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Exploring the thermal stability of α-chymotrypsin in protic ionic liquids

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ABSTRACT

Ammonium based ionic liquids (ILs) are biocompatible co-solvents that stabilize the native state of proteins. Experimentally, we have explored the stability of α-chymotrypsin (CT) in the presence of nine ILs, i.e., diethylammonium acetate (DEAA), diethylammonium hydrogen sulfate (DEAS), diethylammonium dihydrogen phosphate (DEAP), triethylammonium acetate (TEAA), triethylammonium hydrogen sulfate (TEAS), triethylammonium dihydrogen phosphate (TEAP), trimethylammonium acetate (TMAA), trimethylammonium hydrogen sulfate (TMAS), trimethylammonium dihydrogen phosphate (TMAP). Thermodynamic folding properties such as transition temperature (T_m), Gibbs free energy change of unfolding (ΔG_U), enthalpy change (ΔH) and heat capacity change (ΔC_p) of CT in ILs are obtained by fluorescence spectra analysis. Fluorescence and circular dichroism (CD) spectroscopy experiments were performed to probe CT stabilization and structural changes in the presence of ILs. Our experimental results suggest that the ILs act as stabilizers for the CT structure and the stability of CT depends on the structural arrangement of the ions of ILs. Our experimental results reveal that ILs (DEAA, DEAS and DEAP) having more hydrophobic ammonium cations [DEA^+] are weak stabilizers for CT, while trimethyl ammonium cations [TMA^+] ILs having small alkyl chain length such as TMAA, TMAS and TMAP are strong stabilizers and therefore more biocompatible for the native structure of CT.

1. Introduction

The serine proteinases are widely distributed in nature, where they perform a variety of different functions [1–4]. Many proteinases occur as domains in large multifunctional proteins, but others are independent small peptide chains [5]. Bacterial serine proteinases share the chymotrypsin like bilobal β-barrel structure; however, they are more distantly related due to their shorter sequences and structural differences in surface loops [5,6]. One of the most predominant families of serine proteases is the S1 family. α-Chymotrypsin (CT) belongs to the S1 family that contains a catalytic triad system consisting of aspartate, histidine, and serine that work together to control the nucleophilicity of the serine residue during catalysis.

CT is one of the valuable biological substances for understanding the mechanism of protein folding or unfolding with the addition of co-solvents [7]. A solvent-surface image of the CT crystal structure shows that out of the eight tryptophan residues of the enzyme; Trp 172, Trp 207 (46%), Trp 215, and Trp 237 (49%) are completely exposed to the solvent [8–10]. The latter three residues are situated in direct proximity to the active site residues. Trp 5l and Trp l41 are completely buried in the globule core, and Trp 27 and Trp 29 are partly exposed to the solvent. The enzyme surface is initially responsible for the interaction with the environment of the solvent molecules. However, understanding the structure–function relationship is still one of the challenging tasks in biochemical and biotechnology studies.

The exhaustive structural information and function of biomolecules in ionic liquids (ILs) are fascinating and crucial for understanding their metabolic role as well as their use in chemical and pharmaceutical industry. The stability of native proteins shows marginal changes in the solvent environment affecting their properties and functional activity dramatically [11–24]. ILs have recently emerged as a novel class of solvents for a wide range of chemical industrial applications [25]. Apparently, the physicochemical properties of ILs are quite sensitive towards the structure and nature of cations and anions [24–29]. They are thermally and chemically stable, non-flammable, have a low melting point, a large liquid range and favourable solvation behaviour. Apparently, they are considered to be possible replacements for conventional organic solvents to reduce the environmental and economic costs in the chemical industry [25,30,31]. As solvents, they have been used to solubilize proteins, DNA [32], biocatalysis [33], biosensors [34] while preserving enzyme stability [35]. ILs have also been reported to increase protein activity, selectivity, refolding and yield in comparison to other solvent media [36–42]. In view of the growing importance of ILs, it is desirable to explore the ion

