^{13}C FLUX ANALYSIS IN INDUSTRIAL CHO CELL CULTURE APPLICATIONS

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Key Words: Chinese hamster ovary, IgG expression, metabolic flux analysis

Industrial bioprocesses place extraordinary demands on the intermediary metabolism of host cells to meet the biosynthetic requirements for maximal growth and protein expression. Identifying host cell metabolic phenotypes that promote high recombinant protein titer is a major goal of the biotech industry. ^{13}C metabolic flux analysis (MFA) provides a rigorous approach to quantify these metabolic phenotypes by applying stable isotope tracers to map the flow of carbon through intracellular metabolic pathways. We have conducted a series of ^{13}C MFA studies to examine the impacts of IgG expression and other physiological stresses on CHO cell metabolism.

First, we performed ^{13}C MFA to characterize the metabolism of a IgG-expressing DHFR-deficient CHO host during four separate phases of a fed-batch culture. We found that peak specific growth rate during early exponential phase was associated with high lactate production and minimal citric acid cycle (CAC) flux. Conversely, we found that lactate metabolism switched from net production to net consumption as the culture transitioned from peak growth to peak IgG production. During stationary phase when IgG production peaked, energy was primarily generated through CAC and oxidative phosphorylation.

Second, we examined nine CHOK1SV (Lonza) clones cultured in 3-liter fed-batch bioreactors, to assess their metabolism during stationary phase. Three of the clones did not express IgG. Six of the clones used the GS System^{TM} to express one of three different IgGs. Four of the clones were genetically manipulated to be apoptosis-resistant by expressing Bcl-2Δ. Hierarchical clustering was performed to assess correlations amongst flux phenotypes of the nine clones. The six IgG-producing clones clustered together and were separated by host background (Bcl-2Δ or CHOK1SV). The lactate dehydrogenase (LDH) flux was most closely associated with specific IgG productivity: as IgG productivity increased, lactate production decreased. Additionally, elevated CAC fluxes corresponded strongly with increased specific productivity. This study provided further evidence of enhanced oxidative metabolism in high-producing CHO cell lines.

Finally, ^{13}C MFA was used to characterize the metabolic response of CHO cells to a novel medium variant designed to reduce ammonia production. Ammonia production was reduced by manipulating the amino acid composition of the culture medium; specifically, glutamine, glutamate, asparagine, aspartate, and serine levels were adjusted. Parallel ^{13}C flux analysis experiments determined that, while ammonia production decreased by roughly 40%, CHO cell metabolic phenotype, growth, viability, and monoclonal antibody (mAb) titer were not significantly altered by the changes in media composition. This study illustrates how ^{13}C flux analysis can be applied to assess the metabolic effects of media manipulations on mammalian cell cultures. The analysis revealed that adjusting the amino acid composition of CHO cell culture media can effectively reduce ammonia production while preserving fluxes throughout central carbon metabolism.

Taken together, these studies provide several useful examples of how ^{13}C MFA can be applied to assess metabolic responses of CHO cell cultures to high-yield IgG production and changing bioprocess conditions. This presentation will describe the methodology and its application to develop engineering strategies to enhance IgG productivity and titer of industrial CHO hosts.