ABSTRACT

Protein misfolding is considered to be the major pathogenic mechanism in homocystinuria due to cystathionine beta-synthase (CBS) deficiency. The aim of this work was to study molecular mechanisms underlying protein misfolding of CBS mutants.

Firstly, we studied spatial arrangement of normal human CBS protein. Using data from differential covalent labeling of solvent-exposed aminoacid residues, we identified interdomain contact area between the catalytic core and the regulatory domain in human CBS, and we subsequently generated the structural model of the full-length CBS. In the next step, we studied evolutionary divergence of CBS protein structures. We performed phylogenetic analysis that revealed unique spatial arrangement of CBS enzyme in nematodes; the domain architecture of CBS in *Caenorhabditis elegans* was studied experimentally in more detail. Finally, we determined conformational properties of a representative set of human CBS mutants that exhibited in various extent affected formation of tetramers and decreased catalytic activity. Using thermolysin-based proteolytic techniques for analysis of nine mutants expressed in *E.coli*, we found that an unfolded structure is a common intermediate occurring in CBS misfolding. The importance of protein unfolding for pathogenesis of CBS deficiency was further shown by analysis of additional nine purified mutants that were properly assembled into tetramers and possessed normal catalytic activity. These data demonstrated that the altered protein unfolding is a reliable marker of pathogenicity of CBS mutants and proteolysis with thermolysin under native conditions may be an important tool for biochemical assessment of pathogenic variants.

This study advances our understanding of molecular pathology in CBS deficiency and provides knowledge that forms a base for development of chaperone therapy and future improvement in patient care.