Plant Physiol. (1997) 114: 857–862

Changes in the Level and Activation State of the Plasma Membrane H^+-ATPase during Aging of Red Beet Slices[1]

Roberto Papini and Maria Ida De Michelis*

Istituto di Botanica, Università di Genova, Corso Dogali 1c, 16136 Genoa, Italy

The effect of aging on the plasma membrane (PM) H^+-ATPase of red beet (*Beta vulgaris* L.) parenchyma discs was analyzed in PM purified by aqueous two-phase partitioning. Aging increased both the activity and the amount of immunodetectable H^+-ATPase in the PM. The activity assayed at slightly alkaline pH values increased earlier and more strongly than that assayed at acidic pH values, so that the pH curve of the enzyme from aged beet discs was shifted toward more alkaline values. Aging decreased the stimulation of the PM H^+-ATPase activity by controlled trypsin treatments or by lysophosphatidylcholine. After trypsin treatment the pH dependence of H^+-ATPase from dormant or aged beet discs became equal. These results indicate that aging not only increases the level of H^+-ATPase in the PM, but also determines its activation, most likely by modifying the interaction between the autoinhibitory carboxyl-terminal domain and the catalytic site. When the PM H^+-ATPase activity was assayed at a slightly alkaline pH, the tyrosine modifier *N*-acetylimidazole inhibited the H^+-ATPase in the PM from dormant beet discs much less than in the PM from aged discs, suggesting that modification of a tyrosine residue may be involved in the activation of the PM H^+-ATPase induced by aging. The results are discussed with regard to aging-induced development of transmembrane transport activities.

It has long been known that slicing and incubation in aerated solutions of dormant storage tissue results in the progressive increase of various metabolic and transport activities (for review, see Poole, 1976). In red beet (*Beta vulgaris* L.) the increase of K^+ uptake during the first few days after slicing is paralleled by an increase of the membrane potential difference, both parameters being strongly influenced by the extracellular pH. Moreover, the uptake of K^+ correlates with the extrusion of protons. These observations led to the suggestion that K^+ uptake is driven by the activity of an electrogenic H^+ pump (Poole, 1974), before the demonstration that the H^+-ATPase is the principal electrogenic pump in the plasma membrane of higher plant cells (for review, see Serrano, 1989).

Recent work at the biochemical and molecular biological levels have shown that the activity of the PM H^+-ATPase can be regulated both through the control of the expression of different isoforms of the enzyme and through the modulation of their catalytic activity (for review, see Sussman, 1994). With regard to the latter point, it has been shown that the PM H^+-ATPase contains an autoinhibitory C-terminal domain, the interaction of which with the catalytic site can be hampered by the fungal toxin fusicoccin or by lysoPC. Displacement of the C-terminal domain by fusicoccin or lysoPC, or its removal by controlled proteolysis or by genetic deletion, produces an increase of catalytic activity and a shift of pH optimum for enzyme activity toward more alkaline pH values (Palmgren et al., 1991; Johansson et al., 1993; Rasi-Caldogno et al., 1993; Regenberg et al., 1995).

The activity of the PM H^+-ATPase of dormant red beet tissue has been characterized by several research groups (Briskin, 1990, and refs. therein), but the effect of aging on the amount and catalytic activity of the enzyme has not been investigated to date. In this work we show that aging of red beet parenchyma discs increases the level of the enzyme and its catalytic activity. The latter effect can be ascribed to modulation of the interaction of the autoinhibitory C-terminal domain with the catalytic site.

MATERIALS AND METHODS

Red beets (*Beta vulgaris* L.) were purchased at the local market and stored in moist sand in the cold.

Purification of the PM

Discs of parenchyma (8 mm in diameter, 1 mm in thickness) were cut from the storage roots of red beet and incubated for 0 to 96 h in the dark at 21 to 22°C in an aerated medium containing 0.1 mM $CaCl_2$.

Microsomes were extracted as previously described (Rasi-Caldogno et al., 1993) in an extraction medium containing 0.3 M Suc, 3 mM ATP, 3 mM benzamidine, 5 mM DTT, 25 mM Tris, 1 mM EDTA, 1 mM EGTA, 10% (v/v) glycerol, 0.5% (w/v) BSA, and 1 mM PMSF adjusted to pH 7.2 with Mes. Before homogenization, 50 mg of insoluble PVP per gram of tissue was added.

