A H^+/Ca^{2+} Antiporter in Membranes of Microsomal Vesicles from Maize Coleoptiles, a Secondary Energized Ca^{2+} Pump

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Z. Naturforsch. **36 c,** 1009–1012 (1981); received September 2, 1981

Ca^{2+} Pump, H^+-ATPase, Membrane Vesicles, Coleoptile, *Zea mays*

Microsomal vesicles prepared from the ER and the Golgi apparatus of maize coleoptiles possess an ATP-fueled, Cl^--dependent proton pump which can cause an intravesicular acidification of the vesicles (A. Hager and M. Helmle, Z. Naturforsch. **36 c,** 997 (1981); A. Hager *et al.*, Z. Naturforsch. **35 c,** 783–793 (1980)). This acidification is very specifically inhibited by Ca^{2+}. The inhibition is already distinct at concentrations of 30 μM. While 10-fold higher concentrations of La^{3+} produce similar effects, Mg^{2+} remains ineffective even at very high concentrations.

If Ca^{2+} is added after acidification of the vesicles, a rapid H^+-efflux proportional to the amount of Ca^{2+} added characterizes the first reaction phase. In the second reaction phase acidification within the vesicles commences anew, however, at a reduced rate.

If Ca^{2+} is added to vesicles whose proton pump has been inhibited by DCCD, the first reaction phase remains unchanged, while the acidification in the second reaction phase does not set in, and only a leak out of protons is observed.

These results give support for a H^+/Ca^{2+}-antiporter mechanism which can function as a secondary energized Ca^{2+}-pump and regulate the Ca^{2+}-concentration in the cytoplasm.

Introduction

Ca^{2+} participates in the regulation of a variety of cellular processes [3, 4]. In plants, it has been suggested that Ca^{2+} is involved in growth-related mechanisms, in seismonastic movement of leaves, in phototaxis, in microtubuli disaggregation, in phytochrome-mediated chloroplast movement, in regulation of enzyme activities (as quoted by [5]) and in the induction of polarity [6]. The signal for the various Ca^{2+}-regulated systems is usually a transient increase in free cytoplasmic Ca^{2+} concentrations from resting levels of 10^{-6}–10^{-8} M to higher levels [7]. In many of the Ca^{2+}-mediated processes the Ca^{2+}-dependent regulator protein, calmodulin, is the coupling link [8]. Because a low cytoplasmic Ca^{2+} activity has to be maintained against high Ca^{2+} concentrations outside the cell and inside various cell organelles, active Ca^{2+} transport systems are necessary on cell membranes. Such Ca^{2+} pumps have been demonstrated for mitochondria [5, 9, 10] and for microsomal fractions [5, 10–12]. In general, three types of Ca^{2+} pumps have been postulated so far:

(1) $Mg^{2+} + Ca^{2+}$-activated ATPases located in the surface membranes and endoplasmic reticulum of various animal cells [13] and in plant membrane vesicles [5, 10, 11, 14].

(2) Mitochondrial Ca^{2+} pumps utilizing ATP or oxidizable substrates as energy source [4].

(3) Na^+/Ca^{2+} and H^+/Ca^{2+} exchange systems using the electrochemical gradient of Na^+ or H^+ for Ca^{2+} translocation [4, 15–17].

In the preceding paper [1] it was shown that the cytoplasm of maize coleoptiles contains vesicles which are derived from the endoplasmic reticulum or the Golgi apparatus, and which possibly can fuse with the plasmalemma or the tonoplast. Energy-dependent proton pumps which cause an intravesicular acidification are located in the membranes of such "microsomal" vesicles. The amount of acidification is strongly dependent on the Ca^{2+} concentration of the medium. In the present paper it is demonstrated that this Ca^{2+} effect is an expression of a H^+/Ca^{2+} antiporter located within the vesicle membrane. This antiporter may function as a secondary energized Ca^{2+} pump to remove Ca^{2+} from the cytoplasm.

Materials and Methods

The preparation of the vesicles and the determination of pH within the vesicles have been described in a preceding paper [1].

Abbreviations: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; DCCD, N,N′-dicyclohexylcarbodiimide; EGTA, ethylene glycol-bis-(β-aminoethyl ether) N,N′-tetraacetic acid; ER, endoplasmic reticulum.

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0341-0382/81/1100-1009 $ 01.00/0

Results and Discussion

Evidence for ATP-fueled, Cl^--dependent proton pumps in vesicles prepared from the cytoplasm of maize coleoptiles [1] has raised the question concerning their importance and regulation. In the following investigation it is demonstrated that the intravesicular H^+ accumulation is very sensitively regulated by the well known secondary messenger, Ca^{2+}-ion.

