Differential Exposure of Surface Epitopes in the β-Strand Region of LOOP1 of the Yeast H⁺-ATPase during Catalysis*

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The plasma membrane H⁺-ATPase of yeast assumes distinct conformational states during its catalytic cycle. To better understand structural changes in the LOOP1 domain, a catalytically important cytoplasmic loop segment linking transmembrane segments 2 and 3, surface epitopes were examined at different stages of catalysis. A polyclonal rabbit antibody was prepared to a fusion protein consisting of LOOP1 and the maltose binding protein. This antibody was affinity-purified to produce a LOOP1-specific fraction that could be used in competition enzyme-linked immunosorbent assays to assess surface exposure of the LOOP1 epitopes. It was found that in an E₁ conformation stabilized with either adenine 5′-(β,γ-imino)triphosphate (AMP-PNP) or ADP, less than 10% of the LOOP1 epitopes were accessible on native enzyme. However, when the enzyme was stabilized in an E₂-state with ATP plus vanadate, approximately 40% of the surface epitopes on LOOP1 became accessible to antibody. The remaining 60% of the LOOP1 epitopes were fully occluded in the native enzyme and never showed surface exposure. Enzyme-linked immunosorbent assays utilizing fusion proteins consisting of LOOP1 subdomains demonstrated that all of the available epitopes were contained in the β-strand region (Glu-195—Val-267) of LOOP1. The epitopes that were differentially exposed during catalysis were included in regions upstream and downstream of the highly conserved TGES sequence. Our results suggest that during catalysis either the β-strand region of LOOP1 or an interacting domain undergoes substantial structural rearrangement that facilitates epitope exposure.

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1 The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; AMP-PNP, adenosine 5′-(β,γ-imino)triphosphate; MBP, maltose-binding protein; bp, base pairs.
A: Full LOOP1 segment

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\text{malE} \quad \alpha \beta \alpha \quad \text{lacZ}
\]

EcoRI (E162) \quad SpeI (L297)

B: N-terminal half LOOP1

\[
\text{malE} \quad \alpha \beta \quad \text{lacZ}
\]

SmaI (E162-E193) \quad V265 (L297)

C: Fused α-helical region

\[
\text{malE} \quad \alpha \beta \quad \text{lacZ}
\]

D: β-strand region

\[
\text{malE} \quad \alpha \beta \quad \text{lacZ}
\]

EcoRI (E195) \quad Hind III (V267)

**FIG. 1.** Schematic diagram showing construction of LOOP1 fusion proteins. The PMA1 region corresponding to LOOP1 and its partial subdomains were subcloned into the C-terminal portion of the maltose-binding protein (malE) in expression vector pMAL-c2 (see “Experimental Procedures”). The fusion proteins consisted of the full LOOP1 (Glu-162–Leu-297) (A), the N-terminal portion of LOOP1 from Glu-162 to Val-237 (B), the flanking α-helical regions of LOOP1 in which Glu-162–Glu-193 was fused in-frame with Val-263–Leu-297 (C), and the β-strand portion of LOOP1 from Glu-195–Val-267 (D).

epitopes in the β-strand subdomain of LOOP1 at different stages of the catalytic cycle.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Cultures**—The yeast strains utilized in this study were wild type Y55 (HO gal83 MALI SUC1) and a pma1 mutant strain pma11-S368F (22, 23). All yeast cultures were grown in YEP medium (1% yeast extract, 2% peptone, and 2% dextrose, pH 5.7) at 22 °C to mid-log phase (A660 ~ 3).

