

Towards modeling and simulation of Glycolysis and TCA cycle.

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Quantitative analysis of cellular enzymes is one of the fundamental data not only for modeling and simulation of metabolic network but for understanding of physiological network of a cell. To perform this, we selected enzymes in Glycolysis and TCA cycle pathways as the first targets to analyze quantitatively the whole activity including their transcription, translation and metabolic substrates concentration.

To quantify cellular concentration of each enzyme, we first tried to purify all of the enzymes using ASKA library (Kitagawa et al. 2005) which is plasmid clone library fused with His tag at their N terminus of all of the predicted ORF of *E. coli* and applied for antibody production for Western analysis. At the same time, we also develop strains, which are chromosomal fusion of all of the target enzyme genes with GFP reporter gene.

Out of 69 enzymes, 37 antibodies had qualities for Western analysis to quantify the target enzymes.

Using these antibodies, we performed Western analysis to trace the dynamic changes of enzymes concentration of Glycolysis and TCA at every 10 min during exponential growing phase and at every 1 hr during stationary phase in MOPS medium supplemented with 0.4% glucose. Also, we carefully compared the GFP fluorescence to the results using the fusion strains to the results from Western analysis for reliability of quantification by fluorescence.

Simultaneously, those enzymes were measured their transcriptional level by Real Time PCR from the same culture measured by Western analysis.

Finally, we measured the metabolites concentration by CE/MS developed by Soga et al(Soga et al. 2003). We will present 112 metabolites of anion, cation and nucleotides at each time points.

We will show the quantitative profiles in MOPS (0.4% glyucose) and trial to implement the model using Cell Illustrator.

We showed the relative stability of metabolite concentration after elimination of the enzyme genes of Glycolysis or TCA cycle pathways(Ishii et al. 2007). This is “robustness”. And this might be caused by activation of isozymes or alternative pathways. To elucidate the molecular mechanism of the robustness, we are now developing the method and the resources for synthetic lethal or sickness analysis.

We will also show our trial to detect alternative pathways by double knockout mutant analysis and would like to discuss it.

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