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LIGAND DOCKING AND SYSTEMATIC CONFORMATIONAL ANALYSIS IN LOOP MODIFIED PARSLEY PHENYLALANINE AMMONIA-LYASE STRUCTURE

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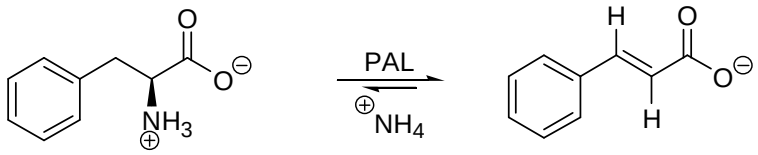
ABSTRACT. The phenylalanine ammonia-lyase (PAL) catalyzes the ammonia elimination from L-phenylalanine to (*E*)-cinnamic acid. A PAL model having more compact active center then the experimental *parsley* PAL (PDB code: 1W27) was constructed on the basis on different PAL and related enzyme structures (plant, bacterial and yeast) and their stability issues. In this PAL model (partial modified crystal structure) conformational analysis of the covalently bounded substrate to the MIO and ligand docking were performed to interpret experimental data.

Keywords: *phenylalanine ammonia-lyase, homology model, conformational analysis, docking*

INTRODUCTION

Phenylalanine ammonia-lyase (PAL EC 4.3.1.5) enzyme catalyzes the deamination from L-phenylalanine to (*E*)-cinnamic acid in the presence of an electrophilic prosthetic group: 5-methylene-3,5-dihydroimidazol-4-one (MIO) (Scheme 1) [1]. (*E*)-cinnamate is the precursor of a large number of plant metabolites, including lignin, coumarins, and flavonoids [2,3]. PAL is a key enzyme in the phenylpropanoid metabolism of plants. Polyethylene glycol-modified PAL can be considered for enzyme supplementation cure for the genetic disorder phenylketonuria [4].



L-Phenylalanin (*E*)-cinnamic acid

Scheme 1. Deamination of L-phenylalanine in the presence of PAL.

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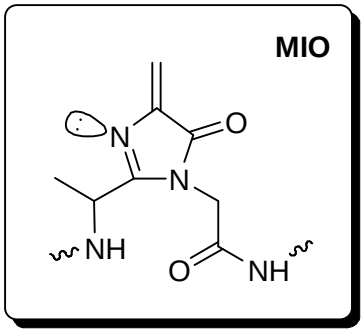
Histidine ammonia-lyase (HAL) and tyrosine ammonia-lyase (TAL) enzymes are analogous of PAL. The structures and functions of these three ammonia-lyases are similar, because every of them contain the autocatalitically formed electrophilic MIO prosthetic group (Scheme 2) which is essential for the reaction [1].



Scheme 2. The 5-methylene-3,5-dihydroimidazol-4-one (MIO) prosthetic group.

Since the catalytically essential Tyr110 residue containing loop region is missing from yeast PAL structure [5] and the parsley PAL (PDB code: 1W27) crystal structure [6] has an inactive conformation [7], we proposed to build a modified parsley PAL structure with a close active center, which can be adequate for mechanistic studies.

RESULTS AND DISCUSSION

Based on HAL structures [8], a modified parsely PAL structure was already built, which has a closer active center than the experimental PAL crystal structure [7]. Recently the TAL crystal structure was resolved [9], in which the essential Tyr-containing loop region has a compact conformation and therefore has a more closed active site. Based on the TAL (PDB code: 2O7B) structure, a new loop-modified PAL model was built. The tetramers of the parsley PAL crystal structure and the PAL model can be seen in Figure 1. A more detailed comparison of the two PAL active sites shows the Tyr110 residue in two different orientations. Tyr110 in our model points in the direction of MIO, whereas in the crystal structure was far from the active site (Figure 1).

Since the mechanism of the ammonia-lyase reactions involves a covalently bound intermediate (the substrate interacts with MIO due to it's N atom), systematical conformational search (CS) can be carried out within the rigid enzyme environment.

