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Characterization and Comparison of Interaction of Capsaicin with Hemoglobin and Bovine Serum Albumin Using Circular Dichroism

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ABSTRACT: Capsaicin, the primary pungent compound in chili peppers (*Capsicum* species), exhibits a wide range of pharmacological and biological activities. Investigating its interaction with proteins is crucial for understanding its behaviour in biological systems and potential physiological effects. This study explores the binding of capsaicin with two model proteins, hemoglobin (Hb) and bovine serum albumin (BSA), using circular dichroism (CD) spectroscopy to evaluate structural changes induced by ligand interaction. Far-UV CD spectra of Hb and BSA reveals characteristic negative bands around 208 nm and 222 nm, consistent with their predominantly α-helical secondary structures. Upon titration with capsaicin, significant changes in the intensity of these bands were observed, indicating partial alterations in α-helical content and conformational adjustments in both proteins. These structural modifications suggest that capsaicin binds to Hb and BSA, likely through hydrophobic interactions and potential hydrogen bonding with specific amino acid residues. Comparative analysis showed differences in the extent of conformational change between Hb and BSA, reflecting variations in their binding affinity and interaction modes with capsaicin. The results highlight the impact of capsaicin on protein stability and secondary structure and demonstrate the utility of CD spectroscopy as an effective tool for probing protein–ligand interactions. This study provides valuable insights into the molecular mechanisms of capsaicin–protein binding, which may inform its physiological and therapeutic relevance.

KEYWORDS: Capsaicin, Serum albumin, Circular dichroism, metalloprotein.

INTRODUCTION

Capsaicin is the principal pungent alkaloid found in chili peppers belonging to the *Capsicum* genus and is chiefly responsible for their distinctive spicy taste. Chemically identified as trans-8-methyl-N-vanillyl-6-nonenamide ($C_{18}H_{27}NO_3$)(figure 1), capsaicin is a hydrophobic phenolic molecule that has gained significant scientific interest owing to its wide range of biological and pharmacological activities. Recent studies have highlighted its therapeutic potential, including analgesic, anti-inflammatory, antioxidant, antimicrobial, and anticancer properties [1]. The growing recognition of these bioactivities has prompted increased investigation into the molecular mechanisms governing capsaicin interactions with biological macromolecules, particularly proteins, which play essential roles in cellular regulation and physiological functions [2]. Protein–ligand interactions are central to numerous biochemical processes such as molecular transport, signal transduction, enzymatic reactions, and drug efficacy. Binding of small bioactive compounds to proteins can trigger structural and conformational modifications that influence protein stability, functionality, and overall biological activity. Consequently, examining small molecule–protein interactions provides important insights into biochemical behavior, transport pathways, and potential physiological outcomes. In biophysical studies, well-characterized model proteins are commonly utilized to explore such interactions. Hemoglobin (Hb) and bovine serum albumin (BSA) are among the most frequently employed proteins due to their well-defined structural features, biological relevance, and easy availability.

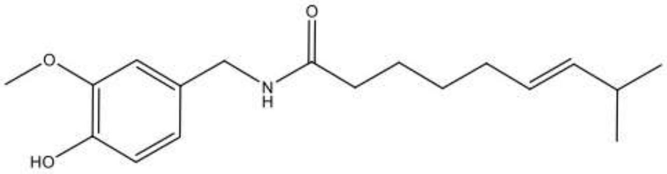

Figure 1. Chemical Structure of Capsaicin


Figure 2. Visuals of Chili with plants


Figure 3. Raw powder of Chili pepper (Capsaicin)

Hemoglobin is an essential metalloprotein found in red blood cells that plays a vital role in oxygen transport. It carries oxygen from the lungs to body tissues and facilitates the transport of carbon dioxide back to the lungs for exhalation. Structurally, hemoglobin is a tetrameric protein composed of two α- and two β-polypeptide chains, each containing a heme prosthetic group capable of binding oxygen molecules. Due to its complex quaternary structure and functional importance, hemoglobin has often been used as a model system to study the effects of ligand binding on protein conformation and stability. The interaction of small molecules with hemoglobin may lead to structural modifications that can influence its functional properties and molecular stability [3].

