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Dynamic model of MAb production and glycosylation for the purpose of product quality control

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Abstract

A combined model of MAb-producing mammalian cell cultures describing cell population kinetics, cell metabolism, and MAb synthesis and secretion has been developed based on previous modelling attempts from the relevant literature (Jang and Barford, 2000; Tatiraju *et al.*, 1999; Bibila, 1991) and is in good agreement with experimental data. This is linked to a modified version of the structured compartmental model of protein glycosylation originally proposed by Umaña and Bailey (1997), which is based on a Michaelis-Menten representation of the oligosaccharide addition/removal reaction network. The dynamic results of the latter model are presented here for the first time. This coupling allows us to examine *in silico* and *a priori* the effect of any feeding strategy on the extent of MAb glycosylation and, therefore, on product quality. Preliminary optimisation results for different feeding strategies are also presented.

Keywords: Dynamic modelling, Bioengineering, Sensitivity analysis, Optimisation.

1. Introduction

Monoclonal antibodies (MAbs) are valuable biopharmaceutical products of mammalian cell cultures with a wide range of diagnostic and therapeutic applications, which reached sales of $7.2 billion in 2003 (Market Research, 2004). The aim of mathematical modelling of these processes is two-fold: to substitute the time-consuming and costly laboratory experiments with *in silico* ones, and to develop model-based algorithms for product quality control. In order to predict MAb production and quality with any accuracy, we need to develop models that sufficiently describe all aspects of cellular behaviour involved in and affecting protein secretion. However, mammalian cells have an extremely complicated internal structure (a simplified schematic can be seen in Figure 1), where several interlinked biochemical processes take place. System complexity is therefore a limiting factor in model building and a careful selection of functions to be incorporated in a mathematical model needs to be made.

Firstly, cell population and MAb production dynamics need to be determined, and, in fact, most modelling attempts are limited to these two outputs (Pörtner and Schäfer,

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1996; Tziampazis and Sambanis, 1994). Nevertheless, MAb secretion by the cells does not guarantee full functionality of the product. To quantitatively determine product quality, the post-translational modification process of MAbs needs to be modelled as well. This process, known as N-linked glycosylation, consists of the addition and removal of oligosaccharides from the protein, while the latter travels through the Endoplasmic Reticulum (ER) and the Golgi apparatus (see Figure 1). Any error degrades the structural integrity of the MAb and, thus, its conformation and bioactivity. In this work, we present a combined model of the overall process of MAb production through mammalian cell cultures. We further present the first dynamic results for protein glycosylation in the Golgi apparatus, which enables us to monitor MAb quality throughout the culture. Finally, we introduce an optimisation strategy to determine the optimal number of feeding intervals for a fed-batch culture.

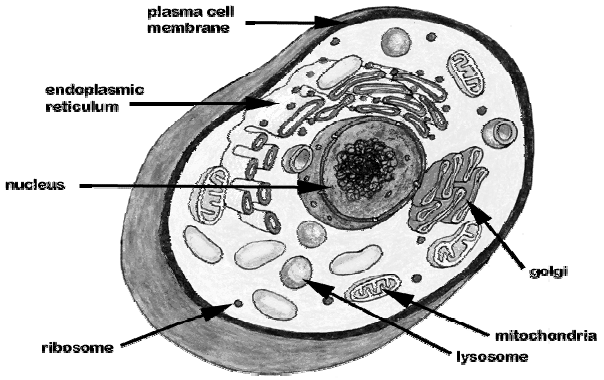

Figure 1. The internal structure of a mammalian cell (http://www.elmhurst.edu)

2. Mathematical model

The proposed model is the first to combine cell population dynamics with MAb synthesis, glycosylation and secretion, in an effort to describe MAb-producing mammalian cell cultures. More specifically, it combines an unstructured model describing cell growth and death (Jang and Barford, 2000) and cell metabolism (Jang and Barford, 2000; Tatiraju *et al.*, 1999), with a structured model of MAb synthesis and production (Bibila and Flickinger, 1992; Tatiraju *et al.*, 1999), and a compartmental model of MAb glycosylation (Umaña and Bailey, 1997). All sections have been modified to facilitate the flow of information between them and to more accurately describe *in vitro* cellular behaviour.

