MEMBRANE POTENTIAL CHANGES DURING TRANSPORT OF GLYCINE AS A NEUTRAL AMINO ACID AND NITRATE IN *LEMNA GIBBA* G 1

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1. Introduction

The active transport of sugars through the plasmalemma of algae, fungi, and higher plants is suggested to be coupled to an H^+ influx. This was concluded from the transient alkalinization of the external medium [1–3] and from the transient depolarization of the membrane [2,4,5] at the onset of sugar transport. The proton-hexose co-transport was supposed to be maintained by an electrochemical proton gradient ($\Delta\tilde{\mu}H^+$) [1–5], in agreement with the co-transport hypothesis proposed [6] for active transport processes through plant membranes in general [6].

The transport of amino acids is known to be accompanied by a proton transport as well, and to be dependent on a H^+ gradient in bacteria and yeast [7–9]. By analogy the same could apply for higher plants.

The electrical membrane potential ($\Delta\psi$) of *Lemna* (duckweed), a eucaryotic autotrophic angiosperm, has a large active component depending on metabolic energy [5]. As the electrochemical proton gradient ($\Delta\tilde{\mu}H^+$) at the plasmalemma of *Lemna* cells seems to be the driving force for sugar uptake [5], it was expected that amino acid transport in *Lemna* cells is related to $\Delta\psi$ as well.

Dedicated to Professor Noe Higinbotham on occasion of his 65th birthday

Not only the uptake of non-electrolytes, but also that of phosphate as an anion was found to be driven by such a mechanism in *Lemna* (C. I. U. E. et al., unpublished) similar to that in yeast [9,10]. Hence it may be expected that also transport of nitrate is coupled to an ATP-driven H^+ extrusion pump.

Amino acid transport in steady-state uptake experiments is not accompanied by an alkalinization of the medium. By contrast, uptake and metabolism of nitrate in algae [11] and in duckweeds [12] lead to a steady-state alkalinization with a stoichiometry of 1 NO_3^- taken up to 1 or 2 OH^- released. Nitrate uptake at the plasmalemma was regarded as an exchange or counter-transport against OH^- (1:1). Excess OH^- is produced by nitrate metabolism; one OH^- is generated by H^+ consumption during NO_2^- reduction to NH_3, a second one in the formation of NH_4^+, if NH_3 is not consumed in protein or nucleic acid synthesis [11,13]. Thus for nitrate the question arises, whether it is also taken up by H^+ co-transport, or by the proposed OH^-counter-transport system. In the case of co-transport with more than 1 H^+ a transient depolarization of the membrane upon addition of nitrate should be observed just as with sugars [5] and amino acids. If nitrate uptake were strictly bound to an OH^- counter-transport, the membrane potential should remain unaffected.

The present results show transient depolarizations of the membrane of *Lemna* cells after the onset of both amino acid or nitrate uptake.

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2. Methods

Duckweed plants (*Lemna gibba* L., strain G 1) were grown as in [14]. Plants for nitrate uptake experiments were transferred to a sterile sucrose-containing nutrient solution without NO_3^-, and were kept in a longday photoperiod at 4.5 klux (HQL lamps) for 6 days. The plants were transferred to 1 mM $CaSO_4$ 7 h before the experiments and were kept in the dark or in continuous light. For glycine uptake experiments the plants were grown in a nutrient solution in the dark either without sucrose for 7 days or with sucrose for 4 days. They were transferred to 1 X solution (1 mM KCl, 1 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, 0.95 mM sodium phosphate, pH 5.7 [15]), 5 h before the experiments.

In order to insert the micropipettes (tip diameter<0.5μm) into the *Lemna* cells, the fronds were mounted in a 4 ml plexiglass chamber, which was perfused (10 ml/min) with 10 mM $CaSO_4$, 10 mM $Ca(NO_3)_2$, or 1 X solution, or 1 X + 50 mM glycine, pH 5.7. The micropipettes were filled with 3 M KCl, and the reference salt bridge with 3 M KCl in 2% agar. They were connected by Ag/AgCl electrodes to an electrometer-amplifier with a line recorder. Additional details concerning the electrical measurements have been described [5]. The temperature was 23°C and the light intensity during the light experiments was 25 klux, obtained from a quartz-halogen bulb, the light being conducted through glass fiber optics.

