ELSEVIER

Biochimica et Biophysica Acta 1420 (1999) 214–222

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Modification of the N-terminal polyserine cluster alters stability of the plasma membrane H^+-ATPase from *Saccharomyces cerevisiae*

Alinda Hasper, Patricia Soteropoulos, David S. Perlin *

Public Health Research Institute, 455 First Avenue, New York, NY 10016, USA

Received 18 March 1999; received in revised form 7 June 1999; accepted 17 June 1999

Abstract

The N-terminus of the H^+-ATPase from *Saccharomyces cerevisiae* contains a serine-rich cluster of 11 serine residues in the first 17 amino acids, including a stretch of eight consecutive serine residues. This cluster is conserved in the weakly expressed *PMA2* gene from the same organism, but it is not present in *PMA* genes from other organisms suggesting that it is not likely to represent a conserved functional motif. To better understand whether this region plays a regulatory role, a series of mutant enzymes were generated in which the serine tract was systematically converted to alanine or deleted. Conversion of the first six serine residues to alanine or deletion of the entire serine tract had little effect on cell growth phenotypes. However, when eight or more serines were converted, the mutant cells displayed prominent hygromycin B-resistant and low pH-sensitive phenotypes indicative of reduced H^+-ATPase function. The mutant enzymes were found to display relatively normal kinetic properties for ATP hydrolysis, but showed significantly decreased abundance in the plasma membrane under stress conditions when eight or more serine residues were converted to alanine. The reduced abundance of the enzyme appeared to be due to degradative turnover, as mutant enzymes with multiple alanine substitutions showed an accelerated rate of turnover relative to wild-type. The polyserine tract in the H^+-ATPase does not appear to be important for catalysis, but may contribute to overall protein stability.

Keywords: Proton ATPase; Polyserine; *PMA1*

1. Introduction

The plasma membrane H^+-ATPase from *Saccharomyces cerevisiae* is an electrogenic proton pump that helps regulate intracellular pH and generates the electrochemical proton gradient necessary for growth and development of the cell. The H^+-ATPase is a typical member of the P-type ion translocating ATPase family that includes the Ca^{2+}-ATPase and Na^+,K^+-ATPase of animal cells, and the H^+-ATPases of plant cells [1]. The gene encoding the *S. cerevisiae* H^+-ATPase, *PMA1*, shows a high degree ($>70\%$) of amino acid sequence similarity with other fungal and plant enzymes, but shows less overall similarity ($<30\%$) with genes encoding higher eukaryotic enzymes [2]. However, all family members show closely related catalytic properties, and these enzymes are typically organized into distinct structural/functional domains. These include a membrane embedded transport region, a cytoplasmic ATP hydrolysis domain consisting of two interacting seg-

* Corresponding author. Fax: +1-212-578-0804;
E-mail: perlin@phri.nyu.edu

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00100-5

ments, and finally, N- and C-termini, which reside in the cytosol and are the most divergent regions, even amongst the most highly homologous P-type ATPases.

These divergent regions are believed to account for some of the diverse functional and regulatory properties of the various P-type enzymes. C-terminal regulation of enzyme activity is well documented for calmodulin binding to the plasma membrane Ca^{2+}-ATPase [3,4] and for glucose regulation of the yeast H^+-ATPase [5]. Less is known about the role of the N-terminus, although modulation of this portion of the enzyme is known to alter kinetic properties of the Na^+,K^+-ATPase [6,7], and the expression of the plasma membrane Ca^{2+}-ATPase [8]. The N-terminus of the *S. cerevisiae* H^+-ATPase contains a notable cluster of 11 serine residues in the first 17 amino acids, including a stretch of eight consecutive serine residues in position 5–12. This cluster of the *S. cerevisiae PMA1* is conserved in the weakly expressed *PMA2* gene from the same organism, despite the fact that other portions of the N-terminal domain are divergent. The serine cluster is unique to the *S. cerevisiae PMA genes* and is not retained in other highly conserved fungal or plant *PMA* genes, suggesting that it is not likely to be essential for catalysis. Consistent with this suggestion is the observation that mutant P-type enzymes containing large deletions encompassing this region show relatively normal rates of ATP hydrolysis [6,9,10], and tryptic cleavage of this region in fully assembled enzymes has little overall effect on enzyme activity [6,11]. However, preservation of this region by *S. cerevisiae* suggests that it might play a cell specific role, perhaps by being important for plasma membrane targeting, assembly or regulation of enzyme turnover.

