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Luminescent study on the binding interaction of bioactive imidazole with bovine serum albumin—A static quenching mechanism

Jayaraman Jayabharathi*, Venugopal Thanikachalam, Natesan Srinivasan, Marimuthu Venkatesh Perumal

Department of Chemistry, Annamalai University, Annamalainagar 608 002, India

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ABSTRACT

Novel bioactive imidazole derivatives were synthesized and characterized by NMR spectra, mass and CHN analysis. The interaction between the imidazole derivative and bovine serum albumin (BSA) was investigated by fluorescence and UV–vis absorption spectroscopy. The fluorescence quenching of BSA by the imidazole derivatives may be due to the formation of imidazole–BSA complex. The fluorescence quenching mechanism of BSA by imidazole was analyzed and the binding constant has been calculated. The binding distance between imidazole and BSA was obtained based on Forester's non-radiation energy transfer (FRET). The effect of some common ions on the binding constant between imidazole and BSA was also examined.

1. Introduction

As a major soluble protein constituent of circulatory system (~60% of the total), serum albumin plays an essential role in binding and transport a wide range of endogenous and exogenous compounds such as fatty acids, heme, bilirubin, metal ions and drugs, in the bloodstream to their target organs [1]. Its functional and physiological properties have been extensively studied in recent years [2]. Moreover, these proteins have long been used as model proteins in both industrial and academic research areas [3].

In this work, we selected Bovine serum albumin (BSA) as our protein model, because of its medically important, abundance, low cost, ease of purification, unusual ligand-binding properties, stability and all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins [4]. BSA has a wide range of physiological functions involving the binding, transport and delivery of fatty acids, porphyrins, steroids, etc. BSA is constituted by 582 amino acid residues and on the basis of the distribution of the disulfide bridges and of the amino acid sequence it seems possible to regard BSA as composed of three homologous domains linked together. The domains can all be subdivided into two sub-domains [5].

Quenching measurements of albumin fluorescence is an important method to study the interactions of compounds with proteins [6,7]. It can reveal accessibility of quenchers to albumin's fluorophores, help to understand albumin binding mechanisms to compounds and provide clues to the nature of the binding phenomenon [8]. The study of the interaction features between small molecules and biological macromolecules such as BSA and discussions regarding the binding force quality, and the mechanism of the interactions play a critical role in promoting research in proteins. The aim of this work is to study the binding and the effects of energy transfer between BSA and the imidazole derivative spectrofluorimetrically.

2. Experimental

2.1. Materials and methods

Butan-2,3-dione (Sigma–Aldrich Ltd.), 2,4-difluoro benzaldehyde (S.D. fine.), 4-methyl aniline, N,N-dimethyl aniline and all other reagents were used without further purification. Bovine Serum Albumin was obtained from Sigma-Aldrich Company, Bangalore). All BSA solution was prepared in Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4) and it 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.

* Corresponding author. Tel.: +91 9443940735.
E-mail address: jtchalam2005@yahoo.co.in (J. Jayabharathi).

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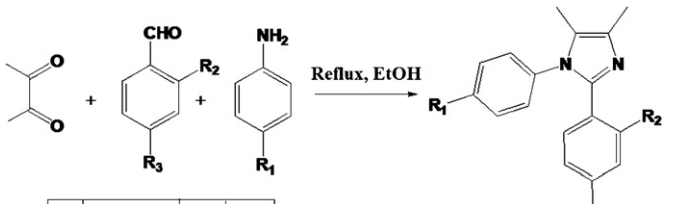


	R_1	R_2	R_3
(1)	CH_3	F	F
(2)	-N(CH_3)(CH_3)	H	H

Scheme 1. Synthesis of imidazole derivatives 1 and 2.

2.2. Optical measurements and composition analysis

NMR spectra were recorded on a Bruker 400 MHz instrument. The UV–vis spectra and photoluminescence (PL) spectra were measured on UV–vis spectrophotometer (PerkinElmer, Lambda 35) and fluorescence spectrometer (PerkinElmer LS55), corrected for background due to solvent absorption. MS spectra were recorded on a Varian Saturn 2200 GCMS spectrometer.

2.3. General procedure for the synthesis of 2-arylimidazole derivatives

The 2-arylimidazole derivatives were synthesized from an unusual four components [9–17] assembling of butan-2,3-dione, ammonium acetate, substituted anilines and substituted benzaldehydes (Scheme 1).

