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Membrane Potential Changes Related to Active Transport of Glycine in *Lemna gibba* G1[1]

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ABSTRACT

Accumulation of ^{14}C-labeled glycine and microelectrode techniques were employed to study glycine transport and the effect of glycine on the membrane potential ($\Delta\psi$) in *Lemna gibba* G1. Evidence is presented that two processes, a passive uptake by diffusion and a carrier-mediated uptake, are involved in glycine transport into *Lemna* cells. At the onset of active glycine uptake the component of $\Delta\psi$ which depended on metabolism was decreased. The depolarized membrane repolarized in the presence of glycine. This glycine-induced depolarization followed a saturation curve with increasing glycine concentration which corresponded to carrier-mediated glycine influx kinetics. The transport of glycine was correlated with the metabolically dependent component of $\Delta\psi$. It is suggested (*a*) that the transient change in $\Delta\psi$ reflects the operation of an H^+-glycine cotransport system driven by an electrochemical H^+ gradient; and (*b*) that this system is energized by an active H^+ extrusion. Therefore the maximum depolarization of the membrane consequently depended on both the rate of glycine uptake and the activity of the proton extrusion pump.

Transient changes of the active component of $\Delta\psi$[3] in *Lemna gibba* which are associated with the transport of sugars, amino acids, nitrate, and phosphate into *Lemna* cells have been reported previously (14, 15, 20). They were interpreted as due to an H^+-substrate co-transport according to the concept of Mitchell (12). The energy-requiring uptake of uncharged solutes and anions is suggested to be coupled to a passive H^+ influx. An inwardly directed free energy gradient of hydrogen ions ($\Delta\tilde{\mu}H^+$), maintained by a metabolically dependent H^+ extrusion pump, is used as the driving force. Similar characteristics of transport phenomena in bacteria (24), fungi (7, 18), algae (9), and higher plants (2, 8, 10, 14) indicate that a common basic process may drive the accumulation of substrates against their chemical and electrical gradients.

In the present paper the correlation between the transient depolarization of the membrane and the uptake of glycine via an assumed H^+-glycine co-transport carrier in *Lemna* cells is reported.

MATERIALS AND METHODS

Plants. Duckweed plants (*Lemna gibba* L., strain G1, obtained from the *Lemna* collection of Prof. Kandeler, Wien) were grown as described earlier, either on sucrose- or nonsucrose-containing media in a photoperiod of 8 or 16 h, respectively (21). Plants were kept in the dark for 4 days and transferred to the bathing solution [1X = 1 mM KCl, 1 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, 0.95 mM Na-phosphate buffer, ref. 4] at least 1 h before experiments. Different pH values were obtained by using various ratios of primary and secondary Na-phosphate. Samples were maintained in the darkness prior to dark experiments or illuminated before light experiments.

[U-^{14}C]Glycine Uptake Experiments. The procedure used by Ullrich-Eberius *et al.* (21) was followed for glycine uptake. Plants were incubated in 1X medium in a final volume of 5 ml. The experiments were started by injection of glycine labeled with [U-^{14}C]glycine (Radiochemical Centre, Amersham, U.K.). The uptake reaction was stopped by rapidly rinsing the fronds and washing for 2.5 min in unlabeled incubation medium (five times 10 ml) in a shaking ice bath. The ^{14}C content was measured on planchets after the plants were mounted upside down in 0.5 ml gelatin solution (2%) and dried at 70 C.

O_2 Gas Exchange Experiments. Rates of respiratory O_2 uptake and photosynthetic O_2 evolution were measured manometrically (19). O_2 evolution rates were not corrected for concurrent respiratory O_2 uptake. The temperature for both experiments, O_2 gas exchange and glycine uptake, was 25 C.

