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Review

Methylation of flavonoids: Chemical structures, bioactivities, progress and perspectives for biotechnological production

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ABSTRACT

Among the natural products, flavonoids have been particularly attractive, highly studied and become one of the most important promising agent to treat cancer, oxidant stress, pathogenic bacteria, inflammations, cardio-vascular dysfunctions, etc. Despite many promising roles of flavonoids, expectations have not been fulfilled when studies were extended to the in vivo condition, particularly in humans. Instability and very low oral bioavailability of dietary flavonoids are the reasons behind this. Researches have demonstrated that the methylation of these flavonoids could increase their promise as pharmaceutical agents leading to novel applications. Methylation of the flavonoids via theirs free hydroxyl groups or *C* atom dramatically increases their metabolic stability and enhances the membrane transport, leading to facilitated absorption and highly increased oral bioavailability. In this paper, we concentrated on analysis of flavonoid methoxides including *O*- and *C*-methoxide derivatives in aspect of structure, bioactivities and description of almost all up-to-date *O*- and *C*-methyltransferases' enzymatic characteristics. Furthermore, modern biological approaches for synthesis and production of flavonoid methoxides using metabolic engineering and synthetic biology have been focused and updated up to 2015. This review will give a handful information regarding the methylation of flavonoids, methyltransferases and biotechnological synthesis of the same.

Contents

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1. Introduction

Polyphenols such as flavonoids, stilbenes are widely presented in plant kingdom and involved in various defense mechanisms including auto-defending against herbivores, stress tolerance, water-lost resistance, etc. Among them flavonoid have gained much interests due to their importantly medicinal and cosmetic properties [1]. Till date around 8000 naturally occurring flavonoids have been identified and characterized which are abundantly deposited in vegetables, stems, fruits, seeds, and other organs [2]. Structurally, flavonoids contain fifteen carbon atoms in their basic nucleus: two six membraned rings linked with a three carbon unit which may or may not be a part of the third ring. For convenience, the rings are labeled A, B, and C [3].

As most plants contain flavonoids, they are considered as traditional herbs to treat various types of diseases for long time such as increasing in immunological system via antioxidant, anti-inflammatory, anti-allergenic, anti-cancer properties [4–6]. Nowadays, several types of flavonoids such as quercetin, rutin, apigenin, etc. have been extensively used in single or mixed form to make functional food, cosmetic or drug and widely commercialized whole the world such as Quercetin B5 Plus Complex (viridian), rutin and vitamin C (Lamberts), etc.

Naturally, flavonoids are often in the type of glycosylated or methylated form in plants due to those structures are more stable, bioavailability as well as bioactivity. Glycosylation of flavonoids have been carried out by a biological tool, glycosyltransferase, in which the enzyme catalyzes for the attachment of sugar molecule into aglycone resulting in glycosides [7,8]. In the similar manner, methylation of hydroxyl group in flavonoids occurs in the presence of methyltransferase that attaches methyl moieties to aglycone to form methoxides. Methylation can occur via oxygen or carbon atom to form *O*-methylated or *C*-methylated compounds, respectively. Experimental data revealed that methylation of flavonoids resulted in dramatic change in pharmacological and biochemical properties of methylated compound in compared with its parent [9,10]. Thereby, it is one of the most effective ways to modify of natural products for drug discovery.

Depending on the reacted substrates, positions and donor groups, glycosylation and methylation may have different effects. Many promising applications of glycosylated flavonoids were not achieved when studies were extended to in vitro biological activity tests. For instance, when nonbenzoquinone geldanamycin was glycosylated, the glycosylated products demonstrated weaker biological activity compared to the original aglycone [11]. Additionally, in our recent preliminary studies, glycosylated genistein was subjected to biological activity tests. Not much improvement was seen in its anti-cancer activity, though it has an added advantage of having higher solubility than its parent compounds [12]. There are many unpublished results due to a lack of significant biological activity related to the glycosylation of flavonoids. This "*-enhances solubility*" tag of glycosylated analogues is true as exemplified by studies focused on the use of sugar conjugation: glycosylated compounds can greatly enhance drug solubility (up to >2-fold) and enhance uptake in vitro[13]. As the motive for various modifications of natural products like flavonoids is to increase their stability and biological activity, and since most of the glycosylated products showed only the increase in solubility and a lack of prominent biological activity (not in all cases though), methylation of these pharmaceutically significant flavonoids may give the compounds a competitive advantage.

Methylation of free hydroxyl groups in flavonoids dramatically increases their metabolic stability and enhances their membrane transport, facilitating absorption and greater oral bioavailability [14,15]. Supporting this, 7-hydroxyflavone, 7,4′-dihydroxyflavone, and 5,7-dihydroxyflavone (chrysin) were undetectable in tissue levels after administration to rats, whereas the corresponding methylated derivatives reached high tissue levels [16]. Mono and dimethylated flavones showed potent antiproliferative activity [17]; they inhibited carcinogenic-activating cytochrome P450 (CYP) transcription and activities [18], benzo[a]pyrene activating enzymes and DNA binding in human bronchial epithelial BEAS-2B cells [19], and aromatase, an important target in hormone-sensitive cancers [20]. Similarly, 7-*O*-methyl genistein and 7-*O*-methyl daidzein significantly inhibited TNF-α-induced invasion of HUVECs at 20 μM, a concentration at which no cytotoxicity was observed [21]. Furthermore, there have been several reports on compounds like rhamnetin [22–24], sakuranetin [25,26] and genkwanin [27,28]. These compounds are the methylated metabolites of quercetin, naringenin and apigenin, respectively, and quercetin is already in the clinical trial phase. These results and current research suggests that methylated forms have higher metabolic stability, oral bioavailabity and biological activity than unmethylated forms. The emphasis is the effect of methylation modification on original compounds, including increase in metabolic stability and enhancement of pharmaceutical properties. In this review, we have summarized the structure, bioactivities as well as strategies for biosynthesis of methylated flavonoids using systematic metabolic engineering.

2. Structure and bioactivity of methylated flavonoids

2.1. O-Methylated flavonoids

O-methylated derivatives are formed via attachment of methyl group with oxygen of hydroxyl moiety in flavonoid skeleton and considered as product of post-modification [29]. Due to numerous hydroxyl groups in flavonoid core, methylation positions of flavonoids are various. For example, structure of several *O*-methylated flavonoids are presented in Fig. 1A, extracted from *Cuphea* and *Diplusodon* [30], *Friesodielsia discolor* [31] or *Piper montealegreanum* [32].

2.2. C-Methylated flavonoids

Numerous *C*-methylated flavonoids have been mined from plant extract such as in *Pisonia grandis* roots [33], in some Myritaceae [34] or *Cleistocalyx operculatus* [35] (Fig. 1B). Bioactivities of *C*-methylated flavonoid have been checked as neuraminidase inhibitors for novel influenza H1N1 [35], antioxidant and radical scavenging effect [36,37].

It will be important to discuss occurrence and biological activities for other selected flavonoids and their methyl con-