TRACKING AMINO ACID METABOLISM IN CHO CELL CULTURES USING STABLE ISOTOPE LABELING ASSISTED METABOLOMICS

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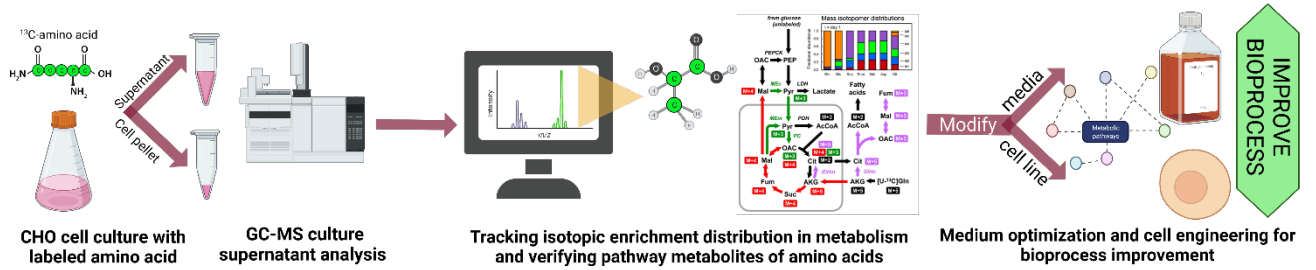
Chinese Hamster Ovary (CHO) cells are the predominant hosts for recombinant protein therapeutics production. CHO cells in high cell density fed-batch cultures exhibit enhanced consumption of nutrients including glucose and amino acids. While these substrates are primarily used for biomass production and protein synthesis, a majority of them are also diverted towards metabolic pathways responsible for secreting by-products. Relatively little is known about the metabolism of amino acids in CHO cell cultures. Here, we applied comprehensive stable isotope (^{13}C) labeling assisted metabolomics and advanced 13C-flux analysis tools to elucidate the metabolic flow of all 20 amino acids and glucose in IgG producing CHO cell cultures using two different cell lines (CHO-K1 and CHO-GS). Custom media formulations each depleted with a single amino acid (e.g., valine) were supplemented with a universally labeled ^{13}C tracer of the depleted amino acid (here, [U-^{13}C] valine). Carbon flows were tracked throughout the growth phase and changes in amino acids metabolism were quantified when cells transitioned from growth phase to stationary phase. GC-MS based analytical methods were used to track the carbon enrichment flows throughout the different phases of CHO cell cultures. As the cells metabolized the labeled amino acids, this resulted in a redistribution of 13C-atoms which we quantified using GC-MS for extracellular metabolites (including lactate, amino acids and TCA cycle metabolites), IgG product and intracellular metabolites (including free intracellular metabolites, biomass and lipids). This allowed us to analyze relative pathway activities, asses nutrient contributions to metabolite production and calculate fraction of each amino acid that was used for cell growth, protein production, lactate formation and energy generation. Mass isotopomer distributions provided information about metabolic pathways and labeling dynamics provide information about changes in metabolism. Notable differences in amino acids utilization were observed between the two different CHO cell lines. Prior studies have employed LCMS based global metabolomics strategies to also identify cytotoxic metabolites that the cells generate via amino acid catabolism. Using ^{13}C labeling assisted GCMS based metabolomics, we were also able to some of these previously identified cytotoxic metabolites such as indolelactate, phenyllactate, isovalerate, lactate and ammonium. Commercially available standards were used to verify the identity of each of these metabolites. Integrating all of this data together, we are building a predictive kinetic and stoichiometric model of CHO cell growth, substrate uptake, by-product secretion and intracellular metabolism. Together, all of this knowledge obtained could help design strategies to modulate amino acids levels in CHO cell cultures such that amino acids levels are sufficient enough for protein synthesis but not high enough to divert the metabolism towards unwanted byproduct synthesis. Such strategies could include optimizing basal media and feed formulations, synthetic biology tools including genetic engineering, and continuous online monitoring of amino acid levels.



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