Methylammonium Transport in *Phaseolus vulgaris* Leaf Slices[1]

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ABSTRACT

Methylammonium (as a nonmetabolized analog of ammonium) transport was studied in leaf slices of *Phaseolus vulgaris* L. var. 'Hawkesbury Wonder.' The relationship of influx to external pH (6.0–10.5) shows that the influx at low external pH is a larger fraction of that at high external pH than would be expected from the pK_a of methylammonium and the assumption that only CH_3NH_2 is entering the cells. The relationship between methylammonium influx and external methylammonium concentration shows some evidence of saturation; this is a function of the transport system rather than of the (limited) methylammonium metabolism in the cells. The "equilibrium" concentration ratio for methylammonium between leaf slices and bathing medium is far higher than can be explained by the transport of CH_3NH_2 alone and the pH of the compartments involved. These three lines of evidence strongly suggest that there is an influx of $CH_3NH_3^+$, possibly by a uniporter driven by the electrical potential of the cytoplasm with respect to the medium, as has been shown for other plant cells. Competitive inhibition of methylammonium influx by ammonium suggests that there is also an ammonium transport system. The significance of this for the recycling of N within the plant and for exchange of gaseous NH_3 between leaves and the atmosphere is discussed.

The leaf apoplast is the terminus for the liquid-phase portion of the transpiration stream. Any solutes in the xylem solution which have not been abstracted by stem or petiole cells (15) must be removed by leaf cells if unacceptable accumulations in the leaf apoplast are to be avoided. Although ammonium[3] is generally a minor contributor to the total N and the total solutes in the transpiration stream (18), it must be removed from the leaf apoplast. Because ammonium generally makes an even smaller contribution to the total mobile N in the phloem than in the xylem (15), it is clear that ammonium delivered to the mature (nongrowing, non-N-accumulating) leaf must be largely converted to organic N before re-export in the phloem; this conversion probably occurs in mesophyll cells (6), although it might also involve phloem companion cells. The mesophyll cells (at least in C_3 plants) have a large capacity for ammonium assimilation related to the photorespiratory carbon oxidation cycle (11); the ammonium flux in this pathway exceeds that of net N assimilation in the leaf even

when all of the nitrate assimilation in nitrate-grown plants occurs in the leaves (2). The immediate application of the work described here relates to the control of gaseous NH_3 fluxes between *Phaseolus vulgaris* leaves and the aerial environment (2). A major determinant of the NH_3 concentration in the intracellular gas spaces in equilibrium with the leaf apoplast solution is the ammonium concentration in the cell wall solution; other important factors are the temperature of the leaf and the apoplastic pH.

The work reported here deals with the mechanism(s) of ammonium transport at the plasmalemma of leaf of *P. vulgaris*; the other important parameter (apoplastic pH) will be dealt with elsewhere (J. A. Raven, manuscript in preparation). Work on a wide range of other cell types has shown that, in addition to the unfacilitated movement of NH_3 through the lipid portion of the cell membranes, the plasmalemma commonly possesses an NH_4^+ uniporter system (4, 16, 18, 20, 22, 23, 25–27; *cf.* ref. 13). The NH_3 diffusion system on its own leads to an ammonium distribution related to the pH on each side of the membrane. The NH_4^+ uniport leads to an equilibrium distribution related to the electrical potential difference across the membrane; however, the involvement of a specific uniporter permits control of net NH_4^+ fluxes (and, hence, of ammonium levels in phases of restricted volume separated by the membrane) via induction/repression and feedback inhibition. The occurrence of the NH_4^+ uniporter system would permit a more precise control of ammonium (and NH_3) concentration in the leaf apoplast than would the occurrence solely of NH_3 diffusion. An elegant demonstration of NH_4^+ transport come from parallel voltage-clamp and net ammonium (methylammonium) flux measurements (25, 26); such experiments were not possible on leaf cells. The (putatively) nonmetabolized analog methylammonium was used; in addition to the ready availability of a convenient isotopically labeled form (^{14}C), the absence of metabolism makes the results of experiments on the relationship between influx and external concentration, and on steady-state distribution between medium and cells, more readily interpretable in terms of CH_3NH_2 or $CH_3NH_3^+$ transport.

