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Poly-pathway model, a novel approach to simulate multiple metabolic states by reaction network-based model – Application to amino acid depletion in CHO cell culture

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ABSTRACT

Mammalian cell lines are characterized by a complex and flexible metabolism. A single model that could describe the variations in metabolic behavior triggered by variations in the culture conditions would be a precious tool in bioprocess development. In this paper, we introduce an approach to generate a poly-pathway model and use it to simulate diverse metabolic states triggered in response to removal, reduction or doubling of amino acids in the culture medium of an antibody-producing CHO cell line. Macro-reactions were obtained from a metabolic network via elementary flux mode enumeration and the fluxes were modeled by kinetic equations with saturation and inhibition effects from external medium components. Importantly, one set of kinetic parameters was estimated using experimental data of the multiple metabolic states. A good fit between the model and the data was obtained for the majority of the metabolites and the experimentally observed flux variations. We find that the poly-pathway modeling approach is promising for the simulation of multiple metabolic states.

1. Introduction

A high demand for the complex biopharmaceuticals produced in mammalian cells continues to stimulate improvement of cell lines and bioprocess conditions. Since the nutritional requirements for optimal growth and productivity vary between production cell lines, the selection of cell culture media and feeds can greatly affect the process performances. The optimization of medium and feed formulations can be a time-consuming and complex task, as it involves balancing the concentrations of numerous, potentially interacting, components (Bibila and Robinson, 2000; Burgener and Butler, 2006). Systematic screening combined with statistical modeling can reduce the development time and costs. Unfortunately, a systematic approach based on the knowledge of the cell metabolism is usually not included in these models.

A better understanding of the metabolic behavior and nutritional requirements of mammalian cell lines can be achieved by the analysis of the metabolite uptake and secretion rates (Xie and Wang, 1994), preferably in combination with metabolic reaction networks and metabolic flux analysis (MFA) (Quek et al., 2010; Ahn and Antoniewicz, 2012; Xie and Wang, 1996; Altamirano et al., 2001, 2006; Sidorenko et al., 2008; Xing et al., 2011; Selvarasu et al., 2012). Furthermore, the possible variations in metabolic behavior have been examined and explained in models of bacterial, yeast and mammalian cells by combining metabolic network analysis with statistical methods such as principal component analysis (Barrett et al., 2009; González-Martínez et al., 2014; Sariyar et al., 2006) and regression techniques (Van Dien et al., 2006; Ferreira et al., 2011).

Kinetic models enable dynamic simulations and potential predictions of the cell culture in response to changes in external conditions, e.g. in the medium composition. Such mechanistic models could provide precious tools for bioprocess optimization. However, modeling the complex and flexible metabolism of mammalian cells is a challenging task for which the strategy will depend on the aim and scope of the model. It involves defining a set of relevant metabolic reactions or pathways, the associated kinetic equations, and the model parameters which can be estimated from literature and/or by fitting to experimental data. The intracellular fluxes in detailed networks can be estimated by e.g. isotopic tracer-based analyses. Such techniques have provided useful insights about the cell metabolism (Zupke and

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Stephanopoulos, 1995; Goudar et al., 2010; Ahn and Antoniewicz, 2011; Sengupta et al., 2011), however they are expensive (Zamorano et al., 2010), and challenging from experimental and computational point of views (Quek et al., 2010). Consequently, most kinetic models are based on simplified networks for which extracellular metabolite and viable cell concentration measurements, attainable by analytical techniques commonly available in laboratories, are used to carry out the flux calculations.

Several kinetic models of CHO cells have modeled intracellular fluxes by defining one kinetic equation for each reaction in which intracellular metabolite concentrations are typically included (e.g. using convenience rate laws, Liebermeister and Klipp, 2006; Nolan and Lee, 2011; Ghorbaniaghdam et al., 2014; Robitaille et al., 2015). In contrast, the present study considers the pathway-based modeling approach at macroscopic level, in which macro-reactions represent metabolic pathways that link the extracellular substrates to products via the reactions of intracellular metabolism (see e.g., Provost, 2004). Thus, different pathways using the same reaction can occur in parallel and are modeled separately, e.g. by generalized Monod- or Michaelis–Menten-type equations including only extracellular metabolite concentrations (Provost, 2004; Provost et al., 2005; Gao et al., 2007; Dorka et al., 2009; Naderi et al., 2011; Zamorano et al., 2013).

The set of possible macro-reactions of a metabolic network can be obtained using pathway analysis tools, e.g., Extreme Pathways (EPs) or Elementary Flux Modes (EFMs) (Schuster and Hilgetag, 1994; Papin et al., 2004; Llaneras and Picó, 2010; Klamt and Stelling, 2003). Both EPs and EFMs define routes through a given metabolic network through sets of non-decomposable vectors that are unique to and that can describe all feasible steady-state flux distributions of the network (Papin et al., 2004). The EFMs provide all the possible non-decomposable routes through the network. Meanwhile, the EPs (which must be systemically independent (Papin et al., 2004)) can miss some important and physiologically meaningful routes found in the EFMs, making EFMs more suitable for certain applications (Klamt and Stelling, 2003). Indeed, most pathway-based kinetic models of hybridoma and CHO cells were based on EFMs rather than EPs (Provost, 2004; Provost et al., 2005; Gao et al., 2007; Dorka et al., 2009; Naderi et al., 2011; Zamorano et al., 2013).