Electrophysiological Measurements. The membrane potential of *Lemna* frond cells was measured as described previously (15). Glass micropipettes filled with 3 M KCl were used as microsaltbridges to Ag/AgCl electrodes. A short polyethylene tube containing 3 M KCl in 2% agar was employed as the reference electrode. Both the microelectrode and the reference electrode were connected to an electrometer-amplifier (Keithley 604) and a chart recorder. Micropipettes were inserted into *Lemna* fronds and observed through a horizontally mounted microscope equipped with a quartz halogen bulb and gooseneck fiber optics (Schott, Mainz). For experiments in darkness impalement was done under a green safelight (combination of two interference filters, $\lambda = 516$ nm and $\lambda = 530$ nm, Schott, Mainz). The measurements were performed at room temperature (about 23 C).

Membrane potential measurements are expressed as mean values ± SE.

RESULTS

Kinetics and Time Course of Glycine Uptake. The rate of glycine uptake into *Lemna* fronds depended on the external glycine concentration (Fig. 1A). Above 10 mM glycine the uptake rate became a linear function of the glycine concentration in the light and in the dark. Evaluation of the experimental data suggests that glycine transport in *Lemna* is a combination of two components. One component which showed saturation kinetics was superimposed on a second linear component. The contribution of each of these parts is visualized by transforming the same data to

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[3] Abbreviation: $\Delta\psi$: membrane potential; v: uptake rate (μmol g^{-1} fresh weight h^{-1}); c: glycine concentration in the external medium (mM).

a Hofstee plot (Fig. 1, B and C), where the horizontal portion of the curve can be interpreted as diffusion and the vertical one as carrier-mediated uptake (13).

The uptake of glycine was linearly correlated with the time of incubation (Fig. 2). At a glycine concentration saturating the carrier-mediated component of uptake (10 mM) and at a nonsaturating concentration (0.5 mM) the uptake rates of 1.96 and 0.59 μmol g^{-1} fresh weight h^{-1} in the dark and 2.89 and 0.85 μmol g^{-1} fresh weight h^{-1} in the light remained constant with time for 30 min.

pH Dependence of Glycine Uptake. Glycine transport into *Lemna* cells was sensitive to pH changes in the external medium (Fig. 3). The pH optimum for glycine uptake was found to be pH 6.0 for both glycine concentrations tested (0.5 and 10 mM).

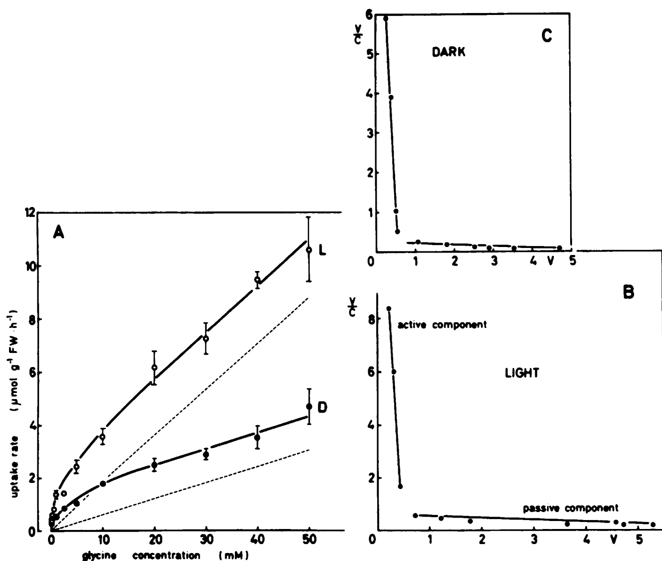

FIG. 1. A: initial rates (5-min incubation) of glycine uptake into *L. gibba* fronds in the light (L), 24 klux, and in the dark (D) in relation to the external glycine concentration (pH 5.7) (mean values of five experiments ± SE); B and C: Hofstee plots of the same data.

