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Interaction of cinnamic acid and methyl cinnamate with bovine serum albumin: A thermodynamic approach

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ABSTRACT

Cinnamic acid (CA) and methyl cinnamate (MC) have attracted interest of researchers because of their broad therapeutic functions. Here, we investigated the interaction of CA and MC with bovine serum albumin (BSA) at pH 3.5, 5.0, and 7.4 using fluorescence spectroscopy, differential scanning nanocalorimetry, and measurements of interfacial tension, size, and zeta potential. BSA formed a complex with the ligands with stoichiometry of approximately 1.0. At pH 7.4, CA-BSA complex formation was entropically driven. The interaction between MC and BSA was more favorable than with CA and was enthalpically driven under the same conditions. The pH played an important role in BSA conformation, which altered the manner in which it interacts with the ligands. Interestingly, both CA and MC had no effect on the surface tension of BSA/air interfaces. These data contribute to the knowledge of CA/MC-BSA interactions and provide important data for application in the food industry.

1. Introduction

Cinnamic acid (CA) (Fig. 1a), like other phenolic acids, is a secondary plant metabolite that is widely found in nature (Bian, Zhang, Yu, Chen, & Liang, 2007; Singh & Mitra, 2011); primarily in cinnamon, cloves, cranberries, and prunes (Patel, Ray, Aswal, & Bahadur, 2014). CA is approved by the Food and Drug Administration (FDA) for use in flavor composition (Zhang, Chen, Zhou, Shi, & Wang, 2011), perfumes, and synthetic indigo dyes (Singh & Mitra, 2011).

CA has been reported to potentially possess broad therapeutic functions, including antimicrobial, antifungal (Patel et al., 2014), and antiparasitic activities (Cury et al., 2015). Interest in CA has also continued to increase following recognition that it demonstrates antitumor activity against human malignant tumors, such as melanoma, glioblastoma, and adenocarcinoma of the prostate and lung (Bian et al., 2007; Zhang et al., 2011). In addition, CA is known to be an anti-diabetic agent (Ganugapati & Swarna, 2014; Singh & Mitra, 2011).

Methyl cinnamate (MC) (Fig. 1b) is a cinnamic acid ester that is produced and released by strawberries during maturation. MC is generally recognized as safe (GRAS) and can be used as a food additive. MC also has importance as an antimicrobial agent, having been shown to possess antimicrobial activity in the recovery of strawberries (Bhatia et al., 2007; Peretto et al., 2014; Stefanovi, Radojevi, & Ljiljana, 2014). Furthermore, MC has been demonstrated to possess anti-adipogenic and vasodilatory activity, and was able to prevent the effects of colitis resulting from acetic acid-induced injury *in vivo* (Chen, Lee, Hsu, Wei, & Tsai, 2012; Lima et al., 2014; Vasconcelos-Silva, Lima, Brito, Lahlou, & Magalhaes, 2014).

Both CA and MC have low water solubility (Liu et al., 2016; Peretto et al., 2014). Enhancing the solubility of these compounds would thus improve their application in the food, pharmaceutical, and cosmetic industries. In this regard, recent studies have shown that CA can be carried by different compounds, such as surfactant micelles, carbohydrates, and proteins. Patel et al. (2014) demonstrated that Triton X-100 micelles could enhance CA solubility at

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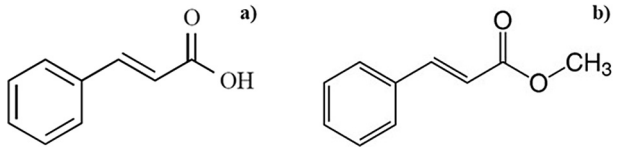

Fig. 1. Representative chemical structures of a) cinnamic acid, and b) methyl cinnamate.

physiological pH by approximately 25 times than water solubility. Cyclodextrins (CDs) were also shown to be efficient at improving CA solubility, forming a 1:1 stoichiometric inclusion complex with β-CD, as determined by fluorescence (Liu et al., 2016). On the other hand, studies on improving MC solubility or encapsulation remain scarce.

Proteins, such serum albumins, can act as drug and bio-active small molecule carriers. Studying the intermolecular interaction between them is thus of immense physiological and industrial importance since these molecules can bind reversibly to albumins, which significantly affects their solubility, stability, absorption, distribution, and metabolism (Kamat, Seetharamappa, & Melwanki, 2004; Zhang, Ma, Wang, Zhang, & Zhou, 2012).

Bovine serum albumin (BSA) consists of a single polypeptide chain of 583 amino acids with no carbohydrate residues. It is made up of three homologous domains (I, II, and III) that, in turn, are divided in two sub-domains. BSA contains two tryptophan residues, one of which is located on the surface of the protein's first domain (Trp-134) and the other is entrapped within the hydrophobic pocket of the second domain (Trp-212) (Bolel, Mahapatra, Datta, & Halder, 2013; Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010).

