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Base uptake, K^+ transport and intracellular pH regulation by the green alga *Chlorella fusca*

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Uptake of permeant bases by cells of *Chlorella fusca* and the concomitant effects on K^+ movements and intracellular pH have been investigated. From various bases tested benzylamine and trimethylamine were selected for measurement and manipulation of the intracellular pH. Uptake of benzylamine and trimethylamine in the light reached an equilibrium distribution after 30–50 min corresponding to an average intracellular pH (pH_i) of 5.6 at lower base concentrations. At higher concentrations base accumulation declined according to an increase of pH_i. The low pH_i was attributed to acidic compartments, mainly thylakoids and microvacuoles. In the dark pH_i was higher but an additional continuous component of base uptake was present which was attributed to respiratory CO_2. Base uptake was accompanied by K^+ release which was equimolecular at conditions of lower base uptake. With increasing base uptake K^+ release lagged behind and reached saturation about half of the cellular K^+ being extruded. High external K^+ concentrations inhibited base accumulation. The distribution of Na^+ was not affected by bases. The pH_i calculated from base distribution was almost constant up to intracellular base concentrations of 20 mM. Then pH homeostasis decreased and finally broke down at internal base concentrations > 80 mM. This concentration corresponds to the maximum amount of K^+ which can be extruded. Kinetics of base uptake were first order in accordance with unspecific diffusion of the free base. K^+ release, however, showed saturation kinetics with a v_{max} of 4–6 $\mu mol \cdot min^{-1} \cdot (ml\ cells)^{-1}$ indicating carrier-mediated efflux. The results strongly suggest the existence of a K^+/H^+ antiporter in the cell membrane of *Chlorella* which is involved in intracellular pH regulation being activated by intracellular alkalization.

Introduction

Studies with the green alga *Chlorella fusca* have shown that this organism regulates intracellular acidity by proton/potassium exchange through specific membrane-bound transport systems. The effects of intracellular acidification on K^+ transport have been studied in detail. It has been found that *Chlorella* posseses an electroneutral ATP-driven transport system which actively extrudes protons in exchange for potassium [1]. Net K^+ uptake by this system is stimulated by a variety of agents which acidify the cell interior: permeant acids [2], uncouplers [3], but also metabolic reactions as glucose assimilation [4] and fixation of CO_2 [5].

The subject of the present study is now the other side of intracellular pH regulation, i.e. the

Abbreviations DCCD, *N,N'*-dicyclohexylcarbodiimide, DMO, 5,5-dimethyloxazolidine-2,4-dione, Pipes, 1,4-piperazinediethanesulfonic acid, Tris, tris(hydroxymethyl)-aminomethane

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compensation of internal alkalization. Preliminary experiments had shown that permeant bases induced a K^+ release [2]. This effect has been attributed to a K^+/H^+ antiport. An exchange of this kind needs no metabolic energy it can be driven by the K^+ gradient alone. It must, however, be strictly regulated, otherwise it would dissipate the cellular K^+. The basic idea is that the antiporter acts as a safety valve that only opens if internal acidification is needed. In bacteria the best evidence for a mechanism of this kind has been obtained with *Vibrio alginolyticus* [6]. Although K^+/H^+ antiport systems are common in bacteria (e.g. the well characterized KHA system in *Escherichia coli* [7,8]) their involvement in intracellular pH regulation is not conclusively established in many cases [9]. In alcalophiles, Na^+ rather than K^+ seems to be responsible for intracellular pH regulation [9]. In respiring bacteria the necessity of an energy conserving proton gradient at the cell membrane is an additional complication [9,10].

Observations of K^+/H^+ antiport in plants are not yet numerous, nevertheless these studies suggest a widespread occurrence of this mechanism. There are reports of K^+ release via specific K^+/H^+ exchange being induced by special treatments in various plant systems: by dithioerythritol in corn roots [11], by ultraviolet irradiation in rose cells [12], and by a pathogenic bacterium as well as by a pectate lyase in tobacco cells [13,14]. All these observations have been interpreted as the activation of a native transport system that is normally suppressed. A role for this system in pH regulation has also been suggested [12]. There is also some in vitro evidence of electroneutral K^+/H^+ antiport in plasmalemma and tonoplast vesicles [15,16].

The present work investigates the uptake of permeant bases by *Chlorella* and their effects on K^+ movements and intracellular pH. Strong evidence is obtained for the existence of a plasmalemma-bound K^+/H^+ antiport system that plays an important role in intracellular pH regulation.