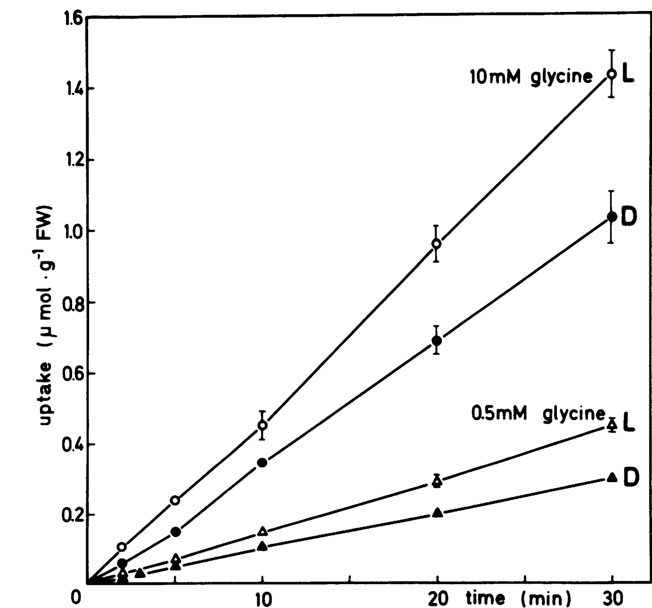

FIG. 2. Time course of glycine uptake at 0.5 and 10 mM external glycine concentration in the light (L) and in the dark (D) (pH 5.7) (mean values of four experiments ± SE).

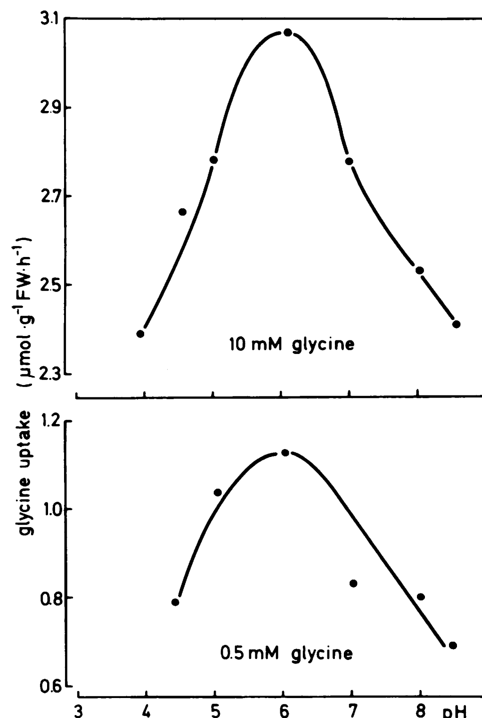

FIG. 3. Effect of pH on glycine uptake at 0.5 and 10 mM concentration. Preincubation in 1X at different pH values for 1 h, experiments were performed in the dark (5-min incubation, mean values of two experiments).

Effect of Glycine on Respiration and Photosynthetic O_2 Evolution. The addition of glycine to *Lemna* fronds stimulated the respiratory O_2 uptake in the dark (Fig. 4). At 50 mM glycine, which saturated the active glycine uptake system, the rate of O_2 uptake of plants grown on a sucrose-containing medium increased from 7.5 to 12.4 μmol O_2 g^{-1} fresh weight h^{-1}. The rate of photosynthetic O_2 evolution at 24 klux, 1.5% CO_2, near saturating light conditions (21), showed a decrease from 145 to 130 μmol O_2 g^{-1} fresh weight h^{-1} in response to 50 mM glycine. *Lemna* plants grown in the absence of sucrose and kept in darkness for 7 days raised their respiratory O_2 uptake in the dark from 4.6 to 8.25 μmol O_2 g^{-1} fresh weight h^{-1} after addition of 50 mM glycine. In the light the photosynthetic O_2 evolution was reduced from 137 to 121 μmol O_2 g^{-1} fresh weight h^{-1}.

Some Characteristics of the Membrane Potential of *L. gibba* G1. When *Lemna* plants were grown in a 16-h daily photoperiod, which enhances growth in the absence of sucrose, $\Delta\psi$ depolarized in the dark within 10 min to a cyanide-insensitive level, *i.e.* to an apparent diffusion potential of about −100 mv (Fig. 5A). Upon switching the light on, $\Delta\psi$ increased within less than 10 min to a high negative level (−236 mv in Fig. 5A). Plants grown in an 8-h daily photoperiod in the presence of sucrose maintained a high resting potential in the light and in the dark (averages: −248 ± 3 mv, $N = 78$, in the light; −222 ± 4 mv, $N = 84$, in the dark). They responded to switching light on or off with transient changes of $\Delta\psi$ (Fig. 5B). These transients are typical for photosynthetically active cells (17); however they seem to occur only when an electrogenic component contributes to $\Delta\psi$ (*cf.* Fig. 5A, refs. 3 and 15).

