STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIII, 2, 2008

MECHANISTIC ASPECTS AND BIOCATALYTIC IMPLICATIONS OF THE MIO-CONTAINING AMMONIA-LYASE / AMINOMUTASE FAMILY

LÁSZLÓ POPPE[a], SAROLTA PILBÁK[a], CSABA PAIZS[b], JÁNOS RÉTEY[c]

ABSTRACT. Histidine, phenylalanine and tyrosine ammonia-lyases (HAL, PAL and TAL) all catalyze ammonia elimination with the aid of a post-translationally formed electrophilic prosthetic group (MIO). MIO occurs also in L-phenylalanine and L-tyrosine aminomutases. Based on the role of PAL as a biocatalyst, the production of β-phenylalanine analogs can be extended with combined use of ammonia-lyases and amino-mutases. For ammonia-lyases two significantly different mechanisms were suggested. One implies an *N*-MIO, whereas the second involves a Friedel-Crafts (FC) type intermediate. A common feature of both mechanisms is the formation of a covalent intermediate which allows systematic confomational analysis of the ligand within the rigid active site. Furthermore QM/MM calculations allowed detailed modeling of the alternative covalent intermediates which demonstrated that the *N*-MIO intermediate has ~140 kcal/mol lower energy than the best FC state.

Keywords: *ammonia-lyase, biocatalysis, covalent intermediate, conformational analysis, QM/MM calculations*

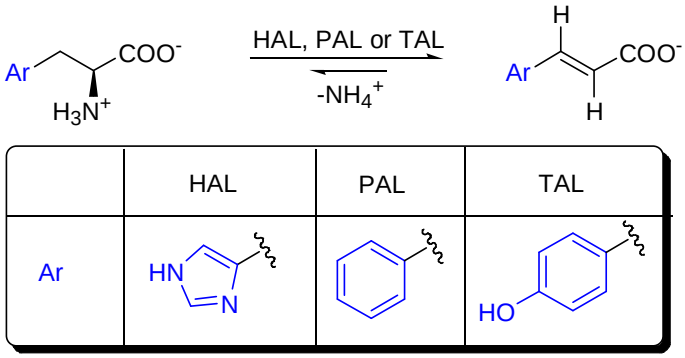
INTRODUCTION

Histidine, phenylalanine and tyrosine ammonia-lyases (HAL, EC 4.3.1.3; PAL, EC 4.3.1.5; TAL, EC 4.3.1.5) catalyze ammonia elimination from L-histidine, L-phenylalanine and L-tyrosine to (*E*)-urocanic acid, (*E*)-cinnamic acid and (*E*)-p-coumaric acid, respectively (Scheme 1).

Under extreme *in vitro* conditions (> 4 M NH_4OH, pH 10) ammonia-lyases catalyze the reverse reaction (amination). The possibility of enantioselective addition to α,β-unsaturated acids made this approach attractive for preparation of L-amino acids by biotransformations [1,2,3,4].

[a] *Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, H-1111 Budapest, Gellért tér 4, Hungary, poppe@mail.bme.hu*
[b] *Department of Biochemistry and Biochemical Engineering, Babeş-Bolyai University, Ro-400028 Cluj-Napoca, Arany János 11, Romania*
[c] *Institute of Organic Chemistry, University of Karlsruhe, D-76128, Fritz-Haber Weg 6, Germany*

In spite of the unexplored biocatalytic potential and substrate and substrate tolerance of L-phenylalanine and L-tyrosine aminomutases (PAM and TAM), these enzymes are potential biocatalysts for preparation of optically active ß-amino acids. Whereas PAM isomerizes (2*S*)-α-phenylalanine to (3*R*)-β-phenylalanine with overall retention of configuration of the *pro*-3*R* hydrogen at C_3[5], TAM converts (2*S*)-α-tyrosine to (3*S*)-β-tyrosine [6,7]. These data indicate that the MIO-containing 2,3-aminomutases may proceed through partially different reaction mechanisms.



Scheme 1. *The HAL, PAL and TAL reactions.*

Due to its potential as biocatalysts and possible application in treatment of phenylketonuria, PAL was choosen as primary target for detailed investigation of the mode of action at molecular level. For these studies, all the previous mechanistic models and the rapidly growing number X-ray structures of various MIO-enzymes have been used. First, based on HAL X-ray structure we constructed a homology model of parsley PAL for mechanistic calculations [8]. Later, we corrected the Y110-loop of the published X-ray structure of parsley PAL to a mechanistically relevant conformation [9].