MATERIALS AND METHODS

P. vulgaris L. var. Hawkesbury Wonder was grown in pots in a glasshouse in Canberra, using a Hewitt nitrate-type nutrient solution. Plants used were 20 to 40 days old (2). Leaflets from trifoliate leaves were detached, and their area and wet weight were measured. They then were sliced into 1-mm thick slices with razor blades (although the original work on solute transport by leaves of *P. vulgaris* L. var. Brittle Wax quoted 8 mm as the thickness of the leaf slices used by Jacoby and Dagan [8], reference to the figures in Jacoby [9], which claims to follow the same technique, suggests a thickness of 0.8 mm). One-hundred-mg portions of randomized slices then were placed in 30-ml screw-topped vials with 10 ml 0.1 mM $CaSO_4$ solution, and the vials were placed in a thermostatted (at 25 C) water bath on a tray shaking at 0.5 Hz, with illumination from above by means of a Xenon arc lamp providing 200 μE m^{-2} s^{-1} at the level of the leaf slices. Leaf slices

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[3] Ammonium will be used to denote (NH_3 + NH_4^+) without specifying the relative quantities of each species or which species is active in a given process. NH_3 and NH_4^+ will be used to denote those chemical species. A similar usage will be employed from the methylammonium system.

intended for dark tracer treatments were wrapped in two layers of aluminum foil. The presence of the screw-caps reduced the irradiance incident on the slices in the "light" treatments below that quoted above, although reflection from the polished sides of the tank and the shaking tray added some irradiation. The purpose of the screw caps was to prevent loss of CH_3NH_2 in treatments at pH values approaching the pK_a (10.44 at 25 C) of methylammonium and, for consistency, they were used in all treatments.

The pretreatment in 0.1 mM $CaSO_4$ was continued for 30 min, with three changes of solution. After the last change of $CaSO_4$, the medium was changed to an appropriate buffer solution containing 0.1 mM $CaSO_4$ and a 10 mM concentration of a zwitterionic buffer adjusted with NaOH solution to the desired pH which was always within 0.5 pH units of the pK_a. Thus, Mes (pK_a 6.1) was used for pH 6.0 and pH 6.5; Hepes (pK_a 7.5) was used for pH 7.0 and pH 7.5; 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (pK_a 8.0) was used for pH 8.0 and pH 8.5; 2-(N-cyclohexylamino)ethanesulfonic acid (pK_a 9.3) was used for pH 9.0 and pH 9.5; and cyclohexylaminopropanesulfonic acid (pK_a 10.4) was used for pH 10.0 and pH 10.5. After a further 15-min incubation in the buffer solution, it was replaced by a similar buffer solution but with the appropriate concentration and specific radioactivity of [^{14}C]methylammonium (unlabeled obtained from Sigma; radioactive form obtained from the Radiochemical Centre, Amersham, United Kingdom) plus any other solutes required in the experiment added. After the desired incubation time (0.5, 1.0, 1.5, 2.0 h) the radioactive solution was removed and replaced by 10 ml 1 mM $CaSO_4$ for 5 min; the $CaSO_4$ treatment was repeated three times in all. It was demonstrated that this procedure removed the "fast phase" of methylammonium in efflux experiments. The leaf slices then were transferred to aluminum or stainless steel planchettes (about 30 mg/planchette); 0.2 ml 10 mM HCl-100 mM sucrose solution then was added, a disc of lens tissue was placed over the slices, and the planchette was dried on a hot plate. Aliquots (0.1 ml) of bathing solution were taken before and after the slices had been incubated in the solution and were treated in the same manner as the leaf slices. Planchettes were counted on an Instrument-Developments Limited gas-flow automatic planchette counter. In experiments in which the extent of methylamine metabolism was determined, the stainless steel planchettes were treated with 0.5 ml 10 mM NaOH solution and dried down again in a fume hood; this treatment volatilized all radioactivity remaining as methylamine (checked with samples of bathing solution), leaving all metabolites, other than those which were ^{14}C-labeled, volatile weak bases (22). The pH of the uptake solution was measured after the experiment.