Glycine-induced Transients of $\Delta\psi$. The membrane of *Lemna* frond cells was depolarized upon addition of glycine (Fig. 6, A and B). This depolarization was only transient. The repolarization was enhanced by removal of glycine from the system, but $\Delta\psi$ partially recovered even in the presence of glycine. When glycine was removed from the medium after almost complete repolarization the membrane was transiently hyperpolarized (Fig. 6B).

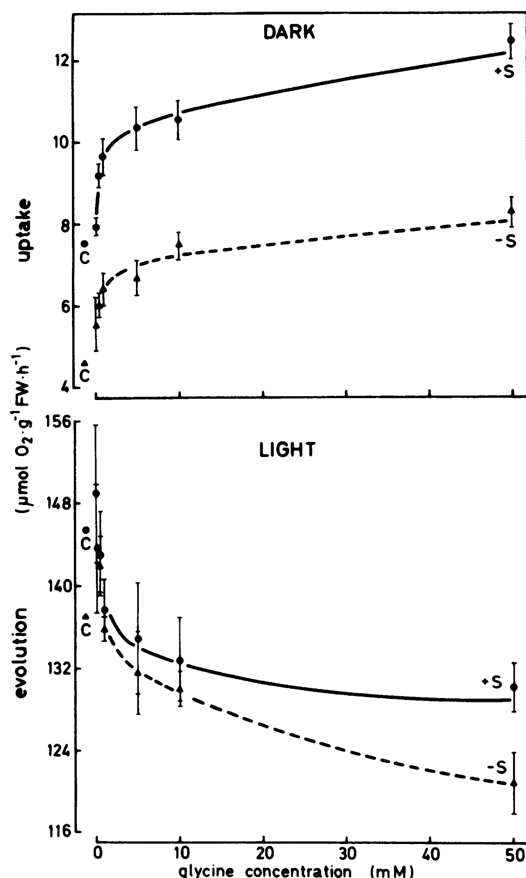

FIG. 4. Effect of glycine on respiratory O_2 uptake in the dark and photosynthetic O_2 evolution in the light (24 klux, 1.5% CO_2) (pH 5.7), (+ S): plants grown in the presence of sucrose in an 8-h photoperiod, (− S): plants grown in the absence of sucrose in a 16-h photoperiod and kept in darkness for 7 days prior to experiments (mean values ± SE, $N = 7$–12).

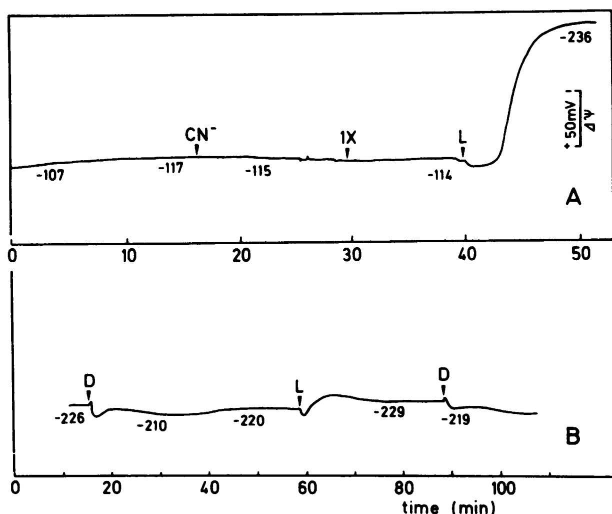

FIG. 5. A: membrane potential of *Lemna* plants grown for 7 days without sucrose in a 16-h photoperiod (−117 mv in the dark) did not respond to 1 mM KCN. Upon switching light on the membrane repolarized immediately to a more negative value of −236 mv; B: light-dependent transients of $\Delta\psi$ in a plant with a large negative resting potential in the light (L) and in the dark (D), grown for 7 days in a 16-h photoperiod on a sucrose-containing medium. Both experiments were performed at pH 7.

