Spectroscopic Characterization of the Active Site Mutation of Cystathionine β-Synthase which causing Homocystinuria

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Abstract

Elevated plasma homocysteine levels have been linked to a variety of human diseases, including heart attack, stroke, Alzheimer's disease and Osteoporosis[1]. Mutations in the cystathionine β-synthase (CBS) gene cause homocystinuria, the most frequent inherited disorder in sulfur metabolism. CBS is unique enzyme using heme and pyridoxal 5-phosphate (PLP) both for activity. Given the importance of CBS in regulating homocysteine levels, major mutation study would be important to understand the pathogenic mechanism on homocysteine metabolism. Among the reported 140 mutations, one of the most common disease causing alteration in CBS is G307S. Generally G307S have a severe form of the disease characterized by pyridoxine non-responsive. Human G307S mutation could not complement the yeast growth with or without PLP presence. G307S enzyme is totally inert. To investigate the pathogenic mechanism of G307S by spectroscopic method, G247S mutation of yeast CBS that is corresponding mutation to human G307S was prepared. Yeast CBS does not contain heme and gave a merit to study the spectroscopic properties. The UV-Visible spectrum of the purified G247S yeast CBS exhibited that the total absence of PLP in the protein. Total loss of activity was found. G307 is suggested to be a key residue for substrate specificity, as they are spatially adjacent to the substrate binding site in the 3-D structure. Our data suggest that G307S makes the distortion of the substrate binding sites and the cofactor PLP can not be fit inside of the active site. This 3-D distortion would made G307S became inert and led to severe clinical phenotype.

Reference

1. Jhee KH, Kruger WD, The role of cystathionine beta-synthase in homocysteine metabolism.(2005) *Antioxidant Redox Signaling.* 7(5-6), 813-822.