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Thermodynamic and kinetic analyses of curcumin and bovine serum albumin binding

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ABSTRACT

Bovine serum albumin (BSA)/curcumin binding and dye photodegradation stability were evaluated. BSA/curcumin complex showed 1:1 stoichiometry, but the thermodynamic binding parameters depended on the technique used and BSA conformation. The binding constant was of the order of 10^5 L·mol^{-1} by fluorescence and microcalorimetric, and 10^3 and 10^4 L·mol^{-1} by surface plasmon resonance (steady-state equilibrium and kinetic experiments, respectively). For native BSA/curcumin, fluorescence indicated an enthalpic and entropic driven process based on the standard enthalpy change ($\Delta H_F^{\circ} = -8.67$ kJ·mol^{-1}), while microcalorimetry showed an entropic driven binding process ($\Delta H_{cal}^{\circ} = 29.11$ kJ·mol^{-1}). For the unfolded BSA/curcumin complex, it was found thatp $\Delta H_F^{\circ} = -16.12$ kJ·mol^{-1} and $\Delta H_{cal}^{\circ} = -42.63$ kJ·mol^{-1}. BSA (mainly native) increased the curcumin photodegradation stability. This work proved the importance of using different techniques to characterize the protein-ligand binding.

1. Introduction

Curcumin is a polyphenolic compound that exhibits various biological activities, such as anti-inflammatory, anticarcinogenic, antioxidative and antimicrobial. However, despite these therapeutic effects, its low solubility and photostability in aqueous systems make its use difficult in food formulations and limits its body absorption (Gupta, Patchva, & Aggarwal, 2013). In addition, curcumin degrades when exposed to light, oxygen and high temperatures (Paramera, Konteles, & Karathanos, 2011).

Because of these limitations, various alternatives have been sought to increase the curcumin solubility and stability in aqueous media, for example the incorporation of curcumin into cyclodextrins, liposomes, and proteins (Barik, Priyadarsini, & Mohan, 2003; Isacchi et al., 2012; Mandeville, Froehlich, & Tajmir-Riahi, 2009; Paramera et al., 2011). Bovine serum albumin (BSA) has been studied as a carrier of various bioactive compounds because of its stability properties, aqueous solubility, and structural homology to human serum albumin (HSA) (Meti, Byadagi, Nandibewoor, & Chimatadar, 2014).

Some studies have described the interaction between curcumin and BSA (Barik et al., 2003; Yang, Wu, Li, Zhou, & Wang, 2013). However, they did not address the influence of the protein conformation on the binding properties, which is important since many food processes lead to the unfolding of proteins; as well as the protection against photodegradation provided to protein-carried curcumin. In addition, most studies only deal with a single technique to define the binding parameters. Fluorescence spectroscopy, for example, is often used to determine the thermodynamic binding parameters between curcumin and BSA (Yang et al., 2013) or HSA (Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010; Mohammadi, Bordbar, Divsalar, Mohammadi, & Saboury, 2009). However, the fluorescence is restricted to the binding occurring directly with fluorophore residues, such as tryptophan (Ghosh, Rathi, & Arora, 2016). In previous studies from our group, we found that the use of multiple techniques is strategic to determine the thermodynamic binding parameters since different techniques are able to view interactions occurring at different sites (Lelis,

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Ferreira, et al., 2017; Lelis, Hudson, et al., 2017).

Therefore, in this work, we present a complete thermodynamic analysis of the binding occurring between curcumin and BSA in its native and unfolded conformation obtained by three sensitive techniques. In addition, we also studied the protection effect provided by BSA (native and unfolded) to the curcumin photodegradation.

2. Materials and methods

2.1. Materials

BSA (> 98% wt), curcumin (> 80% wt), warfarin (reagent grade), ibuprofen (> 98% wt), digitoxin (> 92% wt), and dimethyl sulfoxide (analytical grade) were obtained from Sigma-Aldrich (USA). Sodium phosphate (analytical grade) was acquired from Vetec (Brazil), and 3-(*N*,*N*-dimethylamino)propyl-*N*-ethylcarbodiimide (EDC) (> 99% wt), *N*-hydroxysuccinimide (NHS) (> 99% wt), and sodium acetate (> 99% wt) were purchased from (General Electric Healthcare Company, Uppsala, Sweden).

2.2. Fluorescence spectroscopy

The fluorescence measurements were carried on a LS55 fluorescence spectrophotometer (Perkin Elmer Inc, Waltham, USA), using quartz cells having a 1 cm path length. In each cell 3 ml of native or denatured BSA (15 μM) was added at pH 7, containing different concentrations of curcumin (10–70 μM). A solution of denatured BSA, without any precipitation, was obtained by heating at 80 °C for 10 min. The curcumin stock solution (500 μM) was first prepared in dimethyl sulfoxide (DMSO), and then an aliquot was transferred into a phosphate buffer at pH 7.0, so that the final volume of the solvent would have no effect on the protein.

The fluorescence quenching experiment was performed at five temperatures (20, 25, 35, 45, and 50 °C). The excitation wavelength was 295 nm and slit widths for both excitation and emission were fixed at 5 nm. The emission spectra were recorded in the 296–450 nm range. The effect of DMSO on BSA structure was also evaluated by fluorescence, and at the very low solvent concentration ([DMSO] < 2.0%, w/w), there is no solvent influence on BSA conformation (data not shown).

