Research in Molecular Medicine

Physicochemical Properties, Antioxidant Activity, and Membrane Association of Alpha and Beta Globin Chains of Hemoglobin

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ABSTRACT

Background: This study aimed to predict the physicochemical properties, antioxidant activity, and potential penetration of alpha helices of the alpha- and beta-globin chains of human hemoglobin in membranes of red blood cells (RBCs).

Materials and Methods: The physicochemical properties of hemoglobin's alpha- and beta-globin chains were predicted using the ProtScale tool. Antioxidant activity was predicted using AnOxPePred, and transmembrane regions were identified using TMHMM software, version 2.0. Statistical analysis was conducted using the Kolmogorov-Smirnov test and Pearson correlation in SPSS software, version 27.

Results: Based on the prediction results, a significant direct correlation was observed between hydrophobicity and membraneanchoring tendency for both alpha globin ($r=0.494$, $P<0.001$) and beta-globin ($r=0.869$, $P<0.001$) chains. The highest free radical scavenging (FRS) score in the alpha globin chain was associated with the sequence WGKVGAHAGEYGAEALERMF (residues 14-33), while the highest FRS in the beta-globin chain was linked to the sequence VLVCVLAHHFGKEFTPPV (residues 113-126). Alpha globin chains showed a higher tendency to penetrate the inner layer of the cell membrane of RBCs than beta-globin chains.

Conclusion: Regions with higher FRS scores are more exposed to the cytoplasm, which contains more free radicals.

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Introduction

The hemoglobin tetramer is composed of two alpha-globin chains and two beta-globin chains, with each monomer containing a polypeptide chain and a prosthetic heme group [1]. The structural analysis of human hemoglobin tetramer is performed using various methods, such as X-ray crystallography, nuclear magnetic resonance, and electron microscopy, to examine its behavior under different environmental conditions and in various conformations, including oxygenated and deoxygenated states [2, 3]. This study aimed to explore the biophysical and biochemical properties of this tetramer, focusing on the amino acid sequence that makes up its structure and the molecular model of human hemoglobin (Protein Data Bank [PDB] ID: 7VDE) [1-3].

Materials and Methods

Preparation of the molecular model of hemoglobin (PDB ID: 7VDE)

The molecular model of human hemoglobin, obtained using cryo-electron microscopy, was determined with a resolution of 3.6 angstroms. It has a molecular weight of 65.07 kDa and consists of 4,555 atoms and 574 amino acids, including two alpha-globin chains and two beta-globin chains. It was retrieved from the research collaboratory for structural bioinformatics (RCSB) at RCSB Protein Data Bank [3]. We utilized Molegro Molecular Viewer software, version 2.5 to visualize the model [4].

The biochemical and biophysical properties of the hemoglobin molecule

Utilizing the ProtScale tool, a subset of the Expasy database [5], we generated amino acid-scale profiles and visualized the protein sequences in 2D. ProtScale provides 57 predefined amino acid scales based on physicochemical properties and employs a sliding window algorithm for analysis. Users can adjust the window size and apply edge weighting using linear or exponential models. Standardization options are available for comparing scales, with strong signals indicating structural features that may necessitate validation. The methodology is rooted in biophysical principles, with the workflow encompassing selecting scales, setting the window, and standardizing the data to identify antigenic regions. Limitations of the tool include signal correlation and edge effects on terminal residues. We conducted a predictive analysis of the physical properties, such as residue weight, volume, hydrophobicity, polarity, and average flexibility of each amino acid in the sequence of alpha- and beta-globin of human hemoglobin [6-10].

The distribution of the studied variables was analyzed using the Kolmogorov-Smirnov test, and the correlation between the transmembrane tendency of amino acids in the alpha- and beta-globin chains and their hydrophobicity was investigated using the Pearson Correlation statistical test. All statistical analyses were performed using SPSS software, version 27 [11].

