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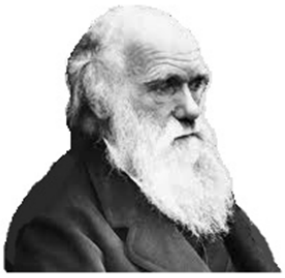

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Investigating the Structural and Spectroscopic Changes in Haemoglobin Variants (HbAA, HbAS, and HbSS) Induced by Sodium Dodecyl Sulphate (SDS) at Varying pH Levels

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ABSTRACT

This study delves into the intricate interplay between sodium dodecyl Sulphate (SDS) and haemoglobin variants—HbAA, HbAS, and HbSS—with a focus on unravelling the structural and spectroscopic alterations induced by SDS at varying pH levels. Employing a range of concentrations (0.8 mM – 4 mM) of SDS, the study conducted a thorough analysis of absorbance changes in the aromatic amino region, Soret region, and oxy-band region. At pH 7.2, increasing SDS concentrations prompted a consistent decrease in absorbance, especially in the aromatic amino and Soret regions. Notable blue shifts were observed, signalling structural modifications. Complete disappearance of peaks in the oxy-band region indicated a potential compromise in oxygen-binding capacity. At pH 5.0, in addition to reduced absorbance, a novel peak emerged at 506 nm, suggesting pH-dependent conformational alterations. The study compared the effects at pH 7.2 and pH 5.0, highlighting distinct changes and indicating the influence of pH on SDS-induced denaturation. The implications of these findings are significant, uncovering the vulnerability of haemoglobin variants to SDS-induced conformational changes and the pH sensitivity of these alterations. This research contributes valuable insights into the structural dynamics of haemoglobin, particularly in the context of haemoglobinopathies. The observed spectral modifications underscore the need for a nuanced understanding of experimental conditions involving SDS and haemoglobin, guiding future research toward a more comprehensive comprehension of the molecular mechanisms at play. This study enhances the understanding of SDS-induced denaturation in haemoglobin variants, providing a foundation for further exploration into potential therapeutic interventions and diagnostic strategies in the realm of haemoglobinopathies.

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Keywords: Haemoglobin Variants, Sodium Dodecyl Sulphate (SDS), Spectroscopic Analysis, pH-dependent Conformation, Hemoglobinopathies

1. INTRODUCTION

Haemoglobin, a crucial protein orchestrating oxygen transport in the human body, exists in diverse genetic variants, each bearing significant implications for human health. The normal adult haemoglobin, HbAA, stands in stark contrast to HbAS, associated with sickle cell trait, and HbSS, responsible for sickle cell disease (1). While extensive studies have unveiled the structural and functional disparities among these variants, their interactions with exogenous substances, particularly Sodium Dodecyl Sulphate (SDS), remain a relatively uncharted domain (2). Sickle cell disease (SCD), a hereditary blood disorder characterized by the abnormal sickling of red blood cells, poses formidable challenges to affected individuals, resulting in complications and a diminished quality of life (3, 4). An in-depth understanding of the underlying mechanisms contributing to the pathophysiology of SCD is imperative for devising effective diagnostic and therapeutic strategies. Despite SDS being a well-studied surfactant with the potential to modify protein structure and function (5-7), limited research has delved into its specific interactions with haemoglobin variants, particularly under varying pH conditions. This knowledge gap impedes the understanding of how exogenous substances, like SDS, might influence the behaviour and implications of haemoglobin variants, including HbAA, HbAS, and HbSS.

The interactions between SDS and haemoglobin variants assume significance due to their potential implications for protein stability, functionality, and overall behaviour (1, 8).

This research contributes to the comprehension of the underlying mechanisms involved in interactions between exogenous substances and haemoglobin variants. Insights into the alterations induced by SDS may uncover the impact of such factors on the stability and functionality of these variants.

Additionally, the study provides valuable information on the pH-dependent effects of SDS on haemoglobin variants, shedding light on factors influencing their behaviour in diverse physiological conditions. Correlating observed structural changes with functional implications, such as oxygen binding, stability, and overall functionality, has broader implications. This knowledge could guide the development of targeted therapeutic interventions and diagnostic approaches for individuals with haemoglobinopathies, including sickle cell disease.

