Molecular dynamics study on the substrate binding free energy of Threonine Synthase

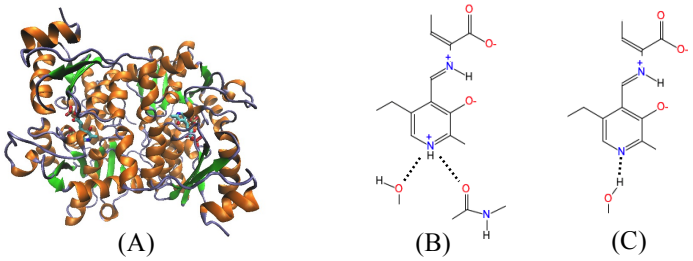
Yuzuru Ujiie[1*], Wataru Tanaka[1], Kyohei Hanaoka[1], Megumi Kayanuma[2], Mitsuo Shoji[1], Katsumasa Kamiya[1], Toyokazu Ishida[3], Kenji Shiraishi[1], Yasuhiro Machida[4], Takeshi Murakawa[5], Hideyuki Hayashi[4]

[1]Graduate School of Pure and Applied Sciences, University of Tsukuba, Japan
[2]Graduate School of Systems and Information Engineering, University of Tsukuba, Japan
[3]Nanosystem Research Institute (NRI), National Institute of Advanced Industrial Science and Technology (AIST), Japan
[4]Department of Chemistry, Osaka Medical College, Japan
[5]Department of Biochemistry, Osaka Medical College, Japan

* yujiie@ccs.tsukuba.ac.jp

Threonine Synthase (TS) catalyzes a L-threonine formation reaction from O-phospho-L-homoserine (OPHS). TS contains a pyridoxal phosphate (PLP) and a phosphate ion in the active site. The reaction of TS is full of regiospecific and stereospecific steps, and the complicated reaction mechanism is not yet elucidated. It is sill unclear even for the protonation form at the PLP-pyridine nitrogen, though it seems to be critically important for the reactivity.

In this study, molecular dynamics (MD) calculations were performed and the substrate binding free energies are evaluated at the protonated and unprotonated state. MM-GBSA method was used for the binding free energy calculations. In the MM-GBSA formulation, total free energy is decomposed into an internal protein energy (G_{gas}) and a solvation free energy (G_{solv}). G_{solv} is the sum of the electrostatics and the nonpolar contributions, which are evaluated by the Generalized Born and surface accessible area calculations, respectively. GROMACS program package was used for MD simulations. By the 20ns MD simulations, the protonated PLP formed a minor hydrogen bond to Ala289 backbone oxygen atom at 5.78%. The unprotonated PLP formed a stable hydrogen bond to Thr317 side chain, which is observed in X-ray structures (Fig.1). These results suggest that the pyridine nitrogen is unprotonated in the X-ray structure. Calculated pKa value is 8.0, which indicates that protonated state is more stabilized in the protein environment.



(A) (B) (C)

Fig.1 structure of TS (A) and molecular structure of pyridoxal phosphate (PLP) at the protonated form (B) and the unprotonated form (C).