Biochimica et Biophysica Acta, 1062 (1991) 157–164

ADONIS 000527369100110E

BBAMEM 75130

Domains of yeast plasma membrane and ATPase-associated glycoprotein

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(Received 12 July 1990)

Key words: Plasma membrane; Yeast; ATPase; Glycoprotein; Membrane domain; Immunogold electron microscopy

In yeast homogenates the plasma membrane H^+-ATPase and a major surface glycoprotein of about 115 kDa are present in two membrane fractions with peak densities in sucrose gradients of 1.17 and 1.22. Immunogold electron microscopy of frozen yeast sections indicates that the ATPase is exclusively (> 95%) present at the surface membrane. Therefore the two ATPase-containing fractions appear to correspond to different domains of the plasma membrane. The 115 kDa glycoprotein is tightly associated with the ATPase during solubilization and purification of the enzyme. However, in a mutant lacking the glycoprotein the activity of the plasma membrane H^+-ATPase is similar to wild type, suggesting that this association is fortuitous. The ATPase and the glycoprotein are difficult to separate by electrophoresis and therefore binding of concanavalin A to the ATPase cannot be unambiguously demonstrated in wild-type yeast. By utilizing the mutant without glycoprotein it was shown that the ATPase band of 105 kDa binds concanavalin A.

Introduction

Most biological membranes contain domains enriched in particular proteins and lipids. For example, the apical and basolateral domains of epithelial plasma membranes [1] and the appressed and non-appressed domains of thylakoid membranes [2]. This lateral asymmetry is important for some membrane functions but it is generated by poorly understood mechanisms [1,2].

The existence of two domains of the yeast plasma membrane has been suggested on the basis of separation by either low pH aggregation [3] or density gradient centrifugation [4]. The fraction that was not aggregated by low pH has a high content of both high molecular weight (150–250 kDa) glycoprotein and ergosterol [3]. The relationship between this domain, the fractions separated on the basis of density [4] and the plasma membrane H^+-ATPase, a major membrane protein [5], were not clarified. In addition, a 115 kDa glycoprotein has been reported to be tightly associated with the ATPase [6], raising the possibility that it is a subunit of the enzyme.

We have reinvestigated these problems by utilizing specific antibodies against the plasma membrane H^+-ATPase and a mutant lacking the associated glycoprotein. Our results indicate that both the ATPase and the 115 kDa glycoprotein are present in two domains of the yeast plasma membrane with different density. We confirm the association of the glycoprotein with the purified ATPase but the activity of the enzyme is normal in the mutant lacking the glycoprotein.

Materials and Methods

Yeast strains and growth conditions. We have utilized two strains of the yeast *Saccharomyces cerevisiae* kindly provided by Prof. H. Riezman, Biozentrum, Basel. Strain RH273-1B is *Mat a, his4, leu2, ura3, bar1* and strain RH273-1A is a derivative where the gene of a surface glycoprotein (GAS1) has been disrupted by insertion of the LEU2 gene [7]. These two strains are referred below as wild type and mutant, respectively. Cells were grown in YPD medium [8] to late-exponential phase (absorbance at 660 nm, 2–3), harvested by centrifugation and washed with water.

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Homogenization and isopycnic gradient centrifugation. Yeast cells were homogenized by shaking with glass beads, the cellular debris and nuclei removed by low speed centrifugation and a crude membrane fraction was prepared by centrifugation for 30 min at 15000 rev/min in a Sorvall SS-34 rotor [9]. Membranes (15 mg protein, 0.5 ml) were applied to 12 ml continuous sucrose gradients (from 33 to 53%, w/w). Gradients were formed by layering 4 ml each of 53, 43 and 33% sucrose and allowing diffusion for 3 h with closed tubes in the horizontal position [10]. After overnight centrifugation at 2°C and 30000 rev/min in a Beckman rotor SW 40 Ti, fractions of 0.7 ml were collected from above.

