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RESEARCH ARTICLE

Fluorescence Report on the Binding Interaction of Imidazole using Bovine Serum Albumin

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ABSTRACT

Nuclear Magnetic Resonance (NMR) spectra, mass and Carbon Hydrogen and Nitrogen (CHN) analyzer synthesize and characterize bioactive Imidazole Derivatives (ID). Fluorescence and ultra violet–visible absorption spectrometry aids in investigating ID and bovine serum albumin. Imidazole–Bovine Serum Albumin (BSA) complex leads to BSA fluorescence quenching, in where ID performs the BSA quenching. Then the calculation of binding constant is made based on quenching analysis. Forester's non-radiation energy transfer determines the binding distance between bovine serum albumin and imidazole, where the influence of certain ions over the binding constant between them has also been carried out.

Keywords: Nuclear magnetic resonance spectra, CHN analyzer, Bio-active imidazole, Forester's non-radiation energy transfer, Bovine serum albumin.

1. INTRODUCTION

Serum albumin, lipoproteins and al-glycoprotein are the proteins that involve in drug delivery system. In the circulatory system of most of the organisms, serum albumin is richly available and it widely contributes to the osmotic blood pressure. Bovine serum albumin is opted for transportation of several compounds [1]. It is given importance due to its structural similarity with that of human serum albumin and the recent studies are based on its functional and physiological characteristics. [2] Such proteins are employed as model proteins in industrial and research sectors [3]. Among the so called proteins, BSA is chosen for our work owing to its medical importance, abundance, availability, cost effectiveness and unique ligand-binding characteristic. It is also stable and easy to purify. In common, the reference papers convey that HSA (Human Serum Albumin) and BSA are homologous in nature [4]. Bovine serum albumin consists of 582 amino acid residues that involves in a series of tasks like binding, transportation of fatty acids and steroids. Conclusion can be drawn in terms of disulfide bridges distribution and amino acid sequencing that three homologous domains are grouped as a whole to make bovine serum albumin, where these domains are classified into two [5]. Carrying out albumin fluorescence quenching mechanism is necessary to determine the influence of compounds with proteins [6, 7]. Fluorescence spectroscopy differs from other mechanisms like calorimetry and far–ultraviolet circular dichroism. It resembles a probing method that sense alteration in the environment, where its function varies from infrared spectroscopy too. The likelihood of structural reorganizations in the fluorophore atmosphere results in same fluorescence, where the results are interpreted in a complicated manner to achieve unique structural and dynamic data [8].

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Extensive researches become vital in accordance with the interaction between the micro and macro molecules like bovine serum albumin and its related binding force mode. Recent studies are focused more on interaction of ID [9-13]. Thus the binding and energy transfer effects between like bovine serum albumin and ID are reported in this article.

2. EXPERIMENTAL PROCEDURE

2.1. Materials and methods

Bovine serum albumin solution was prepared using Tris-HCl buffer solution ($0.05 molL^{-1}$Tris, $0.15 molL^{-1}$NaCl, pH 7.4) and placed at dark by maintaining 303K. The purity of tris base (2-amino-2-(hydroxymethyl)-1, 3-propanediol) is higher than 99.5%. The other solutions such as NaCl and HCl used in this experiment are of analytical purity set, where double distilled water (ddH_20) is used.

2.2. Optical and composition measurements

A record of NMR spectra has been performed on Bruker 400 MHz, followed by the measurement of UV–Vis and Photo Luminescence (PL) spectra using UV–vis and fluorescence spectrophotometer of Perkin Elmer, Lambda 35 and Perkin Elmer LS45 respectively. Further corrections are carried out with respect to solvent absorption. Then the sample mass spectra are acquired by applying thermo Fischer LC-mass spectrometer.

