Subunit a of the Yeast V-ATPase Participates in Binding of Bafilomycin*

Received for publication, August 17, 2005, and in revised form, October 6, 2005 Published, JBC Papers in Press, October 10, 2005, DOI 10.1074/jbc.M509106200

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Bafilomycin and concanamycin are potent and highly specific inhibitors of the vacuolar (H\(^+\))-ATPases (V-ATPases), typically inhibiting at nanomolar concentrations. Previous studies have shown that subunit c of the integral V\(_0\) domain participates in bafilomycin binding, and that this site resembles the oligomycin binding site of the F-ATPase (Bowman, B. J., and Bowman, E. J. (2002) J. Biol. Chem. 277, 3963–3972). Because mutations in F-ATPase subunit a also confer resistance to oligomycin, we investigated whether the a subunit of the V-ATPase might participate in binding bafilomycin. Twenty-eight subunit a mutations were constructed just N-terminal to the critical Arg\(^{735}\) residue in transmembrane 7 required for proton transport, a region similar to that shown to participate in oligomycin binding by the F-ATPase. The mutants appeared to assemble normally and all but two showed normal growth at pH 7.5, whereas all but three had at least 25% of wild-type levels of proton transport and ATPase activity. Of the functional mutants, three displayed \(K_i\) values for bafilomycin significantly different from wild-type (0.22 ± 0.03 nM). These included E721K (\(K_i\) 0.38 ± 0.03 nM), L724A (0.40 ± 0.02 nM), and N725F (0.54 ± 0.06 nM). Only the N725F mutation displayed a \(K_i\) for concanamycin (0.84 ± 0.04 nM) that was slightly higher than wild-type (0.60 ± 0.07 nM). These results suggest that subunit a of V-ATPase participates along with subunit c in binding bafilomycin.

The vacuolar (H\(^+\))-ATPases (or V-ATPases)\(^2\) are ATP-dependent proton pumps widely distributed among intracellular and plasma membranes of eukaryotic cells (1–11). They carry out ATP-driven transport of protons from the cytoplasm to either the lumen of internal compartments or to the extracellular space. V-ATPases present in intracellular membranes are important for membrane traffic processes such as receptor-mediated endocytosis and intracellular targeting of newly synthesized lysosomal enzymes (1). They also provide the acidic environment required for processing and degradation of macromolecules and the entry of certain envelope viruses and bacterial toxins, including anthrax toxin (12), as well as the driving force for coupled transport of small molecules, such as neurotransmitters. Plasma membrane V-ATPases function in a wide variety of normal and disease processes, including renal acidification, bone resorption, K\(^+\) secretion, sperm maturation, and tumor cell invasion (1, 7–11). V-ATPase inhibitors are thus potentially useful therapeutic agents for the treatment of a number of human diseases, including viral infection, osteoporosis, and cancer.

V-ATPases are multisubunit complexes composed of two domains (1–9). The peripheral V\(_1\) domain is composed of eight subunits (A–H) and functions to hydrolyze ATP. The integral V\(_0\) domain is composed of six subunits (in yeast these are subunits a, c, c\(^\prime\), c\(^\prime\prime\), d, and e) and is responsible for proton transport. The V-ATPases thus structurally resemble the ATP synthases (or F-ATPases) of mitochondria, chloroplasts, and bacteria that function in the synthesis of ATP (13–16).

Like the F-ATPases (13–16), the V-ATPases have been shown to operate by a rotary mechanism (17, 18). ATP hydrolysis in the V\(_1\) domain drives rotation of a central stalk composed of subunits D and F (17, 19). These subunits are linked to a ring of proteolipid subunits (c, c\(^\prime\), and c\(^\prime\prime\)) via subunit d (20). Each proteolipid subunit contains a single buried acidic residue that undergoes reversible protonation and deprotonation during the rotational catalysis (21). As the ring of proteolipid subunits rotates relative to subunit a, each buried carboxyl group on the ring is thought to pick up a proton from the cytoplasmic compartment via a hemi-channel located in subunit a (1, 15, 22, 23). Rotation of the ring brings this protonated carboxyl into contact with a second subunit hemi-channel leading to the luminal or extracellular side of the membrane. Interaction between the carboxyl group and the positively charged guanidinium group of Arg\(^{735}\) of subunit a (23) then causes release of the proton into the luminal hemi-channel.