**Construction of Maltose-binding Protein (MBP)-LOOP1 Fusion Proteins**—Fig. 1 shows a schematic map of the construction of different fusion proteins between the MBP and LOOP1. The LOOP1 segment of PMA1 corresponding to amino acids Glu-162–Leu-97 (135 amino acid residues) was excised from plasmid pG2W021 (7) with restriction endonucleases EcoRI and SpeI. The resulting 0.4-kilobase fragment was subcloned into the EcoRI and XbaI sites of commercial vector pMAL-c2 (New England BioLabs). The resulting vector, pMB100, contained the malE gene (encoding MBP) fused with LOOP1. Vector pMB100 was treated with endonuclease SalI to liberate the C-terminal half of LOOP1, and the linearized vector was ligated to produce a new vector, pH100, which contained malE fused with the first half of LOOP1 (Glu-162–Glu-193). Two Smal I sites were introduced into the LOOP1 region of pMB100 at positions 1549 bp (Glu-193) and 1758 bp (Val-263). The intervening fragment containing the β-strand region was excised after digestion with Smal. The linearized vector was ligated to yield a new vector, pDSY100, that contained malE fused to the linked α-helical flanking ends of LOOP1. Finally, new restriction enzyme sites for EcoRI and HindIII were introduced into the LOOP1 region of pMB100 at nucleotide positions 1556 bp (Glu-195) and 1771 bp (Val-267), respectively. The β-strand-containing fragment was excised by digestion with EcoRI and HindIII and cloned into the equivalent site of pMAL-c2 to yield new vector pDSY101.

**Expression and Isolation of the Fusion Proteins**—The various fusion vector constructs were transformed into Escherichia coli strain XL2-Blue ultracompetent cells (Stratagene). Transformants were grown to early log phase in LB medium containing 0.2% glucose and 150 μg/ml ampicillin at 37 °C. Isopropyl-1-thio-β-D-galactopyranoside (3 mM) was added to the medium to induce fusion protein expression, and the cells were grown for an additional 6–7 h at 37 °C (early stationary phase). Cells were harvested by centrifugation at 4000 × g and washed by resuspension in column buffer consisting of 20 mM-Tris-HCl, pH 7.4, 0.2 mM NaCl, 1 mM EDTA, 10 mM mercaptoethanol, and 1 mM NaN3, and centrifuged as above. The washed cells were resuspended in 40 ml of column buffer containing 1 mM phenylmethylsulfonfyl fluoride and lysed in a French pressure cell at 20,000 p.s.i. The lysate was centrifuged for 10 min at 4500 × g to remove the cell debris. NH4SO4 at 0.35 g/ml was added to the supernatant, and the suspension was stirred for 1 h at 4 °C. The precipitate was centrifuged at 4,500 × g for 10 min, and the pellets were resuspended with 5 ml of column buffer. Amylose resin (3 mg of MBP/ml of bed volume of amylose resin) was added to adsorb the MBP fusion protein by incubating at 22 °C for 1 h. The bound amylose resin with MBP fusion protein was washed three times with excess column buffer and brief centrifugation, as above. The bound fusion protein was eluted in a 3-ml suspension with maltose (final concentration). The resin suspension was then centrifuged at 4,500 × g for 10 min. Supernatant containing the MBP fusion protein was centrifuged again to remove traces of residual amylose resin.

**Preparation of LOOP1 Antibodies**—The MBP-LOOP1 fusion proteins were evaluated for purity on a 10% SDS-polyacrylamide gel. A band corresponding to the MBP-LOOP1 fusion protein (M056,000) was excised from the gel and used to inoculate New Zealand White rabbits at Pocono Rabbit Farms. The resulting whole serum contained antibodies to both MBP, as the predominant antigen, and LOOP1. The contaminating antibodies to MBP were removed by affinity chromatography. A MBP affinity resin was prepared by coupling purified MBP (5 mg/ml) to Affi-Gel-10 resin, as described by the manufacturer (Bio-Rad). The coupled Affi-Gel complex was conditioned with 10 ml of 100 mM sodium acetate, pH 4.9, followed by three washes with 10 ml of antibody binding buffer (Pierce IgG Ab Purification Kit). Whole serum (4.5 ml) was added to excess resin along with 10 ml of antibody binding buffer, and the suspension was incubated 1 h at 37 °C. The remaining serum containing unbound LOOP1 antibody was collected, and the IgG fraction was concentrated on a protein A column (Pierce). Antibodies to LOOP1 were further affinity-purified, as described by Seto-Young et al. (24). The LOOP1 antibodies were shown by Western blot analysis to bind to both purified LOOP1 and whole H+–ATPase but not to purified maltose binding protein.