The antioxidant and chelating properties of the alpha- and beta-globin chains of hemoglobin

To assess the antioxidant activity prediction of the alpha and beta globin chains, we utilized the online tool AnOxPePred, available at the Technical University of Denmark (DTU) Health Tech website. AnOxPePred is a computational platform that predicts the antioxidative properties of peptides using convolutional neural networks. It focuses on predicting free radical scavenging (FRS) and metal ion chelation (CHEL) abilities for peptides with 2-30 residues, ideally between 10 and 20. The platform accepts FASTA inputs and can differentiate between intact proteins and pre-digested peptides. It includes a protein mode for simulating cleavage, a proteolysis mode, and a peptide mode for direct analysis. Validation of the predictions relies on accurate datasets. The FRS score indicates the extent of antioxidant activity and free radical neutralization for each sequence. The chelating activity of the alpha- and beta-globin chains was determined using the CHEL score [12, 13].

Prediction of transmembrane alpha helices in globin chain sequences

Initially, we assessed the likelihood of the alpha- and beta-globin chain sequences crossing the cell membrane using the transmembrane tendency prediction tool available in ProtScale tool. Subsequently, we utilized the TMHMM software, version 2.0, available at the Technical University of Denmark (DTU) Health Tech website, to determine the probability of membrane localization for each of the alpha and betaglobin chains. TMHMM software, version 2.0 employs hidden Markov models for predicting transmembrane helices, although it is not as precise as its successor, DeepTMHMM software, version 1.0. It can handle up to 10,000 sequences or 4 million amino acids, providing detailed topology predictions and summaries [13, 14].

