Plant Physiol. (1988) 86, 1038–1043
0032-0889/88/86/1038/06/$01.00/0

Hormone Action on Transmembrane Electron and H^+ Transport[1]

Received for publication July 8, 1987 and in revised form December 2, 1987

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ABSTRACT

A possible involvement of two different systems in proton translocation was investigated by simultaneous measurement of transmembrane electron flow and proton secretion in a pH-stat combined with a redoxstat. The pH gradient between cytoplasm and apoplast is probably maintained by an H^+-pumping ATPase and by a second proton extrusion system, which seems to be linked to a redox chain with NAD(P)H as electron donor. Indole acetic acid inhibits both e^- and H^+ efflux, but only if the 'electron draw' from the outside is not too high. The electron draw depends on the hexacyanoferrate level at the plasmalemma surface and on the Ca^{2+} concentration. The inhibiting effect of auxin on e^- and H^+ efflux in the presence of hexacyanoferrate can be only detected at low levels of bivalent cations and of the artificial electron acceptor. The inhibition of e^- and H^+ efflux by auxin requires high oxygen levels. The influence of auxin on both e^- and H^+ transfer disappears below 2 kilopascals O_2, a level which does not influence respiration. Ethanol and fusicoccin do not increase the e^- flux, probably because the electron transfer from the plasma membrane to HCF III is the limiting step. If electron transfer is reduced by IAA pretreatment, ethanol increases e^- flux. Fusicoccin decreases e^- and increases H^+ efflux if the rates have been lowered previously by indole acetic acid pretreatment. This effect depends on high oxygen levels and is reversible by lowering oxygen pressure. Auxin and Ca^{2+} change e^- flow and H^+ ejection in a 1:1 ratio.

The uptake of ions, sugars, and amino acids depends on the generation of an electrochemical hydrogen ion gradient across the plasmalemma. Such a gradient between cytoplasm and apoplast is possibly maintained by an electrogenic H^+-pumping ATPase and by a second proton secreting system, which seems to be linked to a redox chain with NAD(P)H as electron donor. Evidence for redox-chain-linked proton secretion derives from the following results: (a) Artificial nonpenetrating electron acceptors such as HCF III[2] or HCI IV increase H^+ efflux (5, 15, 23); (b) H^+ extrusion depends on a much higher oxygen level (k_m = 3.2 kPa O_2) (6) than is needed for ATP generation (K_m = 0.008 – 0.08 kPa O_2) (19). This serves as a strong argument as the application of an artificial trigger is avoided. The application of a trigger may induce an artificial pathway. Strong oxidants like HCF III or HCI IV will very likely impair the functions of a variety of plasmalemma proteins, including channels, cotransporters, pumps, and auxin-receptors by oxidizing sulfhydryl groups of the proteins (6). In other words, a number of important transport functions may be impaired which would alter electron and/or proton movement. (c) Alcohols can be used to increase the level of intracellular NADH through the action of the cytoplasmic alcohol dehydrogenase (6, 10, 11). Alcohols, known to be substrates for alcohol dehydrogenase such as propan-1-ol, ethanol, and butan-1-ol increase net H^+ efflux immediately; propan-2-ol and methanol have no effect. This stereoselectivity and the requirement of high O_2 supply for alcohol action exclude an explanation as a membrane effect. Exogenous NADH was found to increase HCF III reduction of protoplasts (21) and of intact roots (29). Komor *et al.* (20) have demonstrated that reduction of externally applied NAD(P)H with concomitant acidificaton in the apoplast is without involvement of transmembrane steps. An independence of transplasma membrane proton gradient from NAD(P)H-HCF III oxidoreduction in maize root microsoms was described (24). We therefore avoided addition of NAD(P)H and tried to distinguish between H^+-pumping ATPase and redox-chain-linked proton extrusion by the use of different O_2 pressures as the ATP generating system and the plasmalemma NAD(P)H oxidase have very different K_m values for O_2.

The aim of the present paper is to investigate the influence of hormones and other effectors on the proton extrusion and its possible linkage to transmembrane electron transfer.

MATERIALS AND METHODS

Plant Material. Seeds of *Zea mays* L., cv Goldprinz, obtained from C. Sperling, Lüneburg, F.R.G., were soaked in running tap water for 2 h and then sterilized by treating with 10% perhydrol for 10 min. The seedlings were grown in moist vermiculite No. 4 in a climatic chamber in the dark at 26°C. Twenty intact seedlings, 3 d old, were carefully selected for uniformity and the seeds placed on the top of a tube with the roots dipping into the nutrient solution. The plants were adapted to hydroculture and constant pH in the nutrient medium for 12 h at an irradiance of 150 μmol m^{-2} s^{-1}.

