Apr 23rd, 9:00 AM

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Characterization of Lysozyme Stability in the Presence of Ionic Liquids

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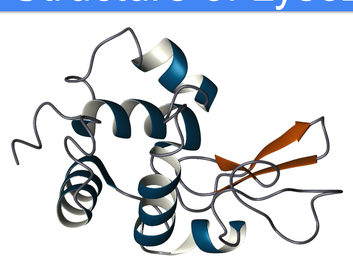
Abstract

Hen egg white lysozyme (Lyz) has been a well studied model system in biophysical investigations for decades. The protein is a small, primarily helical, highly soluble protein that is both commercially available and easily accessible. Ionic liquids (ILs), often referred to as room-temperature ionic liquids or molten salts, have garnered great interest in the last 15-20 years as potential components of electrochemical devices or applications. More recently, the biocompatibility of these molecules has developed an increased interest in the field, especially considering that some IL species can stabilize biomolecular structures while other ILs strongly destabilize 3D structures. In this study, we used fluorescence spectroscopy to characterize the unfolding of lysozyme and the impact imidazolium-based ILs had on this process. The ILs 1-butyl-3-methylimidazolium chloride, 1-hexyl-3-methylimidazolium chloride, and 1-octyl-3-methylimidazolium chloride were first evaluated for their impacts alone, and subsequently on their ability to destabilize the lysozyme when denatured with Guanidinium HCl. Consistent with previous findings, the alkyl chain length had an impact on the destabilization potential of the ILs. Subsequent studies on the denaturation process in using thermal-induced denaturation, as well as quenching studies throughout the process, were also investigated to gain insight into the denaturation process. Overall, longer alkyl chain length ILs more strongly destabilize the lysozyme 3D structure.

3D Structure of Lysozyme



Structure of ILs

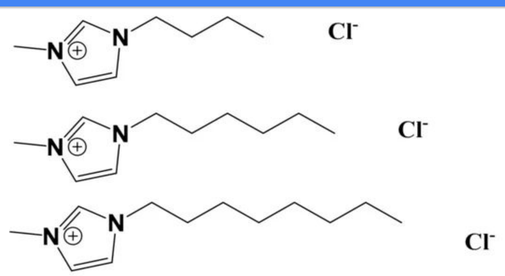


Figure 1: From top to bottom, IL structures: 1-butyl-3-methylimidazolium chloride, [BMIM]Cl, 1-hexyl-3-methylimidazolium chloride, [HMIM]Cl, and 1-octyl-3-methylimidazolium chloride, [OMIM]Cl.

Conclusion

- Partial Denaturation of Lysozyme in the presence of ILs.
- Longer alkyl length chains of ILs more strongly destabilize the 3-D structure of Lysozyme.
- Combined usage of ILs and GuHCl has shown evidence of quenching of the fluorophore.
- Dynamic quenching occurs with Acrylamide, GuHCl, and IL. Increasing the IL diminishes the quenching effects of acrylamide on solutions of 0, 2, and 4 M GuHCl.
- Ionic liquids lower the temperature where lysozyme denatures.

Effects of IL and GuHCl

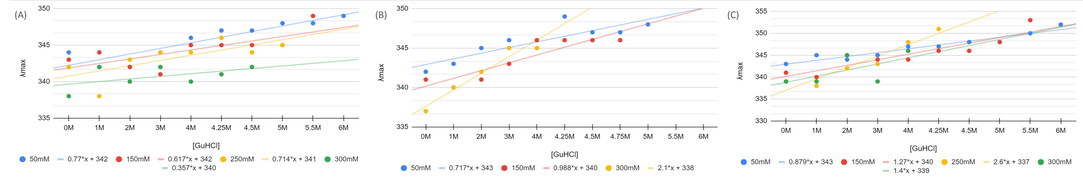


Figure 2: Plots of λ_{max} vs [GuHCl] (M) for solutions of 4 µM Lyz suspended in PBS buffer, treated with varying concentrations of (A) BMICl, (B) HMICl, and (C) OMICl. Values were obtained from fluorescence spectroscopy with excitation at 295 nm and emission measured from 310 to 450 nm.

