Process Biochemistry 49 (2014) 2158–2169

Contents lists available at ScienceDirect

Process Biochemistry

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

A comparative study of myoglobin stability in the presence of Hofmeister anions of ionic liquids and ionic salts

Awanish Kumar, Pannuru Venkatesu*

Department of Chemistry, University of Delhi, Delhi 110 007, India

ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form 5 August 2014
Accepted 5 September 2014
Available online 28 September 2014

Keywords:
Myoglobin (Mb)
Ionic salts (I_s)
Ionic liquids (ILs)
Hofmeister series
Transition temperature (T_m)

ABSTRACT

To reveal the impact of ionic liquids (ILs) on the stability of proteins, a series of ILs possessing same 1-butyl-3-methylimidazolium cation $[Bmim]^+$ with a set of Hofmeister anions such as SCN^-, HSO_4^-, Cl^-, Br^-, CH_3COO^- and I^- were used and their effects on the myoglobin (Mb) structure and stability were studied. For the sake of comparison and also to explore the extent of the stabilization behavior of ILs toward Mb stability, we have chosen a set of ionic salts (I_s) of a fixed sodium cation (Na^+) with the same series of anions such as SCN^-, SO_4^{-2}, Cl^-, Br^-, CH_3COO^- and I^-. UV–vis, fluorescence and circular dichroism (CD) spectroscopic techniques were used in order to investigate the stability behavior of Mb in ionic species (I_s and ILs). The results reveal that both I_s and ILs had a negative influence on the stability of Mb. Apparently, the flexibility in the native structure of Mb gradually increases with the increase in the concentration of I_s and ILs at pH 7.0. Therefore, a sharp decrease in the transition temperature (T_m) of the native Mb is observed in the presence of I_s and ILs.

1. Introduction

Myoglobin (Mb) is an iron- and oxygen-binding protein found in the muscle tissue that generally takes part in cellular respiration as an oxygen reservoir by forming a coordinate bond between imidazole groups of distal histidine (His) residue and oxygen [1]. Interestingly, Mb is a complex protein, which includes a protein and a non-peptide portion (without disulfide linkage) [2]. Mb has often been used as a paradigm for protein folding and unfolding studies because: (1) Mb shows a well defined intermediate unfolded form which still retains some α-helical structure [3]; (2) because of its monomeric behavior in solution [2].

Moreover, Mb is a typical globular protein, which is closely folded and is composed of 8 α-helices (designated A through H from the N terminus side) that make up 85% of the protein sequence [4]. It is a water-soluble globular protein of 150 amino acids (AA_s) containing a heme (iron-containing porphyrin) prosthetic group with a molecular weight of 17 kDa (with heme). Its dimension is approximately $45 \times 35 \times 25$ Å [5]. Inspection of the Mb X-ray structural model reveals that in the native conformation, approximately 14 positively charged side chain amino acid (AA) groups of Arg and Lys residues are paired at the inter charge distances of 2.4–5.0 Å, with an equal number of negatively charged side chain carboxylic groups of (aspartic acid) Asp and (glutamine) Glu residues [6].

Heme, the non-peptide portion of the Mb is composed of two parts, an iron atom and porphyrin. Porphyry is made up of tetrapyrrole, linked together by a methyne bridge [7]. It is already reported by several research groups that the removal of the heme group of Mb destabilizes the native conformation of the protein [8,9]. The structure of Mb without the prosthetic group is provided in Fig. 1. Heme proteins such as Mb provides a better understanding of the efficient electron transfer coupling between a protein and an electrode that can help in understanding the energetic metabolisms in the biological systems that open large possibilities for biotechnological devices in various fields that includes biosensors [10]. In this context, a new field of research has been explored which deals with the study of the heme proteins in the presence of ionic liquids (ILs) [11–15].

