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Mechanism evaluation of the interactions between flavonoids and bovine serum albumin based on multi-spectroscopy, molecular docking and Q-TOF HR-MS analyses

Ling Fu [a,c], Yiqun Sun [a,c], Lina Ding [a,c], Yangyang Wang [a,c], Zhen Gao [a,c], Zhen Wu [a,c], Shaomin Wang [b], Wen Li [a,c], Yuefeng Bi [a,c,*]

[a] Zhengzhou University School of Pharmaceutical Science, Ke Xue Da Dao 100, Zhengzhou 450001, PR China
[b] Zhengzhou University School of Chemistry and Molecular Engineering, Ke Xue Da Dao 100, Zhengzhou 450001, PR China
[c] Collaborative Innovation Center of New Drug Research and Safety Evaluation, Henan Province, Ke Xue Da Dao 100, Zhengzhou 450001, PR China

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Apigenin (PubChem CID: 5280443)
Acacetin (PubChem CID: 5280442)
Tricin (PubChem CID: 5281702)
5,3′,4′-Trihydroxy-6,7-dimethoxyflavone (CAS 34334-69-5)
5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone (CAS 76015-42-4)
Linarin (PubChem CID: 5317025)

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ABSTRACT

The mechanism of interactions between a flavonoid glycoside (linarin) and 6 flavonoids with various hydroxyl and methoxyl substituents (luteolin, apigenin, acacetin, tricin, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone, and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone) and bovine serum albumin (BSA) were investigated by multi-spectroscopy, molecular docking, and quadrupole (Q)-time of flight (TOF) high resolution (HR) mass spectrometry (MS). Fluorescence spectra and molecular docking predicted that each of the flavonoids had only one probable binding site inside the hydrophobic cleft of BSA. The binding constants appeared to correlate positively with the number of hydroxyl groups, and negatively with the number of methoxyl groups. In addition, hydroxyls on ring B bound more easily with BSA than those on ring A. The change in conformation of BSA after binding suggested that the quenching mechanism was static quenching combined with nonradiative energy transfer. The results of Q-TOF HR-MS were consistent with fluorescence quenching and molecular docking.

1. Introduction

Flavonoids are important phytonutrients that are widely distributed in fruits, vegetables, nuts, and beverages (e.g., wine) (Bravo, Silva, Coelho, Boas, & Bronze, 2006) and propolis (Volpi & Bergonzini, 2006). Flavonoids have shown various biological and pharmacological effects, such as improving immunity, and antioxidant (Rodrigues, Pérez-Gregorio, García-Falcón, Simal-Gándara, & Almeida, 2011), anti-inflammatory (Kobori, Masumoto, Akimoto, & Takahashi, 2009), antifungal (Salas, Céliz, Geronazzo, Daz, & Resnik, 2011), and antitumor (Huang et al., 2010) activities, which are believed to be beneficial to our health. These effects are reportedly related to the hydroxylation and methoxylation patterns of flavonoids and the conjugation and resonance between the A- and B-rings (Russo, Toscano, & Uccella, 2000). However, it is not understood definitively how these patterns and effects translate to beneficial biological functions.

Serum albumins are greatly important for transport of agents in the circulatory system, for example forming non-covalent complexes of drug-proteins that affect the absorption, distribution, concentration, metabolism, or excretion of drugs (Li, Zhu, Xu, & Ji, 2011, Bani-Yaseen, 2011; Dufour & Dangles, 2005). The most

* Corresponding author at: Pharmaceutical Science, Zhengzhou University School, KeXueDaDao 100, Zhengzhou 450001, Henan, PR China.
E-mail address: zzubyf@126.com (Y. Bi).

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common and easily obtained serum albumin is bovine serum albumin (BSA). BSA has been used in toxicological studies and the design of medical drugs that incorporate flavonoids (Machicote, Pacheco, & Bruzzone, 2010; Singh & Mitra, 2011). Recent investigations suggest that binding with BSA may affect the absorption, bioavailability, and activities of flavonoids (Bolli et al., 2010), and may depend on the structure of the flavonoid.

The main methods currently used to elucidate interactions between compounds and proteins are multi-spectroscopic analysis, molecular docking, and electrospray ionization (ESI)-mass spectrometry (MS), either singly or in combination. Papadopoulou, Green, and Frazier (2005) used tryptophan fluorescence quenching to rank the binding affinity of studied flavonoids (quercetin > rutin > epicatechin = catechin), and concluded that the binding of flavonoids and BSA did not change the molecular conformation of the BSA. Zhang, Wang, and Pan (2012) used multi-spectroscopic approaches to study the interaction between diosmetin and human serum albumin. This group concluded that individual human serum albumin molecules became larger after interaction with diosmetin, and binding was driven mainly by hydrophobic interactions and hydrogen bonds. Feroz, Mohamad, Bujang, Malek, and Tayyab (2012) used multi-spectroscopy and molecular docking to draw a similar conclusion. Sekar, Kailasa, Chen, and Wu (2014), Ngu-Schwemlein, Lin, Rudd, and Bronson (2014) and Cubrilovic et al. (2012) used ESI-MS to investigate the interaction between metal ions and amoxicillin or multi-cysteinyl peptides.

