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The vacuolar (H^+)-ATPase: subunit arrangement and in vivo regulation

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Abstract The V-ATPases are responsible for acidification of intracellular compartments and proton transport across the plasma membrane. They play an important role in both normal processes, such as membrane traffic, protein degradation, urinary acidification, and bone resorption, as well as various disease processes, such as viral infection, toxin killing, osteoporosis, and tumor metastasis. V-ATPases contain a peripheral domain (V_1) that carries out ATP hydrolysis and an integral domain (V_0) responsible for proton transport. V-ATPases operate by a rotary mechanism involving both a central rotary stalk and a peripheral stalk that serves as a stator. Cysteine-mediated cross-linking has been used to localize subunits within the V-ATPase complex and to investigate the helical interactions between subunits within the integral V_0 domain. An essential property of the V-ATPases is the ability to regulate their activity in vivo. An important mechanism of regulating V-ATPase activity is reversible dissociation of the complex into its component V_1 and V_0 domains. The dependence of reversible dissociation on subunit isoforms and cellular environment has been investigated.

Keywords ATPase · V_1 and V_0 domains · Subunit isoforms

Introduction

The vacuolar (H^+)-ATPases (or V-ATPases) are ATP-dependent proton pumps present in both intracellular compartments and the plasma membrane and function in a variety of normal and disease processes (Forgac 2007; Kane 2006; Wagner et al. 2004). Intracellular V-ATPases are important for receptor-mediated endocytosis and intracellular trafficking, protein processing and degradation, coupled transport of small molecules and ions, and the entry of various pathogens into cells, including viruses such as influenza virus and toxins such as anthrax toxin (Forgac 2007; Abrami et al. 2004). Plasma membrane V-ATPases play an important role in acid secretion in the kidney, bone resorption by osteoclasts, acidification of the male reproductive tract, and in the invasiveness of tumor cells and endothelial cells (Wagner et al. 2004; Toyomura et al. 2003; Pietrement et al. 2006; Sennoune et al. 2004). As a result, V-ATPases have emerged as a possible drug target in treating such diseases as osteoporosis and cancer. This review will focus on recent results from our laboratory dealing with the structure and regulation of the V-ATPases.

Structure of the V-ATPases

The V-ATPases are multisubunit complexes containing a peripheral V_1 domain responsible for ATP hydrolysis and an integral V_0 domain that carries out proton translocation (Fig. 1; Forgac 2007). V_1 contains eight subunits (A–H) organized into a hexameric head of nucleotide binding subunits (A and B) that is attached to the V_0 domain by both a central and peripheral stalk (see below). V_0 contains six subunits (a, c, c′, c″, d, and e) in which the proteolipid subunits (c, c′, and c″) form a ring that sits adjacent to subunit a. Like the F-ATPases, the V-ATPases operate by a rotary mechanism (Imamura et al. 2003; Hirata et al. 2003) in which ATP hydrolysis in V_1 drives rotation of a central stalk connected to the ring of proteolipid subunits. As de-

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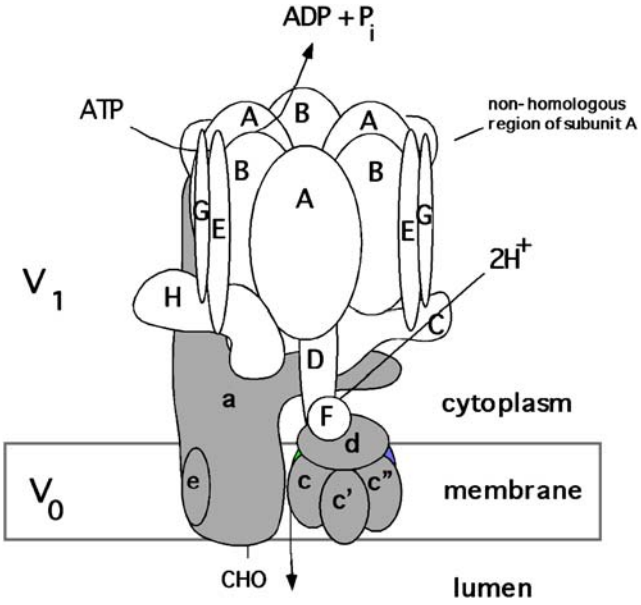

Fig. 1 Structural model of the V-ATPase. The V-ATPase is composed of a peripheral domain (V_1) responsible for ATP hydrolysis and an integral domain (V_0) that carries out proton transport. V_1 and V_0 are connected by both a central stalk (composed of subunits D and F in V_1 and subunit d in V_0) and peripheral stalks (composed of subunits C, E, G, H, and the N-terminal domain of subunit a). ATP hydrolysis in V_1 drives rotation of the central stalk that, in turn, drives rotation of the ring of proteolipids (c, c′, and c″) past subunit a in V_0. It is the movement of the proteolipid subunits past subunit a that drives proton transport across the membrane

scribed below, movement of the ring of proteolipids past subunit a is believed to drive the active transport of protons across the membrane.

