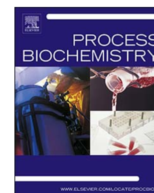
ARTICLE IN PRESS

Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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



Cationic effect of imidazolium-based ionic liquid on the stability of myoglobin

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A R T I C L E I N F O

Keywords:
Myoglobin unfolding
Guanidinium hydrochloride
Ionic liquid
Denaturation
Absorption spectroscopy

A B S T R A C T

The conformational change of myoglobin (Mb) during guanidine hydrochloride (GuHCl)-induced protein unfolding in the presence of various ionic liquids (ILs) in phosphate buffer was investigated using both the Soret band absorption and the fluorescence of tryptophan measurements. The GuHCl-induced denaturation midpoints of Mb derived from the absorption and fluorescence spectra were almost similar in the presence of 150 mM ILs with the same cation 1-butyl-3-methylimidazolium ($Bmim^+$) but different anions (BF_4^-, NO_3^-, Cl^-, and Br^-) in phosphate buffer. In addition, the denaturation midpoints of Mb in the presence of ILs were little lower than those in the absence of ILs in phosphate buffer. For the sake of clarity and comparison, we also measured the GuHCl-induced denaturation midpoints of Mb in the presence of 150 mM sodium salts with different anions (BF_4^-, NO_3^-, Cl^-, and Br^-) in phosphate buffer and found that their corresponding denaturation midpoints of Mb were almost similar to those observed in the absence of sodium salts in phosphate buffer. These experimental data indicate that $Bmim^+$ cation can promote the unfolding of Mb. Further experiments revealed that the denaturation ability of ILs increases with increasing alkyl chain length of imidazolium cation of ILs and that hydroxyl-substituted imidazolium cation could also promote the unfolding of Mb.

1. Introduction

Proteins are one of the most important bio-macromolecules in living beings, and they maintain a three-dimensional structure in vitro through some weak interactions including hydrogen bonds and hydrophobic or ion interactions [1]. A change in the microenvironment surrounding a protein disrupts these interactions, thereby causing denaturation of the protein, which leads to protein inactivity [2]. The empirical Hofmeister effect reflects ion interactions between biomolecules, including proteins, and cations or anions [3]. The inorganic cation or anion interactions with biomolecules have been widely studied [3–5]. Compared with anionic affect, cationic effect on protein is less pronounced [6–9]. Recently, the interaction of biomolecules with ionic liquids (ILs) has attracted considerable attention due to the challenges in finding biocompatible ILs [10–16]. Although IL–protein interactions in aqueous solutions have been found to follow Hofmeister series, the interactions between ILs and protein in aqueous solutions are still not well understood [11,13–15].

Compared with conventional inorganic salts, ILs are low-melting salts, which are generally composed of organic cation and inorganic anion. Thus far, the stabilizing and destabilizing effects of ILs on various proteins have been studied by many researchers [11–15,17–22]. Myoglobin (Mb), a heme-containing protein that stores oxygen in muscles, has been the subject of intense study about protein structure [23]. Miller at al. demonstrated that protein stabilization and destabilization by ILs in aqueous solution are often explained with the Hofmeister series, but Mb may not exactly follow this series [24]. The extent of folding or unfolding of Mb could be monitored by its Soret band absorption or florescence of tryptophan [25–27]. In 1963, Herskovits and Jaillet reported the structural stability and solvent denaturation of Mb, and they found that the denaturing power of water-miscible alcohols increases with increasing chain length of alcohols as judged from the midpoints of the denaturation transition derived from the molar absorbance at the Soret band (409 nm) in the absorption spectrum of Mb [28]. In 1968, Schechter and Epsyein reported both absorption and florescence spectral studies on the denaturation of Mb by urea and guanidine hydrochloride (GuHCl) [29].