In this report, we have systematically altered the serine cluster in *PMA1* by conversion to alanine and examined the effects of these mutations on enzyme activity and assembly at the plasma membrane. The results show that the enzymatic properties of the mutant enzymes are largely unaffected, but the conversion of the serine cluster residues to alanine significantly reduces the steady-state level of assembled H^+-ATPase in the plasma membrane. Turnover of the mutant protein is greatly accelerated suggesting that this N-terminal region may be important for protein turnover by the cell.

2. Materials and methods

2.1. Yeast strains, mutant preparation, and cell culture

Site-directed *pma1* mutants were constructed by the method of Kunkel utilizing uracil-containing phagemid, as previously described [12] or with the QuikChange Site-Directed Mutagenesis Kit (Stratagene). *Pma1*-S1-3A was derived from pGW201 [13] and encodes a *pma1* mutant with the first three serines replaced by alanine. The primer 5′-CAATATGACTGATGCTGCAGCCGCTTCATCATCCTC added a *Pst*I site, which was used to identify the mutation. All additional alanine substitutions were derived from *pma1*-S1-3A in succession as follows: *pma1*-S1-6A was created with the primer 5′-ACTGCAGCCGCTGCCGCGGCCTCTTCAGCATC adding a *Sac*II site; *pma1*-S1-8A was derived with the primer 5′-GCCGCTGCCGCCGCCGCGGCCGCAGCTTCTGTTTCAGC creating an *Xma*III site; and *pma1*-S1-11A was created with the primer 5′-GCGGCCGCAGCCGCGGTTGCAGCTCATCAGCC adding a *Sac*II site. The serine deletion mutant, *pma1*-Δ1-11 was constructed in pGWchim, which consists of vector pGW201 [13] with a N-terminal *Nhe*I site inserted downstream from the serine stretch and an *Mlu*I site at the C-terminus of *PMA1*. *Pma1*-ΔS1-11 was derived using primer 5′-ATGACTGATACAGCTAGCGCTTCATCATCCTC creating an *Eco47*III site. A single-step homologous recombination procedure was used to introduce *URA3*-marked *pma1* mutations into the chromosome of yeast strain SH122 (*HO ade6-1 trp5-1 leu2-1 lys1-1 ura3-1 pma1::LEU2/PMA1*), as described by Harris et al. [14]. Strain GW201 (*HO ade6-1 trp5-1 leu2-1 lys1-1 ura3-1 PMA1::ura3*) was used as a control for all experiments.

All *pma1* mutants were confirmed by PCR amplification of the chromosome region corresponding to *PMA1* and restriction analysis, followed by DNA sequence analysis. Chromosomal DNA of the *pma1* mutants was isolated from 2 ml YPD (1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) dextrose) cultures grown for 18 h at 30°C. PCR amplification of *PMA1* DNA was performed in 100 μl total volume with 100 nmol of each N- and C-terminal primers, 5′-GCTCCCCTCCATTAGTTTCG and 5′-

GCGTGTTGTGAATTGTGC, respectively. The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) or the Wizard PCR Preps DNA Purification System (Promega). The purified DNA was sequenced with the Sequenase PCR Product Sequencing Kit (United States Biochemical) or the Cycle Sequencing Kit (Pharmacia).

Growth on hygromycin B was monitored on YPD agar plates containing 0, 100, 200 and 300 μg/ml hygromycin B. Growth at low pH medium was determined on YPD agar plates at pH 3.0 containing 10–40 mM acetate and in YPD medium containing 15 mM acetate adjusted to pH 2.5–8.5, as previously described [15].

2.2. Plasma membrane isolation and assay of ATP hydrolysis

Plasma membranes were prepared from wild-type and *pma1* mutant strains by centrifugation on a sucrose step-gradient, as previously described [16]. The basic ATP hydrolysis assay medium consisted of 5 mM $MgSO_4$, 5 mM ATP, 25 mM NH_4Cl, 10 mM MES-Tris, pH 6.5. The reaction was performed in a 150 μl volume with 1.0 μg membrane protein. The reaction was stopped by the addition of 100 μl of a combined Stop-Color Development reagent containing 1% SDS, 0.8% ascorbic acid, 100 mM ammonium molybdate and 0.6 M H_2SO_4. After 10 min at 30°C, the OD_{660} was determined in a microtiter plate reader (Tecan U.S. SLT Lab Instruments). K_m and V_{max} values were obtained by measuring ATP hydrolysis in the presence of 0 to 10 mM of $MgSO_4$ and ATP at equimolar concentrations. Vanadate sensitivity was determined by including 0–10 μM vanadate in the basic assay medium.

