Understanding the Effects of Sequence on Monoclonal Antibody Expression in Chinese Hamster Ovary Cells

by

Megan Elisabeth Mason

An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the degree of

DOCTOR OF PHILOSOPHY

Major Subject: CHEMICAL AND BIOLOGICAL ENGINEERING

The original of the complete thesis is on file
In the Rensselaer Polytechnic Institute Library

Examining Committee:

Susan T. Sharfstein, Thesis Adviser

Jonathan S. Dordick, Thesis Adviser

Christopher Bystroff, Member

Peter M. Tessier, Member

Rensselaer Polytechnic Institute
Troy, New York

October 2010
(For Graduation December 2010)

ABSTRACT

Monoclonal antibodies represent a significant fraction of recently approved and pipeline biopharmaceuticals, providing critical new therapies for diseases such as breast cancer and rheumatoid arthritis. As new strategies have emerged for antibody engineering to improve clinical efficiency by improving affinity, potency, pharmacokinetics and pharmacodynamics, it has become apparent that modification to the nucleic acid and protein sequence can also substantially impact manufacturing considerations, including expression levels, purification, aggregation, and stability.

We identified a humanized monoclonal antibody in which a single amino-acid change (from alanine to glycine) in the Kabat position 49 (the heavy chain variable region one residue before the CDR2) led to a four-fold decrease in productivity when expressed in CHO cells. To identify the mechanism for this change in productivity, we created a panel of antibody variants containing all 20 amino acids except glutamine in position 49 and screened for expression level in CHO cells. Small, uncharged residues, particularly with hydroxyl groups (i.e. threonine and serine) gave the highest levels of expression, while bulky substituents and charged groups gave very low levels of expression.

The focus of this thesis was to identify the cellular regulatory mechanisms responsible for differences in recombinant antibody expression of closely related sequences. We showed that the primary effects of sequence on productivity are on the ability to correctly fold and assemble antibody, with the low expressing variants exhibiting higher intracellular degradation, leading to reduced secretion of properly folded proteins. The sequence-induced issues with the protein structure were reflected in the biophysical properties of the secreted antibodies. We also found evidence that CHO cells may possess mechanisms to dynamically regulate exogenous gene expression in proportion to the cellular capacity to produce correctly folded, functional proteins.

The results presented in this study emphasize the importance for proper sequence engineering strategies when humanizing antibodies, especially at the heavy chain-light chain interface. Moreover, for difficult-to-express antibodies where sequence engineering cannot be employed, decreasing culture temperature may be a useful tool for improving the accuracy of co-translational folding and improving the intrinsic stability.

The evidence that expression appears to be limited by the cellular capacity to fold and assemble protein corroborates the use of cellular engineering strategies (e.g. modification of UPR proteins) as an effective method of increasing antibody titers ultimately leading to the production of cheaper, high-quality recombinant antibodies.