

Stabilization of the Globular Structure of Ferricytochrome c by Chloride in Acidic Solvents

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Increasing concentrations of chloride were found to increase the resolution between two visible absorbance spectral transitions associated with acidification of ferricytochrome c. Analysis of a variety of spectral and viscosity measurements indicates that protonation of a single group having an apparent pK of 2.1 ± 0.2 and an intrinsic pK of about 5.3 displaces the methionine ligand without significantly perturbing the native globular conformation. Analysis of methylated ferricytochrome c suggests that protonation of a carboxylate ion, most likely a heme propionate residue, is responsible for displacement of the methionine ligand. Addition of a proton to a second group having an apparent pK of 1.2 ± 0.1 displaces the histidine ligand and unfolds the protein from a globular conformation into a random coil. It is most likely that the second protonation occurs on the imidazole ring of the histidine ligand itself. Chloride is proposed to perturb these transitions by ligation in the fifth c