To determine the equilibrium distribution of methylammonium between the uptake solution and the leaf slices, longer term experiments of a similar design to those described above for uptake times of up to 2 h were carried out, with equilibration times of up to 20 h. In order to test if the same distribution of methylammonium occurred when prelabeled leaf slices were equilibrated with methylammonium-free solution as when methylammonium was being taken up from the uptake solution, samples of leaf slices replicate to those for which uptake equilibration was measured were transferred to methylammonium-free buffer solution, and the bathing medium was sampled at intervals. At the end of the experiment, the leaf slices were assayed for total and non-alkali-volatilized counts. To check on the possibility that any difference between "influx" and "efflux" distributions of methylammonium between slices and medium were a function of the total duration of the experiment (inasmuch as "aging" phenomena occur in leaf slices [14]), influx experiments were run over the same total time as influx/efflux experiments.

The over-all tissue pH was measured by grinding 1 g leaf tissue in 4 ml distilled H_2O and measuring the pH of the resulting homogenate. It is appreciated that this pH is closest to, but not identical with, that of the vacuolar compartment.

RESULTS

Figure 1 shows the time course of [^{14}C]methylammonium influx from 0.1 mM methylammonium chloride solution at pH 7.0 in the light; the influx is essentially linear with time over the 2-h period. Similar results were found in experiments at other pH values and methylammonium concentrations and in the dark; accordingly, the results shown in Figures 1 to 3 and Tables I and II refer to the mean influx computed from 2-h influx periods.

Figure 2 shows methylammonium influx from 0.1 mM methylammonium chloride solution as a function of the pH of the uptake solution. For comparison, the fraction of total methylammonium present as CH_3NH_2 is shown as the portion of the figure below the solid line. It will be seen that, although influx increases with extracellular pH, the increase is nowhere near as great as would be expected if CH_3NH_2 were the sole penetrating species; this agrees with the findings for many other plant tissues (20, 22, 23, 27). The possibility that the influx at the higher external pH values had been underestimated due to the postuptake washing procedure for removal of the free space methylammonium was checked, the argument being that, if sufficient CH_3NH_2 had entered to increase substantially the intracellular pH, there might be a substantial loss of CH_3NH_2 from this high-pH compartment during the washing procedure. We found (results not shown) that more ^{14}C was lost in the washing procedure from leaf slices incubated at high pH than from those incubated at lower pH values. However, correction for this source of error increased the influx into the cells at high pH by less than 2-fold and, thus, could not explain the
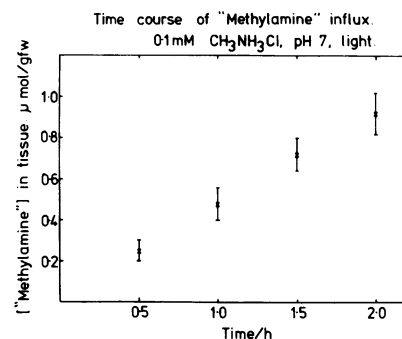

FIG. 1. Time course of [^{14}C]methylammonium influx from 0.1 mM methylammonium; conditions were: pH 7, 200 μE m^{-2} s^{-1}, 25 C.
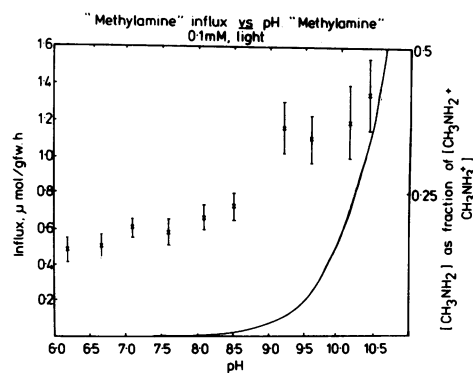

FIG. 2. pH dependence of [^{14}C]methylammonium influx from 0.1 mM methylammonium; conditions were: 200 μE m^{-2} s^{-1}, 25 C; influx was over 2 h. (——), dissociation of methylammonium as a function of pH; area above the line indicates $CH_3NH_3^+$; area below the line indicates CH_3NH_2.

discrepancy between the measured relationship between methylammonium influx and external pH and that predicted for CH_3NH_2 entry as the sole mode of influx; we concluded that $CH_3NH_3^+$ entry must be the dominant mode of methylammonium influx at external pH values below 7.0.

Figure 3 shows the methylammonium influx from external methylammonium concentrations of 10 μM to 30 mM in the light at pH 7.0. There is. no convincing evidence for saturation of the influx within this range but, equally, the influx does not increase in direct proportion to the external concentration. This finding is not without precedent for (methyl-) ammonium influx in a number of organisms or for the influx of a number of solutes in leaf slices.