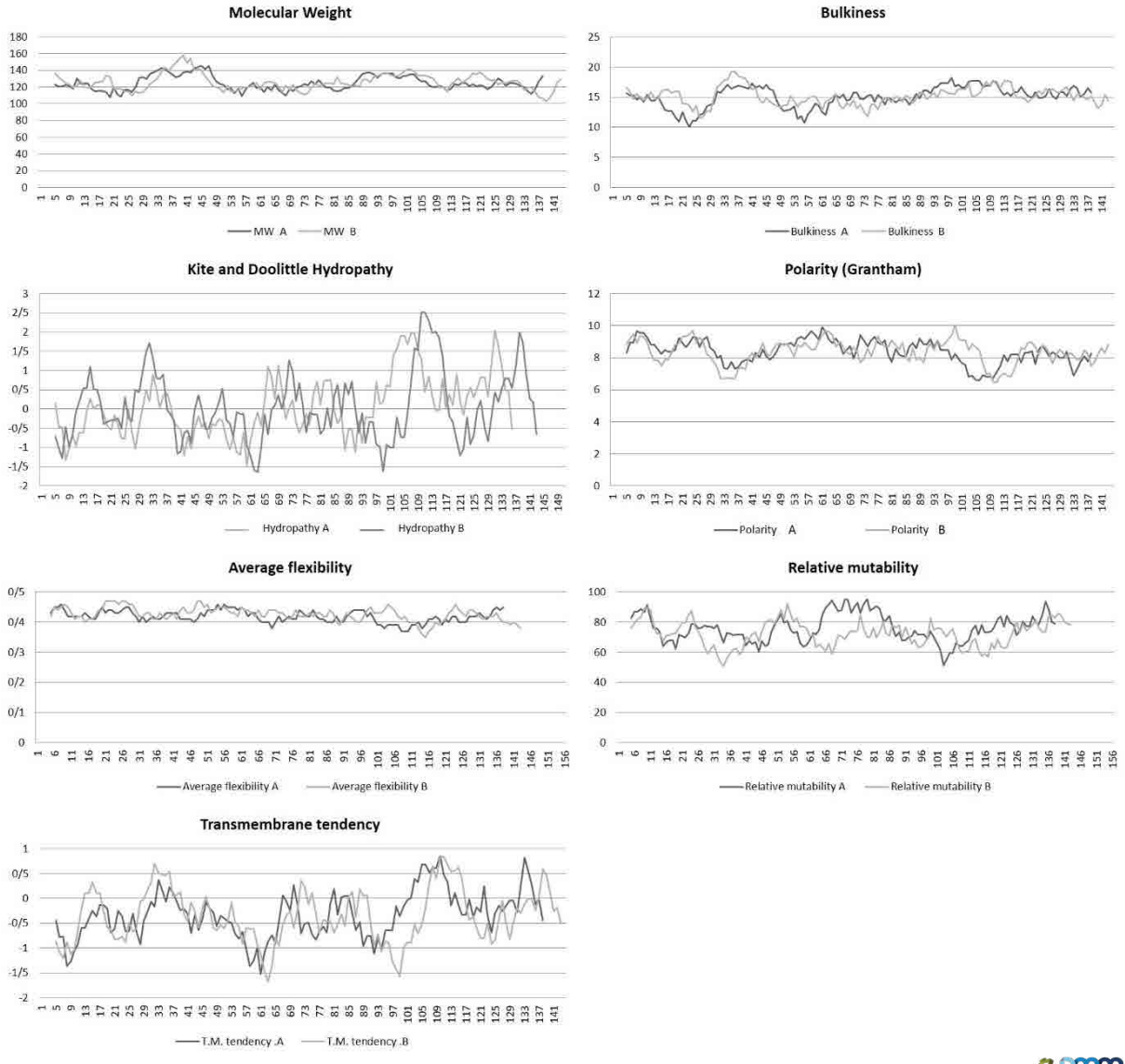

Figure 1. The molecular weight, bulkiness of amino acids, hydrophobicity, polarity, average flexibility, transmembranetendency scores, and relative mutability of amino acids along the sequence of the alpha- and beta-globin chains

Note: The vertical axis represents the ProtScale score for each variable, while the horizontal axis shows the globin chain sequences.

Results

The predicted molecular characteristics of globin chain residues in hemoglobin include weight, volume, hydrophobicity, flexibility, and transmembrane tendency

Initially, we compared the molecular weight and volume of amino acids, their hydrophobicity and polarity, flexibility, membraneembedding tendency, and relative mutability in the sequence of the alpha- and beta-globin chains (Figure 1). We generated diagrams for each of the alpha- and beta-globin chains of hemoglobin to compare polarity with hydrophobicity, as well as hydrophobicity with membranepenetration propensity (Figure 2).

Examination of the normal distribution of amino acid hydrophobicity in the alpha- and beta-globin chains of hemoglobin reveals a normal distribution with significant levels of $P<0.178$ and $P<0.312$. Similarly, the transmembranetendency scores of amino acids in the alpha and betaglobin chains also follow a normal distribution, with P values of $P<0.847$ and $P<0.393$. A significant correlation was found between the hydrophobicity and transmembranetendency scores in the alpha- and beta-globin chains. Specifically, for the alpha chain, $r=0.494$, $P<0.000$, and for the beta-globin chain, $r=0.869$, $P<0.000$.

