BIOREACTOR SCALE INDUCED ALTERATION IN CELL METABOLIC STATE CAN IMPACT AMINO ACID MISINCORPORATIONS IN RECOMBINANT PROTEINS PRODUCED IN CHO CELLS

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Amino acid misincorporations are a type of sequence variants caused by unintended substitutions of non-cognate amino acids in the protein sequence. Technologies like high resolution mass spectrometry have enabled the identification and characterization of several types of amino acid misincorporations in recombinant proteins produced in CHO cells. Many of these misincorporations have been reported in the context of nutrient starvation which greatly enhances the level of misincorporation of the non-cognate amino acid due to reduction in levels of cognate amino acids. It is relatively unknown if the level of amino acid misincorporations can be exacerbated by alterations in the metabolic state of the cells or scale-up of a bioreactor process. During cell culture process development, the metabolic state of cells may be altered if there are scale specific changes in key process parameters like pCO2. We observed higher levels of proline to alanine misincorporations when reproducing a cell culture process at different scales. In this talk, we describe our studies to show that an altered metabolic state can increase levels of alanine and lactate, which can result in an increase in proline to alanine misincorporations when cells are starved for proline. We confirmed this by recreating the altered metabolic state and confirm the increase in proline to alanine misincorporations. Given that process development and GMP manufacturing requires significant scale-up from small scale bioreactor systems, it is key to understand and match the metabolic state of the cells to ensure amino acid misincorporations do not occur at higher frequency due to alterations in bioreactor scale. To our knowledge this is the first report of the metabolic state of cells and bioreactor scale influencing the level of amino acid misincorporations and we would like to share this novel relationship that can influence the development of appropriate cell culture control strategy.