PM-enriched fractions were obtained by two-step aqueous two-phase partitioning in Dextran T500-PEG 3350 in the presence of 5 mM potassium phosphate buffer, pH 7.8, 6.2% (w/w) polymers, 5 mM KCl, 2.5% (v/v) glycerol, and 8.25% (w/w) Suc. The second upper phase was diluted at

[1] This work was supported by the Ministero per le Risorse Agricole, Alimentari e Forestali in the frame of the "Piano Nazionale per le Biotecnologie Vegetali" and by Consiglio Nazionale delle Ricerche, coordinated project "Membrane, Apoplasto e Omeostasi Cellulare." This paper is dedicated to the memory of Franca Rasi-Caldogno.

* Corresponding author; fax 39–10–209–9377.

Abbreviations: Brij 58, polyoxyethylene 20 cetyl ether; BTP, bis-Tris propane (1,3-bis[Tris(hydroxymethyl)methylamino]-propane); lysoPC, lysophosphatidylcholine; NAI, *N*-acetylimidazole; PM, plasma membrane.

least 5-fold with 10% glycerol, 0.1 mM EGTA, 3 mM DTT, 1 mM PMSF, 100 μg mL^{-1} Brij 58, and 1 mM NaOH-Mops, pH 7.0, and collected by centrifugation; the PM-enriched pellets were resuspended at about 1 mg protein mL^{-1}. PM proteins were assayed according to Markwell et al. (1978).

The purity of the PM fraction was tested by assaying the activities of vanadate-sensitive ATPase, NO_3^--sensitive ATPase, oligomycin-sensitive ATPase, and latent IDPase, as described by Olivari et al. (1993). The purified PM fraction contained about 20% of the PM and less than 1% of the endomembranes present in the microsomal fraction. The purification procedure increased the specific activity of the vanadate-sensitive ATPase by about 2-fold, and decreased that of the endomembrane markers by 10-fold (latent IDPase) to 60-fold (oligomycin-sensitive ATPase), so that the specific activity of the endomembrane markers in the purified PM fraction was at least 30-fold lower than that of the vanadate-sensitive ATPase. The purity of the PM fraction was unaffected by the length of aging (data not shown).

All of the experiments were performed on pooled discs that were aged for two or three different time periods before PM isolation. All of the experiments were repeated at least three times, each with three replicates, on different membrane preparations. Unless otherwise specified, reported results are from one representative experiment.

Assay of H^+-ATPase Activity

The activity of the PM H^+-ATPase was measured, unless otherwise specified, by incubating 2 to 5 μg mL^{-1} plasma membrane proteins for 30 to 90 min at 30°C in the presence of 0.2 mM EGTA, 5 mM $MgSO_4$, 50 mM KNO_3, 0.1 mM ammonium molybdate, 5 μM gramicidin, 100 μg mL^{-1} Brij 58, 3 mM ATP, and 40 mM buffer (BTP-Mes, BTP-Hepes adjusted to the specified pH values). The PM vesicles washed in the presence of Brij 58 and frozen and thawed before assay were predominantly inside-out (data not shown); however, Brij 58 was routinely included in the assay medium to avoid possible variations in the proportion of outside-out-oriented vesicles (Johansson et al., 1995). The activity measured in the presence of 100 μM vanadate (less than 5% total activity) was subtracted from that measured in its absence. Released Pi was determined as described by De Michelis and Spanswick (1986).

Trypsin Treatment of the PM

PM vesicles (about 0.5 mg mL^{-1}) were incubated for 8 min at 0°C in the presence of 50 μg trypsin mL^{-1}, as described by Rasi-Caldogno et al. (1993), and the reaction was blocked by the addition of an 80-fold excess of soybean trypsin inhibitor. Controls were treated with trypsin inhibitor either in the absence of or prior to the addition of trypsin, with equal results.

Treatment of the PM with NAI

PM vesicles (0.15–0.4 mg mL^{-1}) were incubated for 20 min at 20°C in the presence of 80 mM BTP-Hepes, pH 7.5, and the specified concentrations of NAI (obtained by diluting 25-fold; freshly prepared stock solutions in dimethylformamide); 4% (v/v) dimethylformamide was added to the controls (De Michelis et al., 1995, 1997). At the end of treatment PMs were diluted at least 10-fold in the assay medium for measurement of the H^+-ATPase activity. Under these conditions carryover of NAI in the assay is negligible, since NAI is very unstable in water (De Michelis et al., 1995, 1997).

