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Spectral characterization of the binding and conformational changes of serum albumins upon interaction with an anticancer drug, anastrozole

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ABSTRACT

The present study employed different optical spectroscopic techniques *viz.*, fluorescence, FTIR, circular dichroism (CD) and UV–vis absorption spectroscopy to investigate the mechanism of interaction of an anticancer drug, anastrozole (AZ) with transport proteins *viz.*, bovine serum albumin (BSA) and human serum albumin (HSA). The drug, AZ quenched the intrinsic fluorescence of protein and the analysis of results revealed the presence of dynamic quenching mechanism. The binding characteristics of drug–protein were computed. The thermodynamic parameters, enthalpy change (ΔH°) and entropy change (ΔS°) were calculated to be +92.99 kJ/mol and +159.18 J/mol/K for AZ–BSA and, +99.43 kJ/mol and +159.19 J/mol/K for AZ–HSA, respectively. These results indicated that the hydrophobic forces stabilized the interaction between the drug and protein. CD, FTIR, absorption, synchronous and 3D fluorescence results indicated that the binding of AZ to protein induced structural perturbation in both serum albumins. The distance, *r* between the drug and protein was calculated based on the theory of Förster's resonance energy transfer and found to be 5.9 and 6.24 nm, respectively for AZ–BSA and AZ–HSA.

1. Introduction

Protein plays a significant role in the living organisms by performing various biological activities. The blood protein, serum albumin has an important role in the transport and disposition of endogenous and exogenous ligands present in blood [1,2]. Among proteins, bovine serum albumin (BSA) and human serum albumin (HSA) are being extensively studied. BSA displays approximately 76% sequence homology with that of HSA [3,4]. Both proteins are divided into three linearly arranged, structurally distinct, and evolutionarily related domains I, II and III. Further, each domain is composed of two subdomains A and B. There are some differences between BSA and HSA. BSA consists of 582 amino acids with 2 tryptophans located at position 134 (located on the surface of domain I) and 214 (located within the hydrophobic pocket of domain II) [5] while HSA contains of 585 amino acids with one tryptophan located at position 214 [6]. Since, tryptophan residues are the main intrinsic fluorophores that are extremely sensitive to their microenvironment, we thought of investigating the binding mechanism of both proteins with AZ in the present study. Such knowledge on the mechanism of interaction between the drug and plasma protein is of crucial importance to understand the pharmacodynamics and pharmacokinetics of a drug [7,8]. Spectroscopy is an appropriate method to investigate the interaction between the drug and protein because of its outstanding sensitivity, selectivity, reproducibility, easy implication and vast theoretical foundation; over conventional approaches *viz.*, affinity or size exclusion chromatography, equilibrium dialysis, ultrafiltration and ultracentrifugation. The conventional methods suffer from either low sensitivity or lengthy operation time or both [5].

Anastrozole (AZ) (Fig. 1), marketed under the trade name, Arimidex, is used to treat breast cancer after surgery and for metastasis in both pre and post-menopausal women. This drug is also frequently used in the treatment of moderate-to-severe pubertal gynecomastia. Side effect of the drug usage is mainly the bone weakness. However, the mechanism of interaction between AZ and BSA/HSA has not been studied so far. This prompted us to carry out the detailed investigations on AZ–protein interactions employing fluorescence, FTIR, CD and UV–vis absorption techniques. This sort of study assumes importance in the fields of life science, chemistry and clinical medicine for drug design.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed on a spectrofluorimeter Model F-2000 (Hitachi, Japan) equipped with a 150 W Xenon lamp and a slit width of 5 nm. The synchronous fluorescence studies were carried out on a RF-5301PC spectrofluorimeter

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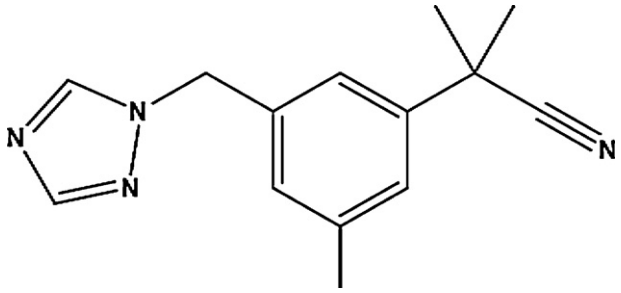

Fig. 1. Structure of AZ.

(Shimadzu). The CD measurements were made on a JASCO-J-715 spectropolarimeter (Tokyo, Japan) using a 0.1 cm cell at 0.2 nm intervals, with 3 scans averaged for each CD spectrum in the range of 200–260 nm. The absorption spectra were recorded on a double beam CARY 50-BIO UV-visible spectrophotometer (Varian, Australia) equipped with a 150 W Xenon lamp and a slit width of 5 nm. FTIR spectra were recorded on a Nicolet Nexus 670 FTIR spectrometer (USA) equipped with a Germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter.