Nutrient availability and metabolite build-up, collectively known as cell metabolism, determine cell population dynamics, which, in turn, are used to calculate MAb production. Each MAb molecule is synthesised by two heavy and two light amino acid chains in the ER, where a precursor oligosaccharide is further added to it. The molecule then travels through the Golgi apparatus, where it is subject to a series of oligosaccharide addition and trimming reactions. The level of these oligosaccharides

when the molecule exits the Golgi apparatus and is secreted to the culture environment determines its functionality. The mathematical equations describing the aforementioned process are briefly outlined below (sections 2.1.1, 2.1.2, and 2.2 can be found in their entirety in Kontoravdi *et al.* (2004)).

2.1 Unstructured model of MAb-producing mammalian cell cultures

2.1.1 Cell growth & death

The total mass balance around the culture bioreactor and the balance on the viable cell population are:

$$\frac{dV}{dt} = F_{in} - F_{out}, \qquad (1);\ \frac{d(VX_v)}{dt} = \mu VX_v - \mu_d VX_v - F_{out}X_v, \qquad (2)$$

where, V is the culture volume, F_{in}, F_{out} are the inlet and outlet flowrates, X_v denotes the viable cell concentration, and μ, μ_d are the specific cell growth and death rates. A similar mass balance can be written for the total cell population. The specific cell growth rate is determined by the concentrations of the key nutrients (glucose and glutamine), and the main metabolites (lactate and ammonia):

$$\mu = \mu_{\max} f_{\lim} f_{inh}, \qquad (3);\ f_{\lim} = \left(\frac{[GLC]}{K_{glc} + [GLC]}\right)\left(\frac{[GLN]}{K_{g\ln} + [GLN]}\right), \qquad (4)$$

$$f_{inh} = \left(\frac{KI_{lac}}{KI_{lac} + [LAC]}\right)\left(\frac{KI_{amm}}{KI_{amm} + [AMM]}\right), \qquad (5)$$

where, μ_{max} denotes the maximum specific growth rate, and f_{lim}, and f_{inh} are the nutrient limitation and product inhibition functions. [GLC], [GLN], [LAC], and [AMM] are the glucose, glutamine, lactate and ammonia concentrations in the culture medium, while K_{glc}, K_{gln}, KI_{lac}, and KI_{amm} denote their respective Monod constants. A similar equation can be written for the specific cell death rate as a function of the concentration of ammonia.

2.1.2 Cell metabolism

The concentration of nutrients and metabolites can be computed by performing mass balances around the bioreactor while taking into account cell metabolism (Jang and Barford (2000)). The mass balance on glucose around the bioreactor is:

$$\frac{d(V[GLC])}{dt} = -Q_{glc}VX_v + F_{in}[GLC]_{in} - F_{out}[GLC], \qquad (6)$$

$$\text{where, } Q_{glc} = \frac{\mu}{Y_{x,glc}} + m_{glc}, \qquad (7)$$

where, Q_{glc}, Q_{gln} are the specific glucose and glutamine consumption rates, and $[GLC]_{in}$, $[GLN]_{in}$ denote the inlet glucose and glutamine concentrations. $Y_{x,glc}$, $Y_{x,gln}$ are the yields of cells on glucose and glutamine, m_{glc}, m_{gln} denote the maintenance coefficients for glucose and glutamine, and α_1, α_2 are constants of glutamine maintenance. Similar equations hold for glutamine, lactate, and ammonia.

2.2 Structured model of MAb synthesis & production

This part of the model consists mainly of mass balances for the synthesis of MAb intermediates and complete molecules in the ER and the Golgi apparatus. For example, the mass balance on MAb molecules in the Golgi apparatus is:

$$\frac{d[H_2L_2]_G}{dt} = K_{ER}[H_2L_2]_{ER} - K_G[H_2L_2]_G, \quad (8)$$

where, $[H_2L_2]_{ER}$, $[H_2L_2]_G$ are the MAb molecule concentrations in the ER and the Golgi, respectively, ε_1 is the ER glycosylation efficiency factor, and K_{ER}, K_G denote the rate constant for ER-to-Golgi and Golgi-to-extracellular medium MAb transport, respectively. The final MAb concentration in the extracellular medium is given by:

