A Review: Sodium-Potassium Adenosine Triphosphatase (Na^+/K^+-ATPase) and its Isoforms

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Abstract **-** Na^+/K^+-ATPase, found in all mammalian cell membranes, is necessary for proper cellular function since it helps to preserve the ionic gradients across the cell membrane and thus the membrane potential and osmotic equilibrium of the cell. The sodium-potassium ATPase (Na^+/K^+-ATPase) is an important ion transporter which pumps sodium out of cells in exchange for potassium, thereby generating large gradients of these ions across the plasma membrane. The isoforms α (αl, α2**,** α3 and α4) and β (β1, β2 and β3) combine to form a number of Na^+/K^+-ATPase isozymes. So in present study paper information regarding molecular regulation of the sodium pump expression and function, as well as role of the sodium pump during heat stress is described.

Keywords **-** *Sodium Pump, ATPase, Protein Expression, Ouabain, Signaling*

I. INTRODUCTION

The sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) is a transmembrane protein, found in all higher eukaryotic cells, that is responsible for the translocation of three sodium ions out of the cell and two potassium ions into the cell for every molecule of ATP that is hydrolyzed [1]. The ion gradients established by the activity of this enzyme allow for the Na^+ coupled transport of various nutrients: glucose and amino acids into cells and movement of ions such as Ca^{2+} and H^+ across the membrane. The nonequivalent pumping of Na^+ and K^+ across the cell membrane is also essential for maintaining the resting membrane potential of the cell, cell volume regulation, osmotic balance, and for the electrical activity of muscles and nerves [2,3].

A. The ATPase family

ATPases are the ion channels bound to the membrane which aid in the ion movement across the cell either by generating or by hydrolyzing a nucleotide. These enzyme utilizes the energy produced from the breakage of the ATP phosphodiester bond and hence creates an ion gradient. Na^+/K^+-ATPase belongs to P type ATPases along with Ca^{+2} ATPase of sarcoplasmic reticulum and plasma membrane, plasma membrane H^+-ATPase and proton potassium ATPase of stomach and colon [4].The widely distributed class of P-type ATPases is responsible for the active transport of a variety of cations across cell membranes. They are found in both prokaryotic and eukaryotic cells, and are used for transporting H^+, Na^+, $Mg^{2+,}$ K^+, Ca^{2+}, $Cu^{2+,}$ and Cd^{2+}. All of these enzymes use the hydrolysis of ATP to drive the transport of cations against an electrochemical potential.

The characteristic feature of P type ATPase is the formation of the phosphorylated intermediate state during the reaction cycle [5]. Hence this features differentiates it from other types of ATPases i.e. F-ATPases (present in mitochondria, chloroplasts and bacterial plasma membranes) and V-ATPases (present in eukaryotic vacuoles). The presence of conserved sequence motifs in the cytoplasmic domains defines the primary structure of P-type ATPases. Nucleotide-binding (N), phosphorylation (P) and an actuator (A) domain are the three domains present in the cytoplasmic domain [6]. A common feature of all known P-type ATPases is the presence of the transmembrane (M) domain having a central core of six alpha helices[4]. These comprise of one polypeptide i.e. α chain which is responsible for ion transport and ATP hydrolysis and a large protein family which pump ions like H^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ag^+ and Ag^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Cu^+ and Cu^{2+} and lipids across cellular membranes [3,7]. P-ATPases bind and transport the ions by cycling between the two different cation-dependent conformations called as E1 and E2 states [3]. The two P-type ATPases that have been studied most are the Na^+/K^+ [8,9] and the Ca^{+2} [10] which are involved in electrolyte and fluid balance and in muscle relaxation respectively [4].

