Properties of an ATP-Fueled, Cl^--Dependent Proton Pump Localized in Membranes of Microsomal Vesicles from Maize Coleoptiles

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Vesicles prepared from the microsomal membrane fraction of maize coleoptiles possess an ATP-fueled H^+-transport into the vesicles. It is highly possible that the vesicles from the microsomal membrane fraction actually are unchanged, native vesicles (originating from the ER or Golgi-apparatus) that can fuse with the plasmalemma or the vacuole. Therefore, they can reflect properties of the vacuole or the plasmalemma.

The energy dependent acidification within the vesicles, which can be completely reverted through the addition of CCCP, was determined on the basis of photometric difference spectra using NR (Hager *et al.*, Z. Naturforsch. **35 c,** 794–804 1980).

The proton pumps possess a very high substrate specificity; only ATP (+ Mg^{2+}) can be used as substrate, while GTP, ITP, UTP and CTP or other nucleoside tri- or diphosphates cannot be used.

DCCD and DES inhibit H^+-ATPase completely, oligomycin has only a slight, orthovanadate no inhibitory effect at all.

The energy dependent transport of H^+ across the membrane takes place only in the presence of Cl^- or Br^- (not as well in the presence of I^-). Other anions (F^-, NO_3^-, SO_4^{2-}, SCN^-, IDA^-, $H_2BO_3^-$ cannot cause an intravesicular acidification through ATP if chloride (or Br^-) is not present.

In the presence of chloride, however, some of these anions inibit the H^+/Cl^--symport (J^-, NO_3^-, SO_4^{2-}, SCN^-, $H_2BO_3^-$). They obviously are in competitive interaction with Cl^- ions for Cl^--binding sites on a carrier or channel without being able to be transported themselves.

Pi, which renders the acidification of the vesicles at a low rate possible without Cl^-, is the only tested anion which can augment the Cl^- dependent acidification.

This supports the idea that either P_i functions as a positive effector on the Cl^--transport or that a Cl^-/P_i-antiporter exists which reduces Cl^- accumulation and therefore facilitates the Cl^- coupled H^+ transport into the vesicles also.

The anion transport inhibitor, DIDS, blocks the ATP-dependent H^+-transport. This again supports the idea of a relatively tight coupling between H^+ and Cl^- transport in a possibly electroneutral system.

The presence of monovalent cations, such as K^+, Na^+, Li^+ and choline$^+$, are not important for H^+ transport.

The dependence of the ATP-fueled acidification of the vesicles on the same anion pattern which seems to regulate elongation growth and stomata aperture speaks for the eminent importance of the H^+-pump and vesicles described in this report for growth and turgor of plant cells.

Introduction

During recent years evidence for the existence of electrogenic pumps in membranes of higher plants has accumulated [1–3, 57]. They are not only necessary for the transport of ions [4–9], sugars [10–18] and amino acids [19–23] across membranes into different cell compartments, but also for the regulation of cytoplasmic pH [24], since excess H^+ ions are produced as by-products of cellular metabolism. Furthermore such energy dependent pumps are postulated to be involved in hormone induced elongation growth [25–29]. It was assumed that the most important and universal electrogenic system coupling metabolic energy to the active transport of solutes is a H^+-pump as well in algae [30, 31, references therein] as in higher plants. Proof of such a system in cytoplasmic membranes of higher plants (coleoptiles) was first given in 1980 [26]. It was shown that vesicles derived from the ER, Golgi and plasmalemma fractions of maize coleoptiles

Abbreviations: IAA, indole-3-acetic acid; BSA, bovine serum albumin (defatted); CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; DCCD, N,N′-dicyclohexylcarbodiimide; DES, diethylstilbestrol; 2,4 D, 2,4 dichlorophenoxyacetic acid; 3,5 D, 3,5 dichlorophenoxyacetic acid; DIDS, 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene; EGTA, ethylene glycol-bis-(β-aminoethyl ether) N,N′-tetraacetic acid; ER, endoplasmic reticulum; HEPES, N-2-hydroxyethylpiperazine-N′-ethanesulfonic acid; IDA^-, iminodiacetate; MES, 2-(N-morpholino)ethane sulfonic acid; 1-NAA, naphthalene-1-acetic acid; 2-NAA, naphthalene-2-acetic acid; NR, neutral red; NRH^+, protonated NR.

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isolated by density gradient centrifugation were able to accumulate protons in the presence of ATP as energy source. The energy dependent transport of H^+ across the membrane could be demonstrated with difference spectra of the lipophilic pH indicator NR.

The present report describes an improved procedure for isolating and stabilizing these vesicles. It will be shown that the H^+ pumps of these vesicle membranes are highly specific for the nucleoside triphosphate ATP and that the H^+ transport into the vesicles is strongly dependent on a Cl^- co-transport. Furthermore a H^+/Ca^{2+} antiporter is present within the membrane.

Materials and Methods

Plant cultivation: The hybrid corn variety " Inrakorn" category 2a (Deutsche Saatgut AG) was washed overnight in running water and set out on damp cellulose. The seedlings were cultivated over a period of 96 h at 26 °C and 90% humidity in the presence of green safety light and 2 h red light daily. Coleoptile segments, 3–5 cm long, were used for the experiments after removal of the primary leaf.