Multi-spectroscopic approaches are reportedly so sensitive that slight changes in conditions can lead to great changes in results (Papadopoulou et al., 2005; Shi, Zhang, Chen, & Peng, 2011). Molecular docking can not only present the structure of proteins visually, but also show 3-dimensionally the positions of the hydrogen bond between flavonoids and proteins. However, quadrupole (Q)-time of flight (TOF) high resolution (HR) mass spectrometry (MS) can reveal the binding between flavonoids and proteins intuitively, by analyzing the molecular weight of the drug–protein complex. Moreover, the complex can be observed directly using very little voltage, making the non-covalent complex lose charge without interfering with the structure of the complex.

Previous studies have investigated the interactions between BSA and various flavonoids that have different substituents. Examples include different quantities or locations of hydroxyls on ring C (e.g., quercetin, luteolin, and taxifolin) (Shi et al., 2011), ring B (catechin,7-hydroxyflavone, chrysin, baicalein) (Xiao et al., 2008), and ring A (galangin, kaempferol, quercetin, and myricetin) (Xiao, Cao, Wang, Yamamoto, & Wei, 2010), methoxyl on ring B (diosmetin) (Zhang et al., 2012), and chlorine and hydroxyl on ring B and ring C (genistein, 8-chlorogenistein, and 3′,8-dichlorogenistein) (Zhang, Wang, Yan, & Xiang, 2011). Most of these studies investigated only one-substituent flavonoids. However, although in natural products hydroxyl, methoxyl, and glycoside groups commonly exist together in one compound, few studies have focused on multiple-substituent flavonoids, especially hydroxyls together with methoxyls or glycoside. Investigations of the interactions between proteins and flavonoids that contain multiple substituents are of great importance to drug design research.

In the current study, to increase the accuracy of results we considered for the first time analyses based on multi-spectroscopy, molecular docking, and Q-TOF HR-MS, to explore the mechanism of interactions between BSA and flavonoids that contain hydroxy, methoxyl, and glycoside substituents in different quantities and at different locations. In this way, we provide more mechanistic support and evidence for the further design of flavonoid-derived leading compounds.

2. Materials and methods

2.1. Chemicals and reagents

Seven flavonoids (Fig. 1) with purities of >98% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). However, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone were separated inhouse. BSA was purchased from Roche (Basel, Switzerland). Methanol (MeOH), ammonium acetate (CH_3COONH_4), and ammonia monohydrate ($NH_3{\cdot}H_2O$) at analytical grade, were purchased from Kermel (Tianjin, China). The water used in all experiments was doubly distilled. The stock BSA solution (10 μM) was prepared in ammonium acetate buffer (0.01 M, pH 7.4) and then diluted to the required concentrations. The 7 flavonoids were initially dissolved in a minimum amount of methanol. All stock solutions were stored at 0–4 °C.

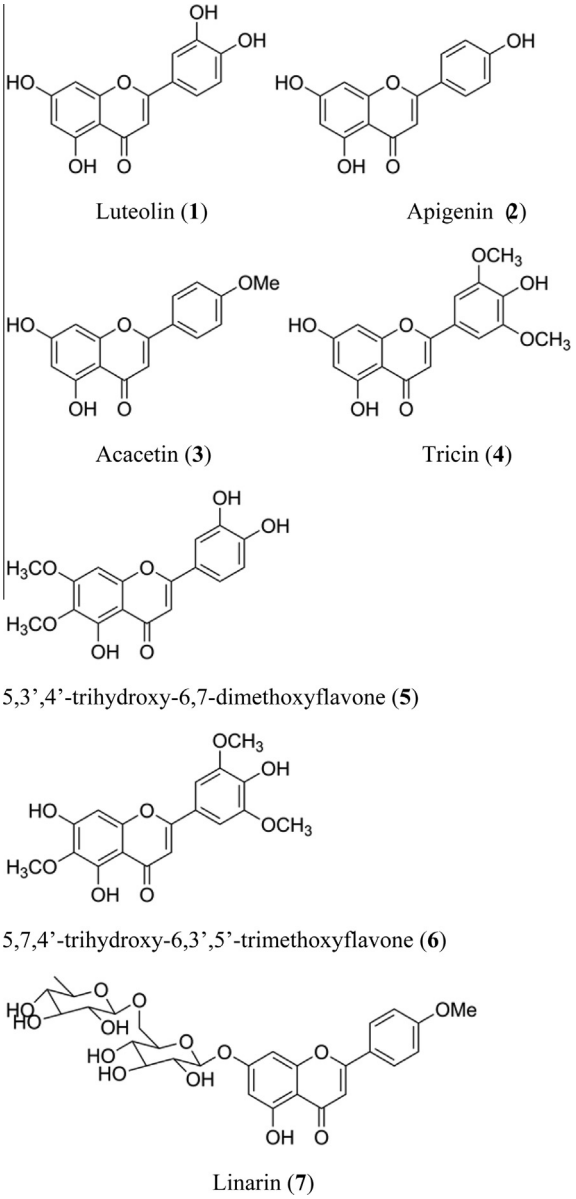

Fig. 1. Molecular structures of luteolin, apigenin, acacetin, tricin, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone, 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone, andlinarin.