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Protein structure analysis of abnormal hemoglobins; Hb D-Los Angeles, Hb S and Hb G-Coushatta

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Abstract

Computational methods have been extensively utilized for protein structure prediction and modeling, including hemoglobin. These methods have provided valuable insights into the three-dimensional conformation of hemoglobin, its allosteric regulation, and its oxygen-binding capacity. For instance, computational modeling has been employed to understand the structural mechanism of hemoglobin, shedding light on the equilibrium between alternative structures, like the tense (T) and relaxed (R) states, which influence oxygen affinity and binding. Abnormal hemoglobins are associated with various genetic and clinical implications. The mutations in the globin genes to cause changes in the molecular structure of hemoglobin, affecting its biological properties such as oxygen carrying capacity and stability. RMSD is the mean displacement of atoms or molecular structures. Therefore, RMSD is a preferred parameter in structural similarity modeling studies. RMSD values calculated for pairwise protein structures are Hb A (Normal Hb)-Hb D-Los Angeles 3.44 Å, Hb A-Hb S 3.339 Å, Hb A-Hb G-Coushatta 3.35 Å. The aim of this study is to discuss the possible structural and electrical properties of abnormal haemoglobin molecules using protein modelling.

Keywords: Hemoglobin, protein models, structural analysis

Introduction

Abnormal hemoglobins are associated with various genetic and clinical implications. Globin gene mutations cause changes in hemoglobin protein properties, affecting its biological properties such as oxygen carrying capacity and stability [1]. The beta globin gene cluster is the most preferred of in human, Because of its association with severe forms of inherited hemoglobin disorders [2]. Comparative structural analysis of normal and abnormal hemoglobins is useful for understanding the causes of genetic diseases. [3,4]. In this study, we aimed to discuss comparative 3D (three-dimensional) protein structure models of abnormal hemoglobins previously published in Türkiye. Computational methods have been extensively utilized for protein structure prediction and modeling, including hemoglobin. These methods have provided valuable insights into the three-dimensional conformation of hemoglobin, its allosteric regulation, and its oxygen-binding capacity. For instance, computational modeling has been employed to understand the structural mechanism of hemoglobin, shedding light on the equilibrium between alternative structures, like the tense (T) and relaxed (R) states, which influence oxygen affinity and binding. Additionally, computational methods have been crucial in predicting the structures of hemoglobin and other proteins, particularly when experimental structure determination methods are not feasible [5-8]. Furthermore, computational softwares have been utilized to study the interaction of hemoglobin with ligands and to investigate the role of specific protein regions, like the α-globin H helix, in the hemoglobin. These studies have provided information on structural features of hemoglobin's oxygen-binding capacity and its functional properties [9-11].

The three-dimensional and conformational structures of hemoglobin variants such as Hb D-Los Angeles, Hb S, Hb G-Coushatta, and normal Hb (Hb A) play a crucial role in understanding their functional properties and clinical implications. The structural differences in these hemoglobin variants are attributed to specific amino acid substitutions, which

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result in alterations in the quaternary structure and functional properties of the hemoglobin molecule [12].

There are more than 250 million Hb molecules in each human erythrocyte. This protects erythrocytes against proteolysis. It ensures their stability and low colloid osmotic pressure, preventing their loss by filtration through the kidneys. Normal Hb concentration is between 120 and 160 mg mL-1 [13]. The molecular structure of Hb consists of 4 chains (2α-2β) and has a total molecular mass (~64 kDa). This structure is characterized by C2 symmetry (Figure 1) [14]. Each chain contains a heme group, a complex structure consisting of an iron ion (Fe2+) coordinated with a porphyrin ring. The iron ion is where oxygen binds, forming oxyhemoglobin. When oxygen binds to a heme group, the oxygen affinity of other heme groups increases Hemoglobin has heme prosthetic groups consisting of a protoporphyrin ring in each subunit and a single iron ion in the center, led by the proximal histidine of α-helix F. Oxygen binds reversibly to these groups (Figure 1). Upon oxygen binding, one αβ-dimer rotates (15°) against the other αβ-dimer. With this conformational change, there is a shift from a tense (T) to a relaxed (R) state [15]. The iron ions in the heme groups can undergo oxidation and reduction reactions, changing the overall charge of the molecule. When oxygen binds to the iron ion, it becomes more positively charged. Hemoglobin has a net negative charge at normal pH due to the amino acid residues on its surface. Hemoglobin exhibits pH sensitivity, known as the Bohr effect. At lower pH (higher acidity), hemoglobin's affinity for oxygen decreases, facilitating oxygen release in metabolically active tissues. This pH sensitivity is essential for the dynamics of O_2 binding and dissociation from hemoglobin. The overall charge of hemoglobin is roughly neutral to maintain electrical balance in the red blood cell. The combination of positive charges on the iron ions and negative charges on the surrounding amino acid residues helps maintain this electroneutrality [16].

