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Regioselective formation of quercetin 5-*O*-glucoside from orally administered quercetin in the silkworm, *Bombyx mori*

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Abstract

The cocoons of some races of the silkworm, *Bombyx mori*, have been shown to contain 5-*O*-glucosylated flavonoids, which do not occur naturally in the leaves of their host plant, mulberry (*Morus alba*). Thus, dietary flavonoids could be biotransformed in this insect. In this study, we found that after feeding silkworms a diet rich in the flavonol quercetin, quercetin 5-*O*-glucoside was the predominant metabolite in the midgut tissue, while quercetin 5,4′-di-*O*-glucoside was the major constituent in the hemolymph and silk glands. UDP-glucosyltransferase (UGT) in the midgut could transfer glucose to each of the hydroxyl groups of quercetin, with a preference for formation of 5-*O*-glucoside, while quercetin 5,4′-di-*O*-glucoside was predominantly produced if the enzyme extracts of either the fat body or silk glands were incubated with quercetin 5-*O*-glucoside and UDP-glucose. These results suggest that dietary quercetin was glucosylated at the 5-*O* position in the midgut as the first-pass metabolite of quercetin after oral absorption, then glucosylated at the 4′-*O* position in the fat body or silk glands. The 5-*O*-glucosylated flavonoids retained biological activity in the insect, since the total free radical scavenging capacity of several tissues increased after oral administration of quercetin.

Keywords: Silkworm; *Bombyx mori*; Mulberry; Morus alba; Moraceae; Flavonoid; Flavonol; Quercetin; UDP-glucosyl transferase; Glucosylation; Antioxidant; Regioselectivity

1. Introduction

Uptake and utilization of dietary flavonoids is widespread in insects, in particular in the Lepidoptera. It has been reported that some insects sequester plant flavonoids into their body cuticles for protection against natural enemies, or into their wings to increase attractiveness to mates (Simmonds, 2003). Larvae of the silkworm *Bombyx mori* sequester flavonoids into their cocoons from the leaves of their host plant, the mulberry tree (*Morus alba*) (Fujimoto et al., 1959). Recently, Tamura et al. (2002) identified three flavonol glucosides, quercetin-5-*O*-glucoside (**5**), quercetin 5,4′-di-*O*-glucoside (**2**), and quercetin 5,7,4′-tri-*O*-glucoside from the cocoon shell. However, these compounds were not present in mulberry leaves, in which flavonol glycosides with a sugar group at the 3-*O* position in the C ring such as isoquercitrin (quercetin 3-*O*-glucoside), rutin (quercetin 3-*O*-rutinoside), quercetin 3-*O*-(6-malonylglucoside), and astragalin (kaempferol 3-*O*-glucoside) are naturally occurring (Doi et al., 2001; Katsube et al., 2006; Onogi et al., 1994). Thus, we can infer that flavonoids absorbed from their diet are modified in the insect for using these compounds to increase fitness. In insects, the formation of glucoside is the predominant pathway for dietary flavonoids (Hopkins and Ahmad, 1991; Lahtinen et al., 2006; Salminen et al., 2004; Wiesen et al., 1994), and the glucosylation of polyphenolics in insects is catalyzed by UDP-glucosyltransferase (UGT) (Ahmad and Hopkins, 1993; Rausell et al., 1997; Real et al., 1991), suggesting the possibility

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that a UGT enzyme that can transfer a glucose moiety to the C-5 position of the flavonols is functioning in *B. mori*.

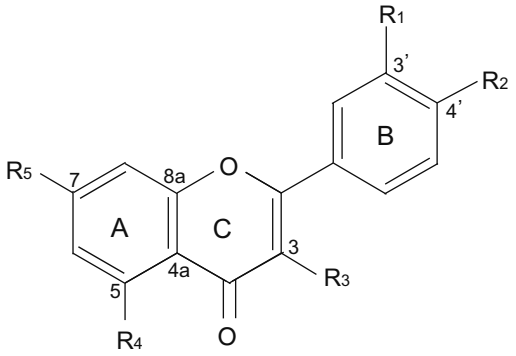
In the present study, we identified flavonoids distributed in the tissues of the silkworms fed a diet supplemented with flavonol quercetin (**10**), and showed that the flavonoids helped increase the anti-oxidative state of the tissues. Further, we determined the in vitro activity of UGT in transferring glucose to each OH group of quercetin (**10**) in the midgut, fat body, and silk glands, in order to establish the metabolic pathway of dietary quercetin (**10**) in the insect. This is the first study on the metabolism of flavonoid through the regiospecific glycosylation pathway in insects and we demonstrate the first example of glycosylation of quercetin (**10**) by a UGT enzyme with the preferred 5-*O* regioselectivity.

