



# Spectroscopic analysis, docking and molecular dynamics simulation of the interaction of cinnamaldehyde with human serum albumin

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## Abstract

Human serum albumin (HSA) is one of the blood plasma proteins, which plays an important role in the transportation of various natural and pharmaceutical compounds in the body. Cinnamaldehyde is the main component of cinnamon extract which has several medicinal properties. In this study, the conformational changes of HSA in the presence of cinnamaldehyde were investigated by UV–Vis, fluorescence and FT-IR spectroscopic methods and the binding parameters of cinnamaldehyde to HSA were determined. The binding site and driving forces of this interaction were calculated using molecular docking softwares. On the other hand, molecular dynamics simulations were also performed with the GROMACS program package. Using the results of the intrinsic fluorescence spectroscopy data, binding constant of  $1.14 \times 10^4 \text{ M}^{-1}$  and number of binding sites 0.883 was obtained. FT-IR measurements have shown that the secondary structure of the protein have been changed by the interaction of cinnamaldehyde with HSA. The free energy of binding ( $\Delta G^\circ$ ) and binding constant ( $K_a$ ) obtained from the intrinsic fluorescence results were  $-5.54 \text{ kcal mol}^{-1}$  and  $1.14 \times 10^4 \text{ M}^{-1}$  respectively, which is in good agreement with molecular docking results. On the other hand, the molecular docking calculations showed that cinnamaldehyde binding site is located in the IA subdomain of HSA. Spectroscopy, molecular docking and MD simulations methods showed that the cinnamaldehyde is bound to the HSA protein and hydrogen bonding and hydrophobic forces play the most important role in this interaction. Excellent agreement was found between the experimental and theoretical results.

**Keywords** Human serum albumin · Cinnamaldehyde · Fluorescence spectroscopy · FT-IR · Molecular docking · Molecular dynamics simulation

## Introduction

Given the concerns of people about the side effects of chemical drugs, including drug resistance, nowadays in the medical sciences, the use of herbal compounds with different properties has been widely considered. Cinnamon is a spice obtained from *Cinnamomum* of the *Laurus nobilis* family, which has many medicinal properties [1]. The main components of the cinnamon essential oil are cinnamaldehyde and eugenol (>90%). The commercial value of cinnamon essential oil depends on the amount of its cinnamaldehyde. Cinnamaldehyde ( $\text{C}_9\text{H}_8\text{O}$ ) is a chemical compound with a

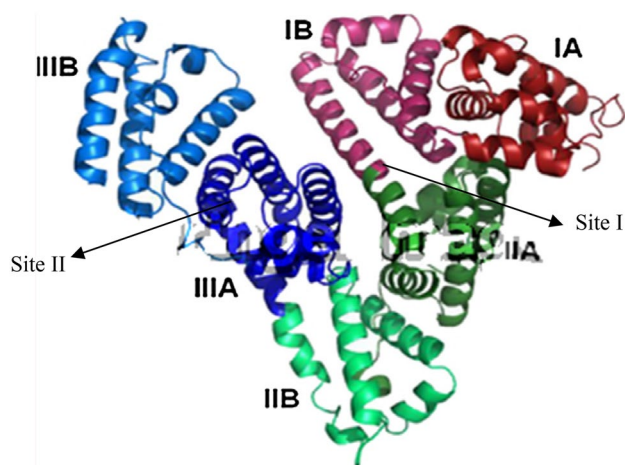
molar mass of  $132.16 \text{ g mol}^{-1}$  [2] and the natural product is trans-cinnamaldehyde. This molecule comprises a phenyl group bound to unsaturated aldehyde [3]. Cinnamon has antioxidant, antimicrobial, antidiabetic, antiviral and anti-spasmodic properties, which increases the body's sweating and stimulates the uterus. Most of cinnamon properties are due to the presence of cinnamaldehyde [4], which can be used as an effective compound in the pharmaceutical and food industries.

Human serum albumin (HSA) is the most abundant plasma protein ( $36\text{--}50 \text{ g L}^{-1}$ ), which is synthesized in the liver and has a half-life of 19 days [5]. HSA consists of 585 amino acids and 17 disulfide bonds with a molecular weight of 67.5 kDa [6]. This protein is involved in the distribution, metabolism and transfer of fatty acids and medicines. In addition, it also contributes to the regulation of osmotic pressure and pH of the blood [7]. The HSA molecule contains three homologous domains I, II and III, which include amino acids 1–195, 196–383, 384–585

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**Fig. 1** X-ray crystallography 3D structure of HSA with its subdomains and binding sites [9]

respectively, and each of its domains consists of two subdomains (A and B) (Fig. 1) [8].

Ligands, which bind to HSA, can alter the conformation of the protein [10]. Initially, it was believed that the ligands bind to HSA in a non-specific manner, but now it has been found that there are a limited number of binding sites. The most important binding sites of HSA are I (Site I) and II (Site II) (Fig. 1). These two binding sites are located in hydrophobic cavities of IIA and IIIA subdomains [11]. In addition to these binding sites, there are some more hydrophilic binding sites for some ligands on this protein [12].