2.3.1. 2.3.1. 2-(2,4-Difluorophenyl)-4,5-dimethyl-1-p-tolyl-1H-imidazole (1)

Yield: 55%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05 (s, 3H), 2.34 (d, 6H), 6.64 (bt, 1H), 6.83 (bt, 1H), 6.98 (d, 2H), 7.16 (d, 2H), 7.41 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.60, 12.74, 21.10, 104.02, 111.38, 116.17, 125.23, 126.98, 129.68, 132.85, 133.87, 134.36, 138.17, 139.83, 144.19, 160.97, 164.06. Anal. calcd. for $C_{18}H_{16}F_2N_2$: C, 72.47; H,5.41; N, 9.39. Found: C, 72.25; H, 5.10; N, 9.09. MS: m/z 298.13, calcd. 298.02.

2.3.2. N,N-Dimethyl-4-(4,5-dimethyl-2-phenyl-1H-imidazol-1-yl)benzenamine (2)

Yield: 47%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (s, 3H), 2.31 (s, 3H), 3.02 (s, 6H), 6.71 (d, 2H), 7.02 (d, 2H), 7.19 (m, 3H), 7.36 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.57, 12.84, 40.40, 112.35, 125.97, 126.53, 127.92, 127.94, 128.39, 131.23, 132.76, 132.99, 145.25, 150.06. Anal. calcd. for $C_{19}H_{21}N_3$: C, 78.32; H, 7.26; N, 14.42. Found: C, 78.11; H, 7.11; N, 14.20. MS: *m/z* 291.17, calcd. 291.01.

2.4. Principles of fluorescence quenching

Fluorescence quenching [18] is described by the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_q\tau_0\,[Q] = 1 + K_{SV}[Q] \quad (1)$$

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, $K_q = K_{SV}$, τ_0, hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F versus [Q].

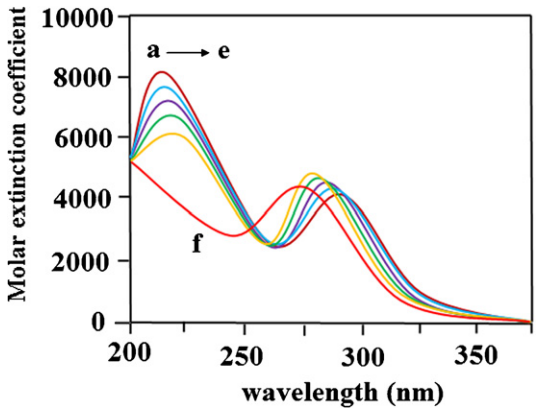


Fig. 1. Absorption spectra of BSA in the presence of imidazole (6) (a–e) and absence of imidazole (6) (f).

2.5. Calculation of binding parameters

Apparent binding constant k_A and binding sites n [19] can be obtained from

$$\log\frac{(F_0 - F)}{F} = \log K_A + n\,\log[Q] \quad (2)$$

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 $(F_0 - F)/F$ versus log [Q], the number of binding sites n and binding constant K_A can be obtained.

3. Results and discussion

3.1. Binding interaction of BSA with bioactive imidazole derivative

The interaction between bioactive imidazole derivative and bovine serum albumin was investigated using UV–vis and fluorescence spectral studies and the fluorescence quenching of BSA by imidazole derivative was the result of the formation of BSA–imidazole complex. The binding site number (n) and apparent binding constant (K_A) were measured. The absorption spectra of BSA in the presence and absence of imidazole were different (Fig. 1). The absorption band of 210 nm of BSA is the

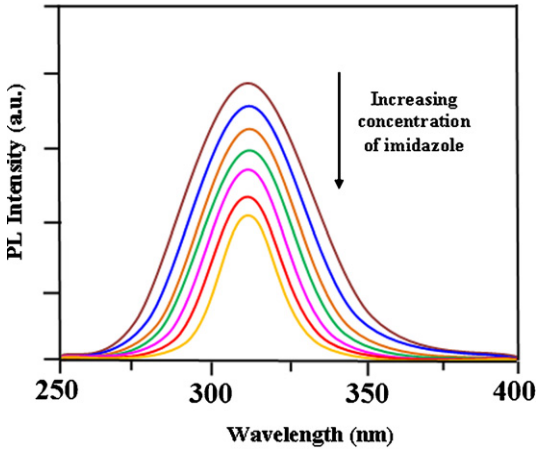


Fig. 2. Fluorescence quenching spectra of BSA at different concentrations of imidazole (2).

