Impact of Competitive Inhibitors on Kinetic Isotope Effects in Reaction Catalysed by Tyrosine Phenol-Lyase

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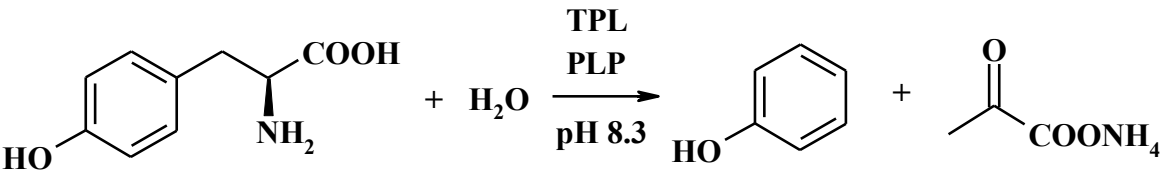
Tyrosine phenol-lyase (TPL, EC 4.1.99.2) is a bacterial enzyme that catalyses the reversible hydrolytic decomposition of L-tyrosine to phenol and ammonium pyruvate. 5'-pyridoxal phosphate is a cofactor of this process.



The mechanism of the reaction consists of several step involving formation of Schiff-base tyrosine-PLP, deprotonation on α-carbon (formation of the quinoid intermediate), subsequent β-elimination of the phenol moiety, and the final rearrangements and dissociation of products.

Our previous research has revealed several phenomena, e.g. the changes of the relative rate of the elementary processes during the reaction course, different behaviour of 3*S* and 3*R* hydrogen atoms in the reactions, involvement of 3',5'- hydrogen atoms in the product dissociation steps, and the impact of 2',6'- hydrogen atoms on the late transition state, etc.

The initial studies have shown the impact of the presence of *S*-methyl-L-cysteine on KIE measured in the reaction. Here, we present data concerning $^{1}H/^{3}H$ KIEs on 2, 3S, 2',6', and 3',5' positions of L-tyrosine. The effects were measured with the addition of *S*-methyl-L-cysteine (a competitive inhibitor undergoing β-elimination) and L-phenylalanine (a competitive inhibitor undergoing α-deprotonation only).

KIEs were measured using radiochemical methods with dual-label approach. Carboxyl ^{14}C was used as a remote label. The results are discussed in the context of our previous results combined with the literature data.