2.3. Relative enzyme abundance and turnover

Relative enzyme abundance was determined by growing wild-type and *pma1* mutants strains under standard conditions at 30°C in YPD at pH 5.7 or under stress conditions at 30°C in YPD with 15 mM acetate at pH 3.0. Cells grown to an OD_{590} ~5 were collected by centrifugation at 5000×*g* for 10 min. Sucrose-gradient purified plasma membranes were prepared, as above. A 10 μg amount of the purified plasma membrane protein was subjected to SDS gel electrophoresis in 10% (w/v) pre-cast minigels (Novex) and either stained with Coomassie blue R-250 or used for Western blot analysis to determine the relative abundance of intact H^+-ATPase [16]. The amount of intact ATPase was analyzed Using Adobe Photoshop and Scion Image (Scion) software. Enzyme turnover was determined by transferring log-phase cultures of wild-type GW201, *pma1*-S1-8, *pma1*-S1-11A and *pma1*-ΔS1-11 grown at 35°C at pH 3.0 to medium containing 20 μg/ml cycloheximide. Cells were incubated at 35°C and aliquots were removed at 0, 2, 4 and 6 h. The level of membrane-associated H^+-ATPase following gel electrophoresis was quantified as above and plotted as a function of time for each strain.

2.4. Other procedures

Protein concentrations were determined by a modified Lowry Protein Assay [17]. Yeast transformants were prepared by the lithium acetate procedure using single stranded carrier DNA, as described in Alkali-Cation Yeast Transformation Kit (BIO 101). Plasmid DNA was sequenced with the Sequenase 2.0 DNA Sequencing Kit (United States Biochemical).

Strain	Residue																
	N-1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Wild Type	M	D	T	D	S	S	S	S	S	S	S	S	A	S	S	V	S
pma1-S1-3A	M	D	T	D	A	A	A	S	S	S	S	S	A	S	S	V	S
pma1-S1-6A	M	D	T	D	A	A	A	A	A	A	S	S	A	S	S	V	S
pma1-S1-8A	M	D	T	D	A	A	A	A	A	A	A	A	A	S	S	V	S
pma1-S1-11A	M	D	T	D	A	A	A	A	A	A	A	A	A	A	A	V	A
pma1-ΔS1-11	M	D	T	D	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 1. Diagram showing N-terminal sequence of *PMA1* and serine mutant constructs. The first 17 amino acid residues from the N-terminus of *PMA1* are shown for wild-type and mutant constructs formed by either substituting serine with alanine or deleting the serine tract.

Table 1
Growth of the *pma1* mutants on hygromycin B

pma1 strains	Hygromycin B			
	0 μg/ml	100 μg/ml	200 μg/ml	300 μg/ml
GW201 (wt)	+++	±	−	−
pma1-S1-3A	+++	+	−	−
pma1-S1-6A	+++	+	−	−
pma1-S1-8A	+++	++	++	−
pma1-S1-11A	+++	+++	+++	+++
pma1-ΔS1-11	+++	±	−	−

3. Results

3.1. Alanine replacement mutagenesis

The cluster of N-terminal serine residues in *PMA1* is shown in Fig. 1 along with the *pma1* mutants generated by sequential substitution of the serine residues with alanine or deletion of residues 5–17. Alanine was chosen because of its small non-polar side-chain and its ability to exist in multiple structural forms [18]. All mutations produced viable cells, indicating synthesis of a functional H^+-ATPase. The *pma1* mutants were tested for typical growth phenotypes associated with defects in H^+-ATPase function including hygromycin B resistance and low pH sensitivity. The growth of wild-type strain GW201 was strongly inhibited on YPD medium containing the aminoglycoside antibiotic hygromycin B at 100 μg/ml. Growth resistance to hygromycin B is linked to the depolarization of the membrane potential generated by the H^+-ATPase [19]. Table 1 shows that normal hygromycin B sensitivity was observed for wild-type and *pma1* mutants, *pma1*-S1-3A and *pma1*-S1-6A, in which the first three and six serines, respectively, were replaced by alanine, and for deletion mutant *pma1*-ΔS1-11. However, low-level growth resistance was observed with *pma1*-S1-8A, in which eight of the 11 serines were converted to alanine, and strong resistance was observed with *pma1*-S1-11A, in which all the serines were replaced by alanine.

