Interaction of Cuminaldehyde with Bovine Serum Albumin and Human Serum Albumin

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Research Article

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Abstract

Cuminaldehyde, an oxidized aldehyde monoterpene, present in green cumin seeds (CuminumcyminumLinn,FamilyApiaceae), is traditionally used for the treatment of abdominal colic, dyspepsia, diarrhoea and jaundice. Also, many studies have reported the antioxidant, antibacterial and antifungal effects of Cuminaldehyde. Serum albumins are the major soluble and small molecule-binding proteins, present in abundance in the circulatory system of a wide variety of organisms. Studies on the interaction of bioactive molecules with Bovine serum albumin(BSA) and Human serum albumin(HSA) have attracted enormous interest due to its direct consequence on drug delivery, pharmacokinetics, pharmacodynamics, therapeutic efficacy and drug designing. Our present study is carried out to understand the mechanism of interaction of pharmaceutically important component of spices, Cuminaldehyde with BSA and HSA. Fluorescence spectroscopic measurements confirmed that Cuminaldehyde interacted with BSA and HSA and quenched its fluorescence intensity via static quenching mechanism. The change in secondary conformation of BSA and HSA upon interaction with Cuminaldehyde was explored by UV-Visible absorption studies. The location of binding site for Cuminaldehyde in BSA and HSA was investigated by site probe displacement experiments and the results indicated that Cuminaldehyde was bound to BSA and HSA at site I. Thermodynamic studies revealed that vander Waal's interaction and hydrogen bonding play a major role in Cuminaldehyde-BSA system while electrostatic interaction plays vital role in Cuminaldehyde-HSA system.

1 Introduction

Cuminaldehyde, a major constituent of green cumin seeds, is a potential drug candidate. Cumin seeds are widely used as spices and have been used for treatment of abdominal colic, dyspepsia, diarrheah and jaundice. Cuminaldehyde has several therapeutic benefits including antidiabetic, anticancer, neuroprotective, anti-inflammatory, antimicrobial and antifungal properties etc.[1] Cuminaldehyde is an active compound involved in the antifibrillation activity. It has been shown that cuminaldehyde inhibits the fibrillation of alpha-synuclein (α-SN) is a critical process in the pathophysiology of several neurodegenerative diseases, especially Parkinson's disease.[2,3] Cuminaldehyde presented antimicrobial and anti-biofilm effects against S. aureus and E. coli.[4,5] Recently many researchers have paid attention in the study of interaction of biomolecules with plasma protein[6–12]. Food or any other substances taken by mouth, is broken down in the process of digestion and small metabolites are absorbed in the small intestine. These metabolites are then delivered to the bloodstream where it binds with the binding proteins and are transported all over the body. Serum albumins are the major soluble and small molecule-binding proteins, present in abundance in the circulatory system of a wide variety of organisms. Many bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Human serum albumin (HSA), present in human plasma, is the most important carrier of exogenous and endogenous molecules. Bovine serum albumin (BSA) can be viable model for nonspecific binding due to its size and collection of binding sites. Studies on the interaction of bioactive molecules with HSA and BSA have attracted enormous interest due to its direct consequence on drug delivery,
pharmacokinetics, pharmacodynamics, therapeutic efficacy and drug designing. The therapeutic action of a pharmacologically important molecule depends upon the extent of binding of these molecules with serum albumins. The interaction of Human Serum Albumin (HSA) with Cuminaldehyde was recently studied and it was found that the interaction was very strong[14]. Another recent study has confirmed the formation of the stable complex between Cuminaldehyde with Bovine Serum Albumin (BSA)[15]. We have designed this study to see the comparative interaction of Cuminaldehyde with BSA and HSA. To accomplish this goal, spectroscopic methods like fluorescence and UV-visible spectroscopy are used. To determine the binding site of HSA and BSA, fluorescent markers are used, which is previously not reported for Cuminaldehyde. This study can provide information on the rational use of Cuminaldehyde as food additives and understanding their physiological effects.

2 Experimental section

2.1. Chemical used

BSA and HSA were purchased from Himedia. Cuminaldehyde, DMSO, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, sodium chloride, potassium chloride, Ibuprofen and ketoprofen were purchased from Sigma-Aldrich. The stock solutions of Cuminaldehyde were freshly prepared by first dissolving in spectroscopic-grade dimethylsulfoxide (DMSO) and then diluted with phosphate buffered saline (PBS), 7.4. The amount of DMSO was kept less than 3% (by volume) for each set of experiment and had no effect on any experimental results. The stock solutions of BSA (1 µM) and HSA (1 µM) were also prepared in PBS.

2.2 Absorption measurements

The UV spectra measurements of all BSA and HSA solutions in absence and presence of Cuminaldehyde were performed on a Agilent-8453 spectrophotometer (Agilent corporate, America). The UV spectra were recorded from 200 to 350 nm at room temperature (297 K).

