

Spectroscopic and Functional Characterization of the Novel Redox Properties of the Heme in Cystathionine β -synthase

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The redox behavior of the heme in human cystathionine β -synthase (CBS) is controlled by pH. At low pH, the heme spontaneously reoxidizes under strict anaerobic conditions, while at high pH, the heme undergoes a redox-mediated ligand switch. In either case, the heme escapes His/Cys(thiolate) coordination in the ferrous state.

Human CBS is a pyridoxal-5'-phosphate-dependent enzyme that catalyzes the condensation of homocysteine and serine to form cystathionine. Human CBS is unique in that heme is required for maximal activity, although the function of heme in this enzyme has yet to be elucidated. The CBS heme is axially coordinated by the sulfur atom of a cysteine thiolate and the N_{ε2} atom of a histidine imidazole. This particular coordination environment is rare among heme proteins; only two other naturally-occurring examples of His/Cys(thiolate) axial coordination have been conclusively identified.

Our work on CBS has revealed that the immediate coordination sphere of the heme in Fe(III) CBS is not altered by changes in pH over a range of pH 6-9. Proton concentration is, however, a determining factor when the ferric enzyme is reacted with reducing equivalents. A variety of spectroscopic techniques, including resonance Raman, magnetic circular dichroism, and electron paramagnetic resonance, reveal that at pH 9 Fe(II) CBS is dominant while at pH 6 Fe(III) CBS is favored. At low pH, Fe(II) CBS forms transiently and can be trapped by addition of CO, but otherwise reoxidizes by an apparent proton-gated electron-transfer mechanism. This regeneration of Fe(III) CBS in the presence of excess reductant does not abolish activity. At high pH, Fe(II) CBS dominates, but is capable of a redox-mediated ligand switch at physiological temperatures. Resonance Raman, electronic absorption, and magnetic circular dichroism spectroscopies suggest that upon reduction at pH 9 and 37 °C, the native cysteinate ligand of the CBS heme is displaced by a neutral donor. Functional assays have shown that this alternate ligand form of CBS, termed "Fe(II)CBS-424" for its Soret absorption maximum, is inactive.

These findings have implications for an existing hypothesis that regulation of CBS activity is controlled by the heme iron redox state. Given that the redox behavior of the CBS heme appears to be affected by pH, interplay of pH and oxidation must occur if CBS activity is redox regulated. We also believe that the rare His/Cys(thiolate) coordination of the CBS heme is responsible for these novel redox properties, which are undocumented in any other heme protein system.