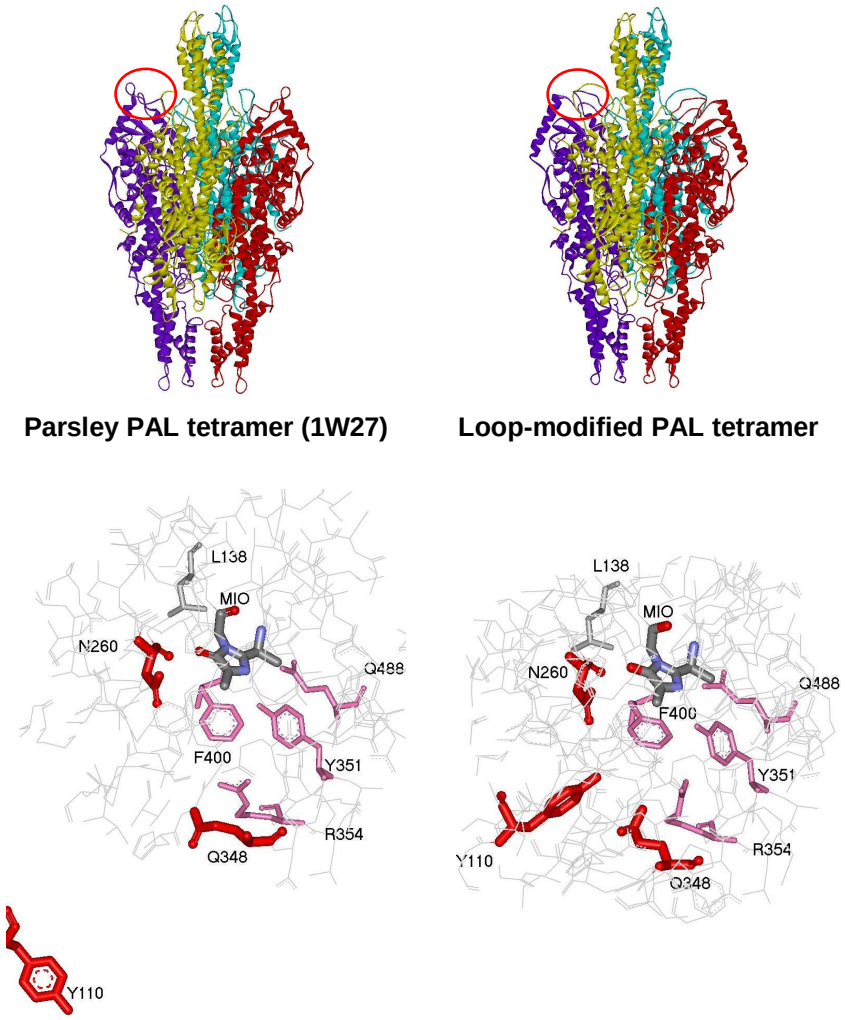
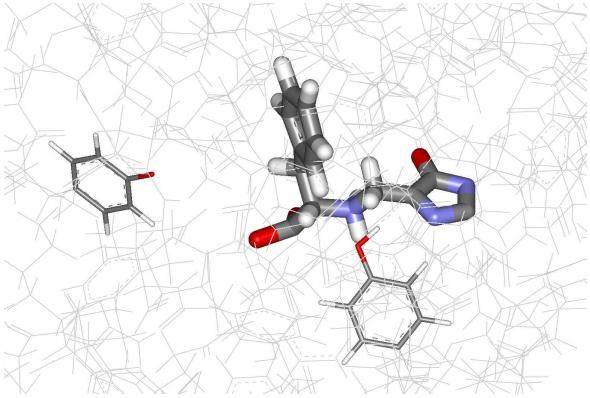


Figure 1. PAL tetramers and their active sites.

In the modified PAL crystal structure conformational analysis and ligand docking was performed. The two different analyses are in concordance, because the best *N*-MIO orientation obtained by CS has very similar orientation with the L-Phe in the docking result (Figure 2).

***N*-MIO conformational search**



L-Phe docking

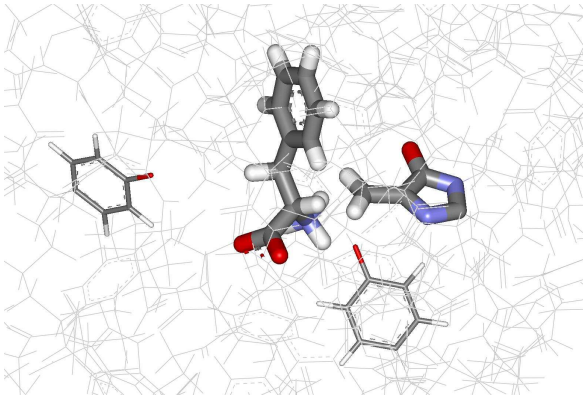


Figure 2. Results of conformational analysis and ligand docking within PAL.

EXPERIMENTAL SECTION

For our studies a 15 Å sphere around the MIO was cut out from the active site model. Next, by the HyperChem [10,11] standard procedure, hydrogen atoms were added to the amino acid residues of this raw active site model (the C- and N-termini at cutting were completed to neutral aldehyde and amino moieties). The MIO group was manually corrected [10]. For the *N*-MIO model three torsion angles of the ligand were varied during the CS [10]. The docking study was performed in ArgusLab.

CONCLUSIONS

Since the parsley PAL crystal structure has an inactive conformation, using homology modeling we built a TAL-like PAL structure, which has a completely closed active center. This gives us the possibility to study the reaction mechanism catalyzed by PAL enzyme.

The best docking poses are in accordance with the conformational analysis result of L-phenylalanine in the modified PAL structure.

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