In recent years, ILs are finding a broader scope within the biochemical sciences, including pharmaceuticals, enzymatic and bioanalytical applications [16]. The basic and unique physical properties of these ILs include extremely low vapor pressure, a wide liquid range, low flammability, high ionic conductivity, high thermal stability, high chemical stability and a wide electrochemical window [17]. Additionally, ILs can be tailored easily to maintain the desired properties such as to polarity, hydrophobicity and solvent miscibility behavior by the appropriate modification of the cations and anions [18]. Due to their exceptional physical properties, ILs are now considered as the most suitable co-solvents for

* Corresponding author. Tel.: +91 11 27666646 142; fax: +91 11 2766 6605.
E-mail addresses: venkatesup@hotmail.com, pvenkatesu@chemistry.du.ac.in, pannuruv@yahoo.com (P. Venkatesu).

http://dx.doi.org/10.1016/j.procbio.2014.09.014
1359-5113/

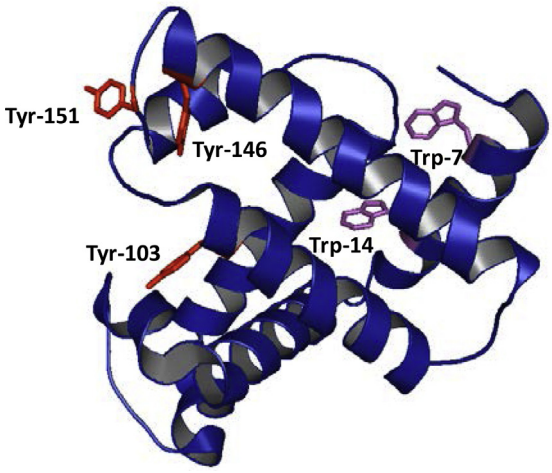

Fig. 1. Schematic diagram of the crystal structure of Mb, which was downloaded from the protein data bank (1 MBN) and processed with the PyMOL viewer software.

various biochemical processes [19,20]. The use of ionic liquids (ILs) as a co-solvent in aqueous medium have improved the biocatalysis reactions [21,22]. For the handling of protein in ILs, a detailed knowledge of the relationship between IL chemistry and protein stability [23–26] is needed. Alternatively, the presence of ILs has improved the properties such as the selectivity of a reaction or the stability of the enzyme during the entire enzymatic reactions [19].

Reports on the use of ILs suggest that only the hydrated IL maintains the metallo-protein structure with a small quantity of water that considerably improved the protein solubility and stability. In this respect, Du et al. [11] investigated the immobilized Mb onto SWNTs coated with ILs such as 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]) and 1-butyl-3-methylimidazolium hexafluorophosphate([Bmim][PF_6]), and observed that the encapsulated Mb retained its bioelectrocatalytic activity toward the reduction of oxygen and hydrogen peroxide. As an interesting application in electrocatalysis, the Pang group [12,13] designed new glassy carbon electrodes by entrapping heme proteins Mb in agarose hydrogel films, and observed high electrocatalytic activities of the Mb–agarose films in both [Bmim][PF_6] and [Bmim][BF_4] solutions containing a small amount of water. The native structures of heme proteins were retained in the agarose film as indicated by UV–vis and Fourier transform infrared (FTIR) spectroscopy. Moreover, the effect of hydrated IL has shown that for low levels of hydration of IL there is a large disturbance in the Mb structure. This is particularly observed in the case of short chained hydrophilic ILs [14]. In this context, Safavi and Farjami [14] studied the Mb-IL interaction by UV–vis spectroscopy and showed that Mb retains its native conformation in the presence of 0.2 M [Bmim][Cl]. With the gradual addition of [Bmim][Cl] up to 0.2 M, no significant change in the spectrum was observed. Additionally, they discovered that the bioactivity of the Mb was retained in the presence of 0.2 M [Bmim] [Cl]. This signifies that Mb is almost stable under these conditions. Sankaranarayanan et al. [15] through spectroscopic investigations predicted that with the change in the native conformation of the Mb in the presence of ILs, there is a large shift in the fluorescence wavelength and the protein transforms to complete β-sheet from its native helical conformation.

