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A study on the binding interaction between the imidazole derivative and bovine serum albumin by fluorescence spectroscopy

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ABSTRACT

The interaction between the imidazole derivative 2-(2,4-difluorophenyl)-1-phenyl-1H-imidazo[4,5-f][1,10]phenanthroline (dfppip) and bovine serum albumin (BSA) was investigated by fluorescence and UV–vis absorbance spectroscopy. From the experimental results, it was found that the imidazole derivative has strong ability to quench the intrinsic fluorescence of BSA by forming complexes. Electrostatic interactions play an important role to stabilize the complex. The binding constants and the number of binding sites have been determined in detail. The distance (r) between the donor and the acceptor was obtained according to fluorescence resonance energy transfer (FRET). Conformational changes of BSA were observed from synchronous fluorescence spectroscopy. The effect of metal ions such as Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+} and Fe^{2+} on the binding constants between the imidazole derivative and BSA were also studied.

1. Introduction

Protein-drug binding greatly influences absorption, distribution, metabolism and excretion properties of typical drugs [1]. Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure [2] are also found in tissues and bodily secretions throughout the body; the extra-vascular protein comprises 60% of the total albumin [3]. In this work, bovine serum albumin (BSA) is selected as our protein model because of its medical importance, low cost, ready availability, unusual ligand-binding properties [4] and the results of all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins [4–6].

Heterocyclic imidazole derivatives have attracted considerable attention because of their unique optical properties [7]. These compounds play very important role in chemistry as mediators for synthetic reactions, primarily for preparing functionalized materials [8]. Imidazole nucleus forms the main structure of some well-known components of human organisms, i.e. the amino acid histidine, Vitamin B_{12}, a component of DNA base structure, purines, histamine and biotin are present in structure of many natural or synthetic drug molecules, i.e. azomycin, cimetidine and metronidazole and also have significant analytical applications utilizing their fluorescence and chemiluminescence properties [9].

Application of the spectral methods can reveal the reactivity of chemical and biological systems in low concentration under physiological conditions and there have been several studies on fluorescence quenching of albumin induced by drugs or other bioactive small molecules [10–13]. In this paper, the quenching of fluorescence of BSA has been used as a tool to study the interaction of dfppip with this transport protein in an attempt to characterize the chemical association taking place.

2. Experimental

2.1. Materials and Methods

1,10-Phenanthroline-5,6-dione (Sigma-Aldrich Ltd.), 2,4-difluorobenzaldehyde (Sigma-Aldrich Ltd), aniline (S.D. fine) and all other reagents were used without further purification. Bovine Serum Albumin (BSA) was obtained from Sigma-Aldrich Company, Bangalore. All BSA solutions were prepared in the Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4) and was kept in the dark at 303 K. Tris base (2-amino-2-(hydroxymethyl)-1,3-propanediol) had a purity of not less than 99.5% and NaCl, HCl and other starting materials were all of analytical purity and doubly distilled water was used throughout.

2.2. Equipments and spectral measurements

NMR spectra were recorded for dfppip on a Bruker 400 MHz instrument. The ultraviolet–visible (UV–vis) spectra were recorded

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on a UV–vis spectrophotometer (Perkin Elmer, Lambda 35) and corrected for background due to solvent absorption. Photoluminescence (PL) spectra were recorded on a (Perkin Elmer LS55) fluorescence spectrometer. Fluorescence quenching spectra were recorded at 301, 310 and 318 K in the range around 300–450 nm. An excitation wavelength of 285 nm was chosen and very dilute solutions were used in the experiment (BSA 2.0×10^{-6} mol L^{-1}, dfppip in the range of $0–10\times10^{-5}$ mol L^{-1}) to avoid inner filter effect. The infrared spectrum was recorded on an Avatar 330-Thermo Nicolet FT-IR spectrometer. Mass spectrum was recorded using Agilant 1100 mass spectrometer.

2.3. Principles of fluorescence quenching

The fluorescence intensity of a compound can be decreased by a variety of molecular interactions viz., excited-state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Such decrease in intensity is called fluorescence quenching. In fluorescence quenching, the decrease in intensity is usually described by the well-known Stern–Volmer equation [14]:

$$F_0/F = 1+K_{sv}[Q] = 1+k_q\tau_0[Q] \quad (1)$$

where, F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. k_q, K_{SV}, τ_0 and [Q] are the quenching rate constant of the bimolecular, the Stern–Volmer dynamic quenching constant, the average lifetime of the bimolecular without quencher ($\tau_0=10^{-8}$ s) and the concentration of the quencher, respectively. Obviously,

$$K_{SV} = k_q\tau_0 \quad (2)$$

2.4. Calculation of binding parameters

When small molecules bind independently to a set of equivalent sites on a macromolecule, the apparent binding constant k_A and the number of binding sites (n) can be obtained from the following equation (3) [15,16]:

$$\log(F_0-F)/F = \log K_A + n\log[Q] \quad (3)$$

where, F_0 and F are the fluorescence intensities before and after the addition of the quencher, [Q] is the total quencher concentration. By the plot of log $(F_0-F)/F$ vs log [Q], the number of binding sites n and binding constant K_A can be obtained.

