

Hydrogen Kinetic Isotope Effects in the Studies of the Mechanism of Action of L-Tyrosine Phenol Lyase

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

Abstract

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

The purpose of our studies was to provide some data about the abstraction of the α -proton and the impact of solvent. In addition, we investigated 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 combinations of hydrogen isotopes placed there, and of solvents (e.g. in water and heavy water). There are also the effects in the 3*R*, 3*S*, and in aromatic moiety reported.

Non-competitive method (e.g. measurements of kinetic parameters of the reactions) of the determination of the $^1\text{H}/^2\text{H}$ kinetic isotope effects was applied, whereas competitive method for the determination of $^{1,2}\text{H}/^3\text{H}$ 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.