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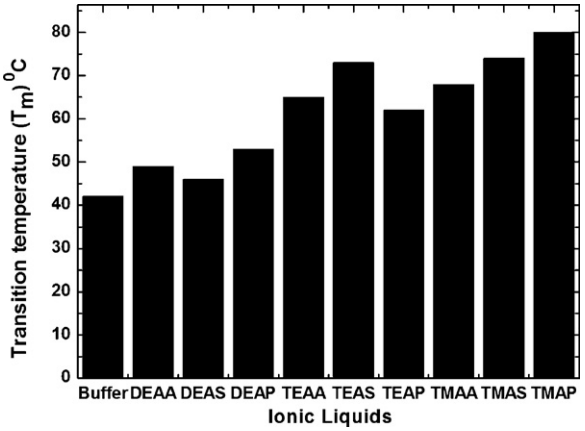

Fig. 1. Influence of ammonium ILs on the structure of CT from the thermodynamic protein stability, which is obtained from fluorescence analysis. The variation in T_m values of CT in buffer and in ILs.

Table 1
Thermodynamic folding profile such as transition temperature (T_m), enthalpy change (ΔH), Gibbs free energy change of unfolding (ΔG_U) and heat capacity change (ΔC_p) of CT in buffer and ILs were obtained by fluorescence analysis.

Sample	T_m (°C)	ΔH (kJ mol^{-1})	ΔG_U (kJ mol^{-1})	ΔC_p[a] (kJ mol^{-1} K^{-1})
Buffer	42.0	424.0	18.15	10.0 ± 0.01
DEAA	49.0	505.5	28.20	10.3 ± 0.01
DEAS	46.0	490.1	24.71	10.6 ± 0.05
DEAP	53.0	584.7	36.54	11.0 ± 0.03
TEAA	65.0	1579.3	129.05	24.0 ± 0.21
TEAS	73.0	1712.0	157.60	23.1 ± 0.03
TEAP	62.0	842.0	64.13	14.0 ± 0.81
TMAA	68.0	1681.1	141.95	24.7 ± 0.01
TMAS	74.0	1742.4	160.40	23.5 ± 0.04
TMAP	80.0	1942.1	192.65	24.2 ± 0.07

[a] Plot of ΔH versus T_m of each ILs provides the value of ΔC_p.

effects on enzyme stability. However, the biocompatibilities of ILs on enzymes are still not fully understood and considerable confusion arises to the quality of biocompatible and particularly in ammonium ILs on biomolecules. In light of these considerations and to gain further deeper insight into the mechanistic basis of the thermal stability in the presence of novel kind of ILs, we have explored the effect of biocompatible ILs on CT structure with the help of fluorescence and circular dichroism (CD) experiments. For the sake of clarity and comparison, we have chosen nine ILs from ammonium family, such as diethylammonium acetate (DEAA), diethylammonium hydrogen sulfate (DEAS), diethylammonium dihydrogen phosphate (DEAP), triethylammonium acetate (TEAA), triethylammonium hydrogen sulfate (TEAS), triethylammonium dihydrogen phosphate (TEAP), trimethylammonium acetate (TMAA), trimethylammonium hydrogen sulfate (TMAS), and trimethylammonium dihydrogen phosphate (TMAP). The schematic chemical structures of the biocompatible ILs are shown in Fig. 1S. Our results clearly demonstrate that subtle variations in the cation and anion structure can dramatically influence CT stability. In this paper we have exemplified that all ILs act as stabilizers for CT using fluorescence and CD studies.

