Journal of Molecular Structure 1167 (2018) 107–117

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Spectroscopic exploration and molecular docking analysis on interaction of synthesized schiff base ligand with serum albumins

Suparna Rudra, Somnath Dasmandal, Chiranjit Patra, Ambikesh Mahapatra*

Department of Chemistry, Jadavpur University, Kolkata, 700 032, India

ARTICLE INFO

Article history:
Received 24 May 2017
Received in revised form
19 April 2018
Accepted 26 April 2018
Available online 27 April 2018

Keywords:
Schiff base
Serum albumin
Docking
Fluorescence

ABSTRACT

The present article reports detailed characterization about interaction between a synthesized Schiff base ligand and serum proteins *viz.*, bovine serum albumin (BSA) and human serum albumin (HSA). The entire interactive phenomena have been diligently investigated through multi spectroscopic studies and quantum mechanical calculations involving density functional theory (DFT) and molecular docking analysis. The ligand induced quenching of intrinsic tryptophenyl fluorescence of the proteins exhibits intriguing characteristics on Stern–Volmer analysis (an upward curvature instead of conforming to a linear regression). Thus, an extensive time-resolved fluorimetric analysis of the quenching process has been carried out in conjugation with temperature-dependent fluorescence quenching studies to divulge the actual quenching mechanism. Using both of these steady state and time resolved fluorescence studies, static and dynamic quenching constants for both the proteins have been calculated appropriately. The thermodynamic parameters, ΔH, ΔS and ΔG for the binding phenomenon as evaluated on the basis of van't Hoff relationship expose the predominance of van der Waal or hydrogen bonding interaction underlying the binding process. The ligand induced modification of native protein conformations have been unmasked using synchronous fluorescence spectra and circular dichroism spectroscopy. Quantum mechanical calculations involving density functional theory (DFT) and molecular docking analysis have been carried out to emphasize the probable binding location of the ligand in protein backbone. Thus this work demonstrates a total assessment of Schiff base-protein interaction.

1. Introduction

Schiff bases are condensation product of primary amines and carbonyl compounds and they are first discovered by Schiff [1] in 1864. The presence of azomethine group makes them biologically important as the presence of lone pair of electrons in a sp^2 hybridised orbital of nitrogen atom have considerable chemical and biological importance [2]. Due to synthetic flexibility, excellent chelating ability and easiness in preparation they are widely used in biology as well as in industries. These imine complexes have wide range application in medicinal industry because of their antibacterial, antifungal, antitumor and antiviral activity [3–5]. So, now a day the interaction of Schiff bases with different biomacromolecules put forth escalating interests to the recent researchers [6–9].

In modern era coumarins have attracted intense interest due to their pharmacological properties. They are widely used in everyday life such as pharmaceutical, cosmetic, perfume and nutrition. They are also very important in drug delivery due to presence of anticancer and antitumor activity. The high potency and low toxicity of the coumarin derivatives make them promising alternatives in conventional therapeutic drugs [10–12].

Serum albumins are the major soluble protein constituent in the circulatory system contributing significant physiological functions as a carrier [13]. Albumins, the principle biomacromolecules in blood plasma (about 52%–60%) are responsible for disposition and transportation of various exogenous as well as endogenous ligands [14]. They maintain blood pressure due to proper distribution of body fluids between body tissues and intra vascular compartments [15,16]. Albumins like bovine serum albumin and human serum albumin can bind a wide variety of compounds like metabolites, drugs etc. due to flexibility in their shape and availability of hydrophobic jackets in their network [17]. BSA is homologous globular protein consisting of 583 amino acid residues and composed of two tryptophan moieties (Trp-134 and Trp-213) [18]. HSA is 76%

* Corresponding author.
E-mail address: ambikeshju@gmail.com (A. Mahapatra).

https://doi.org/10.1016/j.molstruc.2018.04.089
0022-2860/

homologous to BSA and composed of 585 amino acids containing only one tryptophan moiety located Trp-213 [19]. The secondary structure of albumins are composed of almost 67% helix content consisting six turns and 17 disulphide bridges which bring structural flexibility of them [20]. The tertiary structure of proteins are composed of three domains I, II, III with two sub domains A and B. In case of BSA the Trp-134 presents in domain I (sub-domain IB) and exposed towards the surface of the protein molecule, whereas Trp-213 is located in a hydrophobic cavity of the protein in domain II (sub-domain IIA) [21]. In case of HSA Trp-213 is located in sub domain IIA [22].

In our present work we intend to study the binding interaction between a synthesized Schiff base (shown in Scheme 1) consisting coumarin amine and pyrrole-2-carboxaldehyde and the biomacromolecules BSA and HSA. Binding of the ligand with biomolecules may induce considerable changes in the conformation of proteins. The interactive phenomena between the proteins and ligand have been illustrated by using different spectroscopic techniques such as steady state and time resolved fluorescence and circular dichroism measurements. Moreover, molecular docking studies have been executed simultaneously to get a better insight to the binding phenomena.