Biochemical methods. Protein was determined with the Bio-Rad Protein Assay Reagent utilizing bovine gamma-globulin as standard. Plasma membrane H^+-ATPase was assayed at pH 5.7 as the activity resistant to azide, molybdate and nitrate but sensitive to diethylstilbestrol [9]. Plasma membrane ATPase was purified by first isolating plasma membranes in a discontinuous sucrose gradient (43.5/53.5%, w/w) and then solubilizing the ATPase with zwittergent-16 and sedimenting it in aggregated form through a glycerol gradient as described [9].

Polyacrylamide gel electrophoresis in sodium dodecylsulfate [11] was performed in gels 1 mm thick with lanes of 3 mm and either 8 or 6% acrylamide. Samples were prepared by incubation at 37–42°C in sample buffer [11] containing sodium dodecylsulfate and the proteinase inhibitors phenylmethylsulfonylfluoride (1 mM) and chymostatin (50 μg/ml). Boiling in the presence of sodium dodecylsulfate aggregates the ATPase and endogenous proteinases are activated by this detergent at low temperature [9]. Semi-dry electroblotting to nitrocellulose [12], reversible protein staining with Ponceau S [13], staining of concanavalin A-binding proteins with peroxidase [14] and immunodecoration of blotted proteins with a second antibody coupled to alkaline phosphatase [15] were as described. When indicated, 50 mM α-methylmannoside was included with the concanavalin A.

Total carbohydrate was determined by the phenol-sulfuric acid method [16] after precipitation with 15% trichloroacetic acid to remove soluble sugars. Proton pumping in whole cells [17] and in proteoliposomes reconstituted with purified ATPase and soybean phospholipids [9] was measured as described. Treatment with endoglycosidase H (endo-β-*N*-acetylglucosaminidase, Boehringer, 15 mU) was performed with 6 μg purified ATPase and 7.5 μg sodium dodecylsulfate in 20 μl 50 mM succinate-Tris buffer (pH 5.5). After 30 min at 37°C samples were processed for electrophoresis.

Antibodies. A rabbit polyclonal antibody against the 56 carboxyl-terminal amino acids of the yeast ATPase was made by expressing in *Escherichia coli* this portion of the gene [18] as a fusion protein with β-galactosidase [19] (Monk, B.C. and Serrano, R., manuscript in preparation). The antibody was purified by binding to blotted ATPase and elution as described [20]. A mouse monoclonal antibody (AT2-1-A3) was selected from a collection of monoclonals against the yeast ATPase made in collaboration with Prof. E. W. Weiler, University of Bochum, F.R.G. It reacts with an epitope located within the first 300 amino acids of the ATPase (Serrano, R., unpublished data). Standard hybridoma technology [21] was utilized and culture supernatant was employed without purification.

Immunogold electron microscopy. Yeast cells were fixed with 0.5% glutaraldehyde for 15 min at room temperature, centrifuged and resuspended in 2.1 M sucrose. After 1 h the cells were concentrated by centrifugation and a drop from the pellet transferred to a sectioning stub and shock-frozen in liquid nitrogen. Sections 60–100 nm thick were cut with a Reichert Ultracut FC4 at −100°C using glass knives and mounted on 200 mesh formvar carbon-coated grids. Labeling with monoclonal AT2-1-A3 (1/50 dilution) and protein A-gold (9 nm size) was as described by Griffith et al. [22] except that fish-skin gelatin (0.16%) was utilized in all solutions to block non-specific binding [23]. Grids were postembedded in methyl cellulose containing 0.3% uranyl acetate [22] and viewed on a Philips 400 T electron microscope at 80 kV.

Results

The laboratory of H. Riezman at Basel has recently constructed a yeast strain with a disruption of the gene encoding a 125 kDa surface glycoprotein and which exhibits altered levels of other surface glycoproteins [7]. We have found that the 115 kDa glycoprotein associated with the ATPase is missing in this mutant and therefore it offers a direct approach to investigate relationships between surface glycoproteins, domains of the plasma membrane [3] and the plasma membrane ATPase [6].

