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Research review paper

Advances in the biotechnological glycosylation of valuable flavonoids

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ABSTRACT

The natural flavonoids, especially their glycosides, are the most abundant polyphenols in foods and have diverse bioactivities. The biotransformation of flavonoid aglycones into their glycosides is vital in flavonoid biosynthesis. The main biological strategies that have been used to achieve flavonoid glycosylation in the laboratory involve metabolic pathway engineering and microbial biotransformation. In this review, we summarize the existing knowledge on the production and biotransformation of flavonoid glycosides using biotechnology, as well as the impact of glycosylation on flavonoid bioactivity. Uridine diphosphate glycosyltransferases play key roles in decorating flavonoids with sugars. Modern metabolic engineering and proteomic tools have been used in an integrated fashion to generate numerous structurally diverse flavonoid glycosides. *In vitro*, enzymatic glycosylation tends to preferentially generate flavonoid 3- and 7-*O*-glucosides; microorganisms typically convert flavonoids into their 7-*O*-glycosides and will produce 3-*O*-glycosides if supplied with flavonoid substrates having a hydroxyl group at the *C*-3 position. In general, *O*-glycosylation reduces flavonoid bioactivity. However, *C*-glycosylation can enhance some of the benefits of flavonoids on human health, including their antioxidant and anti-diabetic potential.

Contents

Introduction

The natural flavonoids, especially their glycosides, are the most abundant polyphenols in foods and are of great general interest due to their diverse biological activities. Epidemiological and medical data indicate that dietary flavonoids play key roles in the prevention and management of chronic diseases such as cancer, diabetes, and cardiovascular conditions (Andrae-Marobela et al., 2013; Delmas and Xiao, 2012; Deng et al., 2013; Georgiev et al., 2011, 2012; Johnson et al., 2013; Xiao, 2013). The flavonoids (C_6–C_3–C_6) can be grouped into several subclasses such as flavones, flavonols, isoflavones, flavanones and flavan-3-ols, according to their chemical structures (Fig. 1). The flavonoids in plants always exist as α or β glycoside forms such as flavonoid glucosides, galactosides, rhamnosides, arabinosides and

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rutinosides (Fang et al., 2013; Pugliese et al., 2013; Taheri et al., 2013). The most numerous glycosylated flavonoids in food are flavone/flavonol *O*-glycosides and flavone *C*-glycosides (Rayyan et al., 2005, 2010; Zou et al., 2004). Glycosides of dihydrochalcones, aurones, and flavanones are rarely reported (Murata et al., 2013), but chalcone glycosides have received some research attention (Iwashina et al., 2009; Zhao et al., 2011). The flavonoid glycosides mainly occur as 3 or 7 *O*-glycosides, but the *C*-5, 6, 8 and 4′ positions are sometimes glycosylated as well. In some fruits such as apples, flavonols are usually *O*-glycosylated at the *C*-3 position (Tiberti et al., 2007).

Several qualitative reviews on flavonoids have been published since 2011. Veitch and Grayer (2011) described the sources, identification, bioactivities, biosynthesis, and ecological significance of 796 newly identified flavonoid aglycones and glycosides. The numbers of new flavone/flavonol O-glycosides identified between 2007 and 2009 were 31% and 60% greater, respectively, than the numbers of the same compound types reported between 2004 and 2006. However, the number of newly identified flavone *C*-glycosides decreased by 22% over the same period. Moreover, the dietary flavonoid *C*-glycosides have received less attention than their corresponding *O*-glycosides (Brazier-Hicks et al., 2009).

The biotransformation of flavonoid aglycones to glycosides plays a crucial role in flavonoid biosynthesis. As revealed by recent review (Plaza et al., 2014), the glycosylation of flavonoids increases their solubility and stability relative to flavonoid aglycones. In addition, most flavonoid drugs with clinical applications are glycosides, such as rutin (quercetin 3-*O*-rutinoside), which is administered in capsules, and puerarin (daidzein 8-*C*-glucoside) which is administered by injection. The main biological strategies that have been used to achieve flavonoid glycosylation in the laboratory involve metabolic pathway engineering and microbial biotransformation (Fig. 2). Biological approaches for glycosylating aglycones to glycosides have attracted considerable interest because they enable the formation of novel compounds with high stereo- and regio-selectivity under mild conditions. Moreover, biotransformation provides an environmentally friendly option for fine chemical synthesis. However, the biotechnological glycosylation of flavonoids has not previously been systematically reviewed. This review summarizes current knowledge regarding the biotechnological glycosylation of natural flavonoids and the effects of glycosylation on flavonoid bioactivity.