Materials and Methods

Chlorella fusca (211-8b, Göttingen) was cultivated and harvested as described [2]. The algae were suspended in a medium consisting of 10^{-4} M $CaCl_2$, 5 mM Tris and Pipes to give a final pH of 7.0 (standard medium). Cell density was determined by hematocrit and adjusted between 5 and 10 ml packed cells/l suspension, as indicated. The experimental suspensions were aerated at 30 °C and illuminated by fluorescent light (50 watts/m^2), if not otherwise stated. Before starting the experiments the cells were preconditioned for 1.5 h in standard medium in the light. After this time the initial K^+ movements (K^+ release followed by almost complete reuptake) had slowed down and the external K^+ concentrations were between 0.005 and 0.03 mM and remained fairly constant. Then the respective pH values were adjusted by addition of solid Tris. After preconditioning another 1/2 h the experiments were started by addition of the base in labelled or unlabelled form (time zero). K^+ movements and base uptake were followed by analyzing 5-ml samples of the supernatant obtained by 1 min centrifugation at $4000 \times g$. K^+ was determined by atomic absorption spectrometry, the labelled bases were measured by liquid scintillation counting. For determination of the total amounts of K^+ and labelled base a 5 ml sample of the suspension was strongly acidified by addition of 0.1 ml concentrated sulfuric acid. After death of the cells the supernatant was obtained and analyzed as described above. The total value of the labelled bases benzylamine and trimethylamine was independent of time. The bases were added as hydrochlorides from 0.4 M stock solutions which were prepared by neutralizing the pure bases with HCl to pH 6.5. This rather acidic pH was necessary to keep the bases in solution. In order to prevent a pH change of the experimental solutions an appropriate amount of 1 M Tris had to be added together with the hydrochloride.

Oxygen evolution and respiration were measured with a Clark type electrode. The ATP level was determined, as described earlier [3], with the luciferin luciferase method after extraction with perchloric acid.

[7-^{14}C]Benzylamine and [^{14}C]trimethylamine were obtained from the Radiochemical Centre Amersham.

Results

Effects of various bases on K^+ movements

A base that enters a cell only by diffusion of the unprotonated form through the membrane finally reaches equilibrium distribution according to the formula

$$\frac{c_i}{c_e} = \frac{1+10^{(pK-pH_i)}}{1+10^{(pK-pH_e)}}$$

c_i, c_e, pH_i and pH_e being the respective internal and external base concentrations and pH values. Table I shows the theoretical accumulation ratios calculated for bases of various pK values depending on pH_i and pH_e. The accumulation ratios are given for imidazole (pK 6.95), morpholine (pK 8.33), benzylamine (pK9.33), and pK 6.0 and 14.0. The latter pK represents an upper limit for base accumulation under the conditions considered. Although in *Chlorella*, as in most cells, the cytoplasmic pH is near 7.0 [17,3,4,18] we can expect higher accumulations corresponding to lower pH_i values because the bases are concentrated in acidic subcellular compartments, in plants especially the thylakoids and vacuoles. Therefore the pH_i calculated from base distribution can only be an average value of different compartments: cytoplasm, thylakoids, vacuoles, etc. In Table I also the theoretical magnitude of base uptake is given which can be expected in an algal suspension of 5 ml cells/l the internal cell volume being 60% of the packed cell volume [19].

If it is assumed that a K^+/H^+ antiport compensates all pH changes caused by base uptake each base molecule protonated must give rise to the uptake of 1 H^+ and the extrusion of 1 K^+ The amount of K^+ extruded should therefore almost equal the amount of base taken up, provided the base in the inner compartment is practically completely protonated. This should be the case if the base is strong enough and the intracellular pH not too high. As the average pH_i is in most cases < 6 (see later) this condition is fullfilled with imidazole already, weaker bases being not interesting as there is almost no base uptake (see Table I). As K^+ release can be more easily followed than base uptake, for a preliminary test, base-induced K^+

TABLE I

THEORETICAL DISTRIBUTION OF PERMEANT BASES

Theoretical accumulation ratios c_i/c_e as function of pK and pH_e and pH_i (external and internal pH) The value in brackets is the theoretical base uptake (% of base in the cells) by a suspension of 5 ml cells/l 60% of the packed cell volume being internal space [19]

Base pK	pH_e	pH_i 7 0	6 5	6 0	5 5
6 0	7 0	1 0 (0 3)	1 2 (0 4)	1 8 (0 5)	3 8 (1 1)
	8 0	1 1 (0 3)	1 3 (0 4)	2 0 (0 6)	4 2 (1.2)
	9 0	1 1 (0 3)	1.3 (0 4)	2 0 (0 6)	4 2 (1 2)
Imidazole	7 0	1 0 (0.3)	2 0 (0 6)	5 2 (1.6)	15 4 (4 4)
6 95	8 0	1 7 (0.5)	3 5 (1 0)	9 1 (2 6)	26.8 (7 4)
	9 0	1.9 (0.6)	3 8 (1 1)	9 8 (2 9)	28 9 (8 0)
Morpholine	7 0	1 0 (0 3)	3 1 (0 9)	9 6 (2 8)	30 3 (8 3)
8 33	8 0	7 1 (2 1)	21 9 (6 2)	68 5 (17.0)	215 8 (39 3)
	9 0	18 4 (5 2)	56 5 (14 5)	177 0 (34 7)	557 8 (62 6)
Benzylamine	7 0	1 0 (0 3)	3 2 (0 9)	10 0 (2.9)	31 5 (8 6)
9 33	8 0	9 6 (2 8)	30 3 (8 3)	95 6 (22 3)	302 1 (47 6)
	9 0	68 5 (17 0)	215 8 (39 3)	681 6 (67 1)	2154.9 (86 6)
14 0	7 0	1.0 (0.3)	3 2 (0 9)	10 0 (2 9)	31 6 (8 7)
	8 0	10 0 (2 9)	31 6 (8 7)	100 0 (23 1)	316.2 (48 7)
	9 0	100 0 (23 1)	316 2 (48 7)	1000 0 (75 0)	3163 3 (90 5)

extrusion was measured at pH 7.0 and pH 8.0 for a number of different bases (Fig. 1).

