STUDIES OF THE MECHANISM OF ACTION OF L-TYROSINE PHENOL-LYASE FROM KINETIC ISOTOPE EFFECTS

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Abbreviations: TPL - tyrosine phenol-lyase, KIE - kinetic isotope effect

L-tyrosine phenol lyase (β-tyrosinase; TPL) is a bacterial enzyme, which catalyzes the reversible decomposition of L-tyrosine to phenol, pyruvate, and ammonia.

The mechanism of TPL action is a very interesting objective to biochemists, since it occurs via the abstraction of the α-proton and the subsequent elimination of phenol moiety with quite rare disconnection of aliphatic carbon - aromatic carbon bond. This objective has been mainly investigated using spectroscopy (stopped-flow), there is also some isotopic evidence; however, such data is incomplete.

Our goal was to study the mechanism of action of TPL using kinetic and solvent isotope effect method. KIEs of hydrogen (i.e. protium/deuterium and protium/tritium), as well as of carbon (carbon-12/carbon-14) were studied. Non-competitive method (i.e. direct kinetic measurement of labelled and non-labelled substrates to determine and compare their catalytic constants and Michaelis constants in the reaction) was used to determine protium/deuterium KIEs. A competitive method (using dual-label approach) was applied to determine protium /tritium (using carboxyl-^{14}C as a remote label) and carbon-12/carbon-14 (applying 3',5' ^{3}H as a remote). These KIEs were determined by radiochemical means.

New phenomena have been observed; among them are changes of hydrogen and carbon KIEs, as well as quick proton exchange with solvent i) in the methyl group of formed pyruvate; and, initially, ii) of α-proton of the substrate. The rationalization of KIE changes is proposed on the basis of kinetic analysis.

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