The growth sensitivity of the *pma1* mutants to low pH medium in the presence of increasing concentrations of acetate is shown in Table 2. In this assay, weak acid-induced acidification of the cytoplasm is used as a relative measure of the kinetic competency of the H^+-ATPase to pump protons and help regulate intracellular pH. Consistent with the behavior of the *pma1* mutants displaying resistance to hygromycin B, it was observed that *pma1*-S1-8A displayed weak sensitivity to low pH medium, while *pma1*-S1-11A, showed hypersensitivity to cytoplasmic acid loading. Wild-type and *pma1* strains *pma1*-S1-3A, *pma1*-S1-6A, and *pma1*-ΔS1-11 showed normal resistance to low pH medium. In addition, *pma1*-ΔS1-11 exhibited a pH dependent growth profile typical of wild-type, with growth inhibition observed only at pH 2.5, while *pma1*-S1-11A showed a decreased ability to grow below pH 4.5 (data not shown).

These results suggest that defects related to elec-

Table 2
Growth of the serine mutants on low pH medium containing acetate

pma1 strains	Acetate				
	0 mM	10 mM	20 mM	30 mM	40 mM
GW201 (wt)	+++	+++	++	+	−
pma1-S1-3A	+++	+++	++	+	−
pma1-S1-6A	+++	+++	++	+	−
pma1-S1-8A	+++	++	+	−	−
pma1-S1-11A	+++	+	−	−	−
pma1-ΔS1-11	+++	+++	++	+	−

trogenic proton transport by the H^+-ATPase account for the prominent phenotypes observed when eight or more N-terminal serine residues are converted to alanine.

3.2. Properties of pma1 mutant enzymes

To further assess whether altered kinetic properties of the mutant enzymes could account for the various phenotypes observed, the ATP hydrolytic properties of the mutant enzymes were assessed in highly purified plasma membranes obtained by sucrose-gradient centrifugation. Table 3 shows that sequential conversion of all the serine residues to alanine resulted in a progressive decrease in the V_{max} for ATP hydrolysis by the various mutant enzymes. These effects were more prominent when cells were grown under low pH (pH 3.0) stress conditions, as the enzymes separated into two distinct groups with *pma1*-S1-8A, *pma1*-S1-11A, and *pma1*-ΔS1-11 enzymes showing less than 42% of the wild-type V_{max} level. The relatively low total activities obtained for the enzymes from *pma1*-S1-8A and *pma1*-S1-11A, but not *pma1*-ΔS1-11, were consistent with the growth phenotypes observed.

SDS-polyacrylamide gel electrophoresis and Western blot analysis further showed that the alanine mutations caused a reduction in the level of intact assembled (MW ~ 100 000 Da) H^+-ATPase in the plasma membrane from cells grown under standard and stressed conditions (Fig. 2 and Table 3). When the enzyme activities were corrected for the amount of assembled enzyme, it was found that even the most severely perturbed mutant enzyme from strain *pma1*-S1-11A had a rate of ATP hydrolysis that was ~74 and 80% of wild-type for cells grown at pH 5.7 and 3.0, respectively (Table 3). On the other hand, the serine deletion mutant showed reduced ATP hydrolysis rates but a higher level of abundance. The relatively normal turnover rates of ATP hydrolysis for the alanine substitution mutants suggested that the kinetic properties of the mutant enzymes was not altered. This suggestion was further supported by the fact that all the mutant enzymes showed K_m values of 0.7 to 1.1 mM, close to that of wild-type (~0.95 mM). In addition all the mutant enzymes showed a normal IC_{50} of 0.5–1 μM for inhibition by vanadate, a characteristic inhibitor of P-type enzymes. These results indicate that a largely defective enzyme was not responsible for the phenotypes observed. Although, V_{max} levels were lower than expected when taking into account enzyme abundance levels. Overall, the amount of total activity present in the membrane for the mutant strains was significantly reduced relative to wild-type as the number of serine substitutions were increased. This data indicate that the observed growth phenotypes with multiple serine substitutions can be accounted for by a reduction in total transport capacity, as the amount of enzyme in the membrane is reduced.