Although some spectroscopic studies on the interaction of CA with BSA have been reported (Bian et al., 2007; Min et al., 2004; Singh & Mitra, 2011), there remains a lack of evidence regarding the effect the CA-BSA interaction has on the protein's surface properties and on the influence of pH on formation of the CA-BSA complex. To the best of our knowledge, there is also no data on the binding thermodynamics between MC and BSA. Gathering this information may contribute significantly toward gaining key insights into the efficient application of CA and MC into the complex matrices of different food types. In the present study, we therefore report a thermodynamic approach for evaluating the interaction between BSA and CA or MC under different pH conditions using fluorescence spectroscopy, differential scanning nanocalorimetry, interfacial tension, and electrokinetic measurements.

2. Material and methods

2.1. Materials

BSA (heat shock fraction, pH 7, ≥98% wt), CA (>99.0% wt) and MC (≥99.0% wt) were purchased from Sigma-Aldrich (St Louis, USA). BSA solutions were prepared in citrate-buffer (0.1 M, pH 3.5 and 5.0) or phosphate-buffer (0.01 M, pH 7.4). The 10 mM stock solution of MC was prepared in ethanol for subsequent dilution in BSA solutions. CA working solutions were directly prepared in BSA solutions. Experiments with MC were performed with working solutions containing no more than 1% (v/v) of ethanol.

2.2. Fluorescence quenching studies

All fluorescence spectra were measured using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, USA) equipped with a thermostat bath. Temperature was controlled at 15 °C, 25 °C, 35 °C, 45 °C, and 55 °C. BSA Trp residues were excited at 295 nm using an excitation slit of 2.5 nm, an emission slit of 5.0 nm, 650 V for the photomultiplier tube (PMT) voltage, and a scan rate at 600 nm/min to obtain an emission spectra from 296 nm to 450 nm. Fluorescence spectra were obtained at pH 3.5, 5.0, and 7.4, using a fixed BSA concentration (30.1 μM) in the presence of a range of CA or MC concentrations (from 0 to 100 μM).

2.3. Zeta potential and size measurements

For zeta potential and size measurements, six samples containing 30.1 μM BSA were prepared. The first sample was without MC or CA, with the remaining samples ranging in concentration from 10 μM to 100 μM of MC or CA. As with the fluorescence experiments, zeta potential and size measurements were examined at three pH values for native BSA, and at pH 7.4 for denatured BSA. Samples were diluted 10-times in water and incubated at 25 °C for 12 h in an ultra-thermostatic bath. Samples were then transferred to the Zetasizer Nano ZS system (Malvern Instruments Ltd, Worcestershire, UK). The dispersant chosen for the equipment was water at 25 °C, which had a fixed viscosity of 0.8872 cP, a refraction index of 1.330, and a dielectric constant of 78.5.

2.4. Interfacial tension analysis

The pendant drop method was used to determine the interfacial tension of the samples. Samples were prepared in solutions containing 7.5 μM BSA for better curve shape. The first sample contained BSA only in buffer (pH 3.5, 5.0, or 7.4), with the additional samples containing 2.5 μM to 25 μM of MC or CA to maintain the protein:binding ratio of the other experiments. For denatured BSA, the experiment was conducted at pH 7.4. All samples were incubated in an ultra-thermostatic bath for 12 h at 25 °C. An Easy Drop goniometer (Kruss, Hamburg, DE) was used to measure the behavior of the sample drop/air interfacial tension over time by measuring the interfacial tension each second over a total of 20 min at 25 °C (Nunes, 2016).

2.5. Differential scanning calorimetry (DSC) experiments

A Nano DSC (TA Instruments, New Castle, USA) calorimeter, with two platinum cells, was used to conduct the experiments under the following conditions: one round of heating and cooling at 3 atm, at a rate of 1 °C/min from 20 °C to 100 °C. We made use of 30.1 μM BSA solutions at the three studied pH values (3.5, 5.0 and 7.4), and we examined heat treated BSA (85 °C/15 min) at pH 7.4.

3. Results and discussion

3.1. Fluorescence spectroscopy analysis of CA and MC interactions with BSA

When excited at 295 nm, BSA molecules emit a fluorescent signal in aqueous solutions due to the presence of Trp residues. However, the Trp fluorescent signal may change when BSA interacts with other molecules (Bourassa et al., 2010). Thus, fluorescence spectroscopy is a powerful technique to study the interaction between a fluorescent molecule, such as proteins, and small ligands (Wei, Xiao, Wang, & Bai, 2010). Fig. 2 shows the fluorescence spectra of BSA with the addition of increasing concentrations of CA or MC.

The gradual addition of CA or MC into a fixed BSA concentration at physiological pH caused a decrease in BSA fluorescence