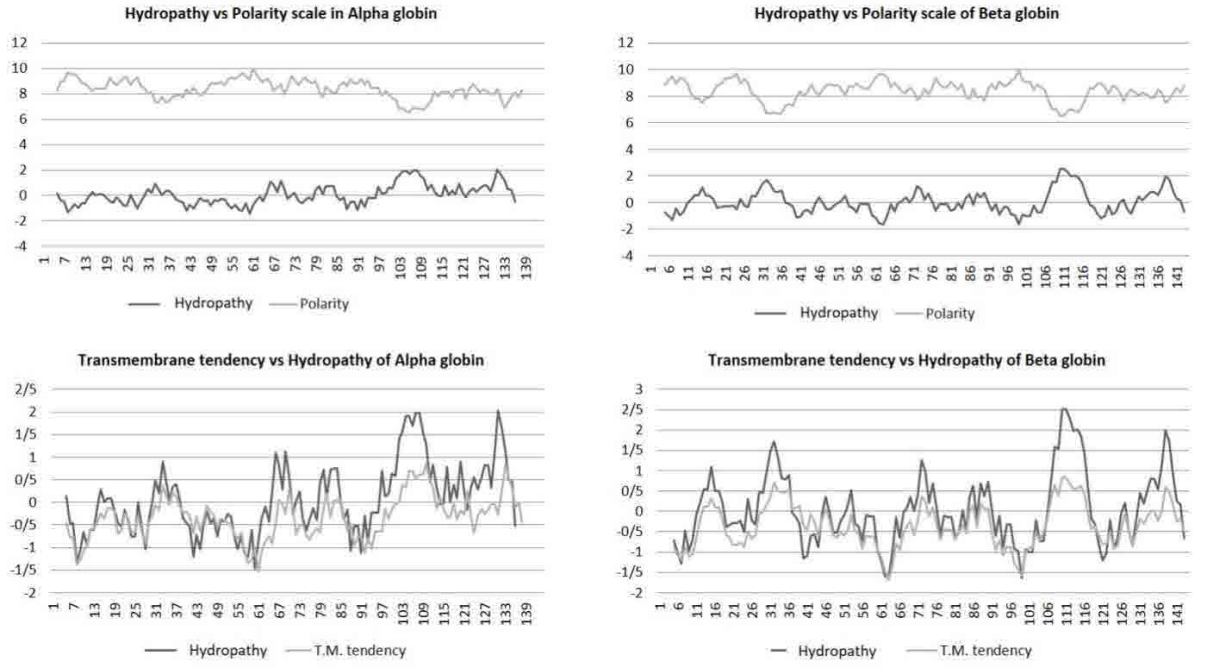

Figure 2. Hydrophobicity and polarity along the sequence of each alpha- and beta-globin chain in the upper row

Note: The lower row shows the relationship between hydrophobicity and membranepenetration propensity along the amino acid sequences of the alpha- and beta-globin chains. The vertical axis represents the ProtScale score for each variable, and the horizontal axis shows the globin chain sequences

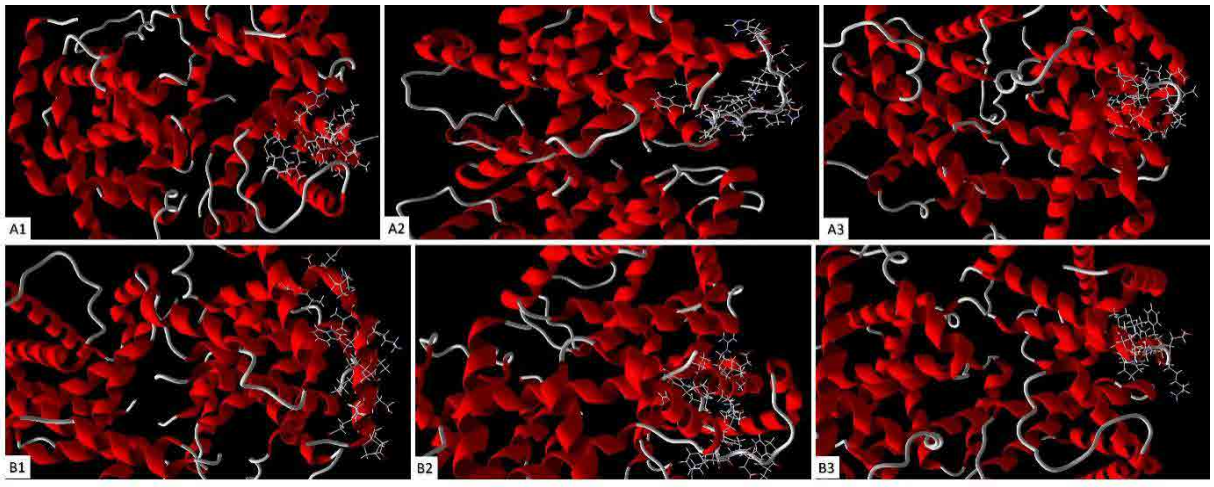

Figure 3. Shows the representation of sequences with FRS scores along the sequences of alpha- and beta-globin chains

Note: The alpha globin chain consists of the sequences A1 (WGKVGAHAGEYGAEALERMF), A2 (SFPTTKTYFPHFDLSHG), and A3 (CLLVTLAAHLPAEFTP). The beta-globin chain consists of the sequences B1 (VLVCVLAHHFGKEFTPPV), B2 (EALGRLLVVYPWTQRFFESF), and B3 (PEEKSAVTALWGKVNVDEVG).