3.3. Accelerated turnover influences enzyme abundance

The reduction in the level of assembled H^+-ATPase in the *pma1*-S1-8A and *pma1*-S1-11A mutant plasma membranes suggested a defect in either the assembly or turnover of the mutant enzymes. Mutant

Table 3
Kinetic properties of serine mutant enzymes

Strain	V_{max} (pH 5.7)[a]	Abundance (pH 5.7)[b]	V_{max} (5.7 corrected)[c]	V_{max} (pH 3.0)	Abundance (pH 3.0)	V_{max} (3.0 corrected)
Wild-type	5.44	100	5.44	5.65	100	5.65
pma1-S1-3A	4.14	106	3.90	4.33	84	5.15
pma1-S1-6A	3.65	108	3.38	3.86	90	4.29
pma1-S1-8A	3.49	93	3.75	2.13	66	3.22
pma1-S1-11A	2.92	72	4.05	1.80	40	4.50
pma1-ΔS1-11	3.22	106	3.03	2.42	80	3.03

[a] V_{max} is in units of μmol Pi min^{-1} mg $protein^{-1}$.
[b] Relative abundance of H^+-ATPase was determined from Western blot analysis of plasma membrane proteins.
[c] Corrected values were obtained by dividing the V_{max} activity by the abundance of intact H^+-ATPase relative to wild-type.

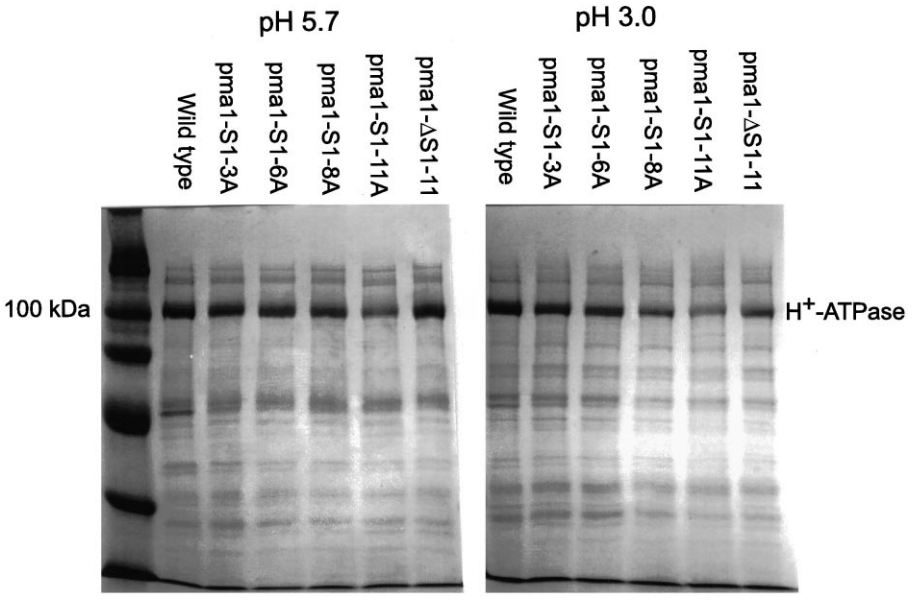

Fig. 2. Abundance of H^+-ATPase in plasma membranes from serine mutants. SDS polyacrylamide gel profile of sucrose gradient-purified plasma membranes obtained from log-phase cells of serine mutants grown under normal (YPD, pH 5.7) and stress conditions (YPD, pH 3.0). The resolved proteins were stained with Coomassie blue. The position of the H^+-ATPase is indicated.

cells grown under standard growth conditions were found to contain wild-type levels of H^+-ATPase in the plasma membrane. Only the *pma1*-S1-11A enzyme showed 72% of wild-type. However, when cells were grown under stress conditions (pH 3.0), the abundance of H^+-ATPase in the membrane was reduced to 66% for *pma1*-S1-8A, 80% for *pma1*-ΔS1-11, and 40% for *pma1*-S1-11A (Table 3).

The H^+-ATPase is known to turn-over relatively slowly in the membrane, and this property was examined for wild-type, pma1-S1-8A, *pma1*-S1-11A and *pma1*-ΔS1-11 strains. To assess turnover of pre-assembled enzyme, cells were grown to mid-log phase and then transferred to fresh media medium containing cycloheximide to block new protein synthesis at pH 5.7 or to a stress medium at pH 3.0 containing 15 mM acetate and cycloheximide at 35°C. Fig. 3A shows that there was little turnover of wild-type enzyme under normal growth conditions after 6 h, consistent with a known $T_{1/2} > 11$ h [20]. In contrast, the *pma1*-S1-11A mutant showed enhanced turnover under these conditions with a $T_{1/2}$ for turnover of $\sim$4.5 h (Fig. 3A). Under stress conditions, wild-type and *pma1*-S1-8 showed somewhat accelerated turnover relative to non-stress conditions. However, the *pma1*-S1-11A mutant enzyme showed considerably enhanced turnover with a $T_{1/2} < 1$ h. Deletion of the serine stretch in *pma1*-ΔS1-11 showed turnover kinetics comparable to wild-type (Fig. 3B). These results suggest that an accelerated rate of H^+-ATPase turnover may account for the reduced abundance of intact assembled enzyme observed in the plasma membrane from highly substituted serine mutants.

