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Thermodynamic analysis of thymoquinone binding to human serum albumin

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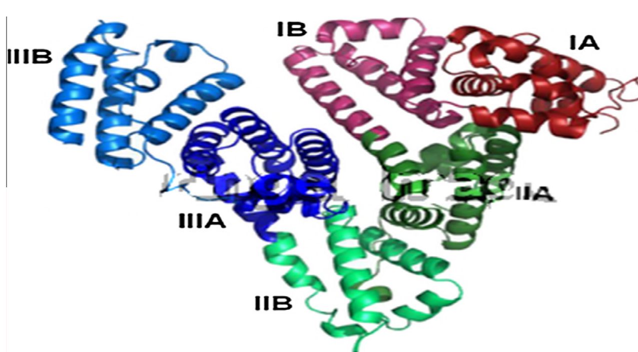
HIGHLIGHTS

- Fluorescence method was used to examine the interaction of human serum albumin with thymoquinone.
- The thermodynamic parameters of the interaction were calculated.
- We examined two mathematical models to analyze the interaction occurred.
- The predominant intermolecular forces affecting the interaction were proposed.
- Fröster energy transfer theory was used to calculate the distance between the donor macromolecule and the acceptor ligand.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of thymoquinone (TQ) with human serum albumin (HAS) in physiological buffer (pH = 7.0) was studied at four temperatures in the range 25–50 °C using fluorescence quenching study. The binding parameters were determined by Scatchard and Stern–Volmer models. Fluorescence quenching data revealed that the binding constants (K_{sc}) are 1.71×10^4, 1.08×10^4, 1.03×10^4 and $0.969 \times 10^4\ M^{-1}$ at 298, 303, 313 and 323 K, respectively (on the basis of Scatchard model).

The thermodynamic parameters ΔG°, ΔH° and ΔS° were calculated the results indicated that the hydrogen bonding and hydrophobic interactions were the predominant intermolecular factors in stabilizing the TQ–HSA complex.

The distance between donor (HSA) and acceptor (TQ) was calculated to be 3.26 nm based on Förster's non-radiative energy transfer theory.

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Introduction

Human serum albumin (HSA) is the most abundant and highly soluble plasma protein in the blood circulatory system in mammals (approximately 60% of the total protein) [1,2]. It is able to bind and thereby transport various compounds such as fatty acids, bilirubin, tryptophan, steroids and many drugs [3]. HSA concentrations in blood plasma ranges from 3.4 to 5.4 g/dL [4]. HSA plays an important role in transporting metabolites and drugs through the vascular system and also in maintaining the pH and osmotic pressure of the plasma [5]. HSA is a globular single polypeptide chain protein of a molecular mass of about 67 kDa and comprises 585 amino acid residues with one cystein residue at position 34 (in domain I) with free sulfhydryl group [6]. Its structure includes three homologous domains (I, II, and III) that

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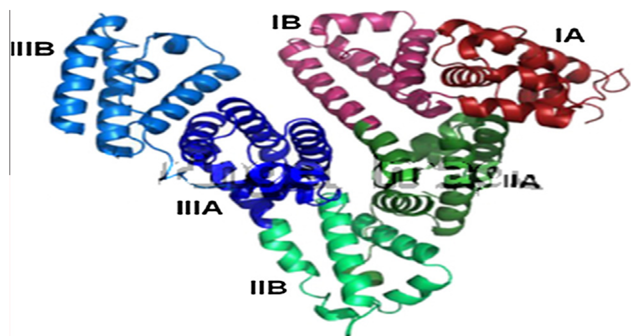

Fig. 1. X-ray crystallographic to 2.5 Å resolution of three dimensional Structure of HSA, with its subdomains [10]. The structure corresponds to the "Protein Data Bank".

assemble a heart shaped molecule (Fig. 1). Each domain is formed by two sub-domains (A and B) which possess common structural motifs by various forces such as salt bridges and hydrophobic interactions [7–9].

Thymoquinone (TQ) is a phytochemical compound present in of a plant called Nigella Sativa possesses important properties such as analgestic and anti-flammatory protection of organs against oxidative damage induced by a variety of free radical generating agents. It is a potent anti tumor agent against human colorectal cancer cells [11–13]. Studies on the binding of drugs to protein are of great importance in biological, biomedical and pharmaceutical science. Plasma protein serve as transport carriers for drug bioavailability, distribution in the body, metabolism and excretion.