Membranes were prepared from both the wild type and the mutant strains and fractionated by isopycnic centrifugation in sucrose gradients. The results of Fig. 1 indicate that in homogenates from wild-type yeast (part A) there are two separate peaks of plasma membrane ATPase at densities 1.173 (39.5% sucrose, w/w, 1/3 of total ATPase activity) and 1.223 (49.5% sucrose, w/w, 2/3 of total ATPase activity). The heavier ATPase peak is coincident with a small peak of protein and the specific ATPase activity is relatively high (0.4–0.5 units/mg). The lighter ATPase peak overlaps with the major protein peak and therefore the specific ATPase activity is relatively low (about 0.1 units/mg). In the mutant devoid of glycoprotein (part B) the heavier peak of ATPase shifts to lower densities (43–45% sucrose,

w/w, density 1.19–1.20) and it spreads as a shoulder of the lighter peak.

Fractions from the sucrose gradients containing equal amounts of protein were analyzed by sodium dodecylsulfate gel electrophoresis and stained for protein with Coomassie blue R-250 (Fig. 2, upper panels) and for carbohydrate with concanavalin A and peroxidase (Fig. 2, middle panels).

The ATPase band of about 105 kDa is easily identified in the Coomassie blue stained gels (upper panels). In the wild-type strain the ATPase is highly enriched in fractions from the high density part of the gradient (fractions 14–18, upper panel, part A), in agreement with the high specific activity of the enzyme in these fractions (see above). A major glycoprotein of about 115 kDa (middle panel, part A) is also enriched in fractions 12–18. This glycoprotein is missing in the gradient from the mutant (middle panel, part B). In this strain the 105 kDa ATPase band is enriched in fractions of lower density than in the case of the wild type (fractions 13–17, upper panel, part B). This is in good accordance with the activity determinations of Fig. 1B. It must be pointed out that the molecular weight of the ATPase as deduced from the sequence of the gene [18] is 99.5 kDa. The apparent molecular weight deduced from its migration in sodium dodecylsulfate gels is slightly higher (105 kDa).

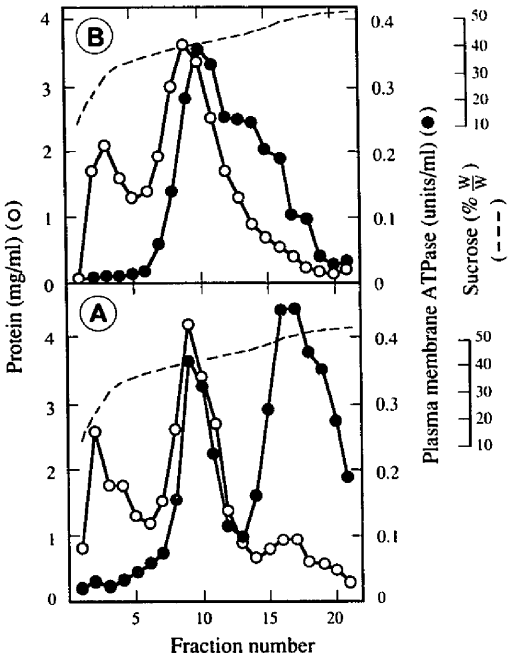

Fig. 1. Distribution of protein and plasma membrane H^+-ATPase after isopicnic centrifugation on sucrose gradients. (A) Membranes from wild-type strain. (B) Membranes from mutant without 115 kDa glycoprotein.

TABLE I

Purification of plasma membrane ATPase from wild-type and mutant strain

Results of a typical experiment starting from 20 g fresh weight of cells is shown. n.d., non determined.

Fraction	Protein (mg)	ATPase [a] (units/mg protein)	Carbohydrate (mg/mg protein)
Wild-type strain			
Crude membranes	510	0.14	nd
Purified membranes [b]	20	0.33	0.85
Purified ATPase	3.8	3.9 [c]	0.50
Mutant wihtout 115 kDa glycoprotein			
Crude membranes	525	0.16	nd
Purified membranes [b]	14	0.27	0.50
Purified ATPase	1.6	3.2 [c]	0.30

[a] Unit of activity correspond to 1 μmol ATP hydrolyzed per min.
[b] Fraction recovered between 43.5 and 53.5% (w/w) sucrose layers [9].
[c] Assayed in the presence of soybean phospholipids [9].

