (sealed under N₂) from Amersham Buchler (Braunschweig, Germany). Portions at 0.2

to 0.3 mg (30–50 μCi) were weighed and stored dry at -70° C until use.

RESULTS AND DISCUSSION

Relationships between Cell and Organelle Volumes and Surface Areas. The calculation of resistance values from data on solute uptake into protoplasts, chloroplasts, or vacuoles requires information on the areas of the biomembranes which must be transversed. Table I shows average volumes and surface areas of mesophyll protoplasts from young barley leaves and of chloroplasts and vacuoles isolated from the protoplasts. The volume of the cytosol was obtained by subtraction. Volumes and surface areas of barley protoplasts and chloroplasts were somewhat smaller than corresponding data from spinach protoplasts and chloroplasts (3, 9–11).

SO₂ Fluxes across the Plasmalemma. Figure 1 shows uptake of radioactivity from a solution containing ³⁵SO₂, H³⁵SO₃⁻ and ³⁵SO₃²⁻ into isolated barley mesophyll protoplasts. The protoplasts were suspended in a slightly hypertonic reaction medium at three different pH values. Uptake of radioactivity was fast at pH 5, slower at pH 6, and very slow at pH 7. Table II shows the distribution of individual radioactive species at different pH. The concentration of SO₂ increased by a factor of about 200 when pH was decreased from 7 to 5. In contrast, the concentration of HSO₃⁻ increased by a factor of only about 2 under the same conditions, whereas that of SO₃²⁻ decreased by a factor of about 50. Since uptake of radioactivity increased almost as much as the concentration of SO₂ when the pH was decreased from 7 to 5 (Fig. 1), we have calculated the plasmalemma resistance R from the rate of SO₂ uptake by the flux equation

$$\phi = (C_0 - C_i) R^{-1}$$

ϕ is the flux of SO₂ into the protoplasts, C_0 the concentration of SO₂ outside, and C_i that inside the protoplasts. Inserting SO₂ concentrations into the flux equation is permissible if the simplifying assumption is made that the neutral solute is the only penetrating molecular species and that the intracellular concentration of SO₂ remained so low during the uptake experiments as to be negligible, so that

$$\phi = C_0 \times R^{-1}.$$

Table III shows plasmalemma resistance R and permeability coefficients P (R^{-1}) for SO₂. It should be noted that resistance values as shown incorporate the membrane resistance to SO₂ penetration and the boundary layer resistance. We did not stir the protoplast suspensions during the experiment in order to make diffusion into the cells as comparable as possible to the situation inside the leaf. Apparent resistance was higher at pH 5 than at pH 6 and 7. There are two possibilities to explain the observations.

(a) It may not be permissible to neglect the accumulation of SO₂ inside the protoplasts during the experiment, particularly when flux is as large as it is at pH 5, although SO₂ entering the cells is rapidly removed by conversion to HSO₃⁻ and SO₃²⁻. Thus, resistance data measured at this pH may be erroneously high. If this were so, SO₂ uptake should decrease with an increasing incubation time. This was not observed within the time span of the experiment of Figure 1. Thus, explanation (a) cannot be correct.

(b) Whereas anion transport by protein carriers is expected to be pH dependent and may be inhibited at low pH values, diffusion of SO₂ across a lipid bilayer should be independent of pH. The plasmalemma resistance of about 8000 s·m⁻¹ as measured at pH 5 reflects resistance to SO₂ flux only, then lower apparent resistance values at higher pH values indicate additional uptake. If extra uptake cannot be attributed to extra SO₂ flux, it

Table I. Volume, Chlorophyll Content, and Surface Area Relations of Isolated Barley Protoplasts, and Chloroplasts and Vacuoles Isolated from Protoplasts

It is assumed that protoplasts contain only one vacuole.

	Protoplasts	Chloroplasts	Vacuoles ^a	Cytosol ^b
Average volume per cell or compartment	19000 μm^3	25 μm^3	15000 μm^3	1500 μm^3
Average area per cell or compartment	3443 μm^2	41.2 μm^2	2940 μm^2	
Number/mg Chl	10 ⁷	10 ⁹	10 ⁷	
Total volume/mg Chl	190 μl	25 μl	150 μl	15 μl
Total surface area/mg Chl	344 cm^2	412 cm^2	294 cm^2	

^a Numbers refer to vacuoles in 10⁷ protoplasts which contain 1 mg Chl.

