Phospholamban (PLB) is a a 52-residue transmembrane peptide which regulates calcium ATPase in the cardiac sarcoplasmic reticulum through an inhibitory association that can be reversed by phosphorylation. We present molecular dynamics simulations which explore the behavior of phospholamban under different conditions on the nanosecond time scale. In methanol and in a membrane environment, PLB remains “L-shaped”, retaining its two characteristic helices, the C-terminal membrane-spanning helix and the smaller N-terminal helix which is known to protrude from the membrane. In methanol solution these helices undergo large amplitude relative motions, which decrease in the membrane environment. Simulations in water show a loss of secondary structure and formation of a globular form, in accord with the water-insoluble nature of the peptide. To study the microscopic effects of PLB phosphorylation, we also perform molecular dynamics simulations of the peptide phosphorylated at Ser 16 and Tyr 17. Finally, we study the dimer and pentamer formation of PLB using umbrella sampling with Generalized Born treatment of electrostatics and replica exchange simulations to accelerate conformational sampling.