Depolarization and recovery could be repeated several times with the same cell (Fig. 6C).

The maximum depolarization of the membrane induced by glycine depended on the external glycine concentration. In the light the recovery of $\Delta\psi$ in the presence of glycine was accelerated (Fig. 7, A and B).

The maximum depolarization could be related to the glycine concentration in the medium by a saturation curve (Fig. 8) (14), just as the rate of glycine uptake (*cf.* Fig. 1A). The depolarization of the membrane was saturated at similar glycine concentrations as the active component of glycine uptake (*i.e.* 7.5–10 mM). The saturation values for the maximum depolarization (10 mM glycine) were 95 ± 7 mv in the dark and 101 ± 7 mv in the light. Since the maximum depolarization depended on $\Delta\psi$ (Fig. 9), the relative maximum depolarization (*i.e.* per cent of $\Delta\psi$) was plotted against the glycine concentration in Figure 8.

DISCUSSION

The kinetics of glycine uptake in *Lemna gibba* suggests that the transport process consists of carrier-mediated and noncarrier-mediated components. Their respective contribution becomes apparent in Hofstee plots of the uptake data (Fig. 1, B and C). The diffusion component was most pronounced at high substrate concentrations ($v/c < 1$ in the light, $v/c < 0.5$ in the dark), whereas the carrier-mediated component of glycine uptake was dominant at low substrate concentrations ($v/c > 1$ in the light and $v/c > 0.5$ in the dark). Similar kinetic data, *i.e.* a biphasic concentration dependence of L-alanine escape from xylem vessels, were reported by van Bel *et al.* (23). In *Lemna* cells the slope of the curves representing diffusional influx differed, surprisingly, between light and dark, suggesting a higher passive permeation of glycine into the cells in light (Fig. 1, A, B, and C). Such changes in diffusion could be explained by an alteration in membrane permeability to glycine by light via direct effects of light on the membrane and possibly involving phytochrome. Phytochrome is known to play an important role in growth and development of duckweed (6) and was suggested to interact with its electric properties based on a red far red reversibility of $\Delta\psi$ changes (11).

In general the $\Delta\psi$ of plant cells is composed of a passive and an active portion [diffusion potential and electrogenic pump(s)] (3, 5). This was also confirmed for *Lemna*, where the apparent diffusion potential is about −100 mv and the total $\Delta\psi$ −222 mv and −249 mv in the dark and in the light, respectively. A rapid growth period in the absence of sucrose led to a low intracellular ATP level and, simultaneously, to a low $\Delta\psi$ in the dark (Fig. 5 and ref. 15). This low $\Delta\psi$ was insensitive to CN^- thereby indicating the lack of a metabolically dependent component. Increased energy supply in the light enabled the cell to establish an active potential; $\Delta\psi$ recovered rapidly to a high negative value. At the low $\Delta\psi$ level glycine was presumably only taken up passively by diffusion (14). An active glycine uptake system was assumed to operate at high $\Delta\psi$ levels, since only at high $\Delta\psi$ levels the membrane potential depolarized upon addition of glycine.

The O_2 exchange studies (Fig. 4) suggested that glycine itself interacts with energy-converting pathways. The stimulation of the respiration in the dark was independent of the endogenous sugar concentration and occurred in *Lemna* plants grown in the presence or the absence of sucrose. The glycine-promoted increase in O_2 uptake cannot be a result of the activation of the amino acid oxidase (16) because it could be inhibited with cyanide (Ullrich-Eberius, personal communication). In the light the photosynthetic O_2 evolution was reduced by an amount which was three times more than the increase of the O_2 consumption in the dark. Glycine, an important metabolite in photorespiration, was shown to be oxidized by intact leaf mitochondria (1). This oxidation was found to be linked to the mitochondrial electron transport chain and thus coupled to the synthesis of ATP. Increased O_2 consumption

