Plant Physiol. (1986) 81, 597–602
0032-0889/86/81/0597/06/$01.00/0

Buffer Capacities of Leaves, Leaf Cells, and Leaf Cell Organelles in Relation to Fluxes of Potentially Acidic Gases[1]

Received for publication February 6, 1986

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ABSTRACT

Since environmental pollution by potentially acidic gases such as SO_2 causes proton release inside leaf tissues, homogenates of needles of spruce (*Picea abies*) and fir (*Abies alba*) and of leaves of spinach (*Spinacia oleracea*) and barley (*Hordeum vulgare*) were titrated and buffer capacities were determined as a function of pH. Titration curves of barley leaves were compared with titration curves of barley mesophyll protoplasts. From the protoplasts, chloroplasts and vacuoles were isolated and subjected to titration experiments. From the titration curves, the intracellular distribution of buffering capacities could be deduced. Buffering was strongly pH-dependent. It was high at the extremes of pH but still significant close to neutrality. Owing to its large size, the vacuole was mainly responsible for cellular buffering. However, on a unit volume basis, the cytoplasm was much more strongly buffered than the vacuole. Potentially acidic gases are trapped in the anionic form. They release protons when trapped. The magnitude of diffusion gradients from the atmosphere into the cells, which determines flux, depends on intracellular pH. In the light, the chloroplast stroma, as the most alkaline leaf compartment, has the highest trapping potential. Acidification of the chloroplast stroma inhibits photosynthesis. The trapping potential of the chloroplast is followed by that of the cytosol. Compared with the cytoplasm, the vacuole possesses little trapping potential in spite of its large size. It is particularly small in the acidic vacuoles of conifer needles. In the physiological pH range (slightly above neutrality), chloroplast buffering was about 1 microequivalents H^+ per milligram chlorophyll per pH unit or 35 microequivalents H^+ per milliliter per pH unit in barley or spinach chloroplasts. This compares with SO_2-generated H^+ production of somewhat more than 1 microequivalent H^+ per milligram chlorophyll per hour, which results from observed SO_2 uptake of leaves when stomata were open and the atmospheric SO_2 concentration was 0.4 microliters per liter (GE Taylor Jr, DT Tingey 1983 Plant Physiol 72: 237–244). At lower SO_2 concentrations, similar H^+ generation inside the cells requires correspondingly longer exposure times.

Gases exchange with cellular systems by diffusion. They enter leaves through stomates and dissolve in the aqueous phase of the apoplast. Depending on the pH of the cell wall (29) potentially acidic gases such as CO_2, SO_2, or NO_2 will react with water to form the anionic species HCO_3^-, HSO_3^-, SO_3^{2-}, NO_2^-, and NO_3^- thereby producing H^+. Diffusion across limiting biomembranes (plasmalemma and intracellular membranes) will be supported by gradients of the dissolved gas, if anion carriers of the membranes do not contribute to flux. After entering into internal compartments, the gases will form anionic species inside the cells, consuming hydroxyl ions there. The consumption of hydroxyl ions will decrease the pH of aqueous phases. The rate of the decrease is a function of the rate of proton production (or hydroxyl ion consumption) and of the buffering capacity of aqueous phases (9, 28, 33, 36), if metabolic reactions aimed at stabilizing intracellular pH are neglected. pH is an important parameter of cellular metabolism. Carboxylation of phosphoenolpyruvate by phosphoenolpyruvate-carboxylase in mesophyll protoplasts of spinach or barley is known to be highly sensitive to a decrease in the pH of the external medium (10). Photosynthesis of isolated chloroplasts is maximal at pH 7.6 to 7.8 and negligible below pH 6.5 of the suspending medium (16, 38). Inhibition of photosynthesis at low external pH is due to stroma acidification which interferes with light activation of photosynthetic enzymes (20). Even light-activated enzymes such as fructose-bisphosphatase and sedoheptulose-bisphosphatase are scarcely active below pH 7 of the stroma (8). Diffusion of neutral gases such as CO_2, SO_2, or NO_2, or of weak acids such as HNO_2 across biomembranes circumvents cellular proton barriers and results in indirect proton import particularly into alkaline compartments which act as anion traps (30, 38). We were interested in obtaining information on the magnitude of acidification of individual cellular compartments in the course of gas absorption and have therefore performed titration experiments with leaves, protoplasts, chloroplasts, and vacuoles. For technical reasons, such experiments are possible at present only with plants such as spinach or barley. To extend information gained from these plants, we have also titrated homogenates of spruce and fir needles.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* var Gerbel) was grown in a growth cabinet. The daily illumination period was 12 h, the temperature 22°C in the light, and 20°C in the dark. For the isolation of protoplasts 10 d old plants were used. Spinach (*Spinacia oleracea L.*) was grown either in a greenhouse or in the field. Needles of spruce (*Picea abies* L.—Karst) and fir (*Abies alba* Mill.) were collected from field-grown trees; only young needles were used. BSA and Ficoll were purchased from Sigma (St. Louis) and erythritol, macerocym R-10, and cellulase "Onozuka R-10" from Serva (Heidelberg, F.R.G.).

