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Myoglobin Unfolding and Protein Stability With TMG

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Abstract

Myoglobin, a widely studied protein in biophysics, is a small, helical, and highly soluble protein that has been investigated for decades. Its heme prosthetic group facilitates easy analysis of its folding state through absorbance spectroscopy. Ionic liquids (ILs), particularly room-temperature ionic liquids or molten salts, have gained significant attention in the past 15-20 years for their potential use in electrochemical devices. Recently, their biocompatibility has become a focal point in research, given that certain IL species can either stabilize or destabilize biomolecular structures. This study employs absorbance and fluorescence spectroscopy to examine how amino acid-based ILs, specifically tetramethyl guanidine and choline as cations and serine, aspartic acid, and proline as anions, impact the unfolding of myoglobin. These amino acids were chosen based on previous findings indicating their varying effects on the protein azurin. The study evaluates the individual impacts of these amino acids and their collective ability to destabilize lysozyme when denatured with guanidinium HCl, monitored through absorbance spectroscopy and fluorescence signals from the heme group, Trp fluorescence, and Trp-heme interactions.

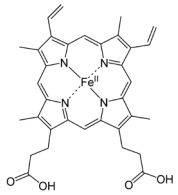
Background

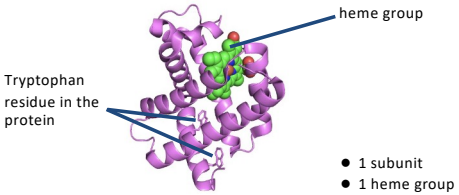
Myoglobin functions as an oxygen storage and transport molecule in muscles, akin to hemoglobin in blood but specialized for muscle cells. Its mechanism involves binding to oxygen in high-oxygen-concentration areas, such as the lungs, and releasing oxygen during muscle contraction in regions of low concentration.

Structure

- Monomeric protein
- 154 amino acid residues long
- 1 oxygen binding site

(heme prosthetic group)





- 1 subunit
- 1 heme group

3D structure of Myoglobin

Myoglobin + TMG-Asp

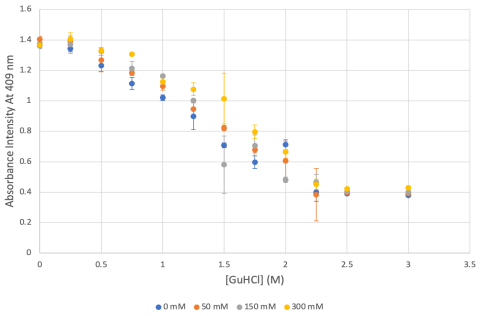


Figure 1: Myoglobin absorbance plot triplicate at 409 nm with TMG-Asp.

Myoglobin + TMG-Asp

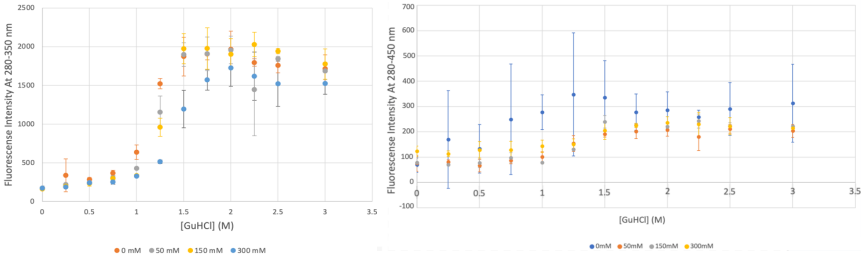


Figure 2: Myoglobin fluorescence plots in varying ionic liquids (A) Fluorescence of Myoglobin in 1M TMG-Asp at excitation 280nm emission 350nm. (B) Fluorescence of Myoglobin in 1M TMG-Asp at excitation 280nm emission 450nm.

Myoglobin + TMG-Ser

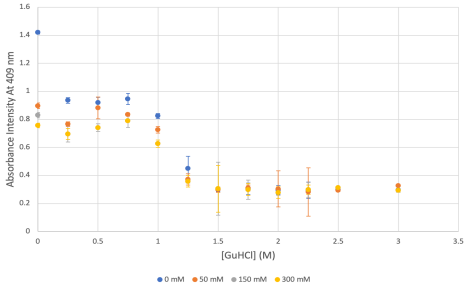


Figure 3: Myoglobin absorbance plot at 409 nm with TMG-Ser.

Myoglobin + TMG-Ser

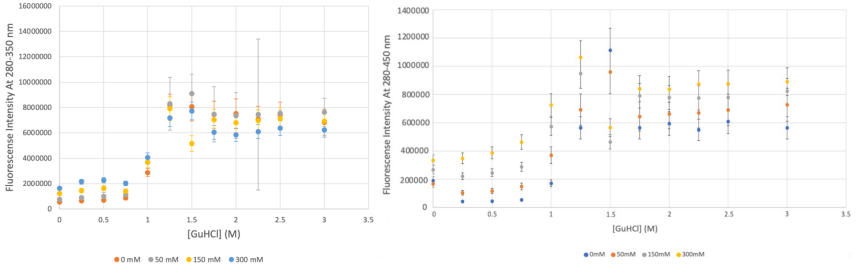
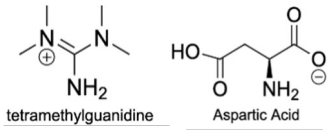


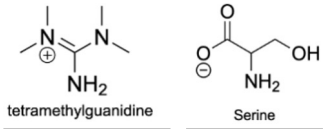
Figure 4: Myoglobin fluorescence plots in varying ionic liquids (A) Fluorescence of Myoglobin in 1M TMG-Ser at excitation 280nm emission 350nm. (B) Fluorescence of Myoglobin in 1M TMG-Ser at excitation 280nm emission 450nm.

Amino Acid Ionic Liquid Structures



tetramethylguanidine

Aspartic Acid



tetramethylguanidine

Serine

Conclusion

Ionic liquids (ILs), notably room-temperature ionic liquids or molten salts, have garnered attention for their electrochemical potential over the last two decades, with recent focus on their biocompatibility and ability to either stabilize or destabilize biomolecular structures. This study delves into the impact of amino acid-based ILs, specifically tetramethyl guanidine and choline as cations, and serine, aspartic acid, and proline as anions, on myoglobin unfolding. By evaluating individual and collective effects, we contribute to understanding protein stability, offering insights applicable to medicine, intramuscular medication, oxygen delivery mechanisms in high-oxygen areas like cardiac and skeletal muscles, and the development of medications for various muscular conditions and disorders. Additionally, our findings hold relevance in food processing, preservation, and the pursuit of environmentally sustainable, healthier meat alternatives.