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Proteome Analysis of *Escherichia coli* K-12 Defective in F1-ATPase Activity

Atsushi Yokota
Laboratory of Microbial Resources and Ecology, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Introduction

For the improvement of fermentation production, emphasis must be put on the enhancement of sugar metabolism of the producer. Our works (1-4) have demonstrated that the introduction of a defect in F1-ATPase activity enhanced glycolytic activity of *Escherichia coli*, which resulted in the hyper production of pyruvic acid. This seems to be the first successful example of the manipulation of energy metabolism for strain improvement.

The above positive effects of the mutation in energy metabolism were considered to be the results of the impaired energy production through oxidative phosphorylation with a defective F1-ATPase. The mechanism underlying this phenomenon seemed to be allosteric activation of the key enzymes of the glycolytic pathway by the decrease in the ATP level of the cells. However, it was revealed in later investigation that the cell composition has been changed qualitatively in the mutant as compared to the parent. Namely, the mutant cells prepared by a glucose-limited chemostat culture in minimal medium showed increases in glycolytic enzyme activities, respiratory enzyme activities, and decreases in TCA cycle enzyme activities. These findings suggested that the enhancement of glycolytic activity cannot be explained simply by the allosteric activation, but the observed qualitative changes in the cell composition must be involved in this event as well.

So far these qualitative changes associated with the F1-ATPase defect have not been reported in detail. Therefore, the clarification of these changes seems to contribute to understand not only the regulation of glycolysis in *E. coli*, but more general issue of how mutation in energy metabolism can affect the gene expression and physiology of the microbial cells. In this study, proteome analyses of the parent and F1-ATPase defective mutant were conducted to investigate the effect of F1-ATPase mutation on the composition of the expressed cell proteins.

Materials and Methods

As the wild type strain of *E. coli* K-12, strain W1485 was used. Strain HBA-1, an F1-ATPase defective mutant, was constructed by P1 transduction of a defective a subunit gene of F1-ATPase, *atpA401*, into strain W1485. Proteome analysis was carried out by two-dimentional (2D) gel electrophoresis of the cells prepared in glucose limited-chemostat cultures using modified M9 minimal medium. The protein spots appeared on the 2D gels were digested with trypsin, and analyzed by the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. For the identification of the protein spot, the obtained combination of the mass numbers of the peptide fragments was queried for the protein database SWISS-PROT.

Results and Discussion

Comparison of the 2D gels of both strains revealed that about 50 spots gave different density, and most of them were identified. In strain HBA-1 increases in the expression levels of several glycolytic enzymes including those of pyruvate dehydrogenase complex, and decreases in the expression levels of most of the TCA cycle enzymes were observed as compared to the wild type strain. These changes were in good agreement with the previously measured enzyme activities. However, it was not possible to detect protein spots of respiratory chain components that showed relatively large increases in enzyme activities in strain HBA-1. Thus, improvement of the solubilization conditions for membrane proteins is necessary to clarify the changes in expression levels of these proteins. Besides these findings, the current analysis revealed the changes in the protein levels of two important enzymes involved in the conversion of acetyl-CoA to acetate, *i. e.*, phosphotransacetylase and acetate kinase. These two enzymes were undetectable in 2D gel of wild-type strain but clearly appeared in that of strain HBA-1. These changes were interpreted to take place to compensate the shortage of energy supply in strain HBA-1, because acetate kinse reaction yields ATP by substrate-level phosphorylation.

The mechanism(s) underlying these changes are not clear. The regulator for glycolytic enzyme expression, CsrA, which can activate several glycolytic enzyme expressions, has been found (5). Most of the TCA cycle enzymes are under *arc* network, and are repressed by ArcA under anaerobic conditions. However, the involvement of ArcA seems not possible because cultures were conducted aerobically in the current experiments. The two enzymes involved in the conversion of acetyl-CoA to acetate are reported to be constitutive, and induced 2 to 3-fold under anaerobic conditions. Therefore, the results obtained in the current study are not explained readily by the previously found regulatory systems for gene expression, suggesting the presence of unknown regulatory system(s) for gene expression in response to the energy status of microbial cells.

References

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