Isolation of Organelles. *Isolation of Protoplasts.* Mesophyll protoplasts were isolated according to Kaiser *et al.* (17). The cellulase concentration was 1% w/v. For purification, a three step gradient was prepared with the lower phase containing 8 ml protoplast suspension (0.4 M sucrose, 7.5% w/v Ficoll, and 5 mM Mes [pH 6]). Phase two was 0.4 M sucrose, 2.5% w/v Ficoll, and 30 mM KCl. On top of phase two a medium containing 3 ml 0.4 M sorbitol was layered. After centrifugation for 7 min at 250*g* (4°C), protoplasts were collected from the upper interphase. Light

[1] Supported by a grant from the Bayerische Forschungsgruppe Forsttoxikologie.

dependent O_2 evolution in a Clark type electrode served as criterion for intactness and viability of the protoplasts. Before titration, intact protoplasts were ruptured by freezing to −20°C for 30 min and violent shaking during thawing; the effect was controlled under a microscope.

Isolation of Chloroplasts. For chloroplast isolation protoplasts were prepared and purified as described above, with 0.1% (w/v) BSA and 5 mM Hepes (pH 7.8) added to phases 2 and 3 for stabilization. Protoplasts were taken from the upper interphase, lysed by the method of Martinoia *et al.* (24), and layered on top of a preprepared step gradient. The harvest phase contained 2 ml 5% (w/v) Ficoll, 0.4 M sucrose, and 30 mM KCl and the following phase 6 ml 2.5% (w/v) Ficoll, 0.4 M sucrose, and 30 mM KCl. Two to 3 ml lysed protoplasts were carefully added. Chloroplasts were pelleted by centrifugation for 4 min at 2100*g* (4°C) and resuspended in a solution containing 0.4 M sucrose and 30 mM KCl. The centrifugation procedure was repeated using the same solutions but omitting BSA. Purified chloroplasts were pooled and intactness was measured according to Heber and Santarius (12). Only chloroplast preparations containing 90% or more chloroplasts with intact envelopes were used for titration experiments.

Vacuole Isolation. For isolation of vacuoles the unpurified protoplast solution was used. Protoplasts were lysed according to the method of Martinoia *et al.* (25) and purified on a slightly buffered three-step gradient: phase 1 (lower phase, with lysed protoplasts) contained 0.4 M sucrose, 2.5% (w/v) Ficoll, 10 mM KCl, 0.1% (w/v) BSA, 2 mM EDTA, 20 mM Hepes (pH 7.8). Phase 2 contained 0.4 M sorbitol, 30 mM KCl, 0.1% (w/v) BSA, 5 mM Hepes (pH 7.8). Phase 3 contained 0.4 M erythritol, 30 mM KCl, 0.1% (w/v) BSA, 5 mM Hepes (pH 7.8).

After centrifugation for 3 min at 200*g* (4°C) and 4 min at 1200*g*, vacuoles were collected from the upper interphase. Vacuoles purified in this way were diluted with a solution containing 0.4 M sucrose, 15% (w/v) Ficoll, mM KCL (adjusted to pH 7.8), to give a final Ficoll concentration of 3% (w/v) and placed on a second three step gradient (analogous to that described above, but without BSA, EDTA, and Hepes) and centrifuged as described above. Thereafter, vacuoles were pooled and frozen to −20°C for 30 min. After thawing, they were titrated. Numbers of vacuoles were determined by measuring α-mannosidase as described by Boller and Kende (3); 10^7 mesophyll protoplasts contained 1 mg Chl. Protoplasts were assumed to release not more than one vacuole per protoplast.

Thylakoid Isolation. Barley leaf tips (5 cm) were rinsed with bidistilled H_2O, dried with muslin, and illuminated for 5 min on ice. The leaves were blended in a Braun mixer (type MX 31, Braun, Frankfurt, F.R.G.) with 150 ml ice-cold isolation medium (200 mM NaCl, 10 mM ascorbate, 3 mM cysteine, 20 mM Tris [pH 8]) for 10 s at the highest speed. The homogenate was squeezed through 6 layers of muslin and a 20 μm nylon gauze and centrifuged for 3 min at 3,200*g*. Four washing procedures followed; the first washing was done with 200 mM NaCl and the following washings with 20 mM NaCl with centrifugation steps at 10,000*g*. The final pellet was resuspended in 20 mM NaCl.