Bovine serum albumin is another extensively studied globular protein commonly used in biochemical and biophysical investigations. BSA is one of the most abundant proteins in blood plasma and primarily functions as a carrier molecule for a wide range of endogenous and exogenous substances, including fatty acids, hormones, metabolites, and pharmaceutical compounds. Because of its structural similarity to human serum albumin, high binding capacity, and remarkable stability, BSA serves as an excellent model protein for studying protein–ligand interactions [4]. Investigating the binding behaviour of bioactive compounds with serum albumins provides valuable insights into their transport, distribution, and bioavailability in physiological systems [5].


Figure 4. Raw material of Hemoglobin (Hb)


Figure 5. Chemical flakes of Bovine Serum Albumin (BSA)

Hemoglobin, in its purified native form, typically appears as a deep red crystalline or powder-like material, a characteristic color arising from the iron–porphyrin (heme) complex present within the protein structure [6]. In contrast, bovine serum albumin (BSA) is a single-polypeptide, heart-shaped globular protein composed of approximately 583 amino acid residues. Its structure is predominantly α-helical and stabilized by multiple disulfide linkages. Functionally, BSA serves as an important transport protein in blood plasma, carrying fatty acids, hormones, and various pharmaceutical compounds. When purified, BSA is generally obtained as a white to off-white powder that readily dissolves in water to form clear solutions [7]. Capsaicin, a hydrophobic phenolic molecule, interacts with both hemoglobin and BSA mainly through hydrophobic forces and hydrogen-bonding interactions. Binding of capsaicin to hemoglobin may induce minor conformational adjustments and influence the stability of the heme microenvironment. In the case of BSA, capsaicin preferentially associates with hydrophobic binding cavities known as Sudlow's sites, which can enhance its solubility, transport, and distribution within biological systems. Such interactions are important for understanding the bioavailability and pharmacological effectiveness of capsaicin [8].

Spectroscopic approaches are extensively employed to investigate protein–ligand interactions and structural modifications in proteins. Among these, circular dichroism (CD) spectroscopy is a highly effective technique for evaluating protein secondary structure and detecting conformational changes following ligand binding. CD spectroscopy measures the differential absorption of left- and right-circularly polarized light by optically active molecules, providing insight into structural elements such as α-helices, β-sheets, and random coils. Variations observed in CD spectra after ligand association can reveal alterations in protein secondary structure and overall conformation [9]. Given the biological relevance of capsaicin and the critical role of protein–ligand interactions, studying its binding behavior with biologically significant proteins is of considerable scientific interest. Hemoglobin and bovine serum albumin serve as suitable model proteins for examining ligand-induced structural changes. Accordingly, the present study aims to spectroscopically characterize the interaction of capsaicin with hemoglobin and BSA using circular dichroism spectroscopy to assess potential modifications in their secondary structural organization upon binding.

MATERIALS AND METHOD

Materials

Capsaicin (purity >96%, molecular weight 305.40 g/mol, CAS No. 404-86-4), hemoglobin (Hb; molecular weight 64,500 g/mol, CAS No. 9008-02-0), and bovine serum albumin (BSA; molecular weight 66,500 g/mol, CAS No. 9048-46-8) were purchased from CDH Chemicals and utilized as received. Stock solutions were prepared in a 10 mM citrate–phosphate buffer adjusted to pH 7.3, containing 5.4 mM Na_2HPO_4 and 0.453 mM citric acid. The concentrations of capsaicin and protein samples were determined using spectrophotometric absorbance measurements at their respective wavelengths. The molar extinction coefficients applied were 3,410 M^{-1} cm^{-1} at 280 nm for capsaicin, 179,000 M^{-1} cm^{-1} at 405 nm for hemoglobin, and 43,824 M^{-1} cm^{-1} at 279 nm for BSA. All experimental procedures involving capsaicin were performed in the same citrate–phosphate buffer system maintained at pH 7.3 to ensure consistency across measurements. The buffer solution was prepared using deionized water and filtered through a 0.22 µm Millipore membrane filter to remove suspended impurities and achieve high clarity. All reagents and chemicals used in the experiments were of analytical grade and were employed without further purification.

