Journal of Biotechnology 216 (2015) 100–109

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Glucosylation of aroma chemicals and hydroxy fatty acids

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
28 September 2015
Accepted 12 October 2015
Available online 19 October 2015

Key words:
Glycosyltransferase
Glycosides
Biotransformation
CaUGT2
SbUGTA1

ABSTRACT

To explore the utility of glycosyltransferases as novel biocatalysts, we isolated the glycosyltransferase genes *CaUGT2* and *SbUGTA1* from *Catharanthus roseus* and *Starmerella bombicola*, respectively and heterologously expressed them in *Escherichia coli*. The purified recombinant proteins were assayed with a variety of small molecule substrates. Carvacrol and its phenol isomer thymol are efficiently glucosylated by CaUGT2. The V_{max}/K_m ratios show that CaUGT2 exhibits the highest specificity towards carvacrol, followed by thymol, geraniol, eugenol, vanillin, menthol, and tyrosol. In contrast, SbUGTA1 accepts ω-hydroxy fatty acids and 1-alkanols as substrates. The V_{max}/K_m ratios indicate that SbUGTA1 exhibits the highest specificity towards 16-hydroxy palmitic acid, followed by octanol, decanol, and hexadecanol. In biotransformation experiments 23, 88 and 99% of octanol, 16-hydroxy palmitic acid, and decanol, respectively is converted into the corresponding β-glucosides by *E. coli* cells expressing *SbUGTA1* whereas those cells expressing *CaUGT2* glucosylate 18, 61, 77 and 97% of applied eugenol, thymol, vanillin, and carvacrol, respectively. To optimize the biotransformation rate, the effects of the concentration of IPTG, glucose, and substrate on the production of glucosides were tested. Taken together, this procedure is a simple operation, environmentally friendly, and is useful for the preparation of glycosides as additives for food and cosmetics.

1. Introduction

A glycoside is a molecule in which a sugar (glycone) is bound to another chemical structure (aglycone) via an acetal bond. In plants, glycosides play an important role in the accumulation, storage, and transport of hydrophobic substances (Franssen and Walton, 1999). Glycosylation regulates many properties of the aglycones, such as their bioactivity, solubility, stability and transport properties within the cell and throughout the plant. The storage of glycosides within the plant vacuole protects the plant from any form of toxicity exhibited by the free aglycone. Glycosides also function as precursors for flower fragrances in plants (Watanabe et al., 1993). Glycosides of flavonoids, such as quercetin and kaempferol, play a role in the UV-B radiation protection of white clover (Hofmann et al., 2000). In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. In addition, glycosides are found to function as a potent antioxidant in rats under oxidative stress (Tsuda et al., 2000).

Glycosyl conjugation of low molecular weight compounds is an efficient tool to enhance water solubility, to improve stability, and thereby to increase bioavailability and modify biological activity. Thus, glycosides find use in various industrial applications. In most cases the type of aglycone determines the industrial applicability of a glycoside. Glycosides of long chain alcohols or acids have surfactant and emulsifying properties, and therefore these glycosides are contained in detergents and cosmetics (de Roode et al., 2003). Terpene and phenolic glycosides are found to have antifungal and antimicrobial activity (Sahari and Asgari, 2013) and have attracted much attention in food industry (Mastelic et al., 2004). Glycosides of peptides and steroids are used in antitumor formulations and as cardiac-related drugs, respectively (Ooi et al., 1985; de Roode et al., 2003). Aroma glycosides are an important class of nonvolatile precursors of volatiles that are currently gaining increased interest and attention for their role in imparting unique aroma to food (Sarry and Günata, 2004; Sanchez Palomo et al., 2006; Schwab et al., 2015). Glycosides of flavors and fragrances are used as water soluble, storage-stable, and odorless pro-aroma molecules that can break down to the desired aroma compounds under controlled conditions (de Roode et al., 2001; Herrmann, 2007). Besides, glycosides of volatile compounds such as aromatic and terpene alcohols have been widely used in folk medicines (Mastelic et al., 2004).

Glycosides can be produced chemically or enzymatically. Currently, chemical preparation of glycosides will not meet EC food regulations, and therefore chemical preparation of glycosides is not applicable in the food industry. Recent legislation in the US and EU have classified "natural" flavors as only those that are extracted from natural sources or involve bioprocesses with precursors isolated from nature (Serra et al., 2005). This classification has led to a

