PROTEIN-PROTEIN INTERACTIONS BY SOLID-STATE NMR SPECTROSCOPY

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We investigated a protein-protein interaction between two integral membrane proteins involved in cardiac muscle contractility, phospholamban (PLB) and sarco(endo)plasmic reticulum calcium pump (SERCA). PISEMA spectra were acquired on a ^{1}H-^{15}N static flat-coil probe with a Bruker DRX600 NMR spectrometer using uniformly labeled ^{15}N PLB reconstituted in mechanically oriented lipid bilayers. These data are the first to be carried out using two membrane proteins reconstituted in oriented lipid bilayers.

The solution NMR structure of PLB has been solved in detergent micelles to be an *L-shape* structure: a cytoplasmic helix (residues 1-17) connected to a transmembrane helix (residues 22-52) via a flexible hinge region (residues 17-21). The PISEMA spectra shown in Fig. 1 rule out the possibility of a continuous α-helical structure for monomeric PLB in both the free and the bound form, and are in agreement with those from EPR studies on the orientation and dynamics of PLB. The well resolved peaks at large dipolar couplings ~4-8 kHz and ^{15}N CSA 150-200 ppm indicate the formation of a PISA wheel. Even from these preliminary data shown in Fig. 1 it is possible to infer that the transmembrane domain undergoes to a remarkable topological change. On the other hand, the cytoplasmic portion of the protein is not very well resolved, hampering the spectral interpretation. Although this can be explained by dynamics in that portion of the protein, further experiments need to be performed using selectively labeled samples.

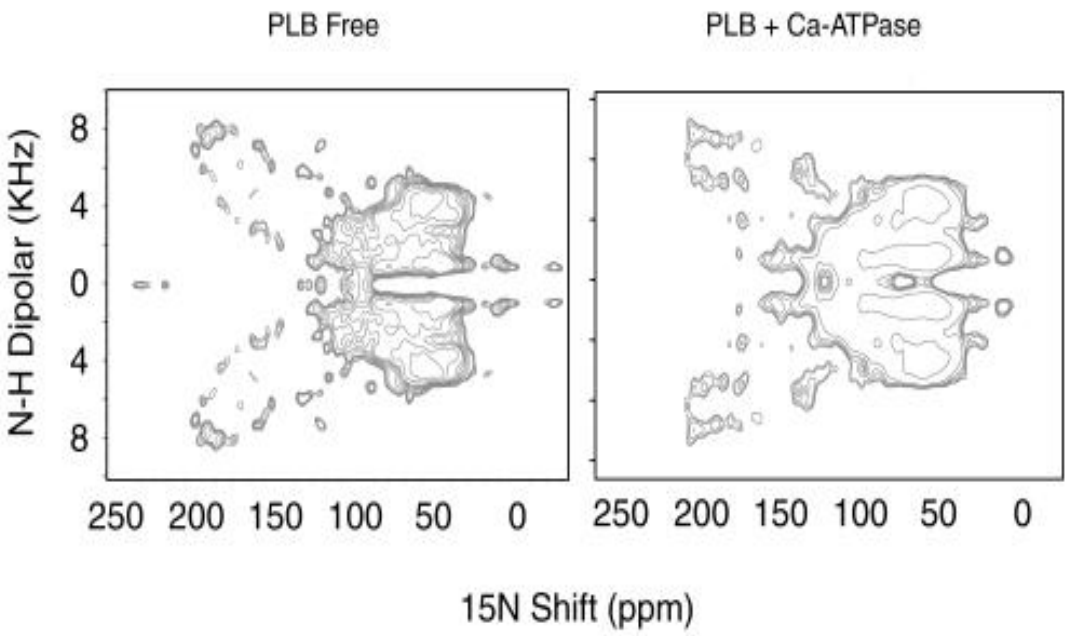

Fig. 1: Two-dimensional PISEMA experiments showing the differences in the topology of free and bound PLB.