Kinetic Isotope Effects as Probes of the Mechanisms of Hydroxylation by Phenylalanine and Tyrosine Hydroxylase

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The reactions catalyzed by the aromatic amino acid hydroxylases can be divided into two half-reactions. In the first, oxygen, a tetrahydropterin, and the active site iron react to form a highly reactive hydroxylating intermediate. In the second, this intermediate reacts with the side chain of the amino acid substrate. We have been using deuterium kinetic isotope effects to probe the mechanism of oxygen addition to the amino acid and to gain insight into the properties of the hydroxylating intermediate. When tryptophan containing deuterium at the sight of hydroxylation is used as a substrate for tryptophan hydroxylase (TrpH), there is an isotope effect on the k_{cat} value of 0.93, consistent with an electrophilic aromatic substitution reaction. However, no significant isotope effect is seen when deuterated tyrosine is used as substrate for tyrosine hydroxylase (TyrH), due to oxygen activation being rate-limiting with that enzyme. This limitation can be overcome by using mutant enzymes in which tetrahydropterin oxidation is partially uncoupled from amino acid hydroxylation. Three mutant forms of TyrH, which are 80-96% uncoupled, exhibit significant deuterium isotope effects with 3,5-D_2-tyrosine. The limiting value of about 0.92 agrees well with the value obtained for TrpH.

Both TyrH and phenylalanine hydroxyase (PheH) will hydroxylate 4-methylphenylalanine to form 4-HO-methylphenylalanine. Use of substrates containing one, two, or three deuterium atoms in the methyl group has allowed determination of the intrinsic primary and secondary isotope effects on this reaction. In the case of TyrH, the primary isotope effect is 9.6 and the secondary isotope effect is 1.2, consistent with a mechanism in which the hydroxylating intermediate abstracts a hydrogen atom from the methyl group and with significant quantum mechanical tunneling of that hydrogen atom. In the case of PheH, the intrinsic primary and secondary isotope effects are 9.4 and 1.13 when there is only one deuterium in the methyl group. Incorporation of a second deuterium alters these values to 7.1 and 1.31, establishing that there is significant coupled motion in the transition state in this case. The temperature dependence of the isotope effect establishes that quantum mechanical tunneling is involved. The differences between the two enzymes suggest that there are slight differences in either the reactivity of the hydroxylating intermediate or in the interactions in the active site.