$$\frac{d(V[MAb])}{dt} = (\gamma_2 - \gamma_1\mu)Q_{MAb}VX_v - F_{out}[MAb], (9); Q_{MAb} = \varepsilon_2\lambda K_G[H_2L_2]_G, \quad (10)$$

where, Q_{MAb} is the specific MAb production rate, [MAb] is the MAb concentration in the culture medium, ε_2 is the Golgi glycosylation efficiency factor, γ_1, γ_2 are constant parameters distinguishing between proliferating and non-proliferating cells in terms of their MAb production rate, and λ is a unit conversion constant.

2.3 Compartmental model of MAb glycosylation

Umaña and Bailey (1997) assumed complete glycosylation in the ER and mathematically described the glycosylation process in the Golgi apparatus as a Central Reaction Network (CRN). This network comprises 33 enzyme-catalysed reactions that take place as the protein is transported through this organelle. The proposed model considers 8 Golgi-localised enzymes and 4 Golgi compartments, namely the *cis*- (compartment 1), medial- (compartment 2), and *trans*-Golgi cisternae (compartment 3) and the *trans*-Golgi network (compartment 4). The mass balance on the amount of glycoprotein in compartment 1 is given by:

$$\frac{dp_1}{dt} = K_{ER}\frac{[H_2L_2]_{ER}}{N}X_vV - K_Gp_1, \quad (11)$$

and in compartments 2, 3, and 4: $\frac{dp_j}{dt} = K_G(p_{j-1} - p_j), 2 \le j \le 4$, (12)

where, p_j is the amount of glycoprotein in compartment j and N is Avogadro's number. As mentioned above, 8 enzymes are thought to catalyse the 33 addition/trimming reactions. As a result, in most reactions multiple substrates compete for one binding site. At this stage, we modified the original model by assuming Michaelis-Menten kinetics for competitive inhibition (Montgomery and Swenson, 1976), and expressed the rates of these reactions as follows:

$$r_{k,j} = \frac{\nu_{m,k,j}X_vVp_jx_{i,j}}{p_jx_{i,j} + K_{m,k,j}V_{G,j}\left[1 + \frac{p_j}{V_{G,j}}\sum_l\left(x_{i_l,j}/K_{m,k_l,j}\right)\right]}, \; 1 \le i \le 33, \; 1 \le k \le 33, \; 1 \le j \le 4 \quad (13)$$

where, $r_{k,j}$ is the rate of reaction k in compartment j, $\nu_{m,k,j}$ denotes the apparent maximal velocity of reaction k in compartment j, and $K_{m,k,j}$ is the apparent dissociation constant for reaction k in compartment j. $V_{G,j}$ is the volume of compartment j and $x_{i,j}$ denotes the mole fraction of oligosaccharide i on the glycoprotein in compartment j. Mass balance on oligosaccharide i in compartment 1:

$$p_1\frac{dx_{i,1}}{dt} = K_{ER}\frac{[H_2L_2]_{ER}}{N}X_vV(ER_i - x_{i,1}) + \sum_k(\vartheta_{i,k}r_{k,1}), \; 1 \le i \le 33, \; 1 \le k \le 33, \quad (14)$$

and in compartments 2, 3, and 4:

$$p_j \frac{dx_{i,j}}{dt} = K_G p_{j-1}\left(x_{i,j-1} - x_{i,j}\right) + \sum_k \left(\theta_{i,k} r_{k,j}\right), \; 1 \le \mathrm{i} \le 33, \; 1 \le \mathrm{k} \le 33, \; 2 \le \mathrm{j} \le 4, \qquad (15)$$

where, ER_i is the mole fraction of oligosaccharide i on the glycoprotein as the latter exits the ER and $\theta_{i,k}$ is the stoichiometric coefficient of oligosaccharide i in reaction k.

