STRUCTURAL DYNAMICS OF PHOSPHOLAMBAN IN A MEMBRANE

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The structural dynamics of phospholamban (PLB), an integral membrane protein that regulates cardiac calcium transport was analyzed by site-directed spin labeling and multifrequency EPR. In order to obtain direct information about peptide backbone dynamics, we used the synthetically-incorporated TOAC spin label, which is rigidly linked to the α carbon. We used derivatives of monomeric PLB (AFA-PLB), containing a single TOAC label at four different positions along the protein's sequence, and reconstituted the spin-labeled protein in lipid bilayers. EPR spectra, acquired at both X and W band, were analyzed by spectral simulation and fitting using the NLSL program utilizing the MOMD model [1].

We assumed either one or two molecular/conformational components, each characterized by a rotational correlation time, an order parameter (orienting potential), and a mole fraction. A and g tensor values were determined directly from low-temperature spectra. To minimize the ambiguity of analysis, grids of spectra were simulated for different rotational correlation times, orienting potentials, and line broadenings, and simulated spectra were compared with experimental spectra in terms of specific diagnostic spectral features, such as outer extrema splitting, line broadening, and relative peak intensities. These were used to establish initial parameters for simultaneous fitting of X and W band experimental spectra. Two motional models were considered: isotropic and anisotropic motion in an orienting potential. Data from probes in each of the major domains of PLB, along with the solution NMR structure, allowed us to construct a model of PLB structural dynamics in the membrane. This establishes the basis for future analysis of the molecular mechanism of phosphorylation-dependent regulation of the Ca-ATPase by PLB.

Acknowledgements

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References

[1] Budil, D. E. *et al.*, J. Magn. Res., **A120**, 155, (1996).