대사흐름분석을 통한 대사특성 파악

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Identification of metabolic characteristics by metabolic flux analysis

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Introduction

Metabolic engineering can be defined as directed modification of cellular metabolism and properties through the introduction, deletion and modification of metabolic pathways by using recombinant DNA and other molecular biological tools [1, 2]. Various metabolic engineering strategies have been widely applied for the more efficient production of desired metabolites and biomolecules. Even though enhanced production of some biomolecules has been successful, many other attempts have failed due to the lack of rational strategies based on predictable technique. Therefore, metabolic flux analysis, which allows calculation of the intracellular metabolic fluxes based on the stoichiometry of intracellular reactions and mass balances around the intracellular metabolites, has become an essential tool for metabolic engineering [3].

Metabolic flux analysis technique is based on the pseudo-steady state assumption, which means no net intracellular accumulation of intermediates, considering the high turnover of intracellular metabolite pools [13, 16]. Metabolic flux analysis has been applied to calculate the maximum theoretical yield of a desired metabolite to be produced, and to identify the rigidity of branch points in the metabolic pathways. Another possible application is the identification of alternative metabolic pathways leading to a desired product.

Succinic acid is a member of the C4-dicarboxylic acid family and can be used as a precursor of numerous products including pharmaceuticals, fine chemicals and biodegradable polymers. Succinic acid has been produced by chemical processes. Recently, much effort is being exerted for the production of succinic acid by microbial fermentation using renewable feedstocks,

because of pollution problems associated with chemical processes. The best known succinic acid producing bacterium is *Anaerobiospirillum succiniciproducens*, which can produce a mixture of succinic acid and acetic acid at a molar ratio of 2:1 from glucose. *Escherichia coli* produces several metabolic products by fermentation: acetic acid, ethanol, formic acid, lactic acid, and also a small amount of succinic acid. The ratio of these fermentation products varies depending on the culture condition employed.
Determination of the achievable maximum yield and optimal metabolic pathways is essential for the engineering of the metabolic pathways and for the redirection of metabolic fluxes towards the desired bioproducts. In this study, we report construction of *in silico* metabolic network of *E. coli*, and as an example, its use in the estimation of the maximum *in silico* yield of succinic acid and the determination of optimal flux distribution.

Materials and methods

Metabolic flux analyses were carried out for the calculation of volumetric rates of formation of intracellular metabolites [2]. Metabolic flux analysis is based on the pseudo-steady state assumption, which means that there is no accumulation of any intermediates.

$$\mathbf{r} = \mathbf{T}^{T}\mathbf{v}$$

The vector $\mathbf{r}$ is defined as the net formation rates of metabolites (mM/g DCW/h), and $\mathbf{v}$ is the internal reaction rates (mM/g DCW/h). $\mathbf{T}$ is the total stoichiometric matrix for all reactants and products of reactions.

Results and discussion

Maximum yields

The maximum capacity for succinic acid production was estimated by metabolic flux analysis. The maximum *in silico* yield of succinic acid is listed in Table 3. The maximum theoretical yield was also calculated for comparison. The maximum *in silico* yield of succinic acid was only 1.65 mole/mole glucose (83% of the maximum theoretical yield). It was found that the CO2 consumption rate was 0.82 mole/mole glucose/h even though the CO2 consumption rate was not restricted. CO2 is required for the carboxylation of C3-compounds (phosphoenolpyruvate and pyruvate) to C4-compounds (oxaloacetate and malic acid), which are further converted to

succinic acid. Therefore, 2 moles of CO2 are theoretically required for each mole of glucose to achieve the maximum yield of succinic acid. These results indicate that there exists a limiting factor. As stated earlier, however, cells are operating metabolic pathways without any nutrient limitation. Examination of metabolic reaction network we constructed suggested that there was no reaction for incorporating external reducing power. It was therefore reasoned that the limiting factor for succinic acid production might be reducing power. To examine this hypothesis, the following reaction was added to the metabolic reaction network.

The electrons generated can be transferred to several different acceptors including FAD and fumaric acid. After incorporating this reaction, intracellular flux distribution was re-estimated. The maximum *in silico* yield of 2 mole succinic acid/mole glucose was achieved. This result is consistent with our previous report showing that succinic acid flux was controlled by reducing power in *E. coli* and succinic acid production could be enhanced by using more reduced carbon substrate such as sorbitol.

Optimal flux distributions

The optimum metabolic pathway for succinic acid production predicted *in silico* is shown in Figure 1. Glucose is converted to pyruvate and finally to succinic acid by sequencial reactions of malic enzyme, fumarase and fumarate reductase. However, the proposed optimal pathway is different from the conventional succinic acid production pathway, which consists of PEP carboxylase, malate dehydrogenase, fumarase and fumarate reductase.

Through this pathway, free energy of PEP is wasted as inorganic phosphate while ATP can be generated by pyruvate kinase. Moreover the optimal pathway indicates that direction of malic enzyme reaction should be opposite of normal direction since usually malic enzyme catalyze decarboxylation of malic acid to pyruvate because of kinetic characteristics (Km = 0.4 mM for malic acid, 16 mM for pyruvate).

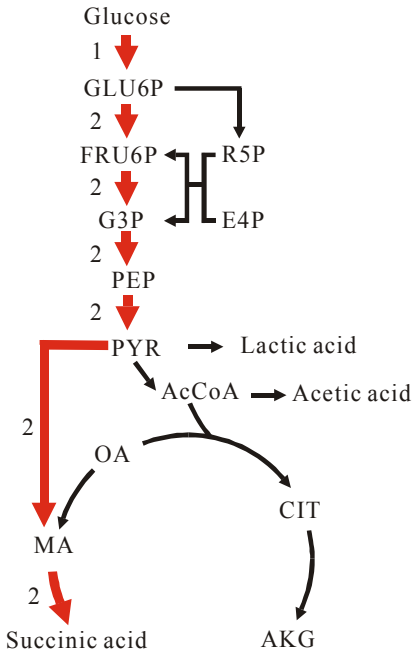

Figure 1. Optimized metabolic flux distributions during the production of succinic acid.

Acknowledgement

This work was supported by the National Research Laboratory Program (2000-N-NL-01-C-237) of the Ministry of Science and Technology (MOST), the Advanced Backbone IT Technology Development Project (IMT2000-C3-1) of the Ministry of Information and Communication (MIC) and MOST, and by the Brain Korea 21 project from the Ministry of Education.

Referances

1. Bailey, J. E., "Towards a science of metabolic engineering", Science, 252, 1668-1674 (1991).
2. Lee, S. Y., Papoutsakis, E. T., "Metabolic Engineering", Marcel Dekker, New York (1999).
3. Nielsen, J., Villadsen, J., "Bioreaction engineering principles", Plenum press, New York (1994).