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# Quantum-Classical Hybrid Simulations of Transamination Enzymatic Reactions in Aqueous Media

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**[Abstract]** We will present some of our recent studies on hybrid molecular dynamics simulations of complex chemical reactions in aqueous solutions. The focus will be on chemical processes which do not take place so easily in normal ab initio molecular dynamics simulations. One such reaction is the process of transamination. Transaminase is a PLP (Pyridoxal 5'-Phosphate) dependent enzyme which reversibly catalyzes the transamination reaction. Aspartate Transaminase (AspTase) is a key enzyme of amino acid metabolism process. In the present talk, we will discuss our recent studies on the mechanism of the transamination reaction in the active site of AspTase using hybrid quantum-classical (QM/MM) molecular dynamics simulation with the aid of the metadynamics techniques. Preliminary results will also be presented for the transimination reaction for the Aspartate substrate which precedes the transamination reaction and also for another transamination reaction involving Serine Hydroxyl Methyl Transaminase (SHMT).

**[Introduction]** Transamination is an important enzymatic reaction where an α-amino acid gets converted to an α-keto acid and another α-keto acid gets converted to a different α-amino acid reversibly [1]. The Aspartate Transaminase, also known as Aspartate Aminotransferase or AspAT, is a dimeric protein which converts aspartic acid to a keto-acid with the help of its co-factor Pyridoxal 5'-Phosphate (PLP) whose structure is shown in Fig.1. Apart from acting as a key reaction for amino acid metabolism and synthesis, this reaction is also involved in many medicinal applications. Hence, it is important to understand the mechanism and energetics of this reaction in aqueous media. The present work makes a contribution toward this end.

AspAT is a dimeric protein comprised of two identical subunits. Each subunit is divided into two domains: one small and one large. AspAT has two active site pockets located at the subunit interface and domain interface. Crystallographic studies have shown that the PLP forms a covalent bond with a lysine residue which is usually referred to as the internal aldemine state. Subsequently, the PLP enzyme complex reacts with the substrate amino acid where the bond between the active site lysine and PLP is broken and a new Schiff base is formed between the PLP and the substrate amino acid which is referred to as the external aldemine state [2]. This step is commonly known as the transimination step which is a prerequisite for the transamination reaction. The majority of the PLP catalyzed reactions are initiated by abstraction of the α-proton of the substrate from the Schiff base by a Lys residue leading to the formation of a carbanionic intermediate which subsequently adopts a quinonoid structure. Subsequently, the reprotonation at the C4A atom of PLP results in the formation of ketamine. The first issue that had to be resolved is the protonation state of PLP as it can stay in two tautomeric keto and enol forms (Fig,1). Subsequently, once this issue is resolved, the transamination reaction is studied and the free energy pathway of the reaction is found out.

**[Methods]** The X-ray crystal structure of AspAT [3] complexed with pyridoxyl-aspartic acid 5' monophosphate was used as the initial structure for the current calculations. The enzyme is a homodimer containing 396 amino acid residues in each subunit. To perform the molecular dynamics simulations, the enzyme system was solvated in a rectangular box containing 29,833 TIP3P water molecules. The resulting system had a net negative charge of -22 and 22 positively charged sodium ions were added to achieve electroneutrality. After proper equilibration, the NVT simulation was carried out for 2.5 ns using generalized AMBER force fields. For studying the chemical reactions, we treated the active site part of the system (PLP-substrate complex together with Lys of the enzyme at its active site) quantum mechanically and the rest through force fields (the so-called QM/MM method [4] with QM part treated through Car-Parrinello method [5,6]). In order to accelerate the chemical reactions so as to see them happening within the finite simulation run length, we employed the metadynamics technique [7] in QM/MM simulation.

**[Results and Discussion]** We first discuss our results of the protonation state of PLP-Asp complex (Fig.1). It was found from the metadynamics simulations that the keto-form of PLP is more stable than the enol form [8]. The activation barrier for conversion from keto to enol form was found to be about 7.5 kcal/mol. Hence, we took the keto form as the starting configuration for our metadynamics studies of the transamination reaction which involves the formation of quinonoid through proton abstraction as an intermediate. Our results show that the transamination reaction takes place through multiple steps. The rate limiting step is the one where Lys258 of the enzyme abstracts the α-proton of the substrate and it is followed by reprotonation at C4A atom of PLP Shiff base from Lys 258. The free energy barrier for this proton abstraction is found to be 17.85 kcal/mol [9]. The present study also revealed interesting conformational changes of the Lys258 residue during the course of the reaction.

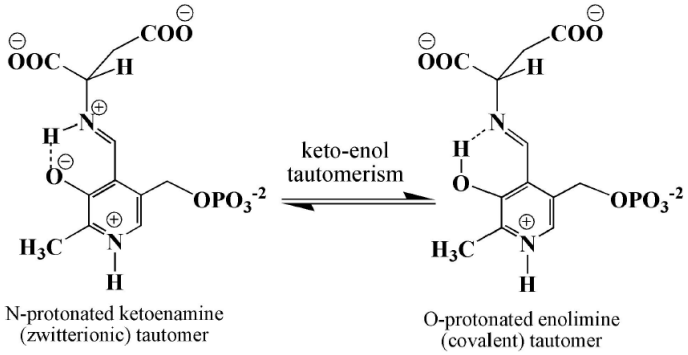


Fig.1 Two tautomeric forms of PLP-Asp complex

## [References]


[1] P. Christen and D.E. Metzler, "Transaminases", 1st Ed (Willey, New York, 1985), pp. 37-101.

[2] A. Okamoto *et al. J. Mol. Bio.* **280**, 443 (1998).

[3] R. Graber *et al. Eur. J. Biochem.* **232**, 686 (1995).

[4] A. Laio *et al. J. Chem. Phys*. **116**, 6941 (2002); *J. Phys. Chem. B* **106**, 7300 (2002).

[5] R. Car and M. Parrinello, Phys. Rev. Lett. **55**, 2471 (1985).

[6] J. Hutter et al. CPMD Program Package. IBM Corp 1990-2011, MPI fuerFestkoerperforschung Stuttgart 1997-2001. See also http:// www.cpmd.org.

[7] A. Laio and M. Parrinello, *Proc. Natl. Acad. Sci.* **99**, 12562 (2002).

[8] S. Dutta Banik and A. Chandra, *J. Phys. Chem. B* **118**, 11077 (2014).

[9] S. Dutta Banik, A. Bankura, and A. Chandra, to be published