Journal of Photochemistry and Photobiology B: Biology 117 (2012) 27–32

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Docking investigation and binding interaction of benzimidazole derivative with bovine serum albumin

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ARTICLE INFO

Article history:
Received 25 July 2012
Received in revised form 21 August 2012
Accepted 27 August 2012
Available online 5 September 2012

Keywords:
Benzimidazole
Molecular docking
BSA
Quenching

ABSTRACT

^{1}H NMR, ^{13}C NMR and Mass spectral analysis have been made for 1-(4-fluorobenzyl)-2-(4-fluorophenyl)-1H-benzo[d]imidazole (FBFPB). The mutual interaction of FBFPB with bovine serum albumin (BSA) was investigated using absorption, fluorescence and synchronous fluorescence spectral studies. The binding distance has been determined based on the theory of Forester's non-radiation energy transfer (FRET). The calculated quenching constants (K_{sv}) were 2.84×10^4, 2.55×10^4 and 2.37×10^4 at 301, 310 and 318 K respectively. The Stern–Volmer quenching constant (K_{sv}), binding site number (n), apparent binding constant (K_A) and corresponding thermodynamic parameters (ΔG, ΔH and ΔS) were calculated. The interaction between FBFPB and BSA have discussed by molecular docking technique.

1. Introduction

Heterocyclic imidazole derivatives have attracted considerable attention because of their unique optical properties [1] and used for preparing functionalized materials [2]. Imidazole nucleus forms the main structure of human organisms, i.e., the amino acid histidine, Vitamin B_{12}, a component of DNA base structure and also has significant analytical applications utilizing their fluorescence and chemiluminescence properties [3,4].

BSA is made up of three homologous domains (I–III), which are divided into nine loops by 17 disulfide bridges. Each domain is composed of two sub-domains (A and B). Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in sub-domain IIA and IIIA, site I and site II [5,6]. BSA has two tryptophans, Trp-134 and Trp-212, embedded in the first sub domain IB and sub-domain IIA, respectively. There is evidence of conformation changes of BSA induced by its interaction with low molecular weight benzimidazole and imidazole ligands [7–10]. It is important to study the interaction of FBFPB with BSA, and hence become an important research field in chemistry, life sciences and clinical medicine. The molecular structure of FBFPB is given in Fig. 1. In the present research article, we have studied the binding interaction of BSA with 1-(4-fluorobenzyl)-2-(4-fluorophenyl)-1H-benzo[d] imidazole.

2. Experimental

2.1. Materials and methods

All BSA solution were prepared in the 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.

2.2. Optical measurements

NMR spectra have been recorded for the FBFPB on a Bruker 400 MHz instrument. The ultraviolet–visible (UV–vis) spectra have been measured on UV–Vis spectrophotometer (Perkin Elmer, Lambda 35) and corrected for background due to solvent absorption. Photoluminescence (PL) spectra have been recorded on a (Perkin Elmer LS55) fluorescence spectrometer. Solvents used for spectral measurements are spectroscopic grade. Mass spectra have also been recorded on a Varian Saturn 2200 GCMS spectrometer.

2.3. Molecular docking

The rigid molecular docking studies were performed by using HEX 6.1 software [11], is an interactive molecular graphics program to understand the drug-protein interaction. The Structure of the FBFPB was sketched by CHEMSKETCH (http://www.acdlabs.com) and converts it into pdb format from mol format by OPENBABEL (http://www.vcclab.org/lab/babel/). The crystal

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http://dx.doi.org/10.1016/j.jphotobiol.2012.08.018

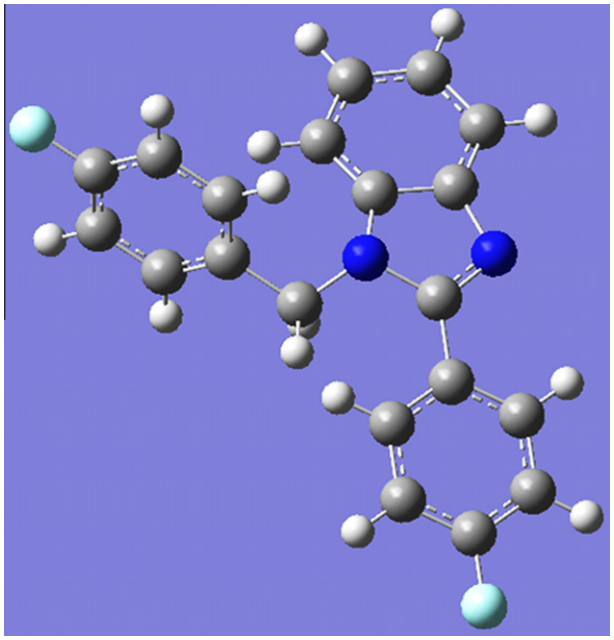

Fig. 1. Molecular structure of FBFPB.

structure of HSA (PDB entry 1AO6) is obtained from the Protein Data Bank (http://www.rcsb.org./pdb). Since the structure of bovine serum albumin (BSA) is unavailable in the PDB, a homology model was used for the docking studies. All calculations were carried out on an Intel pentium 4, 2.4 GHz based machine running MS Windows XP SP2 as operating system. Visualization of the docked pose has been done by using PyMol (http://pymol.sourceforge.net/) molecular graphic program.

2.4. General procedure for the synthesis of ligands

A mixture of 4-fluorobenzaldehyde (2 mmol), *o*-phenylenediamine (1 mmol) and ammonium acetate (2.5 mmol) has been refluxed at 80 °C in ethanol. The reaction was monitored by TLC and purified by column chromatography using petroleum ether: ethyl acetate (9:1) as the eluent.

