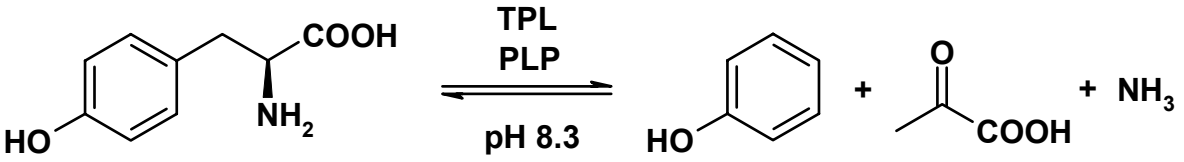
STUDIES OF THE MECHANISM OF ACTION OF L-TYROSINE PHENOL-LYASE FROM KINETIC ISOTOPE EFFECTS

W. Augustyniak, R. Kanski, M. Kanska
Faculty of Chemistry, University of Warsaw
1Pasteur Street, 02-093 Warsaw, Poland

Abbreviations: TPL, tyrosine phenol-lyase; PLP, 5'-pyridoxal phosphate; KIE, kinetic isotope effect; k_{cat}, catalytic constant; K_m, Michaelis constant.

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



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. $^{1}H/^{2}H$ and $^{1}H/^{3}H$), as well as of carbon ($^{12}C/^{14}C$) were studied. Non-competitive method (i.e. direct kinetic measurement of labelled and non-labelled substrates to determine and compare their k_{cat}s and K_ms in the reaction) was used to determine $^{1}H/^{2}H$ KIEs. A competitive method (using dual-label approach) was used to determine $^{1}H/^{3}H$ (using carboxyl-^{14}C as a remote label) and $^{12}C/^{14}C$ (applying 3',5' ^{3}H as a remote position) 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, such explanation has not been known for such systems yet.