Measurement of Net e^- and H^+ Efflux. Proton fluxes were measured by means of a computer assisted pH-stat, consisting of diluters (Microlab M. Hamilton, Bonaduz, Switzerland), AD Converters (type pH 530 D WTW, Weilheim, FRG), and processor (Apple Europlus, 48K). The concentration of HCF III was kept constant by continuous titration. The amount of titrator needed was registered and automatically calculated after measurement of $E_{415-480}$ in a flow-through cuvette by a photometer (Ultrospec II, LKB). N_2 and O_2 were mixed and kept constant by self constructed electronic valve system at a chosen partial pressure which was controlled by a Clark-type gold electrode (type OXI 521, WTW, Weilheim). The gas mixture flowed (6

[1] Substantial help from Deutsche Forschungsgemeinschaft is gratefully acknowledged.

[2] Abbreviations: HCF III, hexacyanoferrate; HCI IV, hexachloroiridate IV (HCl and HCF are the abbreviations of the correct IUPAC nomenclature of complex compounds, the expression ferricyanide is often used, but wrong); DES, diethylstilbestrol; DCCD, *N,N*-dicyclohexylcarbodiimide; FC, fusicoccin.

ml s^{-1}) through the cuvette with the plant material and 25 ml of incubation medium. The bathing solution was kept at pH 5.5 and contained if not otherwise indicated 1 mM $CaCl_2$, 10 mM KCl, and 0.5 mM HCF III. The setup of the pH-redoxstat is shown in Figure 1.

RESULTS AND DISCUSSION

Influence of Oxygen. Adequate aeration is necessary (14) to obtain proper measurements of the auxin-induced pH-drop. Not only the auxin-induced acidification but also the basic proton secretion rate strongly depended on oxygen if HCF III was not present (6, 7). The apparent K_m for proton secretion of coleoptiles has been calculated to be 3.2 kPa O_2. As Figure 2 depicts, H^+ efflux of roots required high oxygen levels if HCF III is not present (Fig. 2, squares). Reduction of O_2 level below 5 kPa led to a strong inhibition in the rate of net H^+ efflux. The rate of H^+ efflux decreased as O_2 pressure declined. The inhibition at a relatively high O_2 level cannot be explained by a lack of ATP, because this O_2 pressure is much larger than the K_m of the ATP generating system (0.008 kPa) (1, 19). Moreover, a lack of ATP as a reason for the decline of net H^+ efflux does not seem likely because the rate of respiration was not changed at an O_2 level higher than 0.5 kPa (7). For roots, the apparent K_m of H^+ efflux for O_2 was calculated to be 3.1 kPa O_2, indicating that there are similar O_2 sensitive proton ejecting systems in coleoptiles and roots (6). Proton extrusion at low O_2 levels is probably not due to efflux of organic acid produced during anaerobiosis as described in the literature (17) because CO_2 production remained unchanged (7) at O_2 levels used in our experiments.

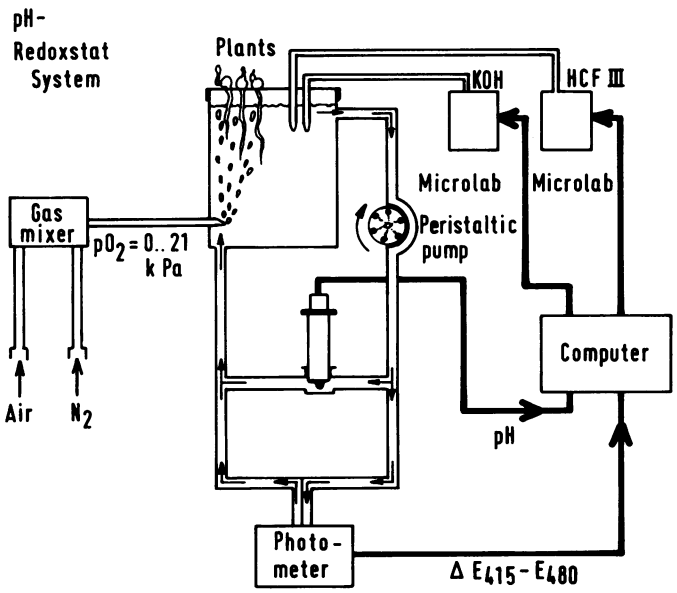

FIG. 1. Setup for continuous and simultaneous measurement of transmembrane H^+ and e^- flow at constant pH and constant concentration of the redox indicator ("pH-redoxstat").

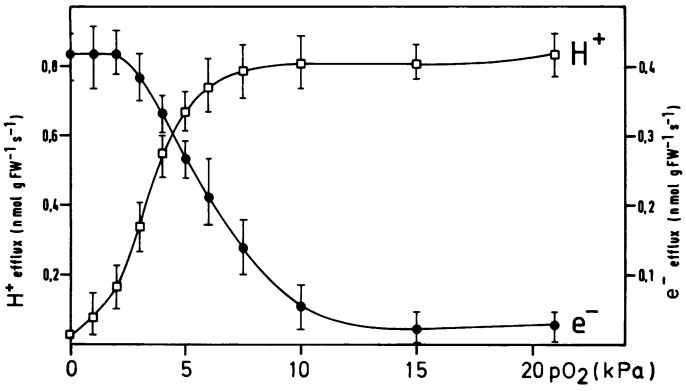

FIG. 2. Dependence of H^+ and e^- flow on oxygen level. Note that there was no further increase of e^- flux at pO_2 lower than 1.5 kPa while the rate of H^+ extrusion still declined. The decrease in the rate of H^+ extrusion appeared already at relatively high O_2 level without respiration was influenced (7). H^+ flux was measured at pH 5.5 without HCF III and e^- flow simultaneously at the same pH in the presence of 0.1 mM HCF III. Vertical bars represent twice a SE.