Since very low concentrations of Ca^{2+} are already able to induce manifold physiological reactions, a prerequisite of any study investigating the effect of Ca^{2+} on membrane fractions is the removal of Ca^{2+} prior to experimentation. This was to some extent achieved by adding 10 mM EGTA to the homogenization medium [II] and repeated washing of the vesicle fraction (centrifugation of the vesicle fraction at 143000 × *g* and resuspending in medium IV).

Fig. 1 shows that the intravesicular ATP-dependent acidification is already inhibited by low Ca^{2+} concentrations. After 10 min 50 μM Ca^{2+} inhibit about 20%, 500 μM Ca^{2+} about 52%.

Plausible mechanisms underlying this Ca^{2+}-induced inhibition are 1. direct inhibition of the proton pump, 2. increased permeability to H^+, or 3. augmented H^+-export due to the presence of a H^+/Ca^{2+} antiporter [15–17]. The course of the curve of ATP-dependent acidification after a later Ca^{2+} addition supports the third possibility: immediately after the addition of Ca^{2+}, the energy dependent acidification is stopped (Fig. 1), and a

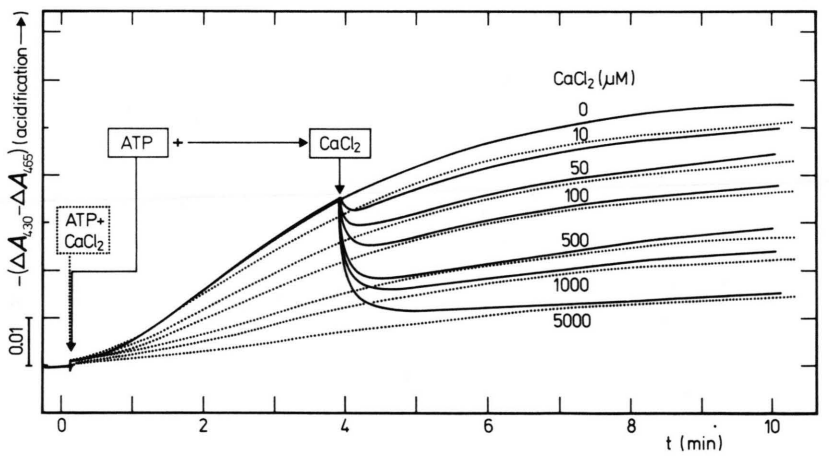

Fig. 1. ATP dependent acidifcation of microsomal vesicles and inhibition by initial and later additions of Ca^{2+}. Conditions as described by Hager and Helmle ([1], Fig. 1) with the exception that remaining EGTA was removed by repeated centrifugation of the vesicles (143000 × *g*, 30 min) and resuspension in medium IV.

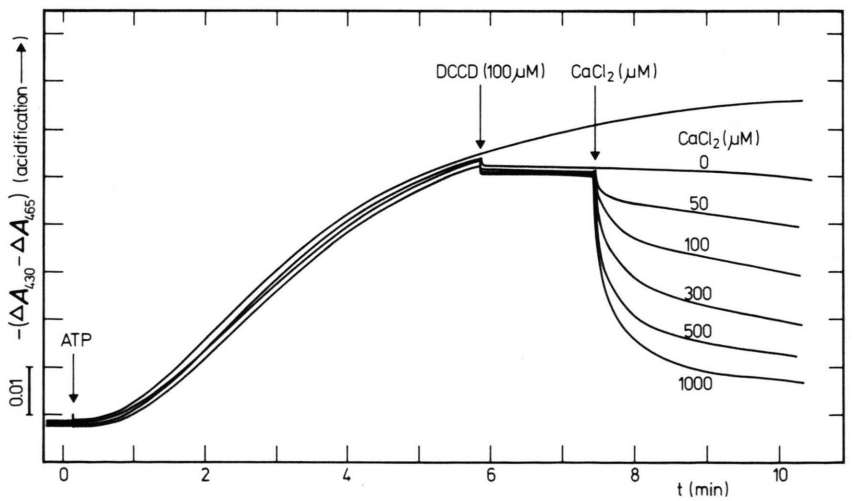

Fig. 2. ATP-dependent acidification within the vesicles; inhibition of the proton pump by DCCD, and H^+-export from within the vesicles after addition of Ca^{2+}, under the conditions described in Fig. 1.

