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Expression, purification and characterization of recombinant L-phenylalanine dehydrogenase

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L-Phenylalanine dehydrogenase (L-phenylalanine: NAD+oxido-reductase, deaminating (EC 1.4.1.20), PheDH), catalyzes the reversible NAD+-dependent oxidative deamination of L-Phe to phenylpyruvate [1]. Even though PheDH is an acknowledged industrial biocatalyst, it is generally known as key component in diagnostic kits and biosensors for detection and disease monitoring of phenylketonuria (PKU), an autosomal recessive inborn error of L-Phe metabolism. Phenylketonuria is characterized by increased levels of L-Phe in biological fluids, which accumulates in central nervous system (CNS) where its toxic effect is manifested. Incidence of classic PKU (L-Phe blood level $\geq$ 1.2 mmol/L) in Republic of Serbia varies between 1:18732 and 1: 39338 [2]. Untreated PKU leads to severe and irreversible intellectual impairment, neurological and behavioral problems. Timely diagnosis, a life long special diet with controlled L-Phe intake and regular monthly monitoring of L-Phe level, are crucial for disease control and prevention of CNS damage. From 1966 a mandatory PKU screening for all neonates is introduced in Serbia at the Institute of Maternal and Child Healthcare „Dr Vukan Čupić" in Belgrade. Recombinant PheDH of bacterial origin was successfully expressed in BL21 strain and characterized. Enzyme is purified to homogeneity by immobilized metal affinity chromatography to the yield of 2.4 mg. Molecular mass, determined by SDS PAGE, is approximately 40 kDa. Maximum catalytic activity in oxidative deamination of L-Phe was exhibited at 37°C and pH 10. Recombinant enzyme was stable in wide pH range of 5.5-11.5, and in temperature range of 25-37°C. Km and V_{max} for L-Phe were 3.3 mmol/L and 0.18 μmol/min, respectively. Furthermore, a standard curve for L-Phe determination with recombinant PheDH showed linearity and high goodness of fit (R^2=0.9963) for the L-Phe concentrations up to 1.5 mmol/L, which encompass both physiological and pathological values of the analyte. These preliminary results indicate that PheDH from our study is a promising candidate for further development of recombinant PheDH assays with real biological samples and PKU biosensor design.

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