

Impact of Competitive Inhibitors on Kinetic Isotope Effects in Reaction Catalysed by Tyrosine Phenol-Lyase

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Layout

1. Introduction:

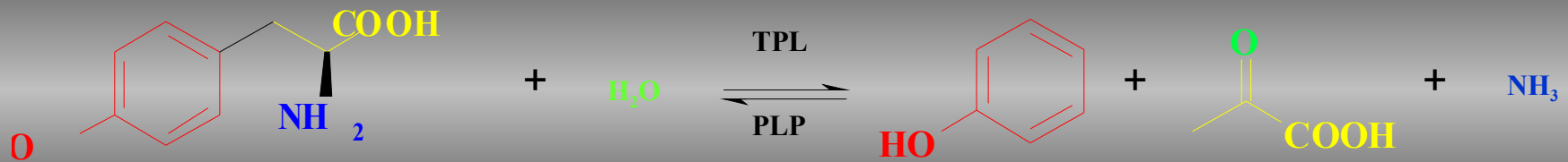
- properties of tyrosine phenol-lyase
- mechanism of action of the enzyme

2. Experimental design of KIE determination

3. Results of the research on impact of solvents (H_2O vs D_2O), inhibitors (phenol, L-phenylalanine, S-methyl-L-cysteine) and Lewis acid (AlCl_3) on KIE values on TPL-catalysed reaction