**ELISA Competition Assay**—Purified LOOP1 fusion antigens (1 pmol) in 50 μl of 94 mM NaCO3 buffer, pH 9.8, and 1 mM NaN3, were added to wells of a microtiter plate (Nunc-ImmuNo-Plate-MaxiSorb™) and incubated at 4 °C for 16 h. The plate was washed several times with TTBS buffer consisting of 10 mM Tris, pH 7.5, 0.05% Tween 20, and 150 mM NaCl. A blocking solution consisting of 100 μl of TTBS plus 3% bovine serum albumin was added to each well, and the plate was incubated for 1 h at 22 °C. Antigen competition was performed in a 100-μl volume containing 20 μl of TTBS, 50 μM MBP-LOOP1, 50 μM MalE, 1 mM NH4Cl, 0.5 mM Na2CO3, and 3% bovine serum albumin. Additions of deoxycholate-enriched H+–ATPase (0–37.5 pmol), affinity-purified LOOP1 antibody, and different hydrolase substrates were made as indicated in the text. The reaction was incubated at the 22 °C for 1 h, and the wells were washed three times with TTBS. A 100-μl suspension of TTBS and 3% bovine serum albumin containing goat anti-rabbit IgG conjugated with alkaline-phosphatase (1/300 dilution) was added to each well and incubated for 1 h at 22 °C. The wells were washed, as described above. LOOP1 affinity antibody bound to the wells was detected by colorimetric assay using Sigma 104-phosphatase substrate (Sigma), as described by the manufacturer. The p-nitrophosphate formation was monitored in a SLT Spectra ELISA microplate reader at 405 nm.

**Other Procedures**—Deoxycholate-enriched H+–ATPase was prepared by sequential extraction of plasma membranes with deoxycholate and washing with KCl medium, as described previously (24). SDS-polyacrylamide gel electrophoresis and semi-dry electrophoretic analysis of LOOP1 and the sub-domain fusion proteins were performed as described previously (24). Protein concentration was determined by a modified Lowry method (25).

**RESULTS**

A fusion protein consisting of the LOOP1 region (Glu-162–Leu-297) of PMA1 fused to the C-terminal end of the MBP was purified and used to raise polyclonal antibodies in rabbits. Antibody specificity to the LOOP1 domain was demonstrated by affinity chromatography, as described under “Experimental Procedures.” The purified anti-LOOP1 antibodies were shown by Western blotting to recognize only the LOOP1 domain of the fusion protein; they did not cross-react with MBP. The antibodies cross-reacted strongly with intact H+–ATPase in both Western blots and ELISA assays, which enabled them to be used as...
be seen that LOOP1 was challenged with increasing amounts of itself. It can to dissect the regions of the LOOP1 that express the changing fusion protein bound in the competition assay, it is also possible reaction cycle. Finally, by altering the LOOP1 portion of the exposure within LOOP1 with distinct stages of the catalytic condition of inhibitors, it is possible to correlate changes in epitope most importantly, since the enzyme can be locked into defined probes of the LOOP1 domain in the native enzyme.

The catalytic reaction cycle of the typical P-type enzymes is characterized by the formation of distinct conformational states, which are known to be manifested by changes in the large cytoplasmic domain (16, 26–28). A competition ELISA assay was developed to examine epitope exposure of the LOOP1 domain at distinct stages of catalysis. In this assay, LOOP1 fusion protein is bound to the well of a microtiter plate. Antibody is added at fixed amount in free solution along with increasing amounts of a challenge protein. Exposed epitopes on the challenge protein compete with epitopes on the bound LOOP1 protein for antibody. The advantage of this system is that it is extremely sensitive and allows the native, catalytically active H\(^{+}\)-ATPase to be evaluated as a challenge protein. Most importantly, since the enzyme can be locked into defined conformations by altering substrate availability or by the addition of inhibitors, it is possible to correlate changes in epitope exposure within LOOP1 with distinct stages of the catalytic reaction cycle. Finally, by altering the LOOP1 portion of the fusion protein bound in the competition assay, it is also possible to dissect the regions of the LOOP1 that express the changing epitopes.