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http://dx.doi.org/10.1016/j.jbiotec.2015.10.011
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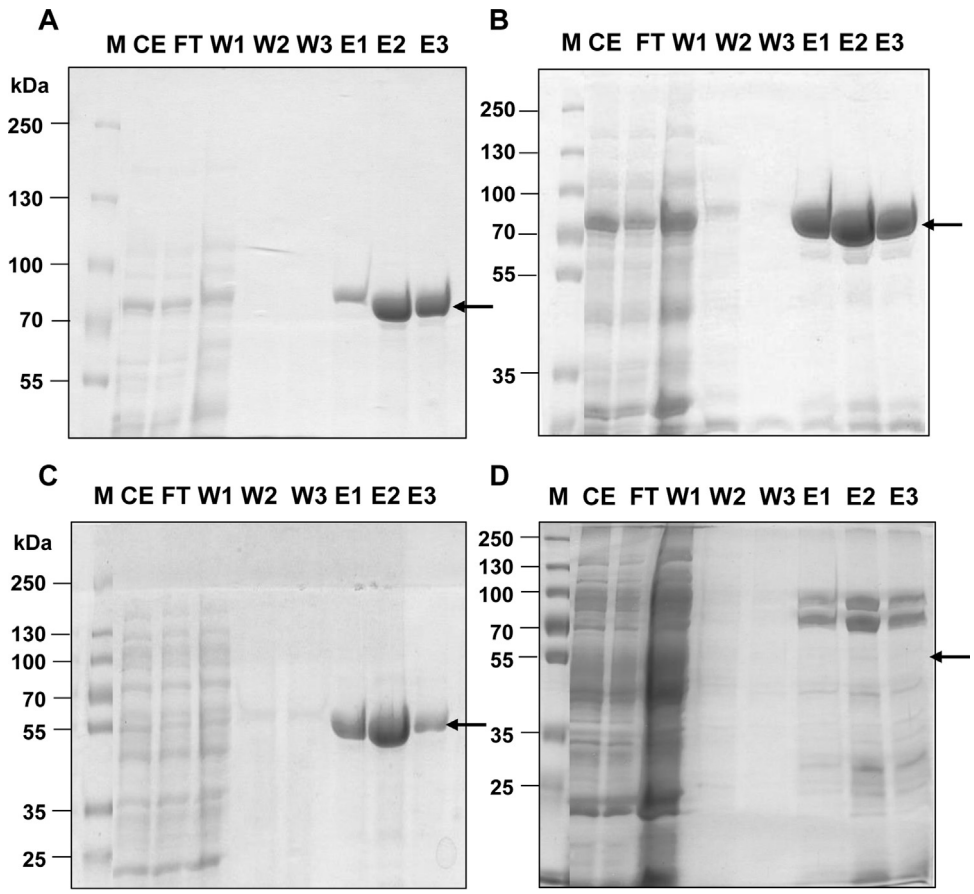

Fig. 1. Coomassie-stained polyacrylamide gel analysis of recombinant CaUGT2 and SbUGTA1 proteins expressed in the pGEX-4T1 and pQE30 vector systems. (A) *CaUGT2*-pGEX-4T1 (B) *SbUGTA1*-pGEX-4T1 (C) *CaUGT2*-pQE30 (D) *SbUGTA1*-pQE30. The crude protein extracts were incubated for 1 hour with the GST resin or IMAC Ni-charged resin to bind fusion proteins. After binding, the resins were washed 3 times with washing buffer (W1-W3). Afterwards, the recombinant proteins were eluted 3 times with elution buffer (E1–E3) and quantified by Bradford solution. M: Marker; CE: crude extract; FT: flow through; W: wash; E: elution. Purified proteins are indicated by arrows (→).

preference for bio-synthetic methods involving biotransformations rather than the use of chemistry. Thus, enzyme-catalyzed reactions are a good alternative to chemical synthesis.

In organisms, glycosylation reactions are catalyzed by glycosyltransferases (GTs) that transfer a saccharide moiety from an activated nucleotide sugar to a nucleophilic acceptor (Lairson et al., 2008). Small molecule GT proteins attach sugars to a wide range of acceptors, from hormones and secondary metabolites to biotic and abiotic chemicals and toxins in the environment (Ross et al., 2001). The enzymes are encoded by large multigene families and can be identified by a signature motif in their primary sequence, which classifies them as a subset of Family 1 GTs (http://www.cazy.org/). The activated sugar donor of plant GTs is typically UDP-glucose (UDP-Glc), although UDP-rhamnose, UDP-galactose, UDP-xylose, and UDP-glucuronic acid have also been identified as activated sugars for the transfer reactions. Single or multiple glycosylation of the acceptors can occur at —OH, —COOH, —NH_2, —SH, and—C groups (Bowles et al., 2006). A remarkably diverse array of GTs is present in plant cells as exemplified by more than 110 GT genes in the Arabidopsis genome (Paquette et al., 2003; Lim et al., 2003). Some GTs have been shown to exhibit broad substrate specificity (Taguchi et al., 2001; Kaminaga et al., 2004; Caputi et al., 2008) which makes them superior candidates to be used in enzyme-catalyzed transfer of sugars to aglycones. This would be an alternative approach to chemical synthesis of useful glycosides.

In the present paper, we describe the cloning and heterologous expression of two GTs, namely *CaUGT2* from *Catharanthus roseus* and *SbUGTA1* from *Starmerella bombicola* in *Escherichia coli*. To gain a better understanding of the molecular mechanism and the biochemical roles of CaUGT2 and SbUGTA1, and to explore the utility of GTs as novel biocatalysts, we analyzed the substrate specificities and kinetic parameters of the recombinant GT enzymes *in vitro* and investigated the optimal parameters for biotransformation. We then employed the UDP-sugar dependent GTs (UGT) as whole-cell biocatalysts in *E. coli* to synthesize a range of aroma and alkyl monoglucosides.

2. Materials and methods

2.1. Chemicals

Commercial chemicals were purchased in analytical grade from the following companies: oleic acid, decanol, octanol, hexanol, 1,6-hexandiol, hexadecanol, ricinoleic acid, 16-hydroxy-palmitic acid, geraniol, eugenol, thymol, tyrosol, menthol, cucurmin, carvacrol, 2-phenylethanol, vanillin (Sigma-Aldrich, Steinheim, Germany); 9(*S*)-hydroxyoctadecadienoic acid (9(*S*)-HOD), 13-hydroxyoctadecadienoic acid (13-HOD; Biozol Diagnostica, Eching, Germany); 18-carboxy-oleic acid (AnalytiCon Discovery GmbH, Potsdam, Germany); and UDP-[U-^{14}C]Glc (300 mCi/mmol; Biotrend, Germany).

2.2. Heterologous expression and purification of CaUGT2 and SbUGTA1

Total RNAs were isolated from *C. roseus* plant by CTAB extraction (Liao et al., 2004) and from *S. bombicola* using Presto SpinR Universal RNA purification kit (Huang et al., 2014). The first-strand