4. Discussion and conclusions

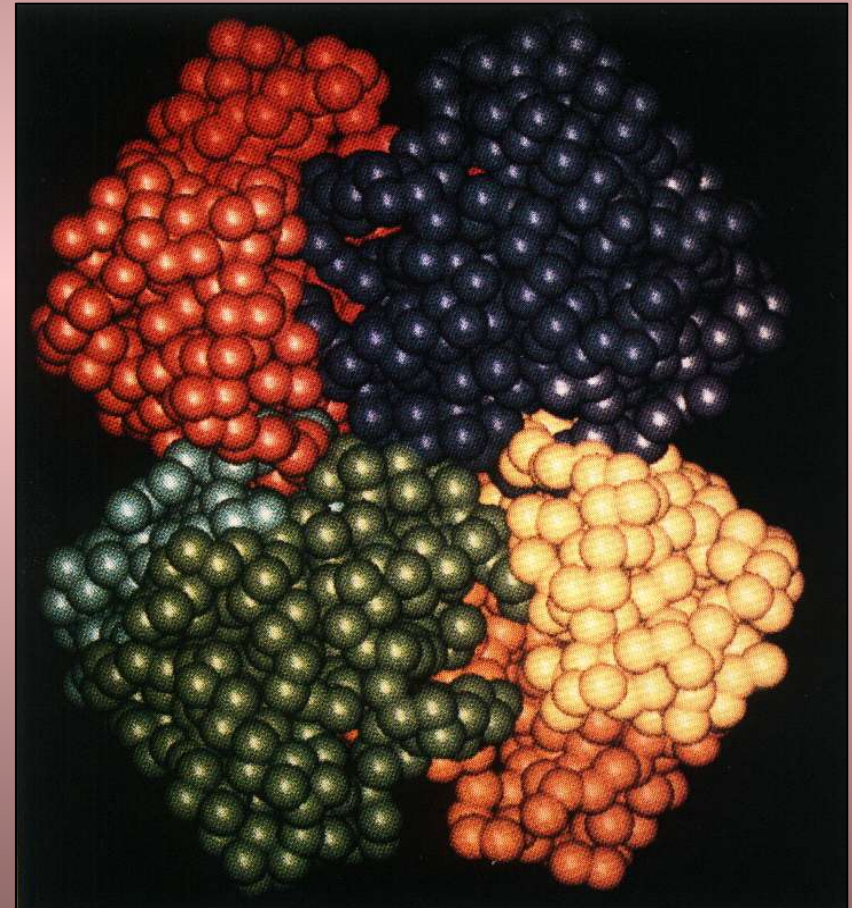
Tyrosine Phenol-Lyase



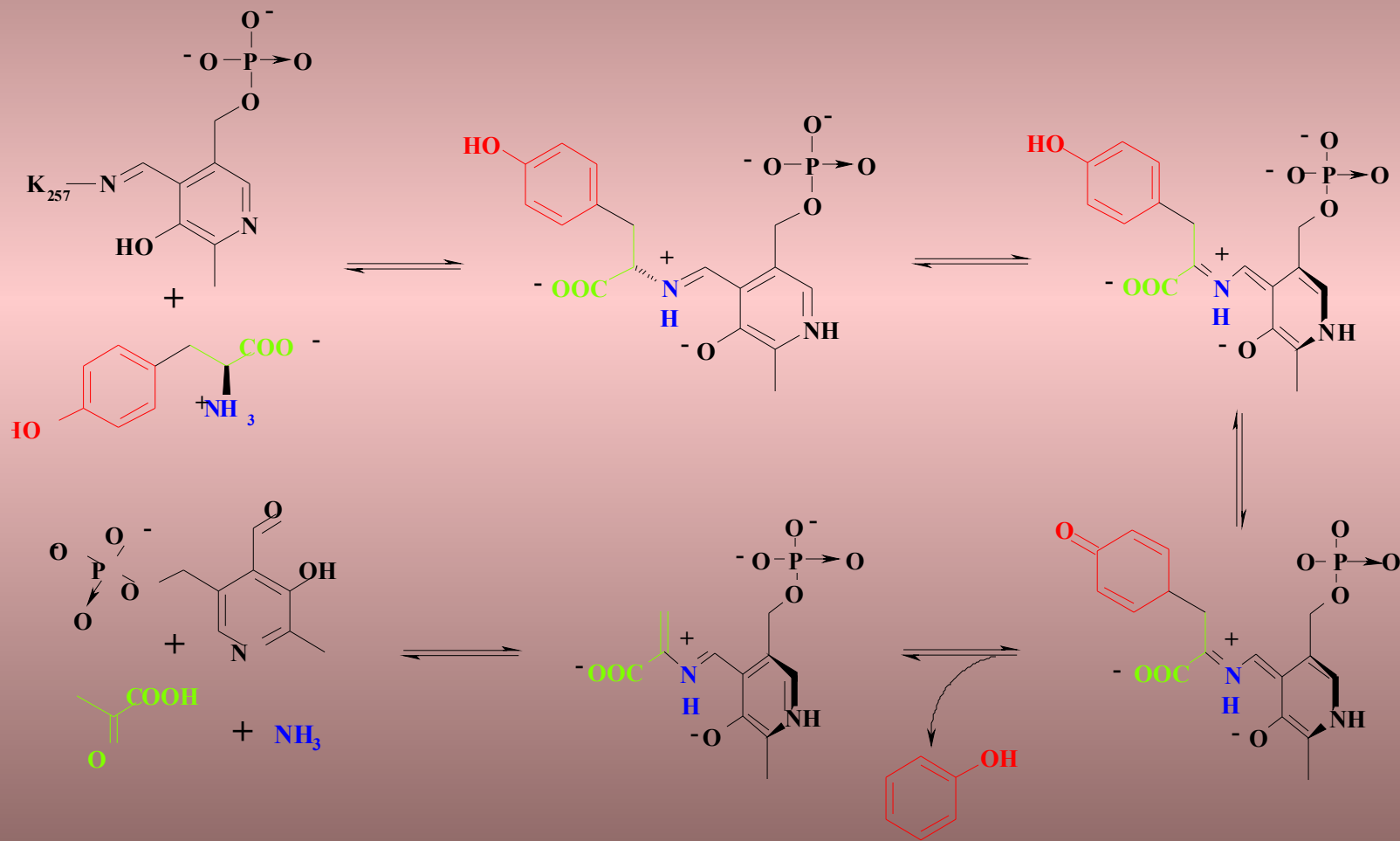
- the enzyme catalyses disruption of aromatic carbon – aliphatic carbon bond
- α -hydrogen is partly transferred to 1' carbon of aromatic moiety
- catalyses formation of L-tyrosine from phenol and pyruvate derivatives, alanine racemisation, α -hydrogen labilisation in amino acid and α -carbon deamination
- monovalent cations (K^+ , Rb^+ , Cs^+ lub NH_4^+) are required for catalytic activity; Na^+ and Li^+ are TPL inhibitors

Tyrosine Phenol-Lyase

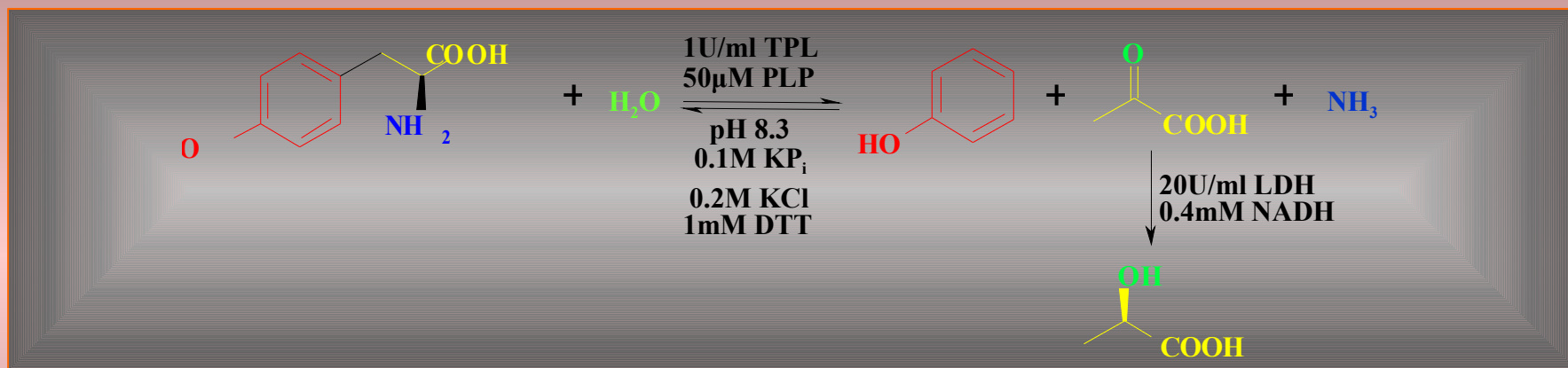
- **other names:** β -tyrosinase, TPL, E.C. 4.1.99.2
- **natural sources:** Gram-negative *Enterobacteriaceae* and some arthropods
- **structure:** α/β protein
135Å x 60Å x 144Å
4 subunits 51kDa
known 3D structure
- **application:**
biotechnological synthesis of dopa
PLP determination in biological samples
phenol waste utilization