^b The nonchloroplast part of the cytoplasm.

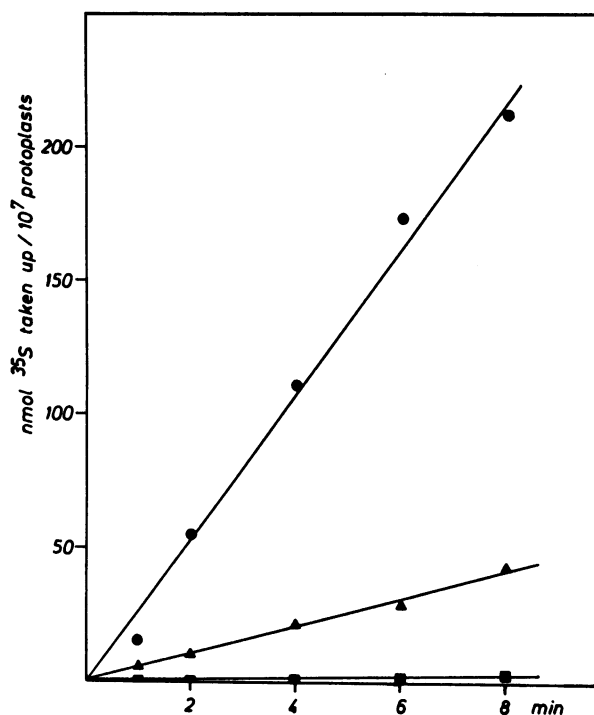


FIG. 1. Uptake of label from solutions containing ³⁵SO₂, H³⁵SO₃⁻, and ³⁵SO₃²⁻ (total concentration 181 μM) into barley mesophyll protoplasts as a function of exposure time at pH 5 (●), pH 6 (▲), and pH 7 (■). The concentration of SO₂ was high at pH 5, intermediate at pH 6, and low at pH 7 (Table II).

Table II. Distribution of SO₂, HSO₃⁻, and SO₃²⁻ as Calculated on the Basis of the Henderson-Hasselbalch Equation at Three Different pH Values

Na₂³⁵SO₃ was added to a final concentration of 181 μM to buffered solutions of pH 5, 6, and 7.

pH	SO ₂	HSO ₃ ⁻	SO ₃ ²⁻
		μM	
5	0.108	179.1	1.8
6	0.0099	164.6	16.8
7	0.00054	89.8	91.8

must be attributed to anion uptake. Since concentrations of the anions are known (Table II), resistance values for HSO₃⁻ or SO₃²⁻ uptake can be calculated from the apparent resistance to SO₂ transfer at pH 6 and 7 (Table III) under the assumption that anion uptake is mediated by electroneutral anion exchange such

Table III. Plasmalemma Resistance to the Penetration of ³⁵SO₂ and Permeability Coefficients for ³⁵SO₂

Calculated from the rate of appearance of label inside isolated protoplasts (Fig. 1), the external SO₂ concentration (Table II), and the surface area of protoplasts (Table I) suspended in solutions of pH 5, 6, and 7. It is assumed that only ³⁵SO₂ crosses the plasmalemma. This assumption is probably correct only for the transport at pH 5 (see text). Each value represents the average of at least 4 measurements.

pH	Plasmalemma Resistance		Permeability Coefficient	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
	$\text{s} \cdot \text{m}^{-1}$		$10^{-4} \text{m} \cdot \text{s}^{-1}$	
5	7750	7870	1.29	1.27
6	3590	3890	2.8	2.6
7	2840	2130	3.5	4.7

as HSO₃⁻/OH⁻ exchange. In the experiment of Figure 1, resistance to HSO₃⁻ penetration ($R_{\text{HSO}_3^-}$) is then $1.2 \cdot 10^8 \text{ s} \cdot \text{m}^{-1}$ at pH 6 and $8 \cdot 10^8 \text{ s} \cdot \text{m}^{-1}$ at pH 7, i.e. four to five orders of magnitude larger than resistance to SO₂ flux.

As the general composition of different biomembranes serving the same function is similar, permeability coefficients which describe diffusion of SO₂ across the lipid bilayer of a barley membrane can be used to describe SO₂ uptake also into cells of other plant species. The rate of anion transport, on the other hand, may be different in different species. Its rate will be governed not by Fick's law, but by carrier characteristics.

