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Vibrational Coherence Spectroscopy Investigation of Cytochrome c equilibrium Unfolding[1] YUHAN SUN, KARUNAKARAN VENUGOPAL, PAUL M. CHAMPION, Department of Physics and Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, MA 02115 — Using vibrational coherence spectroscopy (VCS) we studied guanidinium hydrochloride (GdHCl) induced equilibrium unfolding of ferric cytochrome c excited at 412 nm. Upon unfolding, we observed that the strong 50cm^{-1} mode, which dominates the VCS spectrum of native cyt c, loses its intensity relative to the 80cm^{-1} mode. The 224 cm^{-1} mode (γ_{24}) also shifts to 205 cm^{-1}, reflecting the heme configuration change associated with unfolding. We also compared the amplitude of these unfolding sensitive modes at different GdHCl concentrations using a cyt c-imidazole complex as a model system. The peak of the Soret band does not shift when the cyt c- imidazole complex is unfolded. Because the resonance conditions are invariant, the relative intensities are a direct probe of the heme structural changes. Our results show that the 50 cm^{-1} mode dramatically loses amplitude, while the 80 cm^{-1} mode stays nearly the same. When compared to other Raman studies, which suggest that the heme adopts a more planer structure when cyt c is unfolded, the 50cm^{-1} mode may reflect a similar structural change as the 569 cm^{-1} (γ_{21}) mode. We suggest that these modes are diagnostic of a protein-induced ruffling distortion.

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