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Expression, purification and characterization of isoforms of peripheral stalk subunits of human V-ATPase

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ABSTRACT

The vacuolar-type H^+-ATPase (V-ATPase) is a multi-subunit proton pump that is involved in both intra- and extracellular acidification processes throughout human body. Subunits constituting the peripheral stalk of the V-ATPase are known to have several isoforms responsible for tissue/cell specific different physiological roles. To study the different interaction of these isoforms, we expressed and purified the isoforms of human V-ATPase peripheral stalk subunits using *Escherichia coli* cell-free protein synthesis system: E1, E2, G1, G2, G3, C1, C2, H and N-terminal soluble part of a1 and a2 isoforms. The purification conditions were different depending on the isoforms, maybe reflecting the isoform specific biochemical characteristics. The purified proteins are expected to facilitate further experiments to study about the cell specific interaction and regulation and thus provide insight into physiological meaning of the existence of several isoforms of each subunit in V-ATPase.

Introduction

Vacuolar-type ATPases (V-ATPases) are large multi-subunit, membrane-associated protein complexes that carry out the active transport of protons across the membrane bilayer. V-ATPases are involved in the acidification of intracellular compartments and of the extracellular environment: such as lysosomes, chromaffin granules, other storage vesicles, protein sorting organelles, clathrin-coated vesicles and so on. The multi-subunit V-ATPase has many isoforms of each subunit and those are regulated at several levels, such as at assembly process, gene expression level and so on. Still now we do not know the details about the process of such regulation and differentiation into respective organelles and specific cell membranes. The V-ATPase is made of at least 13 individual components/protein subunits organized into two functional domains: V_0 and V_1 (Fig. 1). The V_0 domain is composed of several transmembrane subunits that are involved in proton translocation across the bilayer, while the V_1 domain is a peripheral catalytic part attaching to the V_0 domain. V_0 is composed of 5 subunits labeled from a to e while V_1 consists of 8 subunits denoted as A to H. All 13 different subunits are encoded by separate genes located throughout the genome [1]. The arrangement of subunits in the stalk regions and the distribution of subunits between the central and peripheral stalks have been investigated using a number of approaches. Cys-mediated crosslinking has provided evidence that subunits C, E, G, H and the N-terminal domain of subunit a all form part of a peripheral stalk, whereas subunits D and F form the central stalk [2].

Generally, mammals express a rich diversity of V-ATPase subunit isoforms and the functions of these various isoforms are not known yet. Unraveling the complex regulatory pathways that control V-ATPase activity, requires a more complete understanding of the structure and function of each subunit of these systems including the diverse isoforms, as well as the array of cellular proteins with which they associate. The crucial function of V-ATPases in many pathophysiological processes indicates that they should be prime targets in the development of therapies for diseases [3]. In higher eukaryotes, several H^+-ATPase subunits have been shown to have multiple isoforms encoded by different genes with

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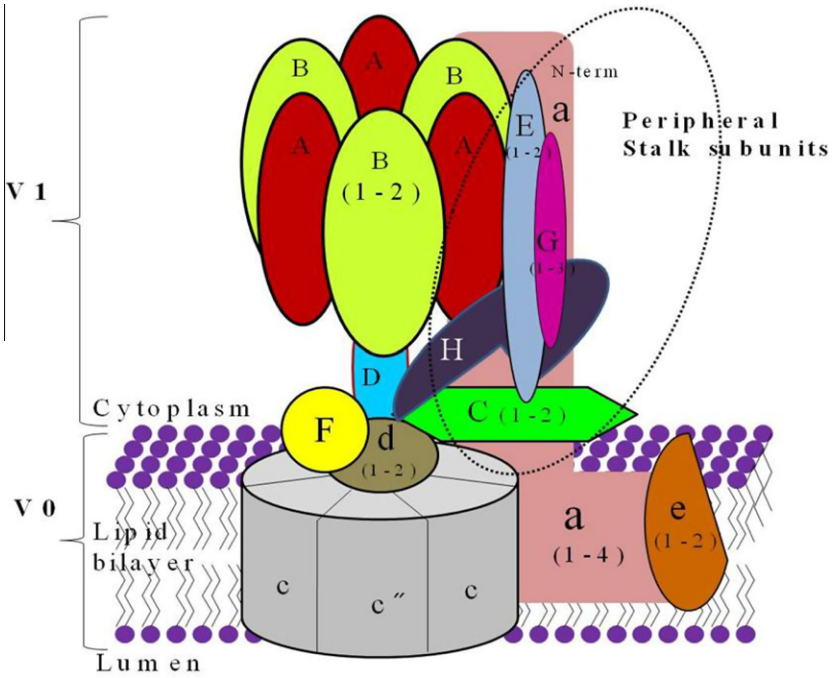