Subunit organization in V_1

To determine the distribution of V_1 subunits between the peripheral and central stalks connecting V_1 and V_0, we have employed photoactivated cross-linking using the cysteine-specific reagent maleimido benzophenone (MBP). In this approach, unique cysteine residues are introduced into structurally defined sites of a particular subunit (the scaffold) of the yeast V-ATPase by site-directed mutagenesis. Both subunit B (for which a molecular model has been constructed) and subunit C (for which a high resolution crystal structure is available) have been used as scaffold proteins. After attachment of MBP to the structurally defined cysteine residues, photoactivated cross-linking is performed followed by Western blotting using subunit-specific antibodies to identify subunits proximal to the initial point of attachment of MBP. Using this approach, we have shown that subunit E is part of the peripheral stalk (or stator) and runs nearly the entire length of the outer surface of V_1 (Arata et al. 2002a, b). Subunit G is also part of the peripheral stalk and is present at least near the top of the complex (Arata et al. 2002b). By contrast, subunit D is located within the interior of the A_3B_3 hexamer and, as such, forms part of the rotary connection between V_1 and V_0 (Arata et al. 2002b).

Subunits C and H are interesting in several respects. First, no homolog exists for these subunits in either the F-ATPases or the more closely related archaebacterial ATPases (or A-ATPases). Next, subunit C is unique among V-ATPase subunits in that it releases from both the V_1 and V_0 domains upon in vivo dissociation of the V-ATPase complex, an important regulatory mechanism (see below; Kane 2006). Release of subunit C has, therefore, been postulated to serve as a trigger for in vivo dissociation of the complex. Subunit H has been shown to serve as the binding site of a variety of non-V-ATPase proteins to the complex, including the HIV protein NEF, the endocytic adaptor protein AP-2 and the Golgi ectoapyrase (Geyer et al. 2002; Zhong et al. 2000). In addition, subunit H has been shown to suppress the ATP hydrolytic activity of the soluble V_1 domain (Parra et al. 2000), an important property to avoid release into the cytosol of an uncoupled ATPase during in vivo regulation. Using the cysteine-mediated cross-linking approach described above, we have shown that both subunits C and H are located near the interface of the V_1 and V_0 domains, with subunit C in contact with both subunits E and G in V_1 and subunit a in V_0 (Inoue and Forgac 2005; Wilkens et al. 2004). Subunit C is, thus, well positioned to control interactions between V_1 and V_0. Moreover, recent cross-linking studies using subunit H as the scaffold (Jefferies and Forgac, manuscript submitted) suggest that subunit H is able to bridge the peripheral and central stalks in free V_1 but not in the intact complex. This latter result suggests that subunit H suppresses the activity of the free V_1 domain by physically preventing rotational catalysis. Localization of subunits C and H to the interface of the V_1 and V_0 domains has also been demonstrated using electron microscopy (Wilkens et al. 2004; Zhang et al. 2006).

Subunit arrangement in V_0

The proteolipid subunits are highly hydrophobic proteins containing either four (subunits c and c') or five (subunit c″) transmembrane helices (TM; Hirata et al. 1997). They are organized into a ring containing single copies of subunits c′ and c″ and four to five copies of subunit c (Arai et al. 1988; Flannery et al. 2004), although a crystal structure of the c ring from the Na^+-translocating A-ATPase of *Enterococcus hirae* reveals a ten-membered ring of identical c subunits (Murata et al. 2005). Each proteolipid subunit contains a single buried glutamic acid residue essential for proton transport. This residue is present in TM4 of subunit c and c′

and TM3 of subunit c″, although a second non-essential glutamic acid residue is also present in TM5 of subunit c″ (Hirata et al. 1997). Subunit d is a hydrophilic protein that sits on top of the c ring (Iwata et al. 2004) and serves as a connector between the central stalk subunits of V_1 (D and F) and the proteolipid ring. The location of subunit e within V_0 has not yet been determined.

Subunit a is a 100-kDa protein containing an amino-terminal hydrophilic domain present on the cytoplasmic side of the membrane and a carboxy-terminal hydrophobic domain containing eight to nine transmembrane helices (Leng et al. 1999). Subunit a contains a critical Arg reside (Arg735 in Vph1p) present in TM7 that is absolutely required for proton transport (Kawasaki-Nishi et al. 2001a). Subunit a is thought to provide access channels (hemi-channels) that allow protons to reach and leave the buried glutamic acid residues on the proteolipid ring (Forgac 2007). In addition, Arg735 of subunit a is thought to displace protons from the protonated carboxyl groups of the c subunits into the luminal hemi-channel and to stabilize the deprotonated form of these residues before their acquisition of a proton from the cytoplasmic hemi-channel. Cysteine-mediated zero-length cross-linking between cysteine residues introduced into subunits a and the proteolipid subunits indicate that TM7 of subunit a containing Arg735 is proximal to both TM4 of subunit c′ and TM3 (but not TM5) of subunit c″containing the essential glutamic acid residues (Kawasaki-Nishi et al. 2003; Wang et al. 2004). Moreover, the results provide evidence for swiveling about an axis normal to the membrane of the essential helices in both subunits a and the proteolipid subunits (Wang et al. 2004). This swiveling may help to control the opening and closing of the proton-conducting hemi-channels in subunit a. Recent results obtained from analysis of the functional properties of chimeric gene fusion constructs containing proteolipid subunits in a defined orientation suggest that these subunits adopt a unique arrangement in functional V-ATPase complexes in which subunits c′ and c″ are next to each other in the ring (Wang et al. in press).