Recently, several groups have reported the structural stability of Mb in the presence of ILs in aqueous solution by spectroscopic methods. In 2010, Safavi and Farjami measured the UV–vis absorption spectra of Mb with the addition of 1-butyl-3-methylimidazolium chloride (BmimCl) up to 0.22 M, and they did not observe significant changes

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http://dx.doi.org/10.1016/j.procbio.2017.05.004
Received 17 February 2017; Received in revised form 18 April 2017; Accepted 3 May 2017
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in the absorption spectra of Mb in the presence of BmimCl and concluded that the presence of BmimCl has no effect on Mb conformation and does not cause any unfolding of Mb [30]. In 2013, Fiebig et al. applied absorption and fluorescence spectroscopic methods to study Mb unfolding in the presence of GuHCl and ethylmethylimidazolium acetate (EmimAc) and 1-butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$) in buffer and found that $BmimBF_4$ significantly destabilized Mb; however, the controlling experimental results finally indicated that the observed denaturation effects were due to the presence of anion species Ac^- and BF_4^- [31]. In 2014, Kumar and Venkatesu comparatively studied the stability of Mb in the presence of ionic liquids or ionic salts at relatively low concentrations and found that both ionic liquids and ionic salts have negligible effect on the stability of Mb [32]. Later, Attri et al. explored the effect of ammonium ILs on Mb structure by using fluorescence and circular dichroism experiments and found that phosphate and sulfate ILs stabilize the native structure of Mb, while acetate ILs destabilize Mb structure [33]. In 2015, Jha et al. reported the cationic effect of 1-ethyl-3-methyl-imidazolium chloride (EmimCl),1-butyl-3-methylimidazolium chloride (BmimCl), 1-hexyl-3-methyimidazolium chloride (HmimCl), and 1-decyl-3-methylimidazolium chloride (DmimCl) on the conformational stability of heme protein hemoglobin (Hb) at different concentrations in buffer, and they found that the destabilization tendency of ILs toward Hb increases with increasing chain length of the cation of ILs at moderate concentration [7]. In 2016, Miller et al. explored the kinetics and mass spectrometric measurements of Mb unfolding in aqueous ionic liquid solutions and found that $BmimBF_4$ destabilizes Mb. These results from the studies of heme protein (Hb and Mb) structural stability in aqueous solution in the presence of various ILs indicate that the main issues for the destabilization of heme proteins are the cation or the anion effect as well as the concentration effect of ILs. In this study, we systematically investigated the cationic effect of imidazolium-based ILs in the presence of BmimX (X = BF_4^-, NO_3^-, Cl^-, and Br^-) at constant moderate concentration on the structural stability of Mb using absorption and fluorescence spectroscopy, and we also explored the effect of the alkyl chain length of the cation in ILs and the effect of the hydroxyl-substituted cation of ILs on the structural stability of Mb in phosphate buffer.

2. Experimental section

2.1. Samples

Equine heart Mb (≥90%), GuHCl (≥99.0%), 1-ethyl-3-methylimidazolium tetrafluoroborate ($EmimBF_4$, ≥99%), 1-butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$, ≥98.5%), 1-butyl-3-methylimidazolium chloride (BmimCl, ≥98.0%), 1-butyl-3-methylimidazolium bromide (BmimBr, ≥98.5%), 1-butyl-3-methylimidazolium nitrate ($BmimNO_3$, ≥95.0%), 1-hexyl-3-methylimidazolium tetrafluoroborate ($HmimBF_4$, ≥97.0%), $NaNO_3$ (≥99.995%), $NaBF_4$ (≥98%), and NaBr (≥99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-hydroxyethyl-3-methylimidazolium tetrafluoroborate ($HOEmimBF_4$, ≥98%) was purchased from TCI China (Shanghai, China). NaCl (≥99.0%) was purchased from Alfa Aesar (Shanghai, China) and used after calcination at 700 °C. Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$) (≥99%) and sodium dihydrogen phosphate dehydrate ($NaH_2PO_4{\cdot}2H_2O$) (≥99.0%) were purchased from Beijing Chemical Reagents Co., Ltd. (Beijing, China). All materials were used without further purification. Pure water (18 MΩ) was used to prepare all solutions. $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}2H_2O$ were used to prepare 20 mM phosphate buffer (pH = 7.4). The stock solutions of 500 mM ILs or inorganic salts and that of 6.0 M GuHCl were prepared in 20 mM phosphate buffer (pH = 7.4).