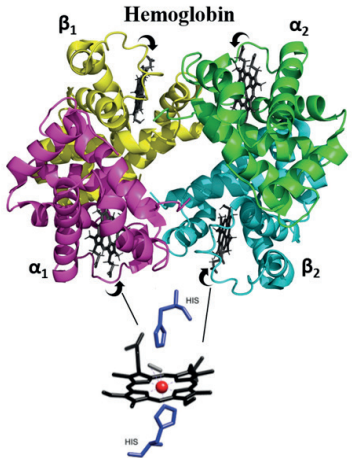

Figure 1. Human hemoglobin structure (α1, α2, β1, β2), heme groups (Fe2+ protoporphyrin IX). Heme groups are marked with arrows. Proximal histidine binds directly with the Fe2+, but distal histidine is thought to form hydrogen bonds with oxygen. Heme group is colored black; iron is colored red; histidines (His) is colored blue

Hb D-Los Angeles [β121 (GH4) Glu→Gln]

Hb D-Los Angeles (HbD) has also been published in the literature under different names. The large number of studies on this abnormal hemoglobin worldwide has been very useful for genetic analysis and mutation research. In this respect, it is one of the most common abnormal hemoglobin (Hb) variants worldwide, including Italy, Belgium, Austria and Türkiye [17]. According to the results published in Türkiye, the haplotype indicating the genetic origin of Hb D-Los Angeles is Mediterranean haplotype I [+ − − − − + +], which is the most frequently observed with 34.6%. The same haplotype also ranked first in the normal population [4]. In the study on haplotype diversity, it was calculated that the Hb D-Los Ageles mutation detected in Denizli region occurred approximately 38,000 ybp. The age of the normal population compared in this study was calculated as 42,000 ybp. Although it is not clinically important, it has structural similarities that may cause diagnostic errors in laboratory screening [18]. Due to it is molecular structure, Hb D Los Angeles electrophoretic mobility at alkaline pH may be similar to other abnormal hemoglobins. Homozygous HbDD and heterozygous HbD alone may not be clinically symptomatic. The combination of this mutation with β-thalassemia or other abnormal hemoglobins is of clinical importance. For example, combinations of HbD variants with HbS show different clinical outcomes. The combination of HbD Punjab with HbS creates clinically severe conditions in sickle cell disease. However, HbD Iran and HbD Ibadan cause clinically benign conditions with HbS [12]. Although HbD is clinically asymptomatic, it is important to investigate conformational structure interactions in terms of possible combinations with other abnormal hemoglobin variants or beta thalassaemias. The structural differences in Hb D Los Angeles, compared to normal Hb, are attributed to specific amino acid substitutions, leading to alterations in the quaternary structure and functional properties of the hemoglobin molecule.

Hb S [β6(A3) Glu→Val]

Hemoglobin S (HbS), which causes serious clinical problems, is caused by a mutation in the 6th codon of the beta-globin gene (GAG→GTG (dbSNP: rs334; NG_000007.3:g.70614A>T). In a study conducted in Denizli, Türkiye, the haplotype type representing this abnormal hemoglobin was reported to be 20.8%, [+ - - - - + +]. Interestingly, this haplotype type does not appear to be included in the normal population haplotype diversity in the region [19]. The dating of the HbS mutation in the region was reported as 26,000 ybp. The normal population was dated as 42,000 ybp [19]. The Hb S mutation causes a conformational change in the formation of hydrophobic bonds between β chains under non-oxygenated conditions. This change causes the tetramers to fiber and red blood cells (RBCs) to become sickle-shaped. This is also the case for variants that cause the formation of bonds that can cause the tetramers to become fibrous. Hb C and Hb O-Arab in combination with Hb S are also clinically symptomatic. The combination of these two variants with Hb S leads to xerocytosis. For this to happen, the intraerythrocytic Hb S concentration increases and enhances sickling [20]. As a result,