Preparation of coleoptiles: 10 g coleoptiles were *plasmolized* (10 min) by vacuum infiltration in 100 ml medium I (400 mM sucrose, 20 mM HEPES, 0.1% BSA, pH 7.5; 2 °C). Thereafter coleoptiles were placed in a mortor or a petri dish with *homogenization* medium (~ 5 ml) (2 ml/g coleoptiles) at 2 °C and cut with razor blades. A further homogenization with a pistill was done gently and only for a very short period. The homogenate was pressed through four layers of mull (cheese-cloth) and diluted with medium II (final volume: 4 ml/g tissue).

Homogenization medium (II): 300 mM sucrose, 100 ml HEPES, 10 mM EGTA, 20 mM ascorbate, 2.5 mM dithiothreit, 1 mM $MgCl_2$, 0.1% BSA; pH 7.5.

The *homogenate* was centrifuged for 15 min at $13000 \times g$ (10500 rpm in Sorvall rotor SS 34) in order to remove whole cells, cell wall fragments, nuclei and mitochondria. The supernatant was either layered on a two-step sucrose gradient (membrane fraction A and B; [26] or centrifuged again for 30 min at $143000 \times g$ (35000 rpm, Beckman rotor SW 27).

The 143 K pellet was taken up in 3 ml medium III (50 mM KCl, 1 mM $MgCl_2$, 0.12 mM NR, pH 7.5) and suspended with a homogenizer (Potter-Elvehjen). Then a further 9 ml of medium IV (50 mM KCl, 10 mM HEPES, 300 mM sucrose, 1 mM $MgCl_2$, 0.1% BSA, pH 7.5) was added (final concentration of NR = 30 µM). In the last experiments medium III was substituted by medium IV.

This *membrane fraction* containing "microsomal vesicles" (or membrane fraction A or B; [26]) was tested for ATP-dependent H^+ pump activity with a difference spectrophotometric method [26]. The difference spectra covered the whole NR-spectrum. In some experiments a dual wavelength method was used to identify proton accumulation within vesicles. The absorption difference $\Delta A_{430\,nm}$ minus $\Delta A_{465\,nm}$ (465 nm ≙ isosbestic point) corresponds to a pH-decrease (see Fig. 1). Because of the sort of experiments, smaller shiftings of the isosbestic point should be taken into consideration.

Results

Determination of ATP-dependent H^+ uptake into the vesicles using the pH-indicator NR; promotion of acidification through EGTA

Fig. 1 shows the difference in the change of absorption of NR after the addition of ATP and AMP respectively to microsomal membrane vesicles prepared from maize. The decrease in absorption at 430 nm in the sample cuvette (ATP) in comparison to the reference cuvette (AMP) is due to acidification through ATP, which signifies a shift to the right of the following equation: $NR + H^+ \rightleftharpoons NRH^+$. Since the vesicles are in a buffered solution, the change in absorption can only account for the change which takes place in closed, vesicular compartments where little buffering capacity is present. Due to formation of NRH^+ an increase in absorption at 525 nm is expected to occur. Corresponding, to the decrease of A_{430} (NR), however the binding of NRH^+ to negative charges of the energized membrane, the formation of dimeres or higher aggregates of the dye with increasing NRH^+ concentrations and stacking of the dye along the polyanions chains [26, 32] bring about a metachromatic shift of the NRH^+ absorbance maximum and a simultaneous decrease of the absorbance co-efficient.

The increase of the NRH^+ concentration within the vesicle is due to an active H^+ uptake; the lipophilic pH-indicator, NR, can permeate the mem-

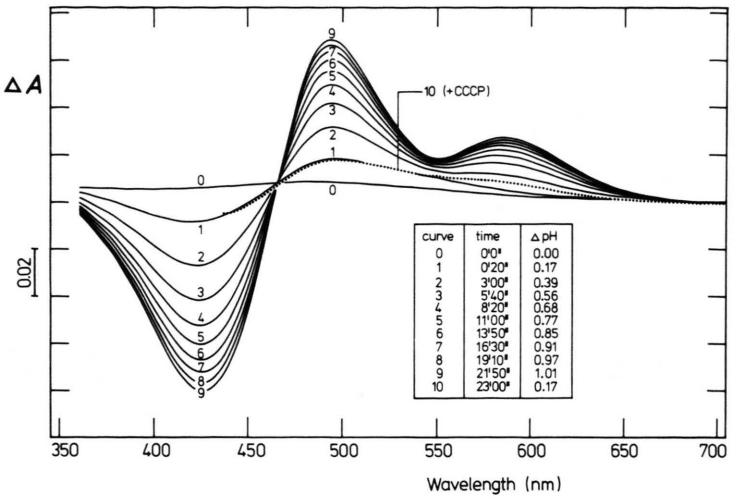

Fig. 1. ATP-dependent H^+ accumulation in microsomal membrane vesicles of *Zea mays* coleoptiles. Curves 0–10: Difference spectra of neutral red absorption. Curve 0: sample and reference equal (medium IV + vesicles). Curve 1: 20 sec after addition of ATP (1 mM) to the sample and AMP (1 mM) to the reference cuvette. Curve 10: addition of CCCP (50 μM) reverts the acidification in the vesicles immediately.

brane, whereas the less lipophilic NRH^+ being formed is trapped within the vesicles.

A further indication of an ATP induced acidification within the vesicles is given by the fact that protonophoric substances totally revert the change in absorption of NR, in other words the H^+ ions flow out of the vesicles and back into the buffered environmental solution. If CCCP is used one must take into account that dissolved CCCP absorbs light under 460 nm. After addition to both cuvettes even small differences in concentration give rise to profound variation in the difference spectra below this wavelength and falsify the NR-absorption, whereas no interference is observed above 460 nm.