The stronger bases benzylamine (pK 9.33) and trimethylamine (pK 9.81) had similar effects. β-Phenethylamine (pK 9.84) had the same effect as trimethylamine but caused strong foaming of the suspensions. With the assumption of equimolecularity between K^+ release, H^+ uptake and base uptake the K^+ extrusion by these bases at pH 8.0 corresponds to a pH_i around 6.0. Also the pronounced pH dependence of the effects is in agreement with the theoretical values of Table I. Accordingly, the weaker bases morpholine (pK 8.33) and especially imidazole (pK 6.95) have a weaker and less pH dependent effect on K^+. Ammonia (pK 9.5) and methylamine (pK 10.5) strongly induce K^+ extrusion. However, this K^+ release is rather pH-independent and eixsts even at pH 6.0 (data not shown). Some plants and bacteria have specific transport systems for ammonium and methylammonium [20,21]. Similarly carrier-mediated exchange of the protonated bases against K^+ seems to prevail here Probably this is also the case with ethylamine (pK 10.8) and dimethylamine (pK 10.7) which caused strong K^+ extrusion at pH 7.0. So ammonia, methylamine, ethylamine and dimethylamine were not useful for the present investigation. Finally, the very weak bases pyridine (pK 5.25) and the picolines (pK 5.7–6.0) had no or very weak effects on K^+ movements, as expected (data not shown). With dimethylaniline (pK 6.61) the cells died and lost all their potassium. Diethanolamine (pK 9.5) penetrating into cells of *Vibrio alginolyticus* [6] had no effect at all, probably this base is too hydrophilic to diffuse through the cell membrane of *Chlorella*.

On the basis of these results finally benzylamine and trimethylamine were selected for further investigation because these substances not only seem to behave as permeant bases in accordance with theory but also are available in radiolabelled form which facilitates measurement of base uptake.

Effect of bases on energy metabolism

The action of bases on photosynthetic oxygen evolution was tested at pH 7.0 and 8.2 (Table II). Methylamine and imidazole had no effect at all, ammonia and trimethylamine caused moderate and benzylamine a stronger inhibition. As expected, inhibition was stronger at higher pH.

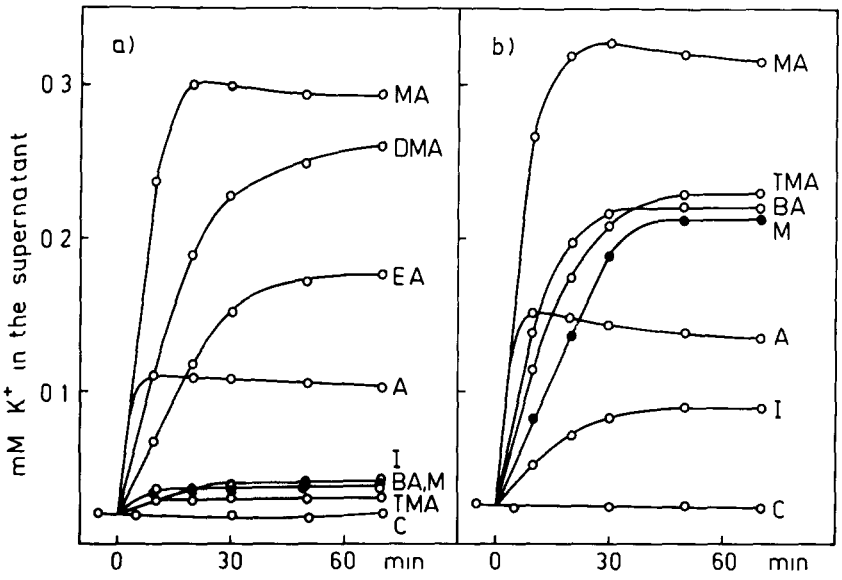


Fig 1 K^+ release induced by various 1 mM bases at pH 7 0 (a) and pH 8 0 (b) 5 ml cells/l, C, control (no addition), MA, methylamine, DMA, dimethylamine, TMA, trimethylamine, EA, ethylamine, A, ammonia; I, imidazole, M, morpholine (●); BA, benzylamine

TABLE II

INHIBITION OF PHOTOSYNTHETIC O_2 EVOLUTION BY BASES

3 ml cells/l, 3 mM $NaHCO_3$ buffer in addition to the usual medium (see Materials and Methods), 50 watt/m^2 Uninhibited O_2 evolution was 16 μmol O_2/min per ml cells

Base	Concentration (mM)	% inhibition	
		at pH 7.0	at pH 8.2
Imidazole	0–10	0	0
Methylamine	0–10	0	0
Ammonia	5	3	14
	10	17	33
Benzylamine	0–0.6	0	0
	1	0	3
	2	0	45, 60 [a]
	3	0	70, 95 [a]
	5	10	85, 100 [a]
	10	22	100
Trimethylamine	2	0	0
	10	13	28

[a] Two values are given where no constant inhibition was reached after 2 min. The first value is the inhibition after 2 min, the second value the final inhibition reached after 15 min.