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when using conventional Hb diagnostic tests, the patient may appear to have only sickle cell trait [20]. The valine substitution promotes the formation of long polymers of Hb S molecules within the red blood cells, leading to the characteristic sickle shape. This structural alteration leads to the characteristic sickle shape of red blood cells and affects the oxygen binding and release properties of hemoglobin (Figure 2 and 3).

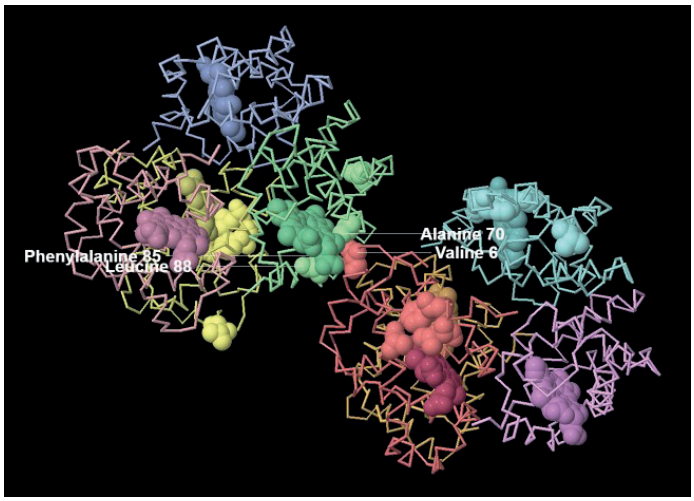

Figure 2. Alpha, Beta, Deoxy-Heme, Mutant Valine 6, Hydrophobic Pocket. The beta-chain mutant valine-6 binds in a hydrophobic pocket that is exposed on the surface of the deoxy-conformation of the beta chain. Leucine 88 and Phenylalanine 85 have been confirmed as crucial for polymerization of sickle hemoglobin by mutational analysis [21-23]

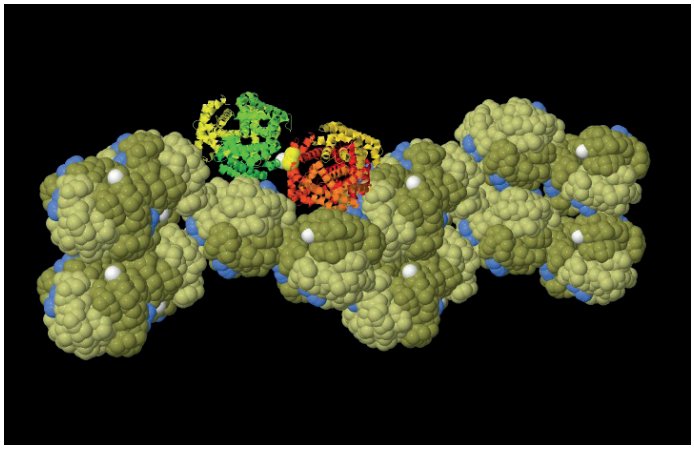

Figure 3. Image show only 2 strands of 8 molecules each, with each amino acid simplified to a single alpha-carbon atom. In reality, each sickle hemoglobin fiber has >1.000 molecules, and many fibers can form in a single cell. Each fiber consists of 7 pairs of strands (14 strands). One "strand" consists of a chain of >100 molecules an extension of the eight-molecule strand shown here [24-27]

Hb G-Coushatta [β22(B4) Glu→Ala]

Hb G-Coushatta [β22 (B4) Glu→Ala] (HBB: c.68A>C) was first described in 1964 [28]. Although there is limited data reported worldwide, haplotypes representing 4 different genetic origins have been published. Hb G-Coushatta data associated with the [− + − + + + +] haplotype in Denizli, Türkiye have been published [4,29]. It has been reported that the Hb G-Coushatta cases in this region date back to 38,000 ybp [30]. Similar to Hb D-Los Angeles, individuals with Hb G Coushatta may experience mild hemolytic anemia, but the clinical severity can vary. Hb G-Coushatta and Hb D-Los Angeles may be confused with Hb S during laboratory identification. The reason for this confusion is that such abnormal haemoglobins show similar electrophoretic and chromatographic properties to Hb S, especially in alkaline media. The aim of this study is to discuss the possible structural and electrical properties of abnormal haemoglobin molecules using protein modelling.