Electrophoresis and Western Analysis

PM proteins were analyzed by SDS-PAGE using Bio-Rad ready gels (7.5% Tris-Gly gel with 4% stacking gel) on a Bio-Rad Mini Protean II cell, according to the method of Laemmli (1970). Before electrophoresis the PM was treated with a cocktail of protease inhibitors (100 μL of the PM suspension was supplemented with 14 μL of a solution containing 5 mg mL^{-1} *N*-α-*p*-tosyl-L-Lys, 1 mg mL^{-1} leupeptin, 20 mM benzamidine, and 25 μg mL^{-1} trypsin inhibitor), and proteins were solubilized for 1 h in 20 mM H_3PO_4, 4% (w/v) SDS, 3% (w/v) β-mercaptoethanol, 20% glycerol, and 1 mM EDTA, pH 2.4. The amount of PM proteins loaded on the gels was 1.5 to 10 μg per lane. Proteins resolved by SDS-PAGE were transferred onto a nitrocellulose membrane by electroblotting using a Bio-Rad Mini Trans Blot, in a Tris-Gly buffer containing 0.1% SDS and 20% methanol; transfer conditions were 250 mA for 75 min in a cold buffer. The blots were saturated in TBS containing 0.2% polyoxyethylenesorbitan monolaurate and 5% (w/v) defatted milk powder for 1 h; blots were washed in TBS and probed by incubating them for 2 h with an antiserum against the N-terminal domain of the PM H^+-ATPase (1:1000 dilution); blots were then washed in TBS and incubated for 1 h with an anti-rabbit IgG alkaline phosphatase conjugate secondary antibody in a 1:3000 dilution (A9919, Sigma). The immunocomplex was detected by adding a precipitating substrate of alkaline phosphatase activity (Fast tablets, Sigma).

Quantification of the immunodecorated bands was performed by computer-assisted densitometric analysis, using GF300 and GS365W software (Hoefer-Pharmacia, San Francisco, CA). The signal increased linearly with the increase of protein load up to at least 10 μg of protein per lane.

The antiserum against the N-terminal domain of the PM H^+-ATPase (a generous gift of Dr. C. Olivari, Università di Milano, Italy) was obtained by inoculating a rabbit with a synthetic peptide corresponding to a highly conserved sequence (amino acids 10–24) of isoform 2 of *Arabidopsis thaliana* PM H^+-ATPase (Harper et al., 1990) conjugated to ovalbumin. The antiserum was saturated overnight with 1% ovalbumin and partially purified by $(NH_4)_2SO_4$ fractionation; the fraction that precipitated between 33 and 50% $(NH_4)_2SO_4$ was suspended in TBS, divided into aliquots, and stored at −30°C.

RESULTS

Effect of Aging on the Concentration of PM H^+-ATPase

To investigate whether the concentration of H^+-ATPase in the PM of red beet parenchyma cells increases during aging in an aerated medium, PMs were purified from red

beet discs incubated for different periods of time in 0.1 mM aerated $CaCl_2$, and solubilized PM proteins were subjected to SDS-PAGE and western blotting with an antiserum against a highly conserved N-terminal sequence of the PM H^+-ATPase. Figure 1 shows that the antiserum specifically recognized a band of about 105 kD, both in the PM from freshly isolated discs and in the PM from discs aged for 48 or 96 h. Within each PM sample, the signal increased with the amount of PM loaded (1.5–6 μg protein per lane). For an equal protein load, the signal increased with the length of aging. The immunodecorated 105-kD band was quantified by densitometric analysis; concentration of immunodetectable PM H^+-ATPase nearly doubled during the first 48 h, and further increased after 96 h of aging. The increase of PM H^+-ATPase activity assayed at the pH optimum of the enzyme (pH 6.3; see Fig. 2) closely matched the increase of immunodetectable PM H^+-ATPase. Similar results were obtained on three independent sets of PMs and also when the blots were probed with an antiserum against the C-terminal domain of the PM H^+-ATPase (data not shown).

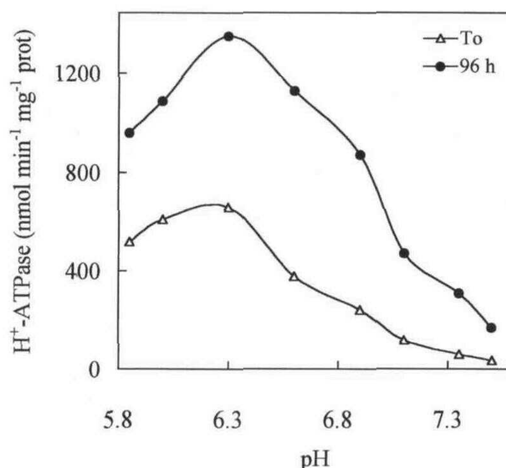

Figure 2. Dependence on the pH of the assay medium of the H^+-ATPase activity in PM isolated from beet discs immediately after cutting (To) or after aging for 96 h. The PM H^+-ATPase activity was assayed at the specified pH values, as described in "Materials and Methods."