Further evidence consistent with a substantial component of CH_3NH_3 influx at the lower pH values investigated is seen in Table I, where the influence of ammonium on methylammonium influx at pH 7 in the light is shown. The inhibition of methylammonium influx by ammonium seems to be competitive; this is consistent with NH_4^+ competing with $CH_3NH_3^+$ for a specific porter, but not with entry of CH_3NH_2 and NH_3 via a lipid pathway. Ammonium has a smaller inhibitory effect on methylammonium influx at an external pH of 10 (results not shown); although this is consistent with a contribution of CH_3NH_2 entry at this pH which is close to the pK_a of methylammonium, the results are inconclusive in that ammonium is above its pK_a, so very high total ammonium concentrations are required to provide
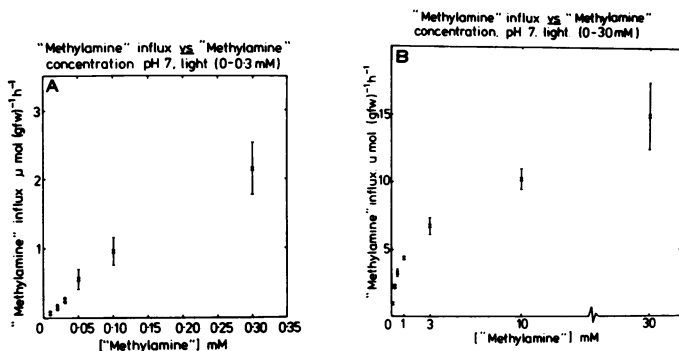

FIG. 3. Dependence of [¹⁴C]methylammonium influx from 0.01 to 0.5 mM (A) and from 0.5 to 30 mM (B) methylammonium; conditions were: pH 7, 200 μE m⁻² s⁻¹, 25 C.

Table I. *Effect of Ammonium Chloride on Influx of [¹⁴C]-Methylammonium Chloride in Leaf Slices of P. vulgaris L. var. Hawkesbury Wonder*

The experiments were run under light conditions; the buffer used was 10 mM Hepes NaOH, 0.1 mM CaSO₄ (pH 7.0).

[¹⁴C]Methyl-ammonium Concentration	Nature and Concentration Added Cation	Influx of [¹⁴C]Methyl-ammonium
mM	*mM*	*$\mu mol\ g^{-1} fresh\ wt\ h^{-1}$*
0.1	None	0.466 ± 0.029
0.1	Ammonium, 0.03	0.311 ± 0.019
0.1	Ammonium, 0.10	0.133 ± 0.015
0.1	Ammonium, 0.30	0.044 ± 0.006
0.3	None	1.117 ± 0.088
0.3	Ammonium, 0.03	1.076 ± 0.079
0.3	Ammonium, 0.10	0.768 ± 0.068
0.3	Ammonium, 0.30	0.281 ± 0.026
0.1	None	0.557 ± 0.052
0.1	Na⁺, 0.10	0.528 ± 0.049
0.1	Na⁺, 0.30	0.571 ± 0.057
0.1	K⁺, 0.10	0.577 ± 0.061
0.1	K⁺, 0.30	0.533 ± 0.050

Table II. *Effect of Light, Darkness, and DCMU on Methylammonium Influx in P. vulgaris Leaf Slices*

Conditions	Influx of [¹⁴C]Methyl-ammonium
	$\mu mol\ g^{-1} fresh\ wt\ h^{-1}$
Light, 200 μE m⁻² s⁻¹	0.388 ± 0.033
Light, 200 μE m⁻² s⁻¹, plus 1 μM DCMU	0.411 ± 0.040
Dark	0.376 ± 0.038

the same NH_4^+ concentration as was used at lower pH values. Table I also shows that the addition of singly charged cations other than ammonium had no significant effect on methylammonium influx at the concentration tested.

Table II shows that the absence of illumination, or the presence of the PSII inhibitor DCMU in the light, had no significant effect on the methylammonium influx. The absence of light stimulation (at least under aerobic conditions) of solute transport in the photosynthetic tissues of vascular land plants is not unexpected (7, 10).

