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Interaction of human serum albumin with sulfadiazine

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ABSTRACT

In this report we have studied the interaction of sulfadiazine (SD), which has recently been found to partially protect the amyloidosis, with human serum albumin (HSA) at physiological conditions of temperature and pH. We have employed several basic and advanced spectroscopic techniques such as UV, fluorescence, circular dichroism (CD) and Fourier transform infra-red (FTIR) spectroscopies. UV spectrum of native HSA was different from the spectrum of HSA in the presence of SD due to the complex formation between albumin and drug. Fluorescence quenching of HSA by SD at 280 nm was due to the formation of HSA–SD complex. The data were analyzed using Stern–Volmer (SV) and the quenching was found to be static with 1:1 binding ratio. Synchronous fluorescence spectra have shown a red shift and revealed that hydrophobicity around both Trp and Tyr residues was decreased. CD results have shown that the conformation of macromolecule remains undisturbed at low concentrations (up to 20 μM of the SD), though, a small change in the secondary structure from 20 to 75 μM of SD was observed followed by a large change and consequent unfolding on further increase in the drug concentration. Both synchronous and CD measurements were consistent to each other. From the FTIR measurement analysis it was found that amide I band also shifted which concluded that the conformational changes take place in the presence of SD.

1. Introduction

Sulfadiazine is an antibacterial drug and belongs to the sulfonamide category. It has been found to protect the rat temporal cortex from amyloid beta peptide-induced alterations of the somatostatinergic system, though, partially [1]. It inhibits the production of folic acid inside the bacterial cell wall causing the elimination of the bacteria [2].

Human serum albumin (HSA) is the foremost protein component of the blood plasma prevalence of which is also found in the interstitial fluids of the body tissues. HSA binds a variety of insoluble endogenous compounds, for instance, unesterified fatty acids, bilirubin and bile acids and, thus, promotes the transport of these substances in the circulatory system [3,4]. The interactions between HSA and xenobiotics are very important in the pharmacology and toxicology of the drug action [5–8]. The availability of the particular drug for its proper action depends upon the affinity of the drug to the albumin. The pharmacological action of the drug is due to its unbound form that diffuses through the circulatory system and interacts with sites of action. HSA accounts for about 60% of the plasma protein and responsible for about 80% of the osmotic pressure of the blood. Being the major ligand binding and transport proteins of circulatory system, it is considered as a model for studying drug–protein interactions in vitro.

The primary structure of HSA contains 585 amino acid residues and is characterized by a low content of tryptophan and a high content of cystine stabilized by a series of nine loops. The secondary structure of HSA is constituted of three structurally similar domains (I, II, and III), each containing two subdomains (A and B) which are stabilized by 17 disulfide bonds [9–11].

In view of the importance of the drug albumin binding in the drug's action, we have studied the binding of sulfonamide drug, sulfadiazine (SD), with HSA. We have selected a broad range of concentration of SD (Scheme 1) for getting full insight into the drug–HSA interaction. Since various drug–albumin binding studies also suggested the consequent partial unfolding of the albumin [12–15], we have selected a high concentration regime in order to understand the effect of high dose of the drug. In our previous studies we have reported the unfolding of albumins in the presence of surfactants. Usually surfactants, when present in millimolar, have a tendency to affect the protein conformation and even completely unfold the macromolecule [16–18]. In the present study it has revealed that SD unfolds the protein more effectively as compared to the conventional surfactants.

2. Materials and methods

2.1. Materials

Human serum albumin, HSA (≥99%, Sigma, USA) was essentially fatty acid free and globulin free. Sulfadiazine, SD (≥99.0%) was also purchased from Sigma and used as received.

Studies of protein folding are normally carried out in buffered dilute aqueous solutions to avoid loss of protein to the aggregation

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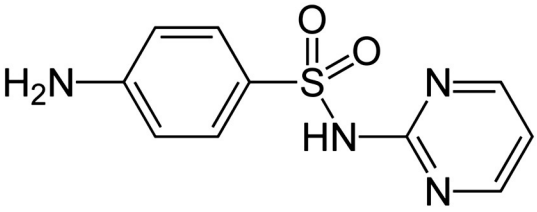

Scheme 1. Chemical structure of sulfadiazine.

phenomenon. Stock solutions of HSA (20 mg/ml) and SD, prepared in phosphate buffer of pH 7.4 (well above the isoelectric point of HSA, 4.7, hence the protein possesses a net negative charge at this pH) using double-distilled water, were utilized to prepare the sample solutions of desired concentrations for all measurements.

2.2. UV measurements

UV absorption spectra, from 240 nm to 310 nm, were recorded on a Perkin-Elmer Lambda 45 Spectrophotometer at 37 °C. Quartz cuvettes of 1 cm path length were used for the measurements.

2.3. Fluorescence measurements

Fluorescence measurements were performed on a Hitachi spectrofluorometer (Model F 7000) equipped with a PC. The fluorescence spectra were collected at 37 °C with a path length cell of 1 cm. The excitation and emission slits were set at 5 nm. The fluorescence spectra were taken with a protein concentration of 0.1 mg/ml. To the 0.1 mg/ml protein solution, different volumes of the buffer were added followed by the requisite volumes of stock additive solutions to obtain the samples of desired additive concentration. Intrinsic fluorescence was measured by exciting the protein solution at 280 nm and emission spectra were recorded in the range of 290–450 nm. Synchronous fluorescence spectra were collected at $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm.