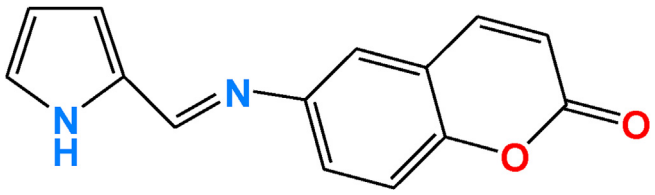
2. Materials and methods

2.1. Materials

Pyrrole-2-carboxaldehyde, bovine serum albumin (BSA), human serum albumin (HSA) have been purchased from Sigma Aldrich (USA) and used as received. All the sample solutions have been prepared in 10 mM *tris*- HCl/NaCl buffer (pH 7.4). The buffer solution has been prepared by dissolving tris base (Merck, India) in deionized ultrapure water from Millipore synergy system (Merck, India). Concentration of buffered protein solutions have been determined by measuring the absorbance value at λ_{max} of 280 nm using their respective molar absorption coefficient (ε) as 43,824 M^{-1} cm^{-1} for BSA and 42,864 M^{-1} cm^{-1} for HSA [22,23]. The Schiff base ligand solution has been prepared in 1:9 (V/V) ethanol-water mixtures.

2.1.1. Synthesis of the ligand

6-amino-coumarin has been prepared according to a literature method [24]. To 6-amino-coumarin (0.161 g, 1 mmol) in methanol (40 mL), pyrrole-2-carbaldehyde (0.095 g, 1 mmol) was added and refluxed for 2 h. A brownish yellow precipitate appeared on slow evaporation in air, filtered and washed by cold methanol to isolate the compound. Then the dried mass has been recrystallized from hot methanol solution in yield 0.167 g (70%). Microanalytical data: $C_{14}H_{10}N_2O_2$; Calcd. (Found): C, 70.58 (70.56); H, 4.23 (4.24); N, 11.76 (11.77) %. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 8.27 (s, imine-H), 7.71 (d, 1H, J = 9.54 Hz), 7.40 (d, 1H, J = 2.07 Hz), 7.37 (d, 1H, J = 2.04 Hz), 7.31 (d, 1H, J = 8.73 Hz), 7.27 (m, 1H), 7.0 (s, 1H), 6.76 (d, 1H, J = 3.27), 6.46 (d, 1H, J = 9.53 Hz), 6.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 160.803, 151.871, 150.165, 148.251, 143.324, 130.530, 124.929, 123.507, 119.280, 119.010, 117.669, 117.669, 117.282, 117.118, 110.828. ESI-MS (M^+ + H), 239.0189. The Mass spectra and NMR (^{1}H-NMR and ^{13}C NMR) have been shown in Fig. S1 (a), (b) and (c) respectively. (S stands for supporting information).



Scheme 1. Molecular structure of synthesized Schiff base ligand.

2.2. Methods

2.2.1. Experimental

2.2.1.1. Absorption spectroscopy. The absorption spectral studies have been performed using UV–Vis Spectrophotometer (Shimadzu, UV-1800) equipped with a Peltier temperature controller, TCC-240A due to sustain a constant temperature of 298 K (±0.1 K). For the spectral analysis a matched pair of quartz cuvettes from Hellma of 1 cm path length has been used.

2.2.1.2. Steady state fluorescence spectroscopy. The steady state fluorometric measurements have been carried out employing Shimadzu RF-5301PC spectrofluorimeter (Kyoto, Japan). The fluorescence spectra of buffered protein solution have been recorded in the wavelength region of 300–450 nm using excitation/emission slit width of 3/5 nm. In case of fluorometric titration protein solution has been maintained at 3.0 μM. The entire experiment has been carried out within 2% dilution of the buffered protein solution.

2.2.1.3. Time resolved fluorometric analysis. To explore conformational dynamics of the proteins time-resolved fluorescence measurements have been done carefully which is very much sensitive to excited state interactions [25]. A nanosecond diode laser (IBH nanoled 07) at 300 nm has been used as excitation source with TBX photon detector and signals have been recorded at magic angle at 54.7°. The number of counts gathered in the channel of maximum intensity has been fixed at 2500. The data have been analyzed using a nonlinear least-squares iterative method utilizing the Fluorescence Analysis Software IBH DAS-6. The quality of fits has been judged from (0.9–1.2) data [26]. The following eq. (1a) [26] has been used to explore the experimental time-resolved fluorescence decays where, *F(t)* is the fluorescence intensity at time t and α_i is the pre-exponential factor representing the fractional contribution to the time resolved decay of the *i*th component with a lifetime τ_i. For multiexponential decays, the average lifetime has been calculated from the following equation where (τ_1, τ_2 and τ_3) are decay times and (α_1, α_2 and α_3) are the normalized pre-exponential factors.

$$F(t) = \sum_i a_i \exp\left(\frac{-t}{\tau_i}\right) \quad (1a)$$

$$\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3 = \langle\tau\rangle \quad (1b)$$

2.2.1.4. Synchronous fluorescence spectroscopy. The synchronous fluorescence spectra (SFS) have been obtained by simultaneously scanning the excitation and emission monochromators. When the Δλ value between excitation and emission wavelength is fixed at a certain value, the SFS can afford characteristic information of the local environment near some specific chromophore. Any alteration in SFS reveals consequent change of micro-environment of the concerned chromophore near the binding site. We have reproduced our results by repeating the SFS experiments for three times.

2.2.1.5. Circular dichroism studies. Circular Dichroism (CD) spectra have been recorded using PC-driven JASCO spectropolarimeter (Japan) equipped with a peltier temperature controller and thermal programmer (PED-425L/15) with a rectangular quartz cuvette of