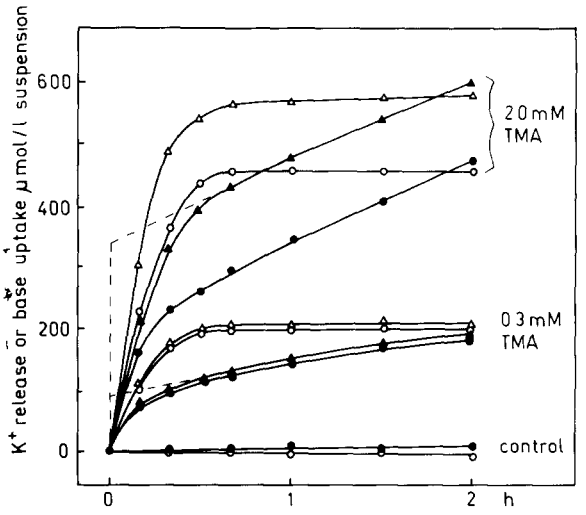


Fig. 2. Uptake of trimethylamine (TMA) and simultaneous K^+ release in the light and in the dark. pH 8.3, 9.5 ml cells/l. The cells were preconditioned in the light as described in Materials and Methods, for the dark experiments the cell suspensions were brought into darkness 5 min before base addition. External K^+ concentration at time zero 0.015 mM. △▲, base uptake and ○●, K^+ release in the light and in the dark. ------ extrapolation of the continuous component of base uptake in the dark to zero. The pH_i values calculated from the saturable components of base uptake are: light 5.75 (0.3 mM base), 6.46 (2 mM base), dark 6.41 (0.3 mM base), 6.73 (2 mM base).

Nevertheless even benzylamine at pH 8.2 had no significant effect on O_2 evolution at concentrations up to 1 mM.

With exception of benzylamine which caused a 30% stimulation the 2 mM bases had no measurable effect on respiration at pH 8.2.

Bases up to 3 mM at pH 8.2 did not significantly affect the ATP level, with 10 mM benzylamine and trimethylamine a 20–40% decrease of the ATP content of the cells could be measured.

Quantitative relation between base uptake and K^+ release

Simultaneous determinations yielded a good molar 1 : 1 relation between base uptake and K^+ release at lower base concentrations (Fig. 2, Table III). At higher base concentrations base uptake surpassed K^+ release as seen in the typical experiment with trimethylamine shown on Fig. 2. In the light base and K^+ movements were finished after 30–50 min, so it was possible to give definite values for integral base uptake and K^+ extrusion under these conditions (Table III). The results with trimethylamine and benzylamine were very similar benzylamine only showing somewhat smaller integral effects but reaching equilibrium distribution more quickly (see also Fig. 3).

In the dark base uptake and K^+ release were also equimolecular at low base concentrations but no constant equilibrium distribution was reached (Fig. 2). By extrapolation of the curves to time zero (Fig. 2) base uptake in the dark can be resolved into two components: (1) a faster component satuable with time corresponding to the base uptake observed in the light but leading to lower base accumulations and (2) a slower but continuous component. Due to this latter component base uptake in the dark can surpass base uptake in the light after some time. Nevertheless the 1 : 1 relation between base uptake and K^+ release was always given for component 2 (see Discussion).

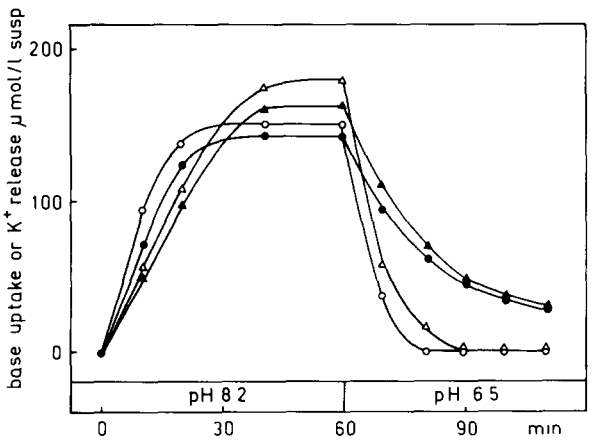
From Table III it can be seen that in the low concentration range base uptake and equimolecular K^+ release increased proportionally with base

TABLE III

BASE UPTAKE, K^+ RELEASE AND pH_i IN DEPENDENCE ON BASE CONCENTRATION AND pH_e

(A) Benzylamine, (B) trimethylamine 9 ml cells/l The data represent the equilibrium distribution reached after 50 min The four figures given are. (a) % of base in the cells, (b) base uptake in μmol base in the cells/l suspension, (c) K^+ release in μmol K^+ released/l suspension, (d) pH_i calculated from base uptake External K^+ concentrations at time zero were 0 005 mM at pH 7 51, 0 01 mM at pH 8 21 and 0.02 mM at pH 8.96 Total K^+ content was 810 μmol K^+/l suspension

