Cloning and expression of human tyrosine hydroxylase gene in Yarrowia lipolytica and analysis of enzyme activities.

ABSTRACT

L-DOPA is a medicine to prevent Parkinson's disease from getting worse, therefore the para-hydroxybenzoate hydroxylase (HBHD) from Pseudomonas aeruginosa PAO1 was chosen as a substrate for tyrosine hydroxylase to transform L-tyrosine into L-DOPA to achieve the treatment. The first part of the Pseudomonas aeruginosa PAO1 wild-type gene was used as the template to amplify the hbhd gene by PCR. Cloning of the hbhd gene into Escherichia coli and expression of enzymatic activities were performed. The second part of the sequence from the wild-type HBHD was used to construct two single mutants of Y310F and Y371F. The length of the hbhd-mut gene was 1,185 bp. The translation product of HBHD was 45 kDa of molecular weight. The P. aeruginosa hbhd-mut (Y310F, Y371F) gene was cloned in pQE30 expression vector and transformed to E. coli Nova Blue, respectively. Finally, Ni-NTA column was used to purify the enzyme. With tyrosine as a substrate, 100 mM pH 7.0 phosphate buffer, 10 mM Ascorbic acid, 830 μM ferrous sulfate buffer, 750 mM 1.5 M 2-ME 6MPPH4 and catalase reaction at 37 ℃, no activity of Y371F was measured by high performance liquid chromatography. Elder said, the site of tyrosine 371 is the most important amino acid. It is a determinant of tyrosine substrate affinity. If we change tyrosine 371 to phenylalanine 371, it won't have activity to substrate tyrosine. Medical treatment for Parkinson's disease (PD) is using the chemical synthesis L-dopa, however, lead to many side effects. Many scientists are focusing on gene therapy and stem cell research to reconstruct the amount of aromatic amino acid decarboxylase (AADC) in the brain or rebuild the substantia nigra, but these therapies are still on the animal experimental phase. In this transitional period, natural biosynthetic L-dopa becomes a researchable direction. Phenylalanine hydroxylase converts phenylalanine to tyrosine, and tyrosine hydroxylase keeps transferring to L-dopa. From BC104967 homo sapiens tyrosine hydroxylase results showed that this protein ORF (open reading frame) is full 1,494 bp, encoding 498 amino acids with a protein molecular weight of about 55 kDa, after glycosylation to 65 KDa, known as a secreted extracellular enzyme with the optimum pH of 7.0 and the optimum temperature at 37 ℃. After analysis of enzyme kinetic parameters, the Lineweaver-Burk plot of the calculated results from the HTH-SC at pH 7.0 and 37 ℃ for 20 minutes, the reaction was stopped by 100 ℃ water bath for 30 minutes. The Km was 2200 μM, Vmax was 111.1 μM/min, Kcat was 9.87 × 10^-3 s^-1, kcat / km was 4.487 × 10^-6 μM/s^-1.

Keywords: tyrosine hydroxylase, Parkinson's disease, L-DOPA
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