Metabolic Engineering 18 (2013) 78–85

Contents lists available at SciVerse ScienceDirect

Metabolic Engineering

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

Dynamic metabolic flux analysis of plant cell wall synthesis ☆

Xuewen Chen [a,*], Ana P. Alonso [b], Yair Shachar-Hill [a]

[a] Plant Biology Department and Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824, United States
[b] Molecular Genetics Department, Ohio State University, Columbus, OH 43210, United States

ARTICLE INFO

Article history:
Received 25 October 2012
Received in revised form
14 March 2013
Accepted 16 April 2013
Available online 30 April 2013

Keywords:
Dynamic metabolic flux analysis
Plant cell wall
Sucrose invertase
Metabolic engineering
Bioenergy crops

ABSTRACT

The regulation of plant cell wall synthesis pathways remains poorly understood. This has become a bottleneck in designing bioenergy crops. The goal of this study was to analyze the regulation of plant cell wall precursor metabolism using metabolic flux analysis based on dynamic labeling experiments. Arabidopsis T87 cells were cultured heterotrophically with ^{13}C labeled sucrose. The time course of ^{13}C labeling patterns in cell wall precursors and related sugar phosphates was monitored using liquid chromatography tandem mass spectrometry until steady state labeling was reached. A kinetic model based on mass action reaction mechanisms was developed to simulate the carbon flow in the cell wall synthesis network. The kinetic parameters of the model were determined by fitting the model to the labeling time course data, cell wall composition, and synthesis rates. A metabolic control analysis was performed to predict metabolic regulations that may improve plant biomass composition for biofuel production. Our results describe the routes and rates of carbon flow from sucrose to cell wall precursors. We found that sucrose invertase is responsible for the entry of sucrose into metabolism and UDP-glucose-4-epimerase plays a dominant role in UDP-Gal synthesis in heterotrophic Aradidopsis cells under aerobic conditions. We also predicted reactions that exert strong regulatory influence over carbon flow to cell wall synthesis and its composition.

1. Introduction

Plant cell wall material is a major component of plant biomass that mostly consists of sugar polymers (Somerville et al., 2004). It is the most abundant sugar resource on earth and a promising feedstock for biofuel production (Pauly and Keegstra, 2010). One major bottleneck in plant biomass based biofuel production is the resistance of plant cell wall to biological, chemical or physical deconstruction (Chundawat et al., 2011). Understanding plant synthesis pathways and their regulation may lead to breakthroughs in bioenergy crop design. Current research on plant cell wall synthesis is mainly focused on understanding how cell wall precursors were assembled to polysaccharides at the gene and protein levels (Bar-Peled and O'Neill, 2011; Carpita, 2012; Keegstra and Cavalier, 2011; Martinez et al., 2009; Zhang et al., 2011). The literature indicates that plant cell wall synthesis has not yet been predictively modeled at the metabolic network level. Metabolic flux analysis (MFA) provides the necessary tools to study the patterns of carbon flow and their regulation, which underpin plant growth and development.

In MFA internal carbon fluxes through metabolic networks are obtained using isotope labeling experiments (Antoniewicz et al., 2007; Nanchen et al., 2007; Sauer et al., 1999; Schmidt et al., 1997; Wiechert and de Graaf, 1997; Wiechert et al., 1997; Wittmann, 2002; Zamboni et al., 2005, 2009). Most MFA studies in plant research are performed at both isotopic and metabolic steady state, where labeled substrates are supplied to plant tissues/cells, carbon rearrangements of metabolites are tracked and fluxes are estimated by optimized fitting of internal fluxes to the measurements of labeling and external fluxes (substrate uptake and biomass production rates). It has been successfully applied in different plant systems to quantify carbon flow through central metabolism (for recent reviews see Allen et al., 2009; Chen and Shachar-Hill, 2012; Kruger et al., 2012; Kruger and Ratcliffe, 2009; Libourel and Shachar-Hill, 2008; Schwender, 2011). However, steady state MFA is limited to metabolic steps and pathways that involve rearrangement of the label (usually carbon). These limitations lead to the need for dynamic metabolic flux analysis.