Fig. 2 shows a typical competition assay in which bound LOOP1 was challenged with increasing amounts of itself. It can be seen that <1 pmol of LOOP1 fusion protein was required to give ~90% competition with bound LOOP1. Complete competition was observed at higher levels of challenge protein (not shown). When purified H\(^{+}\)-ATPase was used as the challenge protein, it was found that the enzyme competed with bound LOOP1, but the extent of competition was highly dependent on the conformational state of the enzyme. In the presence of 2 mM AMP-PNP and 2 mM EDTA, the enzyme is known to adopt an E\(_1\) conformational state (19). Fig. 3 indicates that under these conditions the H\(^{+}\)-ATPase competed poorly with bound LOOP1, showing a saturable competition of ~10% at the highest level. Comparable results were obtained in the presence of 7.5 mM ADP (data not shown). In contrast, when the enzyme was locked in an E\(_2\) conformational state by incubation with 7.5 mM MgATP and 200 \(\mu\)M VO\(_4\), a classical transition state inhibitor of P-type enzymes, the H\(^{+}\)-ATPase competed much more effectively with bound LOOP1, typically showing about 40% competition. MgATP alone also promoted competition but not as effectively as that observed in the presence of vanadate (not shown). This latter result was complicated by the fact that extensive hydrolysis of substrate occurred during the incubation period. The differential effects observed were absolutely dependent on active enzyme, since there was no difference in competition observed when LOOP1 was challenged against itself in the presence of the various conditions that favor either E\(_1\) or E\(_2\). In addition, a S368F mutant enzyme, which has ~500-fold less sensitive to vanadate (29), weakly competed for epitopes on bound LOOP1 relative to wild type enzyme, since it assumes a steady-state conformation that disfavors VO\(_4\) interaction. However, in the presence of acetyl phosphate, which has been shown to promote an E\(_2\) state (18) and to increase the sensitivity of the S368F enzyme to VO\(_4\) (18), the enzyme showed enhanced competition in the presence of VO\(_4\) relative to that observed in ATP-containing medium (Fig. 4). These results support the notion that the E\(_2\) conformational state of the enzyme exposes more epitopes in the LOOP1 than the E\(_1\) conformational state. These experiments further indicate that only ~40% of the epitopes present on the LOOP1 fusion protein are available for interaction in native enzyme. The remaining 60% are most likely occluded within the 3-dimensional structure of LOOP1. The behavior observed for enzyme in the E\(_1\) and E\(_2\) states indicates that a substantial fraction of the surface-accessible epitopes are differentially exposed during the catalytic cycle.

The LOOP1 segment could be dissected to explore more precisely the location of the epitopes involved in conformation-specific binding. The fusion maeE gene constructs with intact LOOP1, and its sub-domains are shown in Fig. 1 (see “Experimental Procedures”). The four constructs include the full LOOP1 (Glu-162–Leu-297), the N-terminal half of LOOP1 (Glu-162–Ala-235), the central \(\beta\)-strand domain (Glu-195–Val-267), and the \(\alpha\)-helical flanking region (Glu-163–Asn-193/Gly-263–Leu-297). All of the fusion proteins except the \(\beta\)-strand domain-containing fragment were expressed at high levels and could be purified to near homogeneity. Special expression conditions involving early log phase cells and short induction times were required to yield a suitable product for the \(\beta\)-strand protein, which even under the best circumstances showed approx-
approximately 50% proteolysis with numerous fragments in Western blots. Fig. 5 shows interaction of the whole anti-LOOP1 antibody with the various fusion proteins in a standard ELISA-type assay. It can be seen that antibody bound strongly to the full LOOP1 and the N-terminal half-LOOP1. The β-strand-containing fragment showed high affinity binding at low protein levels, comparable with the other peptides but rapidly saturated at about 35% of the full LOOP1 level due to proteolysis. In contrast, the α-helical flanking region-containing peptide showed negligible interaction with the antibody, making it unlikely that significant anti-LOOP1 epitopes were present in this region of the protein. This result was confirmed by Western blot analysis, which clearly showed a lack of binding of the anti-LOOP1 antibodies to this peptide. These results suggest that all of the epitopes present in the anti-LOOP1 antibody are present in the 72-amino acid β-strand domain. A destabilized protein is likely to be more susceptible to endogenous cell proteases.

