【Grant-in-Aid for Scientific Research (S)】

Biological Sciences (Agricultural Sciences)

Title of Project : Development of soluble expression technology and utilization of enzymes from plants and animals

Yasuhisa Asano
(Toyama Prefectural University, Faculty of Engineering, Professor)

Research Project Number : 17H06169 Researcher Number : 00222589
Research Area : Enzyme Engineering
Keyword : Gene Expression, Enzyme, Soluble Expression,

【Purpose and Background of the Research】

We named the microbial metabolic pathway of aldoxime and nitriles as the "aldoxime-nitrile pathway", and further proving its occurrence in plants and an animal, by clarifying the structure and functions of various enzymes involved in the pathway. Furthermore, we characterized a new hydroxynitrile lyase (HNL) from an arthropod, millipede. The results of the transcriptomic analyses indicated that the millipede is full of proteins with new structures.

On the other hand, followed by our discovery of a mutation-directed soluble expression in *Escherichia coli* of the gene for a plant HNL, we are discovering that the solubilities are caused by structural alterations found in specific positions of the proteins.

In this research, we will solve two major inter-related problems in enzyme engineering. One is the improvement of the heterologous expression in the soluble form of enzymes of plant and animal origins by mutation of their genes, according to the "α-helix rule" and by a program "INTMSAlign-HiSol". Another is discovery and assignment of the enzymes responsible for cyanide metabolism in the millipede by the genome analyses.

【Research Methods】

By random mutagenesis and the statistical analyses of the mutation sites of the resulting soluble mutations in proteins, we will discover rules among the mutation sites causing the soluble expression. We will then predict how to mutate the enzyme for soluble expression according to the "α -helix rule" and with an aid of a program "INTMSAlign- HiSol". With these methods, it will become possible for many proteins from plants and animals to be expressed in soluble forms in *E. coli*. These technologies will be utilized in the research for the metabolism and enzymology of higher organisms.

We will next search for enzymes participating in the aldoxime-nitrile pathway of the millipede, by its transcriptomic analyses. The reaction mechanism of the millipede HNL will be clarified by studying the relationship between the activity and the structures of the mutants by X-ray crystallography.

【Expected Research Achievements and Scientific Significance】

This research is challenging because there has not been a systematic analyses of the heterologous expression of proteins in soluble forms, by discovering the relationship between the primary structures and how they are expressed.

We will clarify the millipede genes for cyanide and the primary metabolism. It is also challenging to assign the genes of the millipede enzymes by our soluble expression technology.

【Publications Relevant to the Project】

・Y. Asano, M. Dadashipour, M. Yamazaki, N. Doi, and H. Komeda, Functional expression of a plant hydroxynitrile lyase in *Escherichia coli* by directed evolution: Creation and characterization of highly *in vivo* soluble mutants, *Protein Engineering Design and Selection*, **24 (8)**, 607-616 (2011).
・S. Nakano, and Y. Asano, Protein evolution analysis of *S*-hydroxynitrile lyase by complete sequence design utilizing the INTMSAlign software, *Scientific Reports*, **5**, 8193 (2015).

【Term of Project】 FY2017-2021

【Budget Allocation】 157,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.pu-toyama.ac.jp/BR/asano/homepage.html
http://www.jst.go.jp/erato/asano/index.html