Material and Methods

The normal and abnormal hemoglobin protein models were obtained from the protein data bank [31]. 2H35, 2HBS, and 2YRS models were used for Hb A, Hb S, and Hb D-Los Angeles, respectively [25,32,33]. Protein models for Hb G-Coushatta were made using the PyMOL mutation wizard and the backbone-dependent, rotamers function, using the 2H35 protein model as a template. Protein models were visualized with PyMOL [34]. The model comparisons and RMSD values were analyzed using the PyMOL superimpose function.

Results

RMSD is the average displacement of the parameters in the modeled atom or molecule structure with respect to the normal in three-dimensional space. Therefore, the RMSD value is preferred for determining structural similarities and/or differences. In addition, it facilitates interatomic comparison when performing simulation alignment studies on the structure under investigation [35]. Different RMSD profiles are observed in Figure 4 and Figure 5. RMSD values calculated for pairwise protein structures are Hb A (Normal Hb)-Hb D-Los Angeles 3.44 Å, Hb A-Hb S 3.339 Å, Hb A-Hb G-Coushatta 3.35 Å. Figure 6 showed that flexible structure alignment of hemoglobins with surface models. This view were used to highlight the comparison of proteins. In this study, RMSD values were determined for pairwise hemoglobin structure comparisons, analysis of the effects of mutations on ligand binding and oxygenation, analysis of structural differences between the identified hemoglobin variants and identification of common structural domains.

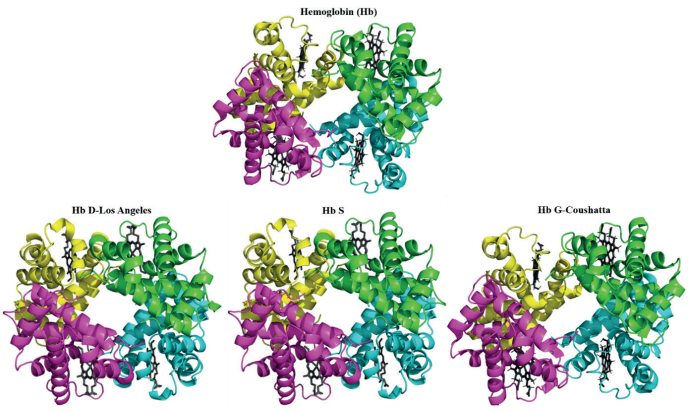

Figure 4. Quaternary structural features of the (Normal Hb, Hb D-Los Angeles, Hb S and Hb G-Coushatta) oxy-hemoglobins tetramer are presented as cartoons. Alpha 1 and 2 chains are coloured pink, green and beta 1, 2 chains are coloured light yellow and cyan, respectively

