ELUCIDATING AMINO ACID METABOLISM IN CHO CELLS

Maciek R. Antoniewicz, University of Delaware
mranton@udel.edu
Jaqueline E. Gonzalez, University of Delaware
Brian O. McConnell, University of Delaware
Harnish Mukesh Naik, Johns Hopkins University
Venkata Gayatri Dhara, Johns Hopkins University
Michael J. Betenbaugh, Johns Hopkins University

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CHO cells require complex media for cell growth and protein production. The major components of industrial media are amino acids, however, relatively little is known about the metabolism of amino acids in CHO cell cultures. Here, we applied advanced ^{13}C-flux analysis tools to elucidate the metabolic flow of the amino acids in a fed-batch CHO culture that overproduced IgG. Carbon flows were tracked throughout the growth phase and changes in metabolism were quantified when cells transitioned from growth phase to stationary phase. In addition, we quantified how changes in amino acids profiles in the medium translated to changes in cell growth, protein production and product quality attributes. To trace each amino acid individually, custom media formulations were used, where each medium formulation was depleted of a specific amino acid. A labeled ^{13}C variant of the depleted amino acid was then added to the medium at the desired concentration. CHO cells were then grown in fed-batch culture. As the cells metabolized the labeled amino acids, this resulted in a redistribution of ^{13}C-atoms which we quantified using GC-MS for both extracellular metabolites (including lactate, amino acids and the IgG product) and intracellular metabolites (including free intracellular metabolites, cell proteins, lipids and carbohydrates). We then estimated metabolic fluxes using state-of-the-art ^{13}C-metabolic flux analysis. This allowed us to calculate the fraction of each amino acid that was used for cell growth, protein production, lactate formation and energy generation. We also investigated the effects of labeling in both the batch and fed-batch stationary phase. Finally, we investigated the effects of varying amino acid concentrations. Each ^{13}C-labeled amino acid was added to the medium at a lower or higher concentration compared to the base medium. ^{13}C-metabolic flux analysis was again performed and changes in fluxes were compared in order to determine the precise impacts of amino acid concentration changes on the flux profiles. Taking all of this data together, we are now building a predictive kinetic model that relates how the metabolism of CHO cells can be predicted from amino acid profiles. In future work, model predictions will be experimentally validated as a means of optimizing the amino acid composition of industrial culture media.