2.2. Reagents

BSA (Fraction V, approximately 99%; protease free and essentially α-globulin free), HSA (fatty acid free) were obtained from Sigma Chemical Co., St. Louis, USA. AZ was obtained as a gift sample from Taj active pharmaceuticals ingredients and chemicals, India. The stock solution of AZ was prepared firstly by dissolving it in alcohol and then diluting with water. The solution of BSA/HSA was prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl based on its molecular weight of 65,000/66,000 Da. All other materials employed were of analytical reagent grade and Millipore water was used throughout.

2.3. Procedures

2.3.1. AZ–protein interaction

Fluorescence spectra were recorded in the range of 300–500 nm for BSA (λ_{ex} = 295 nm) and 280–500 nm for HSA (λ_{ex} = 280 nm). The concentration of protein was kept fixed at 2.5 μM and that of drug was varied from 5 to 45 μM. Quenching experiments were carried out at 289, 299 and 307 K.

2.3.2. Circular dichroism (CD) measurements

The CD measurements of protein (2.5 μM) in the presence and absence of AZ were made in the range of 200–250 nm. The HSA to drug concentration was varied in the ratio of 1:0, 1:1 and 1:2 and the CD spectra were recorded.

2.3.3. Fourier transform infrared studies

IR spectra were taken *via* the ATR method with a resolution of 4 cm^{-1} and using 60 scans. The spectra of protein (2.5 μM) in the presence and absence of the drug (5 μM) were recorded in the range of 1500–1700 cm^{-1}.

2.3.4. Absorbance measurements

The absorption spectra of protein in the presence and absence of AZ were recorded in the range of 240–390 nm. BSA/HSA concentration was fixed at 2.5 μM while that of the drug was varied from 5 to 45 μM.

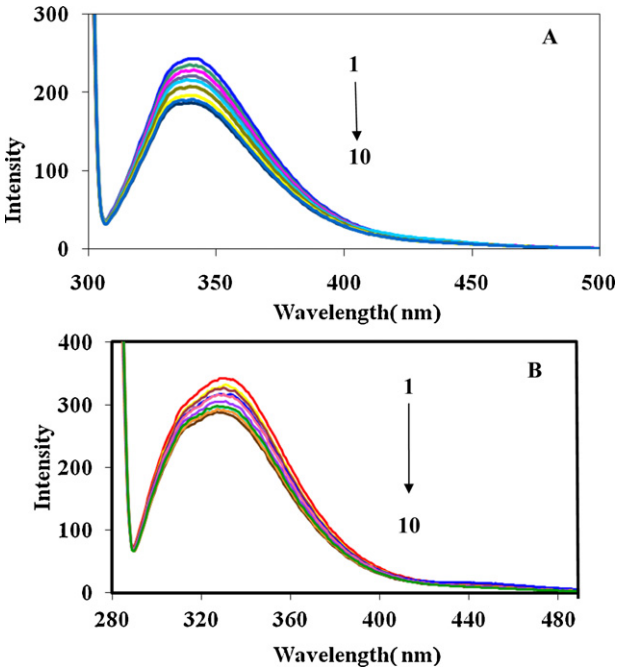

Fig. 2. Fluorescence spectra of: (A) BSA and (B) HSA in the presence of AZ. Concentration of BSA/HSA was fixed at 2.5 μM (1) and that of AZ was maintained in the range of 5–45 μM (2–10).

2.3.5. Synchronous fluorescence measurements

Synchronous fluorescence spectra were recorded with scanning ranges, $\Delta\lambda$ = 15 nm and 60 nm ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) in the absence and presence of AZ and the spectra were recorded in the range of 280–400 nm.

2.3.6. Three dimensional fluorescence studies

The three-dimensional fluorescence spectra of proteins were recorded by scanning excitation wavelength in the range of 200–340 nm and emission wavelength from 200 to 600 nm at an interval of 10 nm. The scanning parameters were just the same as the fluorescence quenching experiments.

3. Results and discussion

3.1. AZ–protein interaction: fluorescence quenching study

The fluorescence emission spectra of BSA/HSA with increasing concentrations of AZ are shown in Fig. 2A and B. It is evident from these figures that the increased concentrations of AZ quenched the intrinsic fluorescence intensity of BSA/HSA. The possible mechanisms of quenching include dynamic quenching, static quenching or both [9,10]. Dynamic and static quenching could be distinguished based on their differing dependence on temperature. For this, we have carried out the quenching studies at different temperatures (289, 299 and 307 K). The quenching data were analyzed using the Stern–Volmer equation shown below:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, [Q] is the quencher concentration and K_{SV} is the Stern–Volmer quenching constant. The plot (not shown) of the values of F_0/F *versus* [Q] yielded the values of K_{SV} (Table 1). The increased K_{SV} values with increase in temperature revealed the presence of dynamic quenching mechanism in the interaction of AZ with protein [11].