ELISA competition assays were performed using fusion proteins containing the N-terminal half-LOOP1, β-strand domain, and α-helical regions. Fig. 6 shows that the N-terminal half-LOOP1 showed the same type of differential effect with AMP-PNP and MgATP plus vanadate as the full LOOP1. It should be noted, however, that the extent of competition was about one-half of the full LOOP1, suggesting that epitope accessibility on LOOP1 may be evenly distributed along the β-strand domain. As expected, the partially degraded β-strand protein showed only a weak competition effect (not shown). The α-helical-containing peptide showed no competition, since antibody binding was barely detectable above base line.

DISCUSSION

Dynamic movements within P-type ATPases are an integral part of the overall catalytic reaction of these enzymes. Numerous studies have defined global changes in enzyme form that are associated with defined conformational states (28, 30). Yet, relatively few studies have dealt with localized changes in protein structure that occur during catalysis (16, 27, 31). An understanding of the partial reactions for nucleotide binding, phosphorylation-dephosphorylation, ion translocation and energy coupling requires an accounting of local dynamic events. The change in electron density observed in the apparent ATP binding groove on the Ca2+-ATPase in the presence and absence of nucleotide pointedly illustrates the need to understand such local changes (32). In this study, we have used antibodies
to the LOOP1 domain, which is bounded by TM2 and TM3, in a competition ELISA assay to examine surface-exposed epitopes that change during catalysis. It was found that epitopes in a 72-amino acid region from Glu-195 to Val-267, comprising the β-strand domain and including the highly conserved signature sequences VPGDII and TGES (1), were differentially exposed at distinct stages of the catalytic cycle. In an E1 conformation stabilized with AMP-PNP and EDTA or ADP, approximately 10% of the epitopes were exposed, indicating that this region was not highly surface-exposed. However, in an E2 state formed with MgATP and vanadate, approximately 40% of the total sites became available, suggesting a major change in this region resulting in surface exposure of epitopes. These results are consistent with a report by Serrano et al. (33) that a monoclonal antibody recognizing an epitope in this region of the yeast H+-ATPase could only react with the native enzyme (in an E2 state) after detergent treatment. Furthermore, they are qualitatively similar to the trypsin proteolysis study of Lutsenko and Kaplan (16) on the Na+-K+-ATPase in which the LOOP1 segment was involved in structural rearrangements upon phosphorylation or ion binding.

Movements in the β-strand region during catalysis are supported by several studies. In the Ca2+-ATPase, it is known that there is conformation-specific exposure of Arg-198 (26) and Glu-231 (31) in LOOP1, as revealed by proteolysis with trypsin and V8 protease, respectively. The role of the LOOP1 domain in catalysis has largely been inferred from the affects of perturbations in this region. It is known that mutations in the LOOP1 β-strand region can alter the progression of catalysis by blocking the interconversion of catalytic intermediates during E1-P-E2-P transition. In the yeast H+-ATPase, scanning alanine mutagenesis of conserved motifs in this domain revealed that overall enzyme function was highly sensitive to mutations in this region. Mutations in LOOP1 of the H+-ATPase also yield vanadate insensitivity (7, 13, 15), which has been linked to changes in phosphate binding (14). In the flanking α-helical regions of LOOP1, mutations I183A and H285Q resulted in partially uncoupled enzymes, suggesting that the LOOP1 participates in the overall coupling reactions (7, 15). These regions have been proposed to form a bundle-type organization, largely because targeted factor Xa proteolysis of a site around residue 275 had no effect on coupled proton transport, suggesting strong intramolecular interactions in this region (13).