This study addresses the knowledge gap regarding the interactions between SDS and haemoglobin variants under varying pH conditions. By investigating structural and spectroscopic changes, exploring pH-dependent effects, and correlating findings with functional implications, this research may advance the understanding of the behaviour and implications of HbAA, HbAS, and HbSS in the presence of SDS. Ultimately, these insights may contribute to improved diagnosis, management, and treatment of haemoglobinopathies, enhancing overall patient care and well-being.

Objectives

1) Characterize the impact of SDS on HbAA, HbAS, and HbSS at varying pH levels, focusing on structural changes and spectroscopic alterations.

2) Investigate the pH-dependent effects of SDS on the spectroscopy of haemoglobin variants, comparing and contrasting changes at pH 7.2 and pH 5.0.
3) Correlate observed structural changes induced by SDS with functional implications, exploring potential effects on oxygen binding and stability in HbAA, HbAS, and HbSS.

2. LITERATURE REVIEW

The prevalence of sickle cell disease (SCD) is particularly pronounced in Africa, with three-quarters of cases occurring on the continent. In Nigeria alone, around 2% of new-borns are affected by sickle cell anaemia, resulting in approximately 150,000 affected children born annually. Izuwa et al. aimed to elucidate the hemorheologic and fibrinolytic activities of HbSS, HbAS, and HbAA subjects to provide insights for proper management (9). Their findings emphasized the need for routine tests, including fibrinogen assay and relative blood viscosity, in the management of sickle cell anaemia patients.

Sickle cell disease (SCD) arises from a mutation in the beta-globin gene, resulting in the production of abnormal haemoglobin. Arishi et al. conducted a review highlighting various techniques for SCD detection, ranging from screening tests to advanced portable point-of-care methods (10). Early detection is crucial for effective disease management, and the review provides an overview of current and emerging techniques, including smartphone-based methods and sensor-based platforms.

Reza et al. explored the effect of sodium n-dodecyl Sulphate (SDS) on haemoglobin autoxidation (11). Their comprehensive study, employing multiple methods such as spectrophotometry and cyclic voltammetry, revealed that low SDS concentrations increased the deoxy form of haemoglobin, suggesting a more stable conformation.

Du et al. investigated the functional and conformational changes in highland barley proteins (HBPs) upon binding with salidroside (12). The study revealed altered secondary structure and improved functional properties in HBPs-salidroside complexes, showcasing the potential of salidroside to enhance the structural characteristics and functionality of HBPs.

Hou et al. delved into the activity-conformation relationship of protein tyrosine phosphatase (PTPase) in the presence of sodium dodecyl Sulphate (SDS) (13). Their study demonstrated that SDS-induced conformational transitions led to PTPase inactivation, shedding light on the intricate interplay between activity and conformation.

Liu et al. explored the interactions of haemoglobin with SDS and dodecyl trimethylammonium bromide (DTAB) (8). Their observations indicated electrostatic and hydrophobic interactions between SDS and haemoglobin, with pH influencing the predominant mode of interaction.

Thom et al. provided a comprehensive review of haemoglobin variants, emphasizing general concepts and illustrative examples (1). The review covered more than 1000 naturally occurring human haemoglobin variants, offering insights into haemoglobin biochemistry and biology with implications for haematology practice.

Otzen et al. addressed the controversy surrounding the interaction of SDS with proteins (7). Their study decisively supported the core-shell model, highlighting the structural evolution of protein-surfactant complexes and the reversibility of SDS denaturation.

Jafari et al. conducted a comparative study on the molecular interactions of SDS with human ubiquitin (5). Their molecular dynamics simulations revealed that SDS disrupted the hydration shell and expanded the hydrophobic core of ubiquitin, inducing complete protein unfolding. Inusa et al. provided a systematic review of sickle cell disease, covering genetic, pathologic, and clinical aspects (4). The review underscored the phenotypic variation in clinical presentation and disease outcomes, emphasizing the importance of understanding the pathogenesis for therapeutic development and intervention.

These diverse studies contribute to the evolving landscape of research on haemoglobin variants, their interactions with substances like SDS, and the broader implications for disease management and detection.

