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Impact of SDS Concentration and pH on Haemoglobin Conformation: A Comparative Spectroscopic Study

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Abstract

This study delves into the intricate interplay between Haemoglobin (Hb) and the anionic surfactant sodium dodecyl sulphate (SDS), focusing on the structural alterations induced by varying SDS concentrations (0.2mM to 1mM) at two distinct pH levels (7.2 and 5.0). Three primary objectives guide the investigation: first, to scrutinize specific structural changes in the aromatic amino region, Soret region, and oxy-band regions; second, to explore aggregations induced by SDS, emphasizing pH-dependent tendencies; and third, to characterize spectral changes, including the impact on absorbance peaks and the absorption peak at 415nm. Results reveal concentration-dependent effects of SDS on Hb conformation, with a notable disappearance of peaks at 273nm and the emergence of new peaks at 537nm. Aggregations are observed, with variations in occurrence and nature between Hb variants (AA, AS, and SS). pH-dependent responses are evident, influencing hyperchromic and hypochromic shifts, as well as the destruction or distortion of specific peaks. Comparisons between pH levels (7.2 and 5.0) demonstrate context-dependent variations in absorbance, with a distinct absorption peak at 415nm. The findings contribute biophysical insights, emphasizing the importance of understanding SDS-induced changes in protein structures. Context-specific aggregations underscore the need for nuanced control in industries where protein stability is crucial. The study's methodology enhances biophysical techniques for studying protein-surfactant interactions. Implications extend to biotechnological applications, clinical relevance, and potential avenues for future research into molecular mechanisms and functional consequences of altered Haemoglobin structures.

Keywords: *Haemoglobin Conformation; Sodium Dodecyl Sulphate (SDS); Protein-Surfactant Interactions; pH-Dependent Structural Changes; Aggregation Dynamics*

Introduction

Haemoglobin, a crucial globular protein found in red blood cells, stands at the forefront of oxygen transport within the human body (Ahmed et al., 2020). Comprising four subunits, each housing an iron-containing haem group, its structural integrity is paramount for effective oxygen binding and subsequent delivery to tissues. The study delves into the dynamic interplay between Haemoglobin, Sodium Dodecyl Sulphate (SDS), and pH, aiming to unravel the subtle changes in protein conformation under varying conditions.

Surfactants, exemplified by SDS, have been extensively scrutinized for their impact on protein structures (Poghosyan et al., 2021; Aguirre-Ramírez et al., 2021). SDS, an anionic detergent, is known to perturb hydrophobic interactions, thereby modifying the conformation of proteins. While the general effects of SDS on proteins are acknowledged, its specific influence on Haemoglobin, and how this impact differs among variants like HbAA, HbAS, and HbSS, remains a frontier demanding comprehensive exploration.

The role of pH as a modulator of protein stability adds an additional layer of complexity to the study. The surrounding pH environment significantly influences the structural stability of proteins. By examining both physiological (pH 7.2) and slightly acidic (pH 5.0) conditions, the study aims to uncover the intricate interplay between pH variations and SDS-induced modifications, offering a holistic understanding of the environmental factors influencing Haemoglobin conformation.

Spectroscopic analysis emerges as a powerful ally in the pursuit of structural insights. UV-Visible spectroscopy, employed in this study, becomes the lens through which the researchers examine specific regions of the Haemoglobin spectrum, including the aromatic amino region, Soret region, and oxy-band regions. The close scrutiny of absorbance peaks and shifts becomes the key to unravelling the subtle alterations in Haemoglobin structure induced by varying concentrations of SDS under different pH conditions.

Beyond the laboratory setting, the study's findings hold promise for diverse biomedical applications. Insights into how SDS and pH intricately mould Haemoglobin conformation may pave the way for innovations in drug delivery systems, diagnostics, and therapeutic strategies. Furthermore, the exploration of variant-specific susceptibilities to structural changes could offer valuable information for the management of Haemoglobinopathies, underscoring the clinical relevance of this fundamental protein science research.

This research embarks on a journey to deepen the understanding of the interwoven influences of SDS concentration and pH variations on Haemoglobin conformation. The seamless integration of these factors, coupled with the study of different Haemoglobin variants, promises to contribute not only to the realm of protein science but also to the realms of medicine and biotechnology.