4. Discussion

Among the various P-Type ATPases that show extensive sequence homology ($>90\%$), like the fungal H^+-ATPases and the α-subunit isoforms of the Na^+,K^+-ATPase, the N-termini frequently show significant diversity [2,7]. Such diversity might be expected to account for altered catalytic properties, enzyme regulation, membrane assembly or intracellular targeting. In the Na^+,K^+-ATPase, removal of the first $\sim$32 N-terminal residues by either trypsin treatment or genetic truncation had no overt affect on overall enzyme activity [6,9]. However, the truncated enzymes showed altered kinetics for K^+ de-occlusion under low ATP conditions, and it was suggested that the N-terminus was involved in regulating this partial catalytic reaction [6,7]. In SERCA2, phosphorylation of Ser-38 by a Ca^{2+}/calmodulin-dependent protein

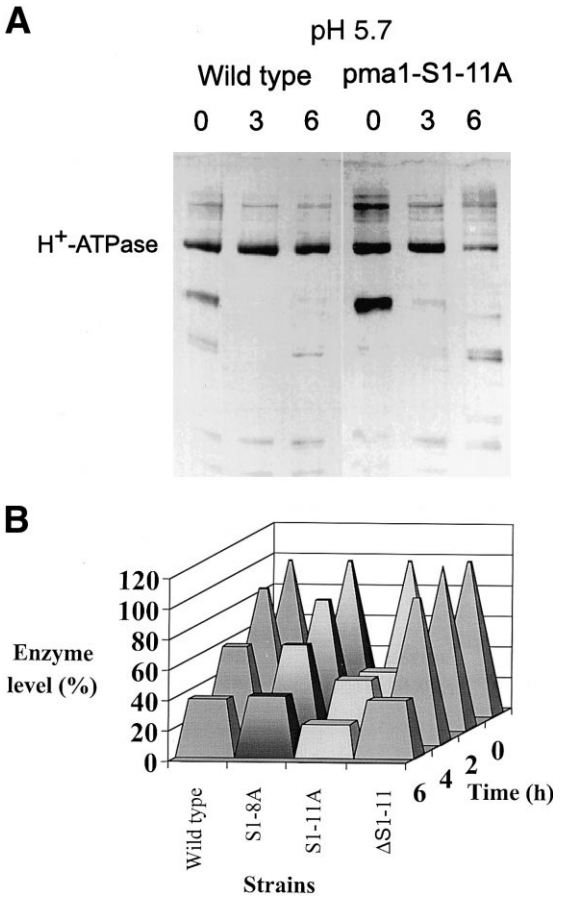

Fig. 3. Turnover of H^+-ATPase from wild-type and serine mutants under normal and stress conditions. (A) Wild-type and *pma1*-S1-11A cells were grown to mid-log phase under normal conditions in YPD medium and then transferred to fresh media containing 10 μg/ml cycloheximide. The cells were incubated for 0, 3 and 6 h under normal and stress conditions, and plasma membranes were isolated from each set of cells. Protein profiles associated with each membrane were evaluated by SDS polyacrylamide gel electrophoresis. (B) Wild-type, *pma1*-S1-8A, *pma1*-S1-11A, and *pma1*-ΔS1-11 strains were grown to mid-log phase in YPD under low pH conditions at 35°C. The cells were transferred to fresh medium containing 20 μg/ml cycloheximide. The cells were incubated for 0, 2, 4 and 6 h at 35°C, and microsomal membranes were prepared. The level of membrane-associated H^+-ATPase following gel electrophoresis was quantified and plotted as a function of time for each strain.