2.3. Synthesis of 2-(Naphthalen-1-yl)-1-phenyl-1H-benzimidazole (NPB)

N-phenyl-o-phenylenediamine (17mmol, 3.128g), napthaldehyde (17mmol, 1.9ml), and ammonium acetate (3g) compound is refluxed at 80°C in ethanol medium by utilizing TiO_2 catalyst for about half an hour. A thin layer and column chromatography are used to monitor and purify the reaction accordingly, where benzene: ethyl acetate mixture at ratio, 9:1 has been used as eluent.

2.4. Fluorescence quenching mechanism

Fluorescence quenching [14] can be defined using Stern-Volmer relation as given in (2.1)

$$F_0/_F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \qquad (2.1)$$

Here let F_0 and F be the fluorescence intensities before and after quencher incorporation correspondingly, K_q be the bimolecular quenching rate constant, K_{SV} be the Stern-Volmer dynamic quenching constant, τ_0 be the bimolecular average lifetime without quencher ($\tau_0 = 10^{-8}$s) and [Q] be the quencher concentration. Since $K_q = K_{SV}\,\tau_0$, K_{SV} is assessed with (1) using linear regression model of F_0/F against [Q] plot.

2.6. Binding parameter assessment

Equation (2.2) defines the apparent binding constant k_A and binding sites n [15].

$$\log\left((F_o - F)/_F\right) = \log K_A + n\log[Q] \qquad (2.2)$$

where, [Q] represents total quencher concentration. The number of binding sites (n) and binding constant (K_A) could be determined using log $(F_0-F)/F$ vs. log [Q] plot.

3. RESULTS AND DISCUSSION

3.1. Binding interaction of bovine serum albumin with bioactive imidazole derivative

The interaction between bioactive ID (NPB) and BSA has been examined using UV-vis and fluorescence spectra. BSA fluorescence quenching by bioactive imidazole derivative has been resulted on the basis of Bovine Serum Albumin and bioactive imidazole derivative (BSA- NPB) complex. As a result, n and K_A are determined. The absorption spectra of BSA with (a-e) and without (f) existence of imidazole tend to vary as shown in figure 1.

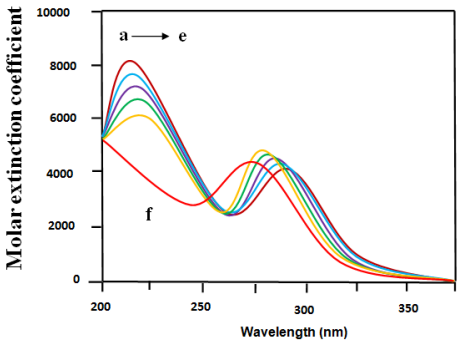

Figure 1.Absorption spectra of BSA

The absorption band of 210 nm of bovine serum albumin is its feature of α–helix form. As the intensity of BSA absorbance decreases, bioactive imidazole derivative concentration gets increased; thereby the peak would be red shifted. It is found out that there lies interaction between imidazole and bovine serum albumin results. [16-18].

Fluorescence quenching spectra of solutions with bovine serum albumin of fixed and varied NPB concentrations is noted as it is shown in figure 2.

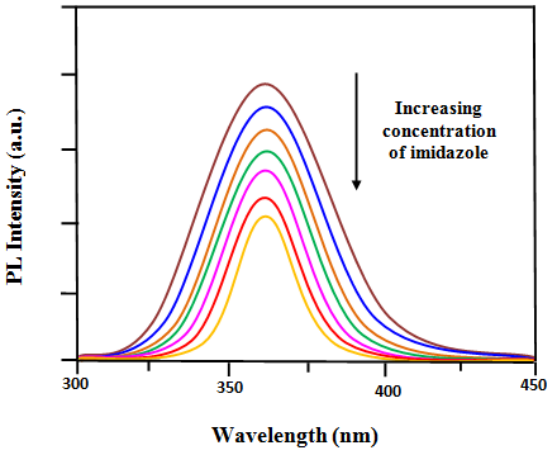

Figure 2.Fluorescence quenching spectra of BSA

Observations are so clear in terms of minimization of BSA fluorescence intensity with that of rise in NPB but the more important emission wavelength shift could not be determined. It is suggested that bioactive imidazole derivative interacts with bovine serum albumin and hence quenches its intrinsic fluorescence. Involvement of Forster type fluorescence resonance energy transfer mechanism in Tyr fluorescence quenching by bioactive ID in BSA- NPB complex is illustrated in figure 3.

