Metabolic Engineering 33 (2016) 1–11

Contents lists available at ScienceDirect

Metabolic Engineering

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

Metabolic engineering of light-driven cytochrome P450 dependent pathways into *Synechocystis* sp. PCC 6803

Artur Wlodarczyk [a,1], Thiyagarajan Gnanasekaran [a,1], Agnieszka Zygadlo Nielsen [a,d], Nodumo Nokolunga Zulu [b], Silas Busck Mellor [a], Manja Luckner [c], Jens Frederik Bang Thøfner [a], Carl Erik Olsen [a], Mohammed Saddik Mottawie [a], Meike Burow [a,e], Mathias Pribil [a], Ivo Feussner [b], Birger Lindberg Møller [a,d], Poul Erik Jensen [a,d,*]

[a] Copenhagen Plant Science Center, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark
[b] Georg-August-University, Albrecht-von-Haller Institute, Department of Plant Biochemistry, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany
[c] Biozentrum der Ludwig-Maximillians-Universität München, 82152 Planegg-Martinsried, Germany
[d] VILLUM Research Center for Plant Plasticity, Center for Synthetic Biology "bioSYNergy", Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark
[e] DNRF Center DynaMo, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form
23 October 2015
Accepted 27 October 2015
Available online 6 November 2015

Keywords:
Cyanobacteria
Synechocystis
Cytochrome P450
Metabolic engineering
Cyanogenic glucosides
Light-driven biosynthesis

ABSTRACT

Solar energy provides the energy input for the biosynthesis of primary and secondary metabolites in plants and other photosynthetic organisms. Some secondary metabolites are high value compounds, and typically their biosynthesis requires the involvement of cytochromes P450s. In this proof of concept work, we demonstrate that the cyanobacterium *Synechocystis* sp. PCC 6803 is an eminent heterologous host for expression of metabolically engineered cytochrome P450-dependent pathways exemplified by the dhurrin pathway from *Sorghum bicolor* comprising two membrane bound cytochromes P450s (CYP79A1 and CYP71E1) and a soluble glycosyltransferase (UGT85B1). We show that it is possible to express multiple genes incorporated into a bacterial-like operon by using a self-replicating expression vector in cyanobacteria. We demonstrate that eukaryotic P450s that typically reside in the endoplasmic reticulum membranes can be inserted in the prokaryotic membranes without affecting thylakoid membrane integrity. Photosystem I and ferredoxin replaces the native P450 oxidoreductase enzyme as an efficient electron donor for the P450s both *in vitro* and *in vivo*. The engineered strains produced up to 66 mg/L of *p*-hydroxyphenylacetaldoxime and 5 mg/L of dhurrin in lab-scale cultures after 3 days of cultivation and 3 mg/L of dhurrin in V-shaped photobioreactors under greenhouse conditions after 9 days cultivation. All the metabolites were found to be excreted to the growth media facilitating product isolation.
© 2015 International Metabolic Engineering Society. Published by Elsevier Inc. All rights reserved.

1. Introduction

Cyanobacteria, the evolutionary ancestor of plant chloroplasts (Cavalier-Smith, 2000), are prokaryotic photosynthetic organisms able to convert inorganic carbon, nitrogen and other mineral nutrients into biomass and a wide variety of fine chemicals. Throughout centuries humanity has used cyanobacteria as a renewable source of fertilizer and as a nutritious food source (Lem and Glick, 1985). However, cyanobacteria have the potential to become the 'green *Escherichia coli*' in synthetic biology (Branco dos Santos et al., 2014; Jensen and Leister, 2014; Møller, 2014).

Over the last decade, several efforts have been undertaken to use genetically modified cyanobacteria as a platform for the bioproduction of fatty acids, biofuels, phenylpropanoids and sugars (Gudmundsson and Nogales, 2015). Increasing availability of well-established tools for genetic manipulation and ease of introducing foreign genes into cyanobacteria render them suitable organisms for synthetic biology and metabolic engineering approaches (Berla

* Corresponding author at: Copenhagen Plant Science Center, Department of Plant and Environmental Sciences University of Copenhagen, Thorvaldsensvej 40 DK-1871 Frederiksberg C, Denmark.
E-mail address: peje@plen.ku.dk (P.E. Jensen).
[1] Equally contributed to the work.

http://dx.doi.org/10.1016/j.ymben.2015.10.009
1096-7176/© 2015 International Metabolic Engineering Society. Published by Elsevier Inc. All rights reserved.