3. MATERIALS AND METHODS

Packing and Equilibration of the Pre-cycled Gel (DEAE-cellulose): The pre-cycled gel was dissolved in 1mM Tris-HCl buffer, pH 8.5. The gel was packed into a column (2.5 (i.d) x 13.0 cm) and equilibrated with 0.001M Tris-HCl buffer pH 8.5. The flow rate was 45 minutes/hour.

Blood Samples: Blood samples from an individual with genotype AS were collected and confirmed by gel electrophoresis. 4ml of blood was collected into a wash bottle containing 0.001M Tris-HCl buffer-saline pH 8.5.

Preparation of Relaxed State Haemoglobin: Blood samples were centrifuged, and the supernatant was recovered as crude Hb. The crude Hb was made 5% NaCl (w/v), and the precipitated anions and erythrocyte proteins were discarded.

Dialysis of Crude Haemoglobin: Dialysis was carried out at 40 °C against 0.05M Tris-HCl buffer, pH 8.5. Dialysis was carried out for 12 hours with a buffer change at the 7th hour.

Purification using Ion-Exchange Chromatography: The crude haemoglobin was separated using DEAE-cellulose ion-exchange chromatography at 40 °C. Eluates were collected, and their absorbencies were measured at 541 nm.

Dialysis of the Eluates: Eluates for HbSS and HbAA with high absorbances were pooled and dialyzed against 0.05M Tris-HCl, pH 7.2, and 0.05M acetate buffer, pH 5.0, respectively.

Spectroscopic Analysis for HbAA, HbAS, and HbSS: The R-state of HbAA, HbAS, and HbSS were used for spectroscopic analysis. A 1 in 3 dilution of crude HbAA, HbAS, and HbSS was prepared and scanned between 250 – 650 nm using a UV-Vis spectrophotometer.

Lipid Peroxidation Determination: Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA).

Preparation of Lipid Peroxidation Standard Curve Using 1,1,3,3-Tetraethoxypropane (TEP): TEP reacts with HCl to release MDA, and a standard curve of absorbance against MDA concentration was plotted.

Incubation of Haemoglobin with Linoleic Acid and Hydrogen Peroxide: Haemoglobin was incubated with linoleic acid and hydrogen peroxide with and without SDS, and the spectra were obtained at the range of 250 nm – 650 nm using a UV-Visible scanned Spectrophotometer.

4. RESULTS

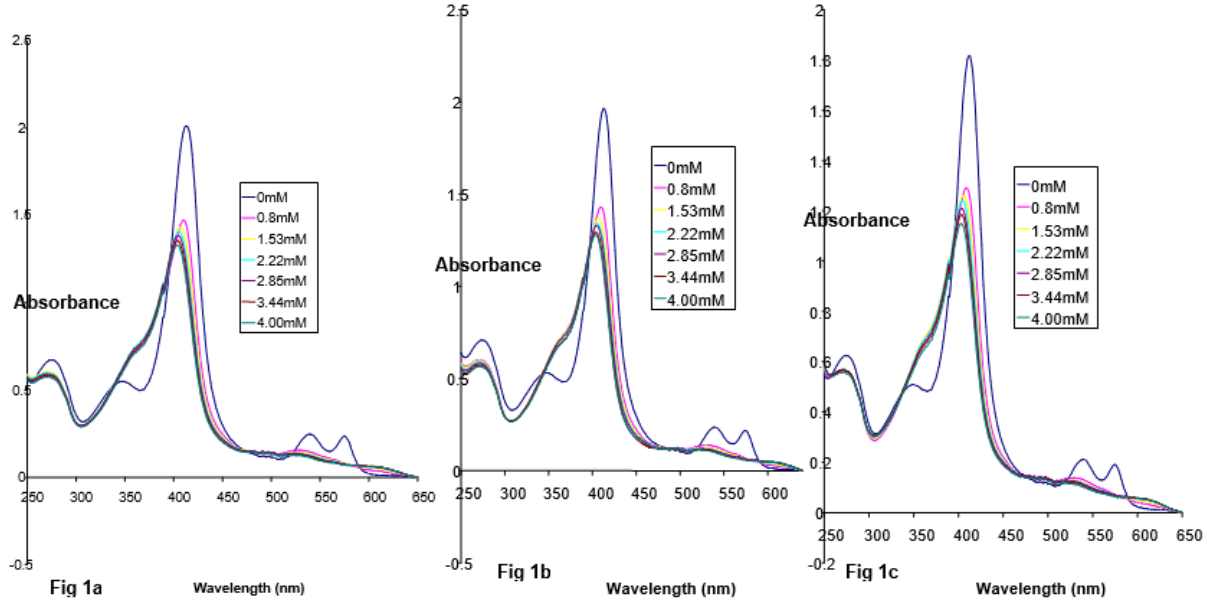

Fig. 1. Effect of SDS (0.8 mM – 4.00 mM) on Haemoglobin at 7.2. (Fig 1a) HbAA, (Fig 1b) HbAS and (Fig 1c) HbSS. 0 mM – no SDS