pH_e	(A) Benzylamine added (μM)						
	10	30	100	300	1000	3000	8000
7.51 a	30 2	28 8	26 0	24 3	19 6	14 7	7 2
b	3 0	8 6	26.0	72 9	196	441	570
c	–	10	25	70	170	320	410
d	5 60	5 63	5 69	5.73	5 85	6 00	6 55
8 21 a	64.1	61 4	56.4	48 9	31 4	18 7	9 1
b	6 4	18 4	56 4	146 7	314	561	730
c	–	20	55	130	275	435	440
d	5 66	5 71	5 80	5 93	6 25	6 55	6 91
8.96 a	87 6	86 6	84 6	77 6	58 5	21 6	11.7
b	8 8	26 0	84 6	233	585	648	935
c	–	25	85	190	430	445	390
d	5 96	5 73	5 80	6.00	6.39	7 10	7 42
pH_e	(B) Trimethylamine added (μM)						
	10	30	100	300	1000	3000	8000
7 51 a	32 5	31.9	30 0	25 3	20 0	14 0	6 5
b	3 3	9 6	30 0	75 9	200	420	520
c	–	10	28	72	180	350	400
d	5 57	5.57	5 62	5 71	5 85	6 03	6 40
8 21 a	71 6	70 7	70 0	61.4	40.0	17 0	7 9
b	7 2	21 2	70 0	184 2	400	510	630
c	–	20	70	170	360	450	430
d	5 54	5 55	5 57	5 73	6 12	6 62	7 00
8 96 a	90 5	90 8	90 5	81 6	76 0	23 1	8 9
b	9 1	27 2	90 5	244 8	760	693	710
c	–	25	90	195	445	430	403
d	5 65	5 64	5 65	5 99	6 35	7 16	7 65



concentration. At higher base concentrations both effects tended to saturate K^+ extrusion increasingly lagging behind base uptake. Under no conditions more than 55% of the cellular K^+ were extruded. As a consequence of the pH dependence of base uptake an increase of the external pH lowered the base concentrations necessary to reach this limit. Any further increase of base con-

Fig 3 Reversibility of base uptake (open symbols) and K^+ release (dark symbols) with 0 4 mM benzylamine (○●) and 0 4 mM trimethylamine (△▲) 10 ml cells/l External K^+ concentration at time zero 0 015 mM

centration and/or external pH could not enhance K^+ release, on the contrary, even a small decrease of K^+ extrusion was sometimes observed.

Base uptake was totally reversible. If after base uptake at pH 8.2 the medium was brought to pH 6.5 all base was chased from the cells. Base release was accompanied by a slower reuptake of the K^+ lost before (Fig. 3)

External K^+ added in concentrations up to 10 mM did not influence the magnitudes of base uptake and K^+ release at pH 8.3. With 100 and 300 mM KCl, however, base uptake was severely inhibited (Fig 4). Under these conditions K^+ release could not be measured because of the high ground level of K^+. The pH_i values calculated from base distribution at the end of the experiment (Fig. 4) were 6.19 for external K^+ concentrations up to 10 mM, 6.45 for 100 mM and 6.97 for 300 mM external K^+.

As a consequence of active Na^+ extrusion intracellular Na^+ concentration in *Chlorella* is only around 2 mM [19]. Although net Na^+/H^+ exchange therefore can only play a minor role the possibility exists that base-induced K^+ release is accompanied by some Na^+ efflux. However, no base-induced net Na^+ movements were found. In a typical experiment at pH 8.2 the Na^+ concentration in the external solution was 0.04 mM and did not change upon addition of 1 mM benzylamine or trimethylamine.

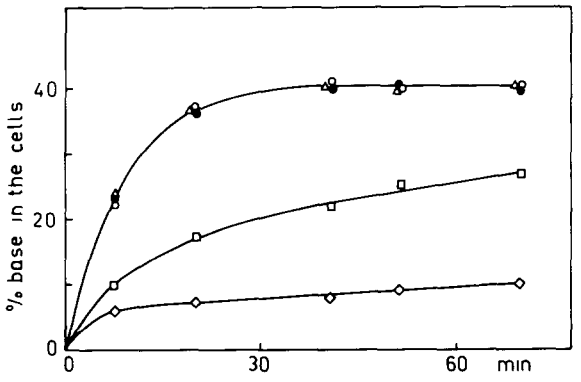


Fig 4 Effect of externa K^+ on benzylamine uptake pH 8 3, 9 5 ml cells/l, 0.8 mM benzylamine K^+ additions immediately before time zero ○, none (external K^+ concentration at time zero 0 02 mM), ○, 1 mM, △, 10 mM, □, 0 1 M, ◇, 0 3 M

Benzylamine and trimethylamine as probes of intracellular pH

Being permeant bases in accordance with theory, benzylamine and trimethylamine must be good probes for the determination of intracellular pH. Table III lists the pH_i values depending on external pH and base concentration. The higher pH_i values with increasing base concentrations evidently indicate that the probe itself influences internal acidity. So, for comparison, all pH_i values obtained under different conditions were plotted against the logarithm of the amount of base taken up by the cells (Fig. 5). The points almost lie on a smooth curve which indicates that the external pH itself has only little effect on pH_i, it is mainly the concentration of the free base which counts. Fig. 5 can therefore be interpreted as a titration curve of