2.4.1. 1-(4-Fluorobenzyl)-2-(4-fluorophenyl)-1H-benzo[d]imidazole

Yield: 55%. mp = 84 °C, Anal. calcd. for $C_{20}H_{14}F_2N_2$: C, 74.98; H, 4.41; F,11.86; N, 8.75. Found: C, 74.49; H, 4.51; F,11.95; N, 9.05. ^{1}H NMR (400 MHz, $CDCl_3$): δ5.41 (s, 2H), 7.02–7.08 (m, 4H), 7.15–7.18 (t, 3H), 7.22–7.23 (d, 1H) 7.26–7.29 (m, 1H), 7.33–7.36 (m, 1H), 7.65–7.67 (m, 2H), 7.87–7.88 (s, 1H). ^{13}C (100 MHz, $CDCl_3$): δ 47.71 (–CH_2 carbon), 110.33, 115.93, 116.03, 116.21, 120.07, 122.92, 123.30, 126.16, 126.19, 127.60, 127.63, 127.67, 131.19, 131.25, 131.93, 131.95, 135.89, 143.06, 153.08, 161.31, 162.78, 163.28, 164.77 (Aromatic carbons). MS: *m/e* 320.1, calcd 321.11 [M + 1].

3. Results and discussion

3.1. Fluorescence spectral studies

The interaction between FBFPB and BSA was investigated by evaluating fluorescence intensity on the BSA before and after the addition of the FBFPB [10,12]. Here, the concentrations of BSA were stabilized at 1.0×10^{-5} mol L^{-1} and the concentration of FBFPB varied from 0 to 3.5×10^{-5} mol L^{-1} at increments of 0.5×10^{-5} mol L^{-1}. The effect of the FBFPB on BSA fluorescence intensity is shown in Fig. 2. The fluorescence intensity of BSA decreases progressively but the emission maximum did not move to shorter or longer wavelength, due to the interaction of FBFPB with BSA and quench its intrinsic fluorescence (*Trp-212*) [13], but there was no alteration in the local dielectric environment of BSA.

The quenching mechanism of FBFPB with BSA was probed using the Stern–Volmer equation [14], which can be applied to determine K_{sv} by linear regression from the Stern–Volmer plot of F_0/F against [FBFPB] (Fig. 3) at different temperatures. Table 1 summarizes the values of K_{sv} and K_A at different temperatures, which shows the values of Stern–Volmer quenching constant K_{sv} and K_A decreases with increase in temperature. According to the literature [15] for dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L mol^{-1} s^{-1} and the fluorescence lifetime of the biopolymer is 10^{-8} s. From Fig. 3, the values of K_{sv} and k_q ($=K_{sv}/\tau_0$) were calculated. The obtained values of k_q were larger than the limiting diffusion rate constant of the biomolecule (2.0×10^{10} L mol^{-1} s^{-1}), which indicate that the fluorescence quenching is caused by a specific interaction between BSA and FBFPB. Therefore, the quenching mechanism mainly arises from the formation of BSA–FBFPB complex rather than dynamic quenching. So it was implied that the static quenching was dominant in the system. From the plot of log[($F_0 - F$)/F] *vs* log[FBFPB], binding constants K_A and the number of binding sites '*n*' were calculated from the intercept and slope.

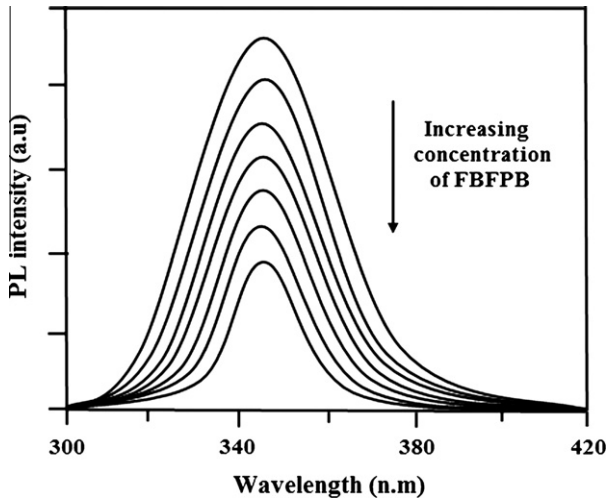

Fig. 2. Fluorescence quenching spectra of BSA at different concentrations of FBFPB.

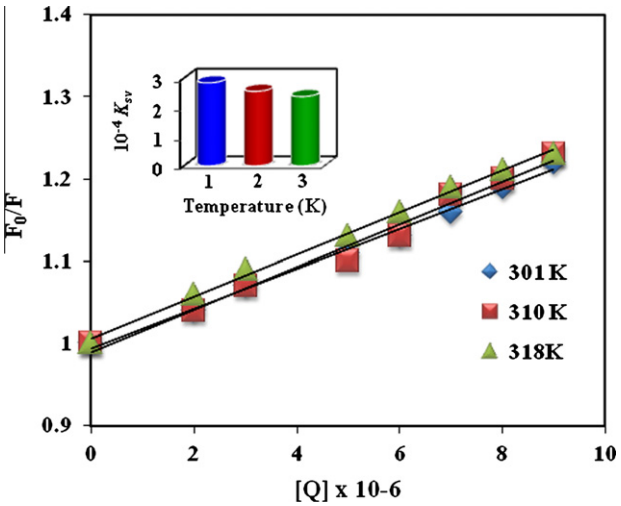

Fig. 3. Stern–Volmer plot of F_0/F against [FBFPB].