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New insights in the catalytic mechanism of tyrosine ammonia-lyase given by QM/MM and QM cluster models

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ABSTRACT

Tyrosine ammonia lyase (TAL) catalyzes the deamination of tyrosine to p-coumaric acid in purple phototropic bacteria and Actinomycetales. The enzyme is used in bioengineering and has the potential to be used industrially. It belongs to a family of enzymes that uses a 4-methylidene-imidazole-5-one (MIO) cofactor to catalyze the deamination amino acids. In the present work, we used a QM/MM and a QM cluster models of TAL to explore two putative reaction paths for its catalytic mechanism. Part of the N-MIO mechanism was previously studied by computational methods. We improved on previous studies by using a larger, more complete model of the enzyme, and by describing the complete reaction path. The activation energy for this mechanism, in agreement with the previous study, is 28.5 kcal/mol. We also found another reaction path that has overall better kinetics and reaches the products in a single reaction step. The barrier for this Single-Step mechanism is 16.6 kcal/mol, which agrees very well with the experimental k_{cat} of 16.0 kcal/mol. The geometrical parameters obtained for the cluster and QM/MM models are very similar, despite differences in the relative energies. This means that both approaches are capable of describing the correct catalytic path of TAL.

Introduction

Tyrosine ammonia lyase (TAL) was first discovered in 2001 from the genome analysis of *Rhodobacter capsulatus*. It catalyzes the deamination of tyrosine to p-coumaric acid with a turnover number of 27.7 s^{-1} [1]. The reaction catalyzed by TAL is of biological, bioengineering, and industrial interest: p-coumaric acid is the chromophore of photoactive yellow protein of purple phototropic bacteria [2] and a precursor in the biosynthesis of caffeic acid in Actinomycetales; [3] the enzyme is also used in bioengineered pathways to produce flavonoids [4] and resveratrol [5]; finally, TAL has the potential to be used in an industrial setting in the production of several industrial chemicals whose precursor is p-coumaric acid [6,7].

Tyrosine ammonia lyase together with phenylalanine ammonia lyase (PAL) and histidine ammonia lyase (HAL) form a small group of enzymes that have a similar active center and catalyze the deamination of amino acids. These enzymes have a common electrophilic cofactor denominated 4-methylidene-imidazole-5-one (MIO) [8] which is formed from the autocatalytic cyclization of three adjacent amino acids, Ala149-Ser150-Gly151 in TAL [9], during the folding of the enzymes [10]. PAL and TAL, specifically, are so similar that a single amino acid, His89, has been identified as selectivity switch between them [11]. In TAL, His89 is making an hydrogen bond with the hydroxyl group of the tyrosine substrate, by mutating this residue to a non-polar (phenylalanine) or more bulky (glutamine) residue, TAL loses the catalytic power over tyrosine but gains it over phenylalanine. The opposite mutation on a PAL makes it capable of catalyzing the deamination of tyrosine. Apart from the MIO cofactor, Tyr60 and Tyr300 have also been identified by mutagenesis studies as essential for catalysis in TAL [12]. In HAL, Tyr53 and Tyr280 are equivalent to the two TAL tyrosine amino acids [8], and in PAL, Tyr371 is in the same position as the Tyr300 of TAL [13]. From these resemblances, it is expected that the reaction mechanism followed by the three enzymes is similar.

Initially, two mechanisms were proposed for the deamination of amino acids by MIO enzymes: a Friedel–Craft type mechanism where the MIO cofactor (thought to be dehydroalanine at the time) forms a covalent intermediate with the aromatic ring of the substrate [14,15]; and a mechanism where the cofactor does a covalent intermediate with the amine group of the substrate (N-MIO) [16]. Recently, a computational study of the TAL reaction

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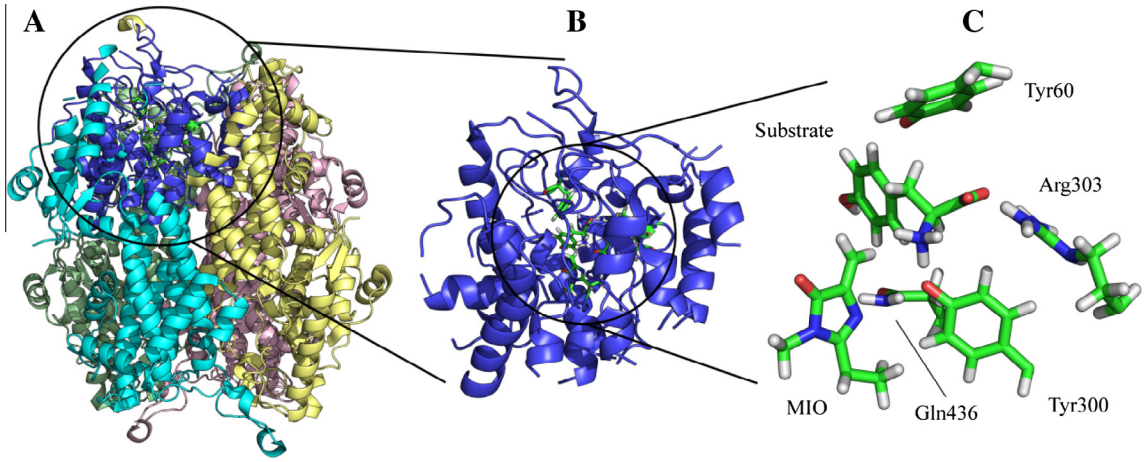