Methods

Circular dichroism (CD) analyses were conducted using a Jasco J-1500 spectropolarimeter at room temperature (≈25 °C) with quartz cuvettes having an optical path length of 0.1 cm (Sterna Cells Inc.). Measurements were performed in the far-UV spectral range of 190–250 nm to examine the secondary structural organization of the proteins. This technique was utilized to investigate structural and conformational changes in hemoglobin (Hb) and bovine serum albumin (BSA) upon interaction with capsaicin under near-physiological conditions (pH 7.3). Protein solutions containing 5 µM Hb and 1 µM BSA were prepared in appropriate buffer media. Separate buffer spectra were recorded under identical experimental conditions and subtracted from the corresponding protein spectra to eliminate background contributions. Spectral acquisition was performed with a bandwidth of 1 nm and a scanning interval of 0.5 nm. Each final spectrum represented the average of three successive scans, thereby improving spectral accuracy and reducing noise.

The far-UV CD spectra revealed two distinct negative ellipticity minima around 208 nm and 222 nm, associated with $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions, respectively, confirming the predominance of α-helical secondary structure in both proteins. These characteristic spectral features were subsequently used for quantitative estimation of α-helical content using established analytical methods. To evaluate structural perturbations induced by ligand binding, Hb and BSA samples were gradually titrated with increasing concentrations of capsaicin ranging from 0 to 100 µM. CD measurements were recorded immediately after each addition to minimize time-dependent effects and accurately monitor conformational changes resulting from capsaicin interaction.

RESULTS AND DISCUSSION

Circular dichroism (CD) spectroscopy was employed to examine structural modifications in hemoglobin (Hb) induced by capsaicin binding. CD spectroscopy is a highly sensitive method for monitoring ligand-induced conformational variations in proteins by detecting alterations in their secondary structural organization. Since capsaicin itself is optically inactive in the far-UV region, any observed spectral changes arise primarily from structural perturbations within the protein framework upon ligand interaction. The far-UV CD spectrum of native Hb displayed two prominent negative ellipticity bands at approximately 208 and 222 nm, which are characteristic signatures of an α-helical protein structure. The band at 208 nm originates from the π→π* electronic transition of the peptide bond within the α-helix, whereas the 222 nm band corresponds to the n→π* transition associated with both α-helical and random coil conformations. These transitions collectively reflect the secondary structural integrity of hemoglobin and are widely used for quantitative estimation of α-helical content [10]. Native Hb exhibited helical content consistent with previously reported structural data [11,12]. Upon gradual addition of capsaicin, noticeable variations in ellipticity at 208 and 222 nm were observed (Shown in Fig. 6), indicating ligand-induced perturbations in the secondary structure of Hb. The decrease in α-helical content at higher capsaicin concentrations suggests partial unfolding or rearrangement of peptide backbone conformations, confirming the formation of a Hb–capsaicin complex. In addition to the far-UV region, the near-visible (Soret band) CD spectrum provided insight into structural changes surrounding the heme environment. Native Hb showed a characteristic positive CD signal in the Soret region, reflecting the asymmetric arrangement of the heme prosthetic group within the globin matrix. Binding of capsaicin resulted in measurable alterations in the intensity and position of this band, indicating modifications in the tertiary structure and microenvironment of the heme moiety. These observations collectively demonstrate that capsaicin interaction influences both the secondary structure and the heme-associated tertiary conformation of hemoglobin, supporting conclusions drawn from complementary spectroscopic analyses.