While H^+ efflux (without HCF III) decreased below 5 kPa O_2 (Fig. 2, squares) a concomitant increase in e^- flux could be observed in the presence of HCF III (Fig. 2, circles). The stimulation of net transmembrane electron flow was reversible at any stage of pO_2. We assume competition between HCF III and O_2 as electron acceptor. Changes of O_2 pressure above 6 kPa did not lead to any change in the rate of HCF III reduction, probably because the O_2 reducing system was saturated with O_2 and transmembrane e^- flow served predominantly for O_2 instead for HCF III reduction. No further increase in e^- flow could be observed below 1.25 kPa O_2. At an oxygen concentration much below the K_m of the NAD(P)H oxidizing system the electrons might be completely transferred to HCF III. This flow is limited by the concentration of the e^- acceptor at the plasmalemma.

The inhibition of H^+ efflux by reduced oxygen pressure disappeared with increasing concentrations of HCF III (Fig. 3). Roots secreted H^+ independent of O_2 level if 5 mM HCF III and 1 mM Ca^{2+} were present in the nutrient solution. But it must be noted that HCF III replaces O_2 only as electron acceptor in one step of H^+ extrusion. Other events, *e.g.* the induction of proton secretion by FC or inhibition by auxin might require O_2 which is then irreplaceable by an artificial electron acceptor such as HCF III.

Figure 4 illustrates the dynamics of the change in e^- and H^+ flow in a typical experiment at low HCF III concentration. Less than 1 mM HCF III has to be used; otherwise, no depression in H^+ extrusion nor increase in e^- flow could be observed. H^+ secretion was at least partially restored after about 2 h (data not shown). This recovery and a following overcompensation is typical and often seen after inhibitor treatments (8). The rates of reduction and H^+ secretion change with time and depend thus on the length of the experiment. Thus, results obtained with 'non-on-line' methods differ from ours due to this compensation

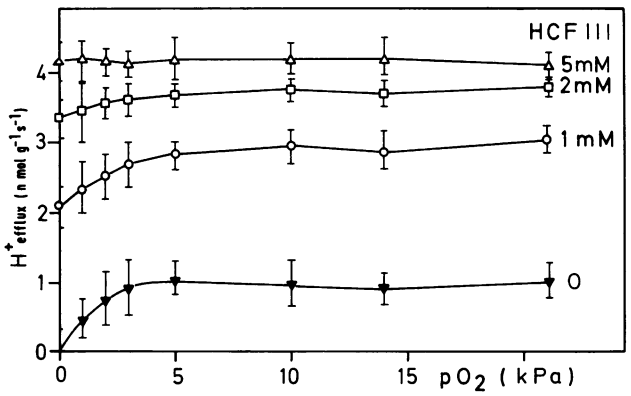

FIG. 3. Increasing the HCF III concentration made proton secretion more independent on oxygen level. H^+ efflux was completely independent on oxygen level at 5 mM (△) of the e^- acceptor. The bathing solution contained HCF III at the concentrations indicated at the right. Bars indicate twice a SE.

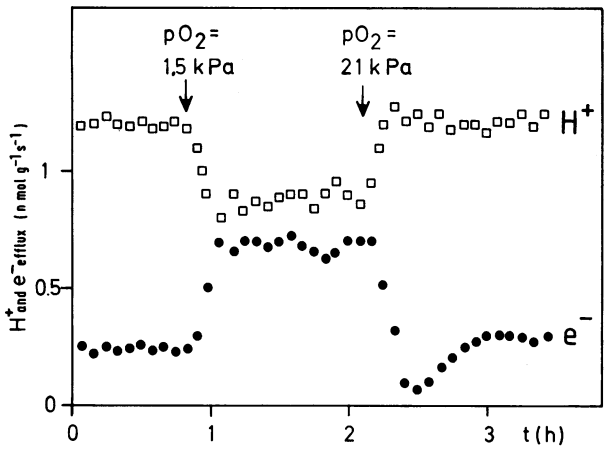

FIG. 4. Reducton of O_2 pressure led to a rapid increase in e^- and decrease in H^+ efflux if a low concentration of the e^- acceptor was present (0.1 mM HCF III, pH 5.5). Respiration was not changed at 1.5 kPa O_2. The increase of e^- flow is explained by a replacement of oxygen by HCF III as electron acceptor. See Figure 11.