It is said that the possibility of fluorescence quenching mechanism of bovine serum albumin by bioactive imidazole derivative (NPB) has not been put forth by dynamic collision rather, from the BSA-imidazole compound generation.

3.2. Concept of fluorescence quenching

Fluorescence quenching can be broadly divided into,

- Static quenching
- Dynamic quenching
- Static and dynamic quenching

In general, these types could participate simultaneously. The fluorescence quenching is identified as a dynamic procedure so as to validate the bovine serum albumin quenching criteria by NPB (bioactive imidazole derivative). At this juncture, the principles are investigated through the typical Stern–Volmer relation [19].

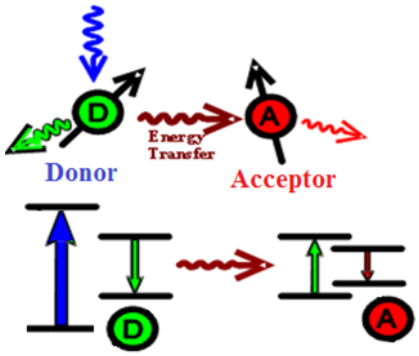

Figure 3.FRET mechanism

In determining K_{SV} by adopting Stern-Volmer graph of F_0/F versus bioactive imidazole derivative based linear regression, equation (2.1) is employed which is represented in figure 4.

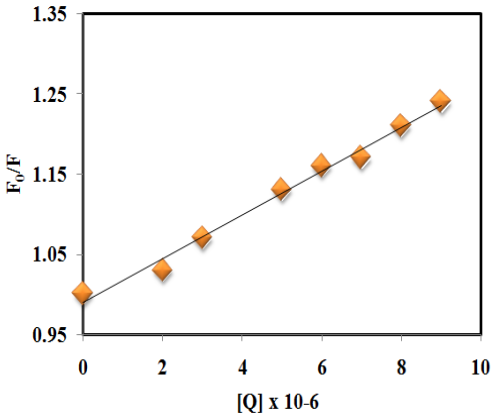

Figure 4.Stern-Volmer graph

Dynamic and static quenching varies in terms of quenching constant K_q. Relating to dynamic type, the utmost scatter collision quenching constant of different quenchers and the fluorescence lifetime in corresponding with biopolymers is $2.0\times10^{10}Lmol^{-1}s^{-1}$ and $10^{-8}s$ accordingly [20, 21]. Using figure 4, K_{SV} and K_q

($=K_{SV}/\tau_0$) readings are determined, where K_q value is greater than $2.0\times10^{10}Lmol^{-1}s^{-1}$. It means that a certain interaction between bovine serum albumin and bioactive imidazole derivative causes fluorescence quenching where it initiates generally due to BSA-NPB complex generation. Here dynamic collision is not at all preferred in the concentration level investigated [22].

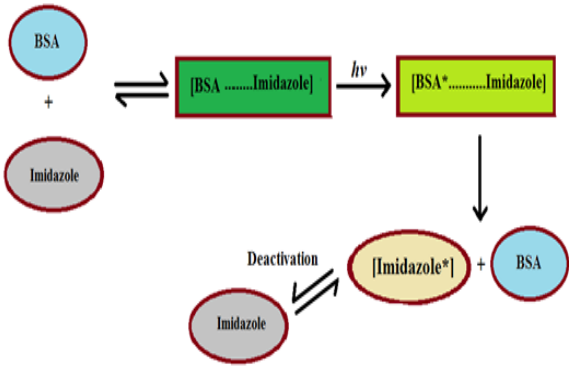

Figure 5.Binding interaction of BSA and imidazole

Figure 5 shows the layout of log [(F_0-F)/F] against log [2-(Naphthalen-1-yl)-1-phenyl-1H-benzimidazole], where K_A and n equals 2.02 x 10^4 M^{-1} and 1.09 of NPB.

