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Spectral and computational features of the binding between riparins and human serum albumin

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ABSTRACT

The green Brazilian bay leaf, a spice much prized in local cuisine (*Aniba riparia*, Lauraceae), contains chemical compounds presenting benzoyl-derivatives named riparins, which have anti-inflammatory, antimicrobial and anxiolytic properties. However, it is unclear what kind of interaction riparins perform with any molecular target. As a profitable target, human serum albumin (HSA) is one of the principal extracellular proteins, with an exceptional capacity to interact with several molecules, and it also plays a crucial role in the transport, distribution, and metabolism of a wide variety of endogenous and exogenous ligands. To outline the HSA–riparin interaction mechanism, spectroscopy and computational methods were synergistically applied. An evaluation through fluorescence spectroscopy showed that the emission, attributed to Trp 214, at 346 nm decreased with titrations of riparins. A static quenching mechanism was observed in the binding of riparins to HSA. Fluorescence experiments performed at 298, 308 and 318 K made it possible to conduct thermodynamic analysis indicating a spontaneous reaction in the complex formation ($\Delta G < 0$). The enthalpy-entropy balance experiment with a molecular modeling calculation revealed that hydrophobic, hydrogen bond and non-specific interactions are present for riparin I–III with HSA. The set of results from fractional fluorescence changes obtained through Schatchard was inconclusive in establishing what kind of cooperativity is present in the interaction. To shed light upon the HSA-riparins complex, Hill's approach was utilized to distinguish the index of affinity and the binding constant. A correspondence between the molecular structures of riparins, due to the presence of the hydroxyl group in the B-ring, with thermodynamic parameters and index of affinity were observed. Riparin III performs an intramolecular hydrogen bond, which affects the Hill coefficient and the binding constant. Therefore, the presence of hydroxyl groups is capable of modulating the interaction between riparins and HSA. Site marker competitive experiments indicated Site I as being the most suitable, and the molecular modeling tools reinforced the experimental results detailing the participation of residues.

1. Introduction

The Lauraceae family is considered to be one of the most fundamental families belonging to the Magnoliophyta division, having become widely distributed throughout tropical and subtropical regions, being composed at approximately 52 genera and 3000 species [1]. Lauraceae are utilized in papermaking, carpentry, construction, perfumery (essential oil), chemicals and folk medicine [2]. Different species of this family are also known worldwide mainly for their use in cooking, such as bay leaf (*Laurus nobilis*) avocado (*Persea americana*) and cinnamon (*Cinnamomum zeylanicu*) [3].

Aniba riparia (Ness) Mez, popularly known in Brazil as *louro*, is a typical plant of the Amazon region [4], commonly used as a spice. Its fruits contain many different chemical components, such as flavonoids, neolignans, stirilpironas and alkamides [5]. Methyl ether of *N*-benzoyl-tyramine (riparin I, insert in Fig. 1a), methyl ether of *N*-2-hydroxy-benzoyl-tyramine (riparin II, insert in Fig. 1b) and methyl ether of *N*-2,6-dihydroxy-benzoyl-tyramine (riparin III, insert in Fig. 1c) have been isolated from the green fruit of this plant [6]. These molecules have important anti-inflammatory, antimicrobial and anxiolytic pharmacological effects, and great therapeutic potential [6,7].

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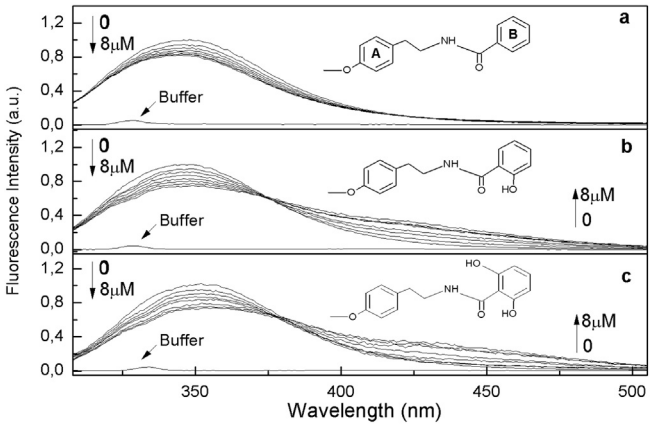

Fig. 1. Fluorescence emission spectra of HSA (4 μM) at different concentrations (0 to 8 μM) of (a) riparin I (b) riparin II and (c) riparin III at 298 K. Inserts shows the correspondent molecular structure.

Riparin I, for example, demonstrated antinociceptive activity [8] and antianxiety effects in animal models [9]. Studies with riparin II revealed that it may inhibit directly or indirectly the activity, production or release of pro-inflammatory mediators involved in the generation of pain associated with inflammation [7], and produces significant antidepressant activity [10]. Riparin III, administered intraperitoneally or orally, also showed anxiolytic and antidepressant activity in rodents [10–12]. Moreover, natural riparins showed antimicrobial activity in multidrug-resistant strains of *Staphylococcus aureus* [13]. However, the interactions of these benzoyl derivatives with specific molecular targets have not been fully understood.

