บทที่ 4 สรุปและเสนอแนะ

In this study, we describe the kinetic analysis of OsBADH1 and OsBADH2 wild-type and mutant enzymes using Bet-ald and GAB-ald as substrates. The kinetic result indicated that the enzyme would prefer GAB-ald to Bet-ald, which is in agreement with previous studies (Bradbury *et al.*, 2008; Mitsuya *et al.*, 2009; Wongpanya *et al.*, 2011). Out of the six mutants (N164A, W172A, W172F for OsBADH1 and N162A, W170A, W170F for OsBADH2), only W172F of OsBADH1 and W170F of OsBADH2 mutants showed a higher catalytic efficiency towards GAB-ald, indicating that this position may be important for substrate specificity towards GAB-ald. In addition to the enzyme kinetics, the binding mode between the enzymes and substrates was carried out by molecular docking followed by MD simulations of the protein–ligand complexes of wild-type OsBADHs bound with Bet-ald and GAB-ald. It is noteworthy that this is the first report to show the structure of a protein–ligand complex of OsBADHs generated by MD. MD simulations suggested that GAB-ald forms hydrogen bonds with these residues, C296, E262, L263, and W461 in OsBADH1 and C294, E260, L261, and W459 in OsBADH2 better than Bet-ald. The decomposition energy was also carried out to determine the interaction energy of the important residues within a 5 Å radius that interact with the substrates. The decomposition energy revealed that W163, N164, Q294, C296 and F397 mainly interacted with Bet-ald in the OsBADH1-Bet-ald complex while E262, L263, C296 and W461 mainly interacted with GAB-ald in the OsBADH1-GAB-ald complex. In the case of the OsBADH2-Bet-ald complex, Y163, M167, W170, E260, S295 and C453 displayed strong interactions in which E260 exhibited the highest interaction with Bet-ald, suggesting that the negatively charged side chain of E260 interacts with the positively charged group of Bet-ald. For the OsBADH2-GAB-ald complex, E260, L261, C294 and W459 are shown to interact with GAB-ald, which is consistent with the hydrogen bonding analysis.

According to our data, the interactions of Bet-ald in the complex are varied in comparison with GAB-ald. This may be accounted for by a positive charge in the molecule and less hydrogen bond formation, leading to movement at various positions which can be observed in our MD result and the crystal structure of BADH in *E. coli* (Gruez *et al.*, 2004). In contrast, GAB-ald can form several hydrogen bonds, leading to a fixed position in the substrate binding site. One interesting point for the substrate recognition of OsBADHs is the difference in the amino acid residues position 290 of OsBADH1 (A290) and position 288 of OsBADH2 (W288). W288 in OsBADH1 interacts with Bet-ald through pi-electron interactions but not with GAB-ald while A290 in OsBADH1 cannot form any interaction with the substrates. In plant PsAMADHs, the position 288 has been proposed to be the key to the differences in substrate specificity in both PsAMADHs isoforms (F288 for PsAMADH1 and

W288 for PsAMADH2) (Tylichová *et al.*, 2010). Therefore, W288 is also the key residue in recognition and substrate specificity in OsBADH isoforms.

Additionally, the conformation of the catalytic residues, Cys and Glu was investigated in comparison to that of PaBADH (PDB codes 2WME) and ALDH2 (PDB codes 1O02). The result revealed that the catalytic Cys residue exists in the "resting" conformation in all structures whereas the catalytic Glu residue exists in the "outside" conformation except for Glu in OsBADH2-Bet-ald, which was found to adopt an "inside" conformation. It is suggested that the negatively charged side chain of catalytic Glu has strong electrostatic interactions with the positive quaternary ammonium of Bet-ald. This difference in the conformation of catalytic Glu residue of OsBADH1 and OsBADH2 may account for the substrate specificity between the two substrates and the enzymes.