Mechanism of Action of TPL

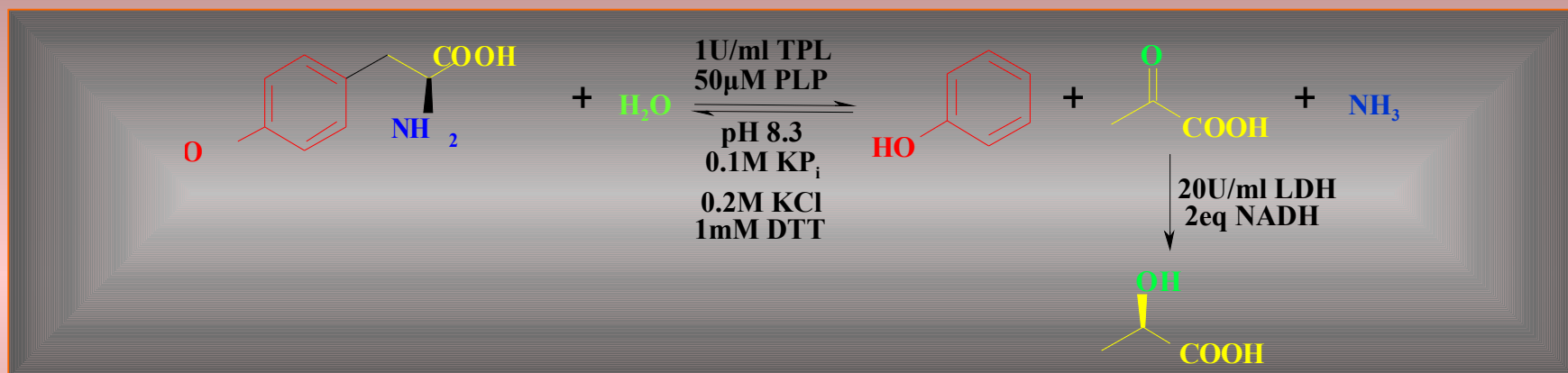


KIE Determination with Non-Competitive Method



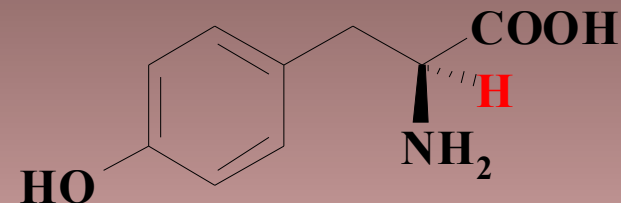
- Steady-state kinetics experiments were performed
- Addition of NADH and LDH makes the reaction irreversible and allows rate determination at 340nm
- Measurement of reaction rates at various substrate concentrations allows to determine V_{\max} i K_m from Michaelis-Menten equation

KIE Determination with Competitive Method



- ❑ Radiochemical method was applied
- ❑ Products and substrate were separated using cation-exchange chromatography on Amberlit IR-120
- ❑ Specific radioactivities of starting substrate (R_0), separated products (R_p) and conversion degree (f) were determined
- ❑ Dual-label approach with $1-^{14}C$ as an internal standard (remote label) was applied
- ❑ NADH and LDH secure reaction irreversibility and convert non-stable pyruvate to stable L-lactate