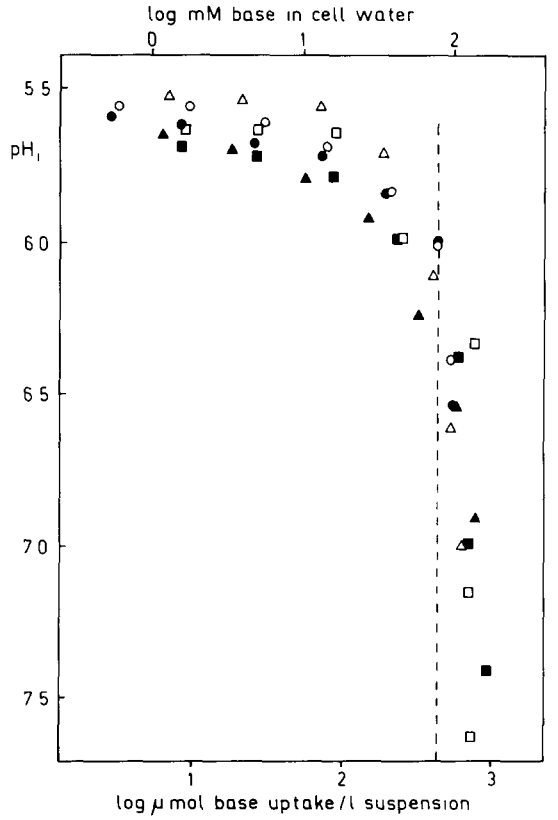


Fig 5 Effect of bases on pH_i Experimental data from Table III Dark symbols benzylamine, open symbols trimethylamine Values obtained at pH 7 51 (○ ●), pH 8.21 (△ ▲), pH 8 96 (□ ■) The dotted line marks the highest K^+ release measured (445 μmol/l, i e 55% of the cellular K^+)

pH_i with base. The lowest concentrations of benzylamine and trimethylamine yield the same pH_i of 5.6, further 'titration' with base initially has only little effect on pH_i. This range of good pH homeostasis is especially pronounced with trimethylamine. At concentrations > 20 mM base in the cell water pH_i rises more steeply and finally breaks down when the internal base concentration exceeds 80 mM which correpsonds about to the maximum amount of K^+ that can be extruded by the cells (dotted line in Fig. 5).

Kinetics of base uptake and K^+ release

The velocities of base uptake and K^+ extrusion were determined during the first 5 min after base addition (Fig. 6). In the concentration range examined from 0.1 to 10 mM the initial velocity of base uptake was proportional to base concentration. At the highest concentrations base uptake was so rapid that it could not be measured equilibrium distribution being reached before taking the first sample after 2 min. This first-order kinetics is in accordance with unspecific diffusion of the free bases through the membrane, benzylamine diffusing somewhat faster than trimethylamine, probably due to its greater lipophilicity. On the contrary, the velocity of K^+ extrusion reached saturation with 3 mM benzylamine and 5 mM trimethylamine higher base concentrations even lowering K^+ efflux a little. This indicates K^+ efflux via a saturable carrier system with a v_{max} of 4–6 $\mu mol \cdot min^{-1} \cdot (ml\ cells)^{-1}$. Fig. 6 also shows that at low base concentrations K^+ efflux keeps in step with base influx, at higher concentrations it increasingly lags behind. But this difference is made up later, at least partially, as base uptake slows down as equilibrium approaches.

Effects of ATPase inhibitors on base uptake and K^+ release

Uptake of 30 μM and 400 μM trimethylamine and the concomitant K^+ release were determined with algae treated with 0.2 mM DCCD for 20 and 90 min. As 30 μM trimethylamine has no effect on pH_i (Table III) the pH_i values obtained with this base concentration show the action of DCCD alone. The rise of pH_i was 0.09 and 0.19 units after 20 and 90 min of DCCD treatment, respectively. With 400 μM trimethylamine the pH_i values were about 0.15 units higher (which is the

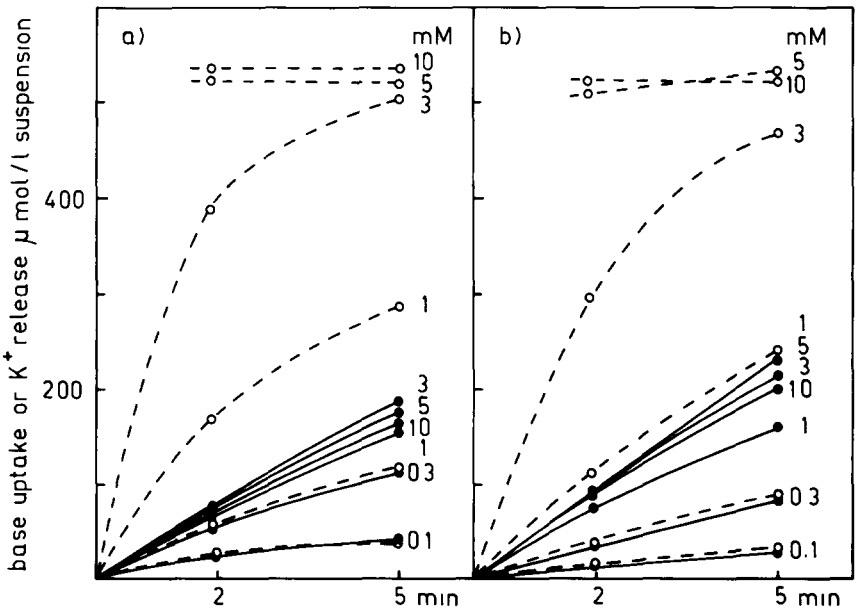


Fig. 6. Initial velocities of base uptake (- - - - - -) and K^+ release (————) with benzylamine (a) and trimethylamine (b). The respective concentrations of bases added at time zero are given in the figure. 9 5 ml cells/l, pH 8 25, external K^+ concentration at time zero 0 005 mM

normal base effect under these conditions (Table III) except with cells treated for 90 min with DCCD where pH_i was raised by 0.40 units. Only under these conditions a significant deviation from the equimolecularity between base uptake and K^+ release was found. This inhibition of K^+ release, however, seems to be rather the consequence of the loss of 35% of the cellular K^+ during the 90 min of DCCD treatment than a direct effect of the inhibitor on K^+ movements.

