



The pressure-temperature phase diagram of hen lysozyme at low pH

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The equilibrium unfolding of hen lysozyme at pH 2 was studied as a function of pressure (0.1-700 MPa) and temperature $(-10^\circ\text{C}\text{-}50^\circ\text{C})$ using Trp fluorescence as monitor supplemented by variable pressure 1H NMR spectroscopy (0.1-400 MPa). The unfolding profiles mon itored by the two methods allowed the two-state equilibrium analysis between the folded (N) and unfolded (U) conformers. The free energ y differences ΔG (=GU-GN) were evaluated from changes in the wavelength of maximum fluorescence intensity (λ max) as a function of pressure and temperature. The dependence of ΔG on temperature exhibits concave curvatures against temperature, showing positive heat capacity changes (Δ Cp=CpU-CpN=1.8-1.9 kJ mol-1 deg-1) at all pressures studied (250~400 MPa), while the temperature TS for maximal ΔG increased from about 10°C at 250 MPa to about 40°C at 550 MPa. The dependence of ΔG on pressure gave negative volume changes (Δ V=VU-VN) upon unfolding at all temperatures studied (-86~-17 ml mol-1 for -10°C~50°C), which increase significantly with increasing temperature, giving a positive expansivity change (ΔG ~1.07 ml mol-1 deg-1). A phase-diagram between N and U (for ΔG =0) is drawn of hen ly sozyme at pH 2 on the pressure-temperature plane. Finally, a three-dimensional free energy landscape (ΔG) is presented on the p-T plane.

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