Localization and Capacity of Proton Pumps in Roots of Intact Sunflower Plants

Volker Römhed*, Christine Müller, and Horst Marschner
Institut für Pflanzenährung, Universität Hohenheim, Postfach 70 05 62, 7000 Stuttgart 70,
Federal Republic of Germany

ABSTRACT

Proton extrusion by roots of intact sunflower plants (Helianthus annuus L.) was studied in nutrient solutions or in agar media with a pH indicator. Proton extrusion was enhanced by either iron deficiency, addition of fusicoccin, or single salt solutions of ammonium or potassium salts. The three types of proton extrusion differ in both localization along the roots and capacity. From their sensitivity to ATPase inhibitors it seems justified to characterize them as proton pumps driven by plasma membrane ATPases.

Enhanced proton extrusion induced by preferential cation uptake from (NH₄)₂SO₄ or K₂SO₄ was uniformly distributed over the whole root system. In contrast, the enhancement effect of fusicoccin was confined to the basal root zones and that of iron deficiency to the apical root zones. Also the rates of proton extrusion per unit of root fresh weight differed remarkably and increased in the order: Fusicoccin < K₂SO₄ < (NH₄)₂SO₄ < iron deficiency.

Under iron deficiency the average values of proton extrusion for the whole root system are 5.6 pmol/mg root fresh weight per hour; however, for the apical root zones values of about 28 micromoles H⁺ per mg root fresh weight per hour was calculated. This high capacity is most probably related to the iron deficiency-induced formation of rhizodermal transfer cells in the apical root zones. It can be assumed that the various types of root-induced acidification of the rhizosphere are of considerable ecological importance for the plant-soil relationships in general and for mobilization of mineral nutrients from sparingly soluble sources in particular.
25 white and 40 w/77 Floura, ratio 3:1). The temperature was
26/22°C; the RH approximately 70 to 75%.

Measurement of the pH Changes with pH Electrodes. For
measuring the root-induced pH changes, individual plants were
transferred to black plastic boxes into which a glass beaker was
inserted filled with 150 ml of either single salt solutions or of
complete nutrient solution. Both solutions were continuously
aerated. The pH changes of the solutions over the time period
were plotted by an automatic pH printer with 6 pH electrodes
(METROHM). In addition, the pH was checked manually with
a portable pH meter.

FC (10 μm) or ATPase inhibitors (DCCD, DES) were added
to these solutions. For this purpose, stock solutions were prepared
of FC and the inhibitors, dissolved in ethanol. The final ethanol
concentration in the experimental solutions never exceeded
0.025% (v/v). To exclude an unspecified effect of ethanol, the
same ethanol concentration was also used in the controls.

The amount of protons released by the roots was calculated
by titration of the solutions at the end of the experiments with
10 mm NaOH to the initial pH value. Prior to the determination
of the fresh weight of roots or of excised root tips, the excess
water was removed by blotting with filter paper.

Measurement of Oxygen Consumption. In some experiments,
proton extrusion and oxygen consumption in the root medium
were simultaneously measured by a pH electrode and an
oxygen electrode (Clark type; E900, WTW, Weilheim, West-
Germany). For this purpose a 150-ml glass vessel with both
electrodes installed was filled with complete nutrient solution
or single salt solution. The inlets for the plant stems and the
electrodes in the lid of the glass container were air sealed with
plastic rubber material. The solution (kept at 25°C) was continu-
ously mixed by a magnetic stirrer and root damage was pre-
vented by shielding with a polyester tissue placed on the bottom
of the glass vessel. Oxygen uptake was expressed in μmol O2/g
fresh weight root-h.

Localization of the Proton Extrusion along Roots. To localize
the proton extrusion along single roots of intact sunflower plants,
an agar technique described elsewhere (9) was applied, using a
pH indicator (bromocresol purple, 0.006%) dissolved in an agar
medium (0.75% agar). The agar medium contained either the
complete nutrient solution or the single salts as indicated.

In measuring pH changes in intact plant systems, the agar
technique is the more sensitive method compared to the com-
monly used pH electrodes. This is also the case even when small
solution volumes (150 ml/plant) were used. Proton extrusion
along the roots was seen (purple → yellow color of the pH
indicator) either after a few minutes (Fe deficiency-induced) or
after 2 to 4 h (FC, K2SO4, or [NH4]2SO4).

Each experiment was carried out two or three times with three
to four replicates. Thus, the results presented are representative
of at least six individual plants.