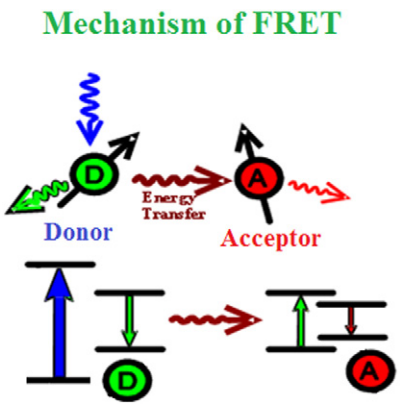


Fig. 3. Fluorescence resonance energy transfer (FRET) mechanism.

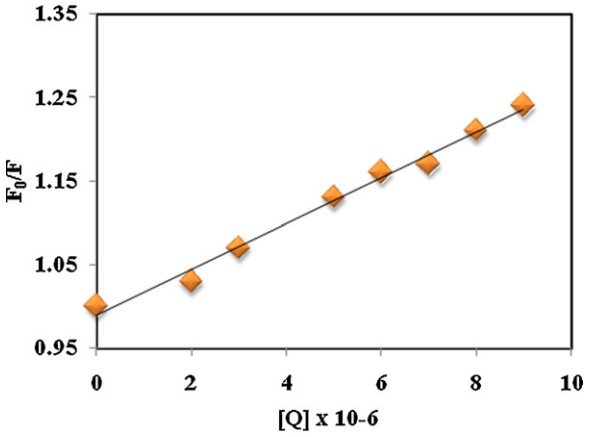
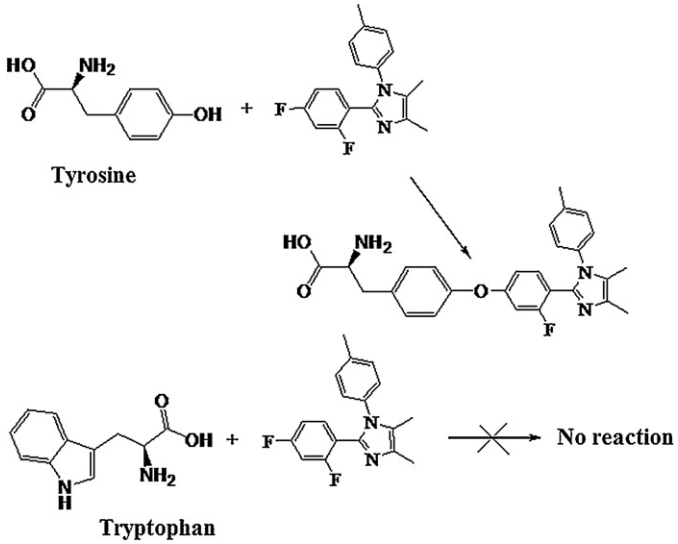


Fig. 4. Stern–Volmer plot of F_0/F against [imidazole].

characteristic of α-helix structure of BSA. The intensity of absorbance of BSA was decreased with increasing concentration of imidazole and the peak was red shifted. The results indicated that there exists interaction between imidazole and BSA results ground state complex was formed [20–22].

Fluorescence quenching spectra of solutions containing BSA of fixed concentration and different concentrations of imidazole were depicted in Fig. 2. It can be observed that the fluorescence intensity of BSA decreases regularly with the increase addition of imidazole but there is no significant emission wavelength shift, suggests that imidazole interact with BSA and quench its intrinsic fluorescence. A Forster type fluorescence resonance energy transfer (FRET) mechanism (Fig. 3) is involved in the quenching of BSA fluorescence by imidazole in BSA–imidazole complex. This means that the



Scheme 2. Schematic representation for the formation of the BSA–imidazole complex.

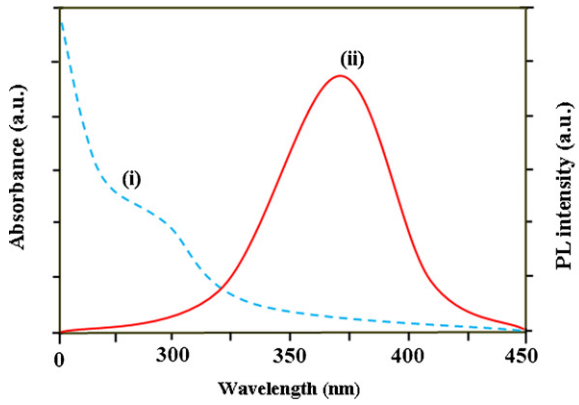


Fig. 6. Overlapping of Fluorescence spectra of BSA with absorption spectra of imidazole.

possible quenching mechanism of fluorescence of BSA by imidazole is not initiated by dynamic collision but from the formation of the BSA-imidazole complex (Scheme 2).