As mentioned above, cinnamaldehyde is a natural product with medicinal properties. On the other hand, HSA carries different ligands in the blood. In this study, structural changes of HSA protein in the presence of different concentrations of trans-cinnamaldehyde is investigated by spectroscopy and molecular docking, to determine the importance of this protein in the transfer of cinnamaldehyde. This study can provide useful information for drug design in the field of medical research and the use of plants in the pharmaceutical and food industries in the future.

## Materials and methods

### Materials

HSA (code: A1653) and trans-cinnamaldehyde (code: C80687) were purchased from Sigma-Aldrich Chemical company. In this study, 20 mM Tris-HCl buffer pH 7.4 was used. The stock solution of cinnamaldehyde (5 mM) prepared in 96% ethanol (purchased from Merck Company).

## Methods

### UV-Vis spectroscopy studies

The absorption spectra were recorded by an EPOCH UV-Vis spectrophotometer in the wavelength range of 235–350 nm at room temperature. HSA solution (2.5  $\mu\text{M}$ ) was prepared in (20 mM) tris buffer of pH 7.4. At first, 2.5 mg of HSA protein dissolved in 1.5 ml of tris buffer. In order to obtain the concentration of this protein solution, HSA adsorption was determined at 280 nm and then Beer-Lambert law was used (Eq. 1), in this equation, A is absorption at 280 nm, L is the length of the light path (1 cm), C is the protein concentration, and  $\epsilon$  is the molar extinction coefficient of HSA, which is 37,500  $\text{M}^{-1} \text{cm}^{-1}$  [13].

$$A = \epsilon CL \quad (1)$$

After preparing HSA solution 2.5  $\mu\text{M}$ , the absorbance of HSA was read in the absence of cinnamaldehyde, and then HSA titrated with a stock solution of cinnamaldehyde, so that the molar ratios of cinnamaldehyde to HSA were 0, 0.5, 1, 3, 5, 7.5, 10 and 12.5. After each step of adding the desired volume of cinnamaldehyde, the sample was incubated for 3 min. Additionally, at each step of the titration, an equal volume of cinnamaldehyde added to control cuvette containing 1500  $\mu\text{l}$  of Tris buffer to reduce the ligand absorbance of the complex.

### Fluorescence quenching studies

Fluorescence spectroscopy is a useful technique for studying structural changes of proteins and obtaining the thermodynamic parameters of ligand binding to the protein in solution. All fluorescence spectra were recorded on a Fluorescence Spectrophotometer from Varian Australia company and a quartz cell of 1 cm width was used for the measurements. The excitation wavelength was adjusted at 280 nm and the wavelength range of emission was 300–500 nm. Slits were 2 nm for both excitation and emission monochromators.

The intrinsic fluorescence emission of HSA (2.5  $\mu\text{M}$ ) was measured in the absence of cinnamaldehyde, and then HSA titrated by the stock solution of cinnamaldehyde with molar ratios of 0, 0.5, 1, 3, 5, 7.5, 10 and 12.5. After each step of titration, the sample was incubated for 3 min, and then the fluorescence emission spectrum was measured.

### FT-IR spectroscopy studies

FT-IR measurements were carried out on a Bruker FT-IR spectrometer. FT-IR spectroscopy has long been used as a powerful method for investigating the secondary structure of proteins and their dynamics. In the IR region, the

frequencies of bands due to the amide I–III vibrations are sensitive to the secondary structure of proteins. Particularly, the amide I band is useful in studying the secondary structure. The amide I band (mainly C=O stretch) position occurs in the region of 1600–1700  $\text{cm}^{-1}$  and the amide II band (C–N stretch coupled with N–H bending mode) in 1500–1600  $\text{cm}^{-1}$ . Amide I band is more sensitive to the protein secondary structure changes than amide II [14]. In this study, we report the FT-IR spectroscopic results on the binding of cinnamaldehyde to HSA, when the concentration of cinnamaldehyde and HSA both were 2.5  $\mu\text{M}$ .