Subunit a is a 100-kDa integral membrane protein containing two domains (24). The N-terminal hydrophilic domain has a molecular mass of 50 kDa and is oriented toward the cytoplasmic side of the membrane (25). Together with subunits C, E, G, H, and the non-homologous domain of subunit A (19, 26–29), the N-terminal domain forms a peripheral stalk or stator that keeps the catalytic domain of the V-ATPase fixed during rotational catalysis. Topological studies suggest that the C-terminal domain of subunit c contains nine transmembrane helices (25), with the critical Arg\(^{735}\) located in TM7 (23). Cross-linking studies have demonstrated that TM7 of subunit a is in close proximity to TM4 of subunit c\(^\prime\) and TM2 of subunit c\(^\prime\prime\) (30, 31), both of which contain an essential glutamic acid residue (21). Moreover, the data are consistent with swiveling of helices within both subunits a and the proteolipids relative to each other.

Bafilomycin and concanamycin are structurally related, highly specific inhibitors of the V-ATPase that inhibit purified preparations in the nanomolar concentration range (32, 33). Mutagenesis studies have demonstrated that mutations in the proteolipid c subunit are able to confer resistance to both bafilomycin and concanamycin (34, 35). Consistent with the participation of this subunit in binding of these inhibitors, photochemical labeling of the insect V-ATPase with a radioactive derivative of concanamycin also resulted in modification of subunit c (36).

One striking conclusion that emerged from the mutagenesis studies was that mutations conferring resistance to bafilomycin were in very similar locations on the V-ATPase c subunit to those on the F-ATPase c...
subunit that conferred resistance to oligomycin (34). This suggested that these two classes of drugs were binding to related sites on the two classes of ATPase. Because mutations on F-ATPase subunit a have been identified that confer resistance to oligomycin (37–39), this suggested the possibility that V-ATPase subunit a, although not homologous to the F-ATPase subunit a, might similarly be participating in drug binding. The results of the present study support this conclusion and provide further insight into how bafilomycin may inhibit the V-ATPase.

**EXPERIMENTAL PROCEDURES**

**Materials and Strains**—Zymolyase 100T was obtained from Seikagaku America, Inc. Concanamycin A and bafilomycin A1 were purchased from Fluka. Protease inhibitors were from Roche Molecular Biochemicals. The mouse monoclonal antibody 10D7 against the Vph1p, followed by horseradish peroxidase-conjugated secondary antibody (Bio-Rad) (43). Blots were developed using a chemiluminescent detection method obtained from Kirkegaard & Perry Laboratories.

**ATPase Activity**—ATPase activity was measured using a coupled spectrophotometric assay (43). The reactions were carried out at 30 °C and vacuolar membrane vesicles were incubated with Me6SO or different concentrations of bafilomycin A1 or concanamycin A (in Me6SO) for 5 min prior to measurement of ATPase activity.

**Other Procedures**—ATP-dependent proton transport was measured by fluorescence quenching with the fluorescence probe 9-amino-6-chloro-2-methoxyacridine in transport buffer (50 mM NaCl, 30 mM KCI, 20 mM HEPES, 0.2 mM EGTA, 10% glycerol (pH 7.0) as described (44) in the presence or absence of 1 μM concanamycin A. SDS-PAGE was performed as described by Laemmli (45). Protein concentrations were determined by the Lowry method (46).

**RESULTS**

**Construction of Mutants of Vph1p**—Fig. 1 shows the location of F-ATPase mutations conferring resistance to oligomycin (37–39). As can be seen, the mutations fall just N-terminal to Arg210 in TM4 (E. coli numbering) and in the polar loop just preceding this transmembrane segment. Although not homologous to F-ATPase subunit a, subunit a of the V-ATPase serves a similar function in proton translocation (23, 47, 48). In particular, Arg210 in TM7 of V-ATPase subunit a plays a similarly critical role in proton transport to Arg210 of the F-ATPase subunit a (23, 49). Site-directed mutations were therefore introduced into the
sequence just N-terminal to Arg735, including residues both within TM7 and in the polar loop connecting TM6 and TM7. Each residue from Glu721 to Thr730 was replaced with alanine and each residue from Thr719 to Thr730 (except Phe722) was also replaced with phenylalanine. In addition, several mutations were constructed on the basis of observed changes in F-ATPase subunit a conferring oligomycin resistance, including I720M, E721K, and S732R (37–39). Finally, three further mutations in this region, L724T, L724C, and S728T, and the double mutation, E721K/L724A (see below), were also constructed. The mutant forms of Vph1p were expressed in yeast strain MM112 disrupted in both Vph1p and Stv1p using the expression plasmid pRS316.