B. Structure of ATPase

The functional Na^+/K^+-ATPase is composed of three subunits: an alpha, beta and gamma subunit. The alpha subunit, molecular weight approximately 112-kDa, is the catalytic component of the Na^+/K^+-ATPase and contains the ATP, Na^+, and K^+ binding sites, as well as the cardiac glycoside (inhibitor of Na^+/K^+-ATPase activity) binding site [2, 3]. The beta subunit, a highly glycosylated protein with a molecular weight of 55- kDa, is necessary for the maturation of the enzyme and localization of the Na^+/K^+-ATPase to the plasma membrane A third protein, called the gamma subunit, has been identified in purified preparations of the enzyme. The gamma subunit is a small, hydrophobic polypeptide of 8-14 kDa that has been shown to be responsible for altering the affinity of the Na^+/K^+-ATPase for cations in certain cell types.

II. ALPHA SUBUNIT

The alpha subunit is the catalytic subunit has a relative molecular mass of 100–113 kDa depending on the presence of different isoforms which are αl, α2**,** α3 or α4 encoded by *ATP1A1*, *ATP1A2*, *ATP1A3* and *ATP1A4* genes respectively.

Alpha subunit is the site for both ATP binding and ion occlusion. Hence it contains the functional sites for ATP, transported cations, and cardiac glycoside inhibitors [3]. The α subunit has ten transmembrane segments which form the transmembrane domains M1 to M10 and both N- and C-termini are confined to the cytosolic side [12]. Analysis of cDNA sequences of the alpha subunit has revealed that there are 10 transmembrane domains (M1–M10) which enclose cation binding sites. Two cytoplasmic loops i.e. major and minor loops are present between the helices M4 and M5 and between M2 and M3 respectively. Major loop contains the site for nucleotide binding and phosphorylation sites. The ouabain binding site is located in a small extracellular domain which is composed of short loops between various transmembrane helices and is present on the extracellular side of the membrane [13].

III. BETA SUBUNIT

The β subunit is highly glycosylated and has a relative molecular mass of about 60 kDa. The β subunit has single membrane crossing i.e. it is type II protein 1[4]. The N-terminus is present on the intracellular side of the membrane. More recent results have shown that the β subunit makes direct contact with the alpha subunit [15] and so helps in the stabilization of the α subunit and also aid in the transportation of alpha subunit from the endoplasmic reticulum to the plasma membrane [16]. It is also required for maturation of the Na^+/K^+-ATPase and alters the activity of the Na^+/K^+-ATPase. Beta subunit is important for ATP hydrolysis, ion transport and for binding of inhibitors such as ouabain [12].

A. FXDY2 (Gamma subunit)

The third subunit of Na^+/K^+-ATPase is the the γ subunit having molecular mass 7–11 kDa. It is a strech of 30 amino acids of α helical structure as seen in electron density map [5]. The γ subunit associates with various isoforms of Na^+/K^+-ATPase [17]. The γ subunit is a single-span membrane protein with the carboxyl terminus exposed to the cytosol [18]. FXYD2 is found to be associated with renal Na^+/K^+-ATPase [19]. The level of similarity between *Xenopus* gamma subunit and other mammalian gamma subunits is 76% [17].

B. Tissue Expression

Isoforms of the alpha and beta subunits, each encoded for by different genes, have been identified and exhibit unique tissue and developmental expression patterns [3, 20]. In mammals, there are four isoforms of the alpha subunit (alpha 1,2,3, and 4) and three isoforms of the beta subunit (beta 1,2,and 3) [2, 21]. The alpha isoforms have been shown to exhibit the following distinct tissue-specific expression patterns: the alpha 1 isoform is expressed ubiquitously, the alpha 2 isoform is mainly expressed in heart, skeletal muscle and brain, the alpha 3 isoform is expressed in neural tissue and in the ovary, and the alpha 4 isoform is expressed in the testis, specifically in sperm [2, 20]. In mammals the three beta subunit isoforms are not as tissue specific as the alpha isoforms. The beta 1 isoform has been shown to be expressed in most tissues, the beta 2 isoform is mainly located in the neural tissue, and the beta 3 isoform is expressed in many, but not all [2, 20, 22].

C. Na/K-ATPase function (Post-Albers Model)

The Na/K-ATPase belongs to P-ATPases (known as E1-E2 ATPases) which has two conformations states; E1 and E2. In the E1 state, cation binding sites facing the cytoplasm have high affinity for Na^+ and vice-versa in E2 state which faces the extracellular side and have high affinity for K^+ and. In E1 state, three sodium ions bind to the pump from the cytoplasmic side of the membrane. ATP phosphorylates the enzyme and the remaining ADP is released. The conformation then changes to E2, and the three sodium ions are released into the extracellular space.

