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Study on Expression of Human Acetyl-CoA Carboxylase 2 and Malonyl-CoA Decarboxylase Using Silkworm-based BmNPV Bacmid Expression System and their Functional Analysis

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

専　　攻：バイオサイエンス　　氏　名：　黄　寅旭
Course：　Bioscience　　Name：　InWook Hwang

論文題目：
Title of　Thesis：
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論文要旨：
Abstract：

Biotin-dependent human acetyl-CoA carboxylases (ACCs) play important roles in homeostatic lipid metabolism. By securing the post-translational biotinylation, ACCs perform its coordinated catalytic function allosterically regulated by several factors including phosphorylation/dephosphorylation and citrate. Due to the patho-physiological relevance of ACCs in lipid metabolic syndrome, the production of authentic recombinant ACCs is an emerging task to provide a reliable information and tool for drug discovery efforts by unmasking their molecular functions.

In this thesis, whether the human ACC2 (hACC2), an isoform of ACC expressed using silkworm BmNPV bacmid system, equips with proper post-translational modifications to carry out catalytic functions as silkworm harbors inherent post-translational modification machinery, was investigated. Purified hACC2 possessed biotinylation probed by biotin-specific streptavidin and biotin antibodies. In addition, phosphorylated hACC2 displayed limited catalytic activity whereas dephosphorylated hACC2 revealed 2-fold enhanced enzymatic activity. Moreover, hACC2 polymerization, analyzed by native page gel analysis and atomic force microscopy imaging, was allosterically regulated by citrate, and phosphorylation/dephosphorylation regulated citrate-induced hACC2 polymerization process.

Decarboxylation of malonyl-CoA to acetyl-CoA by malonyl-CoA decarboxylase (MCD) is an important reaction in the regulation of fatty acid metabolism. This regulation of MCD is affected by phosphorylation/dephosphorylation. Although MCD has several phosphorylation sites, whether it is inhibited or activated by phosphorylation has been not investigated. Therefore, human MCD (hMCD) without N-terminal mitochondria targeting sequence (1-39 amino acids) was over-expressed using silkworm BmNPV bacmid expression system and purified. It was proved by western blot using anti-phophoserine antibody, and native PAGE that hMCD purified silkworm fat body and pupae was phosphorylated and polymerized. The specific activities of hMCD purified silkworm fat body and pupae were 60 and 48 nmol/mg/min, respectively. From LC-MS/MS experiment, Ser204 and Tyr405 might be phosphorylated. To make clarify and analyze the phosphorylation at each site the site-directed mutagenesis was performed. S204G and Y405F mutated proteins purified from silkworm fat body and pupa were found to be phosphorylated, but the level of phosphorylation was decreased in each mutant compared to that of wild type hMCD. The specific activities of S204G and Y405F purified from silkworm fat body were 30 and 34 nmol/mg/min and the specific activities of each mutant were lower by 40–50% than that of hMCD. The specific activity of each mutant purified from pupae was also lower by 35–50% lower than that of hMCD. Moreover, dephosphorylation by mutation leads to slightly attenuated degree of polymerization. These results suggest that dephosphorylation and polymerization of hMCD may be connected with its function. This research demonstrates that silkworm BmNPV bacmid expression system permits large potential value in recombinant eukaryotic protein production with proper post-translational modification such as phosphorylation for functional analysis.

The study might be a helpful guide for medical science or medicine industry to develop the inhibitors. Moreover, the silkworm-based BmNPV bacmid system would provide a reliable eukaryotic protein production platform for structural, functional analysis and therapeutic drug discovery applications implementing suitable post-translational biotinylation and phosphorylation.