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Flavonoid binding to human serum albumin

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ABSTRACT

Dietary flavonoid may have beneficial effects in the prevention of chronic diseases. However, flavonoid bioavailability is often poor probably due to their interaction with plasma proteins. Here, the affinity of daidzein and daidzein metabolites as well as of genistein, naringenin, and quercetin for human serum albumin (HSA) has been assessed in the absence and presence of oleate. Values of the dissociation equilibrium constant (K) for binding of flavonoids and related metabolites to Sudlow's site I range between 3.3×10^{-6} and 3.9×10^{-5} M, at pH 7.0 and 20.0 °C, indicating that these flavonoids are mainly bound to HSA in vivo. Values of K increase (i.e., the flavonoid affinity decreases) in the presence of saturating amounts of oleate by about two folds. Present data indicate a novel role of fatty acids as allosteric inhibitors of flavonoid bioavailability, and appear to be relevant in rationalizing the interference between dietary compounds, food supplements, and drugs.

1. Introduction

Human serum albumin (HSA), the most abundant protein in plasma (reaching a blood concentration of about 7.0×10^{-4} M), provides a depot and carrier for many endogenous and exogenous compounds, affects pharmacokinetics of many drugs, and holds some ligands in a strained orientation which results in their metabolic modification. Furthermore HSA renders potential toxins harmless transporting them to disposal sites, accounts for most of the antioxidant capacity of human serum, and displays (pseudo-)enzymatic properties [1–8].

HSA is a single non-glycosylated all-α chain protein, constituted by 585 amino acids, containing three homologous domains (labeled I, II, and III). Each domain is made by two separate sub-domains (named A and B) connected by random coils. Inter-domain helical regions link sub-domains IB–IIA and IIB–IIIA (Fig. 1) (see [2,6,9,10]).

The structural organization of HSA provides several ligand binding sites (Fig. 1). HSA display seven binding clefts hosting chemically-diverse ligands including fatty acids (FAs), and therefore labeled FA1–FA7 (Fig. 1). In particular, FA3 and FA4 compose the so-called Sudlow's site II (located in sub-domain IIIA) that recognizes preferentially aromatic carboxylates with an extended conformation, and FA7 represents the so-called Sudlow's site I that binds especially bulky heterocyclic anions. Remarkably, warfarin and ibuprofen (commonly used as anti-coagulant and anti-inflammatory drugs, respectively) are considered to be stereotypical ligands for Sudlow's site I and II, respectively [4,8,11–17].

Flavonoids are plant phenolic secondary metabolites widely distributed in the human diet (see [18,19]). Although flavonoid consumption has been associated with the prevention of several degenerative diseases [20], their bioavailability is often poor probably due to their interaction with plasma proteins [21].

Since scarce information is available on HSA recognition by flavonoids and their metabolites [21], the binding mode of isoflavones (daidzein, daidzein metabolites, and genistein), flavanones (naringenin), and flavanols (quercetin) to HSA has been investigated by automated docking simulation. Moreover, values of the equilibrium constant for flavonoid binding to HSA Sudlow's site I (i.e., FA7) have been determined in the absence and presence of oleate. Oleate inhibits allosterically flavonoid binding to HSA, highlighting the role of FAs in modulating ligand binding to HSA. This appears to be relevant in rationalizing the interference between dietary compounds, food supplements, and drugs.

Abbreviations: FA, Fatty acid; HSA, Human serum albumin.

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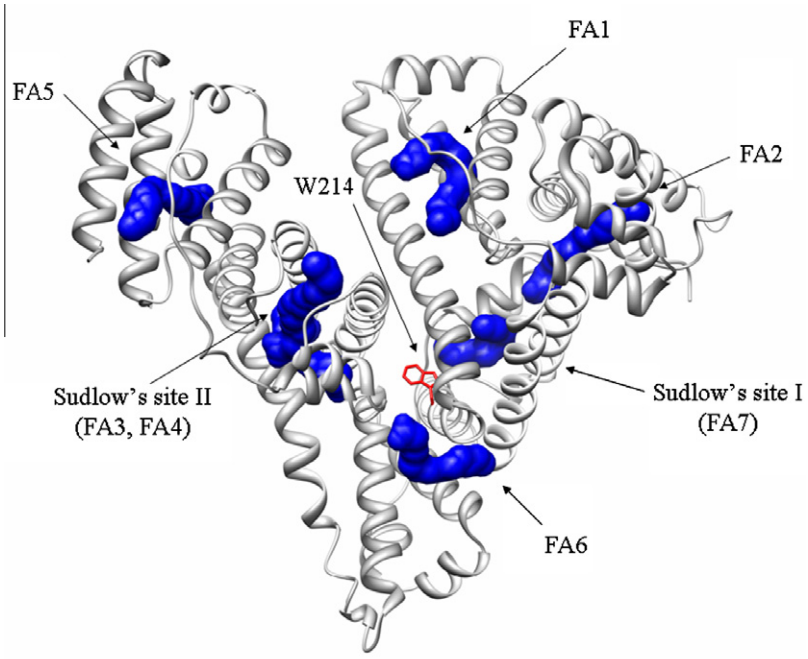