Human serum albumin (HSA) has been used as a model protein for protein folding and ligand – binding studies over many decades [14,15]. The affinity of a drug to a protein would directly influence the concentration of the drug in the binding site and duration of the effectual drug, and consequently contribute to the magnitude of its biological actions in vivo [16].

It is informative to study interaction of bioactive compounds such as TQ with the protein, because the effectiveness of these compounds as pharmaceutical agents based on their binding ability [7].

In the present study, using the spectroflorimetric titration method, the interaction of TQ with HSA has been studied and the results has been interpreted on the basis of different mathematical models leading to estimate the binding parameters.

Materials and methods

Materials

Human serum albumin (HSA, fatty acid free <0.05%) was purchased from Sigma Aldrich company.

Thymoquinone was purchased from Sigma Aldrich and its stock solution was prepared in 5% ethanolic solution.

The other substances were of reagent grade, and were used without further purification. phosphate buffer (20 mM, pH = 7.0) was used through the study.

All solutions were prepared using doubly distilled water.

Fluorescence quenching studies

All fluorescence spectra were recorded on an Perkin–Elmer luminescence Spectrofluorimeter, (series no. 70412) equipped with a water-jacketed cuvette holder maintained at a constant temperature by means of a circulatory water bath. A quartz cell of 1.00 cm width was used for the measurements.

Fluorescence spectra related to the titration of fixed volume of HAS of 1 μM with concentrated stock ethanolic TQ solution were measured on the Spectrofluorimeter at the desired temperature.

The fluorescence intensity measured were corrected for dilution.

All samples were measured after two minutes and all binding data reported here correspond to the average values obtained after two different titrations.

The fluorescence emission spectra were recorded at four different temperatures in the range 25–50 °C in the wavelength range of 250–500 nm with the excitation wavelength at 278 nm and using slits of 10 nm for both excitation and emission monochromators.

Results and discussion

Calculation of binding constants and data analysis

The fluorescence quenching data were assessed using two distinct mathematical models: Stern–Volmer and Scatchard models.

The binding constants (K_{SV} and K_{Sc}) obtained at each temperature in the range of 25–50 °C were estimated from the Stern–Volmer and Scatchard plots.

Fluorescence quenching of single tryptophan residue in HAS (Trp 214) was used to monitor the TQ/HSA interaction and to measure the binding affinity of the interaction occurred. The addition of TQ to HSA caused a decrease in the intrinsic fluorescence emission intensity of the protein upon excitation at 278 nm (Fig. 2).

Fluorescence spectra of HSA alone and complexed with TQ at 25 °C was recorded (Fig. 2). From Fig. 2 it is evident that addition of TQ quenches the fluorescence intensity of HSA and the quenching was accompanied by a small red shift in maximum fluorescence intensity of emission. This behavior could be attributed to an increase in the environmental polarity. The binding curves at different temperatures in the range of 25–50 °C, depecting the change in fluorescence of HSA (ΔF) as a function of molar ratio [HSA]/[TQ] are shown in Fig. 3. Here, it is clear to see that the binding curve displayed saturation at certain values of the molar ratio at each studied temperature, clearly indicating that the binding occurred at specific binding sites on the HSA.

Binding constant and number of binding sites

The apparent binding constant (K_{SV} and K_{Sc}) and number of binding sites (n) for TQ that binds HSA can be obtained using Eqs. (1) and (2) Stern–Volmer and Scatchard Eqs. (1) and (2) [17,18]:

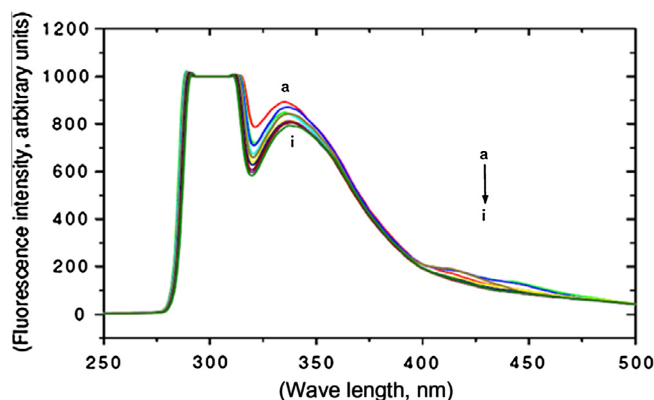

Fig. 2. Emission spectra of HSA (1 μM) in 20 mM phosphate buffer (pH = 7.0) and 25 °C. Excitation carried out at 278 nm. The arrow (with its direction) shows that the increasing TQ concentration is accompanied with fluorescence intensity quenching for HSA.

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