The presence of ATPase antigen in the different fractions of Fig. 1 was investigated with specific antibody against yeast plasma membrane H^+-ATPase. Although we have developed polyclonal antisera against the whole ATPase [18], immunogold labeling of yeast sections indicated that these antisera react with cell wall polysaccharides even after affinity purification (Ferguson, C., unpublished data). The apparent glycosylation of the ATPase (see below) may explain these results. Therefore, two novel antibodies were utilized. One is a rabbit polyclonal against the carboxyl-terminal domain of yeast ATPase expressed in *Escherichia coli* (Monk, B.C. and Serrano, R., unpublished data). The other is a mouse monoclonal recognizing an epitope within the amino-terminal third of the ATPase. As indicated in Fig. 3, both antibodies specifically recognize the 105 kDa ATPase polypeptide of purified preparations and crude membrane fractions.

The immunodecoration of blots from gradient fractions with the monoclonal antibody is shown in the lower panels of Fig. 2. Similar results were obtained with the anti-carboxyl terminus antibody. In order to estimate the total amount of ATPase, equal volumes from each fraction and not equal amounts of protein were applied. The distribution of antigen reacting with anti-ATPase antibody parallels the distribution of ATPase activity (Fig. 1) in both the wild-type and mutant strain.

The two membrane fractions containing plasma membrane ATPase could correspond either to domains of the plasma membrane or to different cellular membranes. Immunogold electron microscopy of frozen yeast

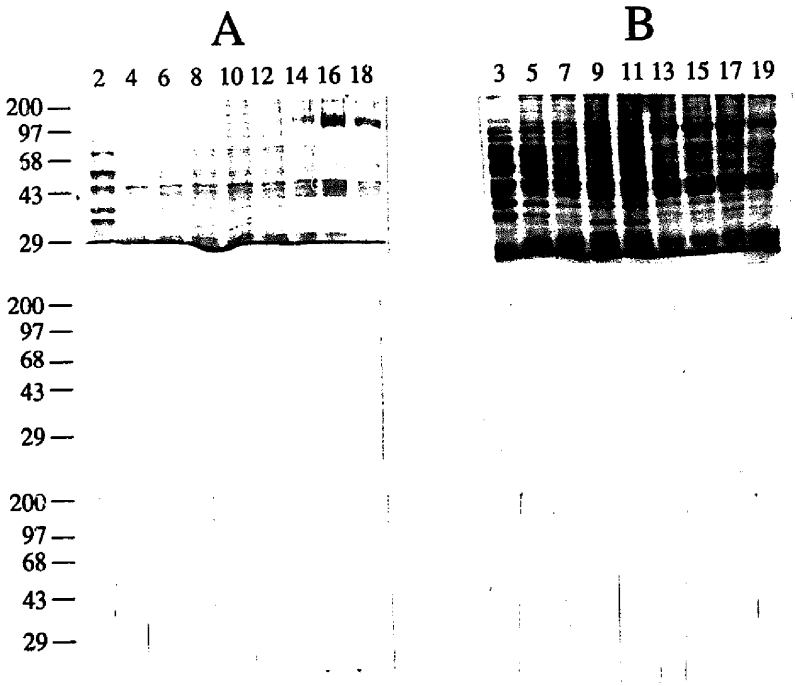

Fig. 2. Distribution of protein (upper panels), carbohydrate (middle panels) and ATPase antigen detected with monoclonal antibody AT2-1-A3 (lower panels) after sodium dodecylsulfate gel electrophoresis of alternate fractions from the gradients of Fig. 1. A and B correspond to the gradients from the wild-type and mutant strains, respectively, like in Fig. 1. The corresponding fraction number in Fig. 1 is indicated on top of the lanes and the position of molecular weight standards is at the left ($M_r \times 10^{-3}$). Gels contained 8% acrylamide. In the upper and middle panels 4 μg total protein were applied from each fraction, with the exception that 2 μg were applied from fractions 18 and 19. Therefore, the enrichment of both the ATPase and glycoprotein bands can be estimated. In the lower panels, 3 μl were applied from each fraction and therefore the total amount of ATPase antigen can be estimated.

sections (Fig. 4) indicates that most (> 95%) of the ATPase is present at the external membrane. There is no significant pool of plasma membrane ATPase in internal membranes and, therefore, the first explanation should apply. It is interesting that frozen sections of whole yeast cells are usually devoid of cell wall (Fig. 4). Cell wall remnants are present in the preparations, suggesting that they are detached during sectioning. No gold particles were observed in these cell wall fragments (not shown).

