

## pH Dependency of the reaction catalyzed by branched-chain amino acid aminotransferase

(<sup>1</sup>Department of Chemistry, Osaka Medical College) ○Hideyuki Hayashi,<sup>1</sup>

**Keywords:** Pyridoxal Phosphate; Enzyme Catalysis; Reaction Mechanism, pH-dependency, Stopped-flow spectrophotometry, kinetics

Pyridoxal 5'-phosphate (PLP)-dependent enzymes are classified to more than five groups based on the fold type of the enzyme protein. Most of the aminotransferases belong to Fold-type I, whereas only D-amino acid aminotransferase and branched-chain amino acid aminotransferase (BCAT) have been known to belong to Fold-type IV. The most striking difference between them is the stereochemistry of the reaction: the 1,3-prototropic shift occurs at the *re*-face and *si*-face of the cofactor C4' in the Fold-type IV and Fold-type I aminotransferases, respectively. The proton-transfer steps in the Fold-type I aminotransferases, especially that of aspartate aminotransferase, have been well established. However, those in Fold-type IV aminotransferases are less characterized. As a first step to explore these steps, the pH dependency of the transient kinetic behavior of BCAT was studied.

On the reaction of BCAT with isoleucine, the absorbance at 415 nm, which is due to the absorption by the PLP–Lys159 Schiff base, decreased with a concomitant increase in the absorbance at 330 nm, reflecting the formation of the pyridoxamine 5'-phosphate form ( $E_M$ ) of BCAT. The spectral changes proceeded in a bi-exponential manner, and the apparent rate constant for the fast phase decreased, whereas that of the slow phase increased, with increasing pH.

These complex kinetic behaviors of BCAT can be interpreted by assuming the following scheme, in which the PLP form of BCAT exists in two forms  $E_L$  and  $E_LH^+$ , and the protonation of the enzyme affects the binding of the substrate, which exists both in the unprotonated form (S) and the zwitterionic form ( $SH^+$ ).

