

Identification, Cloning, and Characterization of Human 2-Keto-4-Hydroxy-Glutarate Aldolase (KHGA), an Attractive Therapeutic Target to Block the Production of Glyoxylate From Hydroxyproline Catabolism

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Primary hyperoxaluria (PH) types I and II are characterized by an elevated synthesis of glyoxylate and oxalate as a consequence of functional defects in alanine-glyoxylate aminotransferase and glyoxylate reductase, respectively. Recent dietary studies in humans have shown that the degradation of hydroxyproline (Hyp) is a significant contributor to increased glycolate, a glyoxylate metabolite, and oxalate synthesis. The final step in Hyp catabolism involves the conversion of 2-keto-4-hydroxyglutarate (KHG) to glyoxylate and pyruvate by KHG aldolase (KHGA), an enzyme unique to this metabolic pathway. Thus, the inhibition of KHGA represents a significant therapeutic opportunity for decreasing oxalate synthesis in PH patients and individuals with idiopathic stone disease. We have identified, cloned, recombinantly expressed, purified, and characterized human KHGA for the first time. Recombinant hKHGA readily converts KHG to glyoxylate with kinetic parameters ($k_{\text{cat}} = 63 \text{ min}^{-1}$, $K_m = 48 \text{ }\mu\text{M}$, $k_{\text{cat}}/K_m = 1.3 \text{ mM}^{-1}\text{min}^{-1}$) nearly identical to the bovine enzyme used to identify the human gene through mass spectrometric-proteomic analyses. Analytical ultracentrifugation experiments and sequence comparisons to the class I and II aldolases support that hKHGA is novel (i.e., possessing the trimeric oligomeric state of the class I enzymes, but possessing some of the active site motifs of the tetrameric class II enzymes. Based on these comparisons, Tyr140, Tyr168, and Lys196 are the putative catalytic residues within the active site.