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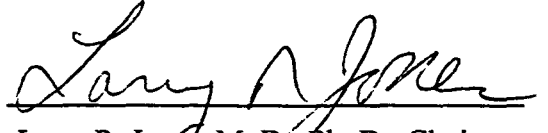
**RESIDUES INVOLVED IN
PHOSPHOLEMMAN
CHANNEL ACTIVITY**

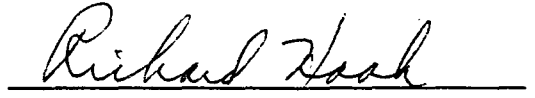
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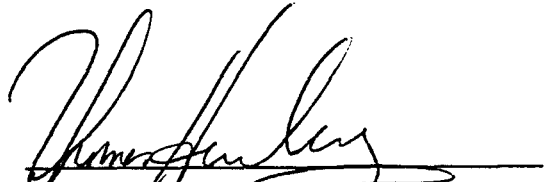

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ABSTRACT

Phospholemman (PLM), a single transmembrane domain protein of 72 amino acids, is the principal sarcolemmal substrate for cAMP-dependent protein kinase and protein kinase C in cardiac cells. PLM is hypothesized to function as an ion channel because it induces Cl^- currents when expressed in oocytes and generates single channel currents when reconstituted into planar bilayers. Further characterization of PLM is important because it is a major target for hormone-stimulated phosphorylation in heart tissue, and because of its unique channel activity, small size, simple structure, and wide distribution. In this thesis, detailed structural and functional analyses of PLM was performed by combining biophysical, biochemical, and molecular biological techniques. Wildtype PLM exhibited complex patterns of channel activity including voltage-dependent closing and selectivity switching in bilayers. To study the role of different region(s) of PLM in channel activity, wildtype PLM and several PLM mutant proteins were expressed in insect cells, purified using antibody affinity chromatography, and characterized electrophysiologically in planar lipid bilayers. By comparison of the channel activities of the wildtype and mutant PLM proteins, it is concluded that the minimum channel requires at least the N-terminal and the transmembrane domains (residues 1-43) for ion conductance. These domains constitute the pore and comprise the “core” of the channel. Moreover, the N-terminus is involved in anion selection, but the transmembrane domain seems to play no role in ion selection or voltage sensing. On the other hand, the C-terminal region of PLM (residues 43-72) appears to be predominantly regulatory. It may plug the pore at certain voltages to generate voltage-dependent closing. Due to these unique properties, PLM appears to belong to a new family of ion channel proteins.

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