Growth Phenotype of Yeast Strains Expressing Mutant Forms of Vph1p

We first determined the effect of each Vph1p mutation on the growth phenotype of the strain expressing the mutant protein. Disrupting MM322 indicates the vph1/H9004 stv1/H9004 strain (MM112) expressing the wild-type form of Vph1p in pRS316, whereas the lane labeled pRS316 indicates the same strain transformed with the vector alone.
tion of V-ATPase function leads to a conditional growth phenotype (vma−) in which cells are able to grow in media buffered to pH 5.5 but not in media buffered to pH 7.5 (50, 51). Of the mutants tested, only S728F severely compromised growth at pH 7.5. The E721F mutant grew weakly at pH 7.5, whereas the remaining mutants all showed near wild-type growth at neutral pH, suggesting the formation of V-ATPase complexes possessing significant levels of V-ATPase activity in vivo.

Assembly of V-ATPase Complexes Containing Mutant Forms of Vph1p—To test whether the mutations introduced into Vph1p caused destabilization of subunit a, Western blot analysis was performed on whole cell lysates using the monoclonal antibody 10D7 specific for Vph1p. All the mutants tested showed levels of Vph1p similar to that observed for the wild-type strain (data not shown). As an indication of the effects of the mutations in Vph1p on assembly of the V-ATPase, partially purified vacuolar membranes were subjected to SDS-PAGE and Western blot analysis was performed using antibodies against both Vph1p and Vma1p (subunit A). Disruption of assembly of the V1 and V0 domains results in the loss of subunit A (and other V1 subunits) relative to subunit a on vacuolar membranes (43). As can be seen in Fig. 2, vacuolar membranes from all of the mutant strains showed near wild-type levels of subunit A, suggesting that none of the mutations resulted in gross defects in assembly of the V-ATPase complex, although more subtle changes in assembly because of loss of particular subunits has previously been observed. Because both proton transport and ATPase activities of each mutant were assessed to determine the effect of the mutations on function (see below), a more detailed analysis of V-ATPase assembly by Western blot using other V-ATPase subunit antibodies was not performed.

ATPase and Proton Transport Activity of Mutants of Vph1p—To determine the effect of the mutations in Vph1p on activity of the V-ATPase, both ATP hydrolysis and ATP-dependent proton transport (using the fluorescent dye 9-amino-6-chloro-2-methoxyacridine) were measured in isolated vacuolar membrane vesicles as described under “Experimental Procedures.” The data shown in Fig. 3 represents the ATPase and proton transport activities sensitive to a high concentration (1 μM) of concanamycin. The activities are expressed relative to those measured for vacuolar membranes isolated from a yeast strain expressing wild-type Vph1p. For ATPase activity this corresponds to 0.81 μmol of ATP/min/mg of protein. As can be seen, all but three of the mutants have at least 25% of wild-type levels of both proton transport and ATPase activity. The three exceptions are S728F (3–5% of wild-type), E721F (7–10% wild-type), and the E721K/L724A double mutant (17–19% wild-type). These data are thus generally consistent with the previous observation that a wild-type growth phenotype requires V-ATPase activities on the order of 20–25% of wild-type (52, 53). In addition, the mutations generally result in parallel changes in both proton transport and ATPase activity, although a partial uncoupling is observed with the S732R mutant.
Because mutations were constructed in a region of subunit a postulated to confer resistance to bafilomycin or concanamycin, it was possible that a decrease in concanamycin-sensitive activity might be coupled with an increase in concanamycin-resistant activity. To determine whether this was the case, the ATPase activity that is either sensitive or resistant to concanamycin, which did not vary significantly relative to the absence or presence of the indicated concentrations of bafilomycin A1 as described under “Experimental Procedures.” All activities were corrected for ATPase activity resistant to 1 mM concanamycin A. Values are expressed relative to those measured in the absence of bafilomycin A1, which for wild-type (MM322) was 0.81 μmol of ATP/min/mg of protein, E721K was 0.22 μmol of ATP/min/mg of protein, L724A was 0.24 μmol of ATP/min/mg of protein, and N725F was 0.40 μmol of ATP/min/mg of protein. Values shown are the average of three measurements on two independent vacuole preparations, with error bars corresponding to standard deviations. Half-maximal inhibition by bafilomycin A1 was observed at 0.22 nM for MM322, 0.38 nM for E721K, 0.40 nM for L724A, and 0.54 nM for N725F.