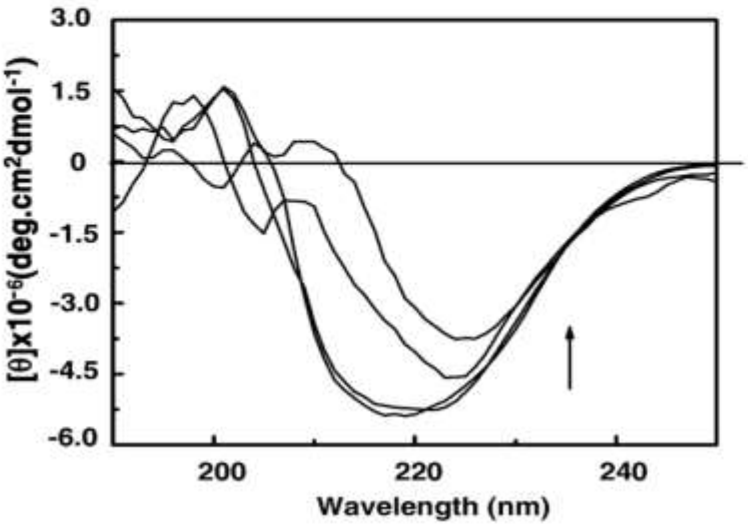

Figure 6. Far-UV circular dichroism spectra of hemoglobin recorded during stepwise titration with capsaicin at concentrations of 0, 0.5, 1.0, 2.0, and 4.0 µM.

The far-UV circular dichroism (CD) spectrum of native bovine serum albumin (BSA) recorded under experimental pH condition exhibited two characteristic negative ellipticity minima near 210 and 222 nm (observed in Fig. 7), confirming the predominance of an α-helical secondary structure, consistent with earlier literature reports [13,14]. Analysis of the CD data using Jasco secondary-structure estimation software revealed that BSA consists of approximately 56% α-helix, ~30% β-sheet (including both parallel and antiparallel arrangements), and nearly 13% random coil conformation. The secondary and tertiary structures of BSA remain largely stable across the condition.

Capsaicin itself does not exhibit intrinsic CD activity in the far-UV region, which also discussed in above paragraph; therefore, any spectral alterations arise from ligand-induced structural perturbations in the protein. Progressive titration of BSA with increasing concentrations of capsaicin resulted in a gradual reduction in the intensity of the CD bands at 210 and 222 nm, suggesting partial disruption of the α-helical framework. The decrease in ellipticity indicates conformational rearrangements of the peptide backbone upon ligand binding. At higher capsaicin concentrations approaching saturation, a measurable decline in α-helical content was observed, accompanied by a relative increase in β-sheet and random coil contributions, reflecting partial unfolding of the protein structure. These findings demonstrate that capsaicin binding induces notable secondary structural modifications in BSA, supporting the formation of a stable protein–ligand complex and highlighting the sensitivity of albumin's helical domains to hydrophobic ligand interactions.

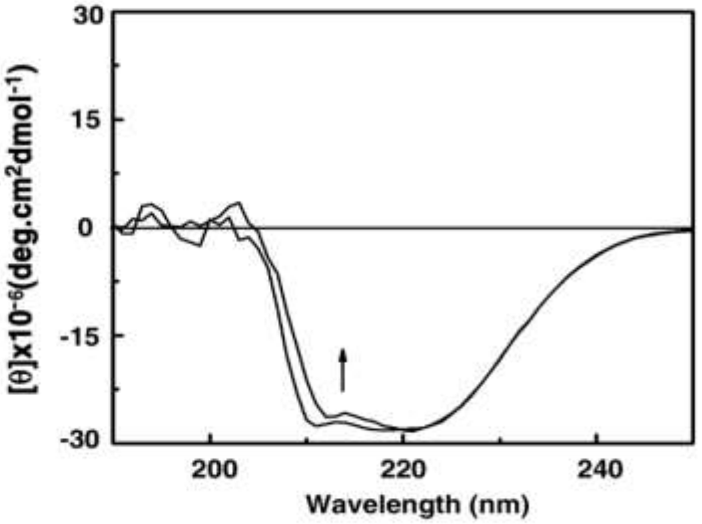

Figure 7. Far -UV CD spectral changes of BSA (1 µM) interaction on several concentration of capsaicin 0, 50 and 100 µM.