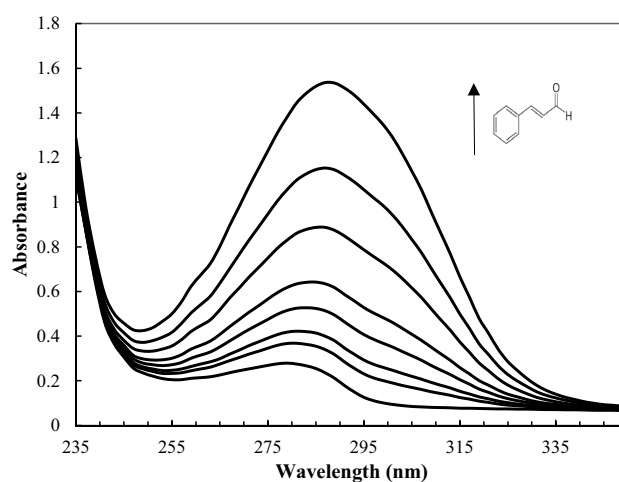
### Molecular docking

The crystal structure of the HSA was obtained from a Protein Data Bank with a PDB ID: 1E7I. At the protein preparation stage, with the help of the Arguslab 4.0.1 software, additional structures such as ligands and all water molecules were removed, hydrogen atoms were added to the protein, and the structure was optimized [15]. The structure of the ligand was drawn up in the HyperChem software and then the structure optimization was done [16]. In this study, molecular docking was done by AutoDock4.2 and AutoDockTools-1.5.6 in the PMV-1.5.6<sup>1</sup> software environment [17]. The molecular docking was performed with the Lamarckian genetic algorithm, so that the protein and ligand are considered rigid and flexible, respectively. HSA protein was enclosed in ten boxes with a number of grid points in x, y and z directions, 100, 90 and 60 and a grid spacing of 0.375 Å. For each of the docking cases, the energy of docked conformation was calculated according to the autodock scoring function, and a rank with the lowest energy was selected as the binding mode, then the interaction was shown with LigPlot [18].

VMD1.8.7 software was used to model the complex and obtain the distance between cinnamaldehyde and HSA chromophore (Trp 214) [19]. For this purpose, the PDB file obtained from cinnamaldehyde and HSA docking was visualized in VMD software and cinnamaldehyde distance from tryptophan residue was reported.

### Molecular dynamics simulations

The obtained molecular docking results of ligand–HSA allowed us to propose a general binding mode. The MD simulation can be used for further investigation of binding modes of ligands and to explain the effects of ligand binding on the conformation of the protein. In this research, the MD simulation was done by the GROMACS software version 5.1.2 and the Ubuntu Linux operating system, and



**Fig. 2** Absorption spectra of HSA in the absence and presence of cinnamaldehyde in 20 mM Tris buffer at pH 7.4. Concentration of HSA: 2.5  $\mu\text{M}$ ; containing cinnamaldehyde with molar ratios of 0, 0.5, 1, 3, 5, 7.5, 10 and 12.5. (The arrow shows the increase of the cinnamaldehyde concentration)

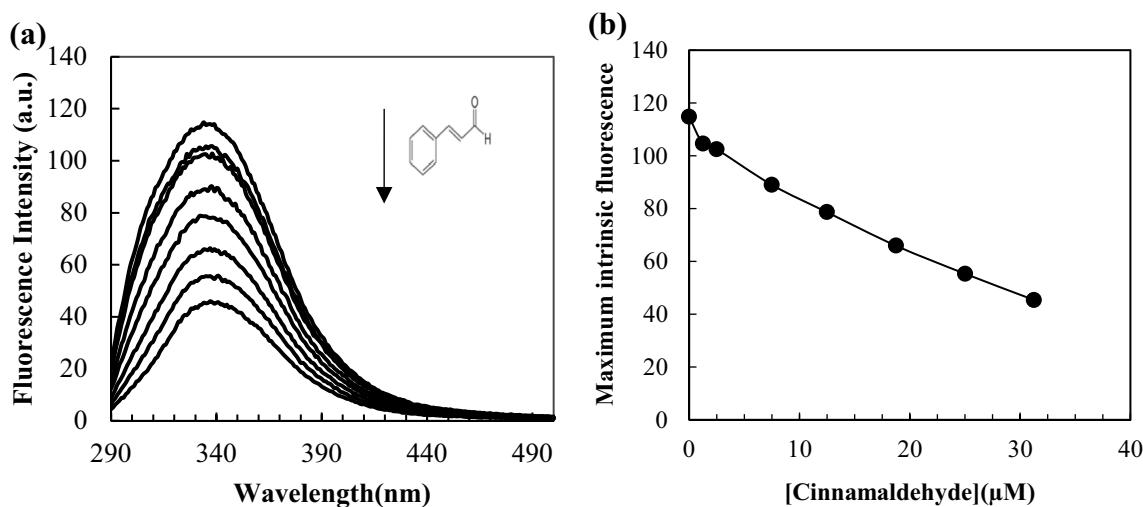
the HSA topology parameters were created [20]. The interaction parameters and long-range electrostatic interactions were calculated using CHARMM-27 force field and by the Particle-Mesh Ewald (PME) method, respectively. The initial atomic velocity was produced by a Maxwellian distribution at an absolute temperature [21]. The coordinate of ligand (cinnamaldehyde) to Gromacs topologies were transferred using the SWISSPARAM server. Then the set was immersed in a triclinic box of extended simple point charge (SPC) water molecules [22]. After adding water molecules, the solvated system was neutralized by adding  $\text{Na}^+$  and  $\text{Cl}^-$  in the simulation. Then the energy was minimized using the steepest descent minimization algorithm of 1295 steps with a cutoff 1 nm for van der Waals and Coulomb forces. In order to prevent the deviation of the protein position during molecular dynamics simulation, the simulation was performed using NVT and NPT ensembles. For this purpose, in the NPT phase, pressure and temperature, and in the NVT phase, the volume and temperature were fixed. Finally, the full system was subjected to 20 ns MD at 1 bar pressure and 298 K temperature.