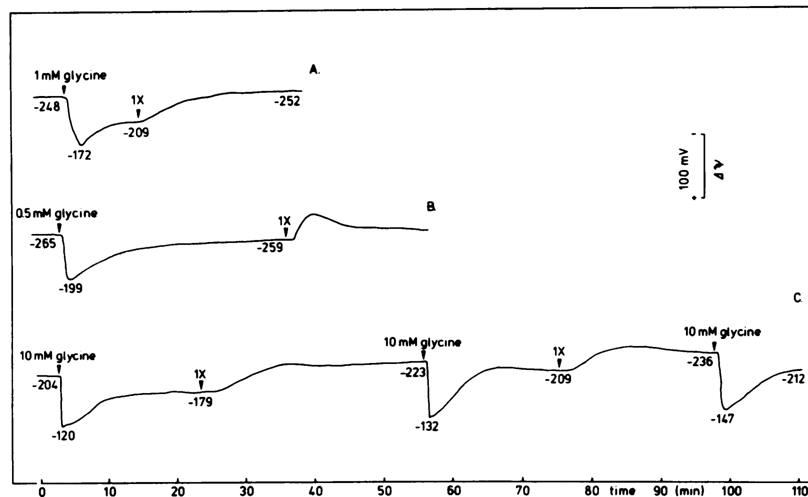

FIG. 6. A: depolarization of the membrane after addition of 0.5 mM glycine followed by enhanced recovery of $\Delta\psi$ after removal of glycine; B: depolarization at the onset of 10 mM glycine and repolarization in its presence. The removal of glycine after recovery of $\Delta\psi$ caused a remarkable transient hyperpolarization; C: repetitive depolarization and repolarization of the same cell after addition of glycine followed by washing with 1X (all measurements represented in Figure 6 were performed in the dark at pH 5.7).

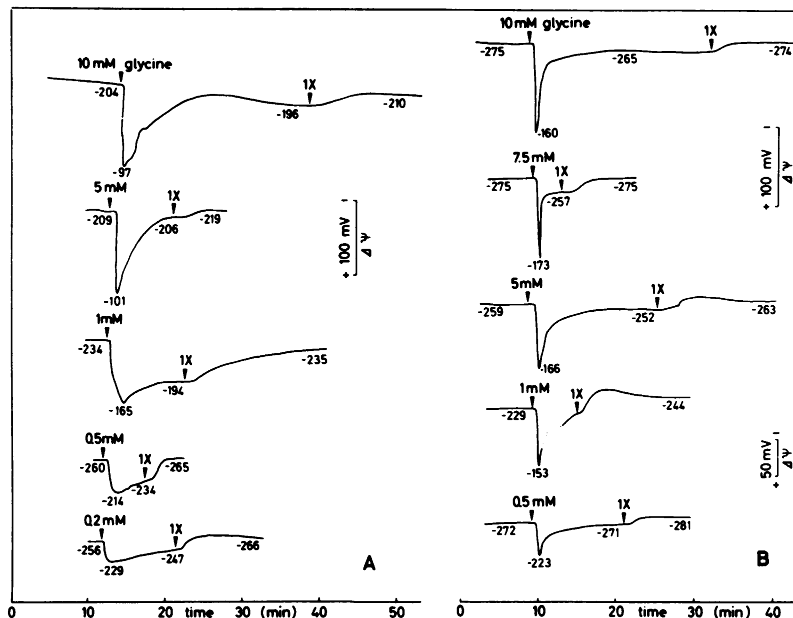

FIG. 7. Glycine-induced depolarization of the membrane in the dark (A) and in the light (B). Slope and maximum depolarization were correlated to the glycine concentration in the medium, repolarization was enhanced by light in the presence of glycine (pH 5.7).

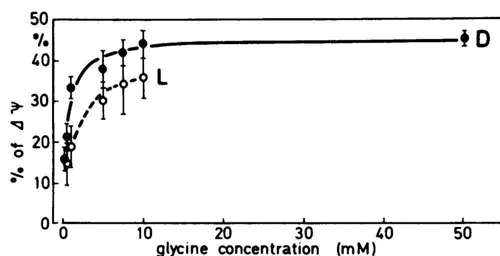

FIG. 8. Relative maximum depolarization in per cent of $\Delta\psi$ in the light (L) and in the dark (D) in relation to external concentration of glycine (mean values ± SE, $N = 4$–18).

and production of nonphotosynthetic ATP may account for the decrease in photosynthetic O_2 evolution upon addition of glycine. However, the results presented here cannot be unequivocally evaluated.