The same type of experiment carried out with 0.2 mM diethylstilbestrol resulted qualitatively similar but far weaker effects the loss of cellular K^+ being 3% and pH_i rising by 0.06 units only after 90 min of inhibitor treatment. In this case the molecular ratio between base uptake and K^+ release was not affected.

With 0.1 mM orthovanadate it was not possible to detect any significant effects on base uptake, pH_i and K^+ movements.

Discussion

Benzylamine and trimethylamine were found to be permeant bases suited for measurement and manipulation of intracellular acidity of *Chlorella* cells. Despite their chemical diversity the two bases behave almost identically, their accumuation ratios being constant in the low concentration range. At higher concentrations accumulation decreases indicating alkalization of the cell interior. The fact that the bases can be chased completely from the cells by external acidification (Fig. 3) shows that no significant metabolization and/or irreversible binding occurs.

Benzylamine and trimethylamine also have very similar effects on K^+ movements (Table II). The clear quantitative relation between base uptake and K^+ extrusion shows that base-induced K^+ release is not the consequence of some general toxic or 'uncoupling' effect of the bases on energy metabolism and K^+ transport. If there are any effects of this kind they exist at rather high base concentrations and/or high external pH values only. It must be assumed that the bases dissipate the pH gradient at the (internal acidic) thylakoids, nevertheless even high base concentrations had only little effect on the ATP level. Probably under these conditions the mitochondria being less sensitive to bases due to their inverse pH gradient can still produce ATP. All in all, benzylamine seems to have a little more 'side effects' than trimethylamine as indicated by the stronger effect on O_2 evolution and respiration. Also pH homeostasis of the cell is not so good with benzylamine than with trimethylamine (Fig. 5) which is also reflected by the lower accumulation ratios at higher benzylamine concentrations. It may be that benzylamine dissipates the thylakoidal pH gradient more effectively being more membrane-soluble. Moreover, it probably has a direct inhibitory action on photosynthetic O_2 evolution.

Measurements of the intracellular pH of *Chlorella* with the weak acid DMO resulted values around 7.0 [17,18,3,4] contrasting with the pH_i of 5.6 found here with the basic probes (Fig. 5). The reason for this discrepancy must be small acidic compartments which cannot be detected by the acidic probes. More recent determinations of the intrathylakoidal pH in the light resulted in values around 5.4 [22]. As the thylakoid volume is only a small fraction of the intracellular volume this pH is not acidic enough to account for the high base accumulations even if we assume some binding of bases within the cell [23]. So other small but strongly acidic compartments should exist. Although, in this respect, little is known of subcellular physiology of *Chlorella*, microvacuoles occupying about 10% of the cell volumes [24] seem to be the most likely candidates for such compartments.

Fig. 7 shows a model of the ion movements caused by base addition. The free base entering the cytoplasm is protonated and a change of the cytoplasmic pH is prevented by K^+/H^+ antiport. In addition, base diffuses into the acidic interior of the thylakoids and microvacuoles. If these compartments also restore their internal pH (by proton uptake in exchange against a cation or in symport with an anion) the cytoplasm looses protons which also must be replaced from outside by K^+/H^+ antiport at the plasmalemma. Recent investigations on plant membrane vesicles actually show that K^+/H^+ and Na^+/H^+ exchange are common in vacuolar membranes [15,25]. So as long as the intracllular compartments maintain their internal acidity the 1 : 1 relation between base uptake and K^+ release is given. At higher intracellular base concentrations pH regulation by

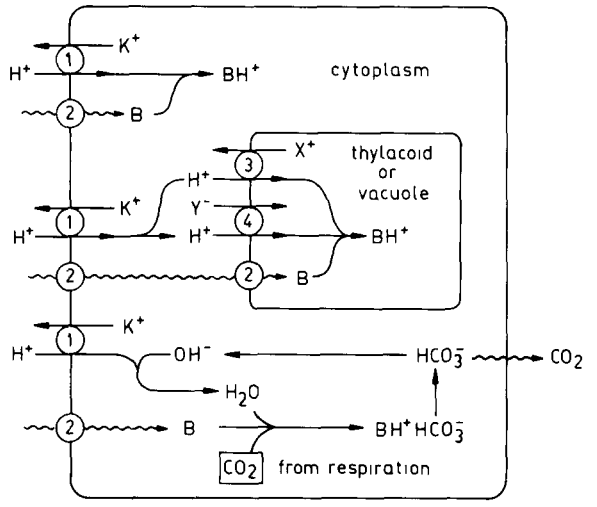


Fig. 7. Model of base-induced K^+ release Mechanisms involved (1) K^+/H^+ antiport across the plasmalemma, (2) passive diffusion of the free base through membranes, (3) proton/cation exchange and (4) proton/anion symport across the thylakoid or vacuolar membranes

cation/proton exchange is overloaded, probably because the pools of counterions are exhausted, and base uptake surpasses K^+ release. Consequently pH_i rises and also base accumulation begins to 'saturate' at higher base concentrations (Table III).

