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An integrated approach with experimental and computational tools outlining the cooperative binding between 2-phenylchromone and human serum albumin

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ABSTRACT

2-Phenylchromone (2PHE) is a flavone, found in cereals and herbs, indispensable in the human diet. Its chemical structure is the basis of all flavonoids present in black and green tea, soybean, red fruits and so on. Although offering such nutritional value, it still requires a molecular approach to understand its interactions with a specific target. The combination of experimental and computational techniques makes it possible to describe the interaction between 2PHE and human serum albumin (HSA). Fluorescence spectroscopy results show that the quenching mechanism is static, and thermodynamic analysis points to an entropically driven complex. The binding density function method provides information about a positive cooperative interaction, while drug displacement experiments indicate Sites 1 and 2 of HSA as the most probable binding sites. From the molecular dynamic study, it appears that the molecular docking is in agreement with experimental data and thus more realistic.

1. Introduction

Flavonoids are polyphenolic secondary metabolites that are widely distributed in higher plants and are ingested by humans in their food (Bravo, 1998). They have attracted considerable attention in the scientific community, owing to a biological and physiological interest. The literature shows that flavonoids can present antioxidant, anticancer, antiviral, anti-inflammatory and heart disease protective activities (Grotewold, 2006). Flavones are one of the major classes of flavonoids, and are mainly found in cereals and herbs (Bravo, 1998). 2-Phenylchromone (2PHE) is a flavone which has the basic structure of all flavonoids, as shown in Fig. 1a. In addition to its antioxidant activity, 2PHE also exhibits the inhibitory property of estrogen without binding to the estrogen receptor, but acting as a competitive agonist for the aryl hydrocarbon receptor, which means that it is anti-estrogenic flavonoid (Jung, Ishida, Nishikawa, & Nishihara, 2007). 2PHE present in *Primula macrophylla* (Primulaceae) is the major compound responsible for the antileishmanial activity of the plant (Najmus-Saqib, Alam, & Ahmad, 2009).

Human serum albumin (HSA) is the main extracellular protein, and is highly concentrated, in blood plasma. HSA is a monomeric globular protein composed of three structurally similar domains (I, II and III), each containing two subdomains (A and B). Aromatic and heterocyclic ligands bind to HSA primarily within two hydrophobic pockets in subdomains IIA and IIIA, namely Sites 1 and 2, respectively. Site 1 is the primary binding site for drugs like warfarin and phenylbutazone analogs, whereas diazepam and ibuprofen are bound primarily to Site 2 (Peters, 1996). Studies have indicated that the subdomain IB can also be a binding site for compounds such as bilirubin, hemin and methyl orange. Subdomain IB has been called Site 3 (Zsila, 2013). The exceptional capacity of HSA to interact with several organic and inorganic molecules turns this protein into an important regulator of intercellular fluxes and the main carrier for many drugs to different molecular targets. Up until now most of the studies with several kinds of flavonoids (Caruso, Vilegas, Fossey, & Cornélio, 2012; Caruso, Vilegas, Souza, Fossey,

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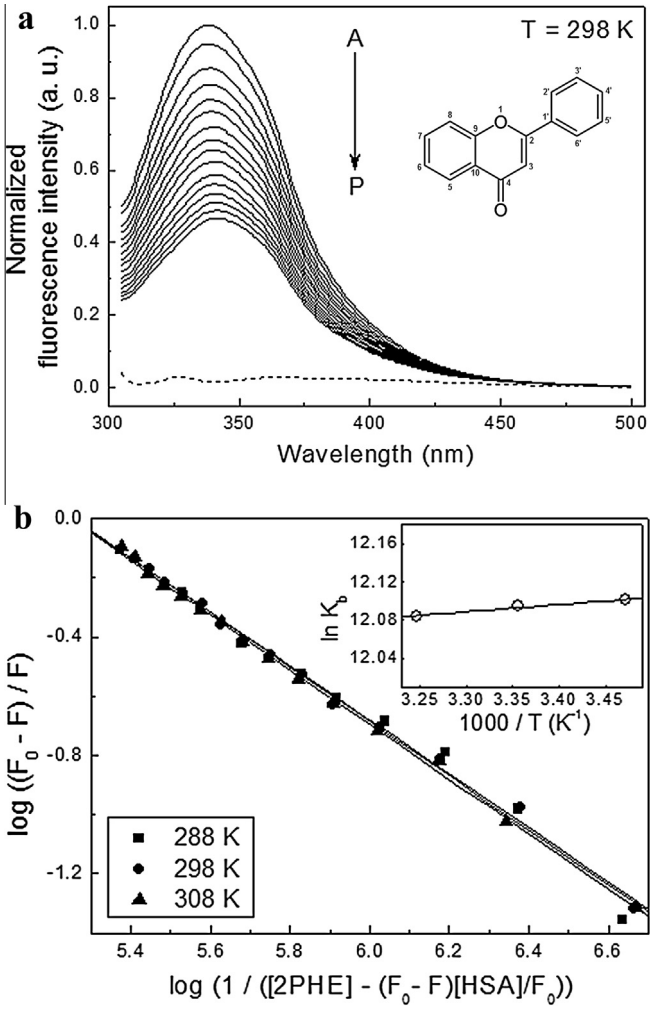

Fig. 1. (a) Emission spectra of HSA obtained with the increments of the 2PHE concentration (pH 7.0, T = 298 K, λ_{ex} = 295 nm). [HSA] = 4.0 μM; [2PHE] (μM), A–P: from 0 to 6.0 with the increments of 0.4. The dotted line corresponds to the emission spectrum of 2PHE in phosphate buffer (6.0 μM). The insert corresponds to the chemical structure of 2-phenylchromone. (b) Double-log plots for the fluorescence quenching of HSA by 2PHE at pH 7.0 and 288, 298 and 308 K. The insert corresponds to the van't Hoff plot for the HSA–2PHE complex.

