Mutational analysis of structure-function relationship of transmembrane segment M1 of the Ca^{2+}-ATPase and the Na^+,K^+-ATPase

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ABSTRACT

The present PhD dissertation is based on experimental work carried out at the Institute of Physiology and Biophysics, University of Aarhus. The study was conducted to gain further insight into the molecular transport mechanism of the Ca^{2+}-ATPase and the Na^+,K^+-ATPase.

The Ca^{2+}-ATPase and the Na^+,K^+-ATPase, which belong to the superfamily of P-type ATPases, are membrane-bound enzymes that use the energy liberated by ATP-hydrolysis to transport Ca^{2+}/H^+ and Na^+/K^+, respectively, across the membranes, thereby contributing to the maintenance of cellular ion-homeostasis. They share a common overall fold comprising ten transmembrane segments (M1-M10) and a large cytosolic protrusion. The translocation of ions across membranes is achieved by a series of conformational changes in the enzymes coupled to the formation and breakdown of a phosphorylated intermediate. It is believed that during their passage through the membrane, the ions are kept in an occluded state with no access to the medium on either side of the membrane, their escape being prevented by so-called "gates". At present, the nature of the gating mechanism is poorly understood.

The present study aimed at elucidating and comparing the functional role of transmembrane segment M1 of the Ca^{2+}-ATPase and the Na^+,K^+-ATPase. For that purpose, an approach combining mutant construction by site-directed mutagenesis and expression in mammalian cells with kinetic analysis was used to examine the role of amino acids in M1. The latter was achieved using, among others, a rapid mixing technique, which allows measurements in the millisecond range. The results identified residues in the middle part of M1 of the Ca^{2+}-ATPase – in particular a leucine (Leu65) – that is critical to the binding and occlusion of Ca^{2+}. Furthermore, specific residues in the middle section of M1 the Na^+,K^+-ATPase were found important for the interaction with extracellular K^+ as well as for K^+-occlusion. In the Na^+,K^+-ATPase, the most prominent effects were observed for mutants with alteration to a leucine (Leu99 – the equivalent of Leu65). The findings could be accounted for by a model for ion-occlusion in which Leu65 of the Ca^{2+}-ATPase and Leu99 of the Na^+,K^+-ATPase interact and cooperate with a conserved ion-binding glutamate in M4 in the gating of the ion-binding sites. Besides Leu99, a glycine (Gly94) in the Na^+,K^+-ATPase was shown to be crucial to K^+-binding. Structural modeling of the Na+,K+-ATPase suggested that Gly94 forms a contact point with an isoleucine in M3 that serves as a pivot for the movement of M1 in connection with binding of extracellular K^+.

In conclusion, this dissertation contributes new knowledge to the field of P-type ATPases by implicating M1 of the Ca^{2+}-ATPase and the Na^+,K^+-ATPase in ion-translocation – a role of M1 that may well pertain to all P-type ATPases.