As a profitable target, human serum albumin (HSA) is one of the most important and abundant proteins in the circulatory system. It is a 585-residue monomer and consists of three homologous domains (denoted I, II and III), each of which has two sub-domains, A and B, possessing common structural motifs [14]. HSA has an exceptional capacity to interact with many molecules, playing a crucial role in the transport, distribution, and metabolism of a wide variety of endogenous and exogenous ligands [15]. Site I and site II, located respectively in subdomains IIA and IIIA are two major ligand binding sites for small organic molecules. Recently, subdomain IB (site III) has been identified as the primary or secondary binding site for some compounds [16]. Structural features of compounds, specifically the number and configuration of hydroxyl radical on polyphenols, influence binding characteristics with this transport protein. The —OH moiety is also associated with the reactivity of aromatic molecules and is important for their biological activities [17–19].

In that sense, an investigation of the interaction between riparin I, II and III and HSA contributes to an understanding of the riparin-protein affinity, disposition, transportation and metabolism of these benzoyl-tyramines at molecular level. In the present study, fluorescence quenching was employed to probe the affinity of riparins to HSA under simulative physiological conditions. Particularly, the influence of the hydroxylation pattern, present in riparin II and III, on binding characteristics with HSA was analyzed, as was the specific binding site for each riparin on HSA. As part of the strategy to tackle the problem computational methods were applied with experimental parameters as references to perform the docking.

2. Materials and Methods

2.1. Chemicals

Riparins were isolated by one of our collaborators in pure form for the study. Human serum albumin, warfarin, ibuprofen, methyl orange, dibasic sodium phosphate, methanol and sodium chloride (NaCl) were purchased from Sigma Aldrich Chemical Company (USA). All other chemicals were analytical grade and Milli-Q ultrapure water was used throughout the experiment. HSA was dissolved in a phosphate buffer solution of 50 mM containing NaCl (150 mM, pH 7.0). The protein concentration was determined spectrophotometrically using the molar extinction coefficient of 36,500 M^{-1} cm^{-1} at 280 nm. Riparin I, riparin II and riparin III stock solutions were prepared in methanol with 23.8, 5.42 and 1.23 mM, respectively.

2.2. Fluorescence Spectroscopy

Fluorescence measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer equipped with 1.0 cm quartz cells and Carry Eclipse thermostat bath. Both excitation and emission slits were set at 5.0 nm. The fluorescence spectra were recorded in the range of 300–505 nm and the excitation wavelength of 295 nm was chosen, since it provides excitation of the single tryptophan residue (Trp 214) and avoids absorption of tyrosine residues. All fluorescence intensities were corrected for the buffer and inner filter effects (Eq. (1)) [20–22].

$$F_{corr} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

F_{corr} and F_{obs} are the fluorescence intensity corrected and observed and A_{ex} and A_{em} are the absorbance of the system at the excitation and emission wavelengths, respectively.

In fluorescence quenching experiments, titration was performed by adding appropriate quantities of riparin I–III stock solution to the HSA solution (2.0 mL) at constant concentrations of 2.0, 4.0 and 8.0 μM. In experiments to determine the Stern-Volmer constant and Binding Data Analysis the HSA concentration remained constant at 4.0 μM, and the riparin concentrations varied from 0 to 8 μM with increments of 1.0 μM at 298, 308 and 318 K. For the binding density function (BDF) method, titration was performed at HSA concentrations of 2.0 and 8.0 μM and the range of riparin concentrations were 0–12 μM, with increments of 1.0 μM, at 298 K. The effect of methanol as a co-solvent was verified by adding aliquots to the HSA solution (2.0, 4.0 and 8.0 μM, at 298 K) within the volume variation of previous titrations. The final volume of methanol in the buffer was <1%, in all experiments.

2.3. Site Marker Competitive Experiments

Competitive experiments were carried out by the use of warfarin, ibuprofen and methyl orange which bind to site I, site II, and site III, respectively [16,23]. The ratio of site marker to HSA solution was 1:1 (4.0 μM each). After 1 h incubation of the mixtures, amounts of riparin I, II and III solution, varying from 0 to 10 μM with increments of 1.0 μM, were added to each of HSA-marker solutions as previously. The fluorescence spectra were recorded at 298 K under the same experimental conditions described above.

2.4. Ab Initio Calculation

The Gaussian 09 program [24] provided by Núcleo de Computação Científica da Universidade Estadual Paulista (NCC/GridUNESP) was applied to the calculation of riparin structures. The optimized geometry was calculated in the gas phase with riparin molecules isolated by using DFT/B3LYP/6-31G(d,p) method. The next step, the vibrational frequency calculation, was performed to check the optimized riparin molecules. The molecular electrostatic potential (MEP) map was calculated to investigate the distribution of charge density on the molecular surface of riparin I, II and III.