Thermophoresis: The case of apomyoglobin

Binny A. Rudani[1], Johan Buitenhuis[1], Andreas M. Stadler[2,3] , Simone Wiegand[1,4]
[1] IBI-4:Biomacromolecular Systems and Processes, Forschungszentrum Jülich GmbH, D-52428 Jülich, Germany
E-mail: s.wiegand@fz-juelich.de,
[2] Jülich Centre for Neutron Science (JCNS-1), Forschungszentrum Jülich GmbH, D-52428 Jülich, Germany
[3] Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, D-52056 Aachen, Germany
[4] Department für Chemie - Physikalische Chemie, Universität zu Köln, 50939 Cologne, Germany

Introduction

This study investigates the thermodiffusive behavior of apomyoglobin (Apo-Mb) at different pH levels using the thermal diffusion forced Rayleigh scattering (TDFRS) technique, highlighting hydration changes associated with its conformational states. It is an extensively studied model system for understanding protein folding, the heme-free form of myoglobin. At near-neutral pH (∼6), Apo-Mb adopts a compact, native structure; at slightly acidic pH (∼4-4.5), it shifts to a partially folded "molten globule" state; and at pH∼2, it reaches an acid-unfolded state. As the pH decreases, the net positive charge of the protein increases, leading to protein unfolding [1]. The predominantly α-helical conformation of Apo-Mb is stabilized by strong intramolecular hydrogen bonding, which enhances its structural stability and exhibits a hydrophilic character on the surface of the protein. In the unfolded state, hydrophobic exposure reduces electrostatic interactions and effective hydrophilicity, which decreases solvation stability and promotes aggregation because unfolded proteins minimize water contact [2]. In addition, anions interact preferentially with the positively charged regions of the protein and effectively shield the repulsive forces between positive charges by binding to them, thus reducing internal repulsion. In particular, anions with higher charge and affinity towards protein are more effective than anions with lower affinity in inducing the transition from unfolded to folded proteins [3]. The stability of intermediate states of Apo-Mb strongly depends on the net charge of the protein, emphasizing that loss of positively charged residues generally increases stability by reducing internal charge repulsion and vice versa [4].

For aqueous systems, the Soret coefficient S_{T} of the solute changes its sign from negative to positive with increasing temperature [5]. Previous studies on aqueous systems have shown that the temperature sensitivity of S_{T} (difference at two temperatures) $\Delta S_{\mathrm{T}}(\Delta T)$ decreases with increasing temperature which has been attributed to the disruption of hydrogen bonding at higher temperatures [5, 6]. Thus, a highly hydrophilic solute exhibits high temperature sensitivity compared to a hydrophobic one. However, the thermophoretic behavior of proteins is a complex interplay of surface properties, as the solvent-accessible surface area also depends on the ionic strength and surface charge[7].

Thermophoresis of Apomyoglobin

Figure 1 shows the temperature dependence of S_{T} for Apo-Mb solutions measured at different pH values with and without buffer. At lower temperatures, Apo-Mb is thermophilic at pH 2 and pH 6, but switches to thermophobic behavior when the temperature increases above ∼20°C. In contrast, Apo-Mb remains thermophobic at pH 4 over the entire temperature range studied. However, the value of S_{T} increases with increasing temperature in all solution conditions [7]. Interestingly, in the presence of sodium phosphate buffer (NaP), the light scattering intensity increased exponentially at 45°C due to protein aggregation, obscuring the cuvette window and preventing data collection. According to the Hofmeister series, the phosphate anions in the buffer are very hydrophilic and act as strong water structure makers, resulting in a more compact protein structure and its aggregation [3, 8].

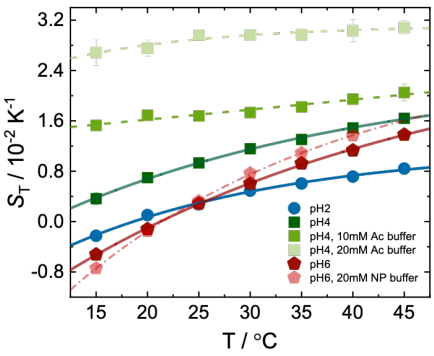

Figure 1: Temperature dependence of S_{T}, measured for 7 mg/ml Apo-Mb with and without buffer at different pH values corresponding to the different folding states of Apo-Mb

Correlation between Circular Dichroism (CD) and TDFRS

Prior to TDFRS measurements, the secondary structure content and charge of Apo-Mb under different solution conditions were confirmed by CD. In absence of buffer, the α-helical content decreases progressively with decreasing pH. In case

of buffers, NaP buffer stabilizes the α-helix at pH 6, whereas sodium acetate (NaAc) buffer at pH 4 promotes partial unfolding by decreasing the α-helix content. At pH2, a small fraction of the α-helical content is still retained in its unfolded state [1].

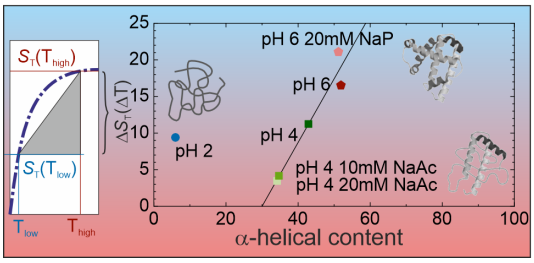

Figure 2: The plot shows a strong correlation between temperature sensitivity, $\Delta S_{\mathrm{T}}(\Delta T)$ and α-helix content.