The obtained difference spectra indicates a considerable ATP-dependent acidification within the vesicles corresponding to a pH-value of 1 after 22 min. The very high activity of the proton pump is brought about by EGTA present in the homogenizing medium or in the incubation medium, which removes remaining Ca^{2+} ions from the preparation by chelation. Without EGTA the drop in pH within the same time span is only 70% (Fig. 2). This itself already points out the important role of Ca^{2+} in the regulation of energy dependent acidification within the vesicles [33].

The effect of DCCD, DES, oligomycin and vanadate on ATP-dependent proton transport

DCCD blocks the proton channel of ATPases by binding to a glutamyl residue of the lipophilic protein helix [34]. An addition of 100 μM DCCD to vesicles prepared from maize brings the activity of the pump to an arrest already after 2 min or to a steady state between a remaining H^+-influx and a proton leak out (Fig. 3).

DES is regarded as a specific inhibitor of ATPases which are not located in mitochondria or chloroplasts [35–38]. Fig. 4 shows the inhibition of ATP-fueled H^+ pumps on microsomal membranes through DES. Like in the case of DCCD, 100 μM DES lead to a total standstill of acidification within the vesicles.

Similar to DES, $H_2VO_4^-$ should inhibit non-mitochondrial ATPases, especially plasmalemma ATPases

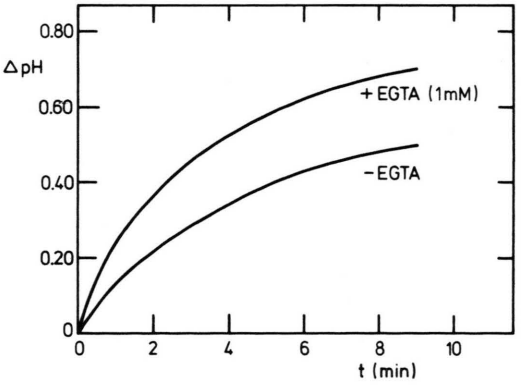

Fig. 2. Promotion of ATP-dependent acidification in microsomal vesicles through EGTA. Conditions as described for Fig. 1 except that the homogenization medium (II) is without and medium IV with or without EGTA (1 mM).

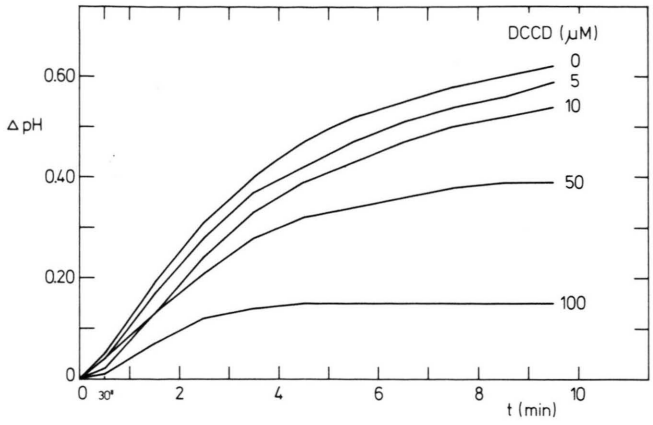

Fig. 3. Inhibition of ATP-dependent acidification in microsomal vesicles by DCCD. Conditions as described for Fig. 1.

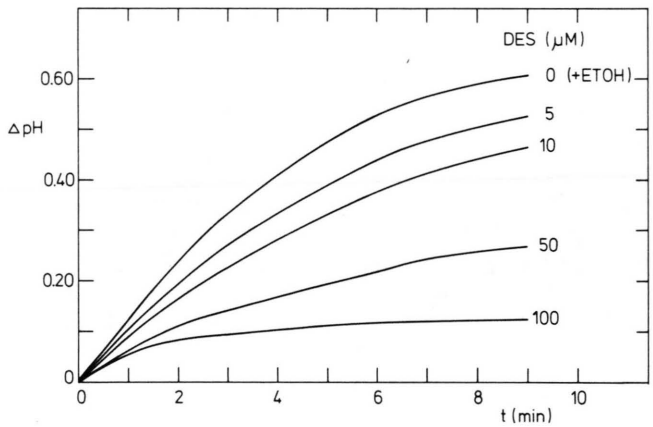

Fig. 4. Inhibition of ATP-dependent acidification in microsomal vesicles by DES (conditions as described for Fig. 1.).

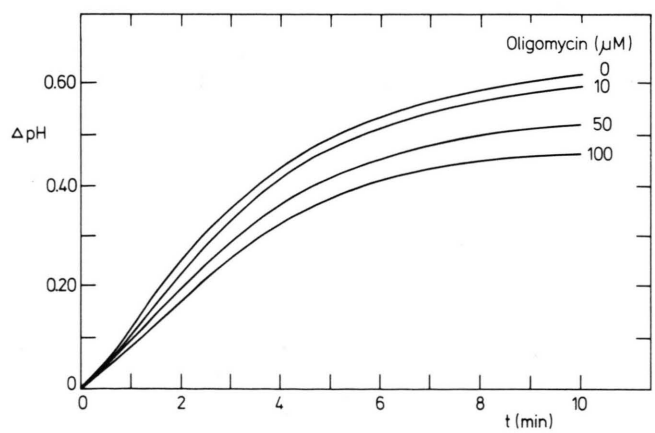

Fig. 5. Marginal influence of oligomycin on ATP-dependent acidification within vesicles.

[39, 40], but it does not have any effect under the given conditions (not shown). Oligomycin, an inhibitor of mitochondrial ATPases, shows little influence (Fig. 5).