Table III shows the results of experiments in which equilibrium distributions of methylammonium were measured in experiments in which equilibrium was approached from either the influx or from the efflux direction. Also shown is the extent of methylammonium metabolism (*i.e.* the radioactivity not released from the planchettes by treatment with alkali). The external concentration fell from 2 to 0.3 μM in the first 11 h of the influx experiment; over the next 8.70 h, the concentration fell from 0.302 to 0.225 μM. In the efflux part of the experiment, the external concentration increased from 0 at 15.00 h to 0.39 μM at 16.50 h and to 0.050 μM at 19.70 h. Inasmuch as the influx external concentration of methylammonium was still slowly falling at 19.70 h while the efflux external concentration was still slowly rising at 19.70 h, we concluded that the final equilibrium concentration ratio C_i/C_0 must lie between the ratios computed from the influx experiment (367) and that derived from the efflux experiment (1,382). Even the lower estimate of C_i/C_0 (that derived from the influx experiment) cannot be explained in terms of a passive distribution of CH_3NH_2 across the plasmalemma in response to the pH values on the inside and outside of the plasmalemma. The external pH at the end of the influx experiment was 6.65 ± 0.003, whereas the mean intracellular pH was 6.22 ± 0.04. Inasmuch as the pK_a of methylammonium is 10.44, such pH difference could only account for a C_i/C_0 for methylammonium of 3.39 at passive flux equilibrium of CH_3NH_2 if no transport of $CH_3NH_3^+$ took place. Plausible assumptions as to the volume and pH of the cytoplasmic and vacuolar phases of the cell such as would yield a mean intracellular pH equal to the observed value of 6.22 cannot give a computed C_i/C_0 for methylammonium of even 0.01 of the measured minimum value of 367. The observed C_i/C_0 for methylammonium can be readily explained if the major flux of methylammonium at the plasmalemma is of $CH_3NH_3^+$, with the tonoplast flux being entirely as CH_3NH_2, provided there is a substantial inside-negative ψ_{co}.[4] The relevant measurements of ψ_{co} do not seem to have been made on *P. vulgaris*, but measurements on mesophyll cells of leaves of other flowering plants give values more negative than −120 mv (*e.g.* the work of Tattar [24] on the legume *Vigna sinensi* in which a ψ_{co} of −181 mv was found). A ψ_{co} of −120 mv would give a cytoplasmic [$CH_3NH_3^+$] of 100 times the external [$CH_3NH_3^+$] at passive flux equilibrium. Assuming a cytoplasmic pH of 7.2 (21) and with the measured external pH value of 6.75, the [$CH_3NH_3^+$]$_{cyt}$/[$CH_3NH_3^+$]$_{out}$ of 100 corresponds to a [CH_3NH_2]$_{cyt}$/[CH_3NH_2]$_{out}$ of about 280. This outwardly directed concentration gradient of CH_3NH_2, together with the substantial

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[4] Abbreviation: ψ_{co}, electrical potential of the cytoplasm with respect to the medium. EPPS, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid.

Table III. *Equilibration of Internal and External Concentrations of $[^{14}C]$-Methylammonium during Influx Experiments*

Influx experiments started with a known fresh weight (about 250 mg) of leaf slices in 10 ml 0.1 mM $CaSO_4$, 2 μM methylammonium chloride, 10 mM Hepes-NaOH (pH 7). Efflux experiments started with the prelabeled leaf slices placed in 10 ml 0.1 mM $CaSO_4$, 10 mM Hepes-NaOH (pH 7). The nonmethylammonium radioactivity in the leaf slices amounted to 19.3 ± 2.9 μM in the influx experiment and 17.1 ± 2.8 μM in the efflux experiment. The equilbrium C_i/C_o in the influx experiment was 367; in the efflux experiment, the ratio C_i/C_o was 1,382.

Time from Start of Experiment	Influx Experiment		Efflux Experiment	
	C_o	C_i	C_o	C_i
h	μM		μM	
11.00	0.302 ± 0.028 (12)[a]			
13.25	0.228 ± 0.026 (12)			
15.00	0.236 ± 0.028 (12)			
16.50	0.248 ± 0.027 (6)		0.039 ± 0.002 (6)	
18.50	0.217 ± 0.022 (6)		0.044 ± 0.003 (6)	
19.70	0.225 ± 0.014 (6)	85.2 ± 9.6 (16)	0.050 ± 0.004 (6)	69.1 ± 7.8 (6)

[a] Values in parentheses are number of replicates for the various treatments.