Figure 2 shows a correlation between temperature sensitivity, $\Delta S_{\mathrm{T}}(\Delta T)$ and α-helix content. The α-helix content is a reliable indicator of the hydrophilicity of proteins. This is because in a α-helix conformation, the hydrophobic residues tend to be buried in the core of the protein, while the hydrophilic residues are exposed to the aqueous environment. Therefore, a higher α-helix content corresponds to a more hydrophilic protein. In addition, previous studies show that the α-helix content of a protein mainly determines the protein diffusion [1]. This correlation is also observed in our results. In particular, the temperature sensitivity of the thermodiffusive behavior, expressed by $\Delta S_{\mathrm{T}}(\Delta T)$, decreases in the following order as the hydrophilicity of Apo-Mb decreases (which is achieved by lowering the pH): ΔS_{T} (pH 6) $>$ ΔS_{T} (pH 4) $>$ ΔS_{T} (pH 2). However, NaP buffer makes the protein more compact, resulting in increased hydrophilicity and a higher value of $\Delta S_{\mathrm{T}}(\Delta T)$ [8]. NaAc buffer enhanced protein solubilization at pH 4, reducing hydrophilicity by exposing hydrophobic regions. This resulted in a significantly lower $\Delta S_{\mathrm{T}}(\Delta T)$ value compared to the unbuffered acidic solution at the same pH [9]. At pH 2, the protein structure was largely disrupted, with a significant decrease in the α-helix content and an increase in the net positive charge. This perturbation led to a deviation from the observed trend between $\Delta S_{\mathrm{T}}(\Delta T)$ and α-helix content.

Conclusions

Our study investigates the influence of structural conformational changes on the thermodiffusion behavior of Apo-Mb using the TDFRS technique. We found that the α-helix content is strongly correlated with hydrophilicity and thus influences the thermodiffusion behavior. Reducing the α-helix content led to a decrease in hydrophilicity (pH6 $>$ pH4 $>$ pH2) and a decrease in the temperature sensitivity of $\Delta S_{\mathrm{T}}(\Delta T)$. ($\Delta S_{\mathrm{T}}(\Delta T)$ (pH6) $>$ $\Delta S_{\mathrm{T}}(\Delta T)$ (pH4) $>$ $\Delta S_{\mathrm{T}}(\Delta T)$ (pH2)). The type of buffer also plays a significant role in modulating the structural and diffusional properties of apo-Mb. At pH 6, NaP buffer preserves the α-helix but promotes aggregation of the protein due to electrostatic screening, as evidenced by a lower diffusion coefficient. Whereas acetate buffer at pH 4 decreases the α-helix content of the protein and the temperature sensitivity of S_{T} and increases the solubility of the protein in solution. Increasing the concentration of acetate buffer at pH 4 further decreases the temperature sensitivity of S_{T}.

Overall, we observed a strong correlation between $\Delta S_{\mathrm{T}}(\Delta T)$ and α-helix content; $\Delta S_{\mathrm{T}}(\Delta T)$ increases steadily with increasing hydrophilicity and α-helix content of Apo-Mb. These results highlight the complex interplay between the structural state of Apo-Mb, pH, buffer composition and thermodiffusion behavior and provide valuable insights into protein hydration.

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References

1. A. M. Stadler, F. Demmel, J. Olliver, T. Seydel. Picosecond to nanosecond dynamics provide a source of conformational entropy for protein *Phys. Chem. Chem. Phys.*, 18, 21527, (2016)

2. A. Kffel, J. Zielkiewicz. Why the Solvation Water around Proteins Is More Dense than Bulk Water *J . Phys. Chem. B*, 116, 12113, (2012)

3. Y. Goto, A. L. Fink. Effect of Buffer on Protein Stability in Aqueous Solutions: A Simple Protein Aggregation *J . Phys. Chem. B*, 125, 2504, (2021)

4. M. S. Kay, R. L. Robert. Alternative Models for Describing the Acid Unfolding of the Apomyoglobin Folding *Biochemistry*, 37, 7859, (1998)

5. D. Niether, S. Wiegand. Thermophoresis of biological and biocompatible compounds in aqueous solution *J. Phys. Condens. Matter*, 31, 503003, (2019)

6. S. Iacopini, R. Rusconi, R. Piazza. Universal temperature dependence of thermal diffusion in aqueous colloidal suspensions *Eur. Phys. J. E*, 19, 59, (2006)

7. R. Piazza, S. Iacopini, B. Triulzia. Thermophoresis as a probe of particle-solvent interactions *Phys. Chem. Chem. Phys.*, 6, 1616, (2004)

8. A. Salis, B. W. Ninham. Models and mechanisms of Hofmeister effects in electrolyte solutions, and colloid and protein *Chem. Soc. Rev.*, 43, 7358, (2014)

9. S. Liese, M. Gensler, et al. Hydration Effects Turn a Highly Stretched Polymer from an Entropic into an Energetic Spring *ACS Nano*, 11, 702, (2017)