To determine the binding site for curcumin on BSA, the marker ligands warfarin, ibuprofen, and digitoxin were used. In this experiment, curcumin was added to the native BSA solution containing each of the binding site markers with a 1:1 stoichiometry, and the fluorescence quenching experiment was conducted as described above.

All experiments were performed in triplicate and the error associated with each determined parameter was between 1 and 3%.

2.3. Isothermal titration microcalorimetry (ITC)

The ITC experiments were performed at 25 °C, using a TAM III microcalorimeter controlled by the ITCRun software (TA Instruments, New Castle, DE, USA), following the method of Lelis, Hudson, et al. (2017). An aqueous BSA (native or unfolded) solution at pH 7 (2.7 ml) was prepared at 2.2 μM, degassed, and put into the sample cell. The titration solution (curcumin at 100 μM) was degassed and loaded into the injection syringe. Then, 25 aliquots of the curcumin solution (10 μl each) were added into the BSA solution. A dilution experiment was carried out by replacing the BSA solution with a pH 7 buffer. The samples were constantly stirred at 300 rpm to ensure thorough mixing. The raw data obtained from a plot of heat flow vs. injection number were transformed using the instrument software to construct a plot of enthalpy change vs. molar ratio. The values of enthalpy change of curcumin dilution (ΔH_{dil}) were subtracted from those acquired from the curcumin addition to the BSA solution (ΔH_{obs}). The apparent interaction enthalpy change ($\Delta H_{app\text{-}int}$) was calculated following the relationship $\Delta H_{app\text{-}int} = (\Delta H_{obs} - \Delta H_{dil})/n_{mols}$, where n_{mols} is the curcumin amount added to the sample cell at each injection. The resulting data of $\Delta H_{app\text{-}int}$ versus [curcumin] were fitted to a one to one binding model (Eq. (1), (Lelis, Hudson, et al., 2017)) in order to obtain the binding constant (K_b), the binding stoichiometry (n), and the calorimetric standard enthalpy change (ΔH°_{cal}) for the BSA/curcumin complex formation:

$$\Delta H_{app\text{-}int} = \frac{V_c \Delta H^{\circ}_{lig}}{2K_b} 1 + K_b[Cur]_t + nK_b[BSA]_t - [(1 + K_b[Cur]_t + nK_b[BSA]_t)^2 - 4nK_b^2[Cur]_t[BSA]_t]^{1/2} \quad (1)$$

where V_c is the cell volume, K_b is the binding constant, $[Cur]_t$ is the total curcumin concentration in the cell, n is the number of independent sites, and $[BSA]_t$ is the total BSA concentration in the cell.

All experiments were carried out in triplicate and the relative errors were between 1 and 3%.

2.4. Surface plasmon resonance (SPR)

SPR analyses (thermodynamic and kinetic) were conducted using a Biacore X100 instrument (General Electric Healthcare Company, Uppsala, Sweden). For the steady-state thermodynamic experiments, native BSA was immobilized on a CM5 sensor chip (GE Healthcare Company) by amine coupling according to the recommended protocol in the Biacore X100 Handbook BR-1008-10 Edition AC. The flow cells were formed when the sensor chip was docked in the instrument, and it was activated for 7 min with a 1:1 mixture of 0.1 mol·L^{-1} 3-(*N*,*N*-dimethylamino)propyl-*N*-ethylcarbodiimide (EDC) and 0.1 mol·L^{-1} *N*-hydroxysuccinimide (NHS) at a flow rate of 20 μl·min^{-1} and 25 °C. Then, 30 μg·ml^{-1} of BSA in 10 mmol·L^{-1} of sodium acetate at pH 7 was injected for 7 min, resulting in a high density BSA immobilization around 7000 resonance units (RU). A 7 min pulse of 1 mol·L^{-1} ethanolamine hydrochloride at pH 8.5 was then used to deactivate the excess of hydroxysuccinimidyl groups on the surface that did not react with the protein. For the kinetic measurements, a similar procedure to the one described above was repeated, with the only difference that the CM5 chip surface was recovered with a lower density of immobilized BSA and an RU equal to 3800.

The BSA-curcumin interaction experiments were carried out at 25 °C and pH 7. For the steady-state equilibrium experiments, the curcumin concentration ranged from 25 to 200 μM; for the kinetic experiments, 10 μM < [curcumin] ≤ 32 μM. For each experiment, the curcumin solution at a determined concentration was flowed over a chip with two channels, one with (ligand channel) and another without (reference channel) immobilized BSA, respectively. Before each curcumin binding cycle, a buffer was injected in order to obtain the baseline.

The experiments were performed in triplicate and the relative error associated was between 1 and 3%.

2.5. Degradation kinetics of curcumin by exposure to light

Curcumin solutions (25 μM) in buffer and native and denatured BSA solutions, at concentrations ranging from 0 to 45 μM at pH 7, were stored in glass bottles and placed inside a light chamber containing two fluorescent lamps, that corresponded to the daylight (Hudson, 2017). Absorbance readings were performed at 425 nm using a UV–Vis spectrophotometer (Shimadzu UV-2550) at 25 °C and 30 min intervals, for 6 h.

All experiments were performed in triplicate and the error associated with determined parameters was lower than 5%.