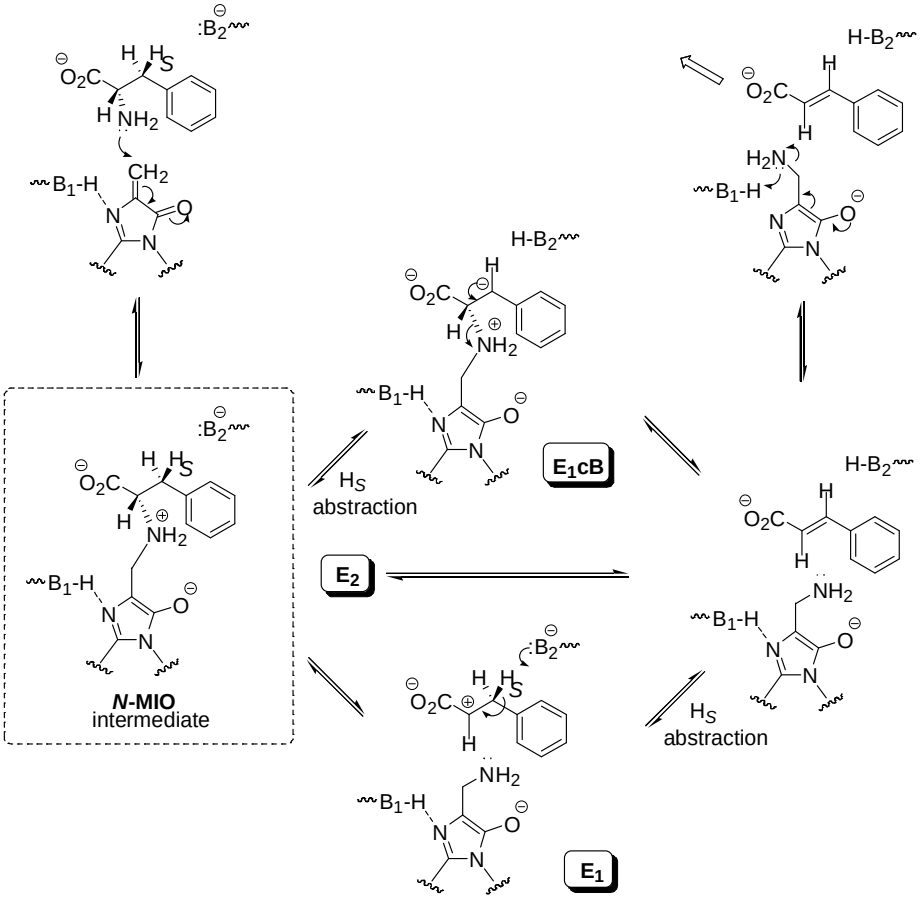
RESULTS AND DISCUSSION

The QM/MM calculations were performed within the active site of the well defined experimental structure of *Rhodosporidium toruloides* TAL (*Rs*TAL) [10]. The PAL activity of the analogous His89Phe mutant of *Rs*TAL proved that the TAL and PAL reactions have the same mechanism.

The comparison of the calculated structures and energies of the TAL intermediates with the substrate and product binding states of the enzyme revealed that the *N*-MIO state has a 139 kcal/mol lower energy

than the more favorable one of the diastereomeric FC states. Owing to serious distortions the difference for the (*S*)-FC structure is even higher (>10,000 kcal/mol).

In addition, comparison of the calculated structures with the 2-aminoindan-2-phosphonate inhibited *Rs*TAL structure showed that the arrangement of L-tyrosine ligand in the *N*-MIO intermediate is consistent with the overall arrangement found experimentally in the inhibited *Rs*TAL structures [10] i.e. the aromatic moiety points towards His89 and the carboxylate group is to close Arg303.