The effect of NH_4^+ on the intravesicular acidification

Based on the manifold proven "uncoupling" effect of NH_4Cl (penetration of lipophile NH_3 molecules from the buffered environmental solution (pH 7.5) into the acidic vesicular compartment and formation of NH_4^+), an addition of 1 mM neutralizes the internal acidification of the vesicles very fast (Fig. 6). The effect of NH_4^+ is further evidence for the acidification of the interior of the vesicles.

ATP-specifity of the energy dependent proton transport into the vesicles

The ATPases which have been described upto now on microsomal membranes show no high substrate specifity [8]. Tognoli and Marrè [41] described a divalent cation-activated ATP-ADPase prepared from pea stem microsomes which also hydrolysed CTP, GTP, UTP, further ADP and to a lesser extent CDP, UDP and IDP.

Unlike observations on pea stem microsomes, the H^+-transport across the membrane of our described microsomal vesicles can occur only through ATP, not however through other nucleoside triphosphates (Fig. 7).

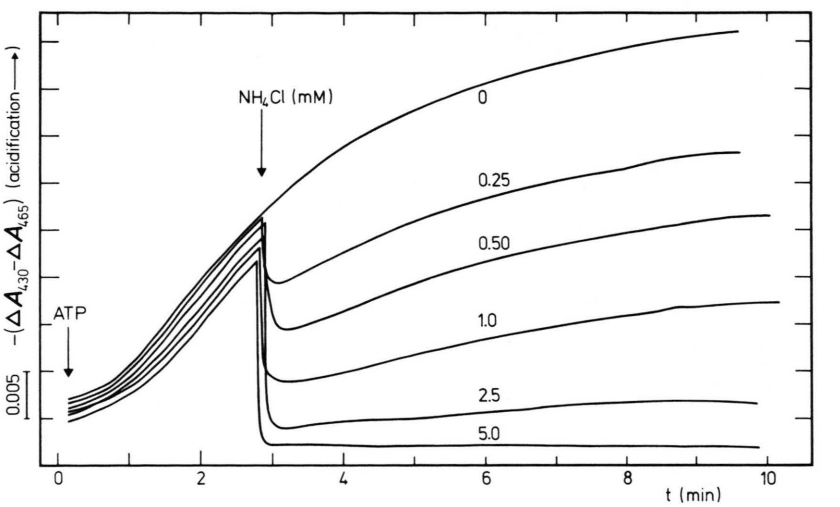

Fig. 6. Reversal of the ATP-induced drop in pH in vesicles through NH_4Cl ("uncoupling effect").

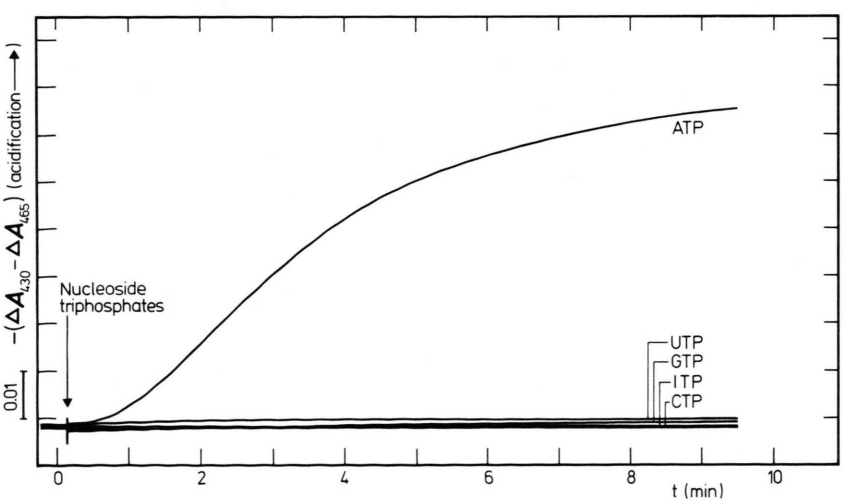

Fig. 7. Substrate specifity of the proton pump; only ATP causes an intravesicular acidification.