Isotope Effects on Position 2



H/D KIE

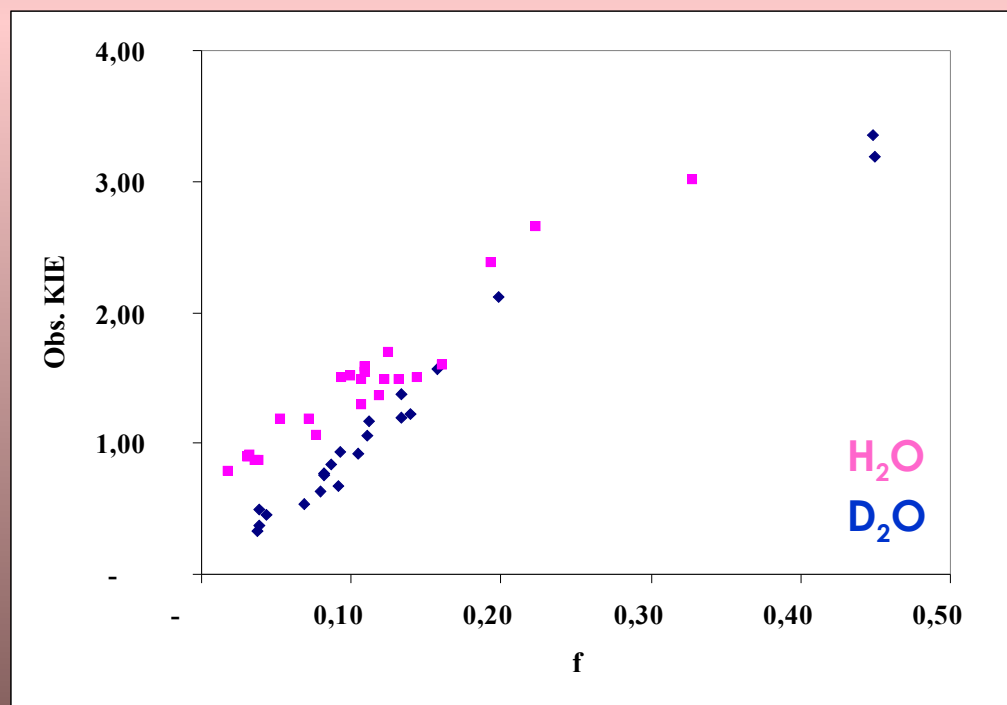
	α_V	$\alpha_{V/K}$
H ₂ O	3.34 ± 0.25	2.42 ± 0.29
D ₂ O	3.28 ± 0.13	2.24 ± 0.61

- α -hydrogen atom and water molecule participate in the same transition state
- α -hydrogen abstraction is more rate-limiting than its subsequent exchange with solvent

H/D SIE

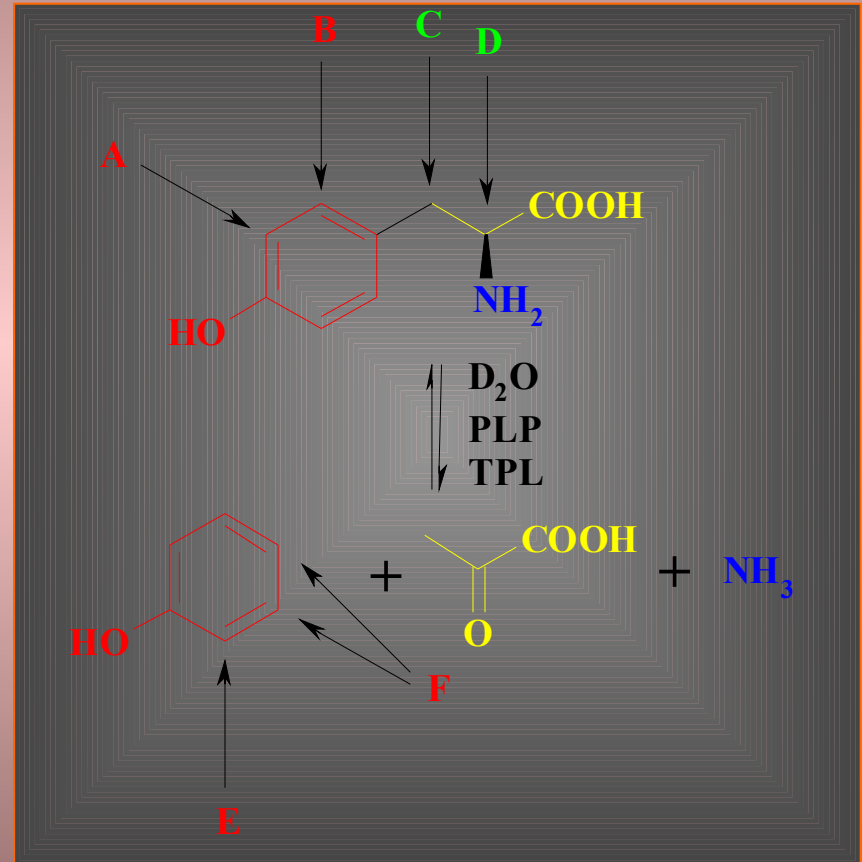
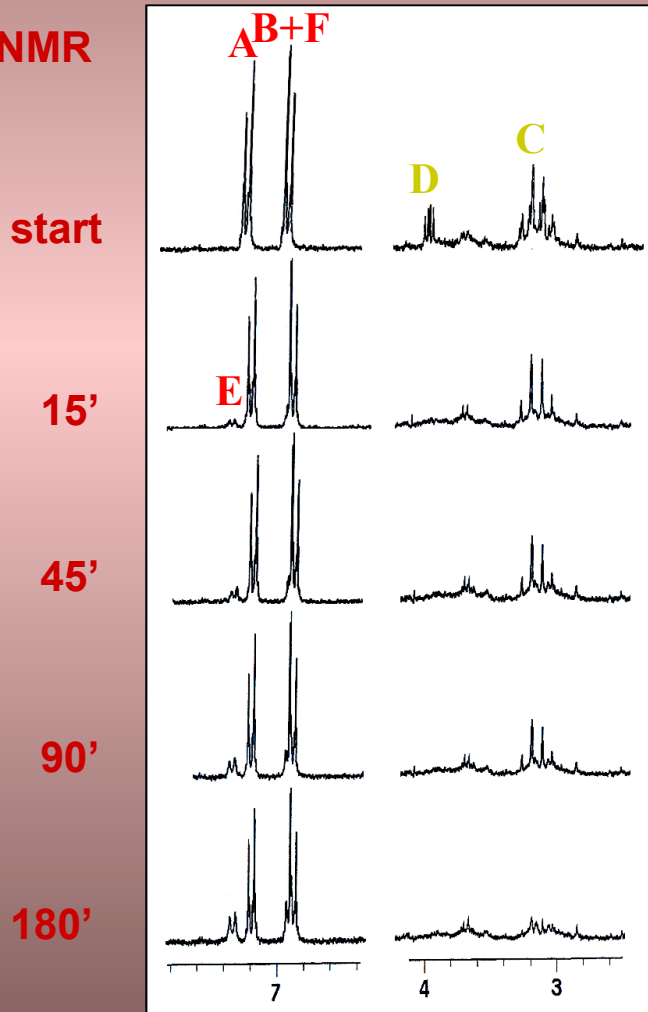
	α_V	$\alpha_{V/K}$
L-tyrosine	1.49 ± 0.06	1.81 ± 0.25
[2- ² H]-L-tyrosine	1.46 ± 0.11	1.67 ± 0.31