Scheme 2. *Mechanisms of the PAL reaction via an N-MIO intermediate (B_1-H: Tyr351/Tyr300 in PAL/ TAL; B_2-H: Tyr110/Tyr60 in PAL/TAL).*

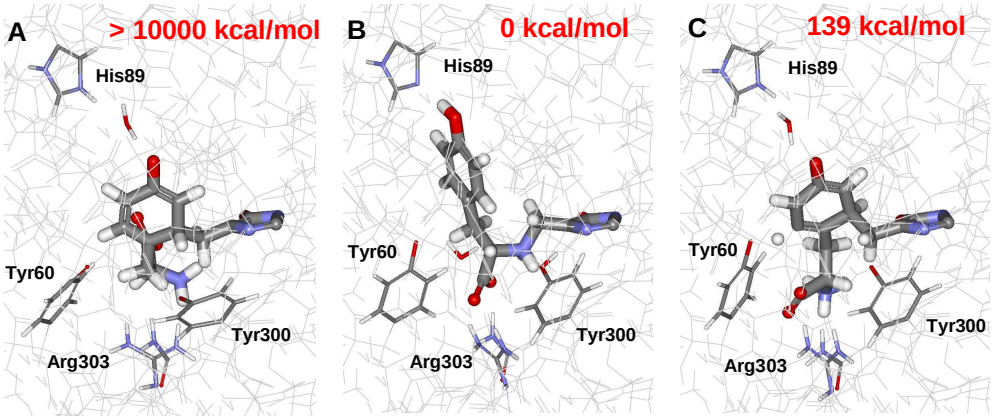


Figure 1. Comparison of the (*S*)-FC (A), *N*-MIO (B) and (*R*)-FC (C) intermediates within the *Rs*TAL active site calculated at PM3/UFF level.

CONCLUSIONS

PAL is a suitable biocatalyst for production of optically pure arylalanines, which can be extended to enantioselective synthesis of β-phenylalanine analogs with combined use of ammonia-lyases and aminomutases.

The QM/MM calculations for the TAL reaction indicated, that a Friedel-Crafts type mechanism can be excluded solely by energetics. The roles of the most important active site residues of *Rs*TAL were explored. His89 and Arg303 anchor the L-tyrosine substrate by hydrogen bonds to its phenolic OH and carboxylate groups, respectively (Figure 1). Tyr300 (B_1) and Tyr60 (B_2) are the two most important enzymic bases (Scheme 2). In the mechanism involving an *N*-MIO intermediate Tyr300 liberates the nucleophilic amino group from its protonated state, whereas Tyr60 has primary importance as the enzymic base responsible for deprotonation at the C_3 *pro-S* side of the substrate.

EXPERIMENTAL SECTION

For the two covalently bound intermediates systematical conformational search (Hyperchem) [11] and QM/MM calculations (Gaussian) [12] were performed within the active site of *Rs*TAL.

REFERENCES

1. JP 63148992, **1988**, ASAHI Glass Co. Ltd.
2. A. Gloge, B. Langer, L. Poppe, J. Rétey, *Arch. Biochem. Biophys.*, **1998**, *359*, 1.
3. A. Gloge, J. Zoń, Á. Kővári, L. Poppe, J. Rétey, *Chem. Eur. J.,* **2000**, *6*, 3386.
4. C. Paizs, A. Katona, J. Rétey, *Chem. Eur. J.*, **2006**, *12*, 2739-2744.
5. W. Mutatu, K. L. Klettke, C. Foster, K. D. Walker, *Biochemistry,* **2007,** *46,* 9785.
6. S. D. Christenson., W. Liu, M. D.Toney, B. Shen,. *J. Am. Chem. Soc.,* **2003**, *125*, 6062.
7. C. V. Christianson, T. J. Montavon, S. G. Van Lanen, B. Shen, S. D. Bruner, *Biochemistry,* **2007,** *46,* 7205.
8. D. Röther, L. Poppe, G. Morlock, S. Viergutz, J. Rétey, *Eur. J. Biochem.,* **2002**, *269*, 3065.
9. S. Pilbák, A. Tomin, J. Rétey, L. Poppe, *FEBS J.,* **2006**, *273*, 1004.
10. G. V. Louie, M. E. Bowman, M. C. Moffitt, T. J. Baiga, B. S. Moore, J. P. Noel, *Chem. Biol.,* **2006**, *13*, 1327.
11. Hyperchem 7.5, Hypercube, Inc., **2003**.
12. Gaussian 03, Revision B.05, M. J. Frisch, *et. al*, Gaussian, Inc., Wallingford CT, **2003**.