Metabolic Engineering 13 (2011) 307–318

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Escherichia coli responds with a rapid and large change in growth rate upon a shift from glucose-limited to glucose-excess conditions

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ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form
24 February 2011
Accepted 18 March 2011
Available online 23 March 2011

Keywords:
Amino acid metabolism
Central metabolism
Intracellular metabolites-fluxes
In vivo kinetics
Stimulus response experiments

ABSTRACT

Glucose pulse experiments at seconds time scale resolution were performed in aerobic glucose-limited *Escherichia coli* chemostat cultures. The dynamic responses of oxygen-uptake and growth rate at seconds time scale were determined using a new method based on the dynamic liquid-phase mass balance for oxygen and the pseudo-steady-state ATP balance. Significant fold changes in metabolites (10–1/10) and fluxes (4–1/4) were observed during the short (200 s) period of glucose excess. During glucose excess there was no secretion of by-products and the increased glucose uptake rate led within 40 s to a 3.7 fold increase in growth rate. Also within 40–60 s a new pseudo-steady-state was reached for both metabolite levels and fluxes. Flux changes of reactions were strongly correlated to the concentrations of involved compounds. Surprisingly the 3.7 fold increase in growth rate and hence protein synthesis rate was not matched by a significant increase in amino acid concentrations. This poses interesting questions for the kinetic factors, which drive protein synthesis by ribosomes.

1. Introduction

In metabolic engineering studies, kinetic models help in the quantitative understanding of the cellular metabolism and in this way they can be used (by selecting genetic engineering targets) to improve the product yield (Stephanopoulos, 1994). A kinetic metabolic model consists of mass balance-based differential equations, which require the enzyme level, enzyme kinetics information and extracellular metabolite concentrations. These kinetic models predict the fluxes and intracellular metabolite levels as function of enzyme levels and extracellular concentrations, and therefore allow selecting for the enzymes, which for example mostly affect the product rate. Subsequently these selected changes in enzyme levels can be implemented by genetic engineering techniques.

Traditionally, kinetic models are set up using published *in vitro* kinetics of enzymes. This approach suffers from several problems: (i) for many enzymes, e.g. in product pathways, the *in vitro* kinetics have not been studied, (ii) enzymes that catalyze the same reaction in different organisms do seldom have the same kinetics due to specific mutations, (iii) *in vitro* kinetics might not apply to *in vivo* conditions (Teusink et al., 2000). These problems can only be avoided by focusing on the *in vivo* kinetics for all enzymes in the organism of interest.

The information on *in vivo* kinetics can be obtained from stimulus–response experiments in which cells, grown at steady-state, are perturbed by an external stimulus, and dynamic responses of the intracellular metabolites are monitored in a time window of tens to a few hundred seconds. In such a set-up the enzyme levels can be assumed constant and is therefore preferred compared to steady-state perturbations, which require additional measurements of changes in enzyme levels. Recent developments on modeling offer promising results on getting *in vivo* kinetics information from such data (Nikerel et al., 2009). Such rapid stimulus response experiments have been applied to *Saccharomyces cerevisiae* and *Penicillium chrysogenum* (Nasution et al., 2006; Theobald et al., 1997; Visser et al., 2004; Kresnowati et al., 2008).

Also for *Escherichia coli* these studies were performed, applying a sudden addition of a concentrated glucose solution to a glucose-limited aerobic chemostat culture (Buchholz et al., 2002; Chassagnole et al., 2002; Hoque et al., 2005; Schaefer et al., 1999; Schaub and Reuss 2008). In these previous experiments with *E. coli*, there are some critical issues: (i) the measurements were depending on either only broth sampling (Chassagnole et al., 2002; Schaub and Reuss 2008) or separation of extracellular metabolites by centrifugation (Buchholz et al., 2002; Chassagnole et al., 2002; Hoque et al., 2005; Schaefer et al., 1999), which leads to unreliable intracellular metabolite data (Taymaz-Nikerel et al., 2009); (ii) only glycolytic intermediates and adenine nucleotides were measured; (iii) the intracellular metabolites were analyzed using enzyme based

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1096-7176/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymben.2011.03.003

assays and MS-methods without metabolite specific internal standards; (iv) the limited (sometimes absence of) quantification of changes in fluxes after the pulse, such as possible secretion of by-products, possible changes in storage metabolism and especially the change in growth rate.

In this present work, the first issue is overcome by applying the differential method, which is required for proper intracellular metabolome analysis due to leakage problems in cold quenching of *E. coli* (Taymaz-Nikerel et al., 2009). Second and third issues are tackled by employing extended MS analytical protocols and the isotope dilution mass spectrometry (IDMS) method, which effectively decreases errors due to possible variations taking place in the sample processing and analysis (Wu et al., 2005), for a broader range of metabolites.

Related to the fourth issue mentioned above, it has been shown that in rapid pulse experiments fluxes such as growth rate in *S. cerevisiae* (Wu et al., 2006) and *P. chrysogenum* (Nasution 2007) can be quantified using the combination of off-gas (O_2/CO_2) measurements together with element (carbon and degree of reduction) and ATP balances. In the previous *in vivo* kinetic studies of *E. coli*, such an approach was not applied and assumptions were made such as the absence of product secretion and a non-changing growth rate after the perturbation. Such flux assumptions have significant consequences on elucidating the *in vivo* fluxes and therewith kinetics of enzymes. Finding ways to quantify growth rate changes in rapid pulse experiments within a time frame of seconds is essential but challenging because changes in biomass dry weight cannot be detected in this short time period. Even the isotope labeling techniques would not be suitable, because the time required for labeling of biomass macromolecules will be longer than the actual experiment time.