Enzyme pathways leading to flavonoid glycosylation and metabolic engineering approaches

Flavonoids primarily occur in plants and the human diet in the form of their *O*- and *C*-glycosides (Fang et al., 2013; Pugliese et al., 2013; Rayyan et al., 2010; Zou et al., 2004). Glycosylation is typically the final step in flavonoid biosynthesis and tends to significantly improve or facilitate the solubility, storage and stabilization of flavonoid aglycones (Jiang et al., 2008). Glycosyltransferases (GTs) catalyze the coupling of activated monosaccharide units to aglycones. At present, 94 GT families have been identified with different substrate specificities and levels of sequence similarity (CAZy database, April 2013). UDP-glucose glucosyltransferases (UGTs) belong to GT superfamily 1, and play vital roles in the glucosylation of flavonoids and other small molecules (Byoung et al., 2007; Cheynier et al., 2013; Jeong et al., 2012; Ji et al., 2006; Paquette et al., 2003; Thuan and Sohng, 2013). The UGTs with identified substrate specificities that have been described in the last 7 years are listed in Table 1.

Several UGTs that are known to have general roles in plant secondary metabolism exhibited broad substrate specificity *in vitro*, recognizing a wide range of substrates as acceptors. However, they also displayed high stereo- and regio-selectivity (Cheynier et al., 2013). For instance, two UGTs from *Allium cepa* exhibited different substrate- and regiospecificity profiles (Kramer et al., 2003). Some GTs can also convert flavonoid glycosides to other glycosides. For example, *Xanthomonas campestris* glucosylated hesperetin to its 3′-, 5-, and 7-*O*-glucosides, and cyclodextrin glucanotransferase can further convert hesperetin glucosides to hesperetin 3′-*O*-α-maltoside, hesperetin 5-*O*-α-maltoside, and hesperetin 7-*O*-α-maltoside (Shimoda and Hamada, 2010; Fig. 3).

UGT73G1 uses various flavonoids as substrates, glucosylating them selectively at the *C*-3, *C*-7 and *C*-4′ hydroxyl groups to yield flavonoid mono- and diglucosides. Conversely, UGT73J1 is only active towards isoquercitrin and genistein, and is regiospecific for the *C*-7 hydroxyl group (Kramer et al., 2003). UDP-flavonoid-3-*O*-glucosyltransferase from the seed coats of black soybean exhibits strict regio-selectivity for the *C*-3 hydroxyl moiety and exclusively glycosylates anthocyanidins and flavonols (Kovinich et al., 2010).

UGT78G1 from *Medicago truncatula* can glycosylate many flavonoids, including kaempferol, myricetin, formononetin, pelargonidin and cyanidin (Modolo et al., 2009). Moreover, it also catalyzes the reverse reaction, *i.e.* the removal of sugar moieties from glycosides. For example, it can glycosylate quercetin to quercetin 3-*O*-glucoside and also deglycosylate the resulting glucoside. The structures of UGT78G1 bound to UDP and myricetin have been determined at a resolution of 2.1 Å, revealing the nature of the interactions between the enzyme and its substrates (Modolo et al., 2009). Glutamate 192 was identified as a key amino acid in the reverse reaction by means of comparative structural analysis and mutagenesis (Modolo et al., 2009).

The enzyme UGT71G1 from *M. truncatula* is involved in saponin biosynthesis, although flavonoids such as quercetin and genistein are its preferred substrates *in vitro* (Achnine et al., 2005). UGT85H2 is a multifunctional GT that can use isoflavones, flavonols, and chalcones as substrates (Li et al., 2007). Jeon et al. (2009) predicted the substrates of a UGT (BsGT-3) from *Bacillus subtilis* based on molecular modeling and docking studies using a range of simulated test substrates

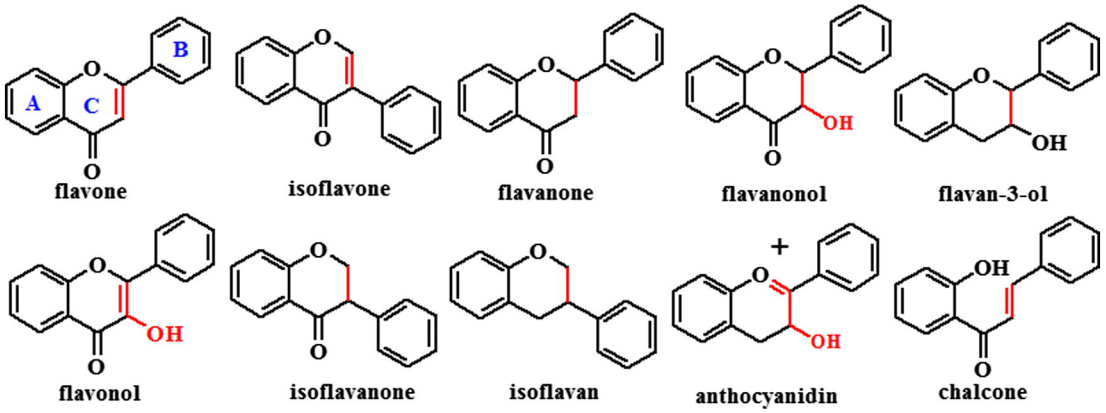

Fig. 1. Flavonoid skeletons and ring designations.

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