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Dynamics of cytochrome *c* in surface active ionic liquid: A study of preferential interactions towards denaturation

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ABSTRACT

Surface active ionic liquid (SAIL) 1-butyl-3-methylimidazolium octyl sulfate ($[C_4mim][C_8OSO_3]$) having octyl tail on anionic moiety have been very less investigated with proteins. Herein, we present a study of the structural change of horse heart cytochrome *c* (h-cyt *c*) in aqueous solutions of SAIL ($[C_4mim][C_8OSO_3]$) by optical spectroscopy and molecular docking methods. Time-resolved fluorescence, UV–vis and circular dichroism (CD) spectra indicated that the addition of up to 25 mM of $[C_4mim][C_8OSO_3]$ induces structural changes of h-cyt *c* resulting from disruption of tertiary structure and after 25 mM of $[C_4mim][C_8OSO_3]$, h-cyt *c* denatures completely and loses its tertiary structure. Thermodynamic parameters for denaturation of h-cyt *c* by $[C_4mim][C_8OSO_3]$ were also obtained having ΔG°_D and *m*-value of the unfolded state. The values were found to be lower when compared to the reported for urea denatured h-cyt *c*. The midpoint concentration of unfolding of h-cyt *c* by $[C_4mim][C_8OSO_3]$ was found ~25 mM. The results showed that the presence of alkyl chain on the anionic moiety destabilizes the protein. The docking results confirm the preferential interactions of ions of SAIL to residues of h-cyt *c* and suggest the dominant role of anion $[C_8OSO_3]^-$ by the electrostatic interaction that drives local destabilization of the h-cyt *c*.

1. Introduction

A quest for liquids that possess such fascinating properties of molecular liquids and provide a unique possibility to modulate protein stability are ionic liquids (ILs), a reason for this burst of interest include their wide tunable properties [1–8]. Ionic liquids have found applications in many fields of science and technology such as biocatalysis, nanoparticle synthesis, polymer, surfactants, dyes, cryopreservation, extraction, biodiesel production, Li-ion batteries, heterocyclic synthesis etc. [9–18]. ILs have been reported to maintain the catalytic activity of enzymes, like lipases where enzyme is in a dispersed state and acts as heterogeneous catalyst. ILs have served the purpose of protein stabilization quite well, however contrary effects have been witnessed with some of them [2,19]. The interaction pattern of protein and ILs is not the same in neat conditions compared to that in dilute aqueous medium [4,8]. ILs act as tunable additives, therefore their refolding effect can be tailored according to the target protein. ILs with short alkyl chain has been reported as an excellent refolding additive and those with long alkyl chain shows concentration dependent effect [20–22] ILs were found to increase stability, solubility and activity of the proteins and extensively used for extraction, purification, separation, liquid formulation of protein based therapeutics [23,24]. ILs solubilize the protein and increase their activity and the stability by preventing their irreversible aggregation [25]. Solubility of many proteins including metalloproteins is observed in aqueous solution of ILs [26,27].

The altered environment around proteins affect their stability and hence their function. Significant advances in the ability of ILs to impart high thermal stability to protein and ultimately conserve their structure have been made [1,3,4,6,28–30]. Several studies relating to protein stability by ILs on timescale have also been reported [5,7]. ILs display their properties according to the type of cation, anion and length of alkyl chain attached to the head group of ILs. Certain ILs having short chain are known to be good refolding additives and those with long chain shows concentration dependent effect [20–22].

ILs enhance the pharmaceutical application of the protein by modifying the solubility of protein and peptides antigen. Recently, ILs were used for transcutaneous delivery of hydrophilic protein (ovalbumin) [31], transdermal and topical drug delivery of sparingly soluble drugs [32]. Williams et al. demonstrated IL based oral drug delivery of poor water soluble drugs, danazol and itraconazole. They found that drug contain self-emulsifying IL formulation provide 4.3-fold higher exposure than the crystalline drug and prolonged exposure compared with a lipid formulation [33]. ILs can also use as active pharmaceutical ingredient to increase the biological role of drugs [34,35]. For example, the anti-inflammatory drug sodium ibuprofen can be paired with didecyldimethylammonium bromide IL having anti-inflammatory and anti-bacterial properties to produce an IL-active pharmaceutical

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ingredient, that contain dual biological function [34]. Hence, study of protein-IL interaction is very important for their chemical, biochemical and pharmaceutical applications.

