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Spectroscopic and docking studies on the interaction between pyrrolidinium based ionic liquid and bovine serum albumin

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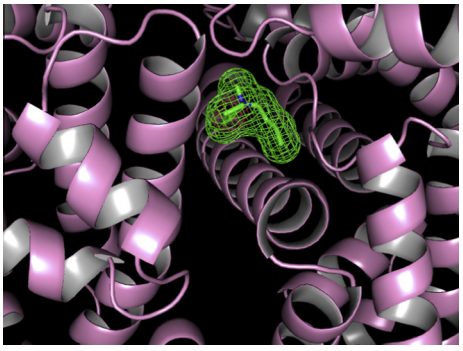
HIGHLIGHTS

- BMOP quenches the fluorescence intensity of BSA and changes its conformation.
- Quenching follows the dynamic mechanism.
- BMOP are first used to investigate their effects on BSA.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of synthesized ionic liquid, 1-butyl-1-methyl-2-oxopyrrolidinium bromide (BMOP) and bovine serum albumin (BSA) was investigated using UV–Vis, FT-IR, steady state and time resolved fluorescence measurements and docking studies. Steady state spectra revealed that BMOP strongly quenched the intrinsic fluorescence of BSA through dynamic quenching mechanism. The corresponding thermodynamic parameters; Gibbs free energy change (ΔG), entropy change (ΔS) and enthalpy change (ΔH) showed that the binding process was spontaneous and entropy driven. It is also indicated that hydrophobic forces play a key role in the binding of BMOP to BSA. The synchronous fluorescence spectroscopy reveals that the conformation of BSA changed in the presence of BMOP. The shift in amide I band of FT-IR spectrum of BSA suggested unfolding of the protein secondary structure upon the addition of BMOP. In addition, the molecular modeling study of BSA–BMOP system shows that BMOP binds with BSA at the interface between two sub domains IIA and IIIA, which is located just above the entrance of the binding pocket of IIA through hydrophobic and hydrogen bond interactions in which hydrophobic interaction are dominated.

Introduction

Ionic liquids (ILs) are solvents that are often liquid at room temperature and composed of organic cation and appropriate anion. Their fascinating properties like negligible vapour pressure, non-flammability, high thermal stability, wide liquid range and large electrochemical window draws key interest of chemists [1,2]. ILs behaves as green surface active agents and can be superior over conventional surfactants in various chemical and biological applications. Till now no reliable rules exist so far that predicts the properties of any novel IL, even though some guidelines are

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zbrooming up, and quantitative structure–property relationships are being explored [3].

Currently, the research interest towards the biological application of ILs, grown up so rapidly because they provided exceptionally interesting solvent media for various biological processes viz. biocatalytic reaction [4], biosensor [5], protein separation, extraction and biopreservation [6,7]. The unusual solvating property and high thermal stability make them a novel protein stabilizing solvent. The spectroscopic analysis showed that [bmpy][NTf_2] provide high thermal stability to the monellin [8]. Similarly, higher thermal stability of α-chymotrypsin, was observed in ammonium based ILs [9]. Beside these unique properties they also have prominent role in miscellaneous industrial applications, where high surface areas, modification of the inter-facial activity or stability of colloidal systems are required. Recently, the temperature dependent self-assembly of amphiphilic drug in [C_8mim][Cl] have been reported [10]. Due to these interesting and useful applications of ILs herein, we examine the effect of synthesized pyrrolidinium based IL on BSA. The molecular interactions between them were studied systematically by fluorescence, time resolved fluorescence, UV–Vis, and FTIR spectroscopic techniques. In addition molecular docking was used to get better understanding of the interaction of BMOP with BSA.

The most abundant proteins in plasma are serum albumins [11]. Serum albumin functions as the major transporter of non-esterified fatty acids and different drugs and metabolites to different tissues [12]. Serum albumin is synthesized in liver and exported as non-glycosylated protein. Bovine serum albumin (BSA) is one of most studied protein and used as a model in protein–ligand binding studies because of its abundance, low cost, easy purification [13,14], as well as of its structural homology with human serum albumin [15]. BSA is a globular protein of 582 amino acid residues [16]. The secondary structure of BSA consists mostly of α-helix, loops and disulphide bridges which unite to form a 3D heart shaped structure [17]. BSA consist of homologous domains I, II and III. Each domains are further subdivided into two subdomains A and B [18]. Among them, IIA and IIIA subdomains being hydrophobic in nature and serve as principle binding sites. BSA has two tryptophan residues, Trp-212 located in subdomain IIA, and Trp-134 in the subdomain IA. Tyrosine residues (Tyr-263) are also present in the subdomains IIA [19].