Effect of Aging on the Dependence of the PM H^+-ATPase Activity on the pH of the Assay Medium

Figure 2 shows that aging of the discs not only increased the PM H^+-ATPase activity, but also modified its dependence on the pH of the external medium; a slightly acidic pH optimum (about pH 6.3) was evident both in the PM from freshly cut red beet discs and in the PM from discs aged for 96 h, but the activity in the PM from discs aged for 96 h decreased much less sharply than that of the freshly cut discs with the increase of pH of the assay medium toward values more representative of the cytosolic pH: at pH 7.0 the activity of 96-h PM was still one-half of that at pH 6.3, whereas that of the freshly cut PM was only about one-forth of that at pH 6.3.

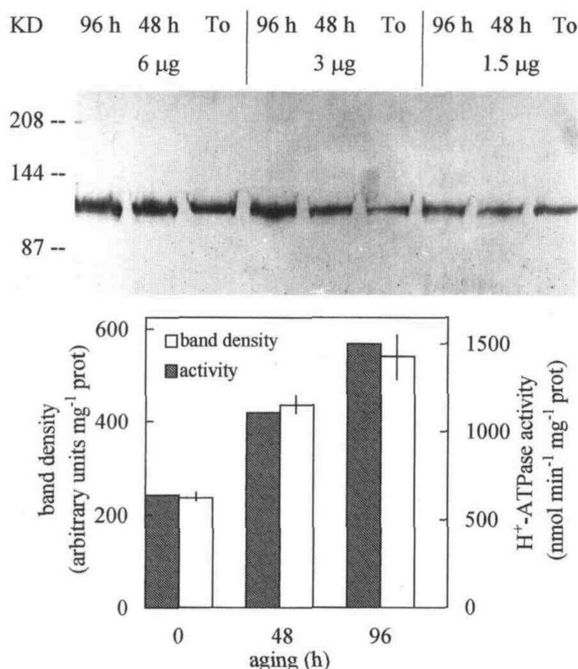

Figure 1. Effect of aging on the concentration of H^+-ATPase in the PM. SDS-PAGE, blotting immunodetection with an antiserum against the N-terminal domain of the PM H^+-ATPase, and quantification of the immunodecorated bands (open bars in the histogram) were performed as described in "Materials and Methods." Densitometry was performed on all of the lanes shown in the figure; bars represent SE. The PM H^+-ATPase activity (shaded bars) was assayed at pH 6.3.

The analysis of the PM H^+-ATPase activity assayed at different pH values, as a function of the length of aging (Fig. 3), shows that the activity assayed at slightly alkaline pH values (7.1 or 7.5) increased not only more steeply, but also much earlier than that measured around the pH optimum of the enzyme. When the ratio between the activity measured at pH 6.3 and that measured at pH 7.1 or 7.5 was calculated to eliminate the influence of the different concentration of enzyme in the PM, it was clear that the change in pH dependence of the enzyme activity was nearly complete during the first 24 h of red beet disc aging (Fig. 3B).

Effect of Trypsin Treatment and of LysoPC on the Activity of the H^+-ATPase in the PM from Freshly Cut or Aged Red Beet Discs

The change in pH dependence of the PM H^+-ATPase activity induced by aging of red beet discs resembles that determined in different plant materials by cleavage or displacement of the autoinhibitory C-terminal domain (Palmgren et al., 1991; Johansson et al., 1993; Rasi-Caldogno et al., 1993; Regenberg et al., 1995).

