บทที่ 1 บทนำ

Fragrant rice *Oryza sativa* is one of the most important cereals in the world and is popular among consumers worldwide. The fragrance in rice is caused by several distinct aromatic volatile compounds such as 2-acetyl-1-pyrroline (2AP). Several reports suggested that betaine aldehyde dehydrogenase 2 (OsBADH2) might be involved in the characteristic aroma of fragrant rice since eight base pair deletion of *OsBADH2* at exon 7 leading to the generation of truncated non-functional protein increases 2AP production (Bradbury et. al, 2005). Rice (*Oryza sativa*) has two *BADH* homologs, *OsBADH1* and *OsBADH2* which are located on chromosome four and eight, respectively. BADH can catalyze the last step in the synthesis of osmoprotectant glycine betaine from betaine aldehyde (Bet-ald) (Figure 1). Since rice is a non-glycine betaine accumulating species, it has been proposed that OsBADHs have a role other than solely in the production of glycine betaine. OsBADH1 can oxidize acetaldehyde in peroxisome and can produce acetate which is then converted into acetyl-CoA (2). According to the pathway of 2AP synthesis, γ-aminobutyraldehyde (GAB-ald) can be converted into 2AP when OsBADH2 is inactive and to γ-aminobutyric acid (GABA) when OsBADH2 is active (3). Several experiments showed that both OsBADHs are more catalytically active towards GAB-ald than Bet-ald (Mitsuya et. al, 2009 and Bradbury et. al, 2008)

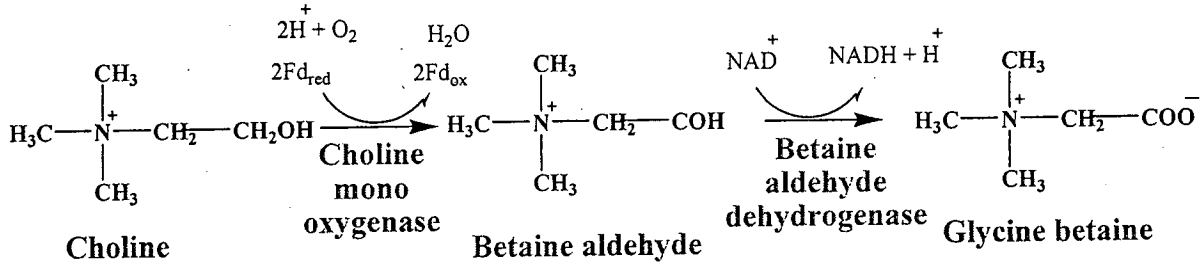

Figure 1 Pathway of choline conversion to glycine betaine.

At present, five crystal structures of BADHs and two crystal structures of plant AMADHs are available in the Protein Data Bank (PDB): BADH from cod liver in apoenzyme form and in complex with NAD^+ (PDB codes 1A4S and 1BPW, respectively) (Johansson *et al.*, 1998), BADH from *Escherichia coli* in apoenzyme form and in complex with NADH and Bet-ald (PDB codes 1WND and 1WNB, respectively) (Gruez *et al.*, 2004) and and BADH from *P. aeruginosa* (PaBADH) in complex with $NADP^+$ and glycerol (PDB code 2VE5) (González-Segura *et al.*, 2009) and two AMADH isoforms from *Pisum sativum* (PsAMADH1 and PsAMADH2) in complex with NAD^+ and glycerol (PDB codes 3IWK and 3IWJ, respectively) (Tylichová *et al.*, 2010). Most of the known structures of BADH are in tetrameric form, except for the plant AMADH that is dimeric. The subunit of BADH comprises a coenzyme binding domain, an

oligomerization domain and a catalytic domain. The catalytic triad of BADH from *P. sativum* contains C294, N162 and E260 which are conserved in BADH from different species (Johansson *et al.*, 1998; Gruez *et al.*, 2004; González-Segura *et al.*, 2009). In the catalytic cycle of BADH (as shown in Figure 2), the catalytic cysteine attacks the aldehyde substrate forming a thiohemiacetal intermediate whereas the glutamate involved in the proton relay system has been proposed to be the general base in the catalysis. The asparagine on the other hand has been implicated in stabilizing the thiohemiacetal intermediate forming the oxyanion hole (González-Segura *et al.*, 2009).

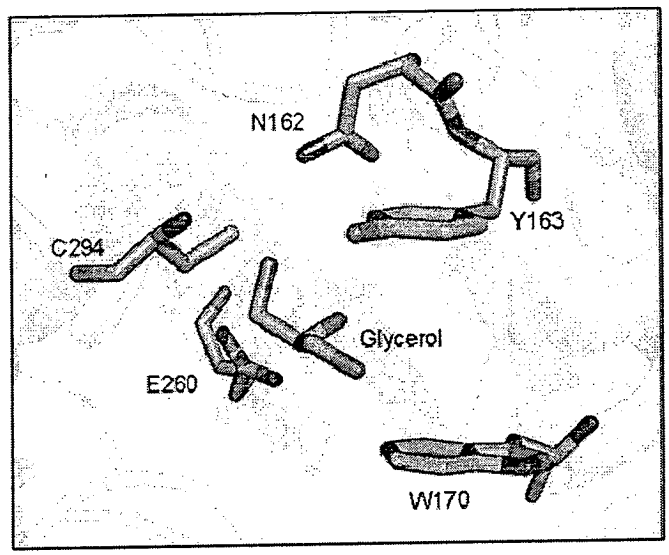

Figure. 2. Substrate binding pocket of PsAMADH1 from *Pisum sativum* (PDB code 3IWK). The amino acid residues are depicted in stick representation and colored by atoms (carbon, deepsalmon). Glycerol is shown in stick and colored by atoms (carbon, green). The red letter represents the catalytic triad residue of PsAMADH1 including C296, E262 and N164. Amino acid residues W170 and Y163 are involved in the substrate recognition.

Although both OsBADHs are very similar in amino acid sequence, their functions might be different. Therefore, it is of our interest to elucidate and understand the substrate specificity of both OsBADHs. In this report, site-directed mutagenesis of amino acids involved in catalysis and substrate recognition was carried out. Then the cofactor binding between the enzymes and NAD^+ was investigated to evaluate the folding of the enzymes. The substrate specificity of the enzymes towards Bet-ald and GAB-ald was also investigated.