Observed H/T KIE



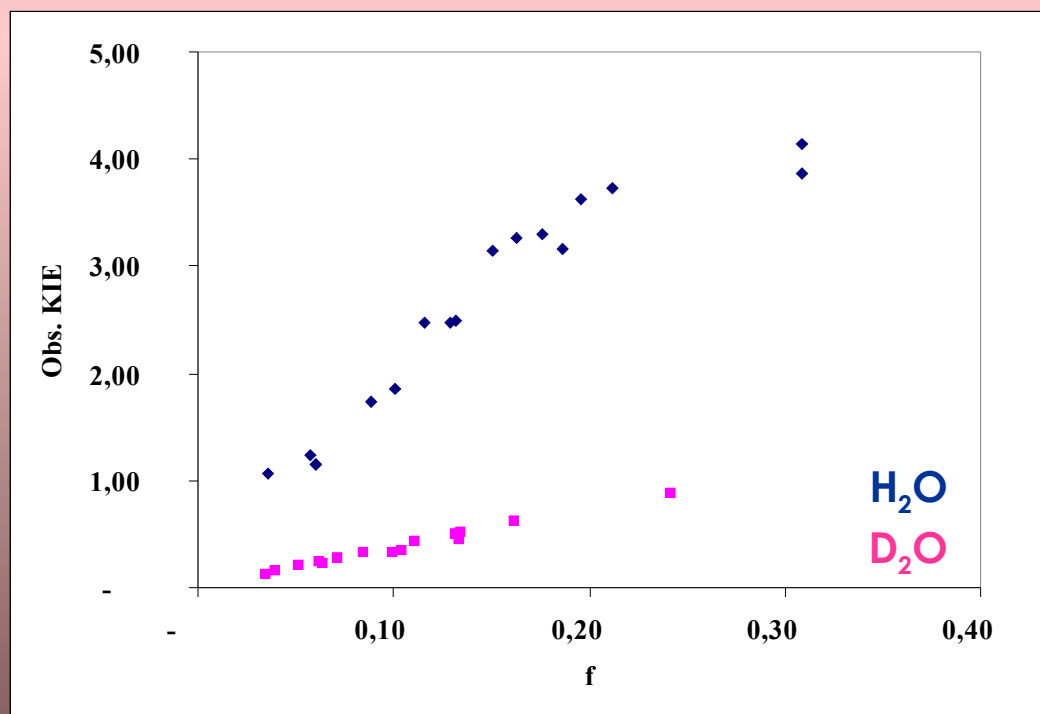
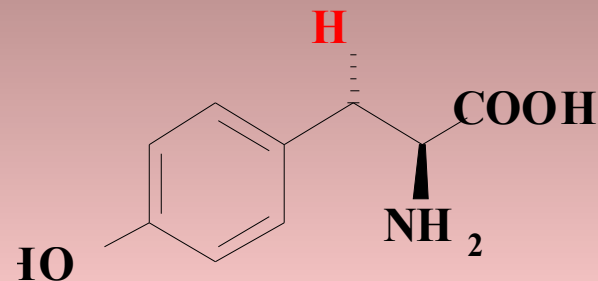
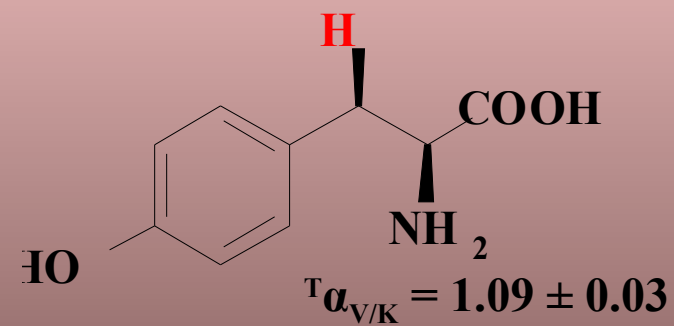
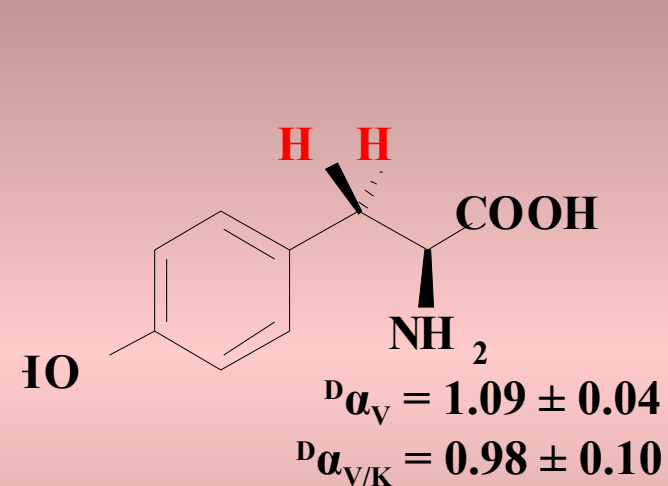
NMR Experiments

