Kultida Jiamsomboon 2012: Protein Engineering of Betaine Aldehyde Dehydrogenases from Rice (*Oryza sativa)* for Substrate Specificity. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Mr. Nonlawat Boonyalai, Ph.D. 151 pages.

Fragrance rice (*Oryza sativa)* contains two isoforms of BADH, named OsBADH1 and OsBADH2. OsBADH1 is implicated in acetaldehyde oxidation in rice plant peroxisomes, while the non-functional OsBADH2 is believed to be involved in the accumulation of 2-acetyl-1-pyrroline, the major compound of aroma in fragrance rice. In the present study, site-directed mutagenesis, molecular docking and molecular dynamics simulation studies were used to investigate the substrate specificity towards betaine aldehyde (Bet-ald) and γ-aminobutyraldehyde (GAB-ald). Consistent with our previous study, kinetics data indicated that the enzymes catalyze GAB-ald better than Bet-ald, and the OsBADH1 W172F and OsBADH2 W170F mutants displayed a higher catalytic efficiency towards GAB-ald than Bet-ald. Molecular docking analysis and molecular dynamics simulations for the first time provided models for aldehyde substrate-bound complexes of OsBADHs. The amino acid residues, E262, L263, C296 and W461 of OsBADH1 and E260, L261, C294 and W459 of OsBADH2 located within 5 Å of the OsBADH active site mainly interacted with GAB-ald forming strong hydrogen bonds in both OsBADH isoforms. Residues W163, N164, Q294, C296 and F397 of OsBADH1-Bet-ald and Y163, M167, W170, E260, S295 and C453 of OsBADH2-Bet-ald formed the main interaction sites while E260 of OsBADH2 showed an interaction energy of -14.21 kcal/mol. Unconserved A290 in OsBADH1 and W288 in OsBADH2 appeared to be important for substrate recognition similar to that observed in amino aldehyde dehydrogenase from *Pisum sativum* (PsAMADH). Overall, the results here help to explain how two homologous rice BADHs recognize the aldehyde substrate differently, which is a key property to their biological roles.

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