## Results and discussion

### UV–Vis spectroscopy

The absorption spectra of HSA in the presence of cinnamaldehyde at different concentrations are shown in Fig. 2. HSA showed one absorption band at about 280 nm which is related to protein chromophores. The HSA chromophores in this range of wavelengths are three amino acids of

<sup>1</sup> Python molecular viewer.



**Fig. 3** Emission spectra (a) and maximum fluorescence emission (b) of HSA (2.5  $\mu\text{M}$ ) containing cinnamaldehyde with molar ratios of 0, 0.5, 1, 3, 5, 8, 10 and 12.5. (The arrow shows the concentration increase of cinnamaldehyde). [Tris-HCl] = 20 mM, pH 7.40

tryptophan, phenylalanine and tyrosine. Upon increasing the concentration of cinnamaldehyde, the absorbance at 280 nm was gradually increased. This indicates that there is an interaction between cinnamaldehyde and HSA and complex may be formed. The binding of cinnamaldehyde to HSA causes structural changes in this protein.

### Fluorescence spectroscopy

As shown in Fig. 3a the maximum of HSA emission was at the wavelength of 334 nm, so it could be said that fluorophores of HSA, especially tryptophan residue (Trp 214), are in a non-polar environment. The maximum emission wavelength has not significantly changed (only about 3 nm blue shift) because of the interaction of cinnamaldehyde with HSA. The fluorescence quenching which is induced by a variety of molecular interactions between fluorophores and quencher molecules can reveal the mechanisms of the binding of small molecules to proteins [23]. Increasing the concentration of the ligand, decreased the HSA intrinsic fluorescence emission. Quenching can appear as result of various inter and intramolecular interactions, i.e. molecular collisions (dynamic quenching), complex formation (static quenching), energy transfer and/or conformational changes [24]. The reason for the quenching of the HSA intrinsic fluorescence emission accompanied by blue shift can be the presence of binding sites near the fluorophores in the protein, and also a conformational change of HSA with the transfer of tryptophan and tyrosine to a less polarized environment.

Figure 3b shows the maximum HSA emission in the presence of different concentrations of cinnamaldehyde.

Fluorescence quenching can be described by Stern–Volmer equation [25, 26]:

$$F_0/F = 1 + K_{sv}[Q] = 1 + k_q\tau_0[Q] \quad (2)$$

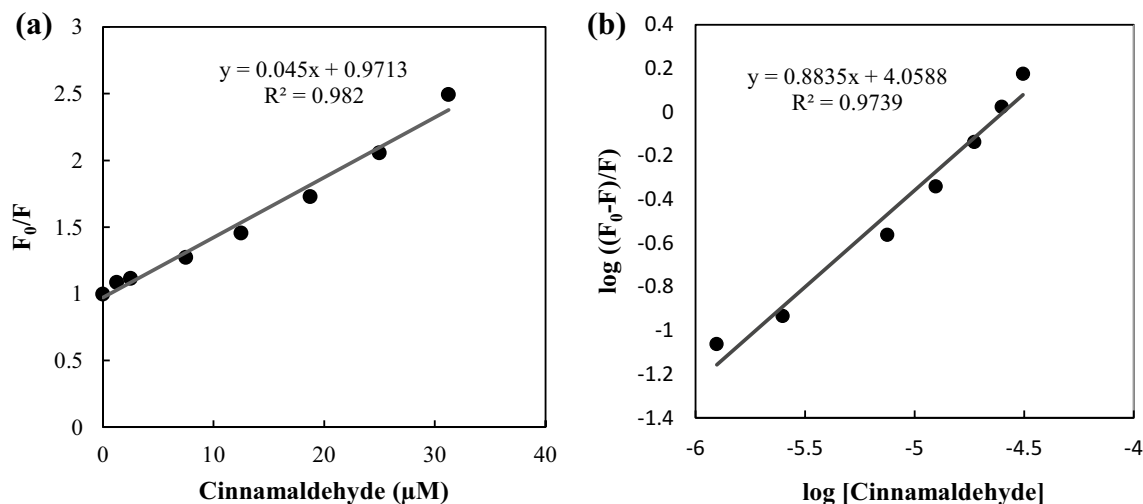
where  $F$  and  $F_0$  are the fluorescence intensities in the presence and absence of quencher (cinnamaldehyde), respectively;  $[Q]$  is the concentration of quencher,  $K_{sv}$  is the Stern–Volmer quenching constant, which measures the efficiency of quenching.  $k_q$  is the quenching rate constant of the protein,  $\tau_0$  is the average lifetime of the fluorophore (tryptophan) in the absence of quencher (5.7 ns) [27]. As shown in Fig. 4a, the Stern–Volmer diagram is plotted on the basis of Eq. 2. The value of  $K_{sv}$  obtained from the Stern–Volmer plot slope  $4.5 \times 10^4 \text{ M}^{-1}$ . The Stern–Volmer constant can be used to calculate the quenching rate constant ( $k_q$ ). Previous studies have shown that if the  $k_q$  value is greater than  $2 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ , the quenching is static and if smaller, the quenching is of a dynamical type [28]. In this study, the  $k_q$  value of  $7.9 \times 10^{12} \text{ L mol}^{-1} \text{ s}^{-1}$  was found. It indicates that the major quenching mechanism is static, so cinnamaldehyde is bound to the HSA.