In the dark the intrathylakoidal pH is about 6.0 [26] and base accumulation should therefore be lower. This is the case with the first component of base uptake in the dark (Fig. 2). As the velocity of the second component about equals the respiration rate it seems probable that respiratory CO_2 production is responsible for continuous base uptake: first the bases are attracted by domains with higher concentrations of carbonic acid then the decomposition of bicarbonate in peripheral parts of the cells yields OH^- which gives rise to K^+/H^+ antiport (Fig. 7). If bicarbonate leaves the cell via a HCO_3^-/OH^- antiport as postulated for microalgae [27] the effect would be the same.

The lack of net Na^+ movements on addition of bases precludes a net pH-compensating role of this ion. Whether Na^+ has some catalytic effect on proton fluxes (e.g. by continuous Na^+ circling via Na^+/H^+ antiport and backdiffusion as postulated for alkalophiles [9]) cannot be decided on the basis of the present data.

Saturation of base-induced K^+ extrusion (Fig. 6) clearly demonstrates its carrier mediated nature. The v_{max} of K^+ efflux is with 4–6 μmol · min^{-1} · (ml cells)$^{-1}$ very high. For comparison: K^+ influx into *Chlorella* cells has dual kinetics [28] and mechanism I of K^+ uptake (the ATP-driven system of high affinity which is responsible for net K^+ uptake in response to intracellular acidification) only has a v_{max} of 0.1 μmol · min^{-1} · (ml cells)$^{-1}$ [29]. Mechanism II, the low affinity component of influx, with a v_{max} of about 1 μmol · min^{-1} · (ml cells)$^{-1}$ at least reaches the same order of magnitude as base-induced K^+ release. Up to now the physiological function of mechanism II is not clear as it seems only to mediate a K^+/K^+ exchange. So it may be speculated that mechanism II represents exchange diffusion of K^+ via the K^+/H^+ antiporter in the nonactivated state.

If the K^+ gradient is the energy source of proton uptake the K^+ gradient needed for K^+/H^+ antiport must at least equal the pH gradient at the plasmalemma. A necessarily rough estimation based on the experiment of Fig. 4 shows that this condition actually must be fullfilled. Here protons are transported against a ΔpH of about 1.0 at the cell membrane. The internal K^+ concentration being around 150 mM, up to an external K^+ concentration of 10 mM the K^+ gradient seems sufficient. Higher K^+ concentrations increasingly inhibit K^+/H^+ antiport and pH_i therefore becomes more sensitive to alkalization.

It was the aim of the experiments with the ATPase inhibitors DCCD, diethylstilbestrol and vanadate (Table IV) to neutralize acidic compartments within the cell in order to obtain simpler experimental conditions for the investigation of the K^+/H^+ antiport. However, this method was not successful because neutralization of pH_i proceeds only very slowly and is accompanied by loss of internal K^+. The more K^+ loss approaches half of the cellular K^+ the more K^+ release is inhibited. This is probably the same effect which limits the magnitude of K^+ release in presence of high base concentrations (Table III). K^+/H^+ antiport itself seems not to be affected directly by the inhibitors.

Finally it is possible to deduce some fundamental regulation properties of the K^+/H^+ exchange system from the present data: The system must be suppressed under normal conditions (perhaps only

TABLE IV

EFFECT OF DCCD ON TRIMETHYLAMINE UPTAKE AND K^+ RELEASE

After 1 5 h preconditioning at pH 7 0 0 2 mM DCCD was added to half of the experimental suspension After 20 and 90 min aliquots of the two suspensions were centrifuged and the algae were resuspended in the same volume of fresh medium of pH 8.05 After 10 min the base was added 8.8 ml cells/l For further explanation see legend of Table III

	Incubation			
	20 min		90 min	
	Control	DCCD	Control	DCCD
Total K^+ per l suspension (μM)	770	710	770	505
External K^+ (μM) before base addition	10	20	10	30
30 μM trimethylamine	61 6	56 6	59 4	48 7
	18 5	17 0	17 8	14 6
	20	20	20	15
	5 56	5 65	5 60	5 79
400 μM trimethylamine	53.7	48 0	50 3	27 3
	215	192	201	109
	205	175	190	70
	5 70	5 80	5 76	6 19

K^+/K^+ exchange taking place). There must be an internal activation site reacting sensitively to cytoplasmic pH elevation. Being near the plasmalemma this site is extremely exposed to the bases diffusing through the membrane so it is understandable that even very low base concentrations whose effects on pH_i seem to be compensable by the mere buffering capacity of the cytoplasm alone nevertheless elicit a response of the antiporter. On the other hand, in the case of extreme alkaline stress K^+ release is cut down, evidently in order to prevent a lethal K^+ loss. This could be the consequence of strongly sigmoidal kinetics at the inner site of the carrier as observed with the K^+/H^+ antiporter of *Chromatium* [30].

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