2.5. Synthesis of 2-(2,4-difluorophenyl)-1-phenyl-1H-imidazo[4,5–f][1,10]phenanthroline

The experimental procedure used was the same as described in our recent research works [17–19]. The imidazole derivative, dfppip, was synthesized from an unusual four components assembling of a mixture of 1,10-phenanthroline-5,6-dione, aniline, 2,4-difluorobenzaldehyde and ammonium acetate in distilled ethanol medium. The reaction was monitored by thin layer chromatography for the completion of the reaction. The reaction mixture was then extracted with dichloromethane and the resultant resinous material was purified by column chromatography using benzene:ethyl acetate (9:1) as the eluent. Yield: 60%, mp. 254 °C, Anal. calcd. for $C_{25}H_{14}F_2N_4$: C, 73.5, H, 3.46, N, 13.72. Found: C, 72.8, H, 3.23, N, 13.17. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01–7.10 (m, 3H) [aldehydic phenyl ring], 7.68–7.73 (m, 3H), 8.27–8.33 (m, 2 ortho protons of aniline ring), 8.57 (d, 2H, J=10.4 Hz), 8.85 (d, 2H, J=8.0 Hz), 9.18 (d, 2H, J=4.0 Hz). ^{13}C (100 MHz, $CDCl_3$): δ 105.65, 112.37, 117.88, 122.82, 123.45,

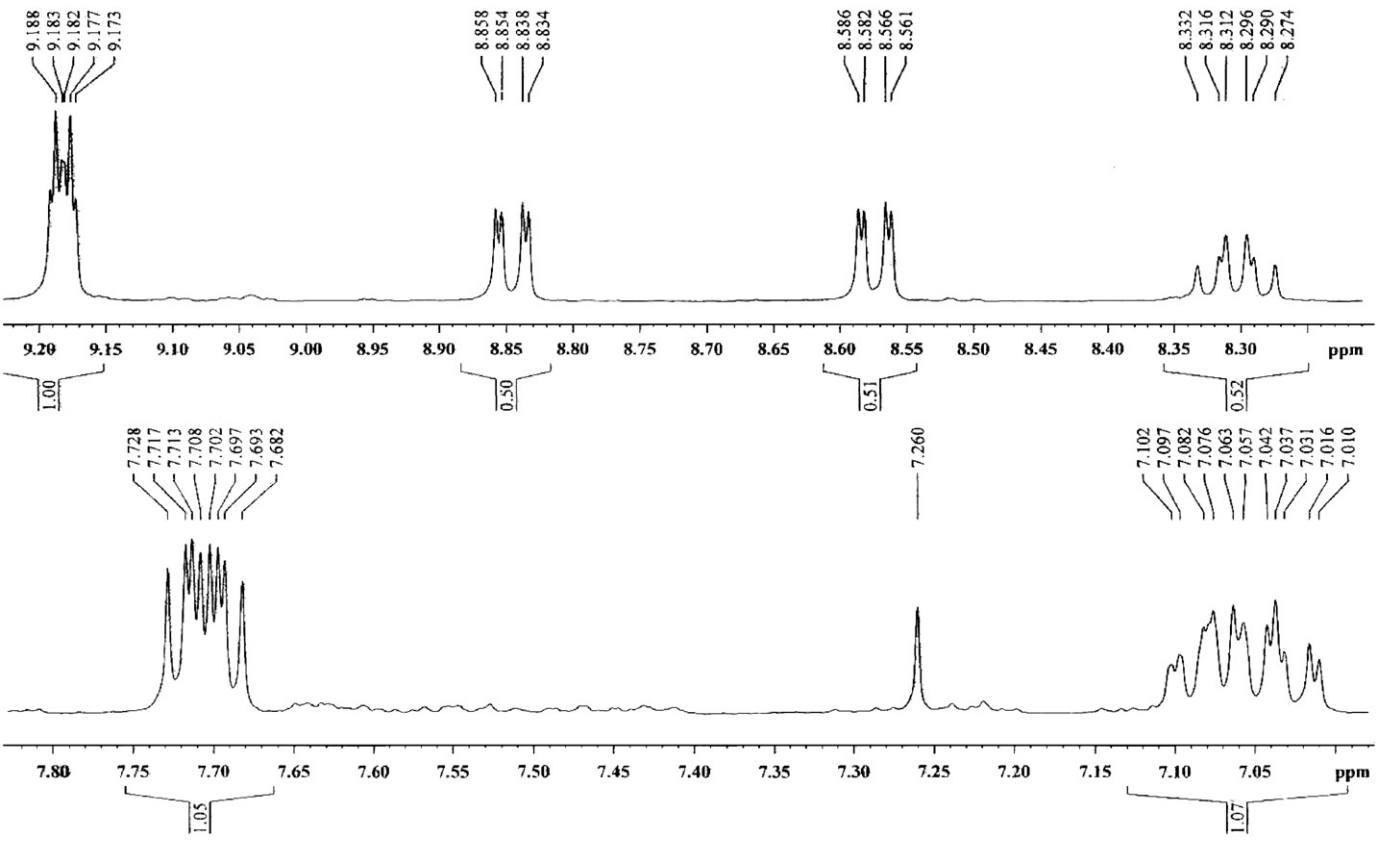

Plate 1. Expanded ^{1}H NMR spectra of dfppip.