SO₂ Fluxes across the Tonoplast. Figure 2 shows uptake of label from an incubation solution containing ³⁵SO₂, H³⁵SO₃⁻ and ³⁵SO₃²⁻ (with 30 mM KCl present) by isolated vacuoles from barley protoplasts. The pH of the suspensions was 6.5 and 7.5. As in the protoplast experiment of Figure 1, uptake of radioactive material was faster at the lower pH. Table IV shows the distribution of the individual labeled species at the different pH conditions. At pH 6.5, the concentration of neutral SO₂ was 32 times higher than at pH 7.5. However, uptake was only 4 times faster at pH 6.5 than at pH 7.5. Tonoplast resistance was 9000 $\text{s} \cdot \text{m}^{-1}$ at pH 6.5 and only 1100 $\text{s} \cdot \text{m}^{-1}$ at pH 7.5, when calculation was made on the assumption that SO₂ is the only penetrating species. Table V lists resistance data from two different uptake experiments. As has been discussed above for uptake across the plasmalemma, decreased resistance at higher pH suggests that not only SO₂ but also anionic species penetrate into the vacuole. The pH of the cytosol which *in situ* encloses the vacuole is about 7.4 (22). If it is assumed that the tonoplast resistance of 9000 $\text{s} \cdot \text{m}^{-1}$ measured at pH 6.5, which is very similar to the plasmalemma resistance of 8000 $\text{s} \cdot \text{m}^{-1}$ measured at pH 5, describes SO₂ transfer across the lipid phase of the tonoplast, then little more than 10% of the total uptake of label observed at pH 7.5 can be attributed to SO₂ flux. The remainder must be caused by

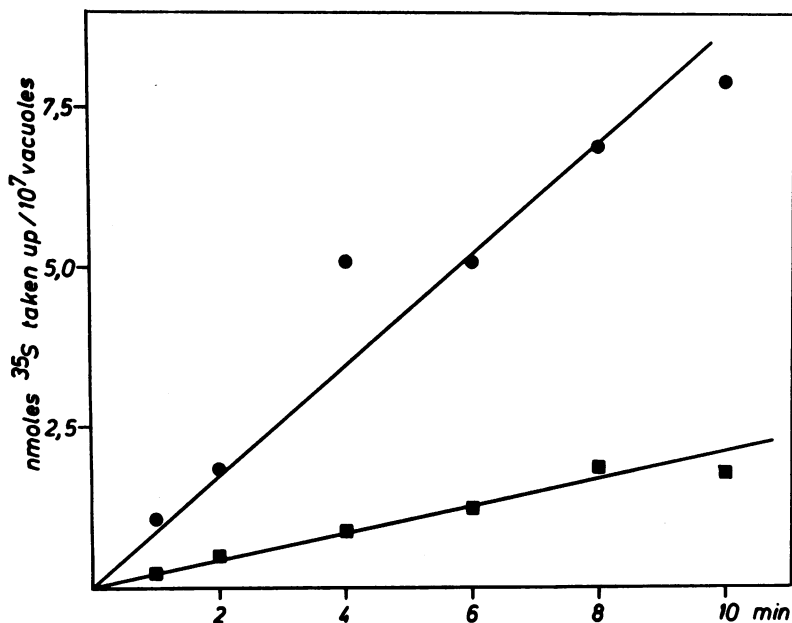


FIG. 2. Uptake of label from solutions containing $^{35}\text{SO}_2$, $\text{H}^{35}\text{SO}_3^-$, and $^{35}\text{SO}_3^{2-}$ (total concentration $311 \mu\text{M}$) into vacuoles isolated from barley mesophyll protoplasts as a function of exposure time at pH 6.5 (●) and pH 7.5 (■). The concentration of SO_2 was higher at pH 6.5 than at pH 7.5 (Table IV).

