Studies on Bacterial β-Asparyl amidohydrolases

Abstract
Amidohydrolase, is an enzyme with a diverse superfamilies which asparaginase (EC 3.5.1.1) and glutaminase (EC 3.5.1.2) belong to. This thesis describes investigations on the exploration of new sources of asparaginase, so-called β-aspartylamidohydrolase. The study also includes the optimization of enzyme production and further extended application of β-aspartylamidohydrolase for the synthesis of peptide compounds and their bioactivity. The exploration bacterial β-aspartylamidohydrolase and plant type β-aspartylamidohydrolase resulted in that all investigated bacteria and cyanobacteria were able to produce intracellular L-β-aspartylamidohydrolase and that Arthrospira (Spirulina) platensis was the best producer. The production optimization through modifications of medium and culture environment exhibited two times higher (0.275 ± 0.005 U) than that normal culture condition (0.127 ± 0.107 U). The exploration on β-aspartylamidohydrolase from Pseudomonas syringae revealed that a novel enzyme, β-aspartyl-γ-glutamyl transferase (BAT-GGT) was found. This enzyme was purified to 13.14 fold with a specific activity of 0.92 U/mg. It is a dimeric form with 37 kDa and 21 kDa as large and small subunits, respectively. This enzyme acts on both hydrolysis of L-glutamine and L-asparagine and transfer its γ-glutamyl and β-aspartyl moieties. It can be used for synthesis of peptides, β-aspartylhydroxamate. β-aspartylhydroxamate exhibited an antioxidant and antibacterial activities. In conclusion, we have been reported a new source of food-grade bacterial and plat-type β-aspartylamidohydrolase with its production optimization. A novel enzyme a name, β-aspartyl-γ-glutamyl transferase (BAT-GGT) was proposed. It could be used to synthesize β-aspartylhydroxamate with several bioactivities.

Objectives
The main objective of this study was to explore, and to characterize the new potential β-aspartyl amidohydrolase for application in food, fine chemical industry or medicines.

To achieve the objective, this study was designed into several chapters and their detail aims as follow, to obtain generally recognized as safe microorganism (GRAS), the present study examined fish-fermented foods as a source of
L-ASNase microorganism, and continued to explore GRAS cyanobacteria for L-asparaginase production. After the best producer of L-asparaginase was achieved, optimization using both medium and culture modification, and cloning of their gene were performed. The other objective was exploration of β-aspartylamidohydrolases which catalyze transfer reaction of β-aspartyl moiety, that their product was expected to be applied for the synthesis of chemical compounds. This synthesized-compound was investigated for their bioactivities.

Summary of chapters

Chapter I, Screening of Selected L-Asparaginase Producing Bacteria and Cyanobacteria.

In section one, the exploration and screening of potentially generally recognized as safe microorganism (GRAS) as a source of L-ASNase was conducted. The two Indonesian fermented foods, namely Terasi and Jambal Roti and well-known food grade cyanobacteria have been explored as sources of intracellular L-asparaginase and plant-type asparaginase bacteria, respectively. In both fermented foods, abundant bacteria which have intracellular L-asparaginase have been isolated. After 16S rDNA analysis, the best L-asparaginase producer which was previously identified as strain T4 is Bacillus cereus strain T4.

In section two, we screened the new source of well-known food grade and recognized as safe cyanobacteria called, Anabaena sp. and Arthospira (Spirulina) platensis which represents plant-type asparaginase. While the best-producing L-asparagine in Cyanopbacteria, is Arthospira (Spirulina) platensis. The production of L-asparaginase is somewhat low; hence in the next chapter we optimize the production through the modification of the medium and culture condition, and overexpression throughout cloning system.

Chapter II, Production of L-Asparaginase from Arthospira (Spirulina) platensis NIES 39.

The best producer of L-asparaginase will be optimized in regard to its production. In section one, the medium and culture condition of A. platensis was modified using statistic methodology. A statistic method called Taguchi experimental design was applied to understand the role of the medium composition and culture condition of A. platensis for the production of L-asparaginase. L-Asparaginase production increased to two times higher than that of standard culture condition. It is revealed that depletion of N and Fe negatively affected ASNase production. NaCl and temperature shock in the dark
condition have contributed to ASNase production because of energy generation, throughout cyanophycin degradation.

In section two, the cloning of A. platensis gene encoding L-ASNase into Escherichia coli was carried out to further enhance the production of L-asparaginase. L-Asparaginase gene derived from A. platensis was overexpressed into E. coli system. Although gene cloning was successfully conducted, the expression of the gene resulted in insoluble aggregates protein.


In our exploration of GGT of Pseudomonas syringae, we have been found unique functional properties of this enzyme. The recombinant enzyme was produced in E. coli cells harboring from pET22b containing putative γ-glutamyltranferase (PsGGT) gene of Pseudomonas syringae. It was purified and its biochemical properties were characterized. The PsGGT was dimer with 37 kDa and 21 kDa of large and small subunits respectively. The striking functional property of this enzyme was that this enzyme is capable of not only hydrolyzing glutamine and asparagine but also transferring its γ-glutamyl and β-aspartyl moieties to hydroxylamine. The β-aspartyl transferring activity was higher compared to γ-glutamyl. We therefore propose this enzyme as β-aspartyl-γ-glutamyl transferase (BAT-GGT).

Chapter IV, Biosynthesis of β-Aspartyl Hydroxamate Using BAT-GGT and Preliminary Investigation of Its Bioactivities.

An optimal reaction condition for production of β-aspartylhydroxamate was achieved. β-Aspartylhydroxamate, as a product of transferring reaction of L-asparagine to hydroxylamine was optimally produced under temperature of 60°C, pH 6 and in the concentration of 80 mM and 40 mM of L-aspargine and hydroxylamine, respectively. The preliminary investigation of BAH bioactivity revealed that this compound has antioxidant activity as well as antibacterial activity against B. cereus, B. pumilus and E. coli.

Conclusion

This thesis described the finding of a new food-grade source, A. platensis, of L-asparaginase along with its optimized culture condition for enhancing enzyme production. On the top of that, the effort to enhance the production by using cloning technology was not satisfied enough due to the formed aggregates protein. On the other hand, we introduced a novel enzyme from P. syringae which is able
to transfer both γ-glutamyl and β-aspartyl moieties. Considering that BAT-GGT is a unique novel enzyme, in the next study, modifying cloning system and/or gene engineering for improvement of enzyme production must be explored. BAT-GGT could be used to synthesize antioxidant and antimicrobial, β-aspartylhydroxamate. This study is the first report on the production of β-aspartylhydroxamate through biosynthesis by using novel enzyme, BAT-GGT.

References