2.3. Fluorescence Measurements

Ligand-induced Tryptophan fluorescence quenching measurements were performed on a Fluoromax-4 spectrofluorometer. For fluorescence titration, protein (1 µM) and varying concentration of ligands were incubated in a PBS solution. In our experiment, Cuminaldehyde and Serum Albumins were let stand for 5 min to reach dynamic equilibrium. During the fluorescence measurement, the slit width was set at 10 nm/10 nm, the excitation wavelength was 285 nm, and the scanning range of the fluorescence emission spectrum was 300–500 nm.

2.4. Combination of fluorescent probes

To determine the displacement percentage of the fluorescent combination probe, according to the method introduced by Sudlow et al., the fluorescence markers for distinct binding sites were chosen: ketoprofen for site I and ibuprofen for site II [16]. For this experiment, Cuminaldehyde (5.0×10⁻⁵ M) and serum
albumins(1.0×10⁻⁵M) were incubated for 5 minutes and then titrated with varying concentrations of ibuprofen and ketoprofen solutions.

3 Results and Discussions

3.1. Determination of binding affinity and mechanism of the ligand to the protein

The endogeneneous fluorescence shown by protein is due to the presence of tryptophan(Trp), tyrosine(tyr) and phenylalanine(phe). The environmentally sensitive Trp moiety mainly contributes to the changed fluorescence of BSA. When excited at 285 nm, BSA had a characteristic band at around 344 nm. Furthermore, when the concentration of the added Cuminaldehyde increased from 0 to 61×10⁻⁶ M, the fluorescence intensity of BSA decreased significantly, indicating there is a binding interaction of cuminaldehyde with BSA and HSA. The reaction temperatures for Cuminaldehyde-Serum albumins system were maintained at 298 K, 303 K and 308K respectively. The fluorescence quenching data are analyzed by Stern-Volmer equation[20]:

\[
\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} [Q] \quad (1)
\]

\(K_{SV}\) is the Stern–Volmer quenching constant with the unit being M⁻¹, and \([Q]\) is the concentration of the quencher. \(k_q\) is the quenching rate constant of BSA, \(\tau_0\) is the average fluorescence lifetime of BSA in the excited state without the quencher (the order of magnitude is 10⁻⁸). \(K_{SV}\) and \(k_q\) value of BSA and HSA triggered by Cuminaldehyde at different temperatures can be determined by calculating the slope of the curve, as shown in Fig. 1B and 1D. In this experiment, the fluorescence intensities of BSA (Fig. 1A) and HSA(Fig. 1C) significantly decreased with increasing concentration of ligands, indicating that the ligand weakens the fluorescence intensities by interacting with protein. In other words, the existence of the ligands would have certain influence on fluorescence chromophore of both BSA and HSA. \(K_{SV}\) were obtained from the Stern–Volmer equation at three different temperatures 298, 304, and 310 K and presented in Table 1. \(K_{SV}\) values decreased as the temperature increases, which indicated that the mechanisms of the two systems were all static quenching.[21, 22] For static quenching, the binding constants (K) can be represented by the double logarithm regression curve

\[
\log (F_0 - F)/F = logK + nlog [Q] \quad (2)
\]

where \(F_0, F,\) and \([Q]\) are the same as in Eq. (1). K is the binding constant, and n is the number of binding sites per HSA. The results(Table 1) showed that the binding constant of the Cuminaldehyde–BSA system decreased and Cuminaldehyde–HSA system increased with the increase in temperature, which may hint a temperature-sensitive complex formation in the binding reaction.[23] In addition, K values in the Cuminaldehyde–BSA system were bigger than that in the Cuminaldehyde–HSA system, indicating the binding ability of cuminaldehyde with BSA was better than HSA.
Thermodynamic parameters and binding forces

The binding forces between macromolecules and ligands include hydrogen bonds, van der Waals forces, hydrophobic forces, and electrostatic interactions.[24–28] The temperature effect was very small and did not result in structural degradation of BSA and HSA; thus, enthalpy change can be regarded as a constant within a little range of temperature. The binding mode was verified by using the Van’t Hoff equation[29]:

\[
\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)
\]

\[
\Delta G = \Delta H - T \Delta S \quad (4)
\]

where \( R \) is the gas constant, \( T \) is the experimental temperature, and \( K \) is the binding constant at the corresponding temperature. The calculated results of the thermodynamic parameters were represented in Table 1. The negative value of \( \Delta G \) indicates the interaction of cuminaldehyde and both BSA and HSA is spontaneous. The negative value of \( \Delta H \) and \( \Delta S \) for Cuminaldehyde-BSA system indicates that vander Waal’s interaction and hydrogen bonding play a major role in binding of the ligand to the protein. On the other hand, positive value of \( \Delta H \) and \( \Delta S \) for Cuminaldehyde-HSA system indicates that electrostatic interaction plays vital role in binding of the ligand to the protein.