Preparation of Leaf Homogenate. One and a half g barley leaves (leaf tips 5 cm long) or leaves of spinach (or needles of spruce and fir) were rinsed twice with bidistilled H_2O, dried with muslin, and homogenized to a fine powder in liquid N_2; 4 ml bidistilled H_2O was added and aliquots were taken for subsequent pH titration.

pH Titration. Calibration and pH measurements were carried out at room temperature (21°C). All solutions that were to be titrated were first brought to pH 10 with KOH. As volumes were small, a micro-pH-electrode (Ingold lot 405-M3, Frankfurt, F.R.G.) connected to a pH-meter (model 701A Orion Research, Cambridge) was used. Titration was performed under stirring by adding aliquots of 0.1 M HCl to the solutions tested and pH-changes were recorded. Chl was determined according to Arnon (2).

RESULTS

Figure 1 shows titration curves for a barley leaf homogenate and barley mesophyll protoplasts, and for vacuoles, chloroplasts, and thylakoids from barley mesophyll protoplasts. The homogenates contained, in addition to soluble constituents, small membrane vesicles, which are less easy to titrate than solutes. Some scattering of the titration data is probably caused by membrane particles. All data are expressed on a unit Chl basis. Reference is made to the Chl content of protoplasts from which vacuoles and chloroplasts were obtained. The curves are, therefore, directly comparable and give an impression of the extent to which chloroplasts and vacuoles contribute to the buffering capacity of mesophyll protoplasts. The thylakoid data also illustrate that the buffering capacity of biomembrane systems can be very significant. Some leakage of solutes may occur from isolated cell organelles, but it is negligible within the time span of isolation. This has been shown for vacuoles by Martinoia et al. (23). Titration curves of nonaqueously isolated chloroplasts from spinach leaves which retain all polar solutes during isolation, and of aqueously isolated spinach chloroplasts were comparable (data not shown) indicating that solute leakage from aqueously isolated chloroplasts was also insignificant. A titration curve for the nonchloroplast part of the protoplasm of barley mesophyll protoplasts (cytosol, nucleus, mitochondria, etc.) was obtained by subtracting chloroplast and vacuolar values from the titration data obtained with protoplasts. It is immediately apparent that buffering is particularly strong at the extremes of pH, apparently

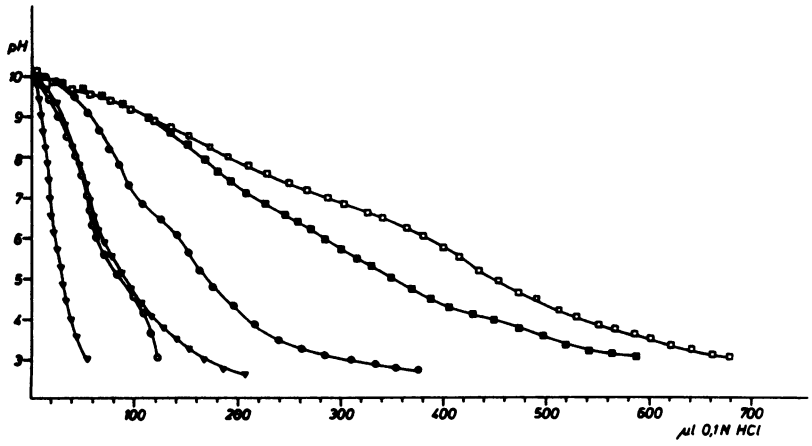

FIG. 1. Titration curves of homogenates of young barley leaves (□), mesophyll protoplasts from barley leaves (■), and vacuoles (●) and chloroplasts (▼) isolated from mesophyll protoplasts. A titration curve of thylakoids (▽) isolated from barley chloroplasts is also shown. The titration curve of cytosolic constituents (○) (including nucleus, ER, etc.) was obtained by substracting values of chloroplasts and vacuoles from mesophyll protoplast values. All data are based on material containing 1 mg Chl or isolated from cells containing 1 mg Chl.