### Binding constant and the binding sites

In the case of static quenching, the binding constant and number of binding sites can be obtained using Eq. (3):

$$\log[(F_0 - F)/F] = \log K_a + n \log[Q] \quad (3)$$

where  $K_a$  is the binding constant of cinnamaldehyde to HSA (Anti log y-intercept) and  $n$  is the number of binding sites (plot Slope) [29, 30]. According to Fig. 4b, the  $K_a$  value is  $1.14 \times 10^4 \text{ M}^{-1}$  at 298 K, indicating strong binding between the HSA and cinnamaldehyde. The number of binding site  $n$



**Fig. 4** Stern–Volmer (a) and Lineweaver–Burk (b) plots for the fluorescence quenching of HSA by cinnamaldehyde at 298 K temperature and excitation wavelength of 280 nm

is about 0.883 indicating that there almost is one site for the binding of cinnamaldehyde on HSA molecule.

Also, after calculating the binding constant, free energy of binding ( $\Delta G^\circ$ ) was obtained by Eq. (4)  $-23151.67 \text{ J mol}^{-1}$  ( $-5.54 \text{ kcal mol}^{-1}$ ) [31]. In this equation, R (gas constant) is equal to  $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$  and T (temperature) is 298.1 K.

$$\Delta G^\circ = -RT \ln K_a \quad (4)$$

### Energy transfer mechanism

Fluorescence resonance energy transfer (FRET) is a reliable method for studying protein–ligand interactions and to determine the distance between the donor (HSA) and the acceptor (cinnamaldehyde). According to Förster's non-radiative energy transfer theory, the energy transfer is possible when the fluorescence emission spectrum of the donor and UV absorption spectrum of the acceptor have suitable overlap, and the donor and the acceptor are within the Förster distance [32]. The efficiency of energy transfer (E) is related to the distance between the donor and the acceptor by Eq. (5).

$$E = 1 - F/F_0 = R_0^6 / (R_0^6 + r^6) \quad (5)$$

where E is the energy transfer efficiency, F and  $F_0$  the fluorescence intensities of HSA in the presence and absence of quencher, r is the donor–acceptor distance and  $R_0$  is the critical distance where the transfer efficiency is 50%.  $R_0$  can be calculated using the Eq. (6):

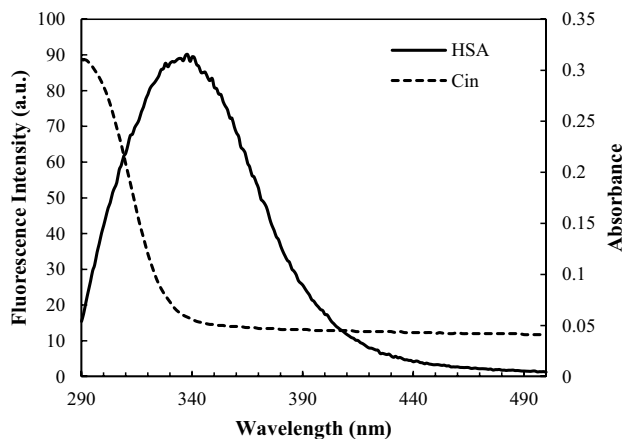
$$R_0^6 = 8.79 \times 10^{-25} K^2 N^{-4} QJ \quad (6)$$

$K^2$  is the spatial factor of orientation (2/3), N is the refractive index of the medium (1.33) and Q is the fluorescence quantum yield of the donor (0.118). Also, J is the effect of

the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor, which can be calculated as Eq. (7):

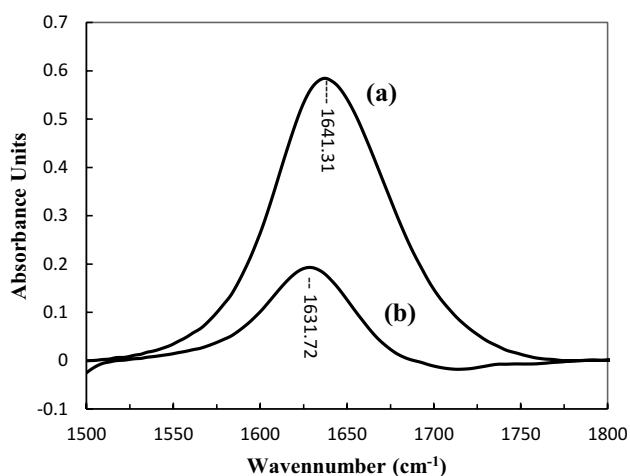
$$J = \frac{[\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda]}{[\sum F(\lambda)\Delta\lambda]} \quad (7)$$