Dynamic metabolic flux analysis (DMFA) is an MFA method based on transient labeling experiments (for reviews see Noack et al., 2011; Nöh and Wiechert, 2011; Wiechert et al., 2005). It involves tracking the propagation of labeling patterns through a metabolic network and fitting a kinetic model (in which ordinary differential equations are used to represent reaction rates) to the

Abbreviations: NDP-sugars, nucleotide-sugars; Api, apiose; Ara, arabinose; Fuc, fucose; Fru, fructose; Gal, galactose; GalA, galacturonic acid; GDP, guanosine diphosphate; Glc, glucose; GlcA, glucuronic acid; Man, mannose; P, phosphate; Rha, Rhamnose; Xyl, xylose; Suc, Sucrose; G(M)1P, glucose(mannose)-1-phosphate; G(M)6P, glucose(Man)-6-phosphate; F6P, fructose-6-phosphate; Gal1P, galactose-1-phosphate

☆Funding for this work was provided by the Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494).

* Corresponding author. Fax: +1 517 353 1926.
E-mail addresses: xwchen@msu.edu, wilson.xw.chen@gmail.com (X. Chen).

1096-7176/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymben.2013.04.006

dynamic labeling measurements obtained from pulse labeling experiments (without chase or washout). It is able to describe metabolic activities of a network *in vivo* and predict the metabolic effects on network fluxes in response to perturbations. Despite its advantages over steady state MFA, DMFA often requires the development of case specific analytical methods for metabolic intermediate measurements as well as the use of mathematical modeling. Computational frameworks for DMFA are emerging and have been demonstrated or applied in different biological systems (Leighty and Antoniewicz, 2011; Nöh et al., 2006; Selivanov et al., 2004, 2006; Young et al., 2008), but none of these frameworks has been implemented as widely applicable software. Due to these difficulties, DMFA in plant research has been mostly performed in small to medium sized metabolic networks that have little or no carbon skeleton rearrangements (Boatright et al., 2004; Colón et al., 2010; Knoke et al., 2009; Matsuda et al., 2003, 2005, 2007; McNeil et al., 2000; Rios-Estepa et al., 2008; Rohwer and Botha, 2001).

Here we have developed a DMFA model to study the regulation of plant cell wall precursor metabolism. We have monitored the time course of ^{13}C labeling in cell wall precursors and related sugar phosphates using a liquid chromatography tandem mass spectrometry method that we previously developed (Alonso et al., 2010). We used the DMFA model based on mass action reaction mechanisms to simulate the carbon flow in the cell wall synthesis network. The kinetic parameters of the model were determined using the time course labeling data of the cell wall precursors. The model allowed us to explore key reactions that control carbon flow to cell wall synthesis and predict *in vivo* metabolic regulation that may affect plant cell wall composition.

2. Materials and method

2.1. Chemicals

UDP-Xyl, UDP-Ara and UDP-GalA were purchased from the Complex Carbohydrate Research Center (University of Georgia). All the other NDP-sugars and hexose phosphates, excluding UDP-Api and UDP-Rha which are not commercially available, were purchased from Sigma. [U-$^{13}C_{Fru}$]Suc and [U-$^{13}C_{Glc}$] were, respectively, purchased from Omicron Biochemicals, Inc and Isotec (Sigma–Aldrich).