3.2. Fluorescence quenching mechanism

The quenching mechanism of imidazole with BSA was probed using the Stern–Volmer equation [23]. Eq. (1) was applied to

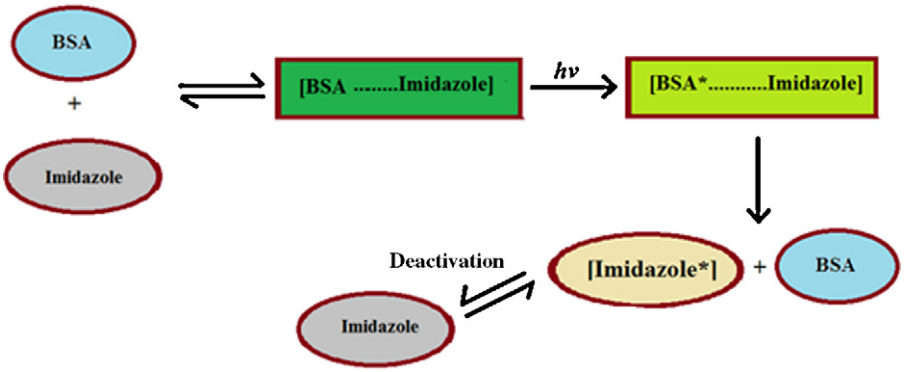


Fig. 5. Schematic representation of binding interaction of BSA with imidazole.

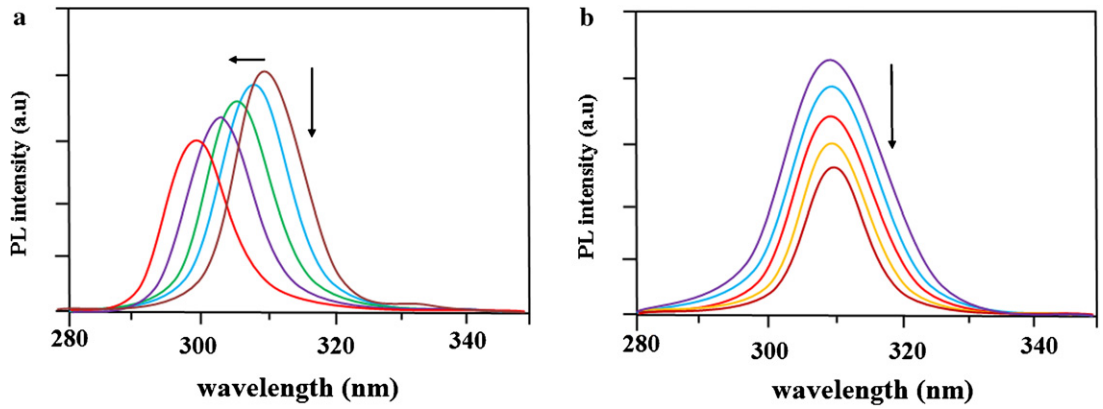


Fig. 7. Synchronous Fluorescence spectra of BSA in the presence and absence of imidazole (a) at $\Delta\lambda = 15$ nm (b) at $\Delta\lambda = 60$ nm.

determine K_{SV} by linear regression from the Stern–Volmer plot of F_0/F against [imidazole] (Fig. 4). Dynamic and static quenching can be distinguished by the quenching constant K_q. According to the literature [24] for dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is $2.0 \times 10^{1\circ}$ L mol^{-1} s^{-1} and the fluorescence lifetime of the biopolymers is 10^{-8} s [25]. From Fig. 4, the values of K_{SV} and K_q ($=K_{SV}/\tau_0$) was calculated. The values of K_q are larger than 2.0×10^{10} L mol^{-1} s^{-1}, which suggest that the fluorescence quenching is caused by a specific interaction between BSA and imidazole (2) and the quenching mechanism mainly arise from BSA–imidazole complex formation, while dynamic collision could be negligible in the concentration range studied [26]. The plot of $\log[(F_0 - F)/F]$ versus log[imidazole] binding constant (K_A) as 2.02×10^4 M^{-1} and binding site (n) as 1.09 of imidazole with BSA from the intercept and slope (Fig. 5).

