Structure, Function and Regulation of Na^+,K^+-ATPase.

Prof. Steven J. D. Karlish

Daniel. M. Tal, Rivka Goldshleger, Eytan Cohen, Maria Füzesi, Moshit Lindzen, Yael Lifshitz, Talya Belogus, Haim Haviv, Elizabeta Dinitz

Department of Biological Chemistry

Contact information

Tel. 972 8 934 2278
Fax. 972 8 934 4118
E-mail: steven.karlish@weizmann.ac.il,

The Na^+/K^+-ATPase or Na^+, K^+-pump utilizes the free energy of hydrolysis of ATP to actively transport Na^+ and K^+ ions across mammalian cell membranes. This function underlies essentially all of mammalian cell physiology. For example, in the kidney the Na^+, K^+-pump controls body Na and K balance, extracellular volume and blood pressure. The Na^+/K^+-ATPase is the receptor of digitalis steroids used to treat heart failure.

Na^+/K^+-ATPase is one of a family of cation pumps, the P-type ATPases, which includes also sarcoplasmic reticulum Ca^{2+}-ATPase, gastric cell membrane H^+/K^+-ATPase, plasma membrane Ca^{2+}-ATPase, plant cell membrane H^+-ATPase, heavy metal-dependent ATPases etc., with selectivity for the other cations. Na^+/K^+-ATPase and H^+/K^+-ATPase consist of a catalytic α subunit with ten trans-membrane segments, and a single trans-membrane glycosylated β subunit, required for stabilization. There are four isoforms of α (1-4) and three isoforms of β (1-3) subunits. $\alpha 1 \beta 1$ is the “housekeeping” isoform.

The field was transformed recently by the publication of molecular structures of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1). Thus, we now have a general idea of the cation pump mechanism (reviewed in (Jorgensen et al., 2003). Since, however, the Ca^{2+}-ATPase structures are incapable of explaining their unique mechanistic, regulatory or pharmacological features, we urgently need molecular structure of Na^+/K^+-ATPase and the other cation pumps.

Purification of Na,K-ATPase expressed in Pichia Pastoris

We have expressed the porcine $\alpha 1/His_{10}\beta 1$ subunits in *Pichia pastoris* (Strugatsky et al., 2003), a methanotrophic yeast which grows to high cell densities and provides a ready source of recombinant Na^+/K^+-ATPase. A recent publication describes 70-80% purification of the n-dodecyl-β-maltoside (DDM) solubilized porcine $\alpha 1$-$His_{10}\beta 1$ complex, by Ni^{2+}-NTA chromatography combined with HPLC (Cohen et al., 2005). The work demonstrated an essential role of phospholipid, including evidence for a direct interaction of dioleoyl phosphatidylserine with Na^+,K^+-ATPase. More recently we developed a simpler procedure, which provides ≈90% pure protein in a single step. Fig. 1 shows the porcine $\alpha 1\beta 1$ complex and for comparison native pig kidney Na^+,K^+-ATPase. The β subunits are expressed as two bands, which are much less glycosylated than in renal Na,K-ATPase.

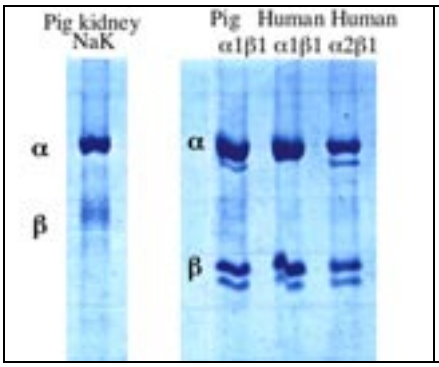

Fig. 1 One-step purification of Porcine $\alpha 1\beta 1$ and human $\alpha 1\beta 1$ and $\alpha 2\beta 1$ isoforms of Na,K-ATPase expressed in Pichia Pastoris

Fig. 1 also shows that human $\alpha 1\beta 1$ and $\alpha 2\beta 1$ isoforms can be readily purified from *P.pastoris* cells (Y. Lishitz and R. Goldshleger). Other isoforms are also being expressed (E.Dinitz)

In a third development FXYD1 (phospholemman), one of the family of FXYD regulatory proteins (Garty and Karlish, 2006), has been expressed in *Pichia pastoris*, and $\alpha 1/\beta 1$/FXYD1 complexes have been purified and characterized (Lifshitz et al., 2006).

The purified Na^+,K^+-ATPase complexes [porcine $\alpha 1/\beta 1$, human $\alpha 1/\beta 1$, $\alpha 2/\beta 1$, porcine $\alpha 1/\beta 1$ /hPLM] can be produced essentially pure, functional and stable in quantities of c.1 mg. Crystalization trials (E. Cohen and H. Haviv). are being initiated, together with Joel Sussman (Structural Biology).