2.2. Arabidopsis cell culture, sampling and extraction of metabolites

Arabidopsis thaliana ecotype Columbia (cell line T87,Jouanneau and Péaud-Lenoël, 1967) were obtained from the Riken BioResource Center. Cell culture and sampling were performed as previously described (Alonso et al., 2010). Briefly, Arabidopsis T87 cells were cultured in a 7 day culture interval under continuous dark conditions at 22 °C and 120 rpm in a liquid minimal media (Alonso et al., 2010). T87 cells at log phase were centrifuged at 2000 rpm for 3 min at 22 °C. Supernatant was removed under sterile conditions. Cells were transferred to the fresh minimal media containing 100% ^{13}C labeled sucrose ([U-$^{13}C_{glc}$]-sucrose or [U-$^{13}C_{fru}$]-sucrose). Cells from 1 mL suspension culture were sampled by fast filtration *via* a nylon membrane filter (Whatman, 0.45 μm pore size and 47 mm diameter) and frozen in liquid nitrogen. Intracellular metabolites were extracted using boiling water (Alonso et al., 2010). The time course of ^{13}C labeling in cell wall precursors and related sugar phosphates were monitored using a liquid chromatography tandem mass spectrometry method (Alonso et al., 2010) until isotopic steady state was reached (Supplementary materials Tables S3 and S4). To measure cellular sucrose, fructose and glucose concentration, 3 mL cell suspension was filtered through the membrane filter and the filtrates on the membrane were washed with 6 mL media lacking sucrose. Cellular sugars were measured using Megazyme® sucrose/fructose/D-glucose kits.

2.3. External flux measurements

T87 cell growth was measured by monitoring the $OD_{600\ nm}$. Cell suspension was collected at different time points. Cells and cultured medium were separated by centrifugation at 13,000 rpm for 5 min

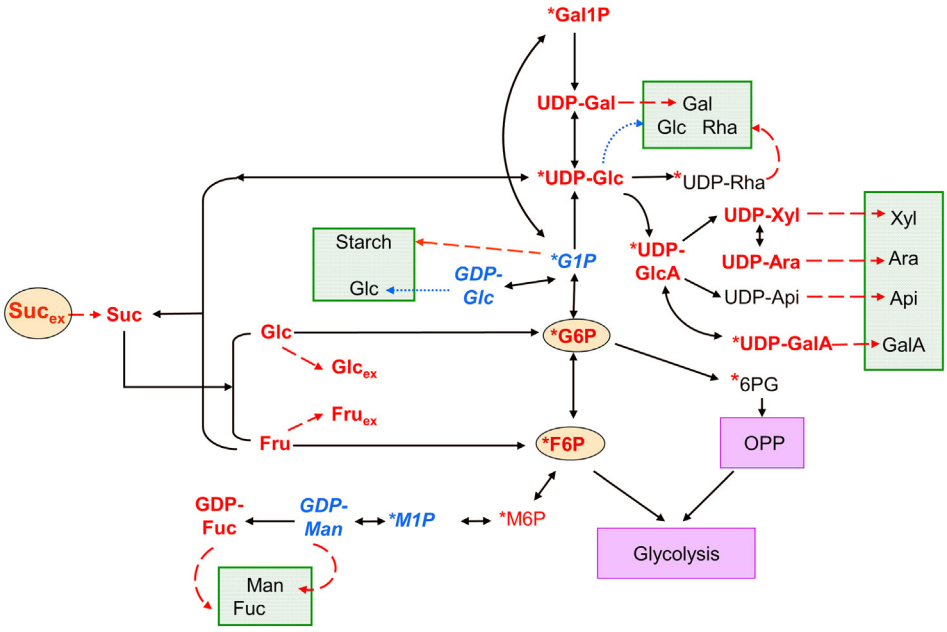

Fig. 1. Metabolic network model of plant cell wall synthesis. Bold font (dash line) represents concentration (flux) measurements were made. Star represents labeling measurements were made. Italic font represents concentration measurements that include indistinguishable quantities (GDP-Man and GDP-Glc; and M1P and G1P) and were measured as GDP-HEX and H1P, Dot line represents flux measurements that include indistinguishable quantities (GDP-Glc -> Glc and UDP-Glc -> Glc). Total glucose monomer content in cell wall was used to obtain the sum of two effluxes that depose glucose into cell wall. Circle represents the measured labeling was treated as inputs to the model. Boxes represents terminal of carbon flow.