Effect of Vph1p Mutations on Affinity of V-ATPase for Concanamycin—To determine whether the mutations in Vph1p conferred changes in sensitivity of the V-ATPase to either bafilomycin or concanamycin, ATPase activity was measured for vacuolar membranes isolated from the wild-type and each of the mutant strains at various concentrations (0.1-10 nM) of either bafilomycin or concanamycin. In each case, the activities were corrected for the activity resistant to 1 μM concanamycin, which did not vary significantly relative to wild-type (Fig. 4). As can be seen from TABLE ONE, only three of the mutants showed Kᵢ values for bafilomycin that were significantly different from wild-type (0.22 ± 0.03 nM). These included E721K (0.38 ± 0.03 nM), L724A (0.40 ± 0.02 nM), and N725F (0.54 ± 0.06 nM). The ATPase activity for each of these mutants and the wild-type as a function of bafilomycin concentration is shown in Fig. 5. When the concanamycin sensitivity of these mutants was compared with wild-type, only the N725F mutant displayed a Kᵢ (0.84 ± 0.04 nM) that was slightly higher than wild-type (0.60 ± 0.07 nM). The other two mutants displayed affinities for concanamycin that were not significantly different from wild-type (Fig. 6). Of the remaining mutants tested, none showed Kᵢ values.

### Table One

| Strain | Kᵢ (nM) Bafilomycin A1 | Kᵢ (nM) Concanamycin A |
|-------|------------------------|------------------------|
| MM322 | 0.22 ± 0.03            | 0.60 ± 0.07            |
| T719F | 0.23 ± 0.03            | 0.41 ± 0.03            |
| T720F | 0.22 ± 0.04            | 0.43 ± 0.03            |
| T720M | 0.24 ± 0.04            | 0.59 ± 0.04            |
| E721A | 0.18 ± 0.03            | 0.65 ± 0.05            |
| E721K | 0.38 ± 0.03            | 0.54 ± 0.04            |
| F722A | 0.19 ± 0.03            | 0.62 ± 0.07            |
| C723A | 0.23 ± 0.06            | 0.57 ± 0.04            |
| C723F | 0.25 ± 0.06            | 0.54 ± 0.05            |
| L724A | 0.40 ± 0.02            | 0.65 ± 0.07            |
| L724F | 0.29 ± 0.03            | 0.52 ± 0.02            |
| L724T | 0.25 ± 0.06            | 0.66 ± 0.06            |
| L724C | 0.23 ± 0.03            | 0.46 ± 0.03            |
| N725A | 0.19 ± 0.04            | 0.63 ± 0.06            |
| N725F | 0.54 ± 0.06            | 0.84 ± 0.04            |
| C726A | 0.22 ± 0.03            | 0.64 ± 0.06            |
| C726F | 0.26 ± 0.03            | 0.58 ± 0.06            |
| V727A | 0.18 ± 0.04            | 0.78 ± 0.07            |
| V727F | 0.24 ± 0.04            | 0.54 ± 0.05            |
| S728A | 0.18 ± 0.04            | 0.62 ± 0.04            |
| S728I | 0.18 ± 0.02            | 0.62 ± 0.05            |
| H729A | 0.23 ± 0.04            | 0.58 ± 0.07            |
| H729F | 0.18 ± 0.03            | 0.66 ± 0.04            |
| T730A | 0.26 ± 0.03            | 0.66 ± 0.05            |
| T730F | 0.28 ± 0.04            | 0.58 ± 0.03            |
| S732R | 0.25 ± 0.03            | 0.56 ± 0.06            |

**FIGURE 5. Effect of mutations in Vph1p on affinity of V-ATPase for bafilomycin A1.** Vacular membranes were isolated from cells expressing wild-type Vph1p (MM322) or the indicated mutant forms of Vph1p, and ATPase activities were measured in the presence or absence of the indicated concentrations of bafilomycin A1 as described under “Experimental Procedures.” All activities were corrected for ATPase activity resistant to 1 μM concanamycin A. Specific activities are expressed relative to those measured in the absence of bafilomycin A1, which for wild-type (MM322) was 0.81 μmol of ATP/min/mg of protein, E721K was 0.22 μmol of ATP/min/mg of protein, L724A was 0.24 μmol of ATP/min/mg of protein, and N725F was 0.40 μmol of ATP/min/mg of protein. Values shown are the average of three measurements on two independent vacuole preparations, with error bars corresponding to standard deviations. Half-maximal inhibition by bafilomycin A1 was observed at 0.22 nM for MM322, 0.38 nM for E721K, 0.40 nM for L724A, and 0.54 nM for N725F.

