Analysis of Hyperosmotic Response of Mammalian Cell Lines

by

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ABSTRACT

Monoclonal antibodies are widely used in therapeutic applications as well as in vitro diagnostic and laboratory assays. As a result of the rapidly expanding market, there is increasing concern about capacity limitations and controlling the cost of goods of the monoclonal antibody production. This study focused on the response of mammalian cells towards hyperosmotic stress, a well known approach capable of increasing specific productivity unfortunately mitigated by decreases in integrated viable cell density of the culture.

Murine hybridoma OKT3 and several industrial CHO cell lines were studied in this work. We initially measured the effect of hyperosmotic stress on the cell growth and specific antibody productivity of a mouse hybridoma (OKT3) and an industrial CHO cell line (B0). In batch culture studies, both cell lines showed substantially increased specific productivity. Not surprisingly, the final titer showed no substantial increase due to the decrease in viable cell density. In fed-batch culture, B0 cells seemed to be more tolerant of osmotic stress than in batch culture. We observed that hyperosmolarity slightly repressed the growth rate of B0 cells in fed-batch culture, but no significant change in productivity or final titer was detected.

The changes in mean cell size and cell-size distribution in response to hyperosmotic stress were studied using flow cytometry and Cedex cell analyzer. Assuming the forward scatter (FSC) signal is proportional to cell size, the flow cytometry analysis suggested cell shrinkage occurred immediately after the initiation of osmotic stress, followed by a regulatory volume increase (RVI). According to the time profile of FSC signal distribution, the degree of cell shrinkage, the starting time of cell size recovery, and the degree of RVI depended on the level of hyperosmolarity. By directly monitoring the cell size distribution by Cedex analysis, we found the increase in a subpopulation of larger cells was a substantial contributor to the increase in the average diameter of stressed cells.

As the first step to relate individual genes to increased productivity and repressed growth, Affymetrix GeneChip® MOE430A and CHO microarrays were used to monitor the transcription profile changes of OKT3 and B0 cells in response to osmotic stress.

After signal normalization, the effectiveness of hyperosmotic stress in altering the expression of each gene was evaluated using ANOVA analysis. Quantitative PCR assays with TaqMan® probes were applied to selected genes to validate the results obtained from microarray analysis. Those differentially expressed genes were classified by extracting their annotations and functionalities from online databases. The pathways involved in hyperosmotic response were revealed using several available pathway analysis tools. The two transcriptome analysis results were compared to find the similarity and dissimilarity in the osmotic response mechanisms between mouse hybridoma and CHO cells. Results of this research will provide guidance for engineering mammalian cells for higher recombinant protein productivity.