Electrogenic Proton Pump in *Nitella* and the Effect of Calcium

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Abstract. The hyperpolarisation of the membrane potential in *Characeae* above that of the diffusion potential is explained by the operation of the electrogenic proton pump. We studied the interaction of calcium with the functioning of the pump. The membrane potential was measured using the standard microelectrode technique. An increase in the calcium concentration resulted in depolarisation, its magnitude increasing with lower proton concentrations. Calcium-induced membrane potential changes, tested in the concentration range of 0.25 mmol/l to 25 mmol/l, were greatest at 0.25 mmol/l $CaCl_2$ and decreased with the increasing calcium concentration. Light-induced initial changes in the membrane potential also showed a dependence on the presence of calcium in the external medium. We conclude that calcium has a role in the regulation of the proton pump in *Nitella*.

Key words: Calcium — Membrane potentials — pH effects — Electrogenic proton pump

Introduction

The membrane potential of Characean cells can reach two different steady states (Hope and Walker 1961; Spanswick et al. 1967). In the depolarised state, which occurs in both the absence of external calcium and the presence of high concentrations of potassium, the potential can be explained by the diffusion potential for potassium and sodium. In the presence of external calcium and at low potassium concentrations, the potential is hyperpolarised above this level. In that case the membrane potential has a diffusion (ψ_D) and an electrogenic component (ψ_P), and can be expressed (Finkelstein 1964) by the equation:

$$\psi = \frac{\psi_P \cdot g_P + \psi_D \cdot g_D}{g_P + g_D} \quad (1)$$

where g_P and g_D are the conductivities of the active and passive channels, respectively.

The membrane potential shows a dependence on the pH of the external medium (Kitasato 1968), and this is explained by the functioning of the electrogenic proton pump expelling protons from the cell. If one assumes that $g_P \gg g_D$, the influence of pH on the membrane potential is then primarily due to its effect on the electrogenic component, according (Spanswick 1972) to the equation:

$$\psi_P = \frac{\Delta \bar{\mu}_P}{\upsilon_H \cdot F} - RT \ln \frac{|H^+|_i}{|H^+|_0} \qquad (2)$$

where $\Delta \bar{\mu}_P$ is the change in the free energy of the driving reaction; υ_H the stoichiometric coefficient; and $|H^+|_0$ and $|H^+|_i$ are the external and internal proton concentrations, respectively. Light has also been shown to have an effect on the membrane potential, illumination after a period of darkness causing first depolarisation (Vredenberg 1970), followed by hyperpolarisation. This hyperpolarisation is similarly explained by the activation of the electrogenic pump (Spanswick 1972).

Calcium seems to be closely associated with the electrogenic pump, as can be seen from its requirement for the occurrence of the hyperpolarised state, as well as from its effect on the initial depolarisation with illumination. In this paper we present the results of our studies of the involvement of calcium with the proton pump of *Nitella*.

Materials and Methods

In these studies we used internodal cells of the green alga *Nitella mucronata*. The plants were grown as described previously (Radenović and Vučinić 1976). The cells were excised 24 hours prior to the experiment and kept in a standard solution consisting of 1.0 mmol/l NaCl + 0.1 mmol/l KCl + 0.25 mmol/l $CaCl_2$. In initial depolarisation experiments, calcium was omitted from the washing medium. The cells were mounted in an experimental chamber and equilibrated for 20—30 minutes in the same solution, before the start of the experiment. The external solution was flowing around the cell at a rate of 5 ml/minute. Vacuolar membrane potentials were measured using the standard microelectrode technique (Radenović and Vučinić 1976). All experiments were performed at room temperature (22—24 °C). In Ca/pH experiments, the cells were illuminated with 10 W/m^2 of white light provided by a 150 W tungsten projector lamp. In the initial depolarisation experiments, the cells were illuminated with 0.5 W/m^2 of red light by a 150 W quartz-halogen lamp and a Schott IL 662 interference filter. The pH of the external solution was maintained in the range between 4.5 and 9.0 using a mixture of two zwitterionic buffers (tris(hydroxymethyl)-aminomethane and 2-(N-morpholino)ethane sulfonic acid) at a total concentration of 2 mmol/l. The buffering capacity of the medium was checked before and after the experiments and proved sufficient. The results shown are the average of at least ten independent experiments for each point, the variability being shown by standard deviation.

Results

The results of our study on calcium and proton dependence of the membrane

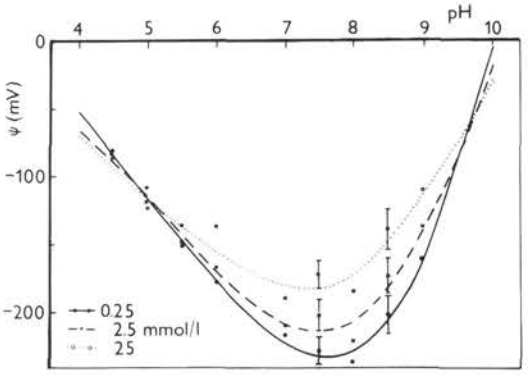

Fig. 1. The effect of $CaCl_2$ on the membrane potential at different pH values. The concentrations of calcium used are shown in the bottom left corner of the diagram.