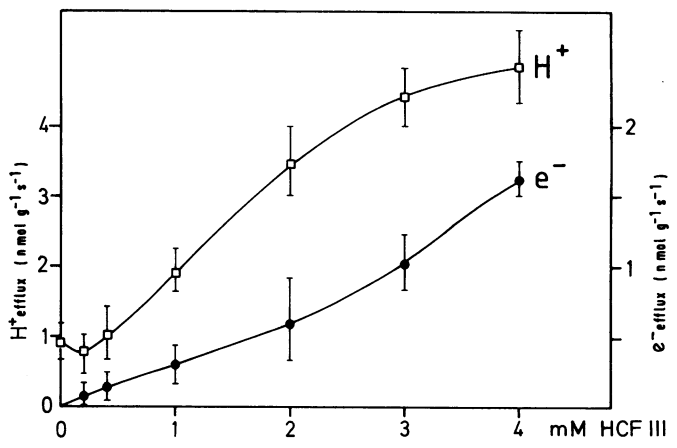

FIG. 5. Dependence of net H^+ at pH 5.5 and e^- flow on HCF III concentration. While the H^+ extrusion system approached a maximum rate, e^- flux increased still with HCF III concentration. Note that most experiments were performed at non-saturating HCF III level. Bars represent twice a SE.

as we are using a system with high time resolution. If O_2 was supplied before restoration started, the response in e^- and H^+ flow to lower O_2 level could be reversed (Fig. 4). The stimulation of net HCF III reduction at low O_2 level is surprising because the electron transfer at the plasmalemma was thought to be the limiting step (10, 26, 29). The access of HCF III anion to the plasma membrane is reduced due to its negative surface charge and the negative membrane potential. Cations such as calcium are able to screen the charge on the plasma membrane surface and therefore increase HCF III reduction. The stimulation might be explained by a decrease of HCF II oxidation, because HCF II oxidation activity has been observed in carrot cell suspension HCF II oxidation activity has been observed in carrot cell suspension cultures (10) and in root segments of maize (28). The dominant reductase activity is localized at the apex and the oxidation of HCF II seems to occur mainly at the basal region of the root (28). One biological function of the Fe-oxidation is to reduce uptake of Fe II by immobilization of iron in the cell wall by conversion into Fe III under anaerobic conditions (4, 16). HCF II (1 mM) reduces proton secretion (6), but the amount of HCF II produced by the plant prior to the reduction of O_2 level was at about 1200 μM and increased at a rate of 72 μM/h under these conditions. The net HCF III reduction rate therefore should decrease with time, depending on the reaction kinetics of the oxidase. Such a behavior could not be observed either because the amount of HCF II produced by the roots was too low or the oxidase activity was saturated even at low HCF II concentrations.

Dependence of H^+ and e^- Transfer on HCF III Concentration. The induction of H^+ extrusion in intact maize roots by HCF III was investigated over the range from 1 μM to 5 mM HCF III. An increase of H^+ secretion could be observed at concentrations greater than 0.2 mM (Fig. 5) with an asymptotic approach to some value, which was not reached at 5 mM. Concentrations lower than 0.2 mM were without influence or inhibited proton secretion. While HCF III reduction increased linearly with HCF III concentration, stimulation of H^+ efflux could be only observed at concentrations higher than 0.2 mM HCF III. It is possible that stimulation of H^+ efflux was superimposed by an inhibition of an another system, which is overcome by stimulation only at higher HCF III concentration. It might be that HCF III reduces the activity of one proton pump (ATPase?) but stimulates another one (redox driven proton pump?).

The dependence of HCF III reduction on HCF III concentration was linear up to 1 mM. The K_m was calculated to be 2.98 mM and V_{max} 2.07 nmol-g fresh weight^{-1} s^{-1}. The average velocity was 0.2 nmol-g fresh weight^{-1} s^{-1} at 0.25 mM. In contrast, Qiu *et al.* (28) found saturation of the reduction rate at 0.3 mM in maize root segments using a cation concentration 3 times lower than in our experiments; and in the presence of high concentration of cations, a saturation was observed at 0.1 mM HCF III in iron deficient roots. The difference might be explained by iron deficiency or by the influence of cut surfaces.

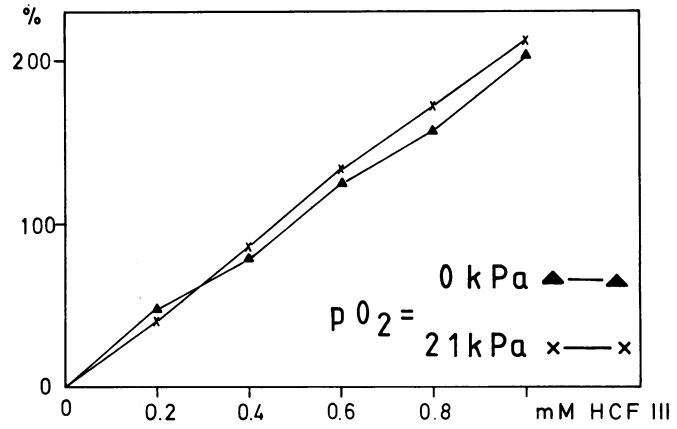

FIG. 6. Relative dependence of net e^- flow on HCF III concentration under pO_2 = 0 kPa (▲) and full aeration (21 kPa [x]). The calculation is based on the e^- flow at 0.5 mM = 100%.