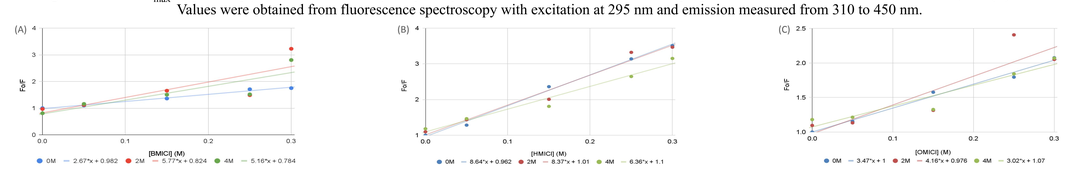


Figure 3: Stern-Volmer plots for the fluorescence of 4 µM Lyz, in PBS buffer, with 0-4 M GuHCl (as depicted in the legends), and varying concentrations of (A) BMICl, (B) HMICl, and (C) OMICl.

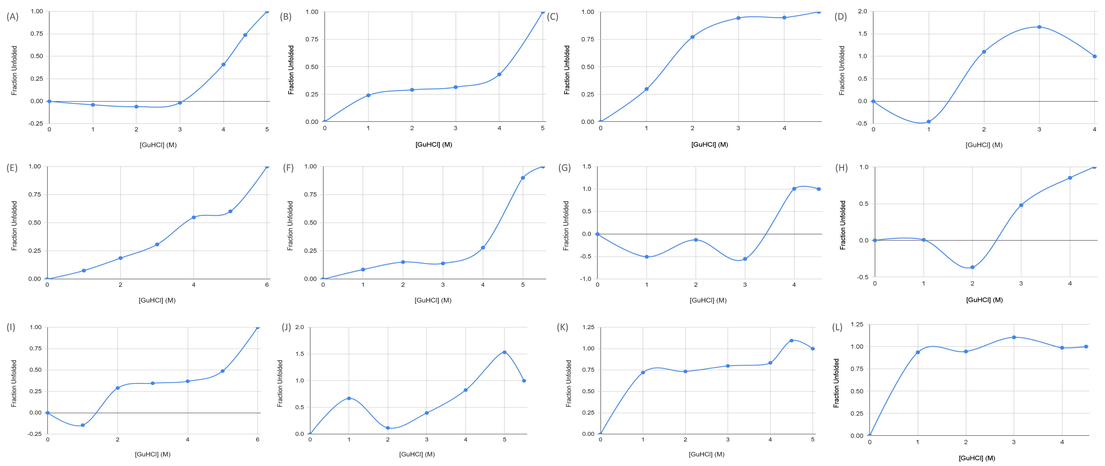


Figure 4: Plots of the fraction of unfolded protein vs [GuHCl] (M) for 4 µM Lyz, in PBS buffer, and varying concentrations of IL. Fraction of unfolded protein was calculated by using the area under the curve for fluorescence of Lyz at the aforementioned conditions, excited at 295 nm and emission measured from 310 to 450 nm. (A) Zero IL (B) 50mM, (C) 150 mM, and (D) 300 mM HMICl; (E) 50 mM, (F) 150 mM, (G) 250 mM, and (H) 300 mM BMICl; as well as (I) 50mM, (J) 150 mM, (K) 250mM, and (L) 300 mM OMICl.

Quenching Experiments

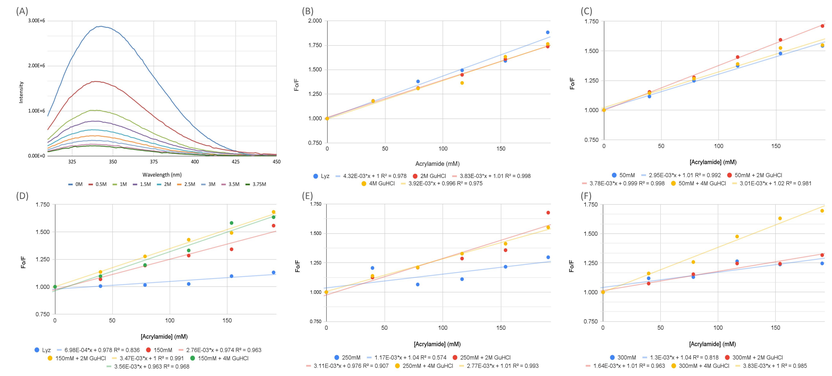


Figure 5: (A) Fluorescence of 4 µM Lysozyme, suspended in PBS, (Excitation: 295 nm; Emission: 310-450 nm) and Acrylamide. Graphs B-F represent Fo/F for the fluorescence of 4 µM Lyz PBS, GuHCl, IL, & Acrylamide. (Excitation: 295 nm; Emission: 345 nm). Samples were treated with Acrylamide in 10 µL increments, up to 50 µL. (B) No IL. (C) 50 mM (D) 150 mM (E) 250mM and (F) 300 mM HMICl