3.3. Energy transfer from BSA to ID

FRET estimates the distance from BSA to interacted NPB. Figure 6 represents the overlap fluorescence and the UV–Vis absorption spectra of imidazole and bovine serum albumin respectively.

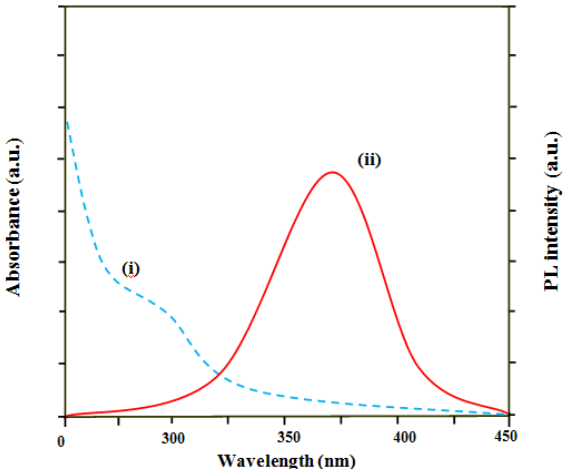

Figure 6.Overlapping of fluorescence and spectra absorption spectra

FRET theory gives rise to the energy transfer efficiency (E) as in (3.1).

$$E = 1 - \frac{F}{F_0} = {R_0^6}\Big/{(R_0^6 + r_0^6)} \quad (3.1)$$

$$R_0^6 = 8.8 * 10^{23}[K^2 n^{-4} \Phi_D J(\lambda)] \text{ in } Å^6 \quad (3.2)$$

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (3.3)$$

where F_D (λ) refers to the corrected donor fluorescence intensity and $\varepsilon_A(\lambda)$ denotes the acceptor molar extinction coefficient at wavelength (λ to ($\lambda + \Delta\lambda$)) and λ respectively. It should be noted that the total intensity has been normalized to 1 with respect to donor fluorescence intensity. Calculation of R_0 (Forster distance) is done with the assumption of donor random orientation and acceptor molecules. Here, K^2 equals 2/3, n equals 1.334 and Φ_D=0.30. Results from the recorded data include $J(\lambda)$ = 2.37 x 10^{-15} cm^3Lmol^{-1}, E = 0.48, R_0 = 2.41nm and r=2.44nm. The donor-to-acceptor distance is not more than 8nm where it denotes that the quenching process is dynamic. Hence it is evident that in static quenching, energy transfer is initiated between bovine serum albumin and bioactive imidazole derivative [23].

3.4. Synchronous fluorescence spectroscopic representation of bovine serum albumin

In relating to the microenvironment of amino acid residual studies, synchronous fluorescence spectra (SFS) plays a significant role, where the researches are carried out by assessing the shift of emission wavelength [24, 25]. It is advantageous in relation with sensitivity, spectral easiness and bandwidth minimization and reduced perturbing factors [26]. It is also beneficial in going ahead with studies corresponding to amino acid residues through λ_{max} (maximum wavelength) shift assessment with respect to the polarity variation of the chromophore. The SFS of bovine serum albumin by considering NPB elements are marked at $\Delta\lambda$=15nm and $\Delta\lambda$=60nm. This representation is shown in figures 7 and 8 accordingly.