The absence of observable circular dichroism (CD) signals in the near-UV region during capsaicin–protein interaction indicates that ligand binding does not significantly perturb the tertiary structure of the protein. Near-UV CD spectra (250–320 nm) mainly arise from the asymmetric environments of aromatic amino acid residues such as tryptophan, tyrosine, and phenylalanine, along with disulfide chromophores. Detectable changes occur only when ligand binding alters the spatial orientation or microenvironment of these groups. Capsaicin predominantly interacts through hydrophobic and surface-level associations, producing limited conformational rearrangement around aromatic residues; consequently, near-UV CD signals remain unchanged despite measurable secondary structural effects in the far-UV region [15].

Comparison of proteins CD spectral analysis on drugs Capsaicin

Comparative analysis of circular dichroism (CD) spectra of hemoglobin (Hb) and bovine serum albumin (BSA) reveals distinct yet related structural responses upon interaction with capsaicin. The far-UV CD spectra of both proteins exhibited characteristic negative ellipticity bands near 208–210 nm and 222 nm, confirming the predominance of α-helical secondary structures, consistent with established protein CD signatures [15]. In Hb, capsaicin binding produced noticeable reductions in ellipticity at these wavelengths, indicating perturbation of the α-helical backbone and partial conformational rearrangement. Additionally, changes observed in the Soret CD region suggested alterations in the heme microenvironment, demonstrating that capsaicin influences not only secondary structure but also tertiary organization around the prosthetic group. In contrast, BSA displayed gradual decreases in CD band intensity without major disruption of its overall structural stability, reflecting moderate unfolding primarily within helical domains. Secondary-structure analysis showed a reduction in α-helix content accompanied by a relative increase in β-sheet and random coil contributions, indicating ligand-induced flexibility rather than extensive structural destabilization. The absence of intrinsic CD activity of capsaicin confirms that all spectral variations originate from protein conformational changes. Overall, Hb appears more sensitive due to heme-associated structural perturbations, whereas BSA undergoes comparatively controlled conformational adjustments, highlighting differences in ligand-binding environments and structural adaptability between globular transport proteins [16].

CONCLUSION

The present circular dichroism (CD) spectroscopic investigation provides clear evidence that capsaicin interaction induces measurable conformational modifications in both hemoglobin (Hb) and bovine serum albumin (BSA), although the extent and nature of structural perturbations differ between the two proteins. Far-UV CD analysis confirmed that native Hb and BSA predominantly possess α-helical secondary structures, as indicated by characteristic negative bands near 208–210 and 222 nm. Upon capsaicin binding, progressive reductions in ellipticity were observed, demonstrating ligand-induced alterations in protein secondary structure. Hb exhibited comparatively pronounced structural sensitivity, including changes in the Soret CD region, suggesting perturbation of the heme microenvironment and modulation of tertiary organization. In contrast, BSA maintained overall structural stability, showing gradual decreases in α-helical content accompanied by increased β-sheet and random coil contributions, indicative of partial unfolding rather than extensive denaturation.

The absence of intrinsic CD activity of capsaicin confirms that observed spectral variations arise exclusively from protein conformational responses. Collectively, these findings highlight the role of hydrophobic interactions and local microenvironmental changes in governing capsaicin–protein binding mechanisms. From a broader perspective, such structural insights improve understanding of capsaicin transport, bioavailability, and protein-mediated distribution in biological systems. Future investigations integrating molecular docking, time-resolved spectroscopy, and physiological models may further elucidate binding dynamics, enabling rational design of capsaicin-based therapeutic and drug-delivery strategies.

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