In this present work we focus on point (iv) by presenting a new method to resolve the dynamics of growth rate in a rapid pulse experiment, which is a prerequisite to calculate the dynamic fluxes. The intracellular metabolite levels were measured to observe the correlation between the calculated dynamic fluxes and the dynamic metabolite levels.

2. Materials and methods

2.1. Organism and chemostat conditions

The *E. coli* K12 MG1655 [λ^-, F^-, rph^{-1}, (fnr^-267)del] was cultivated in aerobic glucose-limited chemostat cultures (at 37 °C, pH 7) with 4 kg working mass in 7 l laboratory fermentors, controlled by weight (Applikon, Schiedam, The Netherlands). The dilution rate (D) was 0.1 h^{-1}. The mineral medium (Taymaz-Nikerel et al., 2009) contained 30 g/l glucose·H_2O, which allowed a steady-state biomass concentration between 10 and 11 gDW/l.

The operating conditions, measurement of the concentrations of O_2 and CO_2 in the off-gas, monitoring of dissolved O_2, medium feeding and steady-state conditions have been described previously (Taymaz-Nikerel et al., 2009). The steady-state was analyzed for biomass, residual glucose, off-gas (O_2/CO_2), possible secreted by-products and total organic carbon (TOC) for quantification of biomass lysis (Taymaz-Nikerel et al., 2009).

2.2. Glucose pulse experiment

To ensure sufficient oxygen transfer during the glucose pulse experiment, the aeration gas (normal air) was blended with pure oxygen as described by De Mey et al. (2010). A few hours before the pulse experiment was carried out, the gas inflow was changed from 1.67 l/min air to 1.67 l/min air+0.5 l/min O_2.

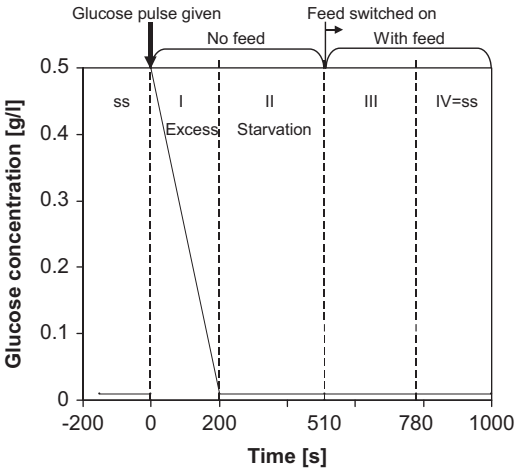

Fig. 1. Scheme of the glucose pulse experiment (ss: steady-state).

Broth and filtrate sampling were carried out to determine the concentration of intracellular metabolites at steady-state before blending (Taymaz-Nikerel et al., 2009). When, after blending, the increased DO concentration in the fermentor reached its new steady-state, broth and filtrate sampling for intracellular metabolites was repeated in order to investigate whether the increased dissolved O_2 and decreased CO_2 levels would have an effect on the metabolism. The pulse experiment was designed such that the initial bulk glucose concentration would be 0.5 g/l. The glucose solution was injected into the reactor (at $t=0$) via a sterile syringe, the volume change caused by the added glucose solution being less than 0.5%. At the same time that the glucose was injected in the chemostat, the feed and outflow pumps were stopped. After the pulse was given, the metabolite dynamics were followed by broth sampling from the fermentor during 20 minutes, using smaller sampling intervals for the first 6 minutes. Culture filtrate for determination of residual glucose and possible secreted by-products was obtained by rapid sampling with immediate cooling to about 1 °C, using cold stainless steel beads, and subsequent fast filtration, as described previously (Mashego et al., 2003). The glucose- feed and broth outflow were restarted 510 s after the pulse. The off-gas concentrations of oxygen and carbon dioxide, dissolved oxygen concentration and pH were monitored on-line during the whole transient. The scheme of the pulse experiment is shown in Fig. 1.

2.3. Rapid sampling

2.3.1. During steady-state

The differential method was used to measure the intracellular metabolite levels (Taymaz-Nikerel et al., 2009). The required broth and filtrate sampling was carried out as described in Taymaz-Nikerel et al. (2009). Briefly, a dedicated rapid sampling device (Lange et al., 2001) was used to rapidly withdraw 1 ml of broth from the fermentor into tubes containing 5 ml of 60% aqueous methanol precooled at −40 °C. The content of each tube was immediately mixed after sampling, by vortexing. Filtrate was sampled from the fermentor by syringe filtration using cellulose acetate filters with a pore size of 0.45 μm (Whatman GmbH, Germany) at room temperature without cold steel beads. After removal of the cells, the obtained filtrate was immediately mixed with 5 ml of 60% aqueous methanol precooled at −40 °C in order to process these samples in the same way as the broth samples. The exact amount of samples obtained (for broth and filtrate) was quantified gravimetrically. For every steady-state condition two