

Mobile loop containing residues 423-426 of Tyrosine hydroxylase optimizes tyrosine binding and substrate specificity.

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Alanine scanning was carried out on positions 423 to 427 in tyrosine hydroxylase (TyrH), a region shown by hydrogen-deuterium exchange to be highly mobile, and seen in the x-ray crystal structure as lying near the opening to the active site cleft. Plasmids to express the variants TyrHY423A, -Q424A, -D425A, -Q426A and -T427A were made by site-directed mutagenesis, and the proteins were overexpressed in *E. coli* using the pET system. Steady-state kinetic analyses were performed on the variants and Michaelis-Menten parameters measured. TyrHD425A was the most altered, with a V_{\max} for DOPA formation of 0.3 ± 0.08 , compared to 150 for the wild-type enzyme. TyrHY423A and TyrHQ424A have higher K_{tyr} values than wild-type, 125 ± 33 and 146 ± 17 μM , respectively, compared to 40 μM for wild-type. Because TyrHD425A was 500-fold diminished in tyrosine hydroxylation activity, its tetrahydropterin oxidation activity was measured and used to determine a K_M value for phenylalanine. Although it is unable to hydroxylate tyrosine, it is fully able to oxidize tetrahydropterin in the presence of phenylalanine, with a V_{\max} value of 20 and a K_{phe} value of 1.2 μM , compared to a V_{\max} value of 100 and a K_{phe} value of 100 μM for wild-type. From these data we conclude that the flexible loop from positions 423-427 is important and in particular aspartate 425 is critical for substrate specificity.