RESULTS

Different Localization of Proton Extrusion along Roots. As
shown in Figure 1, depending upon the type of medium or
pretreatment of plants proton extrusion is localized in different
zones along the roots of intact sunflower plants. In iron-deficient
plants, (NH4)2SO4 (Fig. 1A) and to a lesser extent also K2SO4
(Fig. 1B) and KCl (not shown here) leads to an acidification
along the whole root system. No acidification takes place in iron-
deficient plants in case of solutions of nitrate salts such as KNO3
or Ca(NO3)2 (not shown here) or the complete nutrient solution
with Na source as nitrate only (Fig. 1C). Addition of 10 μM FC to
complete nutrient media of iron-deficient plants induced acid-
ification only in the basal zones of the root system (Fig. 1D) and
not in the elongation zones behind the root tips as had been
expected. In contrast, the acidification of the medium of iron-
deficient sunflower plants is confined to the apical zones of the
roots (Fig. 1E). More or less identical patterns were obtained
when single salts of nitrate were used instead of complete nutrient
solution (data not shown).

Besides the differences in localization of the proton extrusion
along the roots, there are also distinct differences in the rate of
extrusion. Under iron deficiency, acidification (yellow color of
the pH indicator) was clearly visible after 10 to 20 min, whereas
it took about 1 h and 2 h before acidification could be observed
with ammonium nitrogen and FC, respectively.

Rate of Proton Extrusion by Various Types of Proton Pumps. The
different rates of proton extrusion as indicated by the agar
 technique (Fig. 1) were measured directly in solution culture
(Table I). In iron-adequate plants, some proton consumption
took place in complete nutrient solution. Addition of 10 μM FC
decreased proton consumption, but no net proton extrusion
could be measured. The FC-induced acidification—as shown
with the highly sensitive agar technique—is obviously insufficient
to be measured in a larger volume of nutrient solution. In single
salt solutions of KCl or sulfate the net proton extrusion was
between 1.8 and 2.0 μmol H+/g fresh weight-h and thus still
lower than in ammonium salt solutions (3.4–3.6 μmol H+).
In contrast to K2SO4, in Na2SO4 solutions the net proton extru-
sion was negligible.

The highest rate of proton extrusion (5.6 μmol H+/g fresh
weight-h) occurred in roots of iron-deficient plants (Table I).
Taking into account that the iron deficiency-induced proton
extrusion is restricted to the apical root zones (~20% of the
whole root system of 10-d-old sunflower seedlings), actual rates
per unit root weight in these zones can be calculated to be as
high as 28 μmol H+/g fresh weight root tips-h, i.e. about eight
times higher than the rate obtained using ammonium salts with
a uniform acidification along the whole root system (Fig. 1A).

Effect of Various ATPase Inhibitors. As shown in Table I,
proton extrusion, either induced by iron deficiency or potassium
and ammonium salts, is completely inhibited by the ATPase
inhibitors 5 μM DCCD and 50 μM DES. This inhibition occurs
rapidly and is complete within 10 to 20 min (Fig. 2). This
indicates that both types of proton extrusion, driven either by
iron deficiency or imbalance in cation/anion uptake in iron-
adequate plants, are probably ATP-dependent proton pumps.

In contrast to the inhibition of proton extrusion, the ATPase
inhibitor DCCD has no effect on root respiration as measured
by the O2 consumption of the roots (Fig. 2). Even after 3 h and
with the highest chosen DCCD concentration (50 μM), no inhi-
bition of oxygen consumption was detectable (results not shown).

DISCUSSION

Proton extrusion along individual roots of intact plants is well
documented in relation to root extension growth (18, 28) and
genetropism (15). In both instances the proton extrusion is con-
fined to the subapical root zones where extension growth takes
place. In this paper, in roots of intact plants, three other types
of proton extrusion are described which differ in both localization
and capacity. From the sensitivity to inhibitors (Table I; Fig. 2)
it seems justified to characterize them as proton pumps driven
by plasma membrane-bound ATPases.

Enhancement of proton extrusion induced by preferential
cation uptake is a well known phenomenon, especially when
ammonium nitrogen is supplied (20) or in legumes relying on
nitrogen fixation (13). This cation-induced proton extrusion is
more or less uniformly distributed along the whole root system
(Fig. 1) as is to be expected in roots of rapidly growing young
plants with unlimited supply of nutrients at the root surface. In
solid substrate such as soils, however, exhaustion zones of nutri-
tents along growing roots can lead to different rates of proton
extrusion (acidification) along the roots (13).
extrusion of roots is been performed using edge this is the first report on the localization of the FC effect in plants (E). The purple. solution nutrient complete nutrient complete nutrient iron-adequate plants in (NH₄)₂SO₄ solution (0.5 mM) +1.8 ± 0.5 -0.9 ± 0.2 -0.8 ± 0.3 Complete nutrient solution +10 mM FC +2.0 ± 0.5 -0.8 ± 0.1 -0.8 ± 0.2 Complete nutrient solution +10 mM FC +0.1 ± 0.2 ND ND ND +0.1 ± 0.2 ND ND ND +3.4 ± 1.2 -0.6 ± 0.2 -0.6 ± 0.2 +3.4 ± 1.2 -0.6 ± 0.2 -0.6 ± 0.2 Each value represents the mean of six replicates ± SD.

