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Investigations to reveal the nature of interactions of human hemoglobin with curcumin using optical techniques☆

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ABSTRACT

Curcumin (CUR) is an important bioactive compound present in the rhizome of *Curcuma longa*. Herein, we report the interaction of CUR with human hemoglobin (Hb) using various biophysical methods *viz.*, fluorescence, UV absorption, resonance light scattering spectra (RLS), synchronous fluorescence, fluorescence anisotropy, circular dichroism (CD) and three-dimensional fluorescence. There was a considerable quenching of the intrinsic fluorescence of Hb upon binding to CUR through dynamic quenching mechanism. The distance (r) between the donor and acceptor was obtained from the Forster's theory of fluorescence resonance energy transfer (FRET) and found to be 1.55 nm. Alterations in the conformation of Hb due to its interaction with CUR were confirmed by UV absorption and CD spectroscopic methods. The α-helicity of Hb was found to decrease upon binding with CUR.

1. Introduction

Proteins, the biological molecules that are involved in virtually every action of every organism, may themselves move in surprising ways. Hb is a kind of respiratory protein of vertebrate erythrocytes in red blood cell, whose structure, mechanisms of carrying oxygen and transferring electron, enzymatic and antioxidant activities had been realized [1]. Moreover, Hb can reversibly bind many endogenous and exogenous components [2].

Hb exhibits characteristics of both the tertiary and quaternary structures of proteins. Most of the amino acids in Hb form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. In adult humans, the most common hemoglobin type is a tetramer that consists of two α and two β subunits non-covalently bound. Each of α and β subunit comprises 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$ in which α-Trp^{14}, β-Trp^{15} and β-Trp^{37} are located [3]. The subunits are structurally similar and are of about the same size. Each subunit has a molecular weight of about 17,000 Da making the total molecular weight of the tetramer of about 68,000 Da. Concentration of Hb in plasma is about 140 g L^{-1}, which is higher than serum albumin (40 g L^{-1}), an important carrier of biomolecules. Hb plays an important role in the distribution and bioavailability of flavonoids [4].

CUR (Fig. 1) is a natural product found in the rhizome of *Curcuma longa*. Ongoing research and clinical trials provide plenty of evidence that this natural phenolic compound possesses diverse pharmacological activities. Besides its effective antioxidant, antidiabetic, anti-inflammatory and antimicrobial/antiviral properties [5], the compound is also considered as a cancer chemo-preventive agent [6,7].

Like most proteins, intrinsic fluorescence of Hb is sensitive to its microenvironment, especially around Tryptophan (Trp) residues. Some factors such as protein conformational transition, biomolecular binding, denaturation *etc.*, can alter the fluorescence intensity of the protein [8]. Literature survey revealed that no effort has been made to investigate the interaction of Hb with CUR. In view of this, we have studied the mechanism of interaction between Hb and CUR employing different spectroscopic techniques like fluorescence, UV–vis and circular dichroism which are convenient and sensitive.

2. Materials and methods

2.1. Apparatus

Fluorescence intensities were measured on a Hitachi spectrofluorometer Model F-7000 (Hitachi, Japan) equipped with a 150 W Xenon lamp and a slit width of 5 nm. Absorption spectra were recorded on a double beam CARY 50-BIO UV–vis spectrophotometer (Varian, Australia) equipped with a 150 W Xenon lamp and a

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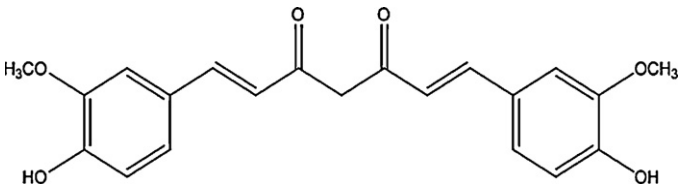

Fig. 1. Structure of curcumin.

slit width of 5 nm. A quartz cell of 1.0 cm was used for the measurements. The CD measurements were made on a JASCO-810 spectropolarimeter (Tokyo, Japan) using a 0.1 cm cell at 0.2 nm intervals, with 3 scans averaged for each CD spectrum.

2.2. Fluorescence and UV absorption studies

Preliminary experiments were carried out to fix the concentration of Hb and CUR. In the present study, the concentration of protein was kept constant at 2.5 μM while that of the CUR was varied from 0 to 22.5 μM for steady state fluorescence, synchronous fluorescence and UV absorption studies. Interactions of CUR with Hb were investigated by recording the fluorescence spectra of Hb in the presence and absence of CUR at 290, 300 and 310 K in the range of 290–450 nm upon excitation at 280 nm. The fluorescence spectra of CUR–Hb system were also recorded in the presence of cations *viz*., K^+, Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} upon excitation at 280 nm. The overall concentrations of Hb and common ions were maintained at 2.5 μM.

2.3. Resonance light scattering

The RLS spectra of Hb in the presence and absence of the CUR were recorded at room temperature in the range of 250–400 nm with $\Delta\lambda = 0$ nm.

