Accumulation of Homocysteine Can Be Alleviated by Altering the Substrate Specificity of Cystathionine Beta Synthase

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Mammals have two strategies to metabolize toxic homocysteine. One is re-methylation pathway to form methionine. The other strategy is transsulfuration pathway to produce cystathionine. The first step of the transsulfuration pathway is catalyzed by the enzyme called cystathionine *beta*-synthase (CBS). CBS catalyzes the pyridoxal 5-phosphate (PLP) dependent *beta*-replacement reaction of L-serine with L-homocysteine to form L-cystathionine. Mutations in the CBS gene cause homocystinuria, the most frequent inherited disorder in sulfur metabolism. I278T mutation is the most common disease-causing alteration in CBS, associated with a less severe form of the disease. I278T patients are also characterized by pyridoxine-responsive homocysteine lowering and clinical improvement. In this study, we show that wild-type human CBS expressed in *S. cerevisiae* and in mammalian cells can catalyze two different homocysteine catabolizing reactions: the canonical reaction using serine as a co-substrate and an alternate reaction using cysteine. In contrast, the I278T enzyme expressed in *S. cerevisiae* has little activity with serine, but has substantial residual activity when using cysteine. Furthermore, this cysteine dependent reaction of I278T has a substantially reduced affinity for pyridoxal 5-phosphate compared to the wild-type enzyme. Thus I278T mutation can alleviate the accumulation of homocysteine by altering the substrate specificity. We are pursing the purification of human I278T protein expressed in yeast. The three dimensional structure of I278T protein will enable us to get more detailed information by comparing it with that of wild type[1].

1. Meier M, Janosik M, Kery V, Kraus J. P, Burkhard P. (2001). Structure of human cystathionine beta-synthase: a unique pyridoxal 5'-phosphate-dependent heme protein, *EMBO J.* **20**(15), 3910-3916.