¹H-NMR

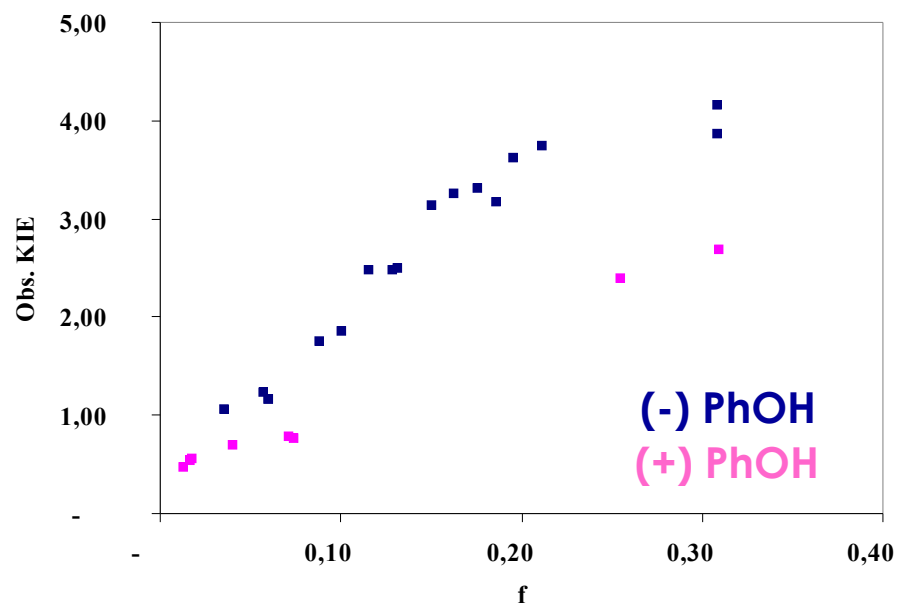
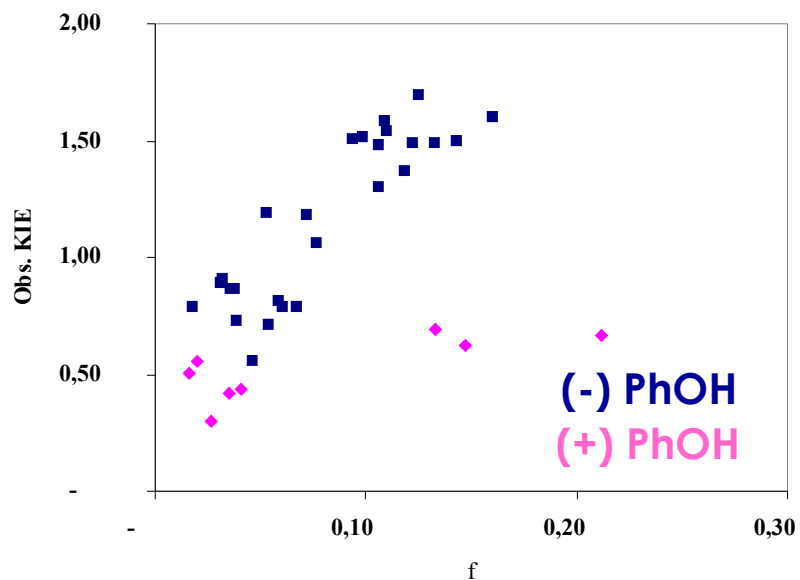
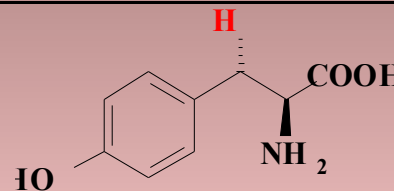
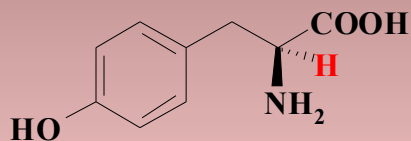