Research Objectives

1. Investigate the specific structural changes induced by varying SDS concentrations (0.2mM to 1mM) at pH 7.2 and pH 5.0 on Haemoglobin conformation, focusing on the aromatic amino region, Soret region, and oxy-band regions.

2. Explore the occurrence and nature of aggregations observed in the presence of SDS, with a particular emphasis on concentration ranges exhibiting aggregation tendencies, and assess the role of pH variations (7.2 vs. 5.0) in the aggregation behaviour.
3. Characterize the distinct spectral changes at different pH levels (7.2 and 5.0) and SDS concentrations, including the impact on absorbance peaks, hyperchromic and hypochromic shifts, and the total destruction or distortion of specific peaks, especially the absorption peak at 415nm.

This research aims to provide a comprehensive understanding of how SDS concentration and pH influence the structural integrity of Haemoglobin, with potential implications for biomedical and clinical applications.

Literature Review

Beginning with Vadas et al. (1986), the exploration of aggregation behaviour in the sea urchin Strongylocentrotus droebachiensis challenged previous hypotheses, revealing that sea urchins aggregated primarily in the presence of food. The study utilized diverse experimental approaches, shedding light on the multifaceted nature of aggregation behaviours in this marine organism.

Moving forward, Kumar et al. (2015) investigated the effects of Sodium Dodecyl Sulphate (SDS) on the fibrillation of α-lactalbumin. The study highlighted concentration-dependent outcomes, with lower SDS concentrations extending the lag time of fibrillation, while higher concentrations accelerated fibril elongation. Notably, concentrations above 2mM led to SDS inhibiting fibrillation, resulting in the formation of amorphous aggregates. This study emphasized differences in SDS effects on proteins with distinct surface charges, suggesting a common mechanism for fibrillation inhibition.

Continuing the exploration of SDS-induced structural changes, Khan et al. (2022) delved into the modulation of amyloid fibrillation and conformational changes in succinyl-Concanavalin A (succinyl-ConA) at low pH. The study revealed concentration-dependent effects, transforming succinyl-ConA from soluble to aggregated states. Far-UV CD results suggested a transition in secondary structure, from β-sheet to cross-β-sheet and ultimately to an α-helical structure. This work shed light on the intricate mechanisms behind SDS-induced succinyl-ConA aggregation.

Alresaini et al. (2023) extended the investigation, examining the effects of SDS on equine skeletal muscle myoglobin (E-Mb) at pH 4.5. The study demonstrated concentration-dependent behaviours of SDS, leading to amorphous aggregates, amyloid-like structures, and an induction of α-helical structure in E-Mb. This research not only contributed to the comprehension of myoglobin aggregation but also offered insights into the diverse behaviours exhibited by SDS, ranging from amorphous aggregates to amyloid fibrils.

In the realm of biomarkers and spectroscopic response, Malyshev et al. (2022) explored pH-induced changes in Raman, UV–vis absorbance, and fluorescence spectra of dipicolinic acid (DPA). This work, crucial for spore detection, highlighted pH-dependent shifts in DPA's spectroscopic response, with distinct peaks corresponding to different ionic forms. The findings

underscored the importance of considering pH variations in spectral analysis, particularly in detection applications.

Lastly, Qu et al. (2020) investigated the effect of pH on fluorescence and absorption of aggregates of chlorophyll a and carotenoids. The study revealed pH-induced changes in the geometry of chlorophyll a, leading to spectral red shifts and fluorescence quenching. The findings suggested the formation of H-type and J-type aggregates under acidic conditions, providing insights into how pH influences the aggregation behaviour of these photosynthetic pigments.

Collectively, these studies, spanning from 1986 to the present, contribute to our evolving understanding of how surfactants, pH variations, and environmental factors intricately influence the aggregation behaviour and structural changes of biomolecules, ranging from proteins to bacterial cells and marine organisms.

Materials and Methods

Objective 1: Investigate the Impact of SDS Concentrations on Haemoglobin Conformational Changes

Preparation of Gel and Equilibration:

i. The DEAE-cellulose gel was dissolved and packed into a column following the method by Scopes (1984).

ii. Equilibration was performed with 0.001M Tris-HCl buffer at pH 8.5.