2. Results and discussion

2.1. Structural elucidation of cocoon shell flavonoids

Tamura et al. (2002) demonstrated that the cocoon shell of the silkworm (the race "Multi-Bi") contained quercetin 5-*O*-glucoside (**5**), quercetin 5,4′-di-*O*-glucoside (**2**), and quercetin 5,7,4′-tri-*O*-glucoside. In the present study, we isolated 9 flavonoids (compounds **1**–**6**, **10**–**12**) from the cocoon shell of the race "Pure-Mysore" reared on mulberry leaves. LC–MS analysis of the isolated flavonoids showed deprotonated ions $[M-H]^-$ with peaks at *m/z* 625 (compounds **1**–**4**), 609 (**11**), 463 (**5** and **6**), 447 (**12**), and 301 (**10**), respectively. In addition, reaction with β-glucosidase gave quercetin (**10**) or kaempferol, suggesting that compounds **1**–**4** were quercetin diglucosides, **5** and **6** were quercetin monoglucosides, **11** was kaempferol diglucoside, and **12** was kaempferol monoglucoside.

To identify the glycosylation sites of the flavonoids, the UV–Vis spectra were analyzed in the presence of the shift reagents as reported by Day et al. (2000). Obtained data identified quercetin 3,7-di-*O*-glucoside (**1**) quercetin 5,4′-di-*O*-glucoside (**2**), quercetin 3,3′-di-*O*-glucoside (**3**), quercetin 5,3′-di-*O*-glucoside (**4**), quercetin 5-*O*-glucoside (**5**), quercetin 7-*O*-glucoside (**6**), quercetin (**10**), kaempferol 5,4′-di-*O*-glucoside (**11**), and kaempferol 5-*O*-glucoside (**12**) (Table 1, Fig. 1). Among these flavonoids, quercetin 5,3′-di-*O*-glucoside (**4**) and kaempferol 5,4′-di-*O*-glucoside (**11**) are novel natural products. To confirm the chemical structures of these novel compounds, their structures were characterized by ^{1}H NMR, ^{13}C NMR, and high-resolution ESI mass spectroscopic analyses. The negative HR-FTICR-MS spectrum of **4** showed a prominent peak at *m/z* 625 $[M-H]^-$ (observed 625.1424, calculated 625.1410 for $C_{27}H_{29}O_{17}$); this value corresponds to a diglucoside



1 $R_1 = R_2 = R_4 = OH$, $R_3 = R_5 = O$-Glucose
2 $R_1 = R_3 = R_5 = OH$, $R_2 = R_4 = O$-Glucose
3 $R_2 = R_4 = R_5 = OH$, $R_1 = R_3 = O$-Glucose
4 $R_2 = R_3 = R_5 = OH$, $R_1 = R_4 = O$-Glucose
5 $R_1 = R_2 = R_3 = R_5 = OH$, $R_4 = O$-Glucose
6 $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = O$-Glucose
7 $R_1 = R_2 = R_4 = R_5 = OH$, $R_3 = O$-Glucose
8 $R_1 = R_3 = R_4 = R_5 = OH$, $R_2 = O$-Glucose
9 $R_2 = R_3 = R_4 = R_5 = OH$, $R_1 = O$-Glucose
10 $R_1 = R_2 = R_3 = R_4 = R_5 = OH$
11 $R_1 = H$, $R_3 = R_5 = OH$, $R_2 = R_4 = O$-Glucose
12 $R_1 = H$, $R_2 = R_3 = R_5 = OH$, $R_4 = O$-Glucose

Fig. 1. Structure of flavonoids either isolated or detected from the silkworm, *Bombyx mori*.

Table 1
Properties of flavonoids isolated from the cocoon shell of the silkworm, *Bombyx mori* (Race: Pure Mysore)

Cocoon flavonoid	Peak wavelength (nm)[a]					Aglycone	$[M-H]^-$[b] (*m/z*)
	MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc		
1 Quercetin 3,7-di-*O*-glucoside	257, 358	266, 402	276, 436	272, 406	263, 415	Q[c]	625
2 Quercetin 5,4′-di-*O*-glucoside	251, 360	269, 317, 394	263, 420	263, 421	273, 382	Q	625
3 Quercetin 3,3′-di-*O*-glucoside	254, 365	272, 431	265, 423	265, 425	262, 383	Q	625
4 Quercetin 5,3′-di-*O*-glucoside	250, 364	272, 324, 414	262, 425	262, 425	274, 394	Q	625
5 Quercetin 5-*O*-glucoside	253, 368	271, 323, 411	272, 451	264, 429	267, 392	Q	463
6 Quercetin 7-*O*-glucoside	256, 373	Decomposed	269, 441	266, 432	259, 384	Q	463
10 Quercetin	256, 372	Decomposed	272, 450	267, 432	273, 385		301
11 Kaempferol 5,4′-di-*O*-glucoside	259, 358	275, 397	266, 418	265, 419	272, 380	K[d]	609
12 Kaempferol 5-*O*-glucoside	259, 362	375, 323, 405	268, 424	267, 423	273, 388	K	447

[a] UV spectral data were recorded in methanol and after addition of various shift reagents NaOMe, $AlCl_3$, $AlCl_3$+HCl, NaOAc.
[b] Deprotonated ion.
[c] Quercetin.
[d] Kaempferol.