The plasma membrane ATPase was purified from both the wild type and the mutant without 115 kDa glycoprotein (Table I). Recovery was lower in the case of the mutant because of the lower density of the plasma membrane. Only membranes equilibrating between 43.5 and 53.5% (w/w) sucrose are recovered during purification to avoid mitochondrial contamination [9]. We confirm the observation [6] that the glycoprotein of about 115 kDa copurifies with the ATPase (Fig. 5 A and B). This glycoprotein could not be detected by Coomassie blue R-250 staining, suggesting a high ratio of carbohydrate to protein. As expected, the glycoprotein was absent in ATPase preparations from the mutant (Fig. 5 C and D). Therefore, the question could be asked whether the tightly-associated glycoprotein is important for ATPase function. The purified ATPases from both strains have similar ATP hydrolytic activities (Table I) and when reconstituted into liposomes exhibited similar proton pumping activities (Fig. 6A). In addition, proton pumping in whole cells, which reflects the 'in vivo' activity of the ATPase [5], was similar in both strains (Fig. 6B).

Some carbohydrate (60% of wild type) is still present in the purified ATPase from the mutant (Table I). As indicated in Fig. 5D, part of this carbohydrate may be attached to the ATPase because it can bind concanavalin A. The 115 kDa glycoprotein migrates during electrophoresis very close to the ATPase and good resolution could only be obtained in gels of 6% acrylamide run for long times (Fig. 5B). Under these condi-

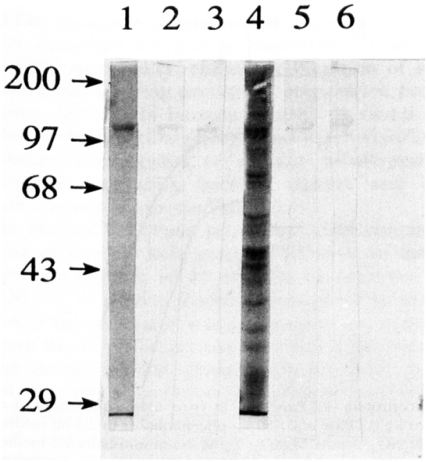

Fig. 3. Specificity of the antibodies against yeast ATPase. Lanes 1–3, 0.5 μg protein of purified ATPase from wild-type strain; lanes 4–6, 5 μg protein of crude membranes from wild-type strain. Lanes 1 and 4, gels stained for protein with Coomassie blue R-250. Lanes 2 and 5, proteins electroblotted from the gel to nitrocellulose and immunodecorated with affinity-purified antibody against the carboxyl-terminal domain of yeast ATPase expressed in *Escherichia coli*. Lanes 3 and 6, proteins electroblotted from the gel to nitrocellulose and immunodecorated with monoclonal antibody AT2-1-A3. The gels contained 8% acrylamide and the position of molecular weight standards is at the left ($M_r \times 10^{-3}$).

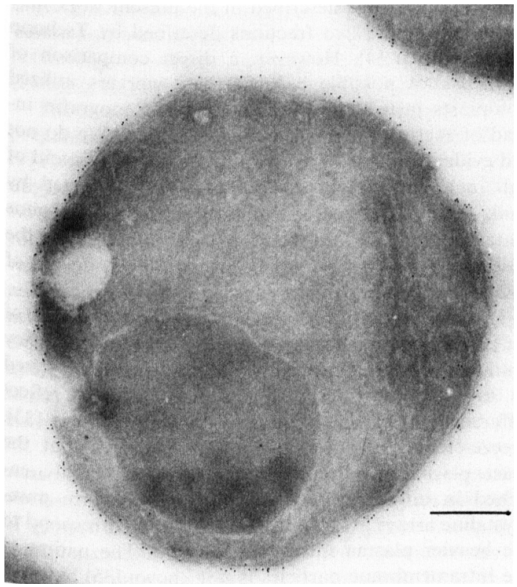

Fig. 4. Subcellular distribution of plasma membrane ATPase as studied by immunogold electron microscopy of frozen yeast sections. Monoclonal antibody AT2-1-A3 was employed. Bar represents 0.5 μm.