**FIGURE 6. Effect of mutations in Vph1p on affinity of V-ATPase for concanamycin A.** Vacuolar membranes were isolated from cells expressing wild-type Vph1p (MM322) or the indicated mutant forms of Vph1p, and ATPase activities were measured in the presence or absence of the indicated concentrations of concanamycin A as described under “Experimental Procedures.” All activities were corrected for the ATPase activity resistant to 1 μM concanamycin A. Specific activities are expressed relative to those measured in the absence of concanamycin (see legend to Fig. 5). Values shown are the average of three measurements on two independent vacuole preparations, with error bars corresponding to standard deviations. Half-maximal inhibition by concanamycin A was observed at 0.60 nM for MM322, 0.54 nM for E721K, 0.65 nM for L724A, and 0.84 nM for N725F.
for concanamycin that were significantly higher than that observed for wild-type, although two mutants (T719F and L724C) showed $K_i$ values that were slightly lower than wild-type (TABLE ONE). The activities of the S728F and E721F mutants and the E721K/L724A double mutant were too low to accurately determine $K_i$ values for bafilomycin and concanamycin.

**DISCUSSION**

Previous data has strongly implicated subunit c of the V-ATPase in binding of the specific inhibitors bafilomycin and concanamycin. Thus, in Neurospora, single mutations in subunit c increase the $K_i$ for bafilomycin by 3.7–67-fold relative to wild-type (34, 35), whereas double mutants increase the $K_i$ for bafilomycin by 37–325-fold (35). Interestingly, these mutations confer a much smaller resistance to concanamycin, with $K_i$ values 1.6–3.1-fold higher for the single mutants and 5.6–39-fold higher for the double mutants (35). This suggests that the concanamycin binding site, whereas overlapping with the site for bafilomycin, also has unique determinants that are not altered by changes in the bafilomycin site. Consistent with the participation of subunit c in binding of these inhibitors is the report that a photoreactive analog of concanamycin results in labeling of subunit c of Manduca sexta (36).

Subunit c contains four transmembrane helices with a single glutamate residue near the middle of TM4 that is essential for proton translocation (54). The mutations conferring resistance to bafilomycin and concanamycin were found to largely cluster in two regions of subunit c. The first was in TM4, near the essential glutamate residue, and the second was in or near the polar loop connecting TM1 and TM2 (34, 35). A model was proposed locating the bafilomycin/concanamycin binding site at the interface of TM1, TM2, and TM4, with the helices oriented so as to bring most of the mutations conferring resistance into contact with a common pocket located either between c subunits or within a single c subunit (35). It was also suggested that bafilomycin or concanamycin binding to this site may inhibit activity by preventing swiveling of TM4 relative to the other transmembrane helices of subunit c, thus blocking movement of the essential glutamate residue into the correct position for proton transport (35).

Consistent with the idea that helical swiveling occurs within subunit c during catalysis is cross-linking data demonstrating that the transmembrane segment containing the essential glutamate residue in either subunit c’ or subunit c” of the V-ATPase can adopt a number of different orientations relative to TM7 of subunit c containing the essential arginine residue (Arg735) (30, 31). Moreover, these same cross-linking studies suggest that TM7 of subunit a also has considerable rotational mobility relative to subunit c (30, 31). Support for helical swiveling associated with proton transport through the integral domain of the F-ATPase comes from a variety of data, including cross-linking results (55), suppressor analysis (15), and NMR studies of the E. coli F-ATPase (56, 57).

The first suggestion that subunit a of the V-ATPase may also participate in the binding of bafilomycin or concanamycin came from reconstitution experiments on the V-ATPase from clathrin-coated vesicles (58). Purified, reconstituted $V_0$ domain was shown to protect intact V-ATPase from inhibition by submaximal concentrations of bafilomycin. Dissociation of the $V_0$ domain and isolation and reconstitution of the isolated subunits revealed that only the a subunit (alone or in combination with subunit c) showed similar protection against bafilomycin inhibition (58), suggesting that subunit a retained the ability to bind bafilomycin.

