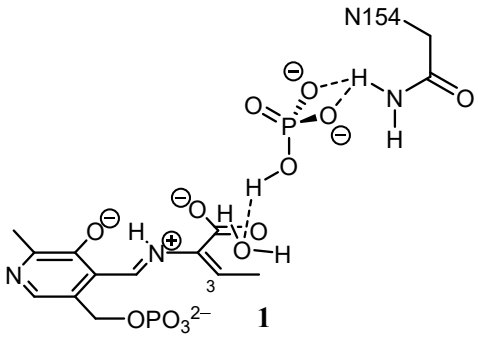
Role of the catalytic phosphate ion in threonine synthase as revealed by molecular dynamic simulation

([1]*Departments of Chemistry*[1] *and Biochemistry*[2]*, Osaka Medical and Pharmaceutical University*)
Yasuhiro Machida[1], Takeshi Murakawa[2], ○Hideyuki Hayashi[1]
Keywords: product-assisted catalysis, phosphate ion, threonine synthase, pyridoxal 5′-phosphate, molecular dynamics

Threonine synthase, a pyridoxal 5′-phosphate-dependent enzyme, undergoes product-assisted catalysis, in which the phosphate ion released from the substrate *O*-phosphohomoserine remains at the active site and acts as a catalyst in the subsequent reaction steps[1)]. The phosphate ion directs a water molecule to attack the (*E*)-2-aminobut-2-enoate–coenzyme intermediate (**1**) to form the L-threonine–coenzyme intermediate[2)3)]. Without the phosphate ion, (*E*)-2-aminobut-2-enoate is released from **1** and hydrolyzed to 2-oxobutanoate as a side reaction. Thus, the phosphate ion is crucial for the reaction specificity of this enzyme. The phosphate ion is held by T88, N154, S155, R160, and N188, through hydrogen bonds and ionic interactions. In order to know the role of these residues, mutant enzymes in which these residues were replaced by Ala were investigated for their catalytic abilities and X-ray structures. R160A showed complete absence of the catalytic ability and distorted orientation of the phosphate ion, indicating the crucial role of this residue for the proper positioning of the phosphate ion. N154A, on the other hand, maintained the position and orientation of the phosphate ion essentially identical to the wild-type and other mutant enzymes, but its reaction specificity was reduced to 50%, that is, the enzyme catalyzed non-specifically the normal and side reactions. As the static structure was unable to explain the peculiar catalytic feature of N194A, we performed molecular dynamic simulations on the reaction intermediate **1** in the wild-type and mutant enzymes. The results showed that N194 makes hydrogen bonds to the two O atoms of the phosphate ion, thereby enabling the phosphate ion to position the nucleophile water molecule near C3 of **1** (Figure). In this way, we can see that the dynamic orientation of the phosphate ion, supported by N194, is important for the product-assisted catalysis.



1

1) Murakawa, T., Machida, Y., and Hayashi, H. (2011) *J. Biol. Chem.* **286**, 2774
2) Shoji, M. et al. *J. Am. Chem. Soc.* **136**, 4525
3) Ujiie, Y., et al. *J. Phys. Chem. B.* **121**, 5536