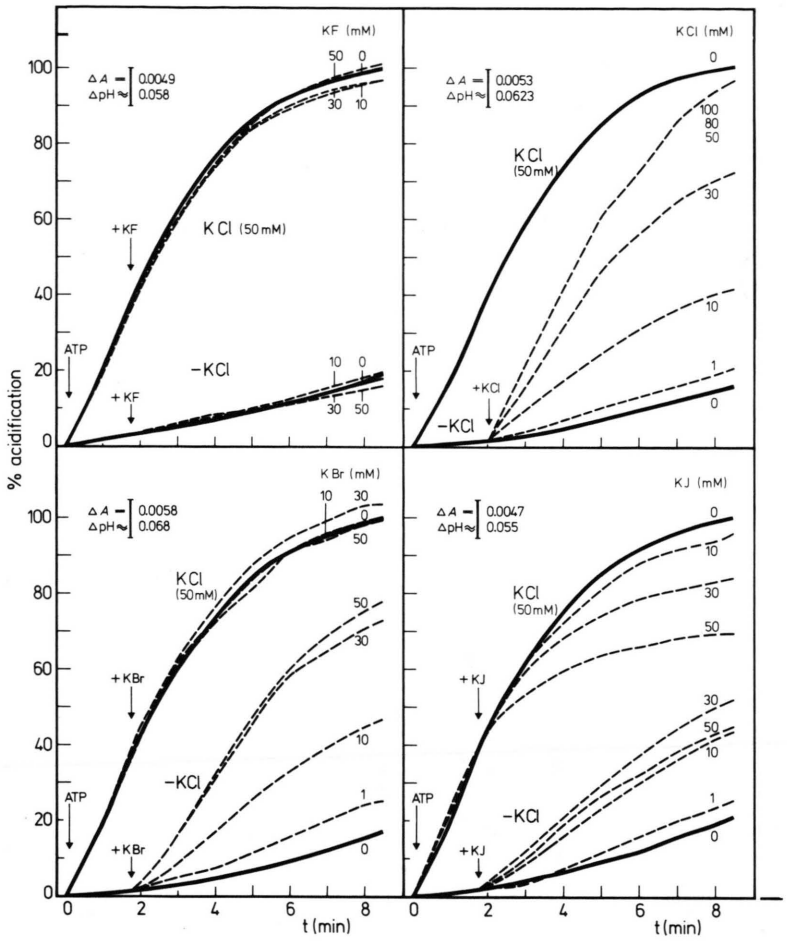

Fig. 8. Influence of the anions, F^-, Cl^-, Br^- and I^- on the ATP fueled, Cl^--dependent acidification in microsomal vesicles. Incubation of vesicles in solutions with and without Cl^-, respectively, and addition of the halogenides after 2 min. Mg^{2+}, essential for ATP-operation, was added as SO_4^{2-} (1 mM).

In addition, Mg^{2+} is necessary and, as will be shown further on, Cl^-, also.

Cl^- as essential factor for the ATP-dependent proton transport; effect of anions and cations on the intravesicular acidification

The ATP-induced acidification within the vesicles is highly Cl^- dependent (Fig. 8). A H^+/Cl^- symport has to be assumed (see Discussion). Among the tested anions only Br^- was able to initiate an ATP-fueled H^+-transport of the same magnitude (Fig. 8). I^- showed a comparatively smaller effect (Fig. 8). Most of the other tested anions (F^-, NO_3^-, SO_4^{2-}, SCN^-, IDA^-, $H_2BO_3^-$) could not themselves catalyze the energy dependent acidification of the vesicles (Fig. 9). Some of these are however able to reduce the effectiveness of Cl^- (I^-, NO_3^-, SO_4^{2-}, SCN^-, $H_2BO_3^-$). They obviously function as competitors for the Cl^- (Br^-) binding sites on the carrier or Cl^--channel without being able to be transported themselves. Only F^- and the zwitterion IDA^-, which is generally regarded as membrane impermeable [42] do not influence the Cl^-/H^+ co-transport; they are not in interaction with the Cl^--binding site.

A striking behaviour is shown by P_i; it can render acidification of the vesicles possible at a low rate (Fig. 10). It is the only anion which can additionally augment and accelerate the Cl^--induced acidification of the vesicles.

Since Ca^{2+} inhibits acidification within the vesicles [33], it was necessary to reflect whether P_i decreases the activity of Ca^{2+} in the environmental solution, and thus secondarily promotes acidification. This is, however, not the case. Even if Ca^{2+} is completely removed by EGTA, P_i still causes a promotion.

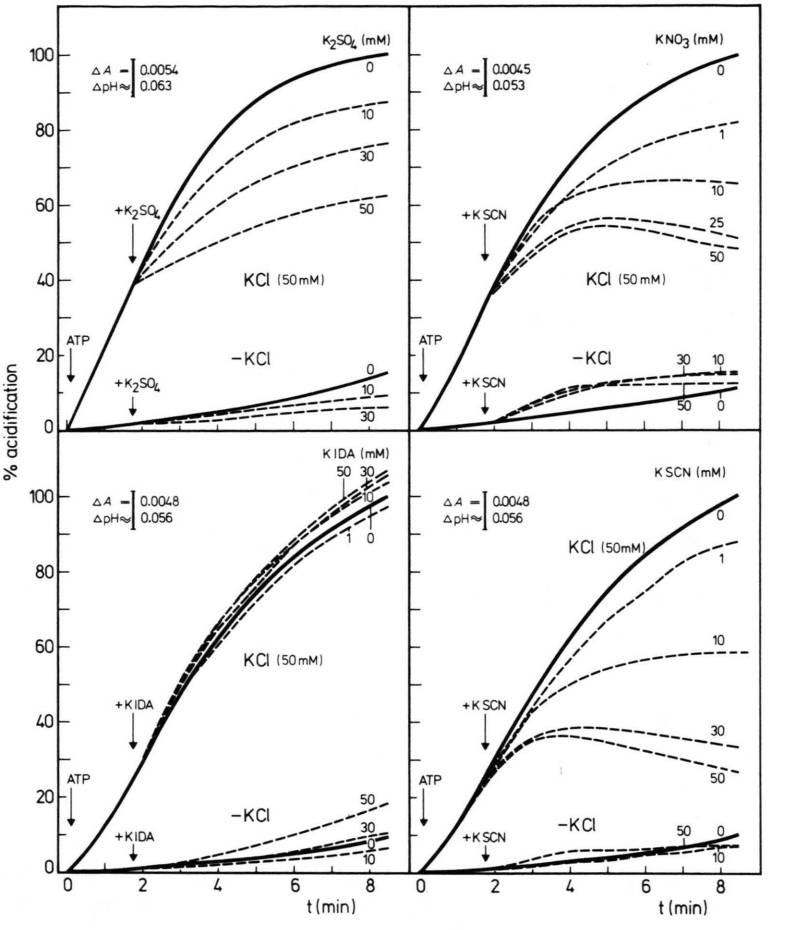

Fig. 9. Influence of the anions, SO_4^{2-}, NO_3^-, IDA^-, and SCN^-, on ATP-fueled, Cl^- dependent acidification of microsomal vesicles. Incubation of vesicles in solutions with and without Cl^-, respectively, and addition of anions after 2 min.