& Cornélio, 2014; Peters, 1996; Sinisi, Forzato, Cefarin, Navarini, & Berti, 2015; Wu, Yan, Wang, Wang, & Li, 2015) investigating the mechanism of interaction have identified a single binding site. Therefore, a more detailed study of the microenvironment of the binding sites of HSA is important in order to achieve a better comprehension of this mechanism.

This paper presents a broad and detailed study on the interaction between the polyphenolic compound considered to be the basic structure of flavonoids (2PHE) and the principal carrier protein in human blood plasma (HSA). The formation of the HSA–2PHE complex was investigated using spectroscopy methods including fluorescence spectroscopy, UV–Vis absorbance, circular dichroism (CD), and computational methods like *ab initio*, molecular dynamic and docking calculations. In particular, this work also made use of the binding density function (BDF) method for data analysis of fluorescence quenching, which is very sparsely explored in the study of protein–ligand interactions. This data set may contribute scientifically to a better understanding of the interaction mechanisms involved in the formation of the protein–flavonoid complex, and the distribution and transportation of flavonoids, which is an important research field in food chemistry.

2. Materials and methods

2.1. Materials and solutions

Human serum albumin fraction V, 2-phenylchromone, warfarin, ibuprofen, methyl orange, dibasic sodium phosphate, citric acid, ethanol and sodium chloride (NaCl) were purchased from Sigma Chemical Co. All other chemicals were of analytical reagent grade and Milli-Q ultrapure water was used throughout the experiments. HSA was dissolved in a phosphate buffer solution of 50 mM at pH 7.0, and adjusted with citric acid, containing 0.15 M of NaCl. The stock solution of 2PHE was prepared in absolute ethanol. The concentrations of the stock solutions of HSA and 2PHE were determined spectroscopically using the molar extinction coefficient of 36,500 and 25,100 $M^{-1}cm^{-1}$ at 280 and 294 nm, respectively. Aliquots of 2PHE applied in the following experiments were carefully evaluated to avoid aggregation of the flavonoid (Pohjala & Tammela, 2012).

2.2. UV–Vis absorbance spectroscopy

UV–Vis absorption spectrum was recorded at room temperature (298 K), controlled by air conditioning and monitored by a thermometer, on a Cary-3E spectrophotometer (Varian, Palo Alto, CA) equipped with a quartz cell with 1.0 cm optical path length. UV–Vis absorption spectra were recorded in the 250–500 nm range, with an integration time of 0.333 s and a spectral bandwidth of 2.0 nm.

2.3. Fluorescence spectroscopy

The fluorescence measurements were performed using an ISS PC1 steady-state spectrofluorimeter (Champaign, IL, USA) equipped with a quartz cell with 1.0 cm optical path length and a Neslab RTE-221 thermostat bath. Both excitation and emission bandwidths were set at 8.0 nm. The excitation wavelength of 295 nm was chosen since it provides no excitation of tyrosine residues, but excites the single tryptophan residue (Trp214) of HSA (Lakowicz, 1999). The emission spectrum was collected in the range of 305–500 nm with increments of 1.0 nm, which was corrected for the background fluorescence of the buffer and for inner filter effects (Borissevitch, 1999). Each point in the emission spectrum is the average of 10 accumulations. In the fluorescence quenching experiments, titrations were performed by adding small aliquots from the 2PHE stock solution to the HSA solution (3.0 ml) at constant concentrations of 1.0, 2.0 and 4.0 μM. In experiments for Stern–Volmer and binding equilibria analysis, the HSA concentration remained constant at 4.0 μM, and the 2PHE concentration varied from 0 to 6.0 μM with increments of 0.4 μM at 288, 298 and 308 K. For the binding density function (BDF) method, the titration was performed at HSA constant concentrations of 1.0, 2.0 and 4.0 μM, and the 2PHE concentration varied from 0 to 2.8, 3.6 and 6.0 μM, respectively, at 298 K. The effect of ethanol as a co-solvent was verified by adding small aliquots to the HSA solution (4.0 μM at 3.0 ml, 298 K) within the volume changes of the previous titrations. In all experiments, the final volume of ethanol in the buffer was <1%.

2.4. Drug displacement experiment

Site-specific marker displacement experiments were carried out by titrating complexes of HSA (4.0 μM at 298 K) with warfarin, ibuprofen and methyl orange at molar ratios of 1:0 and 1:1, with the 2PHE concentration increasing from 0 to 8.0 μM in increments of 1.0 μM. Warfarin, ibuprofen and methyl orange are site-specific