Table IV. Distribution of SO_2 , HSO_3^- , and SO_3^{2-}

Calculated on the basis of the Henderson-Hasselbalch equation at pH 6.5 and 7.5. $\text{Na}_2^{35}\text{SO}_3$ was added to a final concentration of $311 \mu\text{M}$ to the buffered solutions.

pH	SO_2	HSO_3^-	SO_3^{2-}
		μM	
6.5	0.0045	235	76.1
7.5	0.00014	73.4	237.6

Table V. Tonoplast Resistance to the Penetration of $^{35}\text{SO}_2$

Calculations are made under the assumption that transport is mediated exclusively by SO_2 . This assumption is probably incorrect for transport at pH 7.5.

pH	Tonoplast Resistance ^a	
	Experiment 1	Experiment 2
	$\text{s} \cdot \text{m}^{-1}$	
6.5	9000	8300
7.5	1120	500

^a Each value represents the average of at least 4 measurements.

anion uptake (24). If this was electroneutral (*i.e.* accompanied by co-transport of a proton), a $R_{\text{HSO}_3^-}$ of $6.6 \times 10^8 \text{ s} \cdot \text{m}^{-1}$ would describe tonoplast resistance to HSO_3^- uptake at pH 7.5 in the experiment of Figure 2. It should be noted that this resistance is similar to that indicated for HSO_3^- transfer across the plasmalemma at pH 6 and 7.

SO₂ Fluxes across the Chloroplast Envelope. Figure 3 shows uptake of labeled material from a solution containing $^{35}\text{SO}_2$, $\text{H}^{35}\text{SO}_3^-$ and $^{35}\text{SO}_3^{2-}$ into intact chloroplasts at three different pH values. As already shown above for uptake into protoplasts and vacuoles, uptake was strongly pH dependent. It was fast at low and slow at high pH. From initial rates of uptake (see slopes in Fig. 3) envelope resistance was calculated under the simplifying assumption that all uptake is caused by SO_2 diffusion. It was $940 \text{ s} \cdot \text{m}^{-1}$ at pH 5.5, $310 \text{ s} \cdot \text{m}^{-1}$ at pH 6.5, and $35 \text{ s} \cdot \text{m}^{-1}$ at pH 7.5.

A comparison with plasmalemma and tonoplast resistance to transfer shows agreement insofar as apparent resistance decreases as the pH is increased. Absolute resistance values, however, differ. Chloroplast envelope resistance is much lower than plasma-

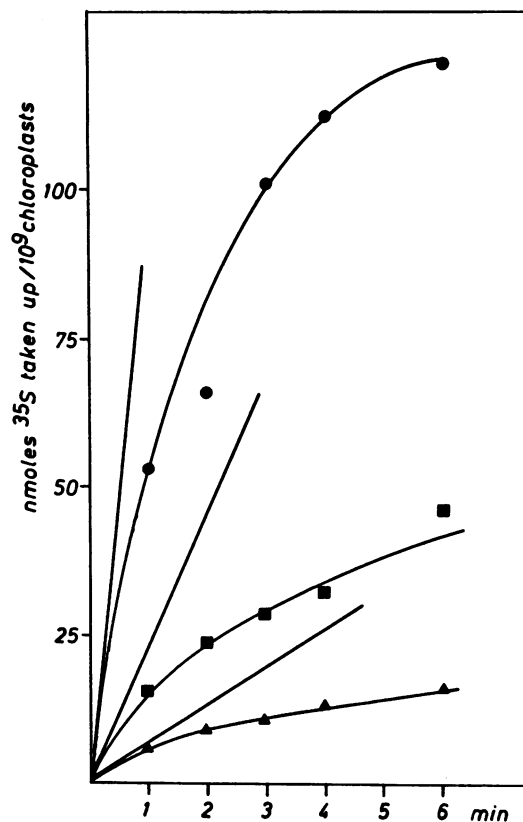


FIG. 3. Uptake of label from solutions containing $^{35}\text{SO}_2$, $\text{H}^{35}\text{SO}_3^-$, and $^{35}\text{SO}_3^{2-}$ (total concentration $200 \mu\text{M}$) into chloroplasts isolated from barley mesophyll protoplasts as a function of exposure time at pH 5.5 (●), pH 6.5 (■), and pH 7.5 (▲). The concentration of SO_2 was high at pH 5.5, intermediate at pH 6.5, and low at pH 7.5. Linear lines indicate the initial rate of uptake, *i.e.* uptake that was not yet slowed down by the decrease in the concentration gradients of labelled solutes across the chloroplast envelope.

lemma or tonoplast resistance. Since it is unlikely that resistance to diffusion of a neutral lipid-soluble molecule across a lipid bilayer is very different for different biomembranes, the data suggest that anion transfer plays a considerable role in chloro-