Unlike anions, monovalent and organic cations only slightly effect the activity of the proton pump in the presence of Cl^-; the accumulation of H^+ is nearly as high in the presence of K^+, Na^+, Li^+ or choline$^+$ (Table I).

These facts do not support the existence of a very specific H^+/K^+ antiporter, but do support a symport of H^+/Cl^-, probably with an integral Cl^- carrier or channel most likely structurally associated with the proton pump (see Discussion).

Inhibition of the energy dependent H^+-transport through the anion channel blocker, DIDS

The importance of anion transport during acidification of the vesicle is supported by the fact that the well-known inhibitor of anion transport, DIDS, arrests acidification at a concentration of 50 µM and at higher concentration even leads to a complete standstill of H^+ transport; a slow leak out of H^+ can be observed. Such an leak out can also be observed at 50 µM, if some of the H^+-pumps have already been inhibited by DCCD (100 µM) (Fig. 11).

In this connection we investigated the possible presence of an ATP-fueled Cl^--pump. If so, the Cl^--transport would have to be accompanied by a co-

Table I. Influence of different monovalent cations on the ATP-fueled, Cl^--dependent acidification (10 min after addition of ATP) in microsomal vesicles.

50 mM	intravesicular acidification [%]
KCl	100
NaCl	95
LiCl	92
choline Cl	90

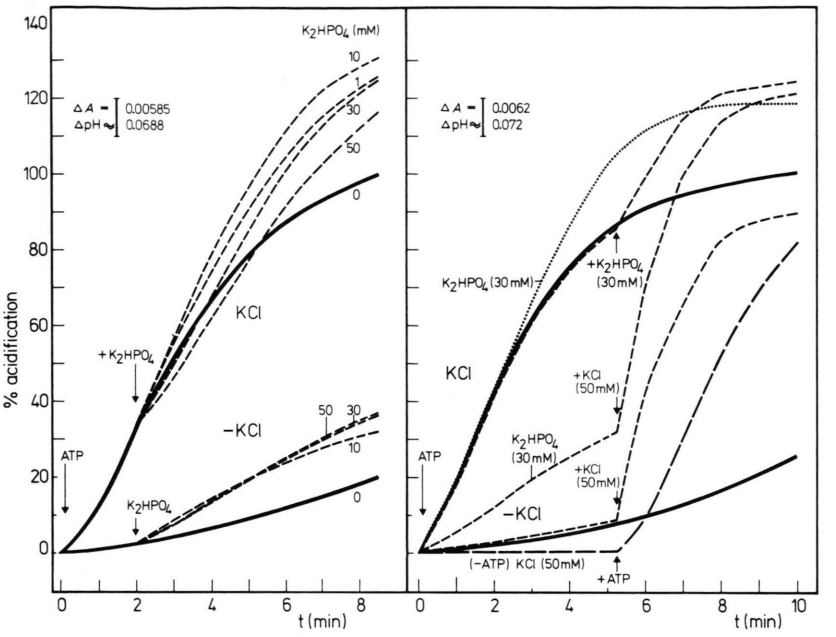

Fig. 10. Influence of $HPO_4^{2-}/H_2PO_4^-$ (P_i) on the ATP-fueled, Cl^--dependent acidification of microsomal vesicles with and without Cl^-, and addition of P_i, ATP and Cl^- at different points in time.

transport of H^+ giving rise to the measurable acidification. A membrane relativley impermeable to K^+ would be prerequisite, since the concentration in the suspension (50 mM) is very high. The K^+-ionophore, valinomycin, would have to make the membrane permeable to K^+, so that a K^+/Cl^- cotransport could take place instead of a H^+/Cl^--symport. An acidification is actually prevented, but valinomycin (10 μM) leads to a sudden efflux of H^+-ions already accumulated within the vesicles. Valinomycin not only makes the membranes permeable to K^+, but also to H^+. The effect of valinomycin does not give evidence for the presence of a Cl^--pump. Moreover, the effect of CCCP, which immediately reverts intravesicular acidification, does not support a Cl^--pump. The presence of a Cl^- pump would demand that the protons which were cotransported via "proton channels" passively would have to remain within the vesicles, even if CCCP were added.

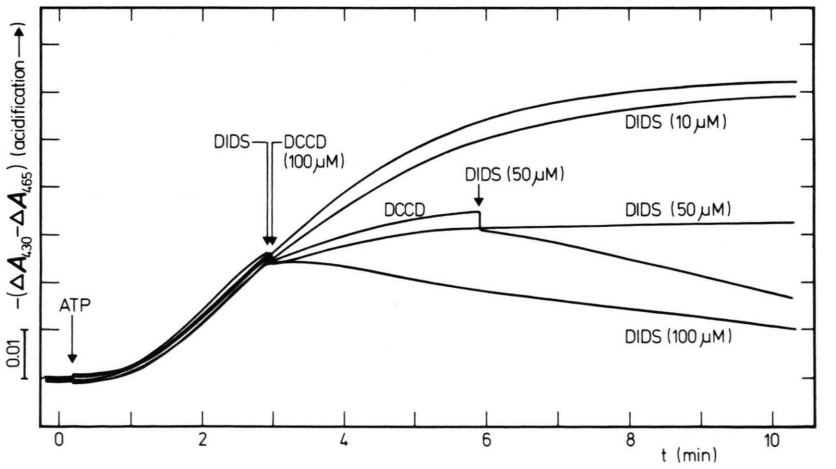

Fig. 11. Inhibition of the Cl^--dependent, ATP-fueled acidification by the anion transport inhibitor, DIDS. After partial blockage of the ATPase through DCCD, DIDS causes a complete standstill of the activity of the pump and a leak out of H^+.