Kinetic models of cell culture processes are often limited to certain prerequisites, e.g., carried out in a certain medium while none or few of the metabolites are depleted. The scenario of amino acid omission and/or depletion is relevant in cell culture (Wahrheit et al., 2014), however rarely accommodated in the kinetic models. Furthermore, diverse metabolic behaviors have been described by multiple models (Nolan and Lee, 2011) in which each scenario is modeled by a distinct set of reactions and/or parameters (Gao et al., 2007; Ghorbaniaghdam et al., 2014) and for which dynamic simulations can be achieved via switching functions (Provost et al., 2005; Zamorano et al., 2013).

The aim of the present work was to develop an approach to capture multiple metabolic states in one single kinetic model. The resulting study is a proof-of-concept focused on metabolic states obtained by varying the amino acid availability in the culture medium for a monoclonal antibody (mAb) producing CHO cell line. The model, named poly-pathway model, uses macro-reactions (i.e. the EFMs of a metabolic network) and kinetic equations with one set of parameters to capture the variations in growth rate and metabolite uptake/secretion rates triggered in pseudo-perfusion cultures when selected amino acids have been omitted, reduced or doubled in the medium.

2. Materials and methods

2.1. Cell line and media

A CHO-K1 cell line producing IgG monoclonal antibody product (mAb) was kindly provided by Selexis (Switzerland) and maintained in HyClone IC3TS SFM4CHO™ medium (Thermo Scientific). Two proprietary and chemically-defined media were kindly provided by Irvine Scientific (USA): IS-C and IS-0. The compositions of IS-C and IS-0 were identical except that IS-0 was obtained without sodium bicarbonate and amino acids, and a lower concentration of NaCl.

2.2. Cell culture maintenance, adaptation and expansion

Cell cultures were performed at 37 °C and 5% CO_2 in Minitron Incubators (INFORS HT, Switzerland). Cryopreserved CHO-K1 cells were thawed into medium supplemented with 8 mM L-glutamine (Irvine Scientific). The cells were routinely passaged in 125 mL shake flasks (Corning, USA) every 3–4 days to maintain them in exponential growth phase. For the experiments, they were weaned to IS-C medium over five passages and expanded in two 500 mL shake flasks during 4 days.

2.3. Preparation of media with varied concentrations of single amino acids

Stock solutions of amino acids (Sigma–Aldrich), except glutamine, were prepared in pure water or 1 M HCl. Concentrated IS-0 was prepared in pure water and supplemented with sodium bicarbonate (Fisher), sterile filtered (0.2 µm) and aliquoted. The amino acid stock solutions were added to create sixteen media with varied concentration of amino acids (Table 1), including a control (Ctrl) with amino acid balance as in IS-C. The pH, osmolality and final volume of each medium were adjusted before sterile filtration (0.2 µm). All the media were supplemented with 8 mM L-glutamine with the exception of Q0 supplemented with water to compensate for the dilution.

2.4. Pseudo-perfusion culture

Suspensions from the two expansion shake flasks were pooled and the cells were harvested by centrifugation at 1000 rpm (equivalent to 180 g) for 5 min (Jouan BR4i centrifuge, VWR International). The cells were seeded in sixteen 50 mL TubeSpin bioreactors (TPP, USA), one bioreactor for each medium, at 2 MVC/mL (MVC = 10^6 viable cells) and 12 mL working volume. The bioreactors were agitated at 200 rpm. After one day, pseudo-perfusion was started and carried out for 10 days to mimic steady-state conditions. The medium in each bioreactor was completely renewed on a daily basis by centrifugation of an appropriate volume of cell suspension followed by re-seeding of the cell pellet at 2 MVC/mL and 10 mL working volume.

2.5. Analytical methods

Samples from the pseudo-perfusion cultures were collected after each medium renewal and before each following medium renewal, and analyzed for viable cell concentration, viability, and concentrations of glutamine, glutamate, glucose, lactate and ammonium (NH_4^+) using a BioProfile analyzer (Nova Biomedical, USA). Sample supernatants were stored at −20 °C for subsequent analyses. The amino acid

Table 1
Media used in pseudo-perfusion culture. The media #1–15 are referred to by code specified by the one-letter abbreviation of the varied amino acid and its concentration level in percent relative to the concentration in #16 (Ctrl).

#	Amino acid	Level (%)	Code	#	Amino acid	Level (%)	Code
1	Alanine	0	A0	9	Glutamate	0	E0
2	Alanine	200	A200	10	Glycine	0	G0
3	Asparagine	0	N0	11	Threonine	200	T200
4	Asparagine	200	N200	12	Serine	0	S0
5	Aspartate	0	D0	13	Serine	200	S200
6	Aspartate	200	D200	14	Proline	200	P200
7	Glutamine	0	Q0	15	Cysteine	50	C50
8	Glutamine	200	Q200	16	–	–	Ctrl

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