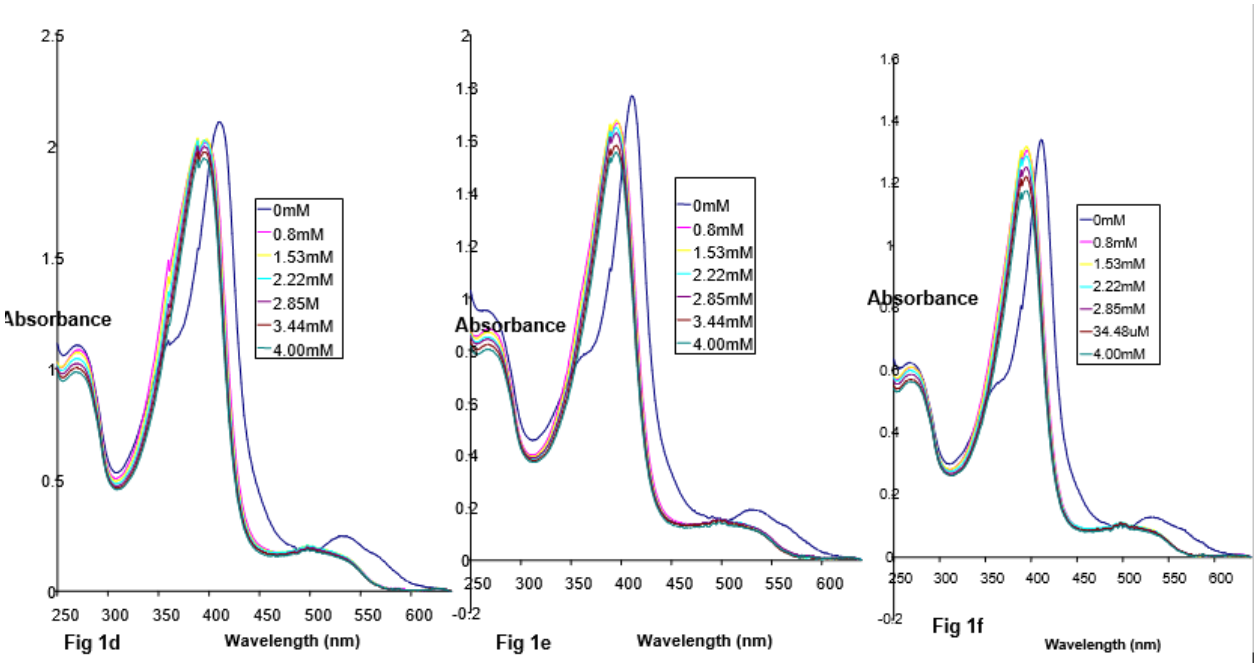

Fig. 2. Effect of SDS (0.8 mM – 4.00 mM) on Haemoglobin at pH 5.0. (Fig 1d) HbAA, (Fig 1e) HbAS and (Fig 1f) HbSS. 0 mM – no SDS.

HbAA (Fig 1a), HbAS (Fig 1b) and HbSS (Fig 1c) at pH 7.2, with increasing concentration of SDS (0.8mM – 4mM), there was a decrease in absorbance at the aromatic amino region and the Soret region. At the Soret region too, for (Fig 1a – c), a blue shift was observed. At the oxy-band region, there was a total destruction of the peaks at 540nm and 577 nm as the concentration of SDS increases (0.8mM – 4mM) for (Fig 1a – c).