Fig. 1. A homotetramer of the tyrosine ammonia lyase enzyme, and the model used in this work. (A) – Homotetramer of TAL colored by chain. The dark blue portion, is the part of the enzyme we used in the ONIOM calculations. (B) – QM/MM model used in this work. In blue ribbons is represent the MM layer of the model, and in sticks is represent the QM layer. (C) – Detailed representation of the QM layer used in the ONIOM calculations.

mechanism showed that the N-MIO mechanism is much more favorable than the Friedel–Crafts mechanism [17]. In that paper, the authors describe two steps of the N-MIO reaction path.

In the present work we used two models of the TAL enzyme to explore its potential energy surface in great detail: a 172 atoms DFT cluster model and a 5216 atoms QM/MM model (see Fig. 1). We were able to describe, with both models, the complete reaction of the N-MIO mechanism, from the state of having a protonated-amine substrate from the leaving of a protonated amine group. Furthermore, we found an additional mechanism that is compatible with the TAL active center and has better kinetics than the N-MIO mechanism. In this new mechanism, there is no covalent intermediate with the cofactor and the reaction happens in a single reaction step with a barrier of 16.6 kcal/mol.

Methods

The QM/MM model of TAL was built from the 2O7B X-ray structure [13]. This structure has the entire TAL homotetramer, and a p-coumarate molecule bonded to the active center. For the QM/MM model (see Fig. 1), we started by selecting all the residues within 20 Å of the substrate. In order to minimize the number of cuts and make the model more cohesive, some residues were added to this selection to fill small gaps in the selection (residues missing in the middle of an alpha helix, for example). The water molecules picked up by the selection where left in the model. The tyrosine substrate was modeled from the p-coumarate molecule by adding the amine group to the α-carbon, with attention made to the ensuing chirality (S at the α-carbon, a L-tyrosine). This model of the enzyme has 5216 atoms. To establish the QM/MM division, residues deemed more important to catalysis where included in the quantum mechanics (QM) layer: the tyrosine substrate, tyrosine 60, tyrosine 300, arginine 303, the MIO cofactor, and glutamine 436; for a total of 99 atoms. The rest of the atoms where included in the molecular mechanics (MM) layer. The selection of the 20 Å sphere around the substrate was done on PYMOL, [18] while the modeling task and the division of the QM/MM system was done on GAUSSVIEW [19].

We used the antechamber program, [20] part of the AMBER10 suite of programs, [21] to parameterize the unbound tyrosine substrate and the MIO cofactor, since the amber force field does not include parameters for these molecules. Antechamber attributes parameters from the GAFF force field [22] to bonds, angles, dihedrals, and van der Waals radii. Atomic charges were calculated from a QM calculation at the HF/6-31G(d) level, [23] to maintain compatibility with the amber force field, followed by a RESP (Restrained ElectroStatic Potential) calculation [24].

The cluster model was built from the optimized structure of the reactants of the QM/MM model in order to ensure the similarity of the initial geometries. The side chains of the most important residues for the catalytic mechanism were maintained: the tyrosine substrate, tyrosine 60, tyrosine 300, arginine 303, the MIO cofactor, phenylalanine 66, histidine 86, phenylalanine 350, methionine 405 (chain B), asparagine 432 and glutamine 436. The modeling task was done on GAUSSVIEW [19].

The QM layer of the QM/MM model and the QM cluster model were treated with the B3LYP [25,26] density functional and the 6-31G(d) basis-set [27]. The MM layer of the QM/MM layer was treated with the AMBER03 force field [28] as implemented in GAUSSIAN09. All calculations were done with the GAUSSIAN09 software [29].

The approach for exploring the potential energy path was the same for both models. Structures of transition states and intermediates were first obtained by scanning appropriate coordinates along the reaction path. Afterwards, all states, including minima and transition states, were freely optimized. During optimizations, the only constrained atoms in the QM/MM model belong to low layer residues were the cuts were made in the preparation of the model. The coordinates of the atoms of these residues were frozen in place during all optimizations. In the cluster model, the atoms of the residue side chains where the cuts were made were also frozen in space to ensure that the structure of the model continued the same throughout the calculations, to simulate the effect of the protein scaffold. Frequency calculations were done to confirm the position (as minima or maxima) of all obtained states. In the QM/MM model, zero point energy and entropic corrections were added to the potential energy path, in order to obtain an approximated free energy path. In order to improve the energy values, single point energy calculations were done for both models at the B3LYP/6-311++G(2d,2p) level [30–32]. Since explicit water molecules near the active center were present in the QM/MM model, and the bulk solvent was at least at 20 Å from of the active center, no additional solvent corrections were required.

Results and discussion

From the early exploration of the reaction space associated with the active center of tyrosine ammonia lyase we found two potential reaction paths. The first one, presented in Scheme 1, had been