where  $F(\lambda)$  is the corrected fluorescence intensity of the donor in the wavelength range of  $\lambda$  to  $(\lambda + \Delta\lambda)$  and  $\varepsilon(\lambda)$  the molar extinction coefficient of the acceptor in the wavelength range of  $\lambda$  to  $(\lambda + \Delta\lambda)$  [33]. Figure 5 shows the overlap of the UV–Vis absorption spectrum of cinnamaldehyde with the fluorescence emission spectrum of HSA. Equations (5–7) gave the following values:  $J = 1.028 \times 10^{-14} \text{ cm}^3 \text{ L mol}^{-1}$ ,



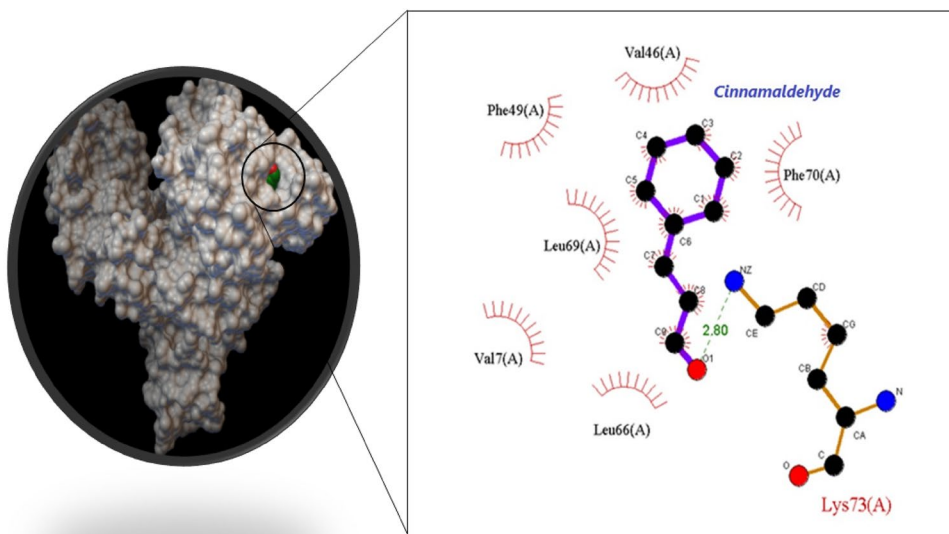
**Fig. 5** Spectral overlap of cinnamaldehyde absorption with HSA fluorescence; [HSA]=2.5  $\mu\text{M}$ , [cinnamaldehyde]=7.5  $\mu\text{M}$ . T=298 K, pH 7.40





**Fig. 6** FT-IR spectra of (a) free HSA; (b) complex [(HSA solution + cinnamaldehyde) – (cinnamaldehyde solution)] in 20 mM Tris-HCl buffer, pH 7.4, at room temperature, [HSA] = 2.5  $\mu$ M; [cinnamaldehyde] = 2.5  $\mu$ M

**Fig. 7** Figure plotted by the LigPlot program of the interaction of cinnamaldehyde and HSA. Hydrogen bond is shown with green color dots and amino acids involved in hydrophobic interactions are shown. (Color figure online)



$E=0.21$ ,  $R_0=2.5$  nm and  $r=3.12$  nm. Since the average distance  $r$  is less than 7 nm and  $0.5 R_0 < r < 1.5 R_0$ , the energy transfers from HSA to cinnamaldehyde occurred with high probability [34].

### FT-IR spectroscopy

The FT-IR spectra of HSA in the absence and presence of cinnamaldehyde is shown in Fig. 6; the peak position of amide I band is shifted from 1641.31 to 1631.72  $\text{cm}^{-1}$  because of cinnamaldehyde binding to HSA. The FT-IR spectra of the cinnamaldehyde-free and cinnamaldehyde-bound form of HSA shows that the secondary structure of the HSA is altered due to interaction with cinnamaldehyde.

