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Deciphering the interaction of a model transport protein with a prototypical imidazolium room temperature ionic liquid: Effect on the conformation and activity of the protein

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ABSTRACT

The present contribution reports the interaction of a prototypical surface-active room temperature ionic liquid (RTIL) *viz.*, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) with a globular transport protein bovine serum albumin (BSA). The BSA–RTIL binding isotherm constructed from conductometric measurements is found to be well reproduced from fluorescence spectroscopy and thus revealing the various interaction zones as a function of the RTIL concentration. The present work delivers particular emphasis to delineate the denaturing action of RTIL on the native protein and in complementarity the effect of RTIL binding on functionality of BSA is explored in terms of esterase-like activity of BSA. The intrinsic time-resolved fluorescence decay and rotational-relaxation dynamics of the protein suggests swelling of the protein rather than aggregation during RTIL-induced denaturation. The result of molecular modeling based on blind docking simulation is found to abet the inferences drawn from experimental results reasonably well. The molecular modeling technique reveals the favorable binding location of the RTIL to be in the hydrophobic domain IIIA (drug site 2) of BSA. The thermodynamic parameters evaluated for the RTIL–BSA binding phenomenon also identifies the pivotal role of hydrophobic force in the interaction.

1. Introduction

Research in the field of protein–surfactant interaction has remained a prospective avenue of investigation for years. This is, however, aptly corroborated by the enormous technical applications of protein–surfactant interaction in various fields e.g., pharmaceuticals, cosmetics, paints and a vista of biochemical processes [1–3]. Further, the application of surfactants in fundamental research to seek acumen into native structure of proteins has been well recognized in which surfactants are used to solubilize proteins and as denaturing or renaturing agents [1,2,4]. Particularly noteworthy in such contexts is the enormous applicability of globular proteins [5]. One of the most widely studied globular proteins for a score of industrial as well as academic interests is bovine serum albumin (BSA) which is a mammalian albumin composed of a single polypeptide chain of 583 amino acid residues, and is structurally analogous to Human Serum Albumin (HSA) [5,6]. The interaction of this globular protein (HSA/BSA) with various surfactants has been extensively addressed in the literature [7–9].

Recently, room temperature ionic liquids (RTILs) are garnering burgeoning interest within scientific community given their special physicochemical properties [10–12] and the scope to tailor them simply through judicious choice of the cationic/anionic counterparts. RTILs having long alkyl chains with their intrinsic amphiphilic character are showing thriving promise as better surrogates of conventional surfactants in a host of industrial, chemical or biochemical applications. This is often argued to be incumbent on the improved surface activity of RTILs compared to analogous conventional surfactants [13]. Naturally, the applications of RTILs in biophysical research is also marking their rapidly growing signature in which of particular interest is the study of interaction of ILs with proteins and nucleic acids [14–16]. Usually, in protein assays, ILs have been employed as neat solvents containing little or no water [17]. Further, the application of ILs as co-solvent for water in biphasic systems has also been tested in which the two aqueous phase systems composed of ILs and buffer are applied for protein separation [18]. In this context, the effective participation of ILs in various biological processes, apart from simply providing a novel

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and efficient reaction medium, has too formed a particularly critical aspect to probe into. The study of interactions of biomolecules with ILs in aqueous medium employing physiological conditions is thus capturing intense attention rather than with neat ILs [14–16].

In spite of the recent thrust in research in the field, interactions between ILs and proteins in aqueous medium remain far from being completely understood and hence demand meticulous exploration of the topic from varied viewpoints. With the topic of protein–RTIL interaction under consideration, apart from simply elucidating the interaction between the concerned parties, it becomes critically important to accentuate issues like effect of RTILs on conformation, stabilization, dynamics and functionality of the protein. To this end, our efforts are delivered to study the interaction between an imidazolium-based archetypal RTIL *viz.*, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) and a model transport protein BSA in the present program. As a case study of investigating the interaction between [BMIM][BF_4] and BSA the present work is arranged with emphasis on the following issues: (i) study of interaction between RTIL and protein using fluorescence spectroscopy; (ii) elucidating the thermodynamics of interaction to delineate the actuating binding force; (iii) effect of interaction with the RTIL on rotational relaxation dynamics of the protein; (iv) effect of RTIL-binding on the conformation of the native protein; (v) effect of RTIL-binding on the functionality (esterase activity) of the globular transport protein (BSA) in physiological conditions; (vi) blind docking simulation to unveil the favorable binding locus of the RTIL within the microheterogeneous protein environment.