kinase resulted in activation of V_{max} for Ca^{2+} transport [21]. Regulation of the Na^+,K^+-ATPase by activation of protein kinase C has also been well documented, and N-terminal phosphorylation of serine and threonine residues in rat kidney α1 subunit has been shown [22,23]. The regulatory role these post-translational modifications impart is unclear, as neither the enzyme activity nor Na^+ affinity were affected by these changes [22]. The polyserine tract in the yeast H^+-ATPase could form a potential phosphorylation site that might be involved in glucose activation of the enzyme. Glucose activation is believed to involve the C-terminus [24] and phosphorylation by casein kinase I enzyme at position Ser-507 [25]. Yet, conversion of the N-terminal serines to alanine had no effect on glucose activation of the enzyme in our studies (data not shown) indicating that the N-terminal serine tract does not contribute to this type of cellular enzyme activation. In the *Neurospora* H^+-ATPase, limited trypsin digestion resulting in deletion of the first 36 amino acids had little effect on enzyme activity [11]. Similarly, genetic truncation of the first 27 amino acids of the yeast H^+-ATPase also had no effect on V_{max} for ATP hydrolysis [10]. Based on studies performed on several P-type ATPases, Lutsenko and Kaplan [1] proposed a consensus profile for the N-terminus in which it does not determine cation affinity or specificity. But rather, it plays a role in the conformational transition associated with cation efflux, and normal enzyme function requires the interaction between the N-terminal segment and other parts of the ATPase. Consistent with previous studies, the results obtained in this study indicate that modification of an N-terminal serine cluster had minimal affect on ATP hydrolysis. However, since partial reactions for proton transport by the H^+-ATPase cannot be readily studied, it cannot be ascertained whether proton affinity had been altered by the N-terminal serine modifications. It appears that the N-terminus has little direct action on overall enzyme function.

Conversion of the serine cluster residues to alanine did not significantly alter the catalytic properties of the enzyme, but it did decrease the V_{max} somewhat and abundance of the H^+-ATPase in the plasma membrane. A reduced abundance of mutant enzyme could result either from degradation of pre-existing enzyme from the cell surface or from degradation of newly synthesized H^+-ATPase. In the last case, the mutant enzyme is not delivered to the plasma membrane, but goes directly to the vacuole, possibly due to the loss of a targeting signal [26]. Incompletely assembled proteins are known to be retained in the endoplasmic reticulum and are not transported to the plasma membrane [27]. However, the data presented

here suggest that accelerated enzyme turnover through the degradative process is most like responsible for the reduced abundance of enzyme in the membrane. The observed turnover effects are significant. Wild-type H^+-ATPase shows a long-lived $T_{1/2}$ $\sim$9 h, consistent with previous studies [20], whereas the pma1-S1-11A mutant showed accelerated turnover from cells grown under both stress and non-stress conditions (Fig. 3). Since the N-terminus can be deleted without significantly affecting enzyme assembly or stability, it is likely that conversion of the serine tract to alanine produces a local aberrant structure that is recognized by the degradative machinery in the cell. The propensity of polyalanine to adopt a stable helical conformation, as shown in model studies, may be a contributing factor [28].

Normal turnover of the pheromone receptors and certain types of permeases occur via vacuolar degradation, in which the cell surface proteins are internalized and transported to the vacuole [29]. The fact that turnover of the mutant H^+-ATPases is accelerated at elevated temperature and occurs following membrane assembly and not before assembly, suggests that proteolysis occurs at the level of the plasma membrane. This turnover may reflect an alternative degradation pathway for the H^+-ATPase, perhaps similar to that observed for other yeast membrane proteins, such as the Ctr1p copper transporter [30].

Is the serine cluster unique to membrane proteins? Numerous proteins show polyserine tracts. A gapped blast search of the NCBI data base with the N-terminal query sequence TDTSSSSSSSSASSVSAHQP identified 108 different genes with high sequence identity ($>$65%) with many showing eight or more consecutive serine residues. The most significant number of genes were from *S. cerevisiae* with 23% of the sequences, while 14% were found in *Drosophila*, 7% in human and 7% in mouse genes. Arrangement of the serine clusters according to distance from the extreme N-terminus indicates that there is no position preference in the gene. The highest degree of sequence homology of the N-terminal serine stretch was found in the N-terminus of the α-subunit of several eukaryotic large conductance calcium-activated potassium channels. These membrane-associated channel proteins consist of two subunits, α and β. The α-subunit is a member of the slow Ca^{2+} activated K^+ channel gene family and forms the ion conduction pore. The N-terminal polyserine stretch is conserved in eukaryotes like mouse, rat, cow and humans, but not in the *Drosophila* or *Caenorhabditis elegans*. Little is known about the serine stretch in these systems, since its deletion does not lead to changes in biophysical properties of the channel when expressed in *Xenopus* oocytes [31].

Serine tracts are found in many diverse proteins, and are especially prevalent in yeast. In the few cases where they have been systematically examined, it appears that they do not play an overt role in protein function. Rather, the serine tract may have a subtle cell biological role, perhaps acting to stabilize local protein structure. It may be that these stretches are phosphorylated, and the degree of phosphorylation is recognized by the cell degradative machinery.

Acknowledgements

The authors wish to thank Dr. Donna Seto-Young for helpful discussions and Padmaja Paderu and David Huang for their expert technical assistance. This work was supported by National Institutes of Health Grant GM 38225 to D.S.P.