3. Results

Glycine, a neutral amino acid, is taken up actively by duckweed cells [16]. The uptake rate is even higher than that of glucose at comparable concentrations [14]. Upon addition of 50 mM glycine the membrane potential difference $\Delta\psi$ decreased within 10 s by 130 mV (fig.1A). $\Delta\psi$ recovered spontaneously still in the presence of glycine, but the kinetics were different from those during hexose uptake [5]. The comparison of fig.1A with fig.1B shows that only the actively maintained component of $\Delta\psi$ was depolarized by glycine, not the passive component. The maximum depolarization was dependent on the external concentration of glycine (fig.2). It was saturated at about 5 – 7.5 mM, and amounted to an

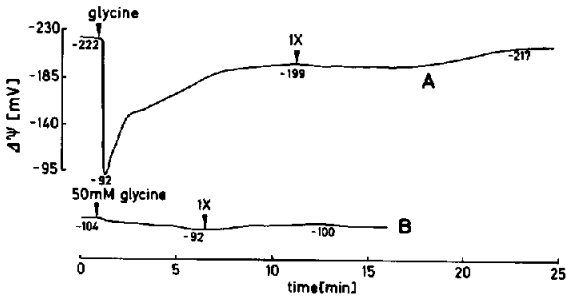

Fig.1. Effect of 50 mM glycine on the active (A) and passive (B) component of the membrane potential $\Delta\psi$ in *Lemna gibba* in the dark at pH 5.7. $\Delta\psi$ measurements in 1 X solution; glycine flow in 1 X reached the electrode chamber 15 s after addition (arrow). Plants were grown without sucrose in the dark for 7 days (A) or in the light (HRIL-lamp) for 12 days (B).

average of 95 mV in these plants. The shape of this curve is similar to that of typical enzyme–substrate saturation kinetics, as was also found for hexose-dependent depolarization of $\Delta\psi$ [5].

Short-time nitrate deficiency did not affect $\Delta\psi$ of

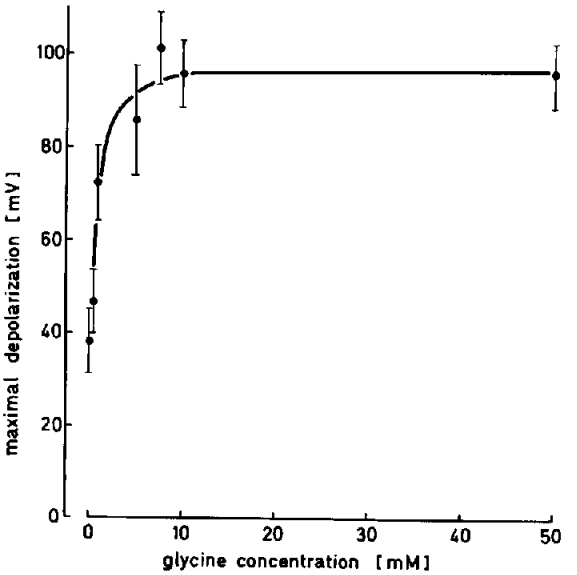

Fig.2. Maximum depolarization of the cell membrane in *Lemna gibba* in relation to the external concentration of glycine. $\Delta\psi$ measurements in 1 X solution, pH 5.7, in the dark. Plants were continuously grown on sucrose and kept in the dark for 4 days before the experiments.