D. Functions of the Isoforms

Recently, it has been suggested that tissue specific pattern of various isoforms of Na^+/K^+-indicates their distinct cellular function. Ubiquitous presence of alpha 1 isoform suggests its role in maintaining the ionic gradient across the cell. The functional role of alpha 2 isoform in the cardiac system (heart) was studied by doing experiments on the mice which had heterozygous null mutation for the alpha 1 or alpha 2 isoform [23]. It was observed that alpha 1 and alpha 2 protein production was reduced by half amount in the animals having null mutations for the alpha 1 or alpha 2 isoform respectively. Increased calcium transients during the contractile cycle were observed in mice with heterozygous alpha 2 hearts which are hypercontractile and heterozygous alpha1 hearts were hypocontractile. This clearly demonstrates that the alpha 1 and alpha 2 isoforms play different roles during the contraction of the heart.

Another example of an isoform specific role comes from a study that examined the rat Na^+/K^+-ATPase alpha 4 isoform, which is only expressed in sperm. This isoform can be selectively inhibited in rat sperm because of its high affinity for ouabain compared to the low affinity of the alpha 1 isoform, the only other isoform present. Using these affinity differences it has been shown that specific inhibition of the alpha 4 isoform, with ouabain, inhibits sperm motility. These results demonstrate a distinct functional role for the Na^+/K^+-ATPase alpha 4 isoform in normal sperm function [21].

E. Association of ATP1A1 with heat stress

The thermal comfort zone is the ambient temperature range within which the metabolic rate of the animal is independent of environmental temperature and any value below or above that range may create discomfort to the animal [24]. At high temperatures, the animal has to consume energy to maintain its body temperature. The buffalo is essentially a shade and water loving animal inhabiting areas containing many water streams. However, they exhibit signs of great distress when exposed to direct solar radiation. Recently, *Na^+/K^+-ATPase* gene has been identified as an important candidate gene during heat shock response. Amongst various isoforms, alpha 1 isoform (*ATP1A1)*, which is the major isoform, has been

suggested to plays a key role in adaptation and heat tolerance. It is known that genotypic variants of different Na^+/K^+-ATPase subunits have different heat tolerance capacities in the cattle. This characteristic has been attributed to its sensitivity to oxidative stress and its role to establish the electrochemical gradient of Na^+ and K^+ across the plasma membrane.

Polymorphism of the *ATP1A1* gene has been examined in Chinese Holstein cattle using polymerase chain reaction low ionic strength single-strand conformation polymorphism [25, 26]. Significant associations of *ATP1A1* gene with physiological parameters like Heat Tolerance Coefficient (HTC) and Respiratory Rate (RR) revealed that *ATP1A1* gene may play an important role in heat shock response. Higher mRNA levels of *ATP1A1* at higher temperatures indicated that it may be promising as a potential candidate for anti-heat stress.

Influence of temperature-humidity variation in winter, spring and summer season on the expression of *ATP1A1* of Tharparker and Vrindavani Cattle was studied and significant fold changes was observed for *ATP1A1* expression in various seasons [27]. It was observed that *ATP1A1* expression showed higher fold change in summer than winter when compared with spring season values in both the cattle.

Hence, Significant positive correlation found between Na^+/K^+-ATPase activity and mRNA level of alpha1 isoforms suggested to plays a key role in adaptation and heat tolerance.

IV. CONCLUSION

Na^+/K^+-ATPase carries out coupled hydrolysis of ATP and hence actively transport the ions across membrane. Na^+/K^+-ATPase performs diverse cellular functions as the regulation of cell volume and pH, nutrient uptake, and membrane excitability. Isoforms of Na^+/K^+-ATPase exhibit tissue specific expression. It is suggested that the bovine *ATP1A1* gene may play an important role in heat resistance.

V. REFERENCES

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