Regulation of V-ATPase activity by reversible dissociation

Reversible dissociation of the V-ATPase complex represents an important mechanism of regulating acidification of intracellular compartments in vivo (Kane 2006; Forgac 2007). This mechanism has been demonstrated to operate in yeast, insect, and mammalian cells (Kane 2006; Beyenbach and Wieczorek 2006; Trombetta et al. 2003). In yeast, dissociation occurs rapidly and in response to nutrient depletion, thus helping to preserve cellular stores of ATP. Dissociation requires catalytic activity and an intact microtubular network but is not dependent upon many of the signaling pathways activated by glucose depletion (Parra and Kane 1998; Xu and Forgac 2001). Reassembly is independent of microtubules but depends upon a novel complex termed RAVE (regulator of the (H^+)-ATPase of vacuolar and endosomal membrane), which is also involved in the normal assembly pathway of the V-ATPase (Seol et al. 2001; Smardon et al. 2002).

Subunit a of the V-ATPases exists in multiple isoforms in all eukaryotes and contains information necessary for targeting the V-ATPase to the appropriate membrane. In mammalian cells, there are four a subunit isoforms, with a1 present in synaptic vesicles; a2 present in endosomes and Golgi; a3 present in lysosomes, insulin-containing vesicles, and osteoclast plasma membranes; and a4 present in the plasma membrane of renal intercalated cells (Sun-Wada et al. 2006; Hurtado-Lorenzo et al. 2006). In yeast, there are two isoforms of subunit a. Vph1p targets the V-ATPase to the vacuole, whereas Stv1p targets the V-ATPase to the Golgi (Manolson et al. 1994; Kawasaki-Nishi et al. 2001c). Overexpression of Stv1p in a strain disrupted in both isoforms results in re-targeting V-ATPase complexes to the vacuole (Kawasaki-Nishi et al. 2001b). We have previously shown that Vph1p-containing complexes localized to the vacuole dissociate in response to glucose depletion, whereas Stv1p-containing complexes localized to the Golgi do not (Kawasaki-Nishi et al. 2001b). For Stv1p-containing complexes localized to the vacuole, however, efficient dissociation is observed. This result suggests that the cellular environment significantly effects dissociation of the V-ATPase complex.

To further investigate this dependence, dissociation of Vph1p- and Stv1p-containing complexes was measured in yeast strains (*vps* for vacuolar protein sorting mutants) disrupted in the normal trafficking of proteins to the vacuole. In strains disrupted in Vps21p, proteins accumulate in a post-Golgi compartment, whereas in Vps27-disrupted strains, proteins accumulate in a pre-vacuolar compartment (Kawasaki-Nishi et al. 2001c). We found that dissociation of both Vph1p and Stv1p-containing complexes depends upon the cellular environment, with dissociation most complete in the vacuole and least complete in the prevacuolar compartment (Qi and Forgac 2007). In addition, dissociation of all V-ATPase complexes was partly blocked by inhibition of activity using either a specific inhibitor (concanamycin) or inactivating mutations, although dissociation does not absolutely require catalytic activity (Qi and Forgac 2007). This result suggests that dissociation requires the V-ATPase to adopt a particular conformational state and that this state is also a function of the cellular environment. Among the environmental factors effecting dissociation are the luminal pH, such that a more alkaline luminal pH partly inhibits dissociation

(Shao and Forgac 2004). One key domain of the V-ATPase that helps to control dissociation is the non-homologous region of subunit A. Mutations in this region are able to block dissociation without affecting catalysis (Shao and Forgac 2004), and this domain is able to bind to V_0 in the absence of other V_1 subunits in a glucose-dependent manner (Shao and Forgac 2004).

In addition to reversible dissociation, the level of assembly of the V-ATPase and the tightness of coupling of proton transport and ATP hydrolysis have been shown to directly modulate acidification. Thus, Vph1p-containing complexes localized to the vacuole are both more assembled with V_1 and more tightly coupled than Stv1p-containing complexes localized to the Golgi (Kawasaki-Nishi et al. 2001b; Qi and Forgac 2007). These differences between a subunit isoforms help to explain the fact that the vacuole is maintained at a more acidic pH than the Golgi. Differences in a subunit isoforms likely contribute to the differences in pH observed between different intracellular compartments in higher eukaryotic cells as well. In addition, mutations in the non-homologous domain of subunit A have been shown to alter the coupling efficiency of the V-ATPase (Shao et al. 2003; Shao and Forgac 2004). Interestingly, the wild-type enzyme does not appear to be optimally coupled (Shao et al. 2003), supporting the idea that changes in coupling efficiency can modulate acidification in vivo.

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