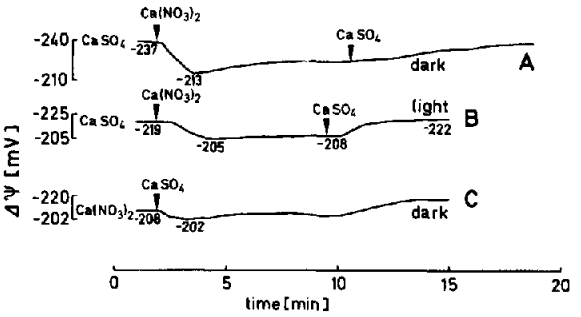

Fig.3. Effect of NO_3^- (20 mM) in the dark (A) and in the light (B) and of SO_4^{2-} (2.5 mM) in the dark (C) on $\Delta\psi$ in *Lemna gibba*, grown for 6 days in longday in the presence of sucrose, without nitrate. $\Delta\psi$ measurements in $CaSO_4$ (10 mM and 2.5 mM).

Lemna fronds. After 6 days of nitrate starvation in the presence of sucrose under longday conditions $\Delta\psi$ was still −215 to −240 mV (interior negative, fig.3) in the majority of the plants. Upon the addition of 20 mM nitrate in the dark, $\Delta\psi$ decreased by 24 mV within 1 min (fig.3A). Recovery to the original $\Delta\psi$ of −235 mV occurred more quickly in the absence of nitrate. In the light, the nitrate-dependent depolarization was smaller (14 mV, fig.3B). Calcium concentration was kept constant at 10 mM throughout the experiments because of its requirement for stabilizing $\Delta\psi$. As compared with nitrate, sulfate exerted a much lesser effect on $\Delta\psi$ (7 mV, fig.3C). This may be due to the lower uptake rates often observed for sulfate. In addition the plants were not starved prior to the experiments.

4. Discussion

The active component of the membrane potential difference $\Delta\psi$ of duckweed cells is maintained by energy-dependent processes at values from −200 − −280 mV (interior negative). A $\Delta\psi$ of −90 mV (at pH 5.6) was found to represent the passive component of the duckweed membrane potential [5]. During the uptake of the neutral amino acid glycine $\Delta\psi$ was depolarized by 130 mV to −92 mV (fig.1A). Severely carbon-starved *Lemna* cells with a steady-state $\Delta\psi$ of −104 mV at pH 5.7 were depolarized by 50 mM glycine only by 12 mV, i.e., to −92 mV as well (fig.1B). These data may suggest that the glycine transport in duckweeds is coupled to a H^+ influx, which may be maintained by an active ATP-driven H^+ efflux. This assumption is supported by the spontaneous recovery kinetics of $\Delta\psi$ even in the presence 50 mM glycine (fig.1A) suggesting an increased activity of the proton extrusion pump upon the glycine-induced H^+ influx.

During NO_3^- uptake of $\Delta\psi$ of duckweeds was depolarized as well. The change of $\Delta\psi$ is also transient (fig.3). In the light the membrane is less depolarized than in the dark (fig.3B), though nitrate uptake is strongly enhanced by light (4–6-fold in *Ankistrodesmus* [17]. According to the hypothesis of active proton extrusion this apparent contradiction could be explained by light stimulation of H^+ extrusion. In carbon-starved *Lemna* fronds a factor of 10 was calculated for the light enhancement of the assumed H^+ extrusion pump [5]. From these results it may be concluded that the NO_3^- uptake process involves a H^+–NO_3^- co-transport step. Hence the steady-state alkalinization of the external medium is due to metabolic H^+ consumption and must be independent of the primary uptake step.

It is suggested that, in spite of various 'carriers' involved, a single basic process may operate in transport through the plasmalemma of eucaryotic plant cells, the transport along $\Delta\tilde{\mu}H^+$ energized by a H^+ extrusion pump. It seems to operate in the transport of sugars [1–5] and amino acids [8,9] as well as that of anions like phosphate ([9,10] and C. I. U. E. et al., unpublished results) and nitrate. This assumption would be in agreement with the hypothesis proposed [6] that the proton extrusion pump is the general driving force for active transport in plant cells.

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Note in proof

After submitting this paper we have learned (personal communication) that B. Etherton and B. Rubinstein found similar depolarization of membrane potential with the amino acid analogue α-aminobutyric acid in oat coleoptiles: Plant Physiol. (1978) in press.

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