Exploring the conformational space of cavity-containing proteins with pH, high pressure, and site-directed spin labeling electron paramagnetic resonance

Abstract. Proteins in pre-existing conformational equilibria sample different conformational states, some of which have important functional roles. Among intermediate conformational states, some proteins adopt a molten globule (MG) structure: a compact conformation that contains a relatively high content of native-like secondary structure, but has fewer tertiary contacts. The plasticity of MG-like states of intestinal fatty acid binding protein (I-FABP), a β-barrel protein, and the T4 lysozyme (T4L) L99A mutant, a predominately α-helical protein, are thought to facilitate binding of a variety of ligands to internal cavities. However, the population of such a state is very low and evades observation by spectroscopy.

Site directed spin labeling (SDSL) paired with electron paramagnetic resonance spectroscopy (EPR) is a sensitive tool for identifying backbone dynamics, conformational exchange, and ligand binding of proteins. In this dissertation, a panel of EPR experiments (continuous wave ((CW)), saturation recovery, and double electron-electron resonance) provides information on nanosecond-microsecond timescale motions and their amplitudes to lay a basis for the structure and dynamics of the I-FABP and T4L L99A MGs.

In addition, as a means to populate rare conformational states, hydrostatic pressure is known to shift conformational equilibria by virtue of respective partial molar volumes. Mechanisms of action for pressure perturbation include structure-relaxation and cavity hydration, for which the latter populates conformational states with strong structural parallels to the MG.

First, the MG-like character of the acid-stabilized and high pressure states of I-FABP is investigated. Second, and in contrast with wild-type T4L, the L99A mutant shows site-specific smooth sigmoidal transitions with pressure that indicate two-state equilibrium between the native state and a new, previously unidentified conformation. The main goal of this dissertation is to characterize the extent of structural heterogeneity of the MG from the SDSL EPR perspective, which encompasses fluctuation amplitudes, motional timescale, and thermodynamic parameters of the MG.

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