Purification using Ion-Exchange Chromatography:

i. Separation of crude haemoglobin was carried out using DEAE-cellulose ion-exchange chromatography at 4°C.

ii. Elution was performed using a pH gradient (8.5 to 6.5), and eluates were collected for further analysis.

Spectroscopic Analysis for Haemoglobin Conformational Changes:

i. The R-state of haemoglobin variants (HbAA, HbAS, and HbSS) was used for spectroscopic analysis.

ii. A 1 in 3 dilution of crude haemoglobin variants was prepared and analyzed at pH 7.2 and 5.0 using a UV-Vis spectrophotometer.

Objective 2: Explore the Influence of SDS on Haemoglobin Aggregation and Structural Changes

Dialysis of Crude Haemoglobin: Dialysis of eluates for HbSS and HbAA with high absorbances was performed against 0.05M Tris-HCl at pH 7.2 and 0.05M acetate buffer at pH 5.0, respectively.

Purification using Ion-Exchange Chromatography:

i. The eluates with high absorbances were pooled and subjected to DEAE-cellulose ion-exchange chromatography at 4°C.

ii. The chromatography aimed to investigate the influence of SDS on haemoglobin aggregation.

Spectroscopic Analysis for SDS Concentrations (0.04mM – 4mM): Spectroscopic experiments were carried out for varying SDS concentrations to analyse the conformational changes in haemoglobin.

Objective 3: Examine the Effect of pH on SDS-induced Haemoglobin Conformational Changes

Dialysis of the Eluates: Dialysis of eluates was performed against 0.05M Tris-HCl at pH 7.2 and 0.05M acetate buffer at pH 5.0, aiming to remove traces of 2,3-bisphosphoglycerate.

Comparative Spectroscopic Analysis at Different pH Levels: Spectroscopic analysis of haemoglobin variants was conducted at pH 7.2 and 5.0 to observe the effects of SDS concentrations on conformational changes under different pH conditions.

Results

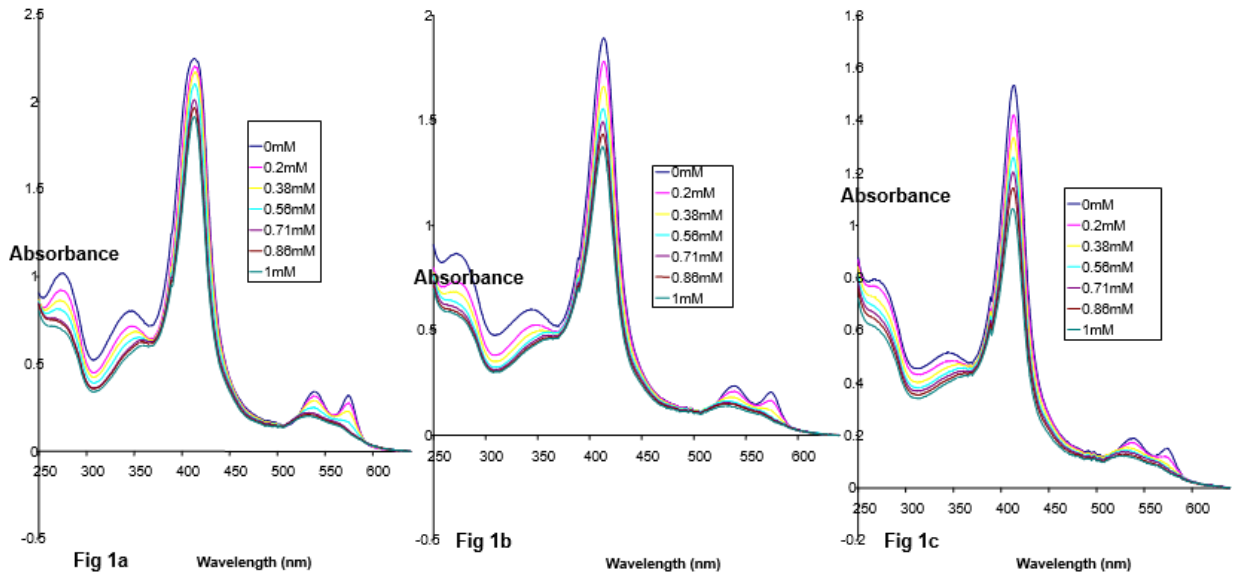

Fig. 1: Effect of SDS (0.2mM – 1mM) on Haemoglobin at pH 7.2. (a) HbAA, (b) HbAS and (c) HbSS.

