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13C flux analysis in industrial CHO cell culture applications

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

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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 metabolic impacts of multiple stressors on CHO cell metabolism.

First, we analyzed the effects of various media compositions and supplementation regimens on CHO cell metabolism. The basal media developed in-house by an industrial collaborator was chemically altered to cause cells to produce less ammonia byproduct. This was tested against the basal media and the basal media supplemented with experimental levels of ammonia. From the comparison of the ^{13}C flux analysis of CHO cells grown identically in the three media types, we have found that neither the chemical composition of the media nor the mere presence of ammonia in the cultures significantly altered cell metabolism. This suggests that the collaborator can use their new medium formulation without altering the metabolic phenotype of their IgG producing CHO cell lines.

We are also implementing ^{13}C MFA studies in several IgG producing cell lines to elucidate metabolic phenotypes associated with high-yield recombinant protein expression. From previous studies, it has been established that there exists a high-productivity metabolic phenotype largely identifiable by an increase in oxidative metabolism. We are engineering these proprietary IgG-producing CHO cells to up-regulate their citric acid cycle (CAC) metabolism to potentially increase IgG productivities. Through ^{13}C stable isotope tracing, we can verify increased flux through the CAC and confirm the rational engineering of a high-productivity phenotype.

These studies prove the value of ^{13}C MFA in assessing the metabolic response to changing medium formulations or rational engineering of the host cell genome. This poster will outline the methodology used to elucidate CHO cell metabolic phenotypes in these studies as well as the potential use for this method in future studies to further increase IgG productivity and titer of industrial host lines.