Characterization of glutamate decarboxylase and industrial production of GABA

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ABSTRACT

Glutamate Decarboxylase 3 (GAD3) can remove carboxyl group and catalyze glutamate into GABA (γ - aminobutyric acid). Glutamate was used to control nerve impulse once changed into GABA which will inhibit nerve impulse. Human brain lacking GAD can't produce GABA normally, and cause hypertension, epilepsy, manic-depressive psychosis, psychomania, insomnia, and end shaking. This shows the important role of GAD in neuron metabolism, and the essential need in the proteomics are of health care system. In this study, the characteristics of the GAD 3 enzyme were analyzed by pH, ion requirements, substrate inhibition, product inhibition , substrate specificity, structure analysis and activity site analysis. These were helpful to promote mass production of GABA in the fermentation tank, and hoping to help mental patients. On the other hand, the random mutation of GAD3 gene was performed by Error prone PCR. However, mutant analysis of GAD3 random mutation was used by the method of TNB with a small amount of crude enzyme expressed after induction . The clones of low activity with L 1, L 24,and high activity with H 20, H 38, H 1, H 26, H 39 and H 46 Were obtined by the method of TNB and sequenced to find out the mutation points. L 1 and L 24 have the same mutation of D 296 A. H 20 has two mutation points of G 297 A and S 491 P. H 38 has a single mutation points of G 297 A. H 1 has two mutation points of V 78 A and F 271 S. H 26 has only one mutation of F 271 S. H 39 has five mutation points of R 50 L, V 78 A, F 271 S, I 375 V and G 515 V. While H 46 has six mutation points of T 16 S, V 78 A, D 128 G, F 271 S , S 471 T and W 423 L. However D 296 and S 491 were found to be the active site suggested by the previous comparison. Using analysis of SDS-PAGE,purified protein of GAD 3 and rest of GAD 3 mutants were found the protein molecular weight of 56 kDa. Y. lipolytica culture encoding glutamate decarboxylase 3 is being actively cooperate with the Vedan in a small 1.2 L fermentation to add 20 μM Pyridoxal phosphate and 20 μM Ferrous fumarate as cofactors. HPLC analysis of the GAD 3 activing has been accurately calculated 5 mM MSG disappeared with the ratio of 61% in 24 hrs,however in the biosynthesis of GABA on the conversion rate of 85 % in 24 hrs. The industrial production of GABA in the 5 L fermenter evaluation was, constantly improved to the conversion rate of 100 % withs 1.42 % solid content analyzed by HPLC. While IN the 1000 L fermentor control conditions were determined to be pH: 5.4 ~ 5.3 and 0.05 ~ 0.018 (%) of glucose concentration. Vedan company recently want to produce a 10 Kg of GABA which can enter in the animal and human trials for health food.

Keywords : glutamic acid decarboxylase enzyme、glutamic acid、amino butyric acid、activity analysis、industry production 、system、after

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