It was proposed previously that glycine uptake by *Lemna* is an H^+-glycine cotransport driven by an electrochemical H^+ gradient at the plasmalemma (14). A pH dependence is often regarded as a criterion for H^+-substrate co-transport, since $\Delta\tilde{\mu}H^+$ is changed with the external pH. This criterion is weak, however, because physiological phenomena in general depend on the pH. The optimum of pH 6 found for glycine uptake (Fig. 3) is close to the isoelectric point of the glycine zwitterion ($pH_I = 6.064$) and may suggest that the pH dependence of glycine transport was due to a preferential transport of the electrically neutral species. On the other hand, glucose and phosphate uptake also operated optimally at a similar pH (Ullrich-Eberius, personal communication) which indicates a pH optimum for H^+-substrate co-transport in *Lemna* cells near pH 6 in general.

More reliable evidence in favor of an H^+-glycine co-transport were the depolarization upon the addition of glycine and the

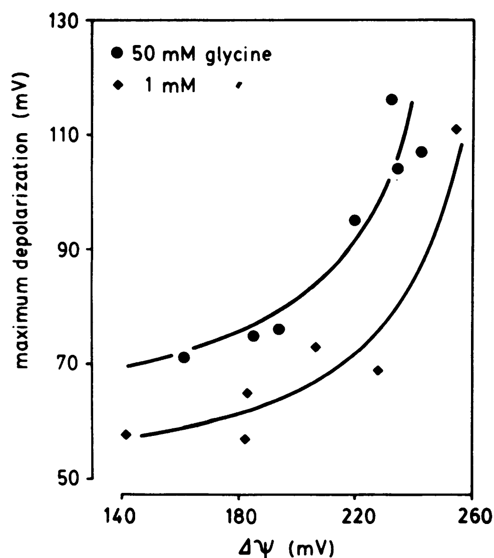

FIG. 9. Dependence of the maximum depolarization on the total value of $\Delta\psi$. Two concentrations of glycine, 1 and 50 mM, were tested (pH 5.7, dark).

transient hyperpolarization when the uptake was terminated after removal of glycine (Fig. 6). The maximum depolarization appeared to be related to the rate of carrier-mediated glycine uptake as shown by similar transport and depolarization kinetics (Figs. 1 and 8). The maximum depolarization was reduced in the light possibly because light increased the rate of the H^+ efflux pump due to additional photosynthetic energy supply. Repolarization in the presence of glycine was explained by an increased activity of the proton extrusion pump which was stimulated by the increased intracellular H^+ concentration at the onset of glycine uptake. The transient hyperpolarization upon removal of glycine could have been due to the cessation of the H^+-glycine co-transport carrier while the H^+-efflux pump still operated at an increased rate.

Since $\Delta\psi$ is a part of $\Delta\tilde{\mu}H^+$ and, therefore, a part of the theoretical driving force of the H^+-glycine co-transport, glycine uptake should depend on $\Delta\psi$. This was demonstrated in comparing the glycine concentration-dependent depolarization of *Lemna* cells in plants with a low $\Delta\psi$ and plants maintaining varying high $\Delta\psi$ (Fig. 9 and ref. 14).

The present paper shows that the characteristics of glycine uptake into *Lemna* cells fulfill the criteria for an H^+-substrate co-transport as far as the $\Delta\psi$ component of $\Delta\tilde{\mu}H^+$ is concerned. To confirm the involvement of an H^+ concentration gradient at the plasmalemma it remains to be demonstrated that at the onset of glycine uptake the external pH shows concomitant transient alkalinization. Transient alkalinization could be measured during glucose uptake in *Lemna* (22). Due to the buffering capacity of glycine these experiments with glycine proved to be difficult.

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