# Structure-guided mutagenesis for increase of enzyme affinity of *Pseudomonas putida* formaldehyde dehydrogenase

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Formaldehyde dehydrogenase (FDH, EC 1.2.1.46) converts the reaction of the formaldehyde into formate by reduction of the NAD+ to NADH. Formaldehyde is considered as an environmental toxin using in the production of industrial particle board and coatings. Volatile formaldehyde is found in some manufactured wood and household products which is able to be readily absorbed from the respiratory and GI tracts into a human body. Exposure to high amounts of formaldehyde is reported that is capable of causing the cancer of the nasopharynx and leukemia as well as sinonasal cancer; hence, monitoring the environmental formaldehyde becomes an interesting issue in public health. To date, there are four assays that use to measure the concentration of the formaldehyde: (1) Nash assay ;(2) 2,4-DNPH spectrometric assay;(3) HPLC quantification; and (4) GFP biosensor methods. Among these methods, the FDH enzyme plays a crucial role that is the limited-step for the reaction of the above method. However, current methods suffer from low sensitivity, complex workflows, or require expensive analytical equipment which results from the low kinetic parameters of that protein. Therefore, we plan to use structure-based protein engineering techniques to improve enzyme activity. In this study, we cloned an FDH plasmid from *P. putida* and followed overexpressed the recombinant *p*FDH protein. The crystal is diffracted and solved by the molecular replacement method. We identify the binding residues interacting

with the cofactor (NAD+) that are Gly47, Ser48, His51, Val197, Asp217, Arg222, Arg267, His269, Pro299, Thr338, and Gly336. In addition, there are two Zinc atoms chelated by the Cys46, His67, and Cys174 residues, coordinated with a water molecule, complete the tetrahedral coordination. A comparison of the active site revealed that the PFDH shared a catalytic mechanism similar to that of the liver alcohol dehydrogenase (LADH). A substrate-entrapped ternary complex as well as site-directed mutagenesis will further realize the substrate location and help us to increase the enzyme activity.