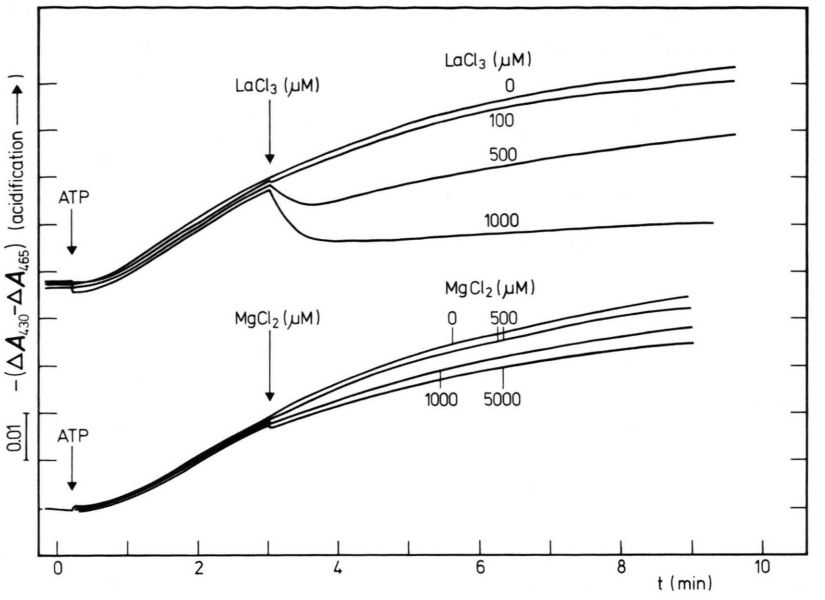

Fig. 3. ATP-dependent acidification within membrane vesicles; release of H^+-ions induced by La^{3+} in comparison to Ca^{2+} (Fig. 1 and 2) lower, Mg^{2+} remains ineffective even at very high concentrations.

fast alkalinization is observed, that is to say a release of H^+ from within the vesicle into the buffered environmental solution. This export is strictly proportional to the added amount of Ca^{2+}. After this first reaction phase lasting about 1–2 min, the energy dependent acidification in the vesicles is restored, but at a reduced speed. The lower rate of acidification in the vesicles determined now corresponds approximately to the rate determined where ATP and Ca^{2+} were simultaneously added at the beginning of the experiment (Fig. 1).

The sudden efflux of H^+ from inside the vesicles after the addition of Ca^{2+} can best be ascribed to a H^+/Ca^{2+} antiporter located in the membrane of the vesicle, which renders a Ca^{2+}-influx into the vesicles in exchange for H^+ until a balance in concentration is reached or a new thermodynamic equilibrium established. Then an ATP-dependent H^+-accumulation takes place anew, however, at a lower speed, since H^+ can still be further exchanged by Ca^{2+}. However, since Ca^{2+} now has to be transported uphill against the diffusion gradient, the antiporter prevents a complete efflux of H^+ and causes an accumulation of H^+ at a lower rate.

If the H^+-pumps on the membrane are inhibited by DCCD (100 μM), an addition of Ca^{2+} again brings about an incipient, fast export of H^+, however, a subsequent re-acidification can not be observed, but only a slow, rather unspecific leak out of H^+ from the vesicles. This slow H^+-efflux is the same for all concentrations of Ca^{2+} added.

While La^{3+} which in various tissues readily displaces Ca^{2+} from its binding sites [18] can first achieve a similar effect as Ca^{2+}-ions at substantially higher concentrations, Mg^{2+} remains completely ineffective (Fig. 3). This speaks in favor of a H^+/Ca^{2+}-antiporter with a very specific binding site for Ca^{2+}.

The H^+/Ca^{2+}-antiporter mechanism can be regarded as a secondary energized Ca^{2+} pump. Such has been detected in the plasmalemma of *Neurospora* [15–17].

Furthermore, a similar mechanism seems to exist in sarcoplasmic reticulum vesicles, since H^+-efflux is fairly tightly coupled with Ca^{2+} uptake utilizing a counter transport mechanism [19–21].

The function *in vivo* of the H^+/Ca^{2+}-antiporter described here could be to keep the cytoplasm free of Ca^{2+} ions or to adjust the Ca^{2+} level according to physiological conditions. Within the vesicles themselves then, an augmented H^+-accumulation could first take place only after a decrease of the Ca^{2+} concentration in the cytoplasm.

The demonstrated Ca^{2+}-pumping mechanism does not seem to be identical with the Ca^{2+}-pump described by Gross and Marmé (1979) [11] on microsomal vesicles from zucchini. The latter Ca^{2+} uptake system cannot be inhibited by CCCP and it shows

no such high specificity for ATP. It is possible that these two Ca^{2+} transport systems are located on different membrane vesicles and that they have different tasks in regulating cell metabolism.

Acknowledgements

We thank Miss Karin Putz for skillfull technical assistance and Dr. J. Gross for critical and stimulating discussions.

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