We compared the effect of controlled tryptic treatment on the activity of the PM H^+-ATPase in PM isolated from red beet discs immediately after cutting or after 96 h of aging. Preliminary experiments showed that for both PM fractions maximal activation was obtained by treatment with 50 to 100 μg of trypsin per milligram of protein (data not shown). Figure 4 shows that, in agreement with what has been observed in other plant materials, in both PM

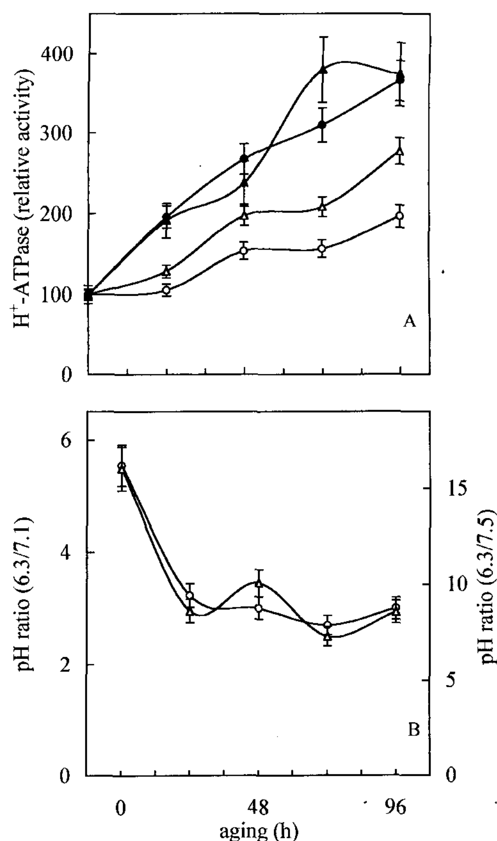

Figure 3. Effect of aging on the pH dependence of the PM H^+-ATPase activity. PMs were isolated after different aging periods and assayed for H^+-ATPase activity at the specified pH values (A). Values of H^+-ATPase activity (nmol min^{-1} mg^{-1} protein) immediately after cutting were 718 at pH 6.3 (open circles), 413 at pH 6.6 (open triangles), 131 at pH 7.1 (closed circles), and 46 at pH 7.5 (closed triangles). pH ratio in B indicates the ratio between activities measured at the indicated pH values. Data are the mean of three to five independent experiments, in each of which pooled beet discs were aged for two or three different periods of time before PM isolation. Bars represent SE.

fractions the stimulation of the H^+-ATPase activity by tryptic treatment of the PM strongly increased with the increase of the H^+-ATPase assay pH. The extent of stimulation of the PM H^+-ATPase following trypsin treatment was very different in the two PM fractions, with the H^+-ATPase in the freshly cut PM being much more stimulated than that in the 96-h PM. As a consequence, the ratio between the activity measured at pH 6.3 and those measured at pH 7.1 or 7.5, which was much higher in freshly cut PM than in the 96-h PM, became very similar in the two PM fractions after trypsin treatment.

Figure 5 shows that the stimulating effect of lysoPC on the PM H^+-ATPase activity assayed at an alkaline pH, which depends on lysoPC-induced displacement of the autoinhibitory C-terminal domain (Palmgren et al., 1991; Rasi-Caldogno et al., 1993; Regenberg et al., 1995; De Michelis et al., 1997), was much stronger in the freshly cut PM (up to 500% at pH 7.1) than in the 96-h PM (less than 150% at the same pH). In both PM fractions the extent of stimulation induced by optimal lysoPC concentrations (80–130 μg mL^{-1} under the conditions of the experiment) was similar to that induced by controlled proteolysis on the PM H^+-ATPase assayed at the same pH (compare Figs. 4 and 5).

Effect of NAI on the Activity of the H^+-ATPase in PM from Freshly Cut or Aged Red Beet Discs

NAI, which selectively reacts with the hydroxyl group of Tyr residues in proteins to form the *O*-acetyl derivative (Riordan and Vallee, 1972), has been shown to inhibit the plant PM H^+-ATPase activity (Ho and Briskin, 1992; De Michelis et al., 1995, 1997). The PM H^+-ATPase activity of freshly cut PM or 96-h PM pretreated with increasing concentrations of NAI is shown in Figure 6. When the PM H^+-ATPase was assayed at pH 6.3, the two PM fractions behaved similarly, 50% inhibition being determined by about 20 mM NAI, similar to what has already been observed in beet (Ho and Briskin, 1992) and radish (De Michelis et al., 1995, 1997). A completely different pattern was evident at pH 7.1. In the 96-h PM inhibition of the PM H^+-ATPase activity was similar to that observed at pH 6.3, as already observed in the PM from germinating radish seedlings (De Michelis et al., 1995, 1997). In contrast, in the

