Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 2632–2642
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Changes in flux pattern of the central carbohydrate metabolism during kernel development in maize

Christian Ettenhuber [a,1], Gertraud Spielbauer [b,1], Lilla Margl [b], L. Curtis Hannah [c], Alfons Gierl [b], Adelbert Bacher [a], Ulrich Genschel [b], Wolfgang Eisenreich [a,*]

[a] Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstr. 4, D-85747 Garching, Germany
[b] Lehrstuhl für Genetik, Technische Universität München, Am Hochanger 8, 85350 Freising, Germany
[c] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States

Received 20 May 2005; received in revised form 15 September 2005
Available online 7 November 2005

Abstract

Developing kernels of the inbred maize line W22 were grown in sterile culture and supplied with a mixture of [U–$^{13}C_6$]glucose and unlabeled glucose during three consecutive intervals (11–18, 18–25, or 25–32 days after pollination) within the linear phase of starch formation. At the end of each labeling period, glucose was prepared from starch and analyzed by ^{13}C isotope ratio mass spectrometry and high-resolution ^{13}C NMR spectroscopy. The abundances of individual glucose isotopologs were calculated by computational deconvolution of the NMR data. [1,2-$^{13}C_2$]-, [5,6-$^{13}C_2$]-, [2,3-$^{13}C_2$]-, [4,5-$^{13}C_2$]-, [1,2,3-$^{13}C_3$]-, [4,5,6-$^{13}C_3$]-, [3,4,5,6-$^{13}C_4$]-, and [U–$^{13}C_6$]-isotopologs were detected as the major multiple-labeled glucose species, albeit at different normalized abundances in the three intervals. Relative flux contributions by five different pathways in the primary carbohydrate metabolism were determined by computational simulation of the isotopolog space of glucose. The relative fractions of some of these processes in the overall glucose cycling changed significantly during maize kernel development. The simulation showed that cycling via the non-oxidative pentose phosphate pathway was lowest during the middle interval of the experiment. The observed flux pattern could by explained by a low demand for amino acid precursors recruited from the pentose phosphate pathway during the middle interval of kernel development.

Keywords: *Zea mays*; Kernel culture; NMR spectroscopy; Starch; Carbohydrate cycling; ^{13}C-glucose

1. Introduction

Grass seeds including wheat, maize, rice, barley and oats are the most important staple for human nutrition and have an important role in animal husbandry. Impressive increases of productivity have been obtained by classical breeding methods. Thus, the productivity of maize can be raised by approximately 1% per year (Duvick, 2001). Whereas plant genetics and biochemistry have been progressing rapidly in recent years, most noticeable with the sequencing of the genomes of *Arabidopsis thaliana* (Arabidopsis Genome Initiative, 2001; Yamada et al., 2003) and rice (Goff et al., 2002), transformation of this molecular information into enhanced yields of crop plants remains problematic.

It is generally agreed that the assessment of metabolite flux could broaden the basis for rational plant breeding. However, the reconstruction of flux parameters from genomic and proteomic data is faced with many uncertainties. Direct and sensitive methods are therefore required for the quantitative assessment of metabolite flux in plants.

Abbreviations: DAP, days after pollination; IRMS, isotope-ratio mass spectroscopy; NMR, nuclear magnetic resonance; PPP, pentose phosphate pathway.

* Corresponding author. Tel.: +49 89 289 13336; fax: +49 89 289 13363.
E-mail address: wolfgang.eisenreich@ch.tum.de (W. Eisenreich).

[1] These authors contributed equally to the work.

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.09.017

Stable isotopolog perturbation analysis can serve that purpose (Dieuaide-Noubhani et al., 1995; Glawischnig et al., 2001; Kruger et al., 2003; Eisenreich et al., 2004; Schwender et al., 2004; Sriram et al., 2004). The quasi-equilibrium state of isotope distribution can be perturbed by the introduction of isotope-labeled compounds into the system under study. Such a perturbation will subsequently spread through the entire metabolic network due to the catalytic activity of thousands of plant enzymes.

Pathways of the intermediary metabolism were identified and quantified in maize root tips growing in the presence of [1-^{13}C]glucose (Dieuaide-Noubhani et al., 1995; Edwards et al., 1998). The ^{13}C-enrichments of free glucose, sucrose, alanine, glutamate, and starch glucose were numerically fitted using a metabolic model including sucrose cycling, glycolysis, the transaldolase reaction of the pentose phosphate pathway (PPP), and the tricarboxylic acid cycle.

Analysis of glycolytic and PPP flux in developing embryos of *Brassica napus* (Schwender et al., 2003) revealed cycling between hexose phosphates and triose phosphates and the reversible transketolase reaction as the largest fluxes, while flux through the reversible transaldolase reaction was very low. More recently, an extended metabolic network model was applied to developing soybean embryos (Sriram et al., 2004), enabling the detection of compartmented parallel fluxes. Soybean embryos also showed intensive cycling between hexose phosphates and triose phosphates, but, in contrast to the situation in *Brassica*, there was a large flux through the oxidative part of the PPP and reversible transketolase and transaldolase fluxes were similar.