Table 1. Comparison of FRS score and CHEL score of alpha- and beta-globin chains sequences

Alpha Globin Sequence: MVLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDL-HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR			
FRS Score	**Sequence of Amino Acids**	**CHEL Score**	**Sequence of Amino Acids**
0.614235	WGKVGAHAGEYGAEALERMF	0.326873	SALSDLHAHKLRVDP
0.612142	KVGAHAGEYGAEALERMFL	0.325368	LSALSDLHAHKLRVDP
0.604754	GKVGAHAGEYGAEALERMFL	0.31599	LSDLHAHKLR
0.605583	SFPTTKTYFPHFDLSHG	0.323707	TYFPHFDLSHGSAQVKGHG
0.596968	LSFPTTKTYFPHFDLSHG	0.319515	TYFPHFDLSHGSAQVKGH
0.591796	LSFPTTKTYFPHFDLSHGS	0.314771	FPHFDLSHGSAQVKGHG
0.572269	CLLVTLAAHLPAEFTP	0.31413	TLAAHLPAEF
0.561153	LLVTLAAHLPAEFTP	0.313206	LVTLAAHLPAEFTP
0.559499	CLLVTLAAHLPAEFTPAVH	0.308934	VTLAAHLPAEFTP
0.565888	ALSALSDLHAHKLRVDPV	0.313666	HFDLSHGSAQ
0.557426	ALSALSDLHAHKLRVDPVN	0.305522	PHFDLSHGSAQ
0.549776	NALSALSDLHAHKLRVDPVN	0.304741	FPHFDLSHGSAQ
0.507374	GSAQVKGHGKKVAD	0.294677	ALSDLHAHKL
0.499947	SAQVKGHGKKVAD	0.290899	LSALSDLHAHKL
0.499679	AQVKGHGKKVA	0.287849	SALSDLHAHKL
0.502413	VADALTNAVAHVDDMP	0.264081	RMFLSFPTTK
0.501917	ALSDLHAHKL	0.244122	LERMFLSFPTTK
0.495854	VADALTNAVAHVDDMPN	0.243577	ERMFLSFPTT
0.372282	MVLSPADKTN	0.22943	MPNALSALSDLHAHKLRVD
0.357551	MVLSPADKTNV	0.222545	MVLSPADKTN
0.317752	MVLSPADKTNVKAAWGK	0.215833	MVLSPADKTNV
0.356995	FLASVSTVLTSKYR	0.207721	VLSPADKTNV
0.349443	SLDKFLASVSTVLTSKYR	0.214373	VSTVLTSKYR
0.345378	LDKFLASVSTVLTSKYR	0.199745	SVSTVLTSKY
0.34921	MPNALSALSDLHAHKLRVD	0.191537	SVSTVLTSKYR