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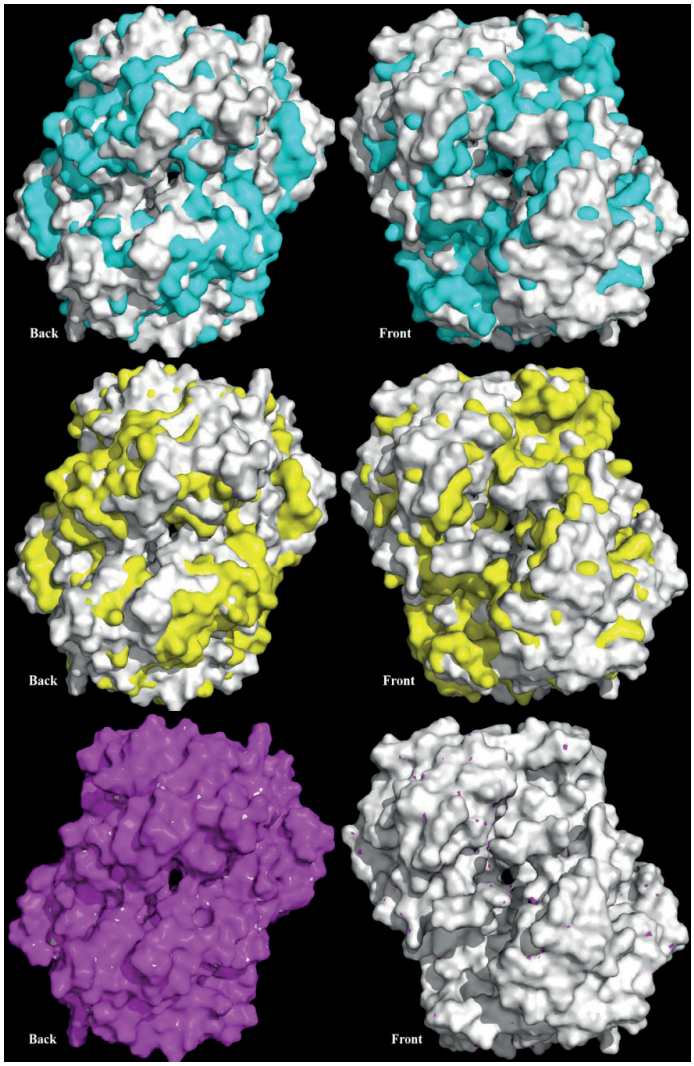

Figure 5. Protein structure alignment of hemoglobins with surface models. This view were used to highlight the comparison of proteins. Hb A (Normal Hb), Hb D-Los Angeles Hb S and Hb G-Coushatta are coloured white, cyan, yellow and pink, respectively

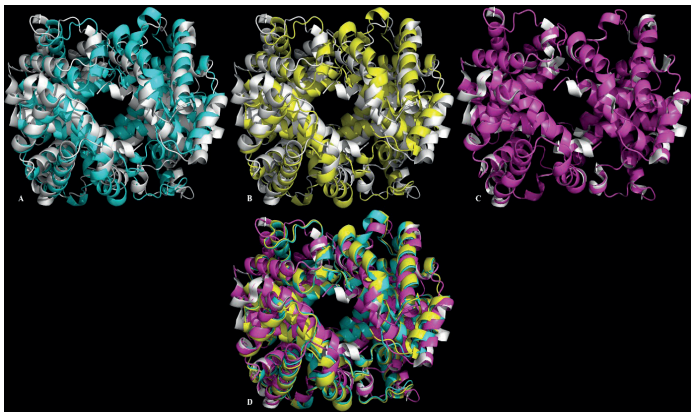

Figure 6. Pairwise protein structure comparison are presented as cartoon. **A**; comparison with Hb A (Normal Hb) (coloured white) and Hb D-Los Angeles (coloured cyan), **B**; comparison with Hb A and Hb S (coloured yellow), **C**; comparison with Hb A and Hb G-Coushatta (coloured pink), **D**; comparison of all samples with each other

Discussion

RMSD values are considered as reliable indicators of variability of structure when applied to very similar conformational proteins, like alternative conformations of the same protein. According to the different RMSD profile values presented in the study, it was determined that the molecular structure similarity rate between Hb S and Normal Hb (Hb A-Hb S 3.339 Å) was high compared to others. Molecular structure similarity was also observed between Hb A-Hb G-Coushatta 3.35 Å. However, Hb D-Los Angeles was found to have structural differences according to both RMSD value (Hb A-Hb D-Los Angeles 3.44 Å) and surface models (Figure 4 and Figure 5). This similarity in RMSD values explains why Hb G-Coushatta and Hb D-Los Angeles can be confused with Hb S in laboratory identification. These abnormal hemoglobins show common electrophoretic characteristics with Hb S due to their identical structural and electrical properties. In Figure 6, the pairwise protein structure comparison is presented as cartoon. Accordingly, Hb G-Coushatta and normal hemoglobin show high structural compatibility. This compatibility is also seen in Figure 5.

Conclusion

In conclusion, the findings presented in this study may provide valuable contributions to the structural analysis and modeling approaches of abnormal hemoglobin for researchers.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The study includes modeling results on protein sequences from open access web databases using bioinformatics software. No analysis was performed using living tissue or material obtained from living organisms.

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