As the concentration of SDS was increased (0.8mM – 4mM) at pH 5.0, HbAA (Fig 1d), HbAS (Fig 1e) and HbSS (Fig 1f) decreased in absorbance at the aromatic amino region and at the Soret region. Complete disappearance of the delta peak and a blue shift was observed at the Soret region. A new peak was observed at 506nm with a complete disappearance of the 540nm peak as the concentration of SDS increased (0.8mM – 4mM) at the oxy-band region.

A comparison of the effects of SDS (0.8mM – 4mM) at pH 7.2 (Fig 1a – c) and pH 5.0 (Fig 1d – f) showed that, at both pH, with increasing concentration of SDS (0.8mM – 4mM), a decrease in absorbance was observed at the aromatic amino region, and Soret region. At both pH, the peaks at the oxy-band region were destroyed. At pH 5.0 (Fig 1d – f), a new peak was formed at 506nm but the oxy-band peaks where completely destroyed at pH 7.2 (Fig 1a – c) as the concentration of SDS increased (0.8mM – 4mM). The blue shift observed at pH 5.0 went outside the control spectrum (no SDS) and formed another peak at 400nm while the blue shift observed at pH 7.2, was still within the control spectrum (no SDS).

5. DISCUSSION OF FINDINGS

1) Absorbance Changes at pH 7.2: The observed decrease in absorbance at the aromatic amino region and the Soret region for HbAA, HbAS, and HbSS at pH 7.2 with increasing SDS concentration aligns with the literature, indicating the denaturing effect of SDS on proteins (5). The blue shift at the Soret region suggests alterations in the haem environment, possibly indicative of conformational changes induced by SDS (1). The total destruction of peaks at 540 nm and 577nm in the oxy-band region implies significant disruption to the oxyhaemoglobin structure. This is consistent with SDS's known ability to unfold protein structures and perturb the haem pocket (11). The disappearance of peaks in the oxy-band region correlates with the unfolding of haemoglobin structures, indicating a loss of oxygen-binding capacity.

2) Effects at pH 5.0: At pH 5.0, the decrease in absorbance at the aromatic amino region and the Soret region, along with the blue shift, suggests a similar denaturing effect of SDS on haemoglobin variants. The disappearance of the delta peak and the emergence of a new peak at 506nm in the oxy-band region indicate structural modifications induced by SDS. This phenomenon may be associated with changes in the tertiary structure of haemoglobin, reflecting the influence of SDS on the protein's environment.

3) Comparison of pH 7.2 and pH 5.0 Effects: Comparing the effects of SDS at pH 7.2 and pH 5.0 reveals interesting differences. At both pH levels, increasing SDS concentration led to a decrease in absorbance at the aromatic amino region and the Soret region, indicative of unfolding and denaturation. However, at pH 5.0, the formation of a new peak at 506nm and the appearance of a peak at 400nm during the blue shift suggest more profound conformational changes. The destruction of oxy-band peaks at pH 5.0 and the emergence of a new peak at 506 nm indicate a more intricate interaction between SDS and haemoglobin variants. The blue shift

extending beyond the control spectrum at pH 5.0 suggests a more pronounced alteration in the haem environment, possibly affecting the overall stability and function of haemoglobin.

4) Comparison with Literature: The findings align with literature on the denaturing effects of SDS on proteins, emphasizing the influence of pH on these interactions (5). The observed alterations in the Soret and oxy-band regions are consistent with studies highlighting SDS-induced conformational changes in haemoglobin (11). The emergence of new peaks and the destruction of oxy-band peaks correlate with SDS-induced modifications in the tertiary and quaternary structures of haemoglobin (12). This study provides valuable insights into the pH-dependent effects of SDS on the spectroscopy of HbAA, HbAS, and HbSS. The observed changes in absorbance and peak positions signify structural alterations induced by SDS, with variations at different pH levels. Understanding these interactions is crucial for unravelling the complexities of haemoglobin denaturation and can inform therapeutic approaches in haemoglobinopathies.