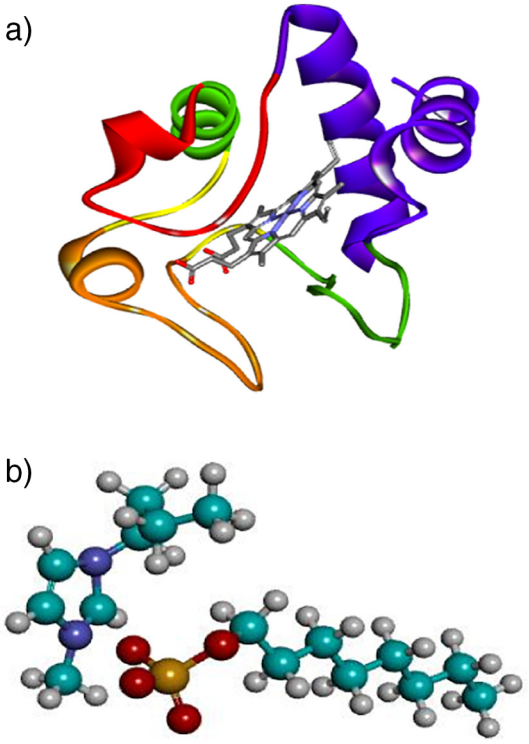
Various studies on ILs with different proteins have been reported on the effect of alkyl chain attached to the cationic moiety [3,29,31–33]. On contrary, very few studies are known to have reported the behavior of proteins with ILs having alkyl chain on their anionic moiety [34]. The properties of ILs differ due to presence of the alkyl chain on the anionic moiety. The anionic tail imparts unique ability to interact in contrast to the cationic tail with different proteins. Very recently Reddy et al. studied the binding and dynamics of imidazole alkyl sulfate based ILs, with two distinct types of proteins, HSA and collagen by using NMR spectroscopy [20–22]. The objective was the selective binding of the anionic and cationic part of the IL with the protein residues. They observed binding affinity of IL to proteins increases with the length of alkyl chain on anionic part of IL, this signifies towards the substantial role of hydrophobic interactions and no structural perturbation in these ILs were observed [35]. In another study, the effect of surface active ILs, 1-dodecyl-3-methylimidazolium chloride [C_{12}mim][Cl] and 1-hexyl-3-methylimidazolium dodecylsulfate [C_6mim][SDS], have been studied on the metalloprotein, hemoglobin. The study reveals that at lower concentration [C_6mim][SDS] monomers form stronger complex with the hemoglobin as compared to the [C_{12}mim][Cl] IL and induces the aggregation of the protein [36]. The studies shows that in addition of other factors (type of IL, length of alkyl chain, concentration of IL, and nature of protein) type of interactions involved in binding of IL with protein also play important role in stabilization and destabilization of protein. Study reported by our group shows remarkable ability of ILs 1-methyl-3-octyl imidazolium chloride [C_8mim][Cl] and 1-decyl-3-methylimidazolium chloride [C_{10}mim][Cl] to sustain the activity and stability of horse cytochrome *c* (h-cyt *c*) for six months at room temperature [32]. Our recent article using biophysical approach show the comparison of the refolding ability of the ILs ([C_8mim][Cl] and [C_{10}mim][Cl]) on denatured h-cyt *c* by urea, GdnHCl and pH 13.0 [37]. The study shows the greater renaturing ability of long chain IL to refold the pH denatured h-cyt *c* with static quenching mechanism and stabilization of the MG state.