owing to the presence of carboxylic groups (pK values between 2 and 5) and amino or guanidino groups (pK values between 8 and 12) in cells and cellular fractions (4, 26, 34). Buffering at pH values between 7 and 8 is weak compared to buffering close to pH 3 or 9. However, it is of considerable physiological importance as pH 7 to 8 is the working range of cytosolic and chloroplast metabolism (27, 31, 34). Especially the latter is known to be pH sensitive (6, 8, 16, 38). In Figure 1 a homogenate from barley leaf tips was more strongly buffered than a homogenate of mesophyll protoplasts. The difference is not due to leakage of solutes from the protoplasts during protoplast isolation (19). It is explained by the presence of Chl-free cells in the leaf homogenate which are not present in preparations of mesophyll protoplasts. Moreover, in contrast to the homogenate made from naked protoplasts, the leaf homogenate includes cell walls with acidic groups of pectins which contribute to buffering at low pH. The vacuole of mesophyll protoplasts accounts for most of the buffering capacity of barley protoplasts. Although the protein content of the vacuoles is very low (less than 3% of the cellular proteins [18]), this is not surprising because the vacuole occupies the main part of the protoplast volume (about 80%) and contains amino acids and organic acids in addition to neutral solutes and inorganic salts (23).

A very significant fraction of the total buffering capacity is localized in the chloroplasts. Since thylakoids have a high protein to lipid ratio (1/1) and also contain phosphatidylglycerol in addition to galactolipids (1), they contribute considerably to buffering in chloroplasts. At a first approximation, about half of their buffering capacity should be added to the buffering capacity of soluble chloroplast constituents to obtain a reasonable estimate of buffering in the chloroplast stroma. Soluble chloroplast constituents can be titrated directly after sedimentation of the thylakoids. Alternatively, buffering by chloroplast solutes can be calculated as the difference of chloroplast and thylakoid titration data.

Table I. *Microequivalents H^+ Required to Change pH by 7 pH Units or by One pH Unit in a Mesophyll Protoplast Preparation Containing 1 mg Chl, or in Organelle Preparations Derived from Mesophyll Protoplasts Containing 1 mg Chl*

	Total Buffer Capacity pH 3–10	pH						
		3–4	4–5	5–6	6–7	7–8	8–9	9–10
Protoplast	57	14	8.5	6.5	7.2	5.2	6.0	9.6
Vacuole	27	8.0	3.7	2.4	4.3	2.5	2.1	4.0
Intact chloroplast	16.1	4.9	2.8	2.2	1.3	1.1	1.2	2.6
Thylakoid	5.3	2.0	0.6	0.8	0.4	0.4	0.4	0.7
Chloroplast stroma[a]	10.8	2.9	2.2	1.4	0.9	0.7	0.8	1.9
Cytosol[a]	13.9	1.1	2.0	1.9	1.6	1.6	2.7	3.0

[a] Obtained by subtraction and possibly not very accurate.

In Table I, the buffering capacity of leaf cells, protoplasts, vacuoles, chloroplasts, and cytosol (*i.e.* the nonchloroplast part of mesophyll protoplasm) is listed as the number of μequivalents H^+ required to decrease pH of the different preparations from 10 to 3, and in steps of 1 pH unit. The Chl basis of the data shown in Figure 1 and Table I permits an easy comparison of the contribution of different leaf compartments to the total buffering capacity of leaves. It does not incorporate volume information. Figure 2 shows some data of Figure 1 recalculated on a volume basis. Chloroplasts containing 1 mg Chl (about 10^9 chloroplasts) are assumed to occupy a volume of 25 μl (13, 27). The volume of the vacuoles of 10^7 protoplasts (also 1 mg Chl) is close to 150 μl (23). When data are plotted on this basis, it becomes apparent that the buffering capacity of chloroplasts (and also of the nonchloroplast part of the protoplasm; not shown) exceed buffering by vacuolar constituents considerably. Because of the volume relationships, this is no contradiction to the data of Figure 1. It is not surprising in view of the high concentration of proteins in the protoplasm. Chloroplasts contain about 70% of the protein of the mesophyll cells (18). At pH values close to neutrality, however, phosphate and phosphate esters also contribute very significantly to buffering. The chloroplast concentration of phosphate and phosphate esters is between 15 and 20 mM in spinach (21), and similar or even higher concentrations were found in the cytoplasm of barley (7, 23). The pK of the couple $HPO_4^{2-}/H_2PO_4^-$ is 7.2 and phosphate esters have similar pK values. There is the question whether the titration data shown for barley mesophyll in Figure 1 are representative also for other plants. For chloroplast and cytosol, fairly little variation is likely (31). Very similar buffering was observed in chloroplasts of spinach (aqueous and nonaqueous chloroplasts) and of barley. The physiological function of chloroplasts from different species is very similar. Phosphate and phosphate esters play a central role in the metabolism of all chloroplasts. Homeostatic mechanisms maintain levels of phosphate/phosphate esters in chloroplasts and cytosol within fairly narrow limits, whereas vacuoles serve as storage pools for phosphate (7, 21). Chloroplast protein concentrations are similarly high in different species, and so is buffering by proteins. Similar relations may hold true for the cytosol and the organelles embedded in the cytosol (31). However, vacuolar constituents appear to differ dramatically in leaves of different plants. This should be expected to lead to different buffering capacities in different plants. Indeed, the titration experiments shown in Figure 3 reveal significant differences in the buffering capacities of needles of *Abies alba* and *Picea abies* and