5. 1. Implications of Findings

1) **Structural Vulnerability to SDS:** The observed alterations in absorbance and spectral peaks indicate that haemoglobin variants (HbAA, HbAS, and HbSS) are structurally vulnerable to the denaturing effects of SDS. This vulnerability is particularly significant as it implies a potential disruption of the normal haemoglobin structure, which may have implications for its physiological functions.
2) **pH Dependency in Denaturation:** The study underscores the importance of pH in determining the extent and nature of SDS-induced denaturation. The variations in spectral changes between pH 7.2 and pH 5.0 suggest that the impact of SDS on haemoglobin is influenced by the environmental acidity. This pH dependency should be considered in any therapeutic or diagnostic applications involving SDS and haemoglobin.
3) **Oxygen-Binding Capacity:** The destruction of peaks in the oxy-band region implies a potential compromise in the oxygen-binding capacity of haemoglobin in the presence of SDS. This finding has critical implications for individuals with haemoglobinopathies, as it suggests that exposure to SDS or similar denaturing agents might affect the oxygen transport function of haemoglobin.
4) **Conformational Changes and Functional Implications:** The emergence of new peaks, blue shifts, and alterations in spectral features indicate conformational changes induced by SDS. These changes may have functional implications for haemoglobin, influencing its stability, oxygen-binding kinetics, and overall performance. Understanding these structural modifications is crucial for predicting how SDS may affect the physiological behaviour of haemoglobin.
5) **Clinical Relevance in Haemoglobinopathies:** Given that HbSS is associated with sickle cell anaemia, the study's findings may have clinical relevance. The structural changes observed in HbSS in the presence of SDS could contribute insights into the molecular dynamics of sickle cell haemoglobin under denaturing conditions. This knowledge could potentially inform therapeutic strategies or diagnostic methodologies for sickle cell disease.

6) **Methodological Considerations:** The study provides insights into the use of SDS in experimental setups involving haemoglobin variants. Researchers and clinicians should be aware of the potential structural modifications induced by SDS, especially when conducting studies that involve spectroscopic analysis or when utilizing SDS as part of experimental protocols. Methodologies involving SDS should be tailored considering the observed pH-dependent effects.

7) **Future Research Avenues:** The study opens avenues for further research, particularly in understanding the detailed mechanisms behind SDS-induced conformational changes in haemoglobin. Future investigations could explore the specific amino acid residues and regions affected, the reversibility of these changes, and the downstream physiological consequences. Additionally, the study prompts further exploration of SDS as a potential tool for probing haemoglobin structure and function.

The implications of these findings extend beyond the laboratory setting, carrying relevance for clinical applications and advancing the understanding of how external agents like SDS interact with haemoglobin, particularly in the context of haemoglobinopathies.

6. CONCLUSIONS

In conclusion, this study investigated the impact of sodium dodecyl Sulphate (SDS) on the structural and spectroscopic properties of haemoglobin variants—HbAA, HbAS, and HbSS—at different pH levels. The findings provide valuable insights into the conformational changes induced by SDS and their potential implications for the functional behaviour of haemoglobin.

The study revealed that increasing concentrations of SDS led to a significant decrease in absorbance at the aromatic amino region and the Soret region for all haemoglobin variants at both pH 7.2 and pH 5.0. A notable blue shift was observed in the Soret region, indicating structural alterations. The oxy-band region exhibited a complete destruction of peaks, suggesting a potential compromise in the oxygen-binding capacity of haemoglobin in the presence of SDS.

Importantly, the pH dependency of SDS-induced denaturation was highlighted, with distinct spectral changes observed at pH 7.2 and pH 5.0. At the lower pH, a new peak at 506nm emerged, indicating pH-dependent conformational modifications.

The implications of these findings extend to the structural vulnerability of haemoglobin variants to SDS, the potential compromise in oxygen-binding capacity, and the importance of pH in modulating SDS-induced conformational changes. These insights are particularly relevant for understanding the behaviour of haemoglobin, especially in the context of haemoglobinopathies such as sickle cell disease.

The study contributes to the existing body of knowledge on haemoglobin structure and function under denaturing conditions. The observed alterations underscore the importance of considering environmental factors, such as pH, in experimental setups involving SDS and haemoglobin. Additionally, the findings pave the way for future research avenues, including a more detailed exploration of the specific molecular mechanisms behind SDS-induced conformational changes and their potential reversibility. This study enhances the understanding of the interplay between SDS and haemoglobin variants, shedding light on the structural

dynamics of haemoglobin under denaturing conditions. These insights have implications for both experimental methodologies involving haemoglobin and the broader context of haemoglobinopathies, opening doors for further investigations into potential therapeutic interventions and diagnostic approaches.

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