2.4. CD measurements

CD measurements were carried out with a Jasco spectropolarimeter, Model J-815, equipped with a microcomputer. The instrument was calibrated with D-10-camphorsulfonic acid. All the CD measurements were carried out at 37 °C with a thermostatically controlled cell holder attached to a Neslab RTE-110 water-bath with an accuracy of ± 0.1 °C. Spectra were collected with a scan speed of 0.2 nm/min and response time of 1 s. Each spectrum was the average of four scans. The far-UV CD spectra were measured at a protein concentration of 0.1 mg/ml at a path length of 1 cm.

2.5. FTIR measurements

FTIR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer (DTGS detector, Ni-chrome source and KBr beamsplitter) from ZnSe windows with a resolution of 16 cm^{-1} using KBr windows at room temperature. To improve signal to noise ratio, 100 scans were averaged for each spectrum. Spectra were analyzed using the OMNIC software provided with the instrument. Protein concentration of samples was 20 mg/ml.

3. Results and discussion

3.1. UV absorption studies

Fig. 1 displays the UV spectra of HSA–SD system. The spectra were collected in the UV range of 240–310 nm (near UV range) which is considered as the most common range for the protein structure determination owing to the prominent absorption bands of three amino acids

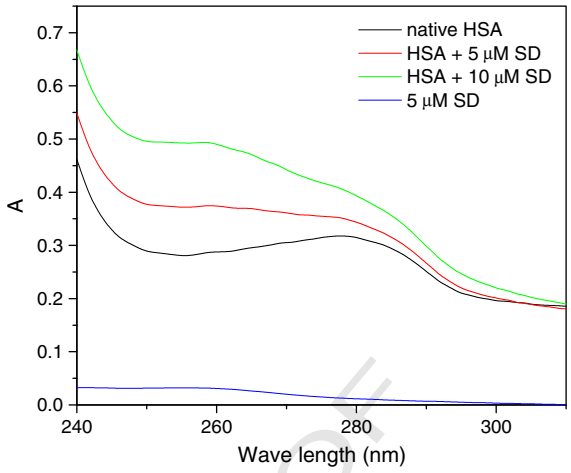

Fig. 1. UV spectra of HSA (2 μM) in the presence of SD.

(tryptophan, tyrosine and phenylalanine) in this region [19]. HSA exhibits a UV absorption peak at around 280 nm. Occurrence of hyperchromic shift (increase in absorption) in the HSA–SD system is a signal of the interaction of macromolecule with the drug. Under our experimental conditions SD has very little absorbance at 280 nm, therefore, the inner filter effect is negligible. However; we have employed fluorescence spectroscopy to determine various binding and thermodynamic parameters for the HSA–SD system.

3.2. Effect of sulfadiazine on the fluorescence of HSA

When excited at 280 nm, fluorescence intensity around 340 nm reflects changes of the microenvironment of the Trp and Tyr residues [20]. HSA possesses a single Trp residue which is located in subdomain IIA (Trp 214) whereas around 18 Tyr residues are distributed all along the macromolecular domain [21].

From the experimental observations, the fluorescence intensity of HSA was found to decrease regularly with increasing SD concentration (Fig. 2). When a molecule or ligand interacts with HSA, its fluorescent properties may change depending on the impact of such interaction on the protein conformation. Although Tyr is more fluorescent than Trp in solution, but when present in proteins, its fluorescence is weaker because protein's tertiary structure inhibits Tyr fluorescence and efficient energy transfer from Tyr to Trp residues occurs in proteins inducing a total or important quenching of tyrosine fluorescence [22,23].

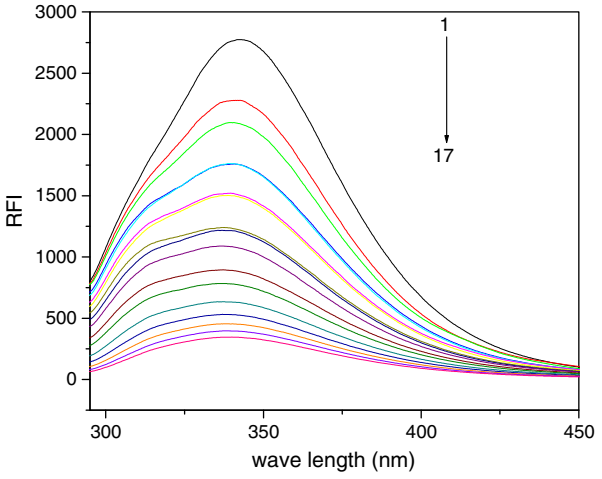

Fig. 2. Fluorescence emission spectra of HSA in the presence of various concentrations of SD. Curves from 1 to 17 corresponding to SD concentrations of 0, 1, 2.5, 5, 10, 15, 20, 25, 35, 50, 70, 90, 130, 170, 200, 230, and 260 μM, respectively when excited at 280 nm. Q2

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