

Intersubunit Binding Domains Within Tyrosine Hydroxylase and Tryptophan Hydroxylase

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Tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin (5-HT) belongs to the aromatic amino acid hydroxylase superfamily, which includes phenylalanine hydroxylase (PAH) and tyrosine hydroxylase (TH). The crystal structures for both PAH and TH have been reported, but a crystallographic model of TPH remains elusive. For this reason, we have utilized the information presented in the TH crystal structure in combination with primary sequence alignments to design point mutations in potential structural domains of the TPH protein. Mutation of a TH salt bridge (K170E) was sufficient to alter enzyme macromolecular assembly. We found that the disruption of the cognate intersubunit dimerization salt bridge (K111–E223) in TPH, however, did not affect the macromolecular assembly of TPH. Enzyme peaks representing only tetramers were observed with size exclusion chromatography. By contrast, a single-point mutation within the tetramerization domain of TPH (L435A) was sufficient to disrupt the normal homotetrameric assembly of TPH. These studies indicate that, although the proposed salt bridge dimerization interface of TH is conserved in TPH, this hypothetical TPH intersubunit binding domain, K111–E223, is not required for the proper macromolecular assembly of the protein. However, leucine 435 within the tetramerization domain is necessary for the proper macromolecular assembly of TPH.

Key words: catecholamines; leucine-zipper; mutagenesis; salt bridge; serotonin