2.4. Circular dichroism

The CD spectra of drug-protein mixtures (0:1, 1:1, 2:1 and 3:1) were recorded in the range of 200–260 nm.

2.5. 3D fluorescence measurements

The 3D-fluorescence spectrum was performed under the following conditions: the emission wavelengths at 200–600 nm, the excitation wavelengths at 200–340 nm, scanning number 15 with an increment 10 nm.

3. Results and discussion

3.1. UV spectroscopic studies

Absorption spectrum of Hb exhibited three peaks at ~210, 275 and ~407 nm (Fig. 2). The band at 275 nm appears due to phenyl group of Trp and tyrosine (Tyr) residues while the sharp peak at 407 nm corresponds to the characteristic absorption of the porphyrin-Soret band. This band originates from the heme group, embedded in a hydrophobic pocket formed by the protein's backbone through appropriate folding [9,10].

Absorption peak noticed at 210 nm arises due to the peptide bond and also represents the microenvironment of α-helix structure in Hb [11]. With successive addition of CUR to Hb solution, the absorbances of peaks at 210 and 275 nm increased regularly indicating that the CUR caused the loss of the Hb skeleton structure, and promoted the exposure of aromatic ring amino acids in the internal hydrophobic region. The peak at 275 nm showed a blue shift from 275 to 255 nm. CUR easily integrates into the hydrophobic pocket of Hb due to spreading of peptide chains. The amino acids in Hb are exposed gradually. The heme groups are released from the hydrophobic cavity of Hb and increased the intensity of the intrinsic absorption peak [12].

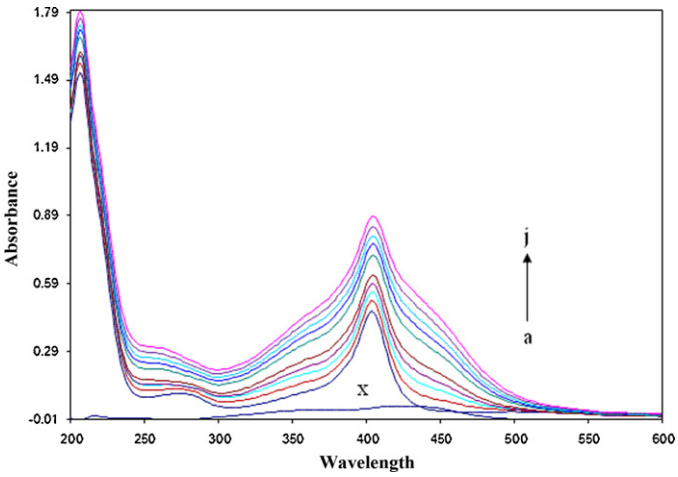

Fig. 2. Absorption spectra of Hb with different concentrations of CUR. Hb concentration was maintained at 2.5 μM [a] and that of CUR was varied- 2.5 [b], 5 [c], 7.5 [d], 10 [e], 12.5 [f], 15 [g], 17.5 [h] and 20 μM [i]. Only CUR 5 μM [x].

3.2. Steady state fluorescence

The intrinsic fluorescence of Hb primarily originates from β-Trp^{37} that plays a key role in the quaternary state change upon ligand binding [13]. These Trps can be used as intrinsic fluorophores to study the binding of small molecules to protein on molecular level. Changes in emission spectra of Trp are common in response to protein conformational transitions, subunit association, substrate binding or denaturation [14].

When a fixed concentration of Hb was titrated with different amounts of CUR, we noticed decreased fluorescence intensity values (Fig. 3). In other words, the CUR quenched the intrinsic fluorescence of Hb.

We have further analyzed the quenching mechanism by carrying out the binding studies at 290, 300 and 310 K. The fluorescence

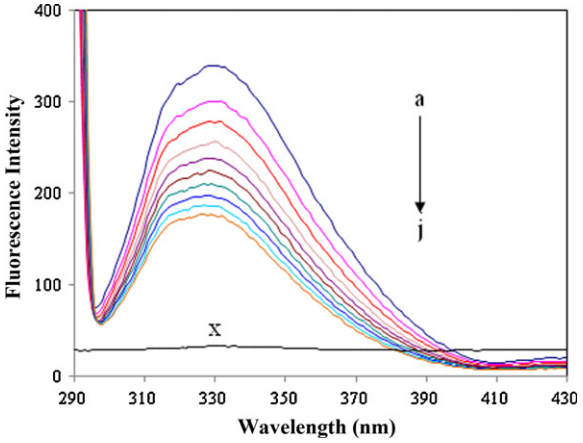

Fig. 3. Quenching of fluorescence intensity of Hb (2.5 μM) upon the addition of increasing amounts of CUR-0 [a], 2.5 [b], 5 [c], 7.5 [d], 10 [e], 12.5 [f], 15 [g], 17.5 [h], 20 [i] and 22.5 μM [j]. Fluorescence emission of free CUR upon excitation at 280 nm.