- 0mM – no SDS.

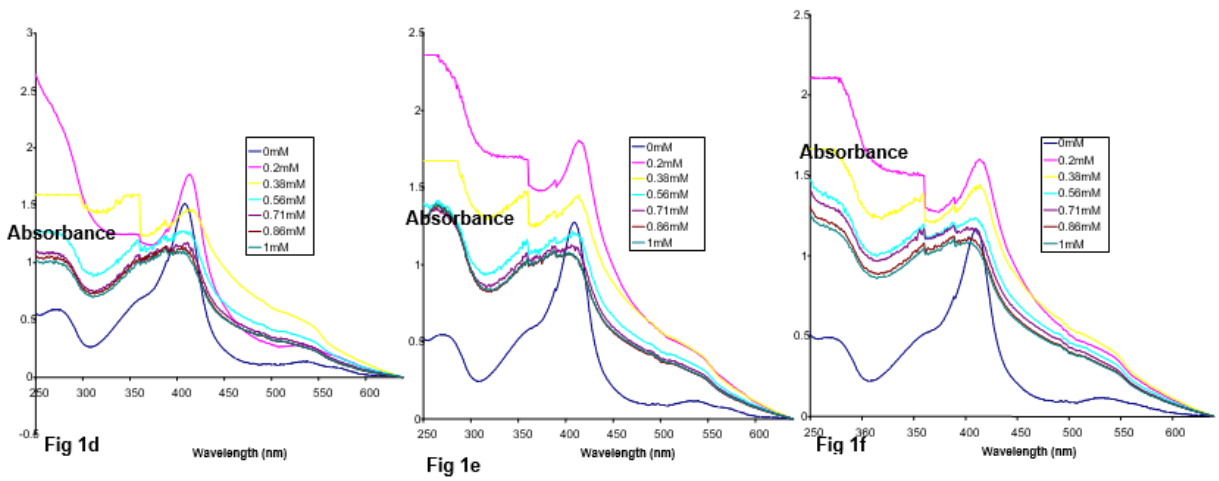

Fig 1: Effect of SDS (0.2mM – 1mM) on Haemoglobin at pH 5.0. (d) HbAA, (e) HbAS and (f) HbSS.

- 0mM – no SDS.

As the concentration of SDS increased (0.2mM – 1mM) pH 7.2, the spectra of HbAA (Fig 1a), HbAS (Fig 1b) and HbSS (Fig 1c), showed a decrease in absorbance until there was a complete disappearance of the peak at 273nm. Also, the delta peak decreased in absorbance as concentration of SDS increased. The absorption peak maximum 415nm was maintained irrespective of the gradual decrease in absorbance as concentration of SDS increased at the Soret region.

Although the spectra for HbAA, HbAS and HbSS were almost identical there were notable differences.

The peaks at 540nm and 577nm at the oxy-band region, for HbAA (Fig 1a) and HbAS (Fig 1b), had a decrease in absorbance within the concentration ranges of (0.2mM – 0.56mM). As concentration of SDS increased to 1mM, a new peak was observed at 537nm with total disappearance of the peaks at 540nm and 577nm. Whereas HbSS (Fig 1c), at the oxy-band region, the peaks at 540nm and 577nm had a decrease in absorbance with SDS concentration ranges of (0.2mM – 0.38mM), and as concentration of SDS continued to increase to 1mM, a new peak was observed at 537nm with a total disappearance of the peak at 540nm and 577nm.

Decrease in absorbance at the aromatic amino region was more for HbAA (Fig 1a), followed by HbAS (Fig 1b) and then HbSS (Fig 1c). Also following the same pattern as above, at the Soret region, the hypochromic shift was also more for HbAA (Fig 1a) than it was for HbAS (Fig 1b) and HbSS (Fig 1c).