2. Experimental

2.1. Materials

Commercially available RTIL 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) was obtained from Sigma Chemical Co., USA and used after proper purification by active charcoal method as described elsewhere [19]. BSA from Sigma Chemical Co., USA was used as received. Tris buffer was purchased from SRL, India, and 0.01 M Tris–HCl buffer of pH 7.4 was prepared in triply distilled deionized water from a Milli-Q water purification system (Millipore). The solvent appeared visually transparent and the purity was also tested by running the fluorescence spectra in the studied wavelength range. Hydrochloric acid was used as received from E-Merck. *p*-Nitrophenylactetate (PNPA) of Analytical Grade was obtained from SRL, India and used as received. A constant BSA concentration of ca. 5×10^{-6} M was maintained throughout all the experiments if not otherwise mentioned.

2.2. Instrumentation and methods

2.2.1. Conductometric measurement

Conductivity measurements were performed on Systronics 304 Digital conductivity meter (employing platinum electrode) using triply distilled deionized water from a Milli-Q water purification system (Millipore).

2.2.2. Steady-state spectral measurements

The absorption and emission spectra were recorded on a Hitachi UV–Vis U-3501 spectrophotometer and Perkin–Elmer LS55 fluorimeter, respectively, with appropriate corrections for instrumental response. The recorded spectra were appropriately background subtracted with IL solutions in aqueous buffer in order to eliminate spectral interferences from the RTIL itself. Experiments were carried out at an ambient temperature of 25 °C, unless otherwise specified. Only freshly prepared solutions were used for spectroscopic measurements. For temperature variation measurements the temperature was kept constant at a given value by recycling water flow accurate up to ±1 °C.

2.2.3. Time-resolved fluorescence decay measurements

Fluorescence lifetimes were obtained by the method of Time Correlated Single-Photon counting (TCSPC) on FluoroCube-01-NL spectrometer (Horiba Jobin Yvon) using a light source of nano-LED at 291 nm and the signals were collected at the magic angle of 54.7° to eliminate any considerable contribution from fluorescence anisotropy decay [20]. The decays were deconvoluted on DAS-6 decay analysis software. The acceptability of the fits was judged by χ^2 criteria. Mean (average) fluorescence lifetimes ($\langle\tau_f\rangle$) were calculated using the following equation [20]:

$$\langle\tau_f\rangle = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} \quad (1)$$

in which α_i is the pre-exponential factor corresponding to the *i*th decay time constant, τ_i.

For time-resolved fluorescence anisotropy decay measurements, the polarized fluorescence decays for the parallel [I_{VV}] and perpendicular [I_{VH}] emission polarizations with respect to the vertical excitation polarization were first collected at the emission maxima of the probe. The anisotropy decay function $r(t)$ was constructed from these I_{VV} and I_{VH} decays using the following equation [20]:

$$r(t) = \frac{(I_{VV} - G \cdot I_{VH})}{I_{VV} + 2G \cdot I_{VH}} \quad (2)$$

in which G is the correction factor for the detector sensitivity of the instrument.

2.2.4. Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded on a JASCO J-815 spectropolarimeter using a cylindrical cuvette of 0.1 cm path-length at 25 °C. The reported CD profiles are an average of four successive scans obtained at 20 nm/min scan rate with appropriately corrected baseline. The concentration of BSA and the RTIL during CD measurements are mentioned in the relevant discussion.

2.2.5. Dynamic light scattering

Dynamic light scattering (DLS) measurements were carried out on a Malvern Nano-ZS instrument employing a 4 mW He–Ne laser (λ = 632.8 nm) and equipped with a thermostatted sample chamber. The sample was poured into a DTS0112 low volume disposal sizing cuvette of 1.5 mL (path-length 1 cm). The operating procedure was programmed by the DTS software in a fashion that there were average of 25 runs, each run being averaged for 15 s, and then a particular hydrodynamic diameter and a size distribution was evaluated.

2.2.6. Esterase activity assay

The effect of the RTIL on the esterase activity of BSA was assayed with the synthetic substrate *p*-nitrophenyl acetate (PNPA) by following the formation of *p*-nitrophenol at 37 °C [21].

2.3. Molecular modeling: Docking study

The native structure of BSA was taken from the Protein Data Bank having PDB ID: 3V03 [22]. Docking studies were performed with AutoDock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein. For docking of the RTIL with BSA, the required file (corresponding to the three-dimensional structure of the cationic $[BMIM]^+$) for the ligand ($[BMIM]^+$) was created through the combined use of Gaussian 03W [23] and