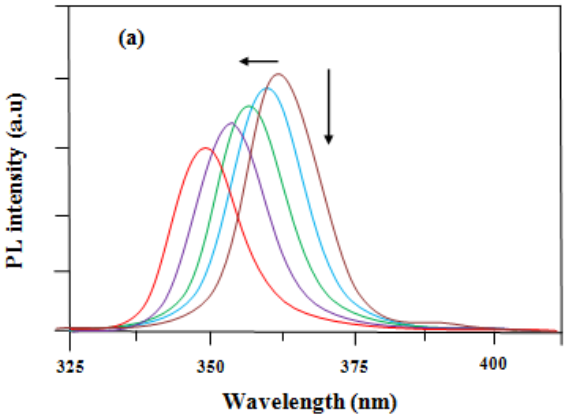

Figure 7.SFS of BSA at Δλ = 15nm

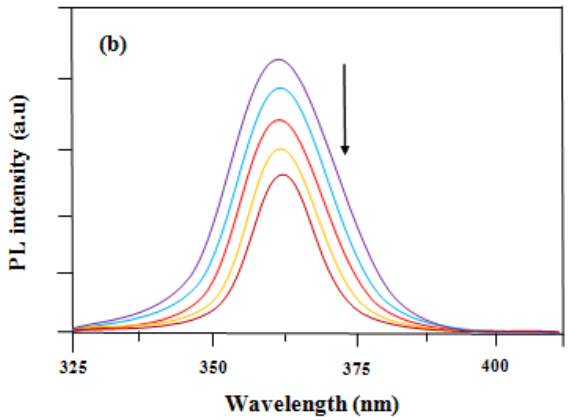

Figure 8.SFS of BSA at Δ λ =60nm

In figure 7, Tyrosine residual emission wavelength is blue-shifted (λ_{max} from 362 to 349 nm with high concentration of bioactive imidazole derivative, where regular reduction of tryptophan fluorescence emission is accomplished simultaneously. Even though tryptophan fluorescence emission is minimized at regular interval, there is no wavelength variation. It means that BSA and NPB interaction does not have any influence over conformation of tryptophan micro-region. Figure 8 shows the SFS of BSA at Δλ=60nm. Change of BSA conformation leads to strengthening of Tyr residual polarity and weakening of hydrophobicity [27]. Tyr is different from tryptophan in where Tyr includes a hydroxyl (HO-) group and it could experience an excited state ionization so that a proton is lost from its HO- group. It results in quenching owing to the dissociation of the excited level, thereby it is the lead factor for BSA and NPB interaction.

3.5. Ionic impact over the binding constants of NPB –BSA compound

In humans, metal ions are important and play a significant role on proteins on the basis of coordinate bonds. It might have influence over the interaction of drugs with bovine serum albumin. The presence of such ions in the blood stream has direct impact upon the drug binding force with proteins.

Metal ionic effect of Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+} and Fe^{2+} on the binding constants is examined at 287K by setting up the fluorescence intensity ranging from 300nm to 500nm upon excitation at 280nm where the bovine serum albumin concentration and metal ions are kept at 2μM as given in table 1.

Table 1.Impact of metal ions on the binding constant

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

The arising competition between the common metal ions and NPB minimizes the binding constant of 2-(Naphthalen-1-yl)-1-phenyl-1H-benzimidazole (NPB) and bovine serum albumin when compared to the binding constant in the absence of metal ions. Hence, the common metal ions reduce the storage time of drug in blood plasma, thus resulting in the requirement of higher NPB dosage in order to obtain the required therapeutic effect [28].

3.6. NPB: ^{1}H and ^{13}C NMR spectra

The ^{1}H-NMR spectra of NPB in $CDCl_3$ the proton chemical shifts are given in table 2. It represents ^{1}H and ^{13}C-NMR chemical shifts (δ, ppm) of NPB. The ^{1}H chemical shifts are quoted after rounding off two decimal points. There is a doublet at 8.12ppm, corresponding to one proton. This should be due to H-9. There is a triplet at 7.83ppm, corresponding to two protons.

This is actually overlapped of two triplets. By careful inspection the chemical shifts of the two triplets were determined as 7.81 and 7.85ppm. These signals are due to the H-7 and H-8 protons. There is a doublet at 7.96 and 7.97ppm corresponding to one proton; this should be due to H-6.