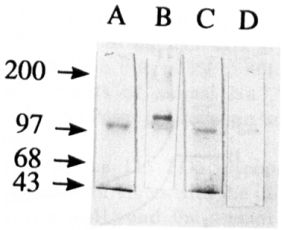

Fig. 5. Presence of glycoprotein in purified ATPase preparations. ATPase was purified from either wild-type yeast (A and B) or 115 kDa glycoprotein-less mutant (C and D) and 1 μg was applied per lane of a 6% acrylamide gel, run for twice the time needed by the Bromophenol blue to reach the end. Lanes A and C were stained for protein with Coomassie blue R-250. Lanes B and D were blotted to nitrocellulose and stained for carbohydrate with concanavalin A and peroxidase. The position of molecular weight standards is indicated at the left ($M_r \times 10^{-3}$).

tions it can also be shown in the wild-type strain that, in addition to the 115 kDa glycoprotein band, the 105 kDa ATPase band also binds concanavalin A.

Fig. 7 demonstrates that all the measured binding of concanavalin A is specific for *N*-glycosylated proteins because it is eliminated by α-methylmannoside (which competes for the lectin) and by pretreatment with endoglycosidase H (which splits asparagine-linked oligosaccharides [24]). In this experiment the ATPase and glycoprotein bands were not as clearly resolved as in the case of Fig. 5 because of shorter electrophoresis time. Treatment with endoglycosidase H generates two novel carbohydrate bands with apparent molecular weights of about 60 and 80 kDa (Fig. 7). The last one is missing in the mutant, suggesting that it is derived from the 115 kDa glycoprotein. The 60 kDa band must derive from glycoproteins present in both the wild-type and mutant strain, such as the ATPase band or some glycoprotein material migrating at the top of the gels. The later is probably underrepresented after blotting to nitrocellulose because of poor transfer (see Fig. 2, middle panel). It can be detected much better by direct staining of the gels with the periodic acid-Schiff reagent and corresponds to a diffuse band of 150–250 kDa [3,25,26].

Discussion

Our results indicate that the yeast plasma membrane is fragmented during homogenization into two domains of different density. Both domains contain plasma membrane ATPase and a major glycoprotein of about 115 kDa. It is important to point out that this conclusion could only be reached after subcellular localization of the ATPase by immunogold electron microscopy. A pool of enzyme in internal membranes, as seems to be the case for chitin synthetase [27,28] could have also explained the dual distribution of the ATPase in sucrose