Moreover, ILs combined with imidazolium cations are highly stable and have deserved special attention in the field of protein stabilities [27]. The combinations of various anions with an imidazolium cation have shown to be both stabilizing as well as destabilizing for the protein structure [24]. In this context, a molecular dynamic simulation (MD) study of the enzyme serine protease cutinase in the presence of 1-butyl-3-methylimidazolium nitrate [Bmim] [NO_3] IL shows destabilization of the enzyme, suggesting that there is a strong interaction between [NO_3] anion with the enzyme donor groups [16]. In addition, Klahn et al. [28,29] concluded that Candida Antartica Lipase B (CALB) destabilized in imidazolium-based ILs through MD simulations. In contrast, The IL N-ethyl-N-methyl imidazolium chloride ([Emim][Cl]) has been described as being very efficient in promoting refolding of the recombinant plasminogen activator rPA [30]. In support, high refolding yields of rPA using 2 or 3 M of [Emim][Cl] was observed by Buchfink et al. [31]. Geng et al. [32] examined the interaction between 1-tetradecyl-3-methylimidazoluim bromide and bovine serum albumin (BSA) and proposed that the secondary structure of BSA was stabilized in the IL. Kaar research group showed that the stability of the proteins in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) and 1-ethyl-3-methylimidazolium ethyl sulfate ([Emim][$C_2H_5SO_4$]) can be improved via modifications that reduce the ratio of positive to negative surface charges on proteins [33,34].

However, the thermodynamic stability of the heme proteins in aqueous solutions of imidazolium-based ILs have not been fully quantified. All of the above discussions on the stability of proteins in ILs indicate that the stability of a protein is particularly based on the ions of the ILs of which it is composed. In this regard, the present literature survey literature reveals that the anions of the IL play a key role in the interactions of ILs with proteins [35–38]. Furthermore; the biomolecular mechanisms of the Hofmeister series of imidazolium-based ILs on various biomolecules especially the metallo-proteins have not been explicitly elucidated. Therefore, in the present work, we have studied the impact of the Hofmeister series of various anions of ILs on Mb. To achieve the goal, we have exploited the structural stability of Mb in imidazolium-based ILs such as 1-butyl-3-methylimidazolium thiocyanate [Bmim][SCN], 1-butyl-3-methylimidazolium hydrogensulfate [Bmim][HSO_4], 1-butyl-3-methylimidazolium chloride [Bmim][Cl], 1-butyl-3-methylimidazolium bromide [Bmim][Br], 1-butyl-3-methylimidazolium acetate [Bmim][CH_3COO], and 1-butyl-3-methylimidazolium iodide [Bmim][I]. Further, in order to compare the results of Mb stability in [Bmim]-based ILs, we have chosen a set of the Hofmeister series of anions of sodium salts such as, sodium thiocyanate (NaSCN), sodium sulfate (Na_2SO_4), sodium chloride (NaCl), sodium bromide (NaBr), sodium acetate ($NaCH_3COO$) and sodium iodide (NaI). Through this work, our aim is to update and enhance the understanding of the effect of the Hofmeister series of ILs on the protein stability. To obtain the goal, we have used UV–vis, fluorescence, circular dichroism (CD) spectroscopy and fluorescence thermal analysis of the protein denaturation curves. Docking studies were also performed in support of our experimental observations.

2. Materials and methods

2.1. Materials

Salt free myoglobin (Mb), 1-butyl-3-methylimidazolium thiocyanate ([Bmim][SCN]) (0.7% water), 1-butyl-3-methylimidazolium hydrogensulfate ([Bmim][HSO_4]) (≤1.0% water), 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) (≤0.2% water), 1-butyl-3-methylimidazolium bromide ([Bmim][Br]) (≤0.2% water), 1-butyl-3-methylimidazolium acetate ([Bmim][CH_3COO]) (≤0.5% water) and 1-butyl-3-methylimidazolium iodide ([Bmim][I]) (≤0.5% water), were purchased from Sigma–Aldrich Chemical Company (USA). High purity and anhydrous sodium salts of anions such as Cl^-, Br^-, I^-, SO_4^{-2}, CH_3COO^- and SCN^- were purchased from Thomas Baker Chemicals Pvt. Ltd. (India). All materials were used without further purification. Tris–HCl buffer solution (10 mM) of pH 7.0