

Abstract View**IDENTIFICATION OF A TRYPTOPHAN ORIENTING RESIDUE IN THE ACTIVE SITE OF TRYPTOPHAN HYDROXYLASE.**

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Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of the neurotransmitter, serotonin (5-HT). Recently, this laboratory has produced a hypothetical 3-D model for human TPH. Rational mutagenesis was performed on TPH based on a crystal model overlay of phenylalanine hydroxylase with bound catechol inhibitor (6PAH) and our TPH model. Computer analysis suggests that tyrosine 235 (Y235) in TPH is 5.6Å from the site of bound substrate in 6PAH. For this reason, Y235 was targeted by site-directed mutagenesis to investigate its role as a potential tryptophan-orienting residue. Mutation of Y235 to alanine and leucine (Y235A and Y235L, respectively) resulted in a 95% reduction in TPH activity ($P < 0.0005$). Steady-state kinetic analyses were performed for wild-type TPH, Y235A, and Y235L. The K_m of tryptophan for Y235A was increased 12-fold (564 μM) over wild type-TPH (44 μM). Y235L exhibited greater than a 2-fold increase in the K_m for BH_4 (96 μM) compared to wild-type TPH. No changes were observed in the Michaelis constant of BH_4 for all proteins tested. Also, mutagenesis of Y235 abolished the substrate inhibition of TPH activity normally observed with high levels of tryptophan (concentrations $> 200 \mu\text{M}$). The use of the hypothetical model of TPH has enabled us to identify functional aspects of the TPH catalytic domain that are mediating substrate specificity of TPH.

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