Structure-function relations.

Although molecular structure is necessary it is rarely sufficient to fully understand complex biological machines. Lower resolution techniques are also required. We have developed specific Fe^{2+}-catalysed oxidative cleavage of Na^+,K^+-ATPase and related P-type pumps mediated by specifically bound Fe^{2+} or ATP-Fe^{2+} (reviewed in Karlish, 2003). This technique provides important

information on proximity of cleavages sites and changes in different conformational states. The most recent application to the recombinant Na^+,K^+-ATPase suggests that ATP is bound with two Mg^{2+} (Fe^{2+}) ions (Strugatsky et al., 2005). This novel feature was not seen in the Ca^{2+}-ATPase structure bound with ADPCP-Mg^{2+}. Fluorescence techniques are being developed to detect conformational changes of recombinant Na^+,K^+-ATPase (T. Belogus).

Regulation of Na^+,K^+-ATPase by FXYD proteins (collaboration with Haim Garty)

FXYD proteins are a group of seven short single span transmembrane proteins termed after the invariant motif FXYD in their extracellular domain. FXYD proteins act as tissue-specific regulatory subunits, which adjust the kinetics properties of the Na^+, K^+-pump to the needs of the particular cell type or physiological state (reviewed in Garty and Karlish, 2006).

Our work has focused mainly on the γ subunit of Na^+,K^+-ATPase (FXYD2), and Corticosteroid Hormone Induced Factor (CHIF or FXYD4), which are expressed in the basolateral membrane of different kidney cells, or colon (CHIF), and are involved in the regulation of the Na^+ and K^+ homeostasis. More recently we turned our attention to FXYD1 (phospholemman, PLM), which regulates the Na^+, K^+-pump in cardiac and skeletal muscle. PLM has PKA and PKC phosphorylation sites and responds to β-adrenergic and other hormonal signals.

We have characterized functional effects of FXYD proteins expressed in mammalian cells, *Xenopus* oocytes (Garty et al., 2002; Lindzen et al., 2003; Lubarski et al., 2005), and *Pichia Pastoris* (Lifshitz et al., 2006). These studies demonstrated different effects of each FXYD protein, which serve the cellular needs in the specific nephron segments. Thus, whereas FXYD2 reduces the apparent affinity for cell Na^+, FXYD4 increases it by up to 3-fold. In *P.pastoris* PLM appears to raise apparent affinity for cell Na^+. In other cell types (*Xenopus* oocytes and *HeLa* cells) PLM has an opposite effect. The difference may be caused by different phosphorylation states of the PLM.

Structural interactions between FXYD proteins and the Na^+/K^+-ATPase have been studied by chemical cross-linking and mutagenesis (Füzesi et al., 2005; Lindzen et al., 2003; Lindzen et al., 2006). Based on these studies we have proposed a molecular model, which places the trans-membrane segment of all FXYD proteins in a groove between trans-membrane segments M2, M6 and M9 and the C-terminal domain of γ in contact with cytoplasmic stalk (S5) and loops (L6/7, L8/9) of the α subunit (Fig. 2). The model makes a number of predictions, which are being tested experimentally.

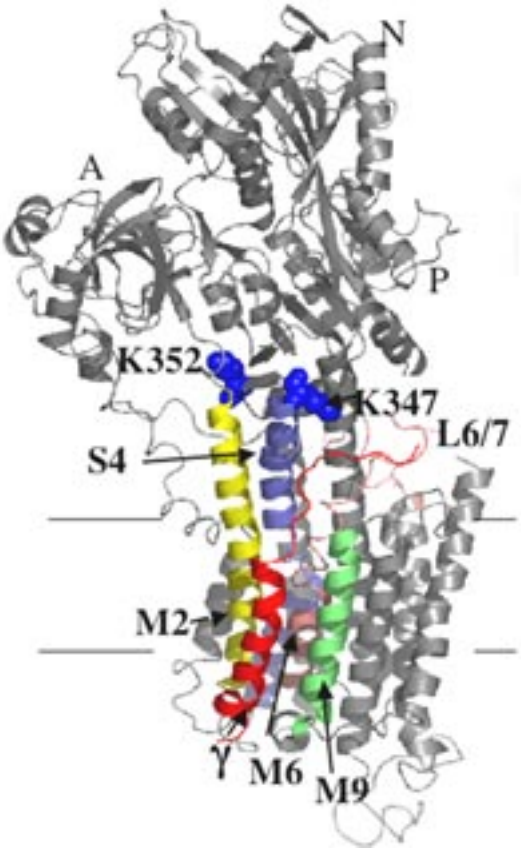

Fig. 2 *Homology model of pig α1 subunit with model for docking of FXYD2 (γ) in the groove between M2, M6 and M9 trans-membrane segments and with cytoplasmic stalk and loops (Fuzesi et al., 2005).*