A comparison of e^- flow under O_2 and N_2 showed an identical type of dependence on HCF III concentration. This gives evidence for one, non-O_2 dependent e^- transfer in the presence of HCF III. O_2 pressure influences energy supply and a different behavior of O_2 and N_2 treated plants is expected if energy supply in form of NAD(P)H or ATP limits redox-chain-linked H^+ extrusion.

The Influence of Calcium. It is already known that calcium stimulates e^- and H^+ efflux and that the removal of divalent ions from the medium by chelators such as EGTA results in inhibition of acidification (3, 15). By the use of antagonists it was concluded that the two systems could be regulated by the Ca^{2+} calmodulin complex (2). Application of Ca^{2+} led indeed to a large increase in both e^- and H^+ efflux (Fig. 7) without any delay.

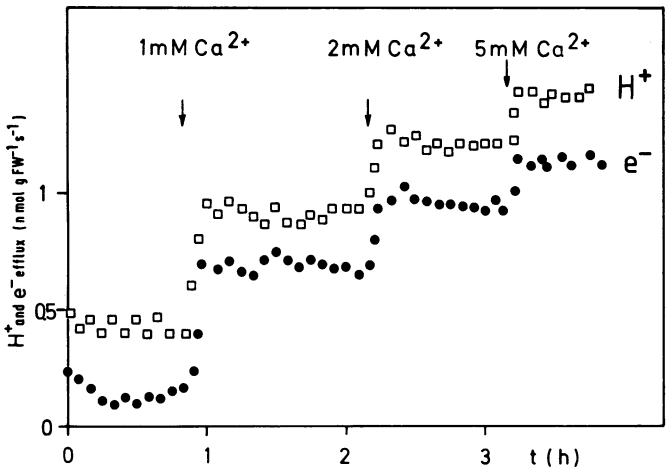

FIG. 7. Influence of Ca^{2+} on transmembrane e^- and H^+ flux. Addition of Ca^{2+} leads to a sudden and long-term increase of both fluxes in a 1:1 ratio. The pH was kept constant at 5.0 and HCF III concentration at 0.25 mM.

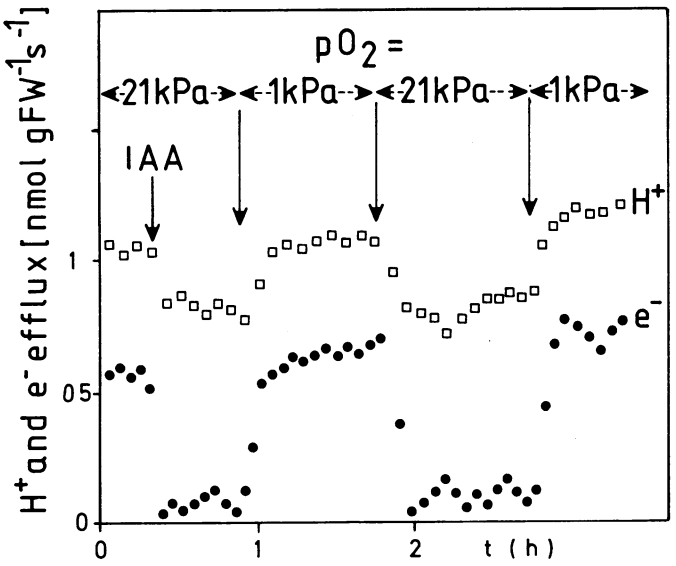

FIG. 8. Simultaneous inhibition of e^- and H^+ flux (at 1 mM HCF III and pH 5.0) by IAA and the reversion by lowering pO_2. The reaction to auxin could be switched out by change of pO_2 from 21 to 1 kPa. The inhibiting effect of auxin reappeared by increase of pO_2 to 21 kPa. Both net fluxes could be reduced to zero by 10 μM IAA if 0.5 mM HCF III without bivalent cations were used.

The maximum stimulation of both fluxes was not reached at 5 mM Ca^{2+} and it is difficult to imagine that calmodulin (2), regulated by calcium at micromolar levels in the cytoplasm would still be stimulated by such high concentrations. Moreover, as the ratio between e^- and H^+ stimulation remains 1:1 over the whole concentration range, we suppose that Ca^{2+} acts via better access of HCF III to the plasma membrane.

