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

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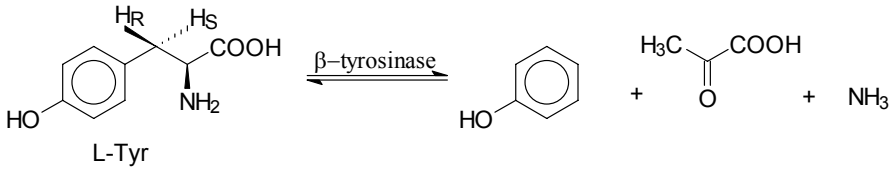
L-tyrosine phenol lyase (TPL) is a bacterial enzyme, which catalyzes the reversible decomposition of L-tyrosine to phenol, pyruvate, and ammonia (Scheme 1).



Scheme 1. Reversible conversion of L-Tyr catalyzed by enzyme β-tyrosinase

The mechanism of its action is a very interesting objective to chemists, since it occurs via the abstraction of the α-proton and the elimination of phenol moiety with quite rare disconnection of aliphatic carbon – aromatic carbon bond. This objective has been mainly investigated using spectroscopy, especially stopped-flow method, there is also some isotopic evidence; however no systematic isotope research has been attempted at this area.

Our research should allow to give some data about the abstraction of the α-proton, and the impact of solvent. It will be also interesting to investigate the effects on 3*R*, and 3*S* hydrogen positions due to their possible asymmetry in the active center of the enzyme.

To gain these objectives, the kinetic isotope effects of hydrogen were determined. The effects on the α position were determined in different solvents (e.g. in water and heavy water). The transfer of the hydrogen to products was also studied. There are the effects in aromatic moiety reported.

Non-competitive method (e.g. measurements of kinetic parameters of reactions) of the determination of the $^{1}H/^{2}H$ kinetic isotopic effects was applied, whereas competitive method for the determination of $^{1}H/^{3}H$ effects was used. In the latter, dual-label method was applied using carbon-14 label of carboxylic group as an internal standard, which is not supposed to be involved directly into the reaction, and thus it does not introduce any significant effect.