2.2. Spectral measurements

The absorption spectra of 1-cm path-length sample cell were recorded on a Shimadzu UV-3600 spectrophotometer (Japan). The fluorescence spectra of 1-cm path-length sample cell were recorded on a Hitachi F-4600 spectrophotometer (Japan). The excitation wavelength was set at 285 nm, which corresponds to tryptophan excitation. In spectral measurements, all samples contained 0.2 mg/ml (11.9 μM) Mb with varying concentrations of GuHCl in 20 mM phosphate buffer (pH = 7.4). The concentrations of ILs and inorganic salts were fixed at 150 mM. All samples were prepared at least 1 h before spectral measurements to ensure that Mb denatured completely. All experiments were conducted at a temperature of 22.0 ± 0.5 °C.

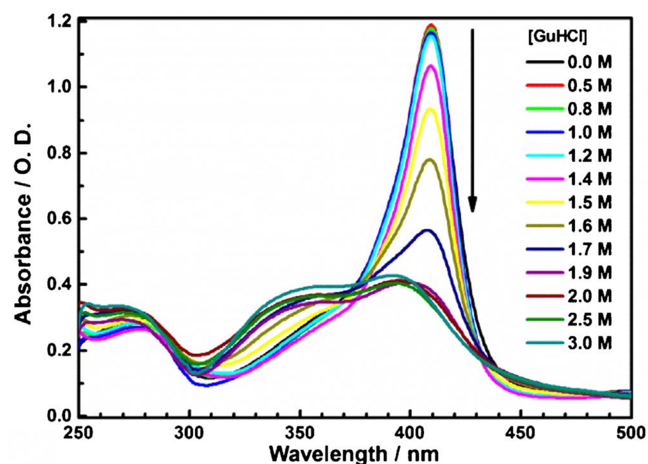

Fig. 1. Absorption spectra of Mb at different GuHCl concentrations in phosphate buffer alone. 0.0 M (black); 0.5 M (red); 0.8 M (green);1.0 M (blue);1.M (cyan); 1.4 M (magenta);1.5 M (yellow); 1.6 M (dark yellow) 1.7 M (navy);1.9 M (purple); 2.0 M (wine); 2.5 M (olive) and 3.0 M (dark cyan). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

Fig. 1 presents the absorption spectra of Mb at different GuHCl concentrations in phosphate buffer. As shown in Fig. 1, a strong absorption band was observed at 409 nm, which denotes the Soret band of the heme moiety of Mb, and a much weaker band at approximately 280 nm that originates from aromatic amino acids (tryptophan, tyrosine, and phenylalanine) in the polypeptide of Mb [34]. It is obvious that the 280-nm band is almost unaffected with the addition of the denaturant GuHCl, but the 409-nm band considerably changes with the addition of the denaturant GuHCl. Thus, the intensity change of the 409-nm spectral band is usually used to probe the conformational transition from folded state to unfolded state upon denaturation of Mb [26,28,29,31]. Briefly, the 409-nm absorbance in the spectrum of native Mb in phosphate buffer alone is correlated to 100% folded Mb and that in phosphate buffer with 3.0 M GuHCl is correlated to 0% folded Mb. Therefore, the folded fraction f of Mb at each GuHCl concentration can be derived using $f = (A_f - A)/(A - A_u)$, where A_f is the 409-nm absorbance of native Mb without the addition of GuHCl; A is the 409-nm absorbance of folded Mb at various GuHCl concentrations in phosphate buffer; A_u is the 409-nm absorbance of Mb at 3.0 M GuHCl concentration in phosphate buffer [24,26]. Using this method, we obtained the folded fraction of Mb from its Soret band absorbance at each GuHCl concentration and then plotted the denaturation fraction curve of Mb as a function of GuHCl concentration in phosphate buffer, as shown in Fig. 2A. The denaturation midpoint (C_M, the concentration of denaturant when f = 0.5 in phosphate buffer) is an indicator of the denaturation degree of Mb [28,31]. From the denaturation fraction curve of Mb in phosphate buffer with GuHCl, we obtained a denaturation midpoint of 1.62 M for equine heart Mb in phosphate buffer, which is a little higher than the reported C_M of 1.5 M for horse skeletal muscle Mb [26] and a little lower than the reported C_M of 1.8 M

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