The exchange of β hydrogen atoms was not observed in the presence of LDH and NADH

Hydrogen KIE on Position 3

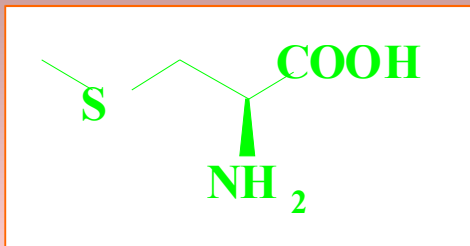


Impact of Products on H/T KIE

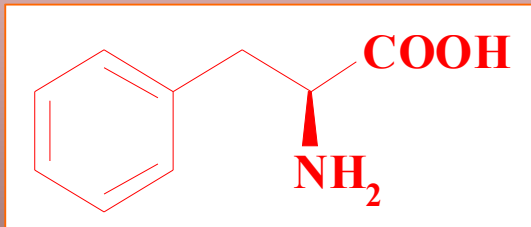


- Observed H/T KIE on 2 and 3S positions does not depend on presence of:
- other products of reaction (pyruvate, ammonia)
 - reaction side-product (*p*-hydroxyphenylpyruvate)

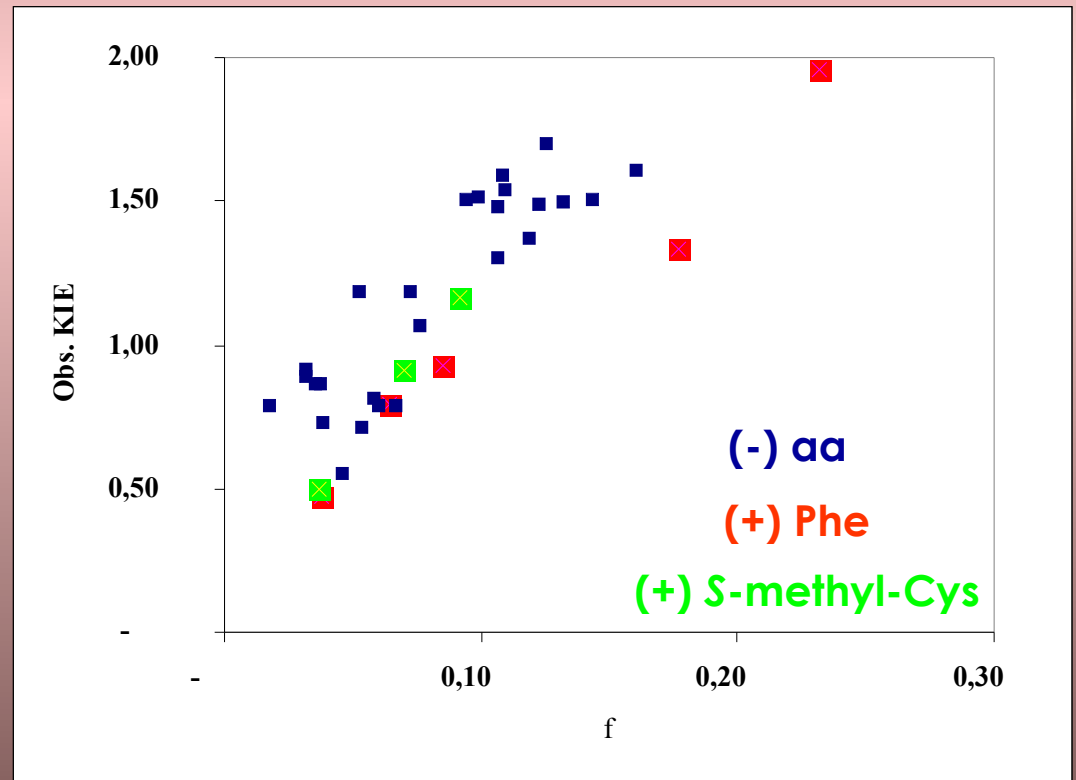
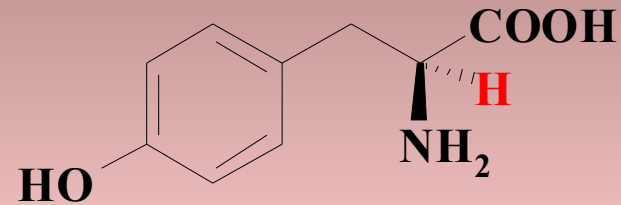
Impact of Amino Acids on H/T KIE



