# Molecular Cloning of Aromatic L-Amino Acid Decarboxylase Gene from Xanthobacter autotrophicus Py2, and Enzyme Characteri


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**ABSTRACT**

Aromatic L-amino acid decarboxylase ( AADC; EC 4.1.1.28 ) catalyses the conversion of L-3,4-dihydroxyphenylalanine ( Levodopa; L-DOPA ) to dopamine. Clinically, medical treatment for parkinson ’ s disease ( PD ) is using the chemically synthetic L-DOPA and dopamine, but that has side effects. So searching for the natural source is becoming more significant and necessary. In our study, AADC gene in the chromosome of Xanthobacter autotrophicus Py2 with the size of 1,425 bp was significantly ligated with pQE30 expression vector and then transformed into E. coli Nova Blue for overexpression. The gene expressed the peptide chain AADC with the molecular weight of 51 kDa. This protein was isolated by 6 His-tag and assayed by TNB ( 2, 4, 6-Trinitrobenzene 1-sulfonic acid ) reagent method in finding activity of the enzyme. After initial incubation of the AADC enzyme at 42 ℃ with 2.5 mM L-DOPA for 15 mins, this solution was added with TNB reagent for further 15 mins. The tube added with toluene was vortexed and centrifuged to separate the top layer of TNP-dopamine ( Trinitrophenyl dopamine ) from the bottom layer of TNP-DOPA ( Trinitrophenyl DOPA ) and then the concentration of TNP-dopamine was determined spectrophotometerically. Following the course, we selected three clones with lower level activity have the same mutation with Q219H from 40 screened clones. In the first screening, we didn ’ t find higher activity so that we changed medium to BHI ( Brain heart infusion ) because BHI is more nutrition for screening higher activity. In the second screening of 40 clones, we found two clones with L325Q and L400R for lower activity, but R406H for higher activity. By the way, we found L400R have growth retardation. Standard curve was made by dopamine reacting with TNB reagent derivation at a high rating of R2 = 0.999 for quantitative determination of AADC enzyme activity. Another standard curve is BSA with Bio-Rad assay for protein quantity. The wild type of AADC enzyme had optimal reaction under 65 mM boric acid pH 8.0 at 42 ℃ which was catalyzing 2.5 mM L-DOPA in 15 mins. The result of specific activity, Km = 209.64 ( μM ), Vmax = 37.17 ( μM / min ), Kcat = 3.8 x 10-3 ( S-1 ) and Kcat / Km = 1.8 x 10-5 were determined . Q219H and L325Q mutants were compared with wild type and resulted in decrease of ( Kcat / Km ) in activity by more than 2.4 and 18 fold for equal quantitative enzyme, respectively. We also added PLP ( Pyridoxal-5`-phosph- ate ) of AADC cofactor that can activate substrate binding more stronger even if original AADC has high activity. The enzymatic activity was increased in 2 fold for purified wild type AADC. However, R406H has lower Km value. These results indicate that both the chemical properties and the shape of these residues are essential for substrate binding in the enzyme catalysis. In addition, the active site of pig kidney AADC was known of H192Q, T246A and K303A, so as found residues at H180, T238 and K295 in the sequence of X. autotrophicus AADC. A null mutant T238A was made, however, its activity was not detectable. Besides, we tried to recovery protein again for mutant L400R using more than one fold LB as culture medium. L400R mutant enzyme was purified, however, its activity was not detectable. We observed modeling of AADC of pig kidney active center is similar to X. autotrophicus AADC. According to modeling of structure from pig kidney active center, corresponding L400R was found very close to the active center. Other mutant AADC, Q219H, L325Q and R406H were on the peripheral of entrance in the active center that might change the original amino acid and influenced substrate binding for AADC activity. In this study, data suggest L400R and T238A are catalytically important active center of X. autotrophicus AADC.



Keywords : Aromatic L-amino acid decarboxylase、L-DOPA、Dopamine、Parkinson ’ s disease、Xanthobacter autotrophicus Py2、Specific activity、active site、active center


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