Influence of Auxin. Auxin-stimulated proton secretion in corn coleoptiles strongly depended on good aeration of the solution (14). This might be due to some O_2 sensitive steps involved in the export mechanism or in the cytoplasmic H^+ generation (6, 7). In roots auxin inhibits proton secretion as has been already described several times (14, 25, 27), but a pO_2-dependence has not been studied carefully. Figure 8 shows that the inhibitory effect of auxin on proton secretion required a high O_2 level. The inhibition by auxin disappeared at an O_2 pressure of 4 kPa (data not shown). At this level, the Cyt *c* oxidase (K_m = 8 × 10^{-3} to 8 × 10^{-2} kPa) should not be influenced (19) and respiration not be changed (7). The inhibition by IAA could be switched off and on by changing pO_2 several times (at least six times).

The effect of low O_2 level on IAA action cannot be explained by an O_2 requirement of the export or generation mechanism of protons as the auxin lowered H^+ extrusion and this inhibition disappeared at low pO_2.

If HCF III was present at low concentrations (less than 0.5 mM), IAA inhibited proton secretion as well as electron transfer. The inhibition of HCF III reduction by IAA was also reversed by moderately low pO_2 and could be switched on and off like proton secretion. An inhibition of HCF III reduction of 20% was observed after auxin treatment (20 μM 2,4-D) in carrot suspension cells (12), but not yet confirmed. The reason might be that IAA does not inhibit at any Ca^{2+} and HCF III concentration.

Table I clearly reveals that HCF III reduction is inhibited only at low HCF III level. If the actual concentration of HCF III at the plasmalemma is too high IAA is not able to switch electron and proton flows in a short cut to an oxidase at the inner side of the membrane (Fig. 11). High concentrations of HCF III may be achieved by high molarity or by addition of Ca^{2+}, which leads to a better access of HCF III to the plasma membrane surface by its screening property. An influence of 2,4-D on e^- flow has already been reported (12). The inhibition of HCF III reduction by 2,4-D in carrot cells was found to be only 20% (12), but the HCF III concentration was 0.5 mM and 10 mM Ca^{2+}. Under these conditions, no inhibition of either H^+ or e^- flow could be observed in our system (Table I).

FC and Ethanol. Addition of ethanol induced a rapid and long-termed increase in net H^+ efflux. However, there was no change in transmembrane e^- transport (Fig. 9A). It has to be remembered that a stimulation of HCF III reduction can only be expected if neither the electron transfer at the plasma membrane nor the diffusion of HCF III through the cell wall represent rate-limiting steps. Only at HCF III levels far higher than the K_m of the reductase, alcohols and FC should increase the reduction rate. Unfortunately, these high HCF III concentration will yield extinctions too high for proper photometry in our system. However, a stimulation of HCF III reduction by alcohol and FC has been detected in carrot cell suspension cultures (10, 12). The increase in NADH level via ethanol may lead to a higher reduction of O_2 because of its higher redox potential compared to HCF III. A decrease of pO_2 should eliminate this competition. Since NADH formation by ethanol does not depend on O_2, a treatment with ethanol should increase HCF III reduction. But ethanol had no effect either on proton secretion or on HCF III reduction rate at low pO_2 (data not shown). The lack of reaction at low pO_2 was evidence that the ethanol influenced H^+ efflux was not due to a leakage of the membrane. It remains to be determined which process in proton secretion depends on high pO_2. The effects of FC resembled those induced by the alcohols.

Table I. *Inhibition of Net Transmembrane e^- Flux by IAA as a Function of Different HCF III—and Calcium—Concentration*

HCF III	0	0.5	1	5 (mM) Ca^{2+}
mM		%		
0.25	94 ± 5[a]	102 ± 7	74 ± 8	27 ± 4
0.5	98 ± 3	85 ± 6	23 ± 5	NS[b]
1	70 ± 6	43 ± 4	12 ± 4	NS
5	10 ± 3	NS	NS	NS

[a] Expressed in percentages relative to inhibition by 10 μM IAA at 0.1 mM HCF III = 100% and SE. [b] Not significant.