The interaction of biomolecules with ILs having long chain cation have been extensively studied whereas the interaction between biomolecules and ILs having long chain anion has received less consideration so far. Hence, understanding the mechanism of their bimolecular interactions on structural basis causing stabilization/destabilization is fundamental to our accumulating knowledge. The h-cyt *c* is the most thoroughly studied model metalloprotein, commonly used for folding/unfolding experiments [38]. It contains heme prosthetic group which has been exploited as probe to monitor structural changes during folding. The h-cyt *c* possesses separable nature of cooperative folding/unfolding units (foldons) [39]. Herein, we have studied the interaction of 1-butyl-3-methylimidazolium octyl sulfate, [C_4mim][C_8OSO_3] (IL having long chain on its anionic moiety) with the model metalloprotein, h-cyt *c* to probe its stability by using various spectroscopic techniques.

2. Experimental

2.1. Materials

Equine heart cytochrome *c* (h-cyt *c*, type IV), sodium monobasic dihydrate, sodium dibasic dihydrate, 1-butyl-3-methylimidazolium octylsulfate ([C_4mim][C_8OSO_3]) and sodium chloride were purchased from Sigma Aldrich. All chemicals and reagents used were of analytical grade. The structure of h-cyt *c* and [C_4mim][C_8OSO_3] is depicted in Scheme 1(a & b). Millipore water was used throughout the experiments. The critical micelle concentration (cmc) of [C_4mim][C_8OSO_3] SAIL was found 34 mM which was calculated from surface tension experiments using DeltaPi-4 Langmuir microtensiometer (Kibron, Helsinki, Finland) based on the Wilhelmy method (data not shown).



Scheme 1. (a) Structure of h-cyt *c* showing helices and loops (PDB ID: 1HRC). The figure depicts the two axial ligands His18 and Met80 covalently linked to heme, its and the single Trp residue at position. (b) Structure of 1-butyl-3-methylimidazolium octylsulfate [C_4mim][C_8OSO_3].

2.2. Preparation of stock solution of h-cyt c

The h-cyt *c* were oxidized to ferric form by adding 0.1% potassium ferricyanide to the solution of h-cyt *c* and dialyzed against 0.1 M NaCl, at pH 7.0 [40]. The oxidized h-cyt *c* solutions were filtered by using 0.22 μm Millipore filter. Concentration of the dialyzed solution of h-cyt *c* were determined experimentally by using molar absorbance coefficient (ε) values of $1.06 \times 10^5\ M^{-1}\ cm^{-1}$ at 410 nm [41]. All the measurements were carried in the degassed sodium phosphate buffer at pH 7.0.

2.3. Addition of [C_4mim][C_8OSO_3]

The aqueous stock solution of [C_4mim][C_8OSO_3] was prepared. To get the final concentration range from 10 to 40 mM, we added different amounts of [C_4mim][C_8OSO_3] from stock solution into the glass vials containing h-cyt *c* for all experimental techniques else mentioned at 25 °C. The samples were incubated in the dark for a period of four hours before each experiment.

2.4. Circular dichroism measurements

CD spectra of h-cyt *c* were carried out through Jasco spectropolarimeter (J-1500), equipped with Peltier type temperature controller (PTC-100). CD spectra of native h-cyt *c* were taken in the absence and presence of [C_4mim][C_8OSO_3] at 25 °C and pH 7.0. For near and Soret spectral measurement cuvette having 1 cm path length were utilized. The raw CD data were converted into $[\theta]_\lambda$, the mean residue ellipticity (deg cm^2 $dmol^{-1}$) at a given wavelength λ using the relation [42]:

$$[\theta]_\lambda = \theta_\lambda Mo/10lc \quad (1)$$

where θ_λ is the observed ellipticity (millidegrees) at wavelength λ, M_o is the mean residue weight of the protein, *c* is the protein concentration (mg/cm^3), and *l* is the path length (centimetres).