Fe-deficient plants

Complete nutrient solution (except Fe) +5.6 ± 1.3 -0.3 ± 0.1 -0.3 ± 0.1

* Not determined.

Enhanced proton extrusion in iron-deficient roots is, on the other hand, confined to the apical root zones. This has certain similarities with the enhanced proton extrusion in the zones of proteoid root formation in Lupinus albus (5). Enhanced proton extrusion has also been described in rape (Brassica napus) in response to phosphorus deficiency (8). However, the exact localization of the proton extrusion in rape roots is not yet known.

With one exception (19), all studies on the effect of FC have been performed using isolated plant tissue, e.g. hypocotyl or root segments (11). From these results, FC-induced proton extrusion was expected to occur in the apical root zones. But, as has been shown (Fig. 1D), using intact plants, the FC-induced proton extrusion of roots is restricted to the basal zone. To our knowledge this is the first report on the localization of the FC effect in roots of intact plants.

Besides localization of the proton efflux pumps, their capacity for proton extrusion is of particular interest. Reports for intact plants are rare in this respect. Rates of proton extrusion in relation to preferential cation uptake between 1.8 and 3.6 𝜇mol H⁺/g fresh weight·h (Table I) are in good agreement with rates reported in literature varying between less than 1.0 𝜇mol H⁺ (14) and 4.0 𝜇mol H⁺ (6, 19). In iron-deficiency roots the rates of proton extrusion are even higher (Table I). Considering the fact that the iron deficiency-induced enhanced proton efflux is confined to the apical root zones (Fig. 1), rates of about 28 𝜇mol H⁺/g fresh weight·h can be calculated for these root zones. These are the highest values reported so far in roots of intact plants. The exceptionally high rate of proton extrusion in these root zones is correlated with the formation of rhizodermal transfer cells (9, 21). The enormous enlargement of the surface area of the plasma membrane at the external surface of rhizodermal transfer cells is most probably the prerequisite for the high rates of proton extrusion in these root zones (21). If proton efflux
rates are calculated for only the rhizodermal cells, values between 80 and 150 μmol H⁺/g fresh weight·h are obtained. This correlation of the formation of rhizodermal transfer cells with the high rate of proton extrusion is therefore a good example of close relationship between structure and function.

Proton efflux pumps in the plasma membrane of root cells are also of ecological importance. Root-induced acidification of the rhizosphere is an important mechanism for mobilization of mineral nutrients from sparingly soluble sources such as phosphate from rock phosphates (20) or iron from inorganic Fe³⁺ compounds (5). The extent of acidification and the corresponding nutrient mobilization depend upon the pH buffering capacity of soils and rate of proton extrusion by the roots. If at a given rate this proton extrusion is not uniformly distributed over the whole root system but confined to certain root zones, the efficiency can be expected to be much higher in terms of nutrient mobilization. Consequently, strong acidification of a small volume of soil in the rhizosphere should be possible even in soils with high pH buffering capacity. This type of local acidification is a typical property of plant species with proteoid roots such as Lupinus albus (5) and an inducible mechanism in roots of dicots in response to iron deficiency (12).

The mechanism of the induction of the proton efflux in response to iron deficiency is still not understood (22). The role of the shoot in the activity of the proton efflux pump induced by iron deficiency will be the subject matter of a following paper.

Acknowledgments.—Fusicoccin was kindly provided by Prof. M. T. Marré, Institute of Plant Sciences, University of Milano, Italy and the sunflower seeds by Saatentandel Hahn u. Karl GmbH, Frankfurt am Main.

LITERATURE CITED

3. DE MICHELS MI, MC PUGLIARELLO, F RASI-CALDONGO 1983 Two distinct proton translocating ATPases are present in membrane vesicles from radish seedlings. FEBS Lett 162: 85-90
21. RÖMHELD V, D KRAMER 1983 Relationship between proton efflux and rhizodermal transfer cells induced by iron deficiency. Z Pflanzenphysiol 113: 73-83