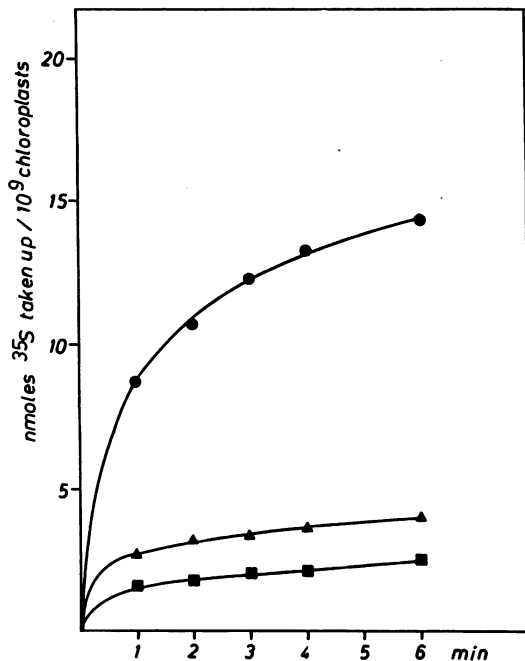


FIG. 4. Decreased uptake of label from a solution containing $^{35}\text{SO}_2$, $\text{H}^{35}\text{SO}_3^-$ and $^{35}\text{SO}_3^{2-}$ (total concentration $200\ \mu\text{M}$) into barley mesophyll chloroplasts in the presence of 20 mM phosphate (\blacktriangle) or 1 mM pyridoxal-P (\blacksquare). The control experiment (\bullet) was performed with 0.5 mM phosphate. pH was 7.5.

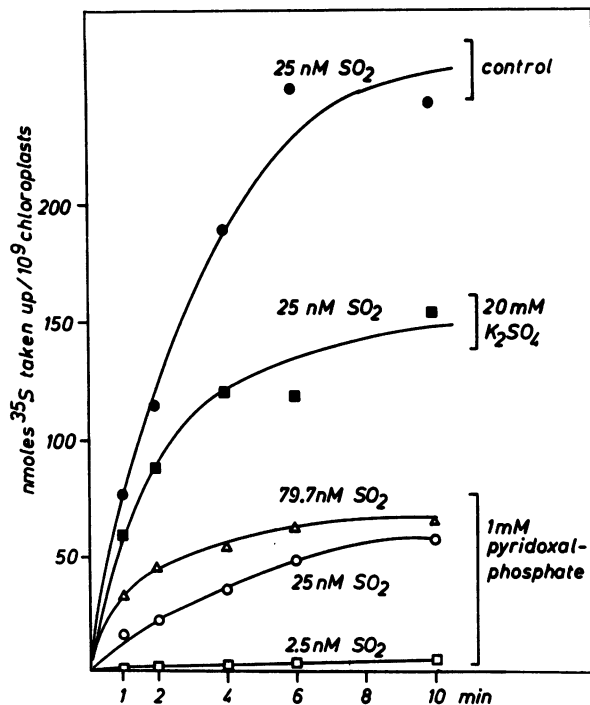


FIG. 5. Decreased uptake of label from solutions containing $^{35}\text{SO}_2$, $\text{H}^{35}\text{SO}_3^-$, and $^{35}\text{SO}_3^{2-}$ into barley mesophyll protoplasts in the presence of 20 mM K_2SO_4 or 1 mM pyridoxal-P (PyP). pH was 6. Only SO_2 concentrations are shown in the figure.

plasts in addition to diffusion of neutral SO_2 . Hampp and Ziegler (5, 6) have indeed demonstrated that the phosphate translocator of the chloroplast envelope also mediates transport of sulfite.

In contrast to SO_2 uptake into protoplasts or vacuoles (Figs. 1 and 2), which was almost linear with time, uptake into chloro-

plasts (Fig. 3) decreased during the time span of the experiment. The decrease is caused by a rapid decrease in the driving force for uptake. After 6 min of incubation, the concentration of labeled material inside the chloroplasts was higher than outside. At pH 5.5, accumulation by a factor of about 28 was observed close to saturation, at pH 6.5 by a factor of 10, and at pH 7.5 by a factor of 4. Accumulation may be explained in two different ways:

(a) If the pH inside of the chloroplasts is higher than outside, HSO_3^- and SO_3^{2-} formed from entering SO_2 will be trapped. The Henderson-Hasselbalch equation predicts that at flux equilibrium with SO_2 a pH difference of 1 unit across the chloroplast envelope will result in an accumulation of HSO_3^- by a factor of 10 and of SO_3^{2-} by a factor of 100. SO_2 influx will decrease the chloroplast pH.

