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## SYNTHESES OF ISOTOPOMERS OF L-TYROSINE AND THEIR USE IN THE STUDIES OF THE MECHANISM OF ACTION OF $\beta$ -TYROSINASE

*Abstract of Ph.D. thesis*

The main objective was to study the mechanism of action of tyrosine phenol-lyase (a bacterial enzyme that catalyses hydrolytic decomposition of L-tyrosine to phenol, pyruvate and ammonia in the presence of 5'-pyridoxal phosphate) using kinetic isotope effects (KIE) of hydrogen and carbon.

The first goal was the synthesis of isotopomers of L-tyrosine specifically labelled with carbon-14 in positions 1, 2, 3 and 1'. Isotopomers of L-tyrosine selectively labelled with deuterium in 2, 3R and 3S positions, and with tritium in 2, 3R, 3S and 2',6' positions were also obtained.

These compounds have been applied in kinetic isotope effects studies of the reaction. Non-competitive method was applied to determine  $^1\text{H}/^2\text{H}$  on maximal velocity (V) and on its ratio to Michaelis constant (V/K) by direct measurements of kinetic parameters (V and V/K) of the reactions with compound enriched with both hydrogen isotopes in c.a. 100%. Deuterium KIE in 2, 3, 2',6' i 3',5' positions, and deuterium solvent isotope effects with L-tyrosine and [2- $^2\text{H}$ ]-L-tyrosine as substrates were determined using non-competitive method.

Competitive method was applied to determine  $^1\text{H}/^3\text{H}$  and  $^{12}\text{C}/^{14}\text{C}$  KIEs with radiochemical means using dual-label approach. 1- $^{14}\text{C}$  was used as a remote label in determination of tritium KIEs, whereas 3',5'- $^3\text{H}_2$  was a remote label in carbon KIE measurements. Tritium KIEs on 2 (in water and in heavy water), 3R, 3S, 2',6' and 3',5' positions were determined using competitive method. Analogously, carbon KIEs on 2, 3 and 1' positions were determined.

These studies yielded in several interesting observations and conclusions concerning studied enzymatic L-tyrosine cleavage:

- relative rates of the elementary processes change during the reaction progress; such phenomenon results in KIE changes with the conversion;
- tritium KIE on 2 position cannot be directly measured due to quick proton exchange with solvent at the very early stage of the reaction;
- hydrogen atom in 2 position and water take part during the same transition state of the reaction;
- the abstraction of 2-hydrogen contributes greater to the reaction rate than subsequent exchange of the abstracted proton with solvent;
- the tritium transfer from 2 position of L-tyrosine to 4 position of formed phenol is lower than 1%;
- tritium KIE on 3S position increases to great primary values with the conversion;
- hydrogen atoms in 3 positions act in a different way during the course of the reaction;
- tritium in 3 both positions undergoes quick exchange with solvent during late, irreversible steps of the reaction;
- hydrogen atoms in 2',6' positions are placed in surrounding similar between the rate-limiting transition state and phenol bound to the enzyme (so-called late transition state);
- hydrogen atoms in 3',5' positions are important to the complex enzyme-phenol dissociation
- carbon KIE on 2 position decreases during the progress of the reaction.