Selected publications

Garty, H., Lindzen, M., Scanzano, R., Aizman, R., Fuzesi, M., Goldshleger, R., Farman, N., Blostein, R. and Karlish, S.J. (2002) A functional interaction between CHIF and Na-K-ATPase: implication for regulation by FXYD proteins. *Am J Physiol Renal Physiol*, **283**, F607-615.[PubMed]

Lindzen, M., Aizman, R., Lifshitz, Y., Lubarski, I., Karlish, S.J. and Garty, H. (2003) Structure-function relations of interactions between Na,K-ATPase, the gamma subunit, and corticosteroid hormone-induced factor. *J Biol Chem*, **278**, 18738-18743.[PubMed]

Jorgensen, P.L., Hakansson, K.O. and Karlish, S.J. (2003) Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol*, **65**, 817-849. [Pubmed]

Karlish, S.J. (2003) Investigating the energy transduction mechanism of P-type ATPases with Fe2+-catalyzed oxidative cleavage. *Ann N Y Acad Sci*, **986**, 39-49.[PubMed]

Strugatsky, D., Gottschalk, K.E., Goldshleger, R., Bibi, E. and Karlish, S.J. (2003) Expression of Na+,K+-ATPase in Pichia pastoris: analysis of wild type and D369N mutant proteins by Fe2+-catalyzed oxidative cleavage and molecular modeling. *J Biol Chem*, **278**, 46064-46073.[PubMed]

Cohen, E., Goldshleger, R., Shainskaya, A., Tal, D.M., Ebel, C., le Maire, M. and Karlish, S.J. (2005) Purification of Na+,K+-ATPase expressed in Pichia pastoris reveals an essential role of phospholipid-protein interactions. *J Biol Chem*, **280**, 16610-16618.[PubMed]

Fuzesi, M., Gottschalk, K.E., Lindzen, M., Shainskaya, A., Kuster, B., Garty, H. and Karlish, S.J. (2005) Covalent cross-links between the gamma subunit (FXYD2) and alpha and beta subunits of Na,K-ATPase: modeling the alpha-gamma interaction. *J Biol Chem*, **280**, 18291-18301.[PubMed]

Lubarski, I., Pihakaski-Maunsbach, K., Karlish, S.J., Maunsbach, A.B. and Garty, H. (2005) Interaction with the Na,K-ATPase and tissue distribution of FXYD5 (related to ion channel). *J Biol Chem*, **280**, 37717-37724.[PubMed]

Strugatsky, D., Gottschalk, K.E., Goldshleger, R. and Karlish, S.J. (2005) D443 of the N domain of Na+,K+-ATPase interacts with the ATP-Mg2+ complex, possibly via a second Mg2+ ion. *Biochemistry*, **44**, 15961-15969. [PubMed]

Garty, H. and Karlish, S.J. (2006) Role of fxyd proteins in ion transport. *Annu Rev Physiol*, **68**, 431-459.[PubMed]

Lifshitz, Y., Lindzen, M., Garty, H. and Karlish, S.J.D. (2006) Functional interactions of Phospholemman (FXYD1) with Na+,K+-ATPase. Purification of alpha1/beta1/PLM complexes expressed in *Pichia Pastoris*. *J. Biol. Chem.*, **In Press**.

Lindzen, M., Gottschalk, K.E., Fuzesi, M., Garty, H. and Karlish, S.J. (2006) Structural Interactions between FXYD Proteins and Na+,K+-ATPase: {alpha}/beta/FXYD SUBUNIT STOICHIOMETRY AND CROSS-LINKING. *J Biol Chem*, **281**, 5947-5955.[Pubmed].

Acknowledgements

This work was supported by research grants from the Israel Science foundation and the US Israel Binational Science Foundation. Steven J. D. Karlish is the incumbent of the William Smithburg Chair of Biochemistry.

INTERNAL support

This work was supported by the Weizmann Institute Renal Research fund, and the Josef Cohen Minerva Center of Biomembranes.

Keywords

Na^+,K^+-ATPase, Karlish, S. J. D
Expression in P.pastoris, Karlish, S. J. D
Structure-function relation, Karlish, S. J. D
FXYD proteins, Karlish, S. J. D
Kidney function, Karlish, S. J. D