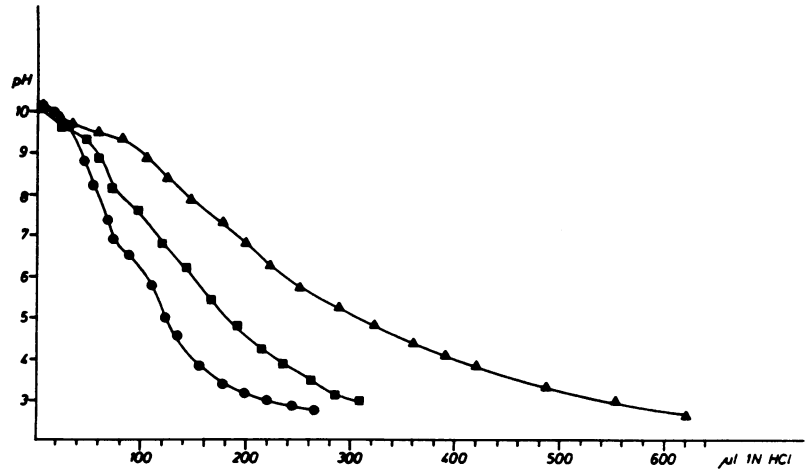

FIG. 2. Titration curves of barley mesophyll protoplasts (■) and of vacuoles (●) and chloroplasts (▼) isolated from mesophyll protoplasts on a unit volume basis (1 ml).

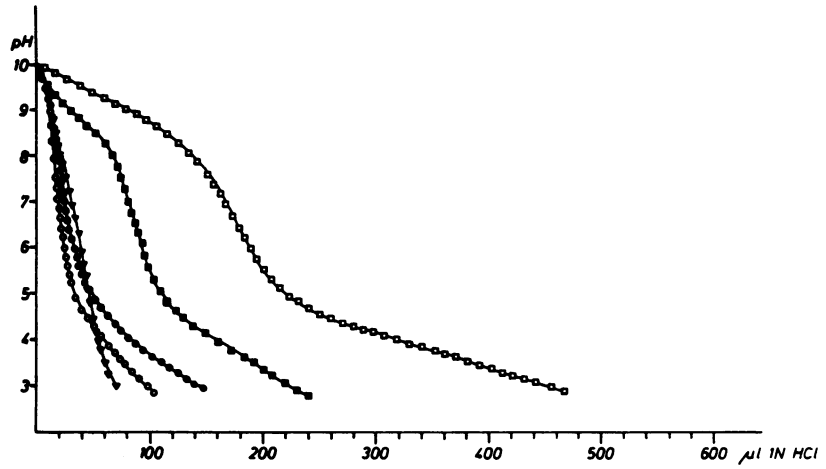

FIG. 3. Titration curves of leaf or needle homogenates from different plants. Data are based on material containing 1 mg Chl. (□), Spruce; (■), fir; (●), spinach grown in the field; (○), spinach grown in the green-house; (▽), barley.

of leaves of *Hordeum* and *Spinacia*, when results are plotted on a Chl basis. Even data from spinach differ to some extent depending on how the plants had been grown. Leaves of spinach rapidly grown in a greenhouse had a lower dry weight content and a lower buffering capacity than leaves of field-grown plants.

When the titration data of Figure 3 were plotted on a unit dry weight basis, the results shown in Figure 4 were obtained. Differences in the buffer capacities of different species were still significant, but much smaller than shown in Figure 3 on a unit Chl basis. Obviously, the fraction of the total dry weight attributable to the chloroplasts is very different in different leaves.

