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Diketo modification of curcumin affects its interaction with human serum albumin

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ABSTRACT

Curcumin isoxazole (CI) and Curcumin pyrazole (CP), the diketo modified derivatives of Curcumin (CU) are metabolically more stable and are being explored for pharmacological properties. One of the requirements in such activities is their interaction with circulatory proteins like human serum albumin (HSA). To understand this, the interactions of CI and CP with HSA have been investigated employing absorption and fluorescence spectroscopy and the results are compared with that of CU. The respective binding constants of CP, CI and CU with HSA were estimated to be 9.3×10^5, 8.4×10^5 and 2.5×10^5 M^{-1}, which decreased with increasing salt concentration in the medium. The extent of decrease in the binding constant was the highest in CP followed by CI and CU. This revealed that along with hydrophobic interaction other binding modes like electrostatic interactions operate between CP/CI/CU with HSA. Fluorescence quenching studies of HSA with these compounds suggested that both static and dynamic quenching mechanisms operate, where the contribution of static quenching is higher for CP and CI than that for CU. From fluorescence resonance energy transfer studies, the binding site of CU, CI and CP was found to be in domain IIA of HSA. CU was found to bind in closer proximity with Trp214 as compared to CI and CP and the same was responsible for efficient energy transfer and the same was also established by fluorescence anisotropy measurements. Furthermore docking simulation complemented the experimental observation, where both electrostatic as well as hydrophobic interactions were indicated between HSA and CP, CI and CU. This study is useful in designing more stable CU derivatives having suitable binding properties with proteins like HSA.

1. Introduction

Human serum albumin (HSA), an important extracellular carrier protein, plays a crucial role in the binding and transport of a wide variety of nutrients and drugs to the cells [1–3]. The concentration of HSA in blood plasma is 5 g/100 ml. It is a monomeric globular protein with a molecular weight of 66.5 kDa consisting of 585 amino acids, composed of three homologous domains (known as Domain I, II and III), each domain is further divided into two sub-domains A and B [1–4]. The secondary structure of HSA results in formation of hydrophilic and hydrophobic pockets that are responsible for the binding of variety of ligands [5,6]. Among the different binding sites, domain IIA (site I) is more significant as hydrophobic drugs such as paclitaxel, warfarin, etc. are bound to this site. Binding of ligands at domain IIA has been found to increase the solubility of hydrophobic drugs, subsequently altering its in vivo pharmacokinetic and pharmacodynamic properties [6–9]. The binding constant thus controls the concentration and residual duration of the drug at the binding sites, thereby influences the magnitude of their therapeutic actions. Alteration in binding nature of the drugs may change the volume of distribution, clearance, and elimination of a drug which may modulate its therapeutic effect. Thus, knowledge of the binding of a drug to HSA is an important aspect for the development of new therapeutics [10,11]. Spectroscopic techniques including absorption and fluorescence are found to be the most versatile tools in exploring the strength and mode of binding between a ligand and macromolecules [7–9,12–15].

Curcumin (CU), a natural pigment obtained from rhizome of Indian spice turmeric (*Curcuma longa*), possesses diverse pharmacological properties like chemopreventive, antioxidant, anti-diabetic, anti-inflammatory, antitumor activities etc. [16–20]. However its applications are limited due to low bioavailability and fast degradation. In order to achieve maximum benefits of CU, extensive research has been carried out to design various CU derivatives [20–27]. One of the strategies to delay the degradation processes, is to convert the diketo group to isoxazole and pyrazole group forming curcumin isoxazole (CI) and curcumin pyrazole (CP), respectively. Chakroborty et al. have

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reported that unlike CU, CI and CP are stable in the presence of strong reducing agents and exhibit comparable antiproliferative activity [28]. Another important attribute of CU is its ability to bind to a number of transcriptional proteins through covalent, non-covalent hydrophobic and hydrogen bonding interactions. Chakroborty et al. have also investigated that CU, CI and CP bind with tubulin at the interface of the α,β-dimers, resulting in inhibition of tubulin self-assembly [29]. Das et al. have studied the binding of CU, CI and CP with novel isomers of protein kinase C (PKC) and observed that the binding interaction of CI and CP with PKC is stronger as compared to CU [30]. Narlawar et al. proposed that CI and CP inhibit formation of fibrillar $A\beta_{42}$ and tau protein aggregation, with 100 folds higher activity as compared to CU [31]. The interaction of CU and its derivatives with different carrier proteins has been investigated and the significance of different structural moiety that participates in binding has been reported [32–35]. The interaction of CU with bovine serum albumin (BSA) and HSA has been reported to be mainly enthalpy driven [32,33]. Bourassa et al. have compared the binding of resveratrol, genistein and CU with BSA and milk α and β-casein proteins and reported that CU exhibits strongest binding interaction and causes higher perturbation of protein conformation than resveratrol and genistein [34,35]. Mohammadi et al. reported that CU binds strongly with HSA and BSA as compared to diacetyl curcumin due to presence of phenolic hydroxyl group [36,37]. Further, Ge et al. compared the binding of CU, demethoxycurcumin and bisdemethoxycurcumin with HSA and proposed that methoxy group in CU, mainly binds at site I, while demethoxycurcumin and bisdemethoxycurcumin binds to site II (domain III A) of HSA [38]. Sahoo et al. have studied the interaction of CI with HSA by spectroscopic methods, and found that the fluorescence quenching of HSA by CI is mainly due to static quenching process [39]. In the above literature reports, the binding was established based on hydrophobic interaction between the drug molecules and protein using absorbance changes, fluorescence quenching and molecular docking studies. With the backdrop of the above literature reports, in the present manuscript we have studied in detail the interaction of CP and CI with HSA and compared the results with those for CU. The binding modes between CU, CI and CP with HSA were investigated systematically by spectroscopic tools like UV–Vis absorption, steady state and time resolved fluorescence spectroscopy. Also time resolved anisotropy studies were performed to understand the global and segmental motion of the protein. Further circular dichroism (CD) and dynamic light scattering (DLS) techniques were used to investigate the conformational changes in the secondary structure of HSA. Finally, docking simulation was carried out to corroborate the binding site as well as binding modes of these compounds with HSA. The chemical structures of CU, CI and CP are given in scheme 1 in the supporting information.