For HbAA (Fig 1d), HbAS (Fig 1e) and HbSS (Fig 1f) pH 5.0, as SDS was added, there was a total destruction of the aromatic amino peak. As the concentration of SDS increased (0.2mM – 1mM), the absorbance at the aromatic amino region decreased. However, in relation to the control spectrum (no SDS), the absorbance increased. The spectra in the concentration ranges (0.71mM –

1mM) showed aggregation for HbAA (Fig 1d) and HbSS (Fig 1f). Aggregations were also observed in the spectra of concentration ranges (0.56mM – 1mM) for HbAS (Fig 1e). Red shift was observed at the Soret region for (Fig 1d – f). For HbAA (Fig 1d), at the Soret region, (0.2mM) was hyperchromic whereas the other concentration ranges from (0.38mM – 1mM) were all hypochromic. Hyperchromic shift was observed with concentration ranges of (0.2mM – 0.38mM) whereas (0.56mM – 1mM) had hypochromic shift for HbAS (Fig 1e). For HbSS (Fig 1f), hyperchromic shift was observed with (0.71mM – 1mM) as the concentration of SDS increased (0.2mM – 1mM). At the oxy-band region, for (Fig 1d – f), there was a total destruction of the peak as concentration of SDS increased (0.2mM – 1mM).

A comparison of the effects of SDS (0.2mM – 1mM) at pH 7.2 (Fig 1a – c) and pH 5.0 (Fig 1d – f) showed that with increasing concentration of SDS (0.2mM – 1mM), there was a decrease in absorbance at the aromatic amino region, Soret region and oxy-band regions at pH 7.2, whereas at pH 5.0, there was a remarkable increase in absorbance and complete flattening out of the spectra as concentration of SDS increased (0.2mM – 1mM). At pH 7.2 (Fig 1a – c), there was a distinct absorption peak at 415nm whereas at pH 5.0 (Fig 1d – f), the peak at 415nm was totally distorted. At pH 5.0 (Fig 1d - f), aggregation was formed with some concentrations whereas at pH 7.2 (Fig 1a – c), no aggregation was observed.

Discussion of Findings

Investigating Structural Changes

The investigation into specific structural changes induced by varying SDS concentrations at pH 7.2 and pH 5.0 provided insightful revelations. In the aromatic amino region, Soret region, and oxy-band regions, the study identified concentration-dependent alterations in Haemoglobin conformation. The decrease in absorbance and the disappearance of specific peaks at 273nm underscored the sensitivity of Haemoglobin to SDS concentration changes.

Exploring Aggregation Behaviour

The exploration of aggregations in the presence of SDS unearthed intriguing phenomena. Concentration ranges (0.2mM to 1mM) exhibited varying degrees of aggregation tendencies. Particularly noteworthy was the role of pH variations (7.2 vs. 5.0) in influencing aggregation behaviour. Lower pH (5.0) accentuated aggregations, showcasing a pH-dependent nature in SDS-induced Haemoglobin aggregation. The study shed light on the dynamic interplay between SDS concentration, pH, and the propensity for Haemoglobin to aggregate.

Characterizing Spectral Changes

The detailed characterization of spectral changes at different pH levels (7.2 and 5.0) and SDS concentrations provided a comprehensive understanding of the Haemoglobin response. The study revealed shifts in absorbance peaks, hyperchromic and hypochromic transitions, and the alteration or distortion of specific peaks. Notably, the absorption peak at 415nm emerged as a focal point, offering insights into the complex interplay of pH and SDS concentrations on Haemoglobin

spectra. The observed differences between pH levels (7.2 and 5.0) emphasized the contextual impact of pH on SDS-induced spectral changes.

In conclusion, this in-depth exploration successfully addressed the three objectives, unravelling the structural intricacies, aggregative tendencies, and spectral nuances influenced by SDS concentrations and pH variations. The findings contribute to our understanding of Haemoglobin behaviour in the presence of SDS, laying the groundwork for further research in the realm of protein conformation and aggregation.