Activity of the H^+ pump under the influence of auxin, fusicoccin and cAMP

Fusicoccin, which is assumed to be able to stimulate plasmalemma H^+-ATPases [43], does not alter the rate of H^+-transport in the system tested. The same holds true for cAMP or cGMP. Auxins such as IAA, 1-NAA, 2,4 D but also the ineffective analogs, 2-NAA and 3,5 D inhibit in higher concentrations the intravesicular acidification. This can be ascribed to an effect anions have; a slight competitive interaction with the Cl^--carrier or the Cl^--channel seems to be present. No direct influence on the activity of the H^+-pump could be observed when incubation took place at physiological auxin concentrations.

Effect of anions during growth of intact maize coleoptile segments

Table II shows the growth of coleoptile segments after 4 h of incubation in MES-buffer (5 mM; pH 5.8) with auxin and in the presence of different anions. It is notable that unlike Cl^- ions, P_i, F^-, I^-, and SO_4^{2-} inhibit growth. It is plausible that an active uptake of Cl^- (which is located in the cell wall or added) is necessary for elongation growth (see Discussion). If the H^+/Cl^--symport is competitively inhibited for instance, by SO_4^{2-}, (on the tonoplast and possibly on the plasmalemma, also), like on microsomal vesicles (Fig. 9), an increase in turgor and an acidification of the vacuole and cell wall respectively are repressed. Contrary to the observations made on vesicles (Fig. 9) NO_3^- does not lead to an inhibition, probably due to a fast reduction and binding to carbon and amino acids.

In the case of F^- though, one must consider that F^- can cause an inhibition of glycolysis.

Table II. Dependence of IAA (20 µM) induced elongation growth of coleoptile segments 1 cm long (4 h, 30 °C) on the anion present (50 mM in 5 mM MES-buffer, pH 5.8) and compared with the dependence of ATP-fueled acidification in microsomal vesicles on the same anion in question (50 mM); intravesicular acidification (%) 8 min after addition of ATP.

50 mM	Elongation [%] of segments	Intravesicular acidification [%]
KCl	100	100
KBr	97	85
KJ	68	45
K_2HPO_4	66	41
K_2SO_4	44	13
KF	7	17
KNO_3	97	19

Discussion

Electron microscopic illustrations of maize coleoptile cells have shown that these possess a large number of vesicles which originate from the ER or Golgi apparatus. Some of these vesicles fuse with the plasmalemma, others seem to participate in the formation of a large central vacuole. The latter would have to be regarded as prevacuoles. It is our opinion that the "microsomal" vesicle fraction tested here predominantly comprises such native vesicles and prevacuoles. There is every reason to believe so, since a mere maceration of the cells with razor blades suffices for a preparation of active vesicles with a highly active H^+-transport; treatment of the membrane suspension with ultrasound or other mechanical homogenization methods is not necessary for the formation of vesicles. The properties of the vesicles tested here can therefore reflect the properties of the vacuole or plasmalemma.

The energy dependent acidification within the vesicles is extremely substrate specific: other than ATP (+ Mg^{2+}) no nucleoside triphosphate is able to drive the proton pump. The ATPases, which have been detected up to now on plant membranes on account of their hydrolisation activity, do not seem to possess any specificity [8, 41] probably because the hydrolytic activity of totally different reaction systems have been measured together. In our preparations, a specific ATP-consuming reaction can be detected with the aid of proton translocation.

The activity of the proton pump can be completely arrested by the ATPase inhibitor, DCCD, which binds specifically to a single glutamyl residue of a hydrophobic segment of the membrane integrated polypeptide chain [33]. The same can be observed with the synthetic estrogene diethylstilbestrol (DES), whose strong inhibitory effect was first observed on plasmalemma ATPases prepared from oat roots and *Neurospora* [35–38].

Oligomycin shows little, while vanadate, which several authors [39, 40] regard as a plasmalemma-ATPase inhibitor, shows no inhibitory effect at all.

It is also surprising to note that an H^+-transport into the vesicle at a high rate can only take place in

the presence of Cl^- (or Br^-), whereas certain monovalente cations such as K^+, Na^+ or choline$^+$ play no important role in the process of acidification. This leads to the assumption that not a countertransport of K^+ compensates for an electrical charge established by the electrongenic transport of H^+, but a symport of Cl^-. This symport could take place by means of a specific carrier for Cl^- (facilitating diffusion) or a Cl^--channel. Such a Cl^--channel was found in the electroplax membrane of the marine ray, *Torpedo californica* [44, 45] and *Narke japonica* [46].

This channel is unusually selective for Cl^-; of all other anions tested, only Br^- is appreciably permeable. This information was obtained from experiments with vesicles or using the planar bilayer system. In addition, the channel of these electroplax membranes is inhibited by stilbene disulfonate (DIDS), a potent and specific inhibitor of anion transport in a variety of systems especially in red blood cells (in blood cells a membrane compound (band 3) is essential for anion transport; this polypeptid interacts with DIDS [47, 48]).