### Molecular docking

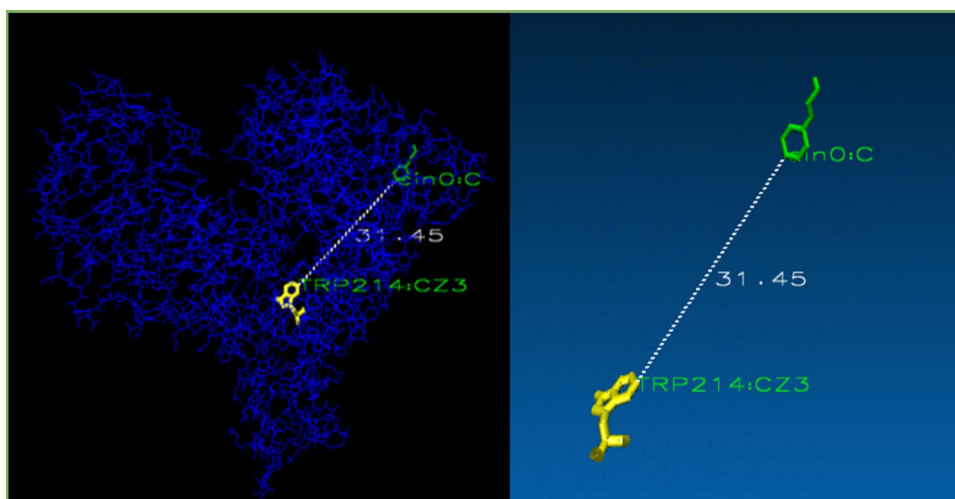
Molecular docking technique is a useful method to understand the ligand–protein interaction which can corroborate our experimental results. In this study, according to autodock 4.2 calculations, the best and most negative free energy of cinnamaldehyde binding to HSA between ten boxes was related to the box that located in the IA subdomain of HSA. According to docking calculations, the number of ten ranks obtained from the molecular docking calculations for this box; the most negative free energy of binding for cinnamaldehyde was  $-5.28$  kcal  $\text{mol}^{-1}$ , which was consistent with our experimental results.

On the other hand, the docked structure (Fig. 7) showed that oxygen atom of cinnamaldehyde has a hydrogen bond with the length of 2.80 Å with the lysine 73 residue from HSA. Also, the cinnamaldehyde has hydrophobic interactions with residues such as valine 7, valine 46, phenylalanine 49, leucine 66, leucine 69, and phenylalanine 70. The results obtained from

molecular docking indicated that the interaction between cinnamaldehyde and HSA was dominated by hydrophobic forces as well as hydrogen bonds.

Also, the distance between Trp 214 of HSA and cinnamaldehyde is obtained by VMD software (shown in Fig. 8). HSA protein has only one tryptophan amino acid residue at position 214, when the cinnamaldehyde interacts with HSA, the distance between cinnamaldehyde and tryptophan is 31.45 Å.

**Fig. 8** The distance between Trp 214 of cinnamaldehyde in minimum energy docking presented by VMD 1.8.7 program



## Results of molecular dynamics simulations

### Root mean square deviations (RMSD)

MD simulations were performed on HSA and HSA–cinnamaldehyde complex to investigate the dynamic properties of protein and complex in water and ions by means of RMSDs of protein and complex with respect to the initial structure of the protein. In RMSD, the average is calculated on the whole particle for every moment. In order to compare the stability of HSA in the absence and presence of cinnamaldehyde, RMSD values of atoms in free HSA and HSA complex were plotted from 0 to 20 ns as shown in Fig. 9a. As shown in Fig. 9a, there are structural changes and deviations from the original structure in HSA, where the RMSD values of atoms were fluctuated for HSA ( $0.28 \pm 0.05$  nm) and complex ( $0.23 \pm 0.04$  nm). The relative decrease in RMSD value of the complex with respect to free HSA also indicates the conformational change and increased rigidity and stability of the protein upon binding of cinnamaldehyde [35].

### Root mean square fluctuation (RMSF)

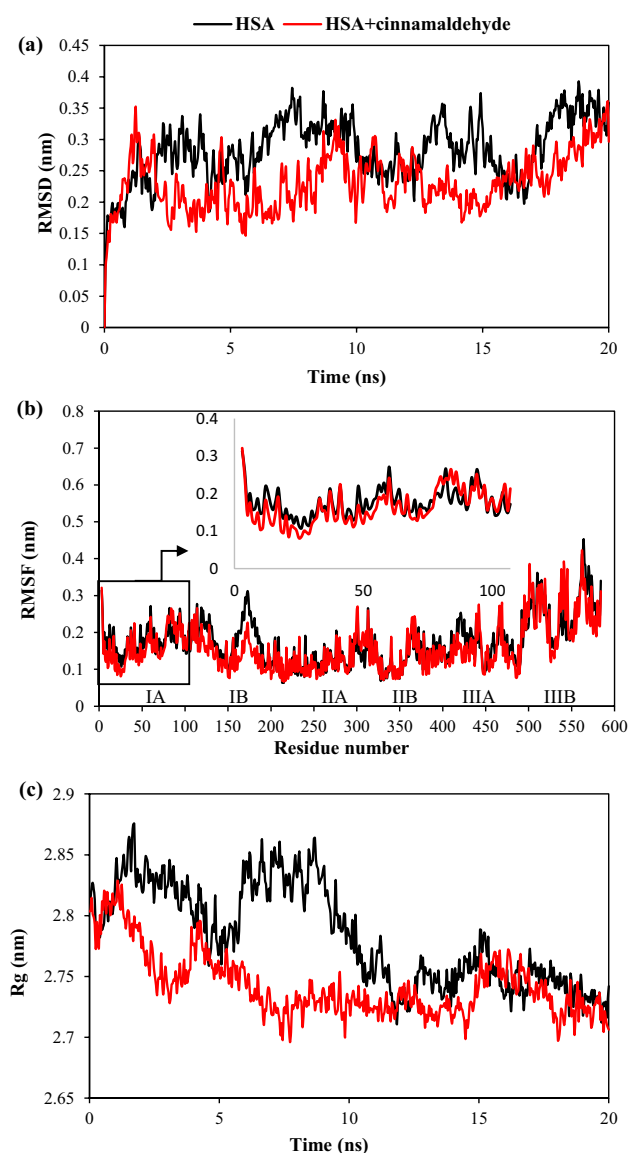
Since RMSD is not an appropriate parameter for reflecting the mobility of structural elements, the RMSF is used to check the flexibility of the protein structure. In the RMSF plot, an average is made on the total time per residue [36]. As shown in Fig. 9b, the HSA flexibility value in the presence of cinnamaldehyde has been decreased and the RMSF value was calculated as an average  $0.17 \pm 0.06$  nm for HSA and  $0.15 \pm 0.06$  nm for the complex. The comparison of RMSF for HSA–Cinnamaldehyde complex in IA subdomain, where the ligand binds, shows that RMSF value of the complex in IA subdomain was less than free HSA, with very little fluctuation.