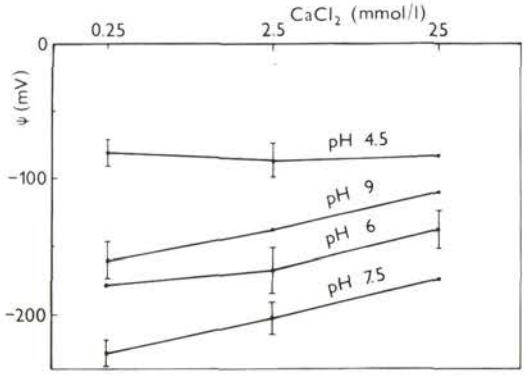

Fig. 2. pH dependence of the membrane potential (ψ) at different $CaCl_2$ concentrations.

potential are shown in Figs. 1 and 2. Maximal hyperpolarisation was observed at pH 7.5 and the protons had effects similar to those observed by other authors (Kitasato 1968; Spanswick 1972; Saito and Senda 1973). Calcium brought about a depolarisation of the steady state potential. The changes induced by calcium were not the same at all pH values and decreased with increasing proton concentrations. From the plot of the difference in membrane potential between maximal values and those at pH 4.5 versus the calcium concentration present (Fig. 3) it can be seen that 0.25 mmol/l $CaCl_2$ induced the greatest change, higher concentrations of calcium being not as effective. Light-induced primary depolarisation was also shown to be dependent on the presence of calcium in the external medium (Fig. 4). Our results are in agreement with those reported elsewhere (Vredenberg 1970) and show that both the amplitude of the initial depolarisation and its kinetics are dependent on

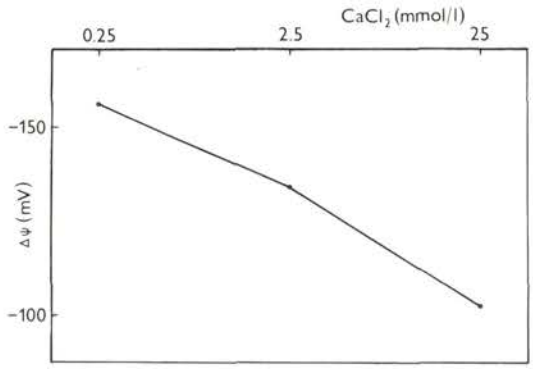

Fig. 3. Membrane potential differences ($\Delta\psi$) between values obtained at pH 4.5 and those at higher pH showing maximal hyperpolarisation, and their dependence on calcium concentration.

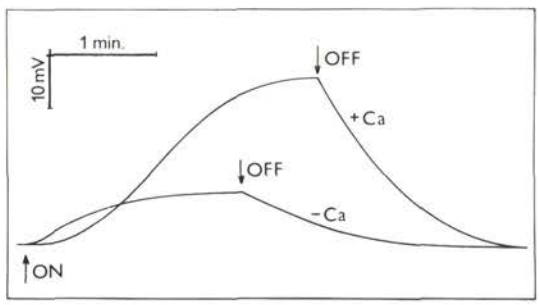

Fig. 4. Kinetics of the light-induced change of the resting membrane potential in the presence and absence of calcium (0.25 mmol/l) in the external medium. Arrows indicate the moment when light was switched on or off.

the presence of calcium in the external medium. Thus the average light-induced depolarisation changes were 23.5 ± 7.3 mV in the presence, and 11.5 ± 4.7 mV in the absence of external calcium. However, there were cases where no depolarisation could be observed when calcium was omitted, and this variability we explain by complete or incomplete washing of the calcium from the cell wall, where it can be ionically tied to the fixed negative charges of the cell wall fibrils. The presence of calcium in the external medium also introduced a delay in the initial depolarisation, from the moment of the light switching on.

Discussion

The electrogenic proton pump of plant cells can be viewed as a primary active transporting mechanism responsible for the creation of an electrochemical gradient, in line with the proposals of Mitchell (1966) and Skulachev (1977). This gradient

in turn can be utilised for driving secondary transporting processes, such as HCO_3^- uptake (Lucas 1982), required for the maintenance of the photosynthesis at adequate rates. Calcium is known to be closely linked to many cellular processes and has important regulatory roles (Duncan 1976). The discovery of calmodulin (Cheung et al. 1978) in cells explained many of these calcium effects by the formation of a complex with this protein, capable of activating many cellular enzymes. Calmodulin has been shown to be also present in plant cells (Charbonneau and Corimer 1979), and has been implicated in the functioning of the proton pump of higher plant cells (Lado et al. 1981).

Our results show that the effect of calcium is greatest at pH values at which the proton pump is expressed to the highest presence. Saito and Senda (1974) have shown that chloride ions are not responsible for such changes. Similarly, calcium has an effect on the initial light-induced depolarisation, which is thought to be a microelectrode registered representation of the process of activation of the electrogenic proton pump, leading to subsequent hyperpolarisation and attainment of a higher potential steady state. Our results differ in that the presence of calcium increased the delay in the appearance of the primary depolarisation on illumination.

Based on the above considerations we conclude that calcium affects the electrogenic proton pump. Whether this effect is due to a direct interaction between the ion and the enzyme, or is an indirect process involving mechanisms which increase calcium concentration in the cytoplasm and bring about a link with calmodulin, cannot be said yet.

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