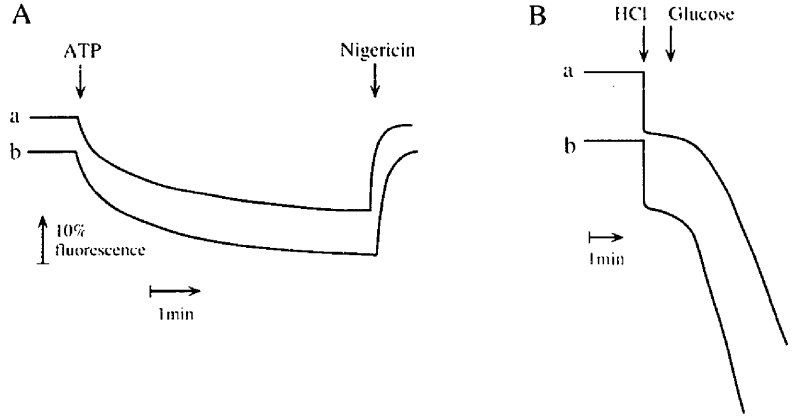

Fig. 6. Proton pumping activities of wild-type (b) and mutant devoid of 115 kDa glycoprotein (a). Panel A: 'in vitro' assay, quenching of acridine dye fluorescence by purified ATPase reconstituted in liposomes. Samples contained 10 μg ATPase and 450 μg phospholipids in 2.5 ml medium with 10 mM 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 6.0 with Tris, 25 mM K_2SO_4, 5 mM $MgSO_4$, 1 μM 9-amino-6-chloro-2-methoxyacridine and 1 μM valinomycin. Fluorescence was recorded at excitation and emission wavelengths of 415 and 485 nm. The addition of ATP (2.4 mM) and nigericin (2.5 μM) is indicated. Panel B: 'in vivo' assay, external acidification by whole cells. Yeast cells (30 mg fresh weight) were incubated in 2 ml medium containing 10 mM glycylglycine (pH 4.1 with HCl) and 0.1 M KCl. The addition of 200 nmoles HCl (for calibration) and 100 μl glucose 20% (to initiate proton pumping) is indicated.

gradients. Although export of the ATPase requires the secretory pathway [29], very few ATPase molecules are present in internal membranes during normal biogenesis.

It is interesting that another plasma membrane enzyme, 1,3-β-glucan synthethase, shows the same dual distribution pattern and the same densities that we have found for the ATPase [27]. All known plasma membrane proteins seem to be present in both domains. The heavier domain corresponds to the plasma membrane fraction initially described by Fuhrmann et al. [30] and utilized as source of purified ATPase [9]. The lighter domain has similar density to other cellular membranes such as mitochondria [30,31] and therefore it cannot be purified by isopycnic centrifugation. Other methods such as partitioning in aqueous polymer two-Phase systems could be employed [32].

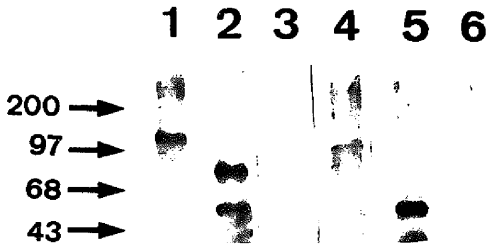

Fig. 7. Sensitivity of concanavalin A binding to α-methylmannoside and endoglycosidase H. Purified ATPase (1 μg) from either wild-type (lanes 1–3) or the mutant strain (lanes 4–6) was subjected to electrophoresis on 6% acrylamide gels, blotted to nitrocellulose and stained for carbohydrate with concanavalin A and peroxidase. Lanes 1 and 4: standard conditions. Lanes 2 and 5: pretreatament with endoglycosidase H. Lanes 3 and 6: α-methylmannoside present during incubation with concanavalin A. The position of molecular weight standards is indicated at the left ($M_r \times 10^{-3}$).

The two domains described in the present work may correspond to the two fractions described by Tschopp and Schekman [4]. However, a direct comparison of results is not possible because these workers utilized protoplasts instead of whole cells and Renografin instead of sucrose for the isopycnic gradients. We do not find evidence for a plasma membrane domain devoid of high molecular weight glycoproteins, as suggested by Rank et al. [3]. We have only investigated domains containing ATPase and therefore we cannot exclude the existence of regions of the plasma membrane devoid of both ATPase and glycoproteins.

The physiological significance and the biogenesis of the two plasma membrane domains is not known. They contain the same plasma membrane proteins analyzed up to now but their different densities may reflect differences in carbohydrate and/or lipid content [33]. Freeze-etching electron microscopy indicates that the yeast plasma membrane contains regions highly enriched in intramembrane particles, which form paracrystaline arrays [34]. These regions could correspond to the heavier plasma membrane domain. The nature of the intramembrane particles is not known [35] but they could correspond to major membrane proteins such as the ATPase.