$P_{CH_3NH_2}$, means that a CH_3NH_2 efflux will partially "short-circuit" the potential-driven accumulation of $CH_3NH_3^+$, *i.e.* [methylammonium]$_{Cyt}$/[methylammonium]$_{out}$ will be less than 100. Even if this ratio is as low as 33, a mean intracellular [methylammonium] 397 times that in the medium can be calculated (assuming equilibration of CH_3NH_2 between the cytoplasm occupying 0.05 of the intracellular volume at a pH of 7.2 and the vacuole occupying 0.95 of the intracellular volume at a pH of 6.1). With otherwise identical assumptions, the efflux value of [$CH_3NH_3^+$]$_{inside}$/ [$CH_3NH_3^+$]$_{outside}$ of 1,382 would require a ψ_{co} of -160 mv. The effect on ψ_{co} of the low concentrations on (methyl) ammonium used in these experiments does not seem to have been investigated in higher plants (5, 13).

DISCUSSION

The relationship between influx and external pH (Fig. 2) is consistent with much of the methylammonium influx at low extracellular pH values being as $CH_3NH_3^+$. The possibility (3) that the higher weak electrolyte influx than is expected for entry solely of the undissociated form, and the concentration of the undissociated form at low pH values, is due to the dissociated form (plus buffered H^+) acting to decrease the significance of unstirred layers for CH_3NH_2 transport to the plasmalemma does not seem to be quantitatively adequate to account for the results shown in Figure 1. Assuming CH_3NH_2 entry alone, and with the measured mg fresh weight cm^{-2} leaf surface (one side) of 20 mg cm^{-2} and the ratio of internal leaf area to external leaf area of 22 measured for *P. vulgaris* L. cf. Kentucky Wonder (12), the CH_3NH_2 influx at pH 6.3 is 0.12 pmol $(cm^2$ internal leaf area)$^{-1}$ s^{-1}, which implies a $P_{CH_3NH_2}$ of at least 1.7×10^{-2} (computed from the relationship $P_{CH_3NH_2}$, cm s^{-1}, equals influx pmol cm^{-2} s^{-1} divided by the CH_3NH_2 concentration in the medium, pmol cm^{-3}). This value is much higher than that found for *Chara corallina* (26), *i.e.* 1.8×10^{-3} cm s^{-1} for the membrane alone (corrected for unstirred layer effects). It is thus likely that, at least at pH values below 7.0, methylammonium influx in *P. vulgaris* leaf slices is predominantly as $CH_3NH_3^+$.

The finding (Fig. 2) that methylammonium influx does not show an obvious "single isotherm," "dual isotherm," or "multiple isotherm" relationship to external concentration is similar to findings for some other solute transport systems in leaf slices (10). Experiments on *P. vulgaris* L. cv. Brittle Wax showed dual isotherms for K^+ (Rb^+), Na^+, and Cl^- influx (9). Ammonium influx in the algae *Hypnea musciformis* and *Macrocystis pyrifera* (4) as a function of external concentration shows a saturable component

at low concentrations with a continued increased in influx at higher concentrations; it is not clear if this nonsaturating component of the influx represents nonmediated NH_3 entry. The results (Fig. 2) are consistent with a mediated influx of methylammonium inasmuch as the small extent of methylammonium metabolism (Table III) could not account (via saturation of the metabolic system with methylammonium, with consequences for the driving force for CH_3NH_2 influx) for the kinetics of methylammonium influx in terms of CH_3NH_2 entry, at least at low concentrations. The ammonium inhibition experiments are also consistent with a catalyzed (methyl-) ammonium transport system. Another very persuasive argument in favor of the predominance of $CH_3NH_3^+$ influx at the plasmalemma comes from the data shown in Table III; the equilibrium distribution of free methylamine between the cells and the medium cannot be explained in terms of the mean intracellular and extracellular pH values and transport of only CH_3NH_2. The equilibrium distribution can, however, be adequately explained in terms of transport of both CH_3NH_2 and $CH_3NH_3^+$ at the plasmalemma and of CH_3NH_2 alone at the tonoplast, granted a substantial inside-negative value of ψ_{co}. The values of ψ_{co} in the mesophyll cells may be less negative than those quoted under "Results" inasmuch as the apoplastic (K^+) is likely to be about 5 mM [Table 12.2 of Lüttge and Higinbotham (14); J. A. Raven, manuscript in preparation]. However, this is not likely to be very important for ammonium transport *in vivo* where the accumulation ratio (cytoplasm:vacuole) is unlikely to exceed 10 to 20, so we do not need to invoke H^+ symport for ammonium entry (19).