Fig. 1. HSA structure. FA binding sites are indicated by arrows and labelled. Oleate molecules bound to FA sites (represented as space filled) are shown in blue. The Trp214 residue is rendered with red sticks. Atomic coordinates have been taken from PDB entry 1GNI [32]. This picture was drawn with the UCSF Chimera software version 1.4.1 [47]. For further details, see text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2. Materials and methods

FA-free HSA (⩾96%), daidzein, genistein, naringenin, quercetin, and oleate were purchased from Sigma–Aldrich (St. Louis, MO, USA). 6,3′-Dihydroxydaidzein, 8-hydroxydaidzein, 8,3′-dihydroxydaidzein, 4′-daidzeinsulfate, 7-daidzeinsulfate, and 7,4′-daidzeindisulfate were purchased as previously reported [22,23]. All products were of analytical or reagent grade and were used without further purification.

The stock HSA solution (=5.0×10^{-4} M) was prepared by dissolving the protein in 1.0×10^{-1} M phosphate buffer (pH 7.0) at 20.0 °C. The flavonoid stock solution (=1.0×10^{-3} M) was prepared by dissolving daidzein, 6,3′-dihydroxydaidzein, 8-hydroxydaidzein, 8,3′-dihydroxydaidzein, 4′-daidzeinsulfate, 7-daidzeinsulfate, 7,4′-daidzeindisulfate, genistein, naringenin, and quercetin in dimethylsulfoxide. The oleate stock solution (=1.0×10^{-2} M) was prepared by dissolving the FA in 1.0×10^{-2} M NaOH.

Values of the dissociation equilibrium constant for flavonoid binding to HSA (i.e., K) were obtained spectrofluorimetrically, in the absence and presence of oleate, at pH 7.0 (1.0×10^{-1} M phosphate buffer) and 20.0 °C. Briefly, small aliquots of the flavonoid stock solutions were added to the buffered HSA solution; the final volume was 600 μL. The final HSA concentration was 5.0×10^{-6} M. The final flavonoid concentration ranged between 1.0×10^{-6} and 7.5×10^{-5} M. The final oleate concentration ranged between 1.0×10^{-6} and 1.0×10^{-3} M.

The flavonoid-dependent changes of the intrinsic tryptophan fluorescence of HSA were recorded after incubation for 20 min, after each addition. Flavonoid-dependent spectrofluorimetric changes were recorded between 300 nm and 500 nm (the excitation wavelength was 280 nm) [24,25]. Test measurements performed after 2 h excluded slow kinetic events. The intrinsic fluorescence of ligands and dimethylsulfoxide (<15%) was subtracted from the flavonoid-induced quenching of HSA intrinsic fluorescence to determine values of K.

Flavonoid binding to HSA was analyzed by plotting the molar fraction of HSA-flavonoid complexes (i.e., α) as a function of the free flavonoid concentration (i.e., [flavonoid]), according to Eq. (1) [26]:

$$\alpha = [\text{flavonoid}]/(K + [\text{flavonoid}]) \quad (1)$$

Data concerning the effect of the oleate concentration (i.e., $1.0 \times 10^{-6}\ \text{M} \leqslant [\text{oleate}] \leqslant 1.0 \times 10^{-3}$ M) on the flavonoid affinity for HSA have been analyzed according to Eq. (2) [26]:

$$\log K^* = \log K\{(H + 10^{[\text{oleate}]})/(H^* + 10^{[\text{oleate}]})\} + \log(H^*/H) \quad (2)$$

where K and K^* are the dissociation equilibrium constants for flavonoid binding to oleate-free and oleate-bound HSA, respectively, and H and H^* are the dissociation equilibrium constants for oleate binding to flavonoid-free and flavonoid-bound HSA, respectively.

Values of the molar fraction of the flavonoid-bound HSA (W), of the molar fraction of the flavonoid-free HSA (X), of the molar fraction of the HSA-bound flavonoid (Y), and of the molar fraction of the HSA-free flavonoid (Z) have been calculated according to Eqs. (3)–(6) [27,28]:

$$W = \{[\text{flavonoid}]/[\text{HSA}] + K/[\text{HSA}] + 1 - \sqrt{(([\text{flavonoid}]/[\text{HSA}] + K/[\text{HSA}] + 1)^2 - 4 \times [\text{flavonoid}]/[\text{HSA}])}\}/2 \quad (3)$$

$$X = 1 - W \quad (4)$$

$$Y = W \times [\text{HSA}]/[\text{flavonoid}] \quad (5)$$

$$Z = 1 - Y \quad (6)$$

Data were analyzed using the MatLab program (The Math Works Inc., Natick, MA, USA). The results are given as mean values of at least four experiments plus or minus the corresponding standard deviation.

Automatic flexible ligand docking simulation for flavonid binding to HSA was performed by using Autodock 4.0 and the graphical user interface AutoDockTools [29–31]. The structure of HSA was downloaded from the Protein Data Bank (PDB entry: 1GNI) [32].