2. Materials and Methods

HSA, sodium hydrogen phosphate (monobasic and dibasic) and sodium chloride were purchased from Sigma and were of the highest purity available. CU, CI and CP were synthesised by the method given in literature [40–42]. The synthetic methodology and spectroscopic characterisation of CU, CI and CP have been provided in supporting information.

Acetonitrile (ACN) was purchased from Sisco Research Laboratories, dialysis membrane-60 of diameter 15.9 mm and molecular cutoff 12–14 kDa was purchased from Himedia. Nano pure water (conductivity of 0.06 μS cm^{-1}) was used from Milli-Q system. All the experiments were performed in 5 mM pH 7 phosphate buffer solution. Due to poor solubility of CU, CI and CP in water, 0.5% ACN was used throughout the investigation.

UV–Visible absorption spectroscopic studies were carried on JASCO V-630 spectrophotometer. Steady-state fluorescence measurements were carried out in a quartz cuvette (1 cm × 1 cm) using a Hitachi spectro-fluorimeter (model F-4500; Tokyo, Japan). CD spectrum was recorded on Biologic MOS450-SFM 300 (France), an automatic recording spectropolarimeter. CD spectra were recorded from 200 to 280 nm in a 1 cm quartz cuvette using a scan rate of 50 nm/min and acquisition period of 0.2 s. For CD spectrum in the range 260 to 500 nm, the acquisition period was 0.5 s under similar experimental setup. Each spectrum was recorded thrice, and the average data was plotted as observed ellipticity (expressed in milidegree) against the wavelength [43].

Time-resolved fluorescence measurements were carried out using a time-correlated single photon counting (TCSPC) spectrometer (Horiba Jobin Yvon, UK). The samples were excited by light pulses from a nano-LED sources and the fluorescence was detected using a PMT based detection module (model TBX4). A deconvolution procedure was used to analyze the observed decays using a proper instrument response function obtained by substituting the sample cell with a light scatterer (turbid solution of TiO_2 nanoparticles in water). The fluorescence decays were analyzed as a sum of exponentials as shown in Eq. (1),

$$I(t) = \sum_i B_i \exp(-t/\tau_i) \quad (1)$$

Here I (t) the time-dependent fluorescence intensity, B_i and τ_i are the pre-exponential factor and the fluorescence lifetime for the i^{th} component of the fluorescence decay, respectively. The quality of the fits and consequently the mono/multi-exponential natures of the decays were judged by the reduced chi-square (χ^2) values and the distribution of the weighted residuals among the data channels. For a good fit, the χ^2 value was close to unity and the weighted residuals were distributed randomly among the data channels. Further time resolved fluorescence rotational anisotropy measurements were carried out by using the same TCSPC setup as discussed above. The time resolved fluorescence decays and rotational anisotropy measurements were employed as per the procedure given in the literature [44–46].

DLS investigations were carried out on a Malvern 4800 Autosizer employing 7132 digital correlator. Here the estimation of average hydrodynamic size of HSA was made by He-Ne laser as a light source at 632.8 nm with a maximum output power of 15 mW and quartz cell of 1 cm × 1 cm at 90° as scattering angle. Moreover the intensity correlation function of scattered light was systematically analyzed by various references given in the literature [47,48].

The optimized structures of CU and its derivatives were docked on crystal structure of HSA (PDB file number 1AO6) using LeadIT 2.1.3-FlexX (BioSolveIT, GmbH, Germany) docking software. The structures of the CU, CI and CP were optimized by density functional theory (DFT) using B3LYP as exchange-correlation functional and basis set at 6–311++G(d,p). SMD (Solvation model based on density) was used for modelling water as solvent at 298 K [49]. Binding site was defined by selecting amino acid sequences present in the given domains, viz. for domain I A & B (amino acids 5–195), domain II A & B (amino acids 196–383) and domain III A & B (amino acids 384–582). Additionally, amino acids within radius 10 Å were also selected along with the above domain. Ligand binding was checked by using hybrid of both enthalpy and entropy based binding. The favoured pose of the ligand on the protein was evaluated by scoring method.

3. Results

3.1. UV–Visible Studies

Interaction of HSA with CP and CI was studied by following the changes in their absorption properties in the UV–visible region. The absorption spectrum of CP and CI exhibited maximum at 325 and 334 nm, respectively, while HSA (50 μM) exhibited characteristic absorption band at 280 nm with no appreciable absorbance beyond 330 nm. On addition of HSA (1–50 μM) to 10 μM CP or CI, the absorption spectrum showed increase in the absorbance at their respective absorption maxima with slight bathochromic shift of 5 nm when the concentration of