Conformational Stability of Horseradish Peroxidase Probed by Spectroscopic Methods

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To better understand the folding and unfolding mechanisms in heme proteins, we are investigating the conformational stability of horseradish peroxidase (HRP). HRP is a member of the important group of heme-containing plant peroxidases that catalyze the oxidation of a variety of organic and inorganic compounds. In addition to the pentacoordinate ferric heme, the x-ray crystal structure of HRP isoenzyme C (HRPC) reveals four disulfide bonds and two calcium binding sites.[1] Binding of Ca^{2+} is essential for catalysis, structural integrity of the heme environment, and overall protein stability.[2]

Peroxidases are ideal for studying conformational stability in proteins because heme serves as a spectroscopic probe of folded, partially unfolded, and completely unfolded forms. In the present work we measured the pH and temperature dependent spectral changes in the heme Soret band of HRPC. Between pH 3 and 11, the Soret band's λ_{max} was 403 nm, although changes in absorbance were observed. At pH 1, λ_{max} blue-shifted to 364 nm. At pH 12 and 13, λ_{max} red-shifted to 420 nm. As pH was further increased to 14, λ_{max} blue-shifted to 393 nm. Interestingly, spectral changes in the Soret band at low pH as a function of time depended on the anion present in solution. Upon dissolving HRPC in water adjusted to pH 3 with HCl, the Soret band remained unchanged after 30 minutes (λ_{max} = 403 nm). In 50 mM potassium phosphate buffer adjusted to pH 3 with HCl, λ_{max} blue-shifted from 409 nm to 370 nm within 30 minutes. This suggests phosphate destabilizes HRPC at pH 3. Preliminary data investigating the reversibility of thermal denaturation of HRPC will be presented. These spectral changes will be discussed in terms of heme environment and overall protein stability.

1. Gajhede, M. D.; Schuller, D. J.; Henriksen, A.; Smith, A. T.; Poulos, T. L. *Nat. Struct. Biol.* **1997**, 4, 1032 – 1038.
2. Howes, B. D.; Feis, A.; Raimondi, L.; Indiani, C.; Smulevich, G. *J. Biol. Chem.* **2001**, 276, 40704 – 40711.