All these studies were based on the analysis of multiple metabolites (e.g., carbohydrates and amino acids). As shown in this paper, highly significant information about metabolite flux can also be obtained by the comprehensive analysis of a single metabolite, in this case glucose isolated from starch hydrolysate. The biosynthesis of starch in the storage tissue of monocotyledonous plants has been studied in considerable detail (for review, see Ball and Morell, 2003; Schultz and Juvik, 2004). In the endosperm, ADP-glucose is predominantly synthesized in the cytosol and transported into the amyloplast, where it is converted into starch by starch synthases, branching enzymes and debranching enzymes (James et al., 2003). However, while the topology of carbohydrate and starch metabolism is understood in some detail, quantification of in vivo fluxes within this metabolic network has been reported in few cases only.

Previously, we showed that [U–$^{13}C_6$]glucose is converted into a variety of ^{13}C-labeled isotopologs of starch glucose by developing maize kernels (Glawischnig et al., 2002). This experimental approach revealed the metabolic history of glucose, i.e., its extensive processing by the network of primary carbohydrate metabolism. Using an improved experimental setup and computational simulation, we now quantified the contribution of individual pathways to the metabolic cycling of glucose in maize kernels between 11 and 32 days after pollination (DAP). During the seemingly uniform process of starch deposition, both the metabolic flux pattern and the intensity of carbohydrate cycling showed appreciable changes.

2. Results

Kernels of the inbred maize line W22 were grown on tissue culture medium in the absence of ^{13}C label until 11 DAP, 18 DAP, or 25 DAP, respectively. Kernels were then transferred onto [U–$^{13}C_6$]glucose-containing medium for 7 days and subsequently harvested (cf. Fig. 1(a)). Starch was isolated from these kernels and hydrolyzed to glucose, which was used for analysis of ^{13}C-labeling patterns. Using the same tissue culture process, the accumulation of dry biomass was found to be approximately linear between 9 and 38 DAP (Fig. 1(b)).

In the ^{13}C-labeling medium, glucose with natural ^{13}C-abundance was present at 29-fold molar excess over [U–$^{13}C_6$]glucose. Total ^{13}C-enrichments of starch glucose from the 11–18 DAP, 18–25 DAP, and 25–32 DAP labeling experiments were determined by ^{13}C-isotope ratio mass spectrometry as 2.53%, 2.08%, and 1.87%, respectively. A successive decrease in the ^{13}C-enrichments is expected because the starch formed during the presence of [U–$^{13}C_6$]glucose is diluted by an increasing amount of preformed natural ^{13}C-abundance starch in the older kernels.

The isotopolog composition of glucose was determined from the quantitative analysis of high-resolution ^{13}C NMR spectra (Eisenreich et al., 2004). Due to ^{13}C^{13}C coupling, all ^{13}C signals of glucose appeared as complex

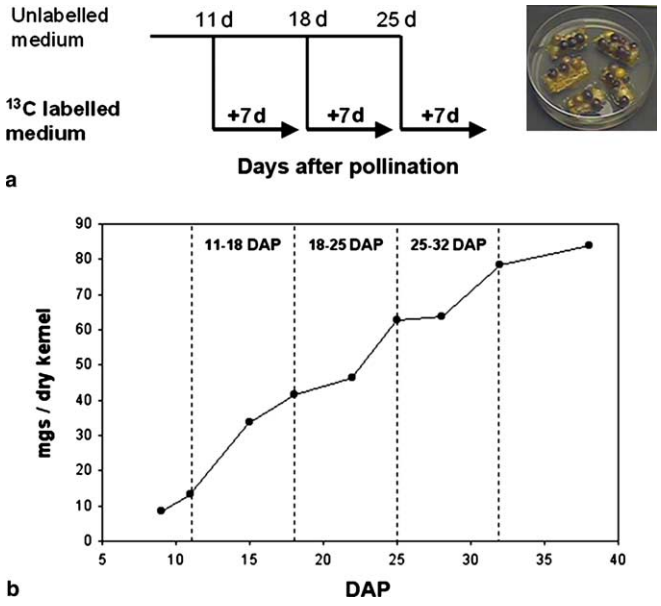

Fig. 1. (a) Experimental schedule for maize kernel culture and labeling scheme. At 11, 18, and 25 days after pollination, maize kernels were transferred onto labeled culture medium for 7 days and then harvested. (b) Increase of kernel dry weight in sterile culture from 9 to 38 DAP. The three intervals chosen for ^{13}C-labeling are indicated.