The reduced density of plasma membranes from the

115 kDa glycoprotein-less mutant corresponds to the density described for plasma membranes from protoplasts [28]. Apparently, enzymatic digestion of surface polysaccharides during protoplast preparation produces the same decrease in buoyant density as found in the absence of the 115 kDa glycoprotein. Carbohydrates are the denser components of cellular membranes and therefore a correlation between density and carbohydrate content is expected [33].

The Na^+/K^+-ATPase of animal cells contains, in addition of the 100 kDa catalytic subunit, an essential glycoprotein subunit of 40–60 kDa of unknown function [36,37]. A similar situation was possible with the yeast ATPase because it was always purified in association with the 115 kDa glycoprotein [6,26]. By utilizing a mutant devoid of this glycoprotein we have demonstrated that this association is fortuitous and that the 115 kDa glycoprotein is not required for activity of the ATPase. The *Neurospora* plasma membrane ATPase is purified free of glycoprotein [6] and the catalytic subunit of 105 kDa is the only requirement for activity [38]. It seems likely that these observations can be generalized to other plasma membrane H^+-ATPases from fungi and plants. The catalytic subunit of 105 kDa is probably the only requirement for activity within this enzyme family, unless other as yet undetected essential species associated with the purified ATPase do exist. The purified ATPase from the mutant is still contaminated by high molecular weight (150–250 kDa) glycoproteins (Fig. 7). Although it is not likely that these glycoproteins are required for activity, this contamination may explain the difficulties encountered in producing two-dimensional crystals of yeast ATPase (Serrano, R. and Leonard, K., unpublished data).

The presence of the 115 kDa glycoprotein in ATPase preparations has made difficult to establish if the ATPase itself is a glycoprotein because of the close migration of both proteins in gels [26]. In fact, using ATPase purified from the mutant devoid of this glycoprotein it is clear that the ATPase band binds concanavalin A. The same result was obtained with ATPase purified from wild-type yeast by utilizing 6% acrylamide gels and long electrophoresis times which resolve the ATPase from the glycoprotein. In addition, the carbohydrate associated with the ATPase band behaves identically to the ATPase antigen in terms of aggregation by boiling in sodium dodecylsulfate (Monk, B.C. and Perlin, D.S., unpublished data). Because of the posibility of contaminating glycoproteins, unambiguous conclusions can only be reached after peptide mapping of the point of glycosylation. In the ATPase sequence there is a single asparagine glycosylation site predicted to be located at the cell surface [18]. Although there is no significant change in electrophoretic mobility during biogenesis of the ATPase [29], given the large size of the polypeptide, minor changes in mobility may not have been detected. The degree of glycosylation of the ATPase, as compared to the 115 kDa glycoprotein, is quite low (Fig. 5B). It is interesting to note that the 105 kDa catalytic subunits of animal Na^+/K^+-ATPases [39] and H^+/K^+-ATPases [40] contain small amounts of carbohydrate.

The function of the 115 kDa glycoprotein is not known. Its synthesis correlates with the initiation of the cell division cycle [6,41] and the mutant without this glycoprotein exhibits a slightly reduced growth rate [7]. As demonstrated by the present work, the growth deffect is not related to its interaction with the ATPase. It is not clear if the 115 kDa glycoprotein associated with the ATPase corresponds to the Gas1p glycoprotein of 125 kDa isolated and disrupted at the gene level by the group of Riezman [7]. This glycoprotein is anchored to the membrane via covalent linkage to an inositol-containing glycolipid. As the ATPase preferentially interacts with acidic phospholipids [42], this could explain its association with the glycoprotein. However, the Gas1p glycoprotein exhibits a greater molecular mass than the ATPase-associated glycoprotein and it can be stained with Coomassie blue R-250, which does not stain the 115 kDa glycoprotein. Disruption of the GAS1 gene affects the expression of other yeast proteins [7] and therefore it is possible that the lack of 115 kDa glycoprotein in the mutant is a secondary effect. Information about the sequence of the 115 kDa glycoprotein is needed to clarify this point.

Acknowledgements

We thank Prof. H. Riezman for providing the yeast strains and for critical reading of the manuscript. M.R. was on leave from Estacion Experimental del Zaidin, CSIC, Prof. Albareda 1, 18008 Granada, Spain.

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