The selectivity of these animal transport systems for Cl^- (and Br^-) are strikingly similar to ours. The vesicular system of coleoptiles demonstrates clearly that unlike Cl^-, a large number of other anions tested (NO_3^-, SO_4^{2-}, F^-, SCN^-) are not able to warrant a symport of H^+ and an acidification respectively. On the other hand, however, these anions lead to an inhibition of the effect of Cl^-. This inhibitory effect can be regarded as a competitive interaction of these anions for Cl^- binding sites (of the carrier or channel).

F^- does not influence the H^+/Cl^--symport nor can it initiate the H^+-transport, in other words it obviously can not compete with the carrier or channel at all (Fig. 8). The same behavior is shown by IDA^-, to which the membrane is considered impermeable (Fig. 9). I^- however, seems to be able to still partially use the Cl^- transport carrier or channel (Fig. 8).

A striking behavior is shown by P_i. It renders the transport of H^+ across the membrane into the vesicles possible, however, at a lower rate than Cl^- (Fig. 10). Together with Cl^- it leads to a considerable acceleration of acidification within the vesicles. This could mean that P_i functions as a positive effector on Cl^- and thus on the H^+-transport, also. A Cl^-/P_i^- antiporter which decreases the intravesicular accumulation of Cl^-, however, is just as plausible. This strict dependence of the H^+ transport on a Cl^- carrier or channel suggest a spatially close association in the membrane, may be even similar in nature to multi-enzyme complexes.

Such a direct coupling of proton to Cl^- (Br^-) transport has already been suggested by Pazoles *et al.* [49] in chromaffin granules; they postulated that the proton-pumping MgATPase and the anion transport site may exist as parts of a macromolecular complex within the granule membrane.

If one assumes, that the isolated vesicles are actually pre-vacuoles, then they can reflect properties of vacuoles and of the tonoplast. In this connection it is interesting to note that a Mg-ATPase has been isolated from a preparation of beetroot vacuoles, whose activity is augmented by Cl^- [50].

An active H^+/Cl^--symport into the vacuoles could be of great importance for cellular turgor regulation. The dependency of the opening of the stomata on the presence of Cl^-, causing an increase in turgor of the guard cells has actually even been shown [51–54, 42]. The osmotic effect of the investigated halogenide anions [55] is strikingly similar to the H^+/halogenide symport pattern in our experiments: KF produced complete stomatal closure; KBr was as effective as KCl in keeping stomata open and KJ reduced stomatal aperture; with KIDA stomata did not open.

Under certain conditions Cl^- in the stomata can be replaced my malate [42, 53]. This exchange could then be explained by the presence of a Cl^-/malate antiporter. Even in this case the primary process would be the accumulation of Cl^-.

Since the degree of potassium accumulation in early stages of opening are much too small to account for the osmotic changes required [56], again indicates the importance of Cl^-.

Even auxin-induced elongation growth is apparently dependent on the presence of Cl^- (Table II); if Cl^- is competitively repressed by other anions, inhibition of growth results. As a matter of fact the pattern of the effect anions have in regulating auxin-induced elongation growth corresponds quite exactly to the pattern of the effect ions have in acidification of the vesicles (Figs. 8–10) and in the previously discussed regulation of stomata aperture (only NO_3^- has no inhibitory effect during growth; it is possible that the NO_3^- taken up is immediately reduced and attached to amino acids, so that it can

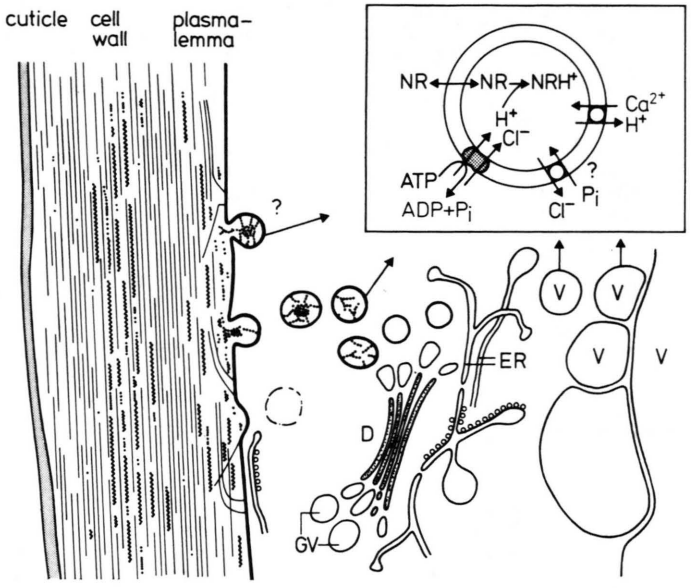

Fig. 12. Possible formation and origin of the vesicles, which were isolated from cells of maize coleoptiles and characterized. The vesicles possess an ATP-fueled, Cl^--dependent proton pump possibly functioning without an electrical potential gradient (H^+/Cl^--symport), a H^+/Ca^{2+}-antiporter and perhaps a Cl^-/P_i-antiporter.

no longer reach the binding site of Cl^- carriers or channels).

In regard to growth, Cl^- could possibly not only function as an osmotic effective substance, but also be responsible for the H^+-transport into the microsomal vesicles, which after fusing with the plasmalemma induces the essential acidification of the cell wall leading to its increased plasticity necessary for elongation growth.

In other words, auxin-induced elongation growth is dependent on the same spectrum of anions as the activity of the ATP-fueled proton pump is. This is a further indication that the Cl^--dependent H^+ pumps on microsomal vesicles (Fig. 12) detected and characterized in this report can be of paramount importance in initiating growth.

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