ELUCIDATING DIPEPTIDE UTILIZATION AND METABOLISM BY CHO CELLS FOR IMPROVED CELL CULTURE PERFORMANCE

Xiangchen Cai, University of Michigan
harrycai@umich.edu
Harnish Mukesh Naik, Johns Hopkins University
Pranay Ladiwala, Johns Hopkins University
Michael J. Betenbaugh, Johns Hopkins University
Maciek R. Antoniewicz, University of Michigan

Key Words: Dipeptides, amino acid metabolism, ^{13}C labeling, growth improvement, metabolic flux analysis

Mammalian cells are widely used in the pharmaceutical industry to produce recombinant protein products such as monoclonal antibodies (mAbs). Currently, about 70% of mAbs are produced by Chinese Hamster Ovary (CHO) cells. In order to improve CHO cell productivity and key product quality attributes, several dipeptides have explored as medium additives in the past. However, there is still a lack of fundamental understanding on how dipeptides are utilized and metabolized by CHO cells. This lack of understanding prevents us from rationally designing cell culture media with optimized concentrations of dipeptides. To address this limitation, over the past years we have performed studies to elucidate dipeptide utilization and metabolism in CHO cell cultures. In this presentation we will report for the first time the results of our studies. First, we will conclusively demonstrate that dipeptides are cleaved both intracellularly and extracellularly in suspension CHO cell cultures. To quantitatively describe dipeptide metabolism, we have developed a kinetic model that describes both the cleavage and utilization of dipeptides by CHO cells. We used experimental data to estimate kinetic parameters using this model. Moreover, we have quantitatively characterized extracellular cleavage of dipeptides using a cell-free experimental setup. Combined with additional 13C-labeled tracer experiments we performed, our model is able to accurately determine net consumption rates of single and multiple dipeptides in cell culture. Taken together, the quantitative data we will present here provides new insights into dipeptide metabolism that can be used to design improved media formulations, optimize feeding strategies, and select appropriate dipeptides for improved CHO cell culture performance.