Involvement of Alpha-Subunit N-Termini in the Mechanism and Regulation of the Na^+,K^+-ATPase

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The Na^+,K^+-ATPase is integral for the maintenance of membrane potential, osmoregulation, and nerve and muscle function. The lysine-rich N-terminus of the Na^+, K^+-ATPase has been designated as a regulatory (R) domain due to its speculated involvement in the acute regulation of ion pumping activity. However, X-ray crystallography studies of the Na^+,K^+-ATPase have been unable to resolve the structure of the N-terminus as it is likely undergoing dynamic motion during the time scale of X-ray structure determination [1]. An initial hypothesis for the regulation of the Na^+,K^+-ATPase suggested the formation and breakage of a salt bridge between Lys30 of the N-terminus and Glu233 in the first M2-M3 cytoplasmic loop of the Na^+,K^+-ATPase, which induces a conformational shift in the protein, affecting overall turnover [2]. However, theoretical studies predicting the structure of the N-terminus with reference to the entire crystal structure of the Na^+,K^+-ATPase later disproved this hypothesis due to the considerable distance between the amino acid residues, rendering it unlikely for direct, sustained interaction to occur between them for ion pump regulation.

We have proposed an alternative hypothesis whereby Na^+,K^+-ATPase regulation is dependent on the electrostatic interaction of positively charged amino acid residues of the N-terminus with negatively charged lipid headgroups, notably phosphatidylserine (PS) on the cytoplasmic leaflet of the neighbouring plasma membrane. We propose that this interaction is potentially governed by an electrostatic switch mechanism in which serine and/or tyrosine residues of the N-terminus are phosphorylated by protein kinases, neutralising the positive charge of the lysines and allowing its subsequent detachment from the membrane. A similar mechanism has been documented in the trafficking of peripheral membrane proteins [3,4]. Electrophoresis with dynamic light scattering detection was used in this study to determine the surface charge density of Na^+,K^+-ATPase - containing membrane fragments the measured zeta potentials (ψ_z) at different ionic strengths of the surrounding solution. The weighted average of the surface charge density of the membranes yielded a value of 0.019 (± 0.001) C m^{-2}, a figure that is consistent with a previous experimental value determined for the surface charge density responsible for the electrostatic interaction that stabilises the enzyme's K^+-selective E2 conformation. Tethered bilayer lipid membranes (tBLMs) in conjunction with AC electrical impedance spectroscopy (EIS) were employed to pinpoint and confirm the N-terminus amino acid residues and membrane phospholipids involved in this hypothesised interaction. Bioinformatic analysis was conducted to search for any evidence of coevolution of the Na^+,K^+-ATPase with different members of the Src Kinase family, which would provide support for the hypothesised electrostatic switch mechanism.