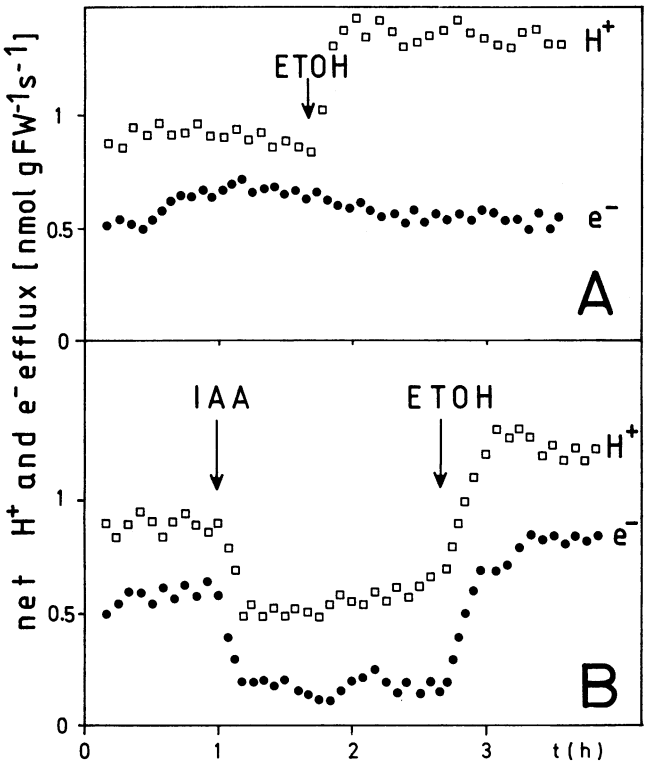

FIG. 9. A, Addition of ethanol (ETOH) induced a rapid increase in net H^+ efflux of roots, but no change of e^- transfer could be observed; B, pretreatment with auxin reduced H^+ and e^- flow in a 1:1 ratio and a subsequent application of ethanol led to a rapid increase in net H^+ efflux and in transmembrane e^- transport. The ethanol treatment affected both parameters in a 1:1 ratio, too. HCF III at a concentration of 0.5 mM and the pH was 5.5.

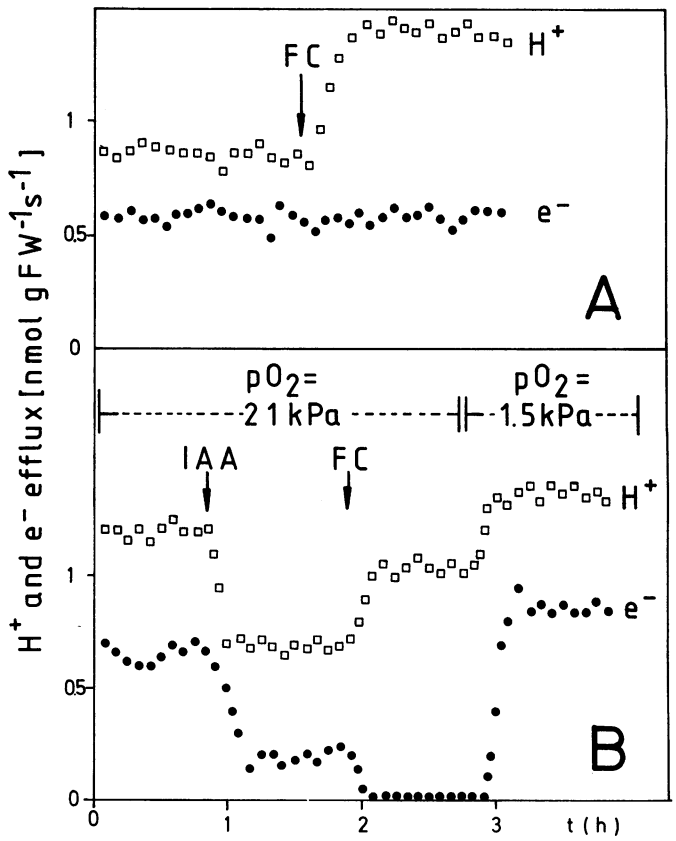

FIG. 10. A, C increased H^+ efflux without any change of transmembrane e^- flow; B, if roots were pretreated with IAA, FC leads to uncoupling of H^+ and e^- flow. The response was pO_2 dependent even in the presence of HCF III as it is true for the effect of other effectors.

Like ethanol, FC increased proton secretion by about 100% and more without influencing the rate of HCF III reduction (Fig. 10A). Like ethanol-induced proton efflux, FC triggered acidification was prevented by a low O_2 concentration.

Auxin Pretreatment. A method for preventing the electron transfer from becoming rate-limiting is to reduce the transmembrane e^- flow by auxin treatment. For inhibition of HCF III reduction, we have a powerful tool: IAA. As Figure 9B depicts, ethanol increased both HCF III reduction and H^+ efflux, if the roots were pretreated with auxin. This again suggests that ethanol does not work by a change of membrane fluidity and that the electron transfer at the plasmalemma can limit HCF III reduction. The treatment with ethanol affects both parameters in a 1:1 ratio.

A similar result should be predicted if FC is applied after auxin pretreatment. Probably due to an interaction between both effectors, FC enhanced the inhibitory effect of auxin on HCF III reduction but stimulated proton secretion. The effect of FC could be suppressed by reducing pO_2 and switched on again by increasing pO_2. It is difficult to explain this kind of interaction between IAA and FC. It might be that FC lowered the K_m of the electron transfer for O_2, but this happened only after pretreatment with IAA. It has to be emphasized that O_2 as an electron acceptor can only be replaced to some extent by a nonpenetrating electron acceptor. H^+ secretion was independent of O_2 level if the presence of high amounts of HCF III allowed transmembrane electron flow, but this was not true for auxin, FC, and ethanol effects. We have no evidence which induction step is dependent on O_2 and cannot be replaced by artificial acceptors.