3.3. Energy transfer from BSA to imidazole derivative

The distance between the BSA and the interacted imidazole was estimated by Forster's non-radiative energy transfer (FRET) and the overlap fluorescence spectra of imidazole and the UV–vis absorption spectra of BSA were shown in Fig. 6. According to Forster's non-radiative energy transfer theory, the energy transfer efficiency (E) is defined by the following equations,

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{(R_0^6/r_0^6)} \quad (3)$$

$$R_0^6 = 8.8 \times 10^{23}[\kappa^2 n^{-4} \Phi_D J(\lambda)] \text{ in } \text{Å}^6 \quad (4)$$

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (5)$$

where F_D (λ) is the corrected fluorescence intensity of the donor at wavelength λ to ($\lambda + \Delta\lambda$), with the total intensity normalized to unity and $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor at wavelength λ. The Forster distance (R_0) has been calculated assuming random orientation of the donor and acceptor molecules. In the present case, $\kappa^2 = 2/3$, $n = 1.334$, $\Phi_D = 0.30$ and from the available data, it results that $J(\lambda) = 2.37 \times 10^{-15}$ cm^3 L mol^{-1}, $E = 0.48$, $R_0 = 2.41$ nm and $r = 2.44$ nm were calculated. The donor-to-acceptor distance is less than the 8 nm, and the long distance indicates that the quenching mechanism is a dynamic one, which is strong evidence for static quenching and energy could transfer from BSA to imidazole [27].

3.4. Synchronous fluorescence spectroscopic studies of BSA

The synchronous fluorescence spectra [28–30] of BSA with various amounts of imidazole were recorded at $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm (Fig. 7a and b), respectively. The emission wavelength of the Tyrosine residues is blue-shifted (λ_{max} from 310 to 300 nm in Fig. 7a) with increasing concentration of imidazole. At the same time, the tryptophan fluorescence emission is decreased regularly, but no significant change in wavelength was observed. It suggests that the interaction of imidazole with BSA does not affect the conformation of tryptophan micro-region. The Tyrosine fluorescence spectrum may represent that the conformation of BSA is somewhat changed, leading to the polarity around *Tyr* residues strengthened and the hydrophobicity weakened [31]. This is because *Tyr* contains one aromatic hydroxyl group unlike tryptophan and *Tyr* can undergo an excited state ionization, resulting in the loss of the proton on the aromatic hydroxyl group. The hydroxyl group can dissociate during the lifetime of its excited state, leading to quenching. Hence the aromatic hydroxyl group present in the *Tyr* residue is responsible for the interaction of BSA with imidazole.

3.5. Effect of common ions on the binding constants of imidazole–BSA complex

In the blood system, there exists many metal ions and these ions can directly influence the binding force of drug with protein. The effect of metal ions Cu^{2+}, Zn^{2+}, Ca^{2+},Mg^{2+}, Ni^{2+}, Co^{2+} and Fe^{2+} on the binding constants was investigated at 287 K by recording the fluorescence intensity in the range from 300 to 500 nm upon excitation at 280 nm and the concentration of BSA and metal ions were fixed at 2 μM (Table 1). The competition between the common metal ions and imidazole makes the decrease in binding constant of imidazole–BSA as compared to the binding constant without the metal ions. Therefore, the common metal ions will shorten the storage time of the drug in blood plasma, which may lead to the need for

Table 1
Effects of metal ions on the binding constant of BSA–imidazole (1).

System	Binding constant (10^4 L mol^{-1})
BSA + (1)	2.06
BSA + (1) + Cu^{2+}	2.02
BSA + (1) + Zn^{2+}	1.96
BSA + (1) + Ca^{2+}	1.89
BSA + (1) + Mg^{2+}	1.71
BSA + (1) + Ni^{2+}	1.51
BSA + (1) + Co^{2+}	1.42
BSA + (1) + Fe^{2+}	1.33

more doses of imidazole to achieve the desired therapeutic effect [32].

4. Conclusion

The interaction between bioactive imidazole derivative and bovine serum albumin was investigated. From the spectral studies it was found that the possible quenching mechanism of fluorescence of BSA by imidazole is not initiated by dynamic collision but from the formation of the BSA-imidazole complex.

Acknowledgments

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