References

[1] S. Lutsenko, J.H. Kaplan, Biochemistry 34 (1995) 15607–15613.
[2] A. Wach, A. Schlesser, A. Goffeau, J. Bioenerg. Biomembr. 24 (1992) 309–317.
[3] J.T. Penniston, A. Enyedi, A.K. Verma, H.P. Adamo, A.G. Filoteo, Ann. New York Acad. Sci. 834 (1997) 56–64.
[4] A. Enyedi, A.G. Filoteo, G. Gardos, J.T. Penniston, J. Biol. Chem. 266 (1991) 8952–8956.
[5] F. Portillo, P. Eraso, R. Serrano, FEBS Lett. 287 (1991) 71–74.
[6] S.E. Daly, L.K. Lane, R. Blostein, J. Biol. Chem. 271 (1996) 23683–23689.
[7] S.E. Daly, L.K. Lane, R. Blostein, J. Biol. Chem. 269 (1994) 23944–23948.
[8] M.E. Grimaldi, H.P. Adamo, A.F. Rega, J.T. Penniston, Ann. New York Acad. Sci. 834 (1997) 452–453.
[9] S.E. Daly, L.K. Lane, R. Blostein, Ann. New York Acad. Sci. 834 (1997) 489–497.
[10] F. Portillo, I.F. De Larrinoa, R. Serrano, FEBS Lett. 247 (1989) 381–385.

[11] S.M. Mandala, C.W. Slayman, J. Biol. Chem. 263 (1988) 15122–15128.
[12] S. Na, D.S. Perlin, D. Seto-Young, G. Wang, J.B. Haber, J. Biol. Chem. 268 (1993) 11792–11797.
[13] G. Wang, M.J. Tamas, M.J. Hall, A. Pascual-Ahuir, D.S. Perlin, J. Biol. Chem. 271 (1996) 25438–25445.
[14] S.L. Harris, D.S. Perlin, D. Seto-Young, J.E. Haber, J. Biol. Chem. 266 (1991) 24439–24445.
[15] D. Seto-Young, S. Na, B.C. Monk, J.E. Haber, D.S. Perlin, J. Biol. Chem. 269 (1994) 23988–23995.
[16] D. Seto-Young, M.J. Hall, S. Na, J.E. Haber, D.S. Perlin, J. Biol. Chem. 271 (1996) 581–587.
[17] M.A.K. Markwell, S.M. Haas, L.L. Bieber, N.E. Tolbert, Anal. Biochem. 87 (1978) 206–210.
[18] J.M. Thornton, S.P. Gardner, in: R.A. Bradshaw, M. Purton (Eds.), Protein: Form and Function, Elsevier Trends Journals, Cambridge, 1990, pp. 153–161.
[19] D.S. Perlin, C.L. Brown, J.E. Haber, J. Biol. Chem. 263 (1988) 18118–18122.
[20] B. Benito, E. Moreno, R. Lagunas, Biochim. Biophys. Acta 1063 (1991) 265–268.
[21] T. Toyofuku, K. Curotto, N. Narayanan, D.H. MacLennan, J. Biol. Chem. 269 (1994) 26492–26496.
[22] M.S. Feschenko, K.J. Sweadner, J. Biol. Chem. 272 (1997) 17726–17733.
[23] M.S. Feschenko, R.K. Wetzel, K.J. Sweadner, Ann. New York Acad. Sci. 834 (1997) 479–488.
[24] P. Eraso, F. Portillo, J. Biol. Chem. 269 (1994) 10393–10399.
[25] E. Estrada, P. Agostinis, J.R. Vandenheede, J. Goris, W. Merlevede, J. Francois, A. Goffeau, M. Ghislain, J. Biol. Chem. 271 (1996) 32064–32072.
[26] A. Chang, G. Fink, J. Cell Biol. 128 (1995) 39–49.
[27] A. de Kerchove, P. Supply, A. Goffeau, Yeast 12 (1996) 907–916.
[28] P. Doruker, I. Bahar, Biophys. J. 72 (1997) 2445–2456.
[29] K. Lai, C.P. Bolognese, S. Swift, P. McGraw, J. Biol. Chem. 270 (1995) 2525–2534.
[30] C.E. Ooi, E. Rabinovich, A. Dancis, J.S. Bonifacino, D.R. Klausner, EMBO J. 15 (1996) 3515–3523.
[31] A. Butler, S. Tsunoda, D.P. McCobb, L. Salkoff, Science 261 (1993) 221–224.