In general, it has been difficult to prepare antibodies to the LOOP1 region (34). Surface exposure of an epitope around Arg-198 (T2 site) in the Ca2+-ATPase has been reported, and this site shows some latent exposure upon denaturation of the enzyme (21). The flexible proline-rich region containing the T2 site in Ca2+-ATPases is absent in partially uncoupled enzymes, suggesting that the LOOP1 participates in the overall coupling reactions (7, 15). These regions have been proposed to form a bundle-type organization, largely because targeted factor Xa proteolysis of a site around residue 275 had no effect on coupled proton transport, suggesting strong intramolecular interactions in this region (13).

Our data suggest that in the E2 state, with the nucleotide bound, the enzyme assumes a state in which most of the β-strand domain is shielded from surface exposure. However, after proton binding and phosphorylation, the LOOP1 structure is altered, and up to 40% of the epitopes in the β-strand domain are exposed. This notion for localized conformational breathing of LOOP1 is fully consistent with a recent model proposed by Lutsenko and Kaplan (16) for the Na+-K+-ATPase, based on a limited trypptic digestion study. It was found that in the presence of ADP or ATP the enzyme forms a compact, protease-resistant form, but when the enzyme is phosphorylated, it forms a less compact protease-sensitive form. It was proposed that in the presence of bound ATP, there is a tight interaction between the ATP binding domain and the membrane resulting in a compact structure. When transported ions bind, these interactions are relaxed, and upon phosphorylation, the cytoplasmic domain moves away from the membrane, resulting in a less compact molecule. After de-phosphorylation and ion release, the interactions increase, and the enzyme again forms a more compact structure.

Interestingly, an alternative model was recently proposed after an analysis of metal-catalyzed cleavage of the Na+-K+-ATPase at different stages of the catalytic cycle. It was suggested that there is an interaction between LOOP1 and the large central domain, and these domains interact strongly in an E2 state but move apart in an E1 state. The implication is that the E2-stabilized enzyme forms a tightly packed structure. Conformation-dependent cleavage at and around the conserved TGES sequence in this study and in previous studies using limited trypptic digestion (26, 35), is consistent with such a suggestion.

Apparent differences between these models may reflect limitations of the probing mechanisms. In our case, only surface exposure of the β-strand domain can be inferred given the large size of the antibody probe. A similar conclusion can also be drawn about limited proteolysis studies with trypsin, given its relative size. On the other hand, Fe2+-catalyzed peptide bond cleavage to a metal binding center should provide excellent information about closely opposed protein structure elements, provided that the reactive chemical species remain localized. To further reconcile the various models, it is possible that movements that expose one portion of the molecule could actually bring other portions closer together in a scissors-like mechanism. As shown in Fig. 7, the LOOP1 and major loop domains could be viewed as a splayed scissors close to the membrane. In the E1 state, the β-strand sector would be occluded because of close contact with the membrane surface, and the metal binding center would be loosely organized. In the E2 state, the domains would interact like a closing scissors, moving away from the membrane and thus exposing the epitopes on LOOP1. The metal binding center would become more organized, as the two domains interact strongly. Thus, surface sites in the β-strand domain would become more exposed in the more organized but less compact E2 state. In contrast, the E1 state appears likely that during catalysis, there is a substantial restricted to a small region around TGES.
state would appear more compact but less organized around the metal binding center.

In conclusion, our data suggest that the region around the highly conserved TGES sequence in the β-strand region of LOOP1 undergoes significant changes in solvent exposure during catalysis. This finding is consistent with recent models for interactions within this domain in which an interacting domain, most likely the large central domain, changes its position relative to LOOP1.