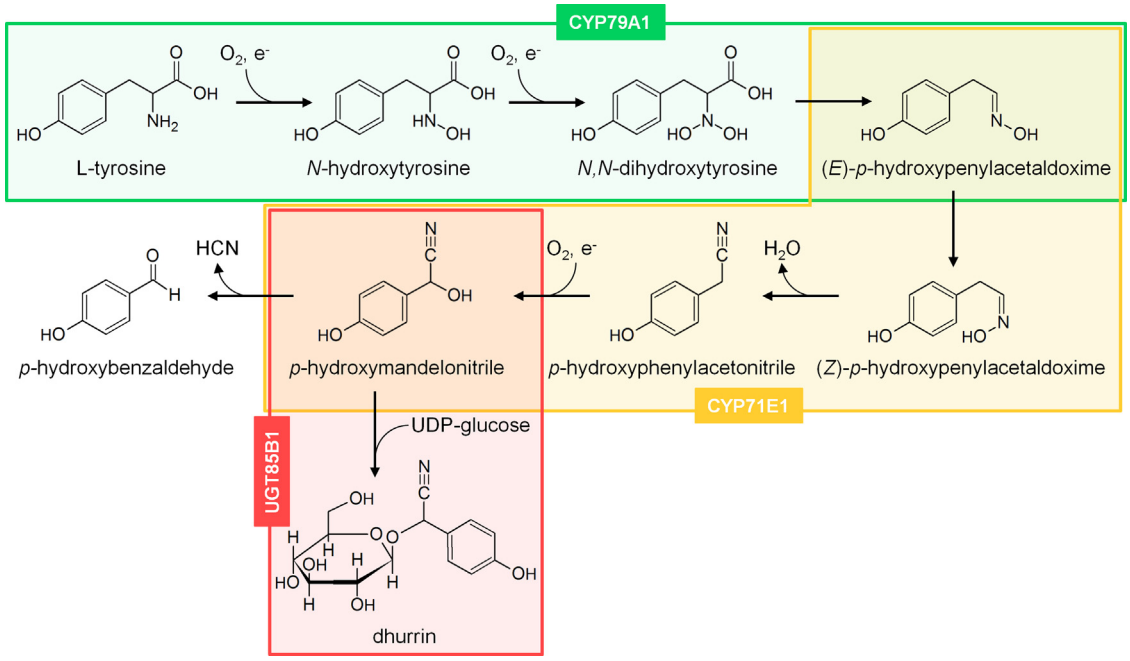

Fig. 1. The biosynthetic pathway leading to dhurrin. The CYP79A1 catalyzes conversion of L-tyrosine into (*E*)-*p*-hydroxyphenylacetaldoxime, which serves as a substrate for CYP71E1. *p*-hydroxymandelonitrile, a labile product of CYP71E1 catalytic activity is glycosylated by the UGT85B1 glycosyltransferase using the activated sugar UDP-glucose. In the absence of the glycosyltransferase, *p*-hydroxymandelonitrile dissociates into *p*-hydroxybenzaldehyde releasing toxic hydrogen cyanide.

et al., 2013). The ability to convert sunlight and carbon dioxide into high value chemicals by photosynthetic organisms is an attractive alternative to heterotrophic organisms like *E. coli* and *S. cerevisiae*, which rely on carbohydrate feed stocks. Currently, *Synechocystis* sp. PCC 6803 is one of the most used model cyanobacterial species for metabolic engineering and synthetic biology purposes but obstacles for efficient production of novel chemicals remain to be overcome (Yu et al., 2013). One challenge is to find strong inducible promoters for expression of gene(s) of interest. Several efforts based on modification of P_{trc} and P_{lac} promoter regions and ribosome binding site sequences have however offered considerable progress (Camsund et al., 2014; Heidorn et al., 2011; Huang et al., 2010). In addition, recent findings show that it is possible to use theophylline inducible riboswitches (Ma et al., 2014) and a synthetic IPTG-inducible promoter has been designed and successfully used in *Synechococcus* (Markley et al., 2015).