3. Results and Discussion

The sensitivity of the parameters of the unstructured model of MAb-producing mammalian cell cultures was investigated using the Sobol' global sensitivity analysis method. Based on the dynamic results of this analysis (Kontoravdi *et al.*, 2005), the values of the 'more sensitive' parameters were estimated using experimental data from the relevant literature (Tatiraju *et al.*, 1999). The combined model was then simulated in gPROMS (PSE, 2002) using the parameter values suggested by Umaña and Bailey (1997) for the glycoform biosynthesis section (parameter values for glycosylation in CHO cells). The good agreement of the simulation results with the data (Figures 2 and 3) verify that the model successfully utilises information from nutrient consumption in order to correctly determine cell viability and MAb production through the intracellular protein-secreting pathway. Predictions for metabolite concentrations and cell viability are also successful (data not shown). Some preliminary results of the scenario-based optimisation study carried out can be found in Figure 4, where the best results for the 5 feeding strategies examined (1, 3, 4, 5, and 10 feeding intervals) are displayed. All five fed-batch strategies greatly improve MAb productivity, with the maximum obtained when 5 feeding intervals are applied (93% increase in final MAb concentration). This is in agreement with general experimental practices, where 4 or 5 intervals are employed. Finally, Figure 5 depicts how these 5 optimal feeding strategies affect the oligosaccharide content of the secreted MAbs. The results show the clear dependence of this content on nutrient availability, as suggested by Tachibana *et al.* (1994). Validation of the glycosylation results is not possible, as no experimental data exist.

4. Conclusions

The proposed model is the first to link cell dynamics and MAb synthesis with MAb glycosylation and can be an excellent starting point for MAb quality control. The simulation results for macroscopic culture variables such as nutrient and product concentrations are in good agreement with experimental data. Moreover, different feeding strategies for maximisation of MAb production and their effect on oligosaccharide content of the secreted protein are evaluated. These dynamic glycosylation results presented here for the first time agree with qualitative experimental observations, but cannot be validated due to lack of experimental data. When obtained, this data will allow us to quantify the efficiency of the glycosylation process in the Golgi apparatus and to examine the effect of different culture conditions on the quality of the product *in silico.*

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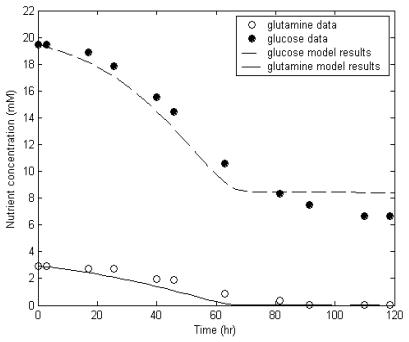
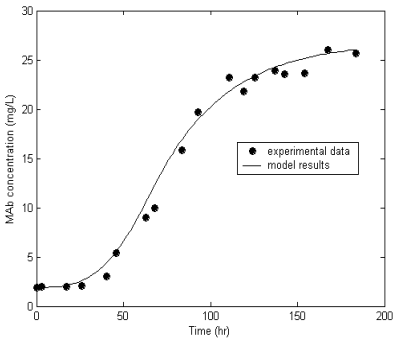
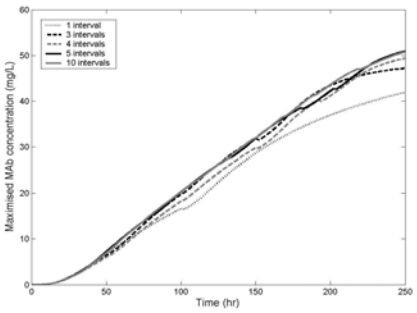
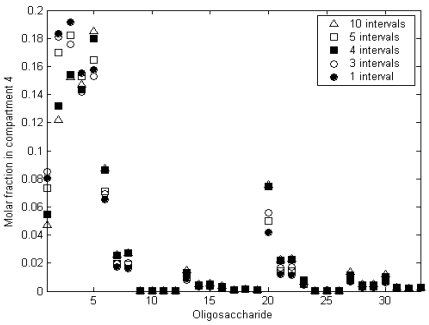




Figures 2 and 3. Agreement of model simulation results for glucose, glutamine and MAb concentrations with experimental data from the relevant literature.





Figures 4 and 5. Preliminary optimisation results for 1, 3, 4, 5, and 10 feeding intervals and the respective oligosaccharide molar fractions on produced MAbs.

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