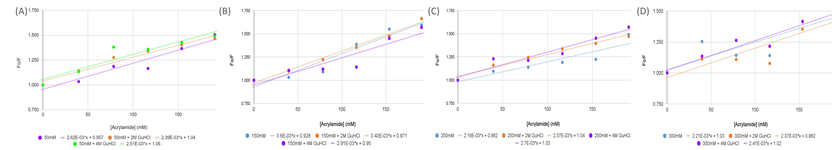


Figure 6: See Figure 5 for experimental parameters. (A) 50 mM (B) 150 mM (C) 250mM and (D) 300 mM HMICl

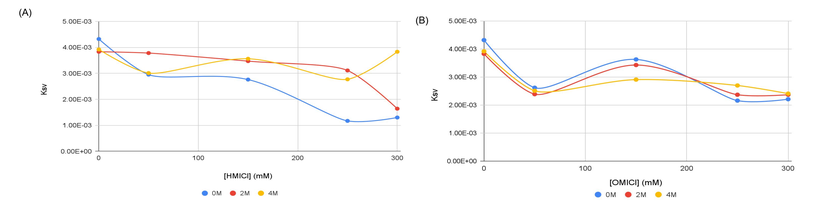


Figure 7: Plots of Quenching Constant (Ksv) vs [IL] for Acrylamide Quenching experiments in Figures 5 and 6. (A) HMICl and (B) OMICl

Temperature Experiments

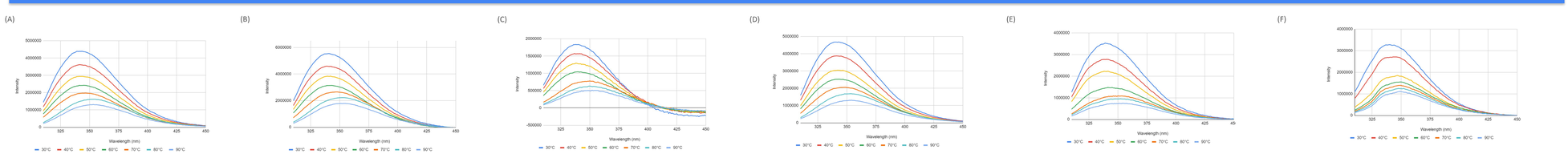


Figure 8: Fluorescence of 4 µM Lysozyme in varying concentrations of HMICl and OMICl, suspended in PBS from 25°C- 90°C, (Excitation: 295 nm; Emission: 310-450 nm). (A) 50 mM HMICl (B) 150mM HMICl (C) 300mM HMICl & (D) 50mM OMICl (E) 150mM OMICl (F) 300mM OMICl.

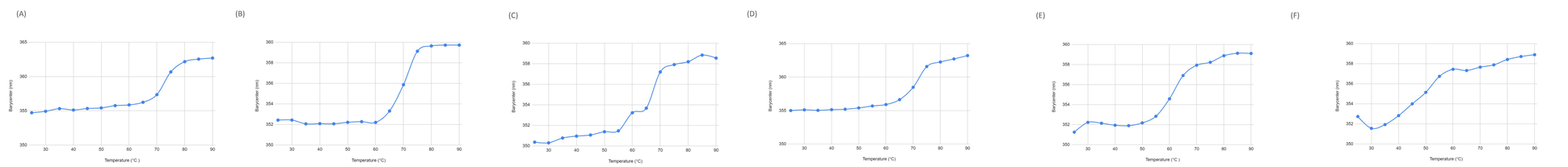


Figure 9: Barycenter values of 4 µM Lysozyme in varying concentrations of HMICl and OMICl, suspended in PBS from 25°C- 90°C, (Excitation: 295 nm; Emission: 310-450 nm). (A) 50 mM HMICl (B) 150mM HMICl (C) 300mM HMICl & (D) 50mM OMICl (E) 150mM OMICl (F) 300mM OMICl.