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Structural effects on the conformational transition of transferrin induced by binding of flavonoids with different numbers and positions of hydroxyl groups

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Abstract

The effects of conformational changes to transferrin induced by the binding of flavonoids with different numbers and positions of hydroxyl groups were explored using spectroscopic and molecular modeling methods. The flavonoid hydroxyl group is not necessary for conformation changes of transferrin. However, the binding ability was found to increase with increasing numbers of hydroxyl groups and further conformational changes were observed. By molecular modeling calculations, intermolecular energy including van der Waals and electrostatic interactions, together with hydrogen bonding are found to have important roles in binding of flavonoids to transferrin. Additionally, the positions of the hydroxyl groups also affect the binding ability because they can alter the relative acidity of the hydroxyl groups, thereby changing the hydrogen bonding ability. Our results have indicated a mechanism for the interactions between flavonoids and transferrin, and provide information for possible flavonoid modification and design of methods to deliver drug molecules via transferrin to target tissues and cells effectively.

Keywords: Transferrin; Flavonoid; Hydrogen bonding; Conformational transition

1. Introduction

Proteins of the transferrin family – serum transferrin, lactoferrin (Lf), and ovotransferrin – have the characteristic ability to bind, tightly but reversibly, two Fe^{3+} ions together with two associated CO_3^{2-} ions [1]. In humans, transferrin is only 30% saturated with iron, and the vacant sites can bind other metals and act as a natural carrier for anticancer metal ions [2,3] and other chemotherapeutic drugs [4], because transferrin receptors are overexpressed on the surface of tumor cells [5,6]. Transferrin has also been used as a carrier or targeted ligand to deliver anticancer drugs in the form of drug conjugates [7]. However, there have been few reports on the direct binding of transferrin with natural drug molecules through non-covalent bonding, which might be the case for most drug delivery in humans.

The flavonoids are a large group of polyphenolic natural products, widely distributed in higher plants [8]. Such compounds have wide biological activities and important therapeutic applications, including anticancer, antitumor, anti-inflammatory, and anticoagulant drugs [9,10]. Interestingly, many biologically active flavonoids can affect various proteins including enzymes. Thus, understanding the interactions of flavonoids with proteins is important to interpret their biological activity [11]. For example, quercetin, which is abundant in the human diet, is among the group of phytoestrogens suggested to reduce risks of certain cancers [12,13]. Several biochemical and molecular biological investigations have revealed that proteins are often the "target" for therapeutically active flavonoids of natural and synthetic origin [14]. Therefore, investigation on the interaction between flavonoids and proteins is needed to explore the distribution, biotransformation, and ultimately the mechanism of action of flavonoids.

Transferrin (Tf) and human serum albumin (HSA) are two serum proteins involved in the transport of drug molecules. Most research has focused on the interaction between drug molecules

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and HSA [15]. However, few reports have assessed the interaction of flavonoids with transferrin at the molecular level, although the binding of metals to transferrin has intensively investigated in the past [16]. Our previous work has shown that the conformational change of Tf could be induced by a natural compound, quercetin [17]. Owing to the contribution of electrostatic forces to the total binding energy (the sum of van der Waals and electrostatic forces) and strong hydrogen bonding between the hydroxyl groups in quercetin and the polar amino acid residues in transferrin, it may be expected that both electrostatic forces and hydrogen bonding would be important for the binding of quercetin to transferrin. In this paper, we expand on our earlier work and further examine the interaction between Tf and flavonoids with different numbers of hydroxyl groups to understand the effect on the binding of flavonoids to Tf. Our aims are to determine (a) whether the hydroxyl group has an important role in the binding of flavonoids to transferrin; and (b) the structure–function relationship of flavonoid and transferrin binding to improve the targeting delivery ability by transferrin.