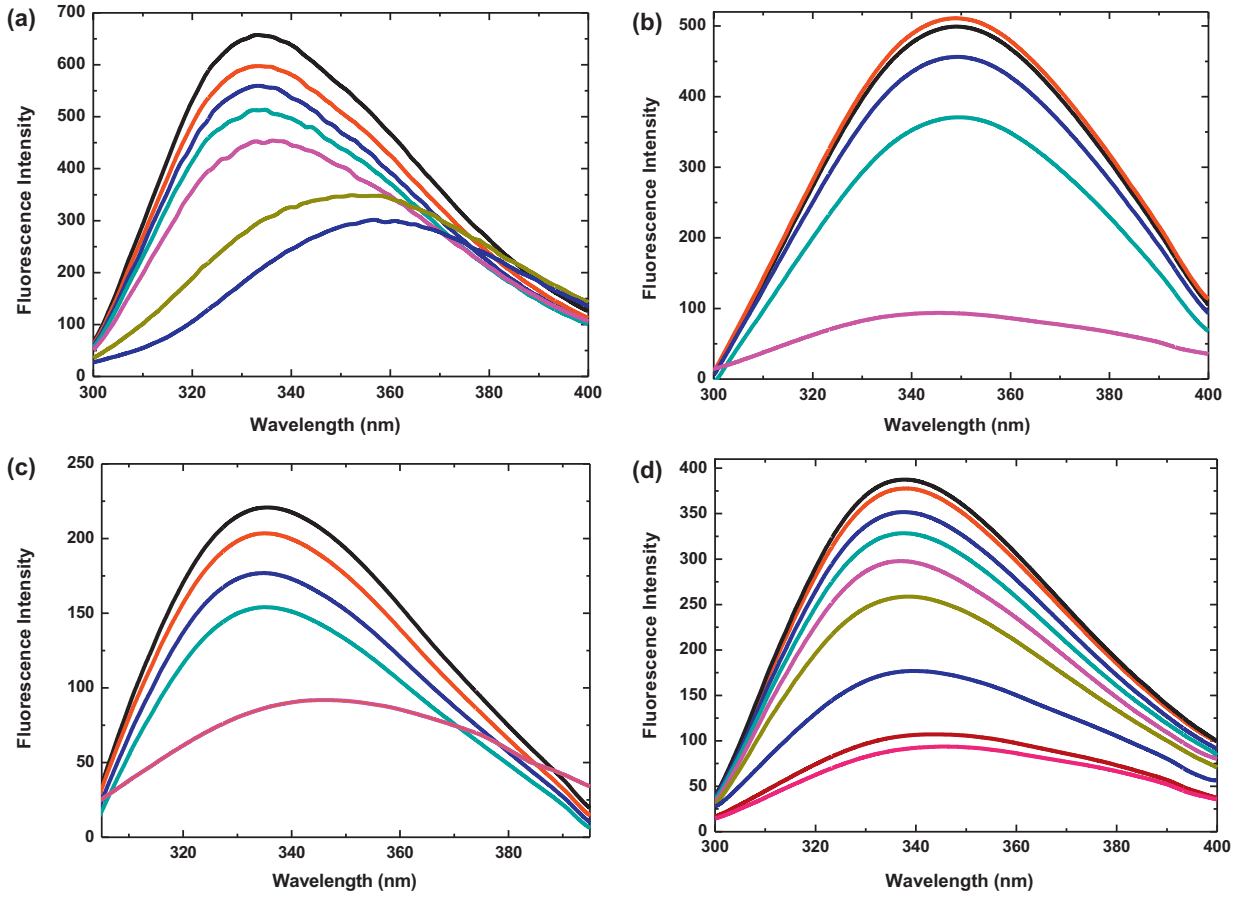

Fig. 2. Fluorescence spectra analysis of the CT in (a) buffer, (b) DEAA, (c) DEAS, (d) DEAP at 25 °C (black), 35 °C (red), 40 °C (blue), 45 °C (dark cyan), 50 °C (magenta), 55 °C (dark yellow), 60 °C (navy), 65 °C (wine) and 70 °C (pink), shows the effect of the ILs on the stability of CT in presence of high temperature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)