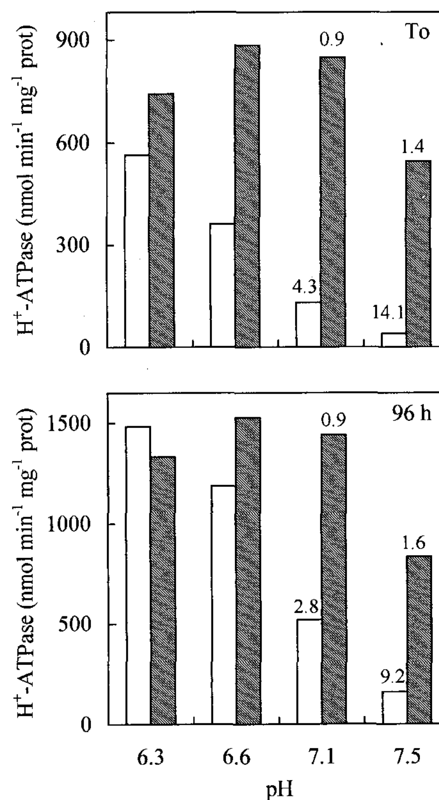

Figure 4. Effect of controlled proteolysis on the H^+-ATPase activity in PM isolated from beet discs immediately after cutting (To) or after aging for 96 h. PM were treated with (closed bars) or without (open bars) 100 μg of trypsin mg^{-1} protein as described in "Materials and Methods." H^+-ATPase activity was assayed at the indicated pH values. Values above the bars represent the ratio between the activity measured at pH 6.3 and that measured at the indicated pH.

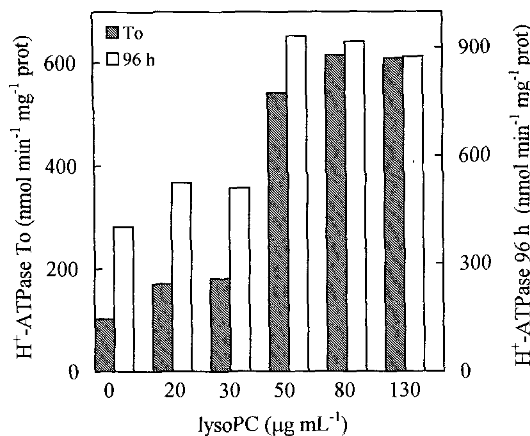

Figure 5. Effect of increasing concentrations of lysoPC on the H$^+$-ATPase activity in the PM isolated from beet discs immediately after cutting (To) or after aging for 96 h. H$^+$-ATPase was assayed at pH 7.1, in the presence of 10% (v/v) glycerol (De Michelis et al., 1996); Brij 58 was omitted in the plus-lysoPC samples.

freshly cut PM inhibition of the PM H$^+$-ATPase activity at pH 7.1 was much less than at pH 6.3, with 50% inhibition being reached only for NAI concentrations greater than 50 mM. As a consequence, in freshly cut PM the ratio between the H$^+$-ATPase activity assayed at pH 6.3 and that assayed at pH 7.1 (Fig. 6, inset) increased with the increase of NAI concentration and became similar to that of the 96-h PM, which was nearly unaffected by NAI.

DISCUSSION

The results reported in this paper show that aging of red beet parenchyma discs in an aerated medium determines, in parallel with the hyperpolarization of the transmembrane potential difference and with the increased ability to take up and accumulate ions, an increase of H$^+$-ATPase concentration in the PM. A similar increase occurs during aging of excised sugar beet leaves, where it has been shown to reflect aging-induced synthesis of PM H$^+$-ATPase protein (Noubhani et al., 1996).

It is interesting that, differently from what happens in sugar beet leaves (Noubhani et al., 1996), aging of red beet parenchyma discs also influences the catalytic activity of the enzyme, by strongly increasing the activity at the slightly alkaline pH values typical of the cytosol compared with the activity measured at the acidic pH optimum of the enzyme. As a consequence, the aging-induced increase of PM H$^+$-ATPase activity measured at an acidic pH closely correlates with the amount of the immunodetectable enzyme (see Fig. 1), whereas the activity measured at slightly alkaline pH values increases much more steeply. The change of pH dependence occurs much earlier than that of H$^+$-ATPase concentration in the PM, being virtually maximal after 24 h, whereas the amount of immunodetectable H$^+$-ATPase increases throughout the time tested, up to 96 h.