Discussion of Findings (Comparing with the Literature)

Investigating Structural Changes

The study delved into specific structural changes induced by varying SDS concentrations (0.2mM to 1mM) at pH 7.2 and pH 5.0, with a focus on the aromatic amino region, Soret region, and oxy-band regions. The findings align with existing literature on SDS's role in modulating protein structures, especially in the aromatic amino acid environment. Previous studies by Khan et al. (2022) and Kumar et al. (2015) have highlighted the concentration-dependent influence of SDS on protein aggregation, corroborating our observation of decreased absorbance and peak disappearance.

Exploring Aggregation Behaviour

The exploration of aggregations in the presence of SDS resonates with studies by Alresaini et al. (2023) and Khan et al. (2022), which emphasize the surfactant's ability to induce various structural forms, from amorphous aggregates to amyloid-like fibrils. Our study's identification of pH-dependent aggregations aligns with the broader literature, emphasizing the critical role of environmental factors in governing protein aggregation kinetics (Burel et al., 2021). This supports the idea that SDS-induced aggregations are context-dependent and influenced by pH variations.

Characterizing Spectral Changes

The detailed characterization of spectral changes aligns with findings in studies by Qu et al. (2020) and Malyshev et al. (2022), illustrating the impact of surfactants on the spectral properties of biomolecules. The alteration of absorbance peaks, hyperchromic and hypochromic transitions, and the distortion of specific peaks are consistent with the literature on surfactant-protein interactions. The observed differences between pH levels (7.2 and 5.0) are in line with studies highlighting pH as a crucial factor influencing protein conformation and stability (Qu et al., 2020).

Comparative Analysis

Our study's findings provide valuable insights into SDS-induced changes in Haemoglobin, contributing to the existing body of literature on surfactant-protein interactions. The observed pH-dependent aggregations and spectral variations are in harmony with established principles in protein biochemistry. The contextual nuances revealed in this study underscore the need for a comprehensive understanding of pH-dependent surfactant interactions in the realm of protein conformational changes.

Future Directions:

While this study significantly advances our understanding of SDS-induced Haemoglobin alterations, further research is warranted to explore the underlying molecular mechanisms and to extend the investigation to other proteins. Additionally, investigating the implications of these structural changes on Haemoglobin function and potential pathological consequences could provide valuable insights for both basic science and clinical applications.

Implications of Findings

1. **Biophysical Insights:**

The study's detailed exploration of SDS-induced structural changes in Haemoglobin offers valuable biophysical insights. Understanding the concentration-dependent effects of SDS on protein conformation at different pH levels contributes to our knowledge of surfactant-protein interactions. This knowledge is crucial for researchers working on protein folding, aggregation, and stability.

2. **Context-Dependent Aggregation:**

The observed pH-dependent aggregations in the presence of SDS highlight the context-specific nature of protein aggregation. This finding has implications for industries and research areas where protein stability and aggregation tendencies are critical factors. Tailoring surfactant conditions based on environmental pH may be crucial for controlling protein behaviour in various applications.

3. **Biotechnological Applications:**

Insights gained from this study could be applied in biotechnological processes involving protein manipulation. Understanding how SDS influences structural changes in Haemoglobin at different pH levels provides a foundation for optimizing protein purification and formulation strategies. This has implications for industries ranging from pharmaceuticals to biopharmaceuticals.

4. **Clinical Relevance:**

Given the centrality of Haemoglobin in oxygen transport, any structural changes can have physiological consequences. The study's findings, especially the pH-dependent alterations, may have implications for understanding how surfactants interact with Haemoglobin in biological systems. This knowledge could be relevant in drug delivery systems or in comprehending the impact of surfactants on blood proteins.

5. **Methodological Considerations:**

The methodology employed in this study, particularly in characterizing spectral changes, adds to the toolkit of biophysical techniques for studying protein-surfactant interactions. Researchers and practitioners can draw upon these methodologies when investigating similar systems, enhancing the robustness and reproducibility of studies in this domain.

6. **Future Research Avenues:**

The observed complexities in SDS-induced Haemoglobin changes open avenues for future research. Further investigations into the molecular mechanisms underlying these changes, coupled with exploring the functional consequences of altered Haemoglobin structures, could deepen our understanding of surfactant-protein interactions.

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