- Undergoes α -deprotonation
- Undergoes β -elimination

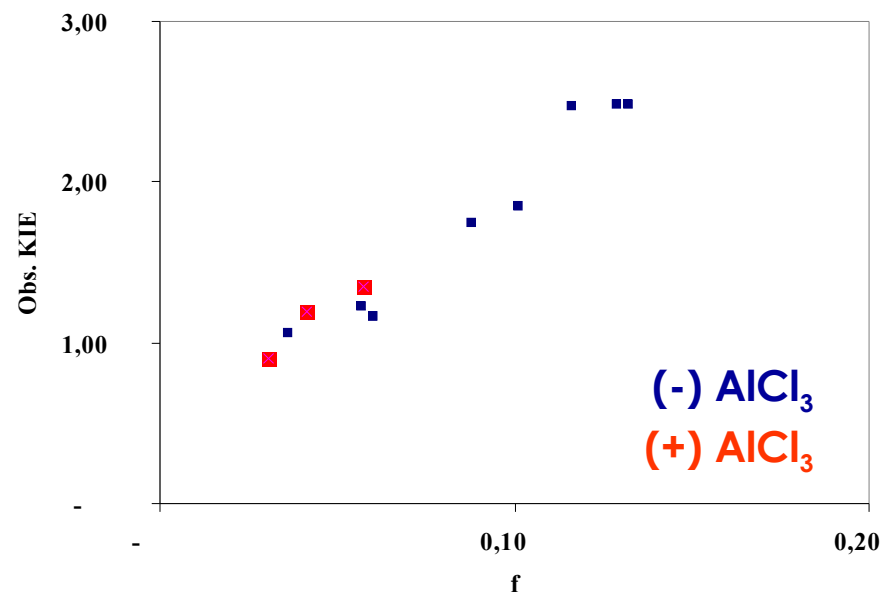
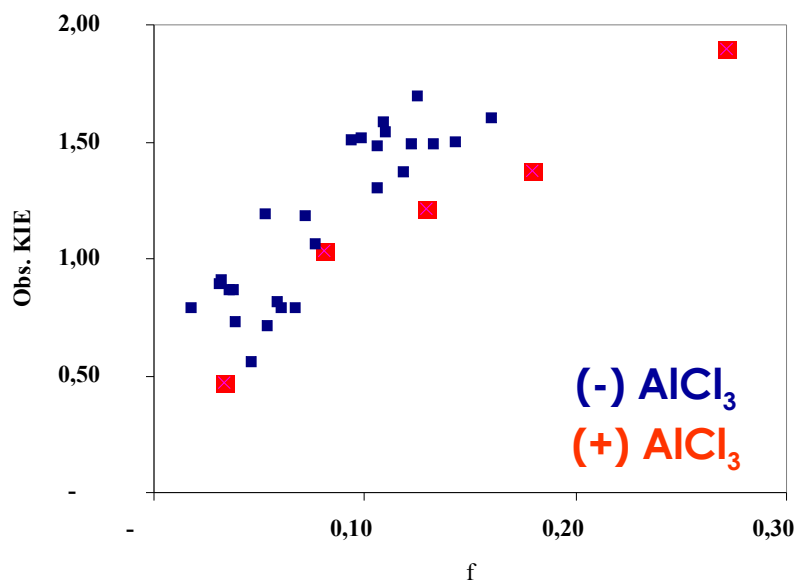
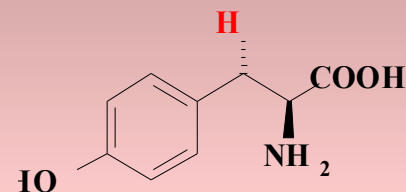
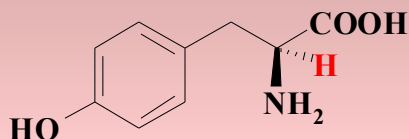


- Undergoes α -deprotonation
- Does not undergo β -elimination



Impact of Lewis Acid on H/T KIE

Lewis acids facilitate Schiff base formation between tyrosine and PLP in solution





Conclusions

- ❑ relative rates of the reactions steps change during the course of the reaction, e.g. the rates of α and β proton exchange with water decrease dramatically
- ❑ heavy water strongly promotes β proton exchange with solvent comparing to water, whereas the isotopic composition of solvent does not significantly affect α proton exchange with solvent
- ❑ changes of relative rates of the reaction steps are not caused by intermediates released by the enzyme
- ❑ the formation of slowly processed intermediate in one of the very last steps of the reaction may explain the observed phenomena
- ❑ Lewis acids inhibit the reaction; however, they do not affect the observed isotope effects

The main conclusion is: we still have a lot of work to do!



Acknowledgements

- prof. Robert Phillips (University of Georgia, Athens, USA) – for providing us with TPL and for helpful discussions
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Thank you for your kind attention!