The spectroscopic techniques, such as UV–Vis spectroscopy [20], fluorescence spectroscopy [21], and Fourier transform infrared spectroscopy [22], are useful techniques to investigate the interaction between ligands and protein due to their non-destructive measurement of substances under physiological conditions with high sensitivity and rapidity [23]. To the best of our knowledge, the binding profile of BMOP with BSA has never been investigated. The aim of this study was to analyse the fluorescence quenching mechanism, binding properties and conformational changes induced by BMOP in BSA.

Experimental

Materials

BSA (96% purity) was purchased from Sigma Aldrich (Batch No. A2153) and was used without further purification. The BMOP was synthesized in the laboratory. All BSA solutions were prepared in phosphate buffer at pH 7.4. Doubly distilled water was used throughout the experiments. The stock solution of BSA was prepared in phosphate buffer (pH 7.4) and its concentration was determined from absorption spectroscopy. The stock concentration (20×10^{-6} M) was calculated by dividing absorbance at 280 nm by the molar extinction coefficients of the BSA ε_{280} = 43,890 M^{-1} cm^{-1} [24]. The working concentration of BSA (5×10^{6} M) was prepared by diluting the stock solution with phosphate buffer.

Synthesis of BMOP

The BMOP as shown in Fig. 1 was synthesized [25,26], by using the following method: in a round bottom flask (250 ml), N-Methyl pyrrolidone (10 mmol) in acetronitrile (50 ml) was taken and bromobutane (10 mmol) in acetronitrile (50 ml) was added to the above solution drop wise. After that the reaction mixture was refluxed for 3 h. Further, it was cooled at room temperature and solvent was evaporated under reduced pressure. It was further distilled under reduced pressure to afford the pure IL and then dried well and degassed at 60 °C for three day. It was well characterized by ^{1}H NMR, ^{13}C NMR, FTIR techniques and the purity of IL was checked by HPLC technique. FTIR (ν = cm^{-1} 2935.05, 1705.87, 1400.25, 1365.89 and 865.49; NMR (δ, $CdCl_3$ ^{1}H NMR aliphatic proton at C_3(2.49, 2H); aliphatic proton at C_4(2.17, 2H); aliphatic proton at C_5(3.19, 2H); aliphatic proton at $C_{1'}$(3.05, 2H); aliphatic proton at $C_{2'}$(1.98, 2H); aliphatic proton at $C_{3'}$(1.56, 2H); aliphatic proton at $C_{4'}$(1.11, 3H); aliphatic proton at $C_{1'}$(2.95, 3H);^{13}C NMR aliphatic carbon at 3, 4, 5, 1′, 2′, 3′, 4′ and 1″ are 35.6, 19.8, 50.2, 54.3, 30.7, 20.9, 15.6, 40.2 respectively and carbonyl carbon at 2 position is 184.9; HPLC purity of the synthesized IL is 98.1%.

Procedures

UV–Vis spectroscopy

The UV–Vis spectra were measured using the Analytik Jena Specord-250 spectrophotometer (USA) using a 1.0 cm cell. The absorption spectra of BSA were recorded at different BMOP concentration at λ_{280}.

Fluorescence spectroscopy

The fluorescence spectra were recorded on a Cary Eclipse spectrofluorimeter (Varian, USA) equipped with a 150W xenon lamp in a 1 cm quartz cell and a thermostat water bath. The emission was measured from 290 nm to 450 nm with an excitation wavelength of 280 nm. Synchronous fluorescence spectra were acquired by the same spectroflourimeter. The difference between excitation and emission wavelength was kept constant ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$). The $\Delta\lambda$ at 15 nm or 60 nm showed by synchronous fluorescence spectra gave characteristic information of Tyrosine (Tyr) residues or Tryptophan (Trp) residues, respectively, with the excitation and emission slit widths at 5 nm. Temperature was controlled during experiments using constant-temperature cell holder connected to constant-temperature water circulator (Varian, USA).

Time-resolved fluorescence spectroscopy

The time-resolved fluorescence measurements were performed at room temperature, using a single-photon counting spectrometer equipped with pulsed nanosecond LED excitation heads at 280 nm (Horiba, Jobin Yvon, IBH Ltd, Glasgow, UK). The fluorescence lifetime data were measured to 10,000 counts in the peak, unless otherwise indicated. The instrumental response function was

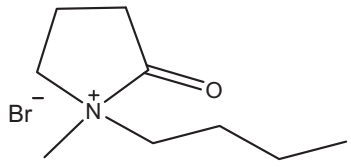

Fig. 1. Schematic structure of the IL (BMOP) used in this work.