2. Materials and methods

2.1. Materials

Human serum transferrin (abbreviated as Tf, Catalog No. T3309, Lot No. 124K0807, 98% purity) was purchased from Sigma–Aldrich Co., and used without further purification. Flavone, 3-hydroxyflavone, galangin, luteolin, and kaempferol were purchased from Sigma–Aldrich Co. and the molecular structures are shown in Fig. 1. Their purities have been confirmed by mass spectrometry (MS) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra. The pH 7.4 phosphate buffer saline (PBS) solution containing 3 mM NaH_2PO_4, 7 mM Na_2HPO_4, and 5 mM NaCl was prepared with double-distilled water. All stock solutions of the above flavonoids were first prepared in spectroscopic-grade ethanol and then diluted in PBS to obtain the final solutions (the final ethanol concentration in buffer was <1%, v/v). Stock Tf solution was prepared in phosphate buffer. All reagents and solvents were of analytical grade.

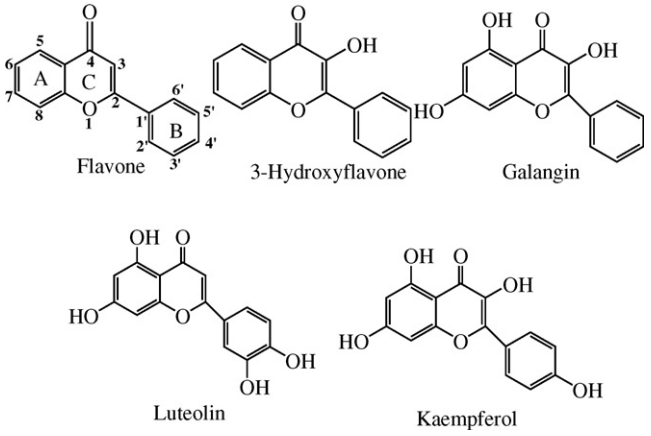

Fig. 1. The structural formulae of flavonoids.

2.2. Spectroscopic measurements

The spectra data were recorded after addition of Tf to flavonoids, and after incubation for 12 h. All the absorption experiments were performed with a 1-cm cuvette on a Shimatzu UV-1601PC spectrometer. The fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrometer with λ_{ex} at 290, 320, 350, 340, and 360 nm for flavone, 3-hydroxyflavone, galangin, luteolin, and kaempferol, respectively. The circular dichroism (CD) spectra were measured using a JASCO J-810 spectropolarimeter, and the spectra were accumulated for six times with a band-width of 1.0 nm, a resolution of 0.025 nm, and a scan speed of 500 nm/min.

2.3. Calculation of association constants

The stereospecific interaction between a ligand (L) and its primary site on the protein (P) can be quantified by the binding constant (K_a):

$$L + P = LP; \quad K_a = \frac{[LP]}{[L][P]} \tag{1}$$

It is evident that

$$[L] = c_L - [LP] \tag{2}$$

and

$$[P] = c_P - [LP] \tag{3}$$

where c_L and c_P represent the total concentrations of the ligand and protein, respectively. Assuming that the quercetin–Tf complex (1:1 stoichiometry) is responsible for the induced CD band, it can be written as [18]

$$CD_{450\,nm}\,(m\mathit{deg}) = k[LP] \tag{4}$$

where $k = 32982.1\Delta\varepsilon l$ ($\Delta\varepsilon$ is the extrinsic molar optical activity at 450 nm of flavonoids bound to Tf in $M^{-1}\,cm^{-1}$ and l is the optical pathlength in cm).

By combination of Eqs. (1)–(4), we obtain

$$CD\,(m\mathit{deg}) = \frac{k}{2}(c_P + c_L + K_a^{-1}) - \sqrt{(c_P + c_L + K_a^{-1})^2 - 4c_P c_L} \tag{5}$$

To calculate K_a, a non-linear regression analysis method was used. The magnitude of negative Cotton Effect at 450 nm was used to calculate the binding constants of all flavonoids.

2.4. Molecular modeling methods

With the aim of characterizing the structure–binding relationship, as well as exploring the mechanism of conformational change, the interaction between flavonoids and human serum transferrin has been investigated. All computations were performed on a SGI silicon graphics fuel workstation using Insight-II 2005 software (Accelrys Inc., San Diego, CA), with