The Stoichiometry of H^+: e^- Fluxes. The calculation of a ratio is rather difficult because the rate of e^- flux across the plasma membrane depends on O_2 level and HCF III concentration. Electrons might reduce intracellular O_2 and HCF III might oxidize compounds in the cell wall or plasma membrane exposed to the outside without proton extrusion. Wall-bound peroxidase could mediate the corn root reaction as well. To reduce the first mentioned side effects, we compared the fluxes under N_2 by increasing HCF III concentration. The stoichiometric ratio at pO_2 = 1.2 kPa was 1:2.3 (H^+:e^-), if H^+ secretion rate was compared before and after addition of HCF III. High N_2 level was used for the calculation to avoid HCF II oxidation and competition between HCF III and O_2. The results are similar to those observed by Federico *et al.* (15)(1:3.3) and Sijmons and Bienfait (30) (1:2). If pO_2 is changed at constant HCF III concentration the ratio was (1:1.6) indicating a competition between O_2 and HCF III as electron acceptors (Fig. 11). Because of the difficulties calculating a ratio, the changes in H^+ and e^- flow were compared after IAA treatment and after the reversion by moderate low O_2 level. Under these circumstances a ratio of 1:1 was obtained. This ratio also was obtained by experiments with Ca^{2+}. These results are much more reliable than the former ones, because side effects on HCF II oxidation and reduction of HCF III without linkage to proton extrusion are avoided.

These results demonstrate that the extrusion of protons and electrons by plant roots is a very complex phenomenon. It is difficult to present a coherent overall model but a functional diagram of a hypothetical proton-redox pump is proposed in Figure 11. We believe that NAD(P)H is oxidized at the cytosolic side of the plasma membrane. Protons and electrons are then

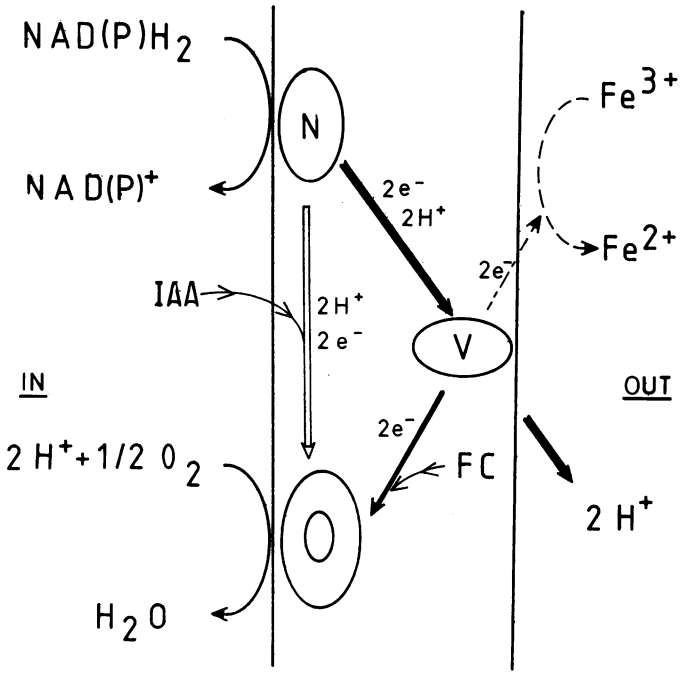

FIG. 11. Hypothetical model of redox-chain-linked proton secretion. N is an unidentified electron carrier and O an oxidase acting in the plasmalemma on the inner surface; V is an electron carrier and a switch exposed at the external surface of the plasmalemma. FC interaction is positioned between V and O, as FC did not increase secretion if oxygen was replaced by HCF III. Application of IAA leads to a short cut of e^- and H^+ between N and O.

transferred to a site at which HCF III can be reduced (V in Fig. 11). There is some evidence that HCF III might compete with oxygen for electrons (Figs. 2–4). If the redox-chain transfers not only electrons but also protons, the oxidase (O) has to be located at the cytosolic side of the membrane. Otherwise, protons excreted by the system would be consumed at the oxidase, so that the system would not cause any net proton efflux. The K_m for O_2 seems to be very high if no artificial electron acceptor is present (6). This might be taken as evidence for its participation in proton pumping.

Effectors like IAA and FC obviously change the mode of action of the proton-electron transfer system. The inhibition of electron and proton secretion by IAA is strongly oxygen-requiring (Fig. 8). One possible explanation is the postulation of a short circuit that transfers protons and electrons directly to the oxidase. This would prevent them from appearing at the apoplastic side.

The promotion of H^+ pumping by FC and the strange interactions between FC and IAA (Fig. 10) are far from being understood. In any case, FC-induced proton pumping is strongly oxygen-requiring, and therefore the oxidase postulated in Figure 11 could be involved.

Acknowledgment—The help of Susanne Krüger is gratefully acknowledged.

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