For a calculation of the extent of an acidification of cellular compartments during absorption of a gaseous solute of acidic nature such as SO_2, CO_2, or NO_2, information on intracellular pH is required in addition to information on intracellular buffer capacities. The rate of influx of a neutral solute into a membrane-surrounded compartment depends on the concentration gradient across the limiting membrane and the permeability coefficient of the solute. The concentration gradient, in turn, is a function of pH which determines the equilibrium between the anionic and the neutral form of a potential acid which diffuses across a membrane. At flux equilibrium, the concentration of a permeable potential acid AH (undissociated) must be the same in different cellular compartments. The concentration of the anion

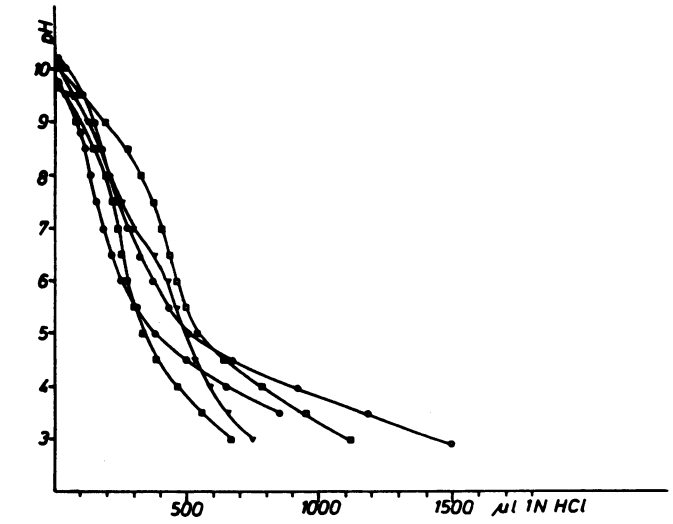

FIG. 4. Titration curves of leaf or needle homogenates from different plants. Data are based on material containing 1 g dry weight. (□), Spruce; (■), fir; (●), spinach grown in the field; (○), spinach grown in the greenhouse; (▽), barley.

A^-, however, differs depending on the H^+ distribution. It is determined by the Henderson-Hasselbalch equation

$$pH = pK + \log \frac{A^-}{(HA)}$$

The consequences are illustrated by an example. If the pH of one compartment is 8 (for instance, the stroma of illuminated chloroplasts) and that of another one 3 (for instance, acidic vacuoles), and if the anionic form A^- cannot readily diffuse from one compartment to the other, its equilibrium concentration will be 100,000 times higher in the alkaline compartment than in the acidic one. If, in turn, the concentration of the anionic form A^- is identical in both compartments, the concentration of the neutral form AH must be 100,000 times lower in the alkaline compartment than in the acidic one. Influx of AH from an external source such as the atmosphere must then be correspondingly faster into the alkaline compartment than into the acidic one because the concentration gradient for AH which supports flux is much steeper. Preferential influx of AH and subsequent dissociation of AH into A^- and H^+ must then decrease the pH particularly of the alkaline compartment. The rate of decrease is a function of the rate of influx of AH and of the buffering capacity of intracompartmental solutes, if metabolic mechanisms to maintain internal pH are not effective. It can readily be seen that influx of AH must, in the long term, decrease and finally abolish internal pH gradients, if metabolic countermeasures are ineffective.

Table II shows pH values measured in a leaf homogenate of barley, in a homogenate of barley mesophyll protoplasts, and in fractions derived from barley mesophyll protoplasts. All pH values were below neutrality, and the most acidic pH was measured in a homogenate made from isolated vacuoles. It is obvious

Table II. *pH Values Measured in Homogenates of Leaves of Barley and Spinach and of Needles of Spruce and Fir (A) and of Barley Mesophyll Protoplasts and Vacuoles, Chloroplasts, and Thylakoids Derived from Barley Mesophyll Protoplasts (B)*

Buffers used during isolation of protoplasts, vacuoles, and chloroplasts were removed carefully by washing in buffer-free media.

	Spinacia	*Hordeum*	*Picea*	*Abies*
(A)				
pH	6.5	6.1	3.9	3.8
	Barley Protoplasts	Vacuoles	Chloroplasts	Thylakoids
(B)				
pH	6.1	5.8	6.3	6.2