It was noted from the mutagenesis studies on the Neurospora subunit c that the mutations conferring resistance to bafilomycin were located in very similar positions relative to the essential buried acidic residue as were mutations on the F-ATPase subunit c that conferred resistance to oligomycin (34). It was thus suggested that these two drugs, whereas specific for inhibition of only one of the two families of ATPase, nevertheless, bound to similar sites in the integral domains of the two classes of proton transporter. It had previously been shown that mutations in subunit a of the F-ATPase also conferred resistance to oligomycin (37–39). These mutations clustered in the transmembrane segment and polar loop region just preceding the critical arginine residue of subunit a. Based upon the previous reconstitution data on the V-ATPase and the mutagenesis data on the F-ATPase, we therefore wished to determine whether mutations in the V-ATPase subunit a are also able to confer resistance to bafilomycin or concanamycin.

The data presented in the current report provide the first evidence indicating that mutations in subunit a can alter the sensitivity of the V-ATPase to bafilomycin or concanamycin. The three mutations conferring partial resistance to bafilomycin (E721K, L724A, and N725F) are located in a 5-amino acid stretch of the polar loop just N-terminal to TM7 (Fig. 7). As can be seen, this is very similar to the region in the F-ATPase subunit a in which mutations conferring resistance to oligo-
mycin are clustered. Consistent with the previous observations on c subunit mutations in \textit{Neurospora} (34, 35), two of the three a subunit mutations had no effect on concanamycin sensitivity and the third (N725F) resulted in only a small change in the \(K_c\) for concanamycin relative to wild-type. Thus, residues in subunit a (or subunit c) that are not involved in binding of bafilomycin are likely to participate in binding of concanamycin.

The magnitude of the resistance observed for mutations in subunit a of yeast (2.0–2.5-fold) is considerably smaller than the resistance observed for single mutations of subunit c of \textit{Neurospora} (3.7–67-fold (34)). Part of this difference may be because of species differences, because it was previously reported that the T32I mutation that confers 67-fold resistance to bafilomycin in \textit{Neurospora} confers only 11–17-fold resistance in yeast (35). In addition, several of the mutations that confer bafilomycin resistance in \textit{Neurospora} were not tolerated in subunit c of yeast, causing severe defects in assembly or activity of the V-ATPase. In summary, we work will be required to completely define the nature of the binding sites for bafilomycin and concanamycin on the V-ATPase. (34, 35). Whereas the structure of the polar loop preceding TM7 of \(E.\ coli\) is not known, it is possible that this region continues as an \(\alpha\) helix after it emerges from the membrane, as has been observed for other transport proteins (59). In fact, the polar loop preceding TM4 of \(E.\ coli\) helical from the results of cysteine modification studies (60). If this is the case for V-ATPase subunit a, all three mutations conferring resistance are predicted to be on the same helical face of TM7 as Arg\(^{265}\). By contrast, the c subunit mutations that confer resistance to bafilomycin are predicted to reside on the helical face of TM4 opposite to the critical glutamic acid residue (34). Cross-linking studies indicate that the \(V_0\) domain can adopt a rotational conformation in which these two helical surfaces are in close proximity (see Ref. 31, Fig. 6, \textit{upper left-hand panel}). Thus, the bafilomycin binding pocket may reside at the interface of subunit a, and the proteolipid ring and bafilomycin may inhibit activity by locking the \(V_0\) domain into a conformation in which the glutamic acid residue of the c subunits cannot swivel to approach the arginine residue on TM7 of subunit a. Alternatively, mutations in subunit a may alter the binding of bafilomycin or concanamycin indirectly, by preventing the \(V_0\) domain from adopting a rotational conformation optimum for binding of the inhibitor. Further work will be required to completely define the nature of the binding sites for bafilomycin and concanamycin on the V-ATPase. In summary, we have identified mutations in yeast V-ATPase subunit a that confer resistance to bafilomycin, providing the first genetic evidence for the involvement of subunit a in the binding of this class of specific V-ATPase inhibitors.

Acknowledgments—We thank Dr. Ayana Hinton as well as Jie Qi and Kevin Jeffries for many helpful discussions.
Bafilomycin-resistant Mutants in a Subunit of the V-ATPase

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