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Using spectral-phasor analysis to assess NADH conformation under pressure LAXMI RISAL, PAUL URAYAMA, Miami University — Reduced nicotinamide adenine dinucleotide (NADH) plays an important role in cellular metabolism, serving as an electron carrier in energy-related metabolic pathways. NADH conformational state – whether it is folded or unfolded – has physiological and biophysical significance because NADH is largely folded in solution and unfolded when protein bound. Previously, we presented measurements of UV-excited NADH fluorescence under pressure up to 50 MPa, observing that the emission spectrum shifts to longer wavelengths upon pressurization. In order to separate shifts due to a conformational change from shift due to changes in solvent coupling, we applied a two-state solvent denaturation model, allowing us to estimate the thermodynamic volume change of the folding-unfolding transition. Here, we further assess this two-state assumption using spectral phasor analysis. A spectral phasor is the first harmonic of a spectrum's Fourier transform; a plot of the phasor's imaginary versus real components is useful for the analytical assessment of a fluorescent sample because phasors from a two-component system lay along a line. We discuss the validity of the two-state model and the volume change estimate in the context of spectral-phasor analysis results.

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