Beta Globin Sequence: MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSEL-HCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH			
FRS Score	**Sequence of Amino Acids**	**CHEL Score**	**Sequence of Amino Acids**
0.692857	VLVCVLAHHFGKEFTPPV	0.307315	TFATLSELHCDKLHVDPE_
0.692782	VLVCVLAHHFGKEFTPPVQ	0.304551	FATLSELHCDKLHVDP
0.687131	NVLVCVLAHHFGKEFTPPVQ	0.301531	FATLSELHCDKLHVDPE
0.671564	EALGRLLVVYPWTQRFFESF	0.300868	VCVLAHHFGKEFTPP
0.66483	ALGRLLVVYPWTQRFFESF	0.299409	LVCVLAHHFGKEFTPP
0.66374	ALGRLLVVYPWTQRFFESFG	0.298896	CVLAHHFGKEFTP
0.557545	PEEKSAVTALWGKVNVDEVG	0.299036	FGDLSTPDAVMGNPKVKAHG
0.553841	EEKSAVTALWGKVNVDEVGG	0.270019	GDLSTPDAVMGNPKVKAHG
0.54652	EKSAVTALWGKVNVDEVGG	0.267141	DLSTPDAVMGNPKVKAH
0.518459	GNVLVCVLAHHFGKEF	0.293641	SDGLAHLDNL
0.511272	LGNVLVCVLAHHFGKEFTP	0.29152	TFATLSELHCDKLHVDP
0.508194	LGNVLVCVLAHHFGKEFT	0.291181	SDGLAHLDNLK
0.498744	TFATLSELHCDKLHVDPENF	0.271553	FFESFGDLSTPDAVMGNP
0.492756	FATLSELHCDKLHVDPE	0.254339	FFESFGDLSTPDAVMGNPK
0.490591	FATLSELHCDKLHVDPENF	0.251697	RFFESFGDLSTPDAVMGNPK
0.494549	VLGAFSDGLAHLDNLKGTF	0.270651	TQRFFESFGDLSTPD
0.493862	FATLSELHCDKL	0.266875	WTQRFFESFGDLSTP
0.49166	KVLGAFSDGLAHLDNLKGTF	0.266717	TQRFFESFGDLSTP
0.48881	VANALAHKYH	0.256981	MVHLTPEEKS
0.440087	VVAGVANALAHKYH	0.249625	MVHLTPEEKSA
0.439115	AGVANALAHKYH	0.233208	VHLTPEEKSA
0.485885	STPDAVMGNPKVKAHGK	0.240519	VANALAHKYH
0.485137	LSTPDAVMGNPKVKAHGKK	0.217814	GVANALAHKY
0.484979	DLSTPDAVMGNPKVKAHGKK	0.21669	GVANALAHKYH
0.441299	MVHLTPEEKSA	0.229083	DPENFRLLGNVLVCVLAHH
0.440729	MVHLTPEEKS	0.218561	NVLVCVLAHHFG
0.409191	MVHLTPEEKSAVT	0.215975	FRLLGNVLVCVLAHHFGKEF
0.396567	RFFESFGDLSTPDAVMGNPK	0.221087	TPEEKSAVTAL
0.392995	FFESFGDLSTPDAVMGNPK	0.213242	TPEEKSAVTA
0.388896	TQRFFESFGDLSTPDAVMG	0.207044	VTALWGKVNVD

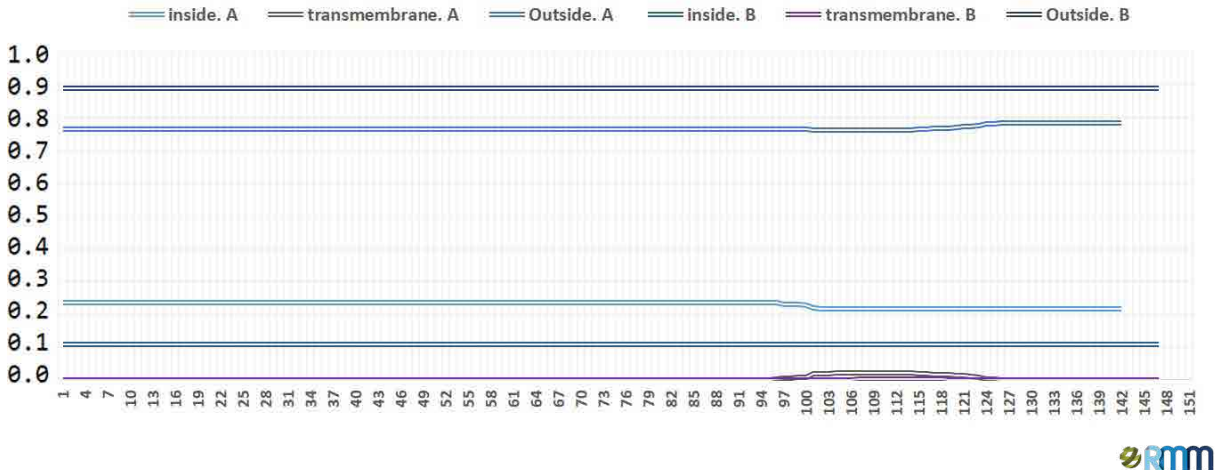

Figure 4. Diagram of the probability of transmembrane helices forming in the sequences of alpha- and beta-globin chains

Note: Inside: The possibility of being placed in the inner layer of the cell membrane. Outside: The possibility of being placed in the outer layer of the cell membrane. Transmembrane: The possibility of crossing two layers of the cell membrane

Peptides From hemoglobin chains are predicted to exhibit high level of FRS and CHEL scores

In the present study, we identified certain amino acids and polypeptide sequences that possess the capacity to neutralize free radicals and oxidizing compounds and to chelate heavy metals. Specifically, we identifies the sequences with the highest FRS score and CHEL scores within the alpha- and beta-globin chains, as shown in Table 1.