Fig. 1. Structure and predicted subunit organization of human V-ATPase. The number of isoforms was shown in parenthesis for each subunit. The peripheral stalk of V_1 domain is composed of Subunit C, E, G, H and N-terminal region of a (encircled with black dots).

differing tissue expression patterns. Two isoforms for each of the B, C, E, d and e, three for G subunit and four for the a subunit allow many possible permutation of the subunit structures in individual proton pumps. It is probable that pumps at different locations have their own unique subunit identities [4,5]. The existence of different subunit isoforms may play an important role in the localization and activity of proton pumps in specific cell types and subcellular compartments [6] (Table 1). We assume that the basic subunit structure is the same among the V-ATPases, and that their diverse cellular localization leading to their unique functions are determined by the specific subunit isoforms [7]. In human, it has been reported that mutation in any isoform specific gene creates disease of that particular isoform specific tissue or organ. For example, mutations in genes encoding a4 and a3 cause kidney disease and osteopetrosis, respectively [2,8]. Different studies suggest that V-ATPase is regulated in a much more complex manner than has been currently assumed [1]. So, having such many isoforms of a specific subunit may be the main cause of creating complexity in understanding human diseases. Therefore, it is necessary to study the functions of different isoforms of human V-ATPase subunits in relation to specific functions in specific tissues, cells and organelles.

In this study we expressed and purified several isoforms of the peripheral stalk subunits using the cell-free expression system: E1, E2, G1, G2, G3, C1, C2, H and N-terminal soluble part of a1 and a2, which were encoded by different genes ATP6V1E1, ATP6V1E2, ATP6V1G1, ATP6V1G2, ATP6V1G3, ATP6V1C1, ATP6V1C2, ATP6V1H, ATP6V0A1 and ATP6V0A2, respectively.

Materials and methods

Expression of E1G1, E1G2, E1G3, E2G1, E2G2, E2G3, C1, C2, H and N-terminal region of a1 and a2 subunit isoforms

Full-length E1G1, E1G2, E1G3, E2G1, E2G2, E2G3, C1, C2, H genes, two DNA constructs for a1 and one DNA construct for a2

Table 1
Characteristics of peripheral stalk subunits of human V-ATPase.

	Human subunit	Isoform	Human gene	Mol. mass (kDa)	Yeast gene	Tissue or cell localization	Subcellular localization	Ref.
V1	C				*VMA5*			[2]
		C1	*ATP6V1C1*	44.4		Ubiquitous		[8]
		C2	*ATP6V1C2*	45		Lung, kidney, epididymus, placenta		[5,8]
	E				*VMA4*			[2]
		E1	*ATP6V1E1*	26.66		Germ cells of testis		[14,8]
		E2	*ATP6V1E2*	26.59		Ubiquitous		[8]
	G				*VMA10*			[2]
		G1	*ATP6V1G1*	14		Ubiquitous	Lysosomal membrane	[8]
		G2	*ATP6V1G2*	14		Neural	Synaptic vesicles	[8]
		G3	*ATP6V1G3*	14		Kidney, inner ear		[8,18]
	H		*ATP6V1H*	56	*VMA13*		Clathrin-coated vesicles	[2]
	a			100	*VPH1*(Vacuole)			[2,8]
					STV1(Golgi)			[2,8]
V0		a1	*ATP6V0A1*			Neuronal, brain	Synaptic vesicles	[19]
		a2	*ATP6V0A2*			Endothelial, neurons	Golgi	[19,8]
		a3	*ATP6V0A3*			Osteoclasts, pancreatic β-cells	Endosomal and lysosomal	[8]
		a4	*ATP6V0A4*			Kidney, epididymis	Plasma membrane	[19,8]