### Radius of gyration (Rg)

The time evolution of the Rg in the course of 20 ns of MD simulation of free HSA and the HSA–cinnamaldehyde complex is shown in Fig. 9c. Initially, the Rg values of both HSA and complex was 2.81 nm. The HSA and HSA–cinnamaldehyde complex were stabilized at about 12 ns, indicating that the MD simulation achieved the equilibrium condition after this time. The HSA and HSA–cinnamaldehyde complex were stabilized at  $2.74 \pm 0.01$  and  $2.73 \pm 0.02$  nm, respectively, after 12 ns of trajectories. An earlier work [37] reported that the Rg value for free HSA, as determined experimentally by neutron scattering in aqueous solution, is  $2.74 \pm 0.04$  nm, which is in satisfactory agreement with that obtained from our MD simulations. The Rg value is a good measure for protein folding and unfolding, so the smaller values indicates the more folded system, and it becomes larger when the system turns from fold to unfold. Therefore, based on Rg results in the Fig. 9c it was concluded that the structure of the HSA in the presence of cinnamaldehyde becomes more folded during MD simulation.

## Conclusion

In the present work, the binding properties of trans-cinnamaldehyde to HSA protein has been investigated using UV–Vis, fluorescence and FT-IR spectroscopy and computational methods. The experimental results indicated that the cinnamaldehyde binds to HSA with moderate affinity and the intrinsic fluorescence of HSA was quenched through static quenching mechanism. The binding parameters were calculated using the modified Stern–Volmer equation. Results indicated that almost one cinnamaldehyde molecule binds to each HSA molecule. According to Förster resonance energy transfer (FRET) theory and



**Fig. 9** **a** Time dependence of RMSDs. RMSD values for HSA (black color) and HSA–cinnamaldehyde complex (red color) during 20 ns MD simulation; **b** root mean square fluctuation (RMSF) around the average MD structure; **c** time evolution of the radius of gyration (Rg) during 20 ns of MD simulation of HSA and cinnamaldehyde. (Color figure online)

fluorescence spectroscopy results, the distance between the tryptophan amino acid of HSA (Trp214) and cinnamaldehyde, which is expressed with “r”, was obtained 3.12 nm that indicates the energy transfer has most probably occurred between cinnamaldehyde and HSA. Also, this interaction was simulated after docking by the VMD software and the r value was obtained to be 3.14 nm, which is consistent with experimental results. FT-IR measurements have shown that the secondary structure of the protein has been changed by the interaction of cinnamaldehyde with HSA. The free energy of binding ( $\Delta G^\circ$ ) and binding

constant ( $K_a$ ) obtained from the intrinsic fluorescence results was  $-5.54 \text{ kcal mol}^{-1}$  and  $1.14 \times 10^4 \text{ M}^{-1}$  respectively, which is in good agreement with the free energy and binding constant obtained from molecular docking results ( $\Delta G^\circ = -5.28 \text{ kcal mol}^{-1}$  and  $K_a = 0.73 \times 10^4 \text{ M}^{-1}$ ). Spectroscopy and molecular docking methods showed that the cinnamaldehyde is bound to the HSA protein and hydrogen bonding and hydrophobic forces play the most important role in this interaction. On the other hand, based on the RMSD, RMSF and Rg values obtained in this work, it can be concluded that the HSA molecule exhibits a slight conformational change when it binds to cinnamaldehyde. Thus, both theoretical and experimental results suggest a slight conformational change, most probably around the binding site of the HSA. Our study showed that the binding site with highest binding affinity for cinnamaldehyde is located in IA subdomain of HSA. This binding site can contribute to transporting drugs and natural substances in the blood by HSA in addition to its well-known sites.

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