Effects of culture conditions on cell growth, production, metabolism and glycosylation of recombinant glycoproteins produced by Chinese hamster ovary cells

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ABSTRACT

With the increase in demand for therapeutic proteins and the shortage of reactor capacity for mammalian cell culture, there has been renewed interest in high cell density cultures, particularly with microcarriers. However, it is not well understood how changes from suspension to microcarrier cultures affect yield and glycosylation of recombinant proteins.

In the first part of this thesis work, a CHO cell line (TR2-255) producing the secreted human placental alkaline phosphatase (SEAP) was investigated in a static culture mode under different culture conditions (adherent, suspension, cells attached to microcarriers). Subsequently, purification and glycan analysis protocols were developed using the static suspension culture. In the second part of the work, the culture conditions were optimized in a Braun Biostat B bioreactor under the various operating conditions including pH control, nutrient feeding, bubble-free culture, bi-phasic culture, low-dissolved oxygen concentration etc. In the third part of the work, the effects of culture conditions (i.e. suspension cultures vs. cells attached to microcarriers) on growth, productivity and metabolic activities of CHO cells producing recombinant proteins was investigated. Particularly, to generalize the changes observed in the SEAP-producing CHO cell line, another CHO cell line (CHO 1-15-500) producing tissue plasminogen activator (t-PA) was investigated under the identical culture conditions as the SEAP-producing CHO cell line. In the fourth part of the work, the effects of hypothermia on the cell growth, productivity and metabolic activities were investigated in a bi-phasic culture under the different culture conditions (suspension, cells attached to microcarriers). In the fifth part of the work, the effects of different culture conditions (suspension, cells attached to

microcarriers and suspension in bi-phasic hypothermia) on N-linked glycan profiles of SEAP was examined using MALDI-TOF mass spectrometry and other methods.

Finally, proposed future work is suggested, including further optimization of the culture conditions, perfusion culture, analysis of the changes in cytoskeletal organization and sub-cellular compartments (e.g. endoplasmic reticulum, Golgi apparatus) between suspension and microcarrier cultures, and finally, analysis of the effects of culture conditions on transcription rates including changes in mRNA levels for recombinant proteins produced by CHO cell.