Cloning and expression of human tyrosine hydroxylase gene in yarrowia lipolytica and analisis of enz

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ABSTRACT

L-DOPA is a medicine to prevent Parkinson ’ s disease from getting worse, therefore para-hydroxybenzoate hydroxylase (HBHD) from Pseudomonas aeruginosa PAO1 was chosen to substrate for tyrosine hydroxylase to transform L-tyrosine become L-DOPA to achieve the treatment. The first part from Pseudomonas aeruginosa PAO1 wild-type gene was used as template to amplify hbhd gene by PCR. Cloning of hbhd gene into Escherichia coli and expression of enzymatic activities were performed. The second part sequence from the wild-type HBHD was used to construct two single mutants of Y310F and Y371F. The length of hbhd-mut performed open reading frame (ORF) was 1,185 bp. The translation HBHD product was 45 kDa of molecular weight. The P. aeruginosa hbhd – mut (Y310F、Y371F)gene was cloned in pQE30 expression vector and trasformated to E. coli Nova Blue, respectively. Fianally, Ni-NTA column was used to purify the enzyme. With the tyrosine as subtrate, 100 mM pH 7.0 phosphate buffer, 10 mM Ascrobic acid, 830 μM ferrous sulfate buffer, 750 mM 1.5 M 2-ME 6MPH4 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 has 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 transition 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) full 1,494 bp, encoded the 498 amino acids with protein molecular weight about 55 kDa, after glycosylate to 65 KDa, known as a secreted extracellular enzymes with the optimum pH of 7.0, and the optimum temperature 37 ℃. After analysis of enzyme kinetic parameters, the Lineweaver-Burk plot of the calculated results from the HTH-SC at pH 7.0, 37 ℃ for 20 minutes, the reaction to 100 ℃ water bath for 30 minutes to stop reaction conditions, 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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