The PM H$^+$-ATPase from dormant beet discs is activated by controlled trypsin treatment or by lysoPC much more than that from aged discs. Notably, after trypsin treatment the pH dependencies of the PM H$^+$-ATPase from dormant and aged discs become very similar. The decreased sensitivity to activation by tryptic treatment or by lysoPC of the PM H$^+$-ATPase from the aged beet discs does not depend on cleavage of the autoinhibitory C-terminal domain, since the molecular mass of the enzyme is unaffected by aging (see Fig. 1), and the enzyme from aged beet discs is recognized by an antiserum against the C-terminal domain as well as that from dormant discs (data not shown). Rather, these results suggest that aging of the beet discs determines an early modification of the interaction between the C-terminal autoinhibitory domain and the catalytic site. To our knowledge, this is the first evidence for a role of such a regulatory interaction in the physiological modulation of the H$^+$-ATPase activity in higher plants.

In yeast it has been shown that carbon nutrition influences the activity of the PM H$^+$-ATPase by modulating the interaction of the C-terminal domain with the catalytic site, through a change of the phosphorylation state of one or a few Ser residues (Chang and Slayman, 1991). Both the mechanism through which the interaction between the C-terminal domain and the catalytic site of the PM H$^+$-ATPase of red beet parenchyma cells is modified by aging, and the signal(s) involved, wait to be elucidated. The observation that the change of pH dependence of the PM H$^+$-ATPase activity induced by aging can be mimicked by the Tyr modifier NAI suggests that the modification (phosphorylation?) of a Tyr residue in the PM H$^+$-ATPase itself, or in another PM protein, may be involved in the aging-induced activation of the PM H$^+$-ATPase. Work is currently in progress in our laboratory to verify this hypothesis.

Taking into account the increase of H$^+$-ATPase in the PM and the increase of its catalytic activity at cytosolic pH values, the rate of H$^+$ pumping in vivo can be estimated

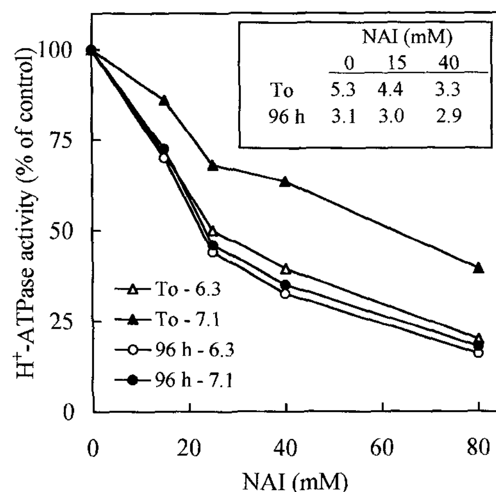

Figure 6. Effect of increasing concentrations of NAI on the H$^+$-ATPase activity in PM isolated from beet discs immediately after cutting (To) or after aging for 96 h. PMs were pretreated with the specified NAI concentrations as described in "Materials and Methods." The PM H$^+$-ATPase activity was assayed at pH 6.3 or at pH 7.1. Values of the H$^+$-ATPase activities of the controls (nmol min^{-1} mg^{-1} protein) at pH 6.3 and 7.1 were 766 and 143 for freshly cut PM and 1566 and 505 for 96-h PM. The values of the ratio between the H$^+$-ATPase activity at pH 6.3 and 7.1 are shown in the inset.

to increase about 4-fold during 96 h of aging. Such an increase is of the same order of that of the capability to take up K^+ (Poole, 1974). Also, the time course of the increase of the rate of H^+ pumping is similar to that reported for K^+ uptake or for the hyperpolarization of the transmembrane potential difference. Moreover, a comparison between the effects of aging on the PM H^+-ATPase activity and on the hyperpolarization of the transmembrane potential difference with those of fusicoccin on the same parameters (for review, see Marrè, 1979; Marrè et al., 1993; Aducci et al., 1995) indicate that the increase of H^+ pumping by the PM H^+-ATPase may well account for the hyperpolarization of the transmembrane potential difference induced by aging in beet discs (Poole, 1974), thus substantiating the early proposal that aging-induced K^+ uptake is driven by the activity of an electrogenic H^+ pump (Poole, 1974).

ACKNOWLEDGMENTS

The authors wish to thank Dr. C Olivari (Dipartimento di Biologia, Università di Milano) for the generous gift of antiserum against the N terminus of the PM H^+-ATPase; Dr. C.F. Cesarone (Istituto di Fisiologia Generale, Università di Genova) for help in quantification of the immunodetected PM H^+-ATPase; and Prof. E. Marrè (Dipartimento di Biologia, Università di Milano) for critical reading of the manuscript.

Received December 2, 1996; accepted March 28, 1997.
Copyright Clearance Center: 0032–0889/97/114/0857/06.