<table>
<thead>
<tr>
<th>T(K)</th>
<th>( K_{SV} \times 10^4 ) (M(^{-1}))</th>
<th>( R^a )</th>
<th>( K \times 10^4 ) (Lmol(^{-1}))</th>
<th>( R^b )</th>
<th>( \Delta G ) (KJ/mol)</th>
<th>( \Delta S ) (J/mol·K)</th>
<th>( \Delta H ) (kJ/mol)</th>
<th>( R^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>1.998</td>
<td>0.992</td>
<td>8.5</td>
<td>0.997</td>
<td>-12.18</td>
<td>-7.61</td>
<td>-14.45</td>
<td>0.979</td>
</tr>
<tr>
<td>BSA</td>
<td>303</td>
<td>1.439</td>
<td>0.987</td>
<td>6.3</td>
<td>0.996</td>
<td>-12.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>1.143</td>
<td>0.988</td>
<td>5.5</td>
<td>0.993</td>
<td>-12.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>1.902</td>
<td>0.998</td>
<td>1.54</td>
<td>0.996</td>
<td>-12.88</td>
<td>189.55</td>
<td>43.6</td>
<td>0.991</td>
</tr>
<tr>
<td>HSA</td>
<td>303</td>
<td>1.89</td>
<td>0.995</td>
<td>3.51</td>
<td>0.995</td>
<td>-13.832</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>0.88</td>
<td>0.966</td>
<td>5.73</td>
<td>0.977</td>
<td>-14.77</td>
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</tr>
</tbody>
</table>

3.2. Conformation investigation

Upon binding of ligand, intermolecular forces sustaining secondary and tertiary structures may be rearranged, resulting in conformational alteration in the protein. In order to ascertain the conformational alteration induced by Cuminaldehyde, UV–vis absorption was performed.[30] The directions and degrees of absorption peak change indicated the disturbance of the hydrophilic and hydrophobic environment. The absorbance intensity of BSA and HSA at 280 nm increased with increasing ligand concentration.
complexes formed between Cuminaldehyde and BSA /HSA and amino-acid residue microenvironment might change upon ligand binding to protein. (Fig. 2). Besides, certain red shifts were observed which indicates that amino-acid residue microenvironment might change upon ligand binding to protein.

**3.3. Combination of fluorescent probes**

BSA and HSA are made up of three linearly arranged structurally homolgous domains(I-III) and each domain in turn is the product of two subdomains(A,B). The two binding sites present on BSA and HSA protein are designated as Site I and Site II, and are present in sub-domains IIA and IIIA, respectively.[31–34] To determine the displacement percentage of fluorescent combination probe, fluorescent markers for distinct binding sites were chosen: ketoprofen for site I and ibuprofen for site-II.[35] The following equation is adopted:

\[
Probedisplacement = \frac{F_2}{F_1} \times 100 \quad (5)
\]

Where \(F_1\) and \(F_2\) represent the fluorescence intensity of Cuminaldehyde-BSA/HSA system in the absence and presence of the probe respectively.

The increase in ketoprofen concentration results in significant decrease in fluorescence intensity(Fig. 3) for both the system indicating that Cuminaldehyde binds at site I of both BSA and HSA.

**4 Conclusions**

The present work provides various methodologies to understand the mechanism of interaction of pharmaceutically important component of spices, Cuminaldehyde with BSA and HSA. Fluorescence spectroscopic measurements confirmed that Cuminaldehyde interacted with BSA and HSA and quenched its fluorescence intensity via static quenching mechanism. The change in secondary conformation of BSA and HSA upon interaction with cuminaldehyde was explored by UV-Visible absorption studies. The location of binding site for Cuminaldehyde in BSA and HSA was investigated by site probe displacement experiments and the results indicated that Cuminaldehyde was bound to BSA and HSA at site I. Thermodynamic studies revealed that vander Waal’s interaction and hydrogen bonding play a major role in Cuminaldehyde-BSA system while electrostatic interaction plays vital role in Cuminaldehyde-HSA system.

**Declarations**

**Acknowledgment**

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References

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Figures
Figure 1

(A) Fluorescence emission spectra ($\lambda_{ex} = 285$nm) of BSA (1.0 $\mu$M) at 298 K with increasing concentration of cuminaldehyde (B) Stern–Volmer plots of F0/F for BSA against [ligand] at three different temperatures. (C) Fluorescence emission spectra ($\lambda_{ex} = 285$nm) of HSA (1.0 $\mu$M) at 298 K with increasing concentration of cuminaldehyde (D) Stern–Volmer plots of F0/F for HSA against [ligand] at three different temperatures.
Figure 2

UV–vis absorption spectra of BSA with various concentrations of cuminaldehyde(A) and HSA with various concentration of cuminaldehyde(B).

Figure 3

The effect of site probe on Cuminaldehyde-BSA and Cuminaldehyde-HSA system.