

Napaporn Thamchuchaowarat 2011: Characterization of BADH2 Mutants from Rice (*Oryza sativa L.*).
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Betaine aldehyde dehydrogenase 2 (BADH2) is believed to be involved in an accumulation of 2-acetyl-1-pyrroline (2AP) in rice. The BADH gene located on chromosome eighth has been found in both fragrant and non-fragrant rice. The 8 bp deletion and 3 single nucleotide polymorphisms (SNPs) at exon 7 of BADH2 gene resulted in loss of its function, which is suggested to be associated with 2AP accumulation in aromatic rice. In this study, BADH2 mutations were constructed by site-directed mutagenesis consisting of tyrosine insertion at residue 420 (Y420 insertion) and alanine replacement at amino acid residues 162, 260 and 294, respectively. Moreover, random mutagenesis was also carried out by exposing to ultraviolet at 10, 30 seconds and 1 minute. The result showed that all mutants were successfully constructed in pET28b plasmid following by transformation into bacterial *E. coli*. The recombinant proteins were then over produced and purified by Ni^{+2} -NTA affinity column chromatography. Then binding affinity between the purified recombinant proteins and cofactors, NAD^+ and NADH, were examined. The results indicated that mutation of Y420, N162A and E260A did not show different binding affinity toward both NAD^+ and NADH comparing to the wildtype. However, C294A mutant resulted in decrease of binding affinity toward NAD^+ that contrasts to toward NADH with increasing. The kinetic study (K_m , k_{cat} and k_{cat}/K_m) of the BADH2 mutants toward aminobutyraldehyde (AB-ald) and Betaine aldehyde (Bet-ald) were then performed. The results indicated that wild type BADH2 showed higher activities towards both substrates than all BADH2 mutants. In addition, Thin Layer Chromatography (TLC) was also performed to detect γ -aminobutyric acid (GABA), an expected product of the enzyme when AB-ald is used as a substrate. The GABA could be only observed in the reaction of wild type BADH2, Y420 and N162A with AB-ald as the substrate. Nevertheless, the random mutagenesis was not successfully constructed.

Student's signature

Thesis Advisor's signature