

Abstract View**FUNCTIONAL ANALYSIS OF THE V177I CODING REGION POLYMORPHISM OF HUMAN TRYPTOPHAN HYDROXYLASE (HTPH1).**

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Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin (5-HT). Recently, Ramaekers *et al.* (2001) have reported the first coding region polymorphism of human tryptophan hydroxylase, a valine to isoleucine substitution at residue 177 (V177I). A hypothetical model of human TPH and the crystal structure of the catalytic domain suggest that the V177 residue points into the active site and may direct interactions with the tryptophan substrate. In an attempt to elucidate the functional effect of the valine to isoleucine substitution at residue 177, site-directed mutagenesis was employed to generate a number of mutants for analysis. Mutation of V177 to isoleucine and alanine (V177I and V177A, respectively) resulted in no significant changes in TPH activity. Substitutions of V177 with leucine, phenylalanine, arginine, glutamate, glycine, serine and threonine resulted in severely reduced enzyme activity. Steady-state kinetic analyses were performed for wild-type TPH, V177A and V177I. The K_M of V177A for tryptophan ($31.6 \mu\text{M} \pm 1.4$) was not significantly different from that of wild-type ($26.4 \mu\text{M} \pm 3.3$). However, V177I exhibited a significant 55% increase in the K_M for tryptophan ($41.1 \mu\text{M} \pm 6.5$). No changes were observed in the Michaelis constant of BH_4 for all proteins tested. Due to insufficient enzyme activity, steady-state kinetic analyses were not performed on the V177G, V177S, V177T, V177L, V177F, V177R, and V177E mutants. These findings suggest that the identity of the residue at position 177 is critical for the proper formation of the substrate pocket. In the case of the naturally-occurring polymorphism, it seems likely that enzyme activity would only be compromised under conditions of reduced amino acid substrate concentrations.

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