Characterization of the Formation of CBS-424 Under Psuedo-Assay Conditions: Effects on Enzyme Activity and Exogenous Ligand Binding

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Mammalian cystathionine β-synthase (CBS), the only known PLP-dependent enzyme which also utilizes heme, catalyzes the condensation of serine and homocysteine into cystathionine as part of the transsulfuration pathway which ultimately converts methionine into cysteine. Deficiency in CBS activity causes an accumulation of homocysteine known as homocystinuria, and is associated with such diverse medical conditions as skeletal and vascular abnormalities, eye lens dislocation, and mental retardation. Understanding the structure, function, and regulation of CBS may prove crucial in improving the treatment of homocystinuric patients.

Although PLP is required to perform the standard β-replacement reaction which generates cystathionine, the heme cofactor does not participate directly in the enzymatic mechanism and may instead serve a regulatory role. Our group has recently discovered that mild heat-treatment of Fe(II)CBS at alkaline pH enables a putative ligand switch that blue-shifts the heme Soret band maximum from 449 nm to 424 nm and irreversibly destroys enzyme activity. Conversion to the 424 nm species (termed CBS-424) is now shown to occur spontaneously when the CBS heme is reduced at 37°C and pH 8.6, pseudo-assay conditions. We have utilized electronic absorption spectroscopy and exogenous ligand binding to identify a kinetic mechanism for the conversion process, while also conducting enzymatic assays to analyze the effects of CBS-424 formation on physiological and assay systems.