that the pH of the leaf homogenate is the average pH of different cellular compartments. Even this is not free from artefacts. Water used for leaf homogenation contained CO_2. Even though the CO_2 concentration was below 20 μM, it shifted pH towards acidity. Because the pK of CO_2 is 6.37, errors caused by CO_2 will not significantly affect the pH of acidic compartments. Therefore, the pH of homogenates from vacuolar fractions will reflect an average of vacuolar pH values almost correctly. The pH of individual vacuoles can vary considerably as indicated by different accumulation of the pH indicating dye neutral red in different vacuoles. Depending on the volume of solutions used for washing aqueously isolated chloroplasts, chloroplast pH values may in contrast to vacuolar pH values be decreased significantly by CO_2. The pH of a concentrated suspension of nonaqueously isolated chloroplasts (50 mg dry weight in 2 ml of water) was indeed higher than the pH of a less concentrated suspension of osmotically ruptured aqueous chloroplasts (pH 6.9 *versus* pH 6.3). Because nonaqueous chloroplasts are not entirely free of vacuolar contaminations, even the pH of suspensions of nonaqueously isolated chloroplasts is likely to be below the pH of the chloroplast stroma *in vivo*. *In vivo* measurements revealed the stroma of chloroplasts in darkened leaves to have a pH close to pH 7.5. On illumination of the leaves, proton pumping into the thylakoid compartment which becomes acidic in the light shifted the stroma pH to 8 (27). The pH measured in a suspension of thylakoids (Table II) is subject to the same criticism which is valid for pH determinations in chloroplast homogenates. Moreover, it does not reflect the intrathylakoid pH which even in the dark is lower than the external pH owing to the Donnan distribution of H^+ (6, 14). Table II (a) compares the pH of homogenates of leaves from different plant species. The homogenates of needles from *P. abies* and *A. alba* were particularly acidic. There is no doubt that this reflects the acidity of the vacuoles. In view of the pH sensitivity particularly of the photosynthetic enzymes, the variability of stroma pH values is limited. Similar conclusions hold for cytosolic pH values.

DISCUSSION

The concentration of potentially acidic gases in the atmosphere, their solubility in aqueous phases, and their dissociation constants (or pK values) are obviously decisive factors in determining their effects on intracellular pH values. This will briefly be considered for CO_2 and SO_2. Although CO_2 has a much higher solubility in water than oxygen, it is much less soluble than SO_2 (5, 15). At 20°C, the concentration of CO_2 in equilibrium with 350 μl/L in the gas phase is 12.8 μM. According to the Henderson-Hasselbalch equation, the equilibrium concentration of bicarbonate at the stroma pH of illuminated chloroplasts is then close to 0.5 mM. The actual bicarbonate concentration will be lower during photosynthesis, as photosynthetic flux maintains a CO_2 gradient and prevents equilibrium of internal bicarbonate with external CO_2. If the external CO_2 concentration is raised, the bicarbonate concentration in the chloroplast stroma will increase correspondingly. Hydroxyl ion consumption during formation of bicarbonate from CO_2 in the chloroplast stroma will decrease the stroma pH. At an equilibrium concentration in the stroma corresponding to 3500 μl/L CO_2 in the gas phase (about 5 mM HCO_3^- in the stroma or 0.15 μmol HCO_3^-/mg Chl), the pH decrease in the stroma is expected to be about 0.15 pH units (see Table I), if there is no active mechanism to maintain the stroma pH constant. At a CO_2 concentration corresponding to 3.5% CO_2 in the atmosphere the decrease in the stroma pH is expected to exceed 1 pH unit. Indeed, photosynthesis of isolated chloroplasts and of mesophyll protoplasts is sensitive to excessive concentrations of bicarbonate (6, 38). CO_2, the substrate of photosynthesis, inhibits photosynthesis at bicarbonate concentrations corresponding to CO_2 levels in the atmosphere of about 3% (16). Photosynthesis of leaves is less sensitive to CO_2 than photosynthesis of isolated chloroplasts and protoplasts, because stomatal resistance increases with increasing CO_2 concentrations in air. It is responsible for significant CO_2 gradients between air and the intracellular space.