(b) The phosphate translocator of the chloroplast envelope, which has been shown to mediate transfer of sulfite (5), catalyzes transport on a strict counterexchange basis (2). Since darkened chloroplasts contain about 20 mM (or 500 nmol/mg Chl) exchangeable phosphate and phosphate esters (8, 10, 14), and since transport equilibrium is reached only when the ratio of the different exchangeable substrates to one another is identical inside and outside the chloroplasts, sulfite uptake will be expected to be driven by phosphate export. At low sulfite concentrations outside the chloroplasts, sulfite will accumulate in the chloroplast stroma. In contrast to SO_2 influx, sulfite import will increase the chloroplast pH as SO_3^{2-} reacts with protons to form HSO_3^- .

The contribution of the phosphate translocator to the uptake of labeled substrate shown in Figure 3 can be recognized when phosphate is added to the medium so that the phosphate gradient between chloroplast stroma and the medium becomes small or is absent (Fig. 4). Also, the phosphate translocator can be inhibited by adding pyridoxal-P, which is known to bind to the translocator (2). Under both conditions, uptake of ^{35}S -labeled substrate was decreased. Thus, the data of Figure 4 shows that a large part of ^{35}S -uptake into isolated chloroplasts must be attributed to sulfite uptake, not to penetration of SO_2 and subsequent trapping of anions formed from SO_2 (5, 6, 23, 26).

It was still of interest to determine envelope resistance to the diffusion of SO_2 . Figure 5 shows that uptake of ^{35}S -labeled material became slow in the presence of 1 mM pyridoxal-P, when compared to a control experiment performed in the absence of pyridoxal-P. Also, there was some degree of linearity which permitted calculation of envelope resistance to diffusion. It was $9000\ \text{s}\cdot\text{m}^{-1}$ at pH 6 in the presence of 1 mM pyridoxal-P and thus very similar to plasmalemma resistance at pH 5 and tonoplast resistance at pH 6.5.

When 20 mM K_2SO_4 was present instead of 1 mM pyridoxal-P, uptake of ^{35}S -labeled substrates was also decreased compared to uptake in a control experiment (Fig. 5). Sulfate has also been reported to be transferred by the phosphate translocator across the chloroplast envelope (5, 6, 18) suggesting it may compete with sulfite for binding sites of the carrier. It did not decrease uptake of label into isolated vacuoles (data not shown).

The different pH values which yield comparable resistances to SO_2 diffusion across different biomembranes are all significantly below the pH the membranes experience *in situ* on that side which is exposed to SO_2 . Stability problems have prevented us from exploring still lower pH ranges which would make it less likely that uptake of anions occurs simultaneously with that of SO_2 .

CONCLUSIONS

When SO_2 enters a leaf through the stomates, it encounters additional barriers. We have determined the resistance to SO_2 diffusion of different cellular biomembranes. Obtained values were similar and close to $8000\ \text{s}\cdot\text{m}^{-1}$. Such values must be

related to the resistance that stomatal barriers constitute for the penetration of SO₂. Since, in many leaves, 30 cm² leaf area contain about 1 mg chlorophyll, and since the mesophyll alone represents a surface area of more than 300 cm² (Table I), resistance of the mesophyll on a leaf area basis is only 800 s · m⁻¹. This relates to dissolved SO₂. As, at equilibrium, about 40 times more SO₂ dissolves in water (at room temperature) than is present in the air, mesophyll resistance to gaseous SO₂ is, on a leaf area basis, only 20 s · m⁻¹. This compares with a resistance to gas flow offered by open stomates of about 250 s · m⁻¹. Naturally, stomatal resistance increases as stomates close. Clearly, stomates are main barriers for the penetration of SO₂ into leaves. Knowing diffusion resistances of biomembranes to SO₂ and pH in different cellular compartments, flux and distribution of SO₂ into mesophyll cells can be calculated. Together with anionic sulfur species, protons are formed from dissolved SO₂. They will decrease the pH particularly in the alkaline cytoplasm. Flux distribution and pH effects will be considered in the following publication (21).

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