LITERATURE CITED

Aducci P, Marra M, Fogliano V, Fullone MR (1995) Fusicoccin receptors: perception and transduction of fusicoccin signal. J Exp Bot **46:** 1463–1478

Briskin DP (1990) The plasma membrane H^+-ATPase of higher plant cells: biochemistry and transport function. Biochim Biophys Acta **1019:** 95–109

Chang A, Slayman CW (1991) Maturation of the yeast plasma membrane H^+-ATPase involves phosphorylation during intracellular transport. J Cell Biol **115:** 289–295

De Michelis MI, Papini R, Pugliarello MC (1995) Effect of N-acetylimidazole on the activity and the regulatory properties of the plasma membrane H^+-ATPase. Giorn Bot Ital **129:** 1053–1054

De Michelis MI, Papini R, Pugliarello MC (1997) Multiple effects of lysophosphatidylcholine on the activity of the plasma membrane H^+-ATPase of radish seedlings. Bot Acta **110:** 63–68

De Michelis MI, Spanswick RM (1986) H^+ pumping driven by the vanadate-sensitive ATPase in membrane vesicles from corn roots. Plant Physiol **81:** 542–547

Harper JF, Manney L, Dewitt ND, Yoo MH, Sussman MR (1990) The *Arabidopsis thaliana* plasma membrane H^+-ATPase multigene family: genomic sequence and expression of a third isoform. J Biol Chem **265:** 13601–13607

Ho IS, Briskin DP (1992) Chemical modification of the red beet plasma membrane H^+-ATPase with N-acetylimidazole. Plant Physiol Biochem **30:** 549–557

Johansson F, Olbe M, Sommarin M, Larsson C (1995) Brij 58, a polyoxyethylene acylether, creates membrane vesicles of uniform sidedness: a new tool to obtain inside-out (cytoplasmic side-out) plasma membrane vesicles. Plant J **7:** 165–173

Johansson F, Sommarin M, Larsson C (1993) Fusicoccin activates the plasma membrane H^+-ATPase by a mechanism involving the C-terminal inhibitory domain. Plant Cell **5:** 321–327

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem **87:** 206–210

Marrè E (1979) Fusicoccin: a tool in plant physiology. Annu Rev Plant Physiol **30:** 273–288

Marrè E, Bellando M, Beffagna N, Marrè MT, Romani G, Vergani P (1993) Synergisms, additive and nonadditive factors regulating proton extrusion and intracellular pH. *In* R Sharp, AJ Novacky, DD Randall, eds, Current Topics in Plant Biochemistry and Physiology. University of Missouri, Columbia, MO, pp 213–230

Noubhani AM, Sakr S, Denis MH, Delrot S (1996) Transcriptional and posttranscriptional control of the plant plasma membrane H^+-ATPase by mechanical treatments. Biochim Biophys Acta **1281:** 213–219

Olivari C, Pugliarello MC, Rasi-Caldogno F, De Michelis MI (1993) Characteristics and regulatory properties of the H^+-ATPase in a plasma membrane fraction purified from *Arabidopsis thaliana*. Bot Acta **106:** 13–19

Palmgren MG, Sommarin M, Serrano R, Larsson C (1991) Identification of an autoinhibitory domain in the C-terminal region of the plant plasma membrane H^+-ATPase. J Biol Chem **266:** 20470–20475

Poole RJ (1974) Ion transport and electrogenic pumps in storage tissue cells. Can J Bot **52:** 1023–1028

Poole RJ (1976) Transport in cells of storage tissues. *In* A Pirson, MH Zimmermann, eds, Encyclopedia of Plant Physiology, Vol 2A. Springer-Verlag, Berlin, pp 229–248

Rasi-Caldogno F, Pugliarello MC, Olivari C, De Michelis MI (1993) Controlled proteolysis mimics the effect of fusicoccin on the plasma membrane H^+-ATPase. Plant Physiol **103:** 391–398

Regenberg B, Villalba JM, Lanfermeijer FC, Palmgren MG (1995) C-terminal deletion analysis of plant plasma membrane H^+-ATPase: yeast as a model system for solute transport across the plant plasma membrane. Plant Cell **7:** 1655–1666

Riordan J F, Vallee BL (1972) O-Acetyltyrosine. Methods Enzymol **25:** 500–506

Serrano R (1989) Structure and function of plasma membrane H^+-ATPase. Annu Rev Plant Physiol Plant Mol Biol **40:** 61–94

Sussman MR (1994) Molecular analysis of proteins in the plant plasma membrane. Annu Rev Plant Physiol Plant Mol Biol **45:** 211–234