Table 2.^{1}H and ^{13}C-NMR chemical shifts

Protons	Chemical shifts	Carbons	Chemical shift
H-9	8.12	C-2	151.9
H-7, H-8	7.81, 7.85	C-4	133.6
H-6	7.96, 7.98	C-5	143.3
o, m, p – H	7.31, 7.33, 7.34, 7.38 & 7.39	C-6, C-9	110.6
H-11	7.25	C-7	123.0
H-12	7.23	C-8	123.5
H-13	7.27	*Ipso carbon* C-10, C-3’	136.1, 136.5
H-15	7.44, 7.45	*o*-C	120.2
H-16	7.16	*m*-C	129.4
H-17	7.18	*p*-C	127.0
H-18	7.46, 7.47	C-11	124.6
		C-12	126.6
		C-13	127.7
		C-14	134.2
		C-15	128.0
		C-16	125.9
		C-17	126.2
		C-18	128.2
		C-19	132.7

The signals around 7.3ppm (7.31, 7.33, 7.34, 7.38 and 7.39) corresponding to five protons. This is due to phenyl protons (o-H, m-H and p-H). There is a signal around 7.2ppm (7.23, 7.25 and 7.27), corresponding to three protons where it is because of H-11, H-12 and H-13 respectively. There is a multiplet at 7.45 ppm, corresponding to two protons. This is actually overlapped by two triplets. By careful inspection, the chemical shifts of the two triplets are determined as 7.44ppm for H-15 and 7.46ppm for H-18 protons. There are two singlets at 7.16 and 7.18ppm each corresponding to one proton. The signal at 7.16 ppm is assigned to H-16 and 7.18ppm to H-17 proton. The ^{13}C NMR spectrum of NPB in $CDCl_3$ and the ^{13}C chemical shifts are given.

3.7. Crystal structure

NPB crystallize in Triclinic geometry with P1space group [29, 30, 31]. The cell dimensions are represented in table 3.

Table 3.Cell dimensions

Cell dimensions	Values
a	8.5529(3) Å
b	9.4517(3) Å
c	11.8936(3) Å
α	86.334(2)°
β	89.838(2)°
γ	75.051(2)°
V	926.92(5) Å^3.

Figure 9 denotes ORTEP structure of Naphthalen-1-yl)-1-phenyl-1H-benzimidazole.

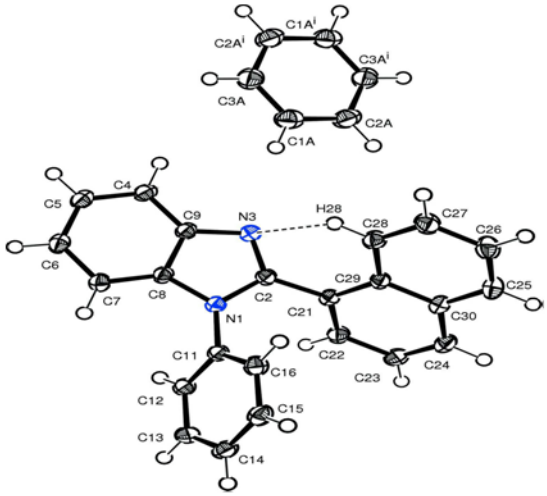

Figure 9.ORTEP diagram of NPB

It is given that the benzimidazole unit [maximum deviation = 0.0258 (6)A˚] and the naphthalene ring mode [maximum deviation = 0.0254 (6)A˚] are planar which form a dihedral angle of 61.955 (17)˚. The imidazole ring develops dihedral angle of 61.73 (4) with the phenyl ring.

DFT performs NPB optimization at B3LYP/6-31G(d,p) using Gaussian-03. The practically resulted values matches with that of the theoretical readings which is tabulated in table 2. Yet several optimized bond lengths along with bond and dihedral angles of XRD reports tend to be greater than the theoretical results. It means that the theoretical assessments are focussed at gaseous phased isolated molecule, whereas the XRD values at the molecules in solid phase.

4. CONCLUSION

The article describes about the interaction between bioactive ID and BSA. The spectral results reveal that the possible fluorescence quenching mode of bovine serum albumin by imidazole has not been negotiated by dynamic collision but through the formulation of the bovine serum albumin-imidazole complex.

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