The solubility of SO_2 exceeds that of CO_2 by a factor of almost 40. SO_2 is a much stronger acid than CO_2. Its pK values are 1.78 and 6.99 (first and second deprotonation reaction). Table III shows that comparable concentrations of HSO_3^- and HCO_3^- are in solution maintained by very different concentrations of SO_2 and CO_2 in the gas phase. Moreover, at pH values not far above neutrality, SO_2 liberates two protons instead of the one liberated by CO_2 under the same conditions. This results in the accumulation of SO_3^{2-}. Whereas CO_2 uptake from HCO_3^- during photosynthesis frees the hydroxyl ion which had been consumed during HCO_3^- formation in the chloroplast stroma, only a small part of the protons released during solubilization of SO_2 is removed as H_2S when sulfite is reduced (35). Other protons accumulate with the SO_4^{2-} which is formed by oxidation of sulfite (11, 32). The extent of possible proton accumulation is shown in Table III. However, the full extent of proton accumulation is not realized, because flux restrictions prevent equilibrium between SO_2 in the atmosphere and in aqueous compartments of leaf cells. For the present purpose it is sufficient to consider briefly measured fluxes of SO_2 in relation to buffering in leaves. Taylor and Tingey (35) have, in the presence of 0.4 μl/L SO_2 in the atmosphere, measured SO_2 fluxes into leaves of *Geranium carolinianum* of about 2500 nanomol/dm^2 · h. Since 1 dm^2 contains about 3 mg Chl, this corresponds to a SO_2 flux of about 0.8 μmol SO_2/mg Chl · h. The stomatal resistance for SO_2 was 6 s/cm. Depending on the pH distribution in the different mesophyll compartments, about 1 to almost 1.5 μequivalents H^+ will be released/mg Chl · h during anion formation from 0.8 μmol SO_2. Since the buffer capacity of a leaf is about 10 μequivalents H^+/mg Chl · pH unit within the physiological pH range, within 10 h exposure to 0.4 μl/L SO_2 the pH inside the leaf might be expected to decrease by 1 pH unit if no pH stabilizing metabolic mechanisms are at work (28). Actually this

Table III. *Bicarbonate, Carbonate, Bisulfite and Sulfite Concentrations in Equilibrium with CO_2 and SO_2 in the Gas Phase at Different pH Values*

To illustrate differences between CO_2 and SO_2 in gas solubility and pK values, equal concentrations of bicarbonate and bisulfate are compared. Solubility data were taken from Refs. (5) and (15). pK Values: CO_2/HCO_3^- = 6.37; HCO_3^-/CO_3^{2-} = 10.25 (37); SO_2/HSO_3^- = 1.78; HSO_3^-/SO_3^{2-} = 6.9.

	CO_2 (Gas)	CO_2 (Solution)	HCO_3^-	CO_3^{2-}	H+ Liberated
	$\mu l/L$	μM	μM	μM	$\mu mol/ml$
pH					
8[a]	350	12	512	2.1	0.52[b]
7.4[c]	350	12	128	0.14	0.13
5.8[d]	350	12	3.2		0.003
3.5[e]	350	12	0.016		
	SO_2 (Gas)	SO_2 (Solution)	HSO_3^-	SO_3^{2-}	H^+ Liberated
8[a]	0.0002	$3.1 * 10^{-4}$	512	5120	10.8[f]
7.4[c]	0.0002	$3.1 * 10^{-4}$	128	321	0.77
5.8[d]	0.0002	$3.1 * 10^{-4}$	3.2	0.2	0.003
3.5[e]	0.0002	$3.1 * 10^{-4}$	0.016	$5*10^{-6}$	

[a] Chloroplast stroma, light (sunflower, barley, spinach). [b] 0.016 μmol/mg Chl. [c] Chloroplast stroma, dark; cytosol. [d] Vacuoles (mesophyll, barley). [e] Vacuoles (needles of fir). [f] 0.33 μmol/mg Chl.

Table IV. *SO_2 Concentrations Measured in 1985 in Warmensteinach (Frankonia, F.R.G.) 800 m above Sea Level*

Data were taken from Lufthygienische Monatsberichte (22).

	January	February	March	April	May	June
Average concentration (μl/L)	0.012	0.017	0.014	0.007	0.0086	0.0038
Maximum during 0.5 h (μl/L)	0.09	0.212	0.152	0.107	0.166	0.098

calculation is too optimistic. The situation is in fact much worse. As has been outlined above, the small neutral and alkaline compartments of the protoplasm will trap much more of the SO_2 than the large acidic compartment of the vacuole because gradients of SO_2 supporting diffusion into these compartments are much larger than gradients into the central vacuole. Quite obviously, photosynthesis is particularly sensitive in this situation as it requires an alkaline pH of the stroma. It is also evident that protonation damage can be minimized only by minimizing diffusion gradients of SO_2. It should be emphasized that, whereas concentrations of SO_2 in the air close to 0.4 μl/L are rare, concentrations of 0.02 μl/L are rather common (Table IV). What has been derived here for 0.4 μl/L SO_2 is valid also for 0.02 μl/L SO_2, if the time factor is taken into consideration: the same proton release will simply require a correspondingly longer diffusion time. The simple conclusion from our analysis is that the concentration of gaseous air pollutants must be decreased to an extent which permits pH-stabilizing reactions of the cells to cope with acidification. The capacity of such reactions is still undefined and requires investigation.

Acknowledgments—We wish to thank Dr. Enrico Martinoia and Michael J. Schramm for help and stimulating comments.

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