

Identification of Substrate Orienting and Phosphorylation Sites Within Tryptophan Hydroxylase Using Homology-based Molecular Modeling

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Tryptophan hydroxylase (TPH) is the initial and rate-limiting enzyme in the biosynthesis of serotonin. The inherent instability of TPH has prevented a crystallographic structure from being resolved. For this reason, multiple sequence alignment-based molecular modeling was utilized to generate a full-length model of human TPH. Previously determined crystal coordinates of two highly homologous proteins, phenylalanine hydroxylase and tyrosine hydroxylase, were used as templates. Analysis of the model aided rational mutagenesis studies to further dissect the regulation and catalysis of TPH. Using rational site-directed mutagenesis, it was determined that Tyr235 (Y235), within the active site of TPH, appears to be involved as a tryptophan substrate orienting residue. The mutants Y235A and Y235L displayed reduced specific activity compared to wild-type TPH ($\approx 5\%$ residual activity). The K_m of tryptophan for the Y235A (564 μM) and Y235L (96 μM) mutant was significantly increased compared to wild-type TPH (42 μM). In addition, kinetic analyses were performed on wild-type TPH and a deletion construct that lacks the amino terminal autoregulatory sequence (TPH $N\Delta 15$). This sequence in phenylalanine hydroxylase (residues 19 to 33) has previously been proposed to act as a steric regulator of substrate accessibility to the active site. Changes in the steady-state kinetics for tetrahydrobiopterin (BH_4) and tryptophan for TPH $N\Delta 15$ were not observed. Finally, it was demonstrated that both Ser58 and Ser260 are substrates for Ca^{2+} /calmodulin-dependent protein kinase II. Additional analysis of this model will aid in deciphering the regulation and substrate specificity of TPH, as well as providing a basis to understand as yet to be identified polymorphisms.

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