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REFERENCES
1. Lutsenko, S., and Kaplan, J. H. (1995) Biochemistry 34, 15607–15613
2. Wach, A., Schlesser, A., and Goffeau, A. (1992) J. Bioenerg. Biomembr. 24, 309–317
3. Cyrrklaff, M., Auer, M., Kuhlbrandt, W., and Scarborough, G. A. (1995) EMBO J. 14, 1854–1857
4. Toyoshima, C., Sasabe, H., and Stokes, D. L. (1993) Nature 362, 469–471
5. Capieaux, E., Rapin, C., Thines, D., Dupont, Y., and Goffeau, A. (1993) EMBO J. 14, 1854–1857
6. Portillo, F., and Serrano, R. (1988) EMBO J. 7, 1793–1798
7. Wang, G., Tamas, M. J., Hall, M. J., Pascual-Ahuir, A., and Perlin, D. S. (1996) J. Biol. Chem. 271, 25438–25445
8. MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985) Nature 316, 696–700
9. Stokes, D. L., Taylor, W. R., and Green, N. M. (1994) FEBS Lett. 346, 32–38
10. Clarke, D. M., Loo, T. W., and MacLennan, D. H. (1990) J. Biol. Chem. 265, 14088–14092
11. Andersen, J. P., Vilsen, B., Leberer, E., and MacLennan, D. H. (1989) J. Biol. Chem. 264, 21018–21023
12. Goffeau, A., de Meis, L. (1990) J. Biol. Chem. 265, 15503–15505
13. Wach, A., Supply, P., Dufour, J.-P., and Goffeau, A. (1996) Biochemistry 35, 883–890
14. Lutsenko, S., and Kaplan, J. H. (1994) J. Biol. Chem. 269, 4555–4564
15. Jorgensen, P. L., and Andersen, J. P. (1988) J. Membr. Biol. 103, 95–120
16. Wang, G., and Perlin, D. S. (1997) Arch. Biochem. Biophys. 344, 309–315
17. Addison, R., and Scarborough, G. A. (1982) J. Biol. Chem. 257, 10421–10426
18. Mandala, S. M., and Slayman, C. W. (1988) J. Biol. Chem. 263, 15122–15128
19. Tunwell, R. E. A., Conlan, J. W., Matthews, I., East, J. M., and Lee, A. G. (1991) Biochem. J. 279, 203–212
20. McCusker, J. H., Perlin, D. S., and Haber, J. E. (1987) Mol. Cell. Biol. 7, 4082–4088
21. Harris, S. L., Perlin, D. S., Seto-Young, D., and Haber, J. E. (1991) J. Biol. Chem. 266, 24449–24455
22. Seto-Young, D., Na, S., Monk, B. C., Haber, J. E., and Perlin, D. S. (1994) J. Biol. Chem. 269, 23888–23895
23. Markwell, M. A. K., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206–210
24. Andersen, J. P., and Jorgensen, P. L. (1985) J. Membr. Biol. 88, 187–198
25. Markwell, M. A. K., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206–210
26. Goffeau, A., and de Meis, L. (1990) J. Biol. Chem. 265, 15503–15505
27. Goldshleger, R., and Karlish, S. J. D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9596–9601
28. Gourmagentigh, E., Vignerone, L., Scarborough, G. A., and Ruysschaert, J.-M. (1994) J. Biol. Chem. 269, 27409–27413
29. Perlin, D. S., Harris, S. L., Seto-Young, D., and Haber, J. E. (1989) J. Biol. Chem. 264, 21857–21864
30. Moller, J. V., Juul, B., and le Maire, M. (1998) Biochim. Biophys. Acta 1286, 1–51
31. le Maire, M., Lund, S., Viel, A., Champeil, P., and Moller, J. V. (1990) J. Biol. Chem. 265, 1111–1123
32. Yonekura, K., Stokes, D. L., Sasabe, H., and Toyoshima, C. (1997) J. Biol. Chem. 72, 997–1005
33. Serrano, R., Monk, B. C., Villaba, J. M., Montesinos, C., and Weiler, E. W. (1993) Eur. J. Biochem. 212, 737–744
34. Colyer, J., Mata, A. M., Lee, A. G., and East, J. M. (1989) Biochem. J. 262, 439–447
35. Torok, K., Trinnaman, B. J., and Green, N. M. (1988) Eur. J. Biochem. 173, 361–367