We predict that the alpha-globin chain of hemoglobin demonstrates the highest antioxidant activity in the amino acid sequence WGKVGAHAGEYGAEALERMF, spanning from amino acids 14 to 33. This sequence is typically located at the junction between helices A and helix B on the outer surface of the hemoglobin tetramer. Another sequence, SFPTTKTYFPHFDLSHG, which includes amino acids 35-51, forms a loop-like structure on the outer surface of the alpha chain in the hemoglobin model (PDB ID: 7VDE). The third sequence, CLLVTLAAHLPAEFTP, encompasses amino acids 104-119 and features loop-like breaks between helices G and H on the outer surface of the alpha-globin chain.

Similarly, our study predicts that the beta-globin chain of hemoglobin exhibits the highest antioxidant activity in the amino acid sequence VLVCVLAHHFGKEFTPPV, covering amino acids 113-126. This sequence often includes the intermediate segment between the G and H helices on the outer surface of the beta-globin chain. Another sequence, EALGRLLVVYPWTQRFFESF, includes amino acids 26-45 and displays a loop-like structure on the outer surface of the beta-globin chain. Lastly, PEEKSAVTALWGKVNVDEVG, containing amino acids 5-24, often involves helix A on the outer surface of the beta-globin chain (Figure 3).

The transmembrane tendency is predicted for globin chains in hemoglobin tetramers

In Figure 4, we have plotted the probability of the sequences of the alpha- and beta-globin chains in hemoglobin being located in the membrane layers or crossing the membrane. For all sequences of the alpha-globin chain, it is likely to be placed in the outer membrane with a probability of over 70%. Additionally, approximately 19%-22% can be located in the inner membrane. The probability of alpha-globin chain passing through two membrane layers in the entire sequence is nearly zero, with the highest probability of passing through two membrane layers found in the sequence F99 to V122 at 1%-3%. For the beta-globin chain sequences, the probability of being placed in the outer layer of the membrane is over 90%, while the probability of being placed in the inner layer of the membrane is less than 10%. The likelihood of crossing the two layers of the cell membrane is almost zero for all alpha-globin sequences. Due to the large number of hydrophobic alpha-helix structures in hemoglobin, it tends to be located in the outer layer of the cell membrane. The alpha globin chain is more likely to be in the inner layer of the membrane than the beta-globin chain.

Discussion

Biophysical and biochemical properties of hemoglobin

The molecular weight, volume, hydrophobicity, polarity, and ability to cross the membrane throughout the sequence are very similar for both alpha- and beta-globin chains. The finding is an inverse relationship between polarity and hydrophobicity in each of the alpha and beta chains. Specifically, as hydrophobicity increases, polarity decreases and vice versa, demonstrating this pattern throughout the sequence of each chain [15, 16].

Another observed relationship is a direct correlation between hydrophobicity and the tendency to be embedded in the membrane. Sequences with higher hydrophobicity are more likely to penetrate the membrane, while in aqueous solutions, the hydrophobic regions of the protein, such as hemoglobin, are typically located in the central regions [9, 13-17].

Antioxidant activity of the alpha- and beta-globin chain sequences

Oxidation is a crucial chemical process that can lead to the formation of free radicals, which are highly reactive and unstable molecules due to their unpaired electrons. These free radicals can function as oxidizing or reducing agents in various cellular processes, such as protein phosphorylation, transcription factor activation, apoptosis, immunity, and differentiation. The accumulation of free radicals can cause damage to cells by reacting with lipids, carbohydrates, proteins, and DNA, resulting in oxidative stress. This stress can be triggered by exposure to air, heat, light, heavy metals, or other sources of free radicals. Certain patterns have been identified in peptides that exhibit antioxidant activity. These include the presence of hydrophobic amino acids, such as leucine or valine at the N-terminal of peptides, sulfur-containing amino acids, such as cysteine and methionine, aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine, and histidine with an imidazole ