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Review

A structural model of the vacuolar ATPase from transmission electron microscopy ☆

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Abstract

Vacuolar ATPases (V-ATPases) are large, membrane bound, multisubunit protein complexes which function as ATP hydrolysis driven proton pumps. V-ATPases and related enzymes are found in the endomembrane system of eukaryotic organsims, the plasma membrane of specialized cells in higher eukaryotes, and the plasma membrane of prokaryotes. The proton pumping action of the vacuolar ATPase is involved in a variety of vital intra- and inter-cellular processes such as receptor mediated endocytosis, protein trafficking, active transport of metabolites, homeostasis and neurotransmitter release. This review summarizes recent progress in the structure determination of the vacuolar ATPase focusing on studies by transmission electron microscopy. A model of the subunit architecture of the vacuolar ATPase is presented which is based on the electron microscopic images and the available information from genetic, biochemical and biophysical experiments.

Keywords: Vacuolar ATPase; Membrane protein; Protein structure; Electron microscopy; Image analysis; Three-dimensional reconstruction; Antibody labeling; Difference imaging

1. Introduction

A vacuolar H^+-ATPase (V_1V_0- or V-ATPase) is found in the membranes of a wide variety of intra-cellular compartments such as clathrin-coated vesicles, chromaffin granules, endosomes, lysosomes, synaptic vesicles, Golgi derived vesicles, the tonoplast of plants and the yeast vacuole. The proton pumping action of the vacuolar ATPase plays a vital role in a large number of intra- and inter-cellular processes including receptor mediated endocytosis, protein trafficking, pH maintenance, storage of metabolites and neurotransmitter release (Nishi and Forgac, 2002; Futai et al., 2000; Nelson and Harvey, 1999; Stevens and Forgac, 1997; Finbow and Harrison, 1997). In higher eukaryotes, V-type ATPases are also found in the plasma membrane of polarized cells like osteoclasts, renal epithelial cells, insect epithelial cells and amphibian skin cells. Here, the V-ATPase pumps protons into the extracellular space. Acidification of the enclosed space between the ruffled membrane of osteoclasts and the bone surface plays an important role in bone resorption and remodeling and in the kidney, a V-ATPase in the plasma membrane of renal intercalated cells functions in blood de-acidification. Defects in the human osteoclast or kidney V-ATPases can lead to osteoporosis or renal tubular acidosis, respectively.

The V-ATPase is a large, membrane bound multisubunit enzyme complex composed of two functional domains: a water soluble V_1 and a membrane embedded V_0. The two domains are joined by a stalk domain, which functions as a structural and functional connection between the V_1 and V_0. The V-ATPase contains at least 13 different subunits with relative molecular masses ranging from 12,000 to 100,000. The V_1 contains eight different subunits A, B, C, D, E, F, G, H and the V_0 is made of five different polypeptides a, c, c′, c″, d. A sixth V_0 subunit, e, originally described for the bovine (Ludwig et al., 1998) and insect (Merzendorfer et al.,

Abbreviations: V_1V_0, proton pumping vacuolar ATPase; V1, water soluble domain of the vacuolar proton pumping ATPase; V0, membrane bound domain of the proton pumping vacuolar ATPase; EM, electron microscopy; MSA, multivariate statistical analysis; MRA, multireference alignment; 2-D, two-dimensional; 3-D, three-dimensional.

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1999) enzyme has now been confirmed in the yeast complex (Sambade and Kane, 2004) which suggests that this subunit is a component of all eukaryotic V-ATPase membrane domains. The total molecular mass of the complex is close to 900,000. The ratio of the individual subunits has been measured for the enzyme from bovine brain clathrin coated vesicles to be $A_3B_3CDEFG_2H_2$ for the V_1 and $a(c,c')_{4\text{-}5}c''d$ for the V_0 (Arai et al., 1988; Xu et al., 1999).

Overall, the structure and mechanism of the V-ATPase is similar to the structure and mechanism of the related F_1F_0-ATP synthase (Müller and Grüber, 2003; Wilkens 2001; Grüber et al., 2001). However, while the F-ATPase can both function as an ATP synthase and an ATP hydrolysis driven proton pump, under physiological conditions, the vacuolar ATPase functions only in the direction of ATP hydrolysis. According to the current mechanistic model of V-ATPase function, ATP hydrolysis taking place on the V_1 A subunits drives rotation of a central domain composed of subunits D and F and the ring of c subunits (protoelipids) against the remainder of the complex (Imamura et al., 2003; Hirata et al., 2003). Proton translocation occurs at the interface of the membrane bound domain of the V_0 a subunit and the lipid exposed glutamate sidechains from the c, c′ and c″ subunits (Arai et al., 1987; Leng et al., 1996). As in the F-ATPase motor, rotation of the static domain during enzyme functioning is prevented by a peripheral stalk connecting the outside of the V_1 to the membrane. It is currently believed that the stator in the V-ATPase is made of the V_1 subunits C, E, G and H together with subunit a of the V_0 (Arata et al., 2002a,b).

Enzymes which are structurally related to the eukaryotic V-ATPase are also found in Archaea (where they are called A-ATPase; Denda et al., 1988; Schäfer and Meyering-Vos, 1992; Ihara et al., 1992) and in certain Eubacteria (Yokoyama et al., 1990; Takase et al., 1994; Speelmans et al., 1994). While the A-ATPase in Archaea functions probably as ATP synthase, the bacterial V/A-type ATPases act as ATP hydrolysis driven proton or sodium pumps. It is believed that the bacterial V/A-type ATPases have been introduced via horizontal gene transfer from Archaea (Hilario and Gogarten, 1993, 1998). In this review, we will use the name V-ATPase only for the eukaryotic enzyme and we will call the bacterial enzyme A/V-type ATPase (Hilario and Gogarten, 1998).

The structure of the V-ATPase and its V_1 and V_0 domains has been studied by electron microscopy and computer assisted image analysis (Radermacher et al., 1999; Wilkens et al., 1999, 2004; Grüber et al., 2000; Wilkens and Forgac, 2001; Domgall et al., 2002; Zhang et al., 2003). Based on these studies and the available biochemical data as well as the enzyme's similarity to the F-ATPase, a structural picture of the vacuolar ATPase is now emerging. The stoichiometry and arrangement of the stalk subunits, however, are still poorly understood and remain a matter of ongoing controversy.

2. Evolutionary relationship between the V-, F- and A-type ATPases

The vacuolar ATPase belongs to a family of ATP hydrolysis driven ion pumps, which are found in Archaea, eubacteria, simple eukaryotes such as yeast and higher eukaryotes including plants and mammals. The family of ion pumps is divided into three sub-families: the F-ATPases (which function mainly as ATP synthases), the eukaryotic vacuolar ATPases (which function solely as ATP hydrolysis driven ion pumps) and the Archaeal A-type ATPases (whose function can be either in the direction of ATP synthesis or hydrolysis). All three members of the family are evolutionary related and it is believed that the three sub-families have arisen from a common ancestor (Gogarten et al., 1989). The similarity is most evident from the overall domain structure of the complexes and the amino acid sequences of the subunits directly involved in ATP synthesis/hydrolysis and ion transport. The bacterial A/V-type ATPases are structurally more related to the Archaeal A-ATPases but their function is more closely related to the eukaryotic V-ATPase as they function exclusively in the direction of ATP hydrolysis driven ion pumping.

2.1. F-ATPase

The F-ATPase or F_1F_0-ATP synthase is found in the inner membrane of mitochondria, the thylakoid membrane of chloroplasts and the plasma membrane of bacteria where it is responsible for synthesis of ATP (Senior, 1988; Boyer, 1997; Fillingame et al., 1997; Junge et al., 1997). The process is driven by the electro chemical gradient across the lipid bilayer generated during electron transport by the enzymes of the respiratory chain or photosynthesis. In certain bacteria, F_1F_0-ATP synthase can function as an ion pumping ATPase to establish an electrochemical gradient across the plasma membrane to drive secondary transport processes. The structure and catalytic mechanism of the F-ATPase has been under intense investigation for more than 40 years and much of what we have learnt about the vacuolar ATPase is based on experiments originally designed for the study of the related F-type ATPase. Atomic resolution crystal structures are available for F_1-ATPase domains from bovine heart (Abrahams et al., 1994; Gibbons et al., 2001) and rat liver (Bianchet et al., 1998) mitochondria, spinach chloroplasts (Groth and Pohl, 2001) and the $\alpha_3\beta_3$ complex from the thermophilic bacillus PS3 (Shirakihara et al., 1997). The only structure showing part of the F_0 (the proteolipid ring containing 10 c subunits) has been obtained for the enzyme from yeast mitochondria at a resolution of 4.5 Å (Stock et al., 1999). According to the crystallographic structures, the F_1 is made of a hexagonal arrangement of alternating α and β subunits around a central cavity. Bound inside the cavity is the γ subunit which is protruding by about 35 Å from the bottom of the $\alpha_3\beta_3$ hexamer to form the central stalk. The ε subunit