The conclusion that the cells (predominantly mesophyll cells) in *P. vulgaris* leaf slices have a system which catalyzes a net influx of $CH_3NH_3^+$ (NH_4^+) at the plasmalemma, probably by a uniporter driven by the inside-negative ψ_{co}, has important repercussions for the analysis of both the role of the leaf apoplast in N recirculation in the plant and in the exchange of gaseous NH_3 between the leaf and the environment.

With respect to N recirculation within the plant, the presence of a NH_4^+ uniporter system at the mesophyll cell plasmalemma permits the removal of ammonium supplied in the transpiration stream in a more controllable manner than would be the case if only NH_3 transport occurred at the plasmalemma. Although methylammonium accumulation may proceed to near thermodynamic equilibrium with ψ_{co}, it is likely that the transport of the natural, metabolized, substrate ammonium is subject to control by products of ammonium assimilation (16). The concentration of ammonium supplied to the transpiring leaf in the xylem is about 0.1 mM in *P. vulgaris* (J. L. Raven, manuscript in preparation);

how much of this is abstracted before the transpiration stream reaches the leaf is not clear. There is likely to be some countercurrent distribution of NH_3 between xylem and phloem (17) if the phloem sap is 1.5 pH units more alkaline than the xylem solution (18) the 0.1 mM ammonium in the xylem would be in equilibrium with 3 mM ammonium in the phloem. The mesophyll apoplast in *P. vulgaris* is, in steady-state transpiration, at a higher pH than that of the xylem sap (as measured in bleeding sap) and is close to pH 6.8 which is the equilibrium pH for *P. vulgaris* leaf slices incubated in aerated, artificial xylem sap (J. L. Raven, manuscript in preparation). This has implications for the transport of ammonium between the apoplast and the mesophyll cells.

The gaseous NH_3 compensation point for *P. vulgaris* leaves at 26 C [2.5 nbar (21)] corresponds to an ammonium concentration in the apoplast of 48 μM at the apoplastic pH of 6.8. If ammonium is being supplied to the leaf apoplast at a rate of 0.6 μmol g^{-1} fresh weight h^{-1} (J. L. Raven, manuscript in preparation) and at a transpiration stream concentration of 0.1 mM, in order to maintain a steady-state apoplastic concentration of 48 μM as described above, the net influx into the mesophyll cells must exceed 0.6 μmol g^{-1} fresh weight h^{-1} at a concentration of 0.1 mM. The methylammonium influx into leaf slices from solutions at pH 6.8 containing 0.1 mM methylammonium is in the range 0.3 to 1.0 μmol g^{-1} fresh weight h^{-1} (Figs. 1-3; Tables I-III). Inasmuch as the data in Table I are consistent with the transport system having a higher affinity for ammonium than for methylammonium, the capacity for ammonium influx from 0.1 mM solution at pH 6.8 probably exceeds 1 μmol g^{-1} fresh weight h^{-1}. The net influx required to maintain the extracellar ammonium concentration at that required to give the observed NH_3 compensation partial pressure could then be a function of regulation by (*inter alia*) the cytoplasmic concentration of metabolites of ammonium, and of any ammonium removal from the transpiration stream before it reaches the mesophyll apoplast.

A final consideration relates to the role of plants in controlling the atmospheric NH_3 level. This partial pressure of NH_3 is lower over the sea than it is over the land areas (1) and suggests that marine plants (mainly phytoplankton) maintain a lower dissolved NH_3 level near the surface of the sea than the leaves of higher plants on land maintain in their leaf apoplast. Because the pH of the sea is at least 1 pH unit higher than that of the leaf apoplast, this requires that the ammonium concentration near the surface of the sea should be less than one-tenth that of the leaf apoplast, *i.e.* about 1 to 2 μM. This is similar to many quoted values of ammonium levels near the surface of the sea (1).

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