In plants, biosynthesis of secondary metabolites (bio-active natural products) such as terpenoids, phenylpropanoids, flavonoids and cyanogenic glucosides often requires highly specific and complex enzymatic reactions that involve a group of monooxygenases named cytochromes P450s (Gleadow and Møller, 2014; Laursen et al., 2015; Morant et al., 2003; Podust and Sherman, 2012). Plant P450s are membrane proteins anchored in the endoplasmic reticulum (ER) that perform a wide variety of stereo- and regio-specific hydroxylations by using electrons transferred from the NADPH-dependent diflavo protein cytochrome-P450-reductase (POR) (Laursen et al., 2011; Munro et al., 2007; Schlichting et al., 2000; Staniek et al., 2013). In plants, the expression levels of P450s and POR are in general very low and their activity is often limited by NADPH and substrate availability (Jensen et al., 2011). Thus, many high value plant secondary metabolites are synthesized at low levels, and typically only at specific developmental stages or following environmental challenges. In addition, their structural complexity often renders them difficult to chemically synthesize, which, due to their catalytic capabilities, has brought P450s in focus as major targets for synthetic biology and metabolic engineering applications towards alternative production of secondary metabolites (Jensen et al., 2012; Lassen et al., 2014b; Renault et al., 2014).

Previously we have demonstrated that P450s from the dhurrin pathway can be targeted to the chloroplast and inserted in the thylakoid membranes of *Nicotiana benthamiana* and remain functional upon transient expression (Nielsen et al., 2013). The relocation of a P450 dependent metabolic pathways to the thylakoid membranes renders the reducing power generated by PSI in the form of reduced ferredoxin (Fd) an abundant source of electrons for the P450s (Lassen et al., 2014b). So far only limited work has been carried out using cyanobacterial strains for expression of plant P450s. The first reported example was expression of *p*-coumarate-3-hydroxylase (CYP98A3) from *Arabidopsis thaliana* in *Synechocystis* sp. PCC 6803 for the production of caffeic acid (Xue et al., 2013). Recent work in our laboratory has shown that CYP79A1, the signature P450 in the dhurrin pathway in the monocotyledonous plant *Sorghum bicolor*, can be stably expressed in *Synechococcus* sp. PCC 7002 and directed to the thylakoid membranes by fusing the protein with the PsaM subunit of photosystem I (PSI) (Lassen et al., 2014a).

In this work, as a proof of concept, we engineered the entire dhurrin pathway from *S. bicolor* (Tattersall et al., 2001) comprising two membrane bound cytochromes P450s (CYP79A1 and CYP71E1) and a soluble glycosyltransferase (UGT85B1) into the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*). Dhurrin is a bio-active natural product belonging to the class of cyanogenic glucosides (Gleadow and Møller, 2014; Møller, 2010). The first enzyme in the pathway, CYP79A1 catalyzes conversion of tyrosine to (*E*)-*p*-hydroxyphenylacetaldoxime (Fig. 1) (Sibbesen et al., 1995), which is further converted to a cyanohydrin *p*-hydroxymandelonitrile by CYP71E1 (Kahn et al., 1997) and glycosylated by the glycosyltransferase (UGT85B1) to form the cyanogenic glucoside dhurrin (Fig. 1) (Hansen et al., 2003; Jones et al., 1999). By successfully targeting the dhurrin pathway P450s to the *Synechocystis* membranes we illustrate the potential of transferring eukaryotic P450s that are usually residing in the endoplasmic reticulum membranes to the prokaryotic membranes without the need to use targeting signals or change the native membrane regions. The P450 activity is supported directly by the photosynthetic reducing power for the biosynthesis of bioactive compounds. Expression of P450s did not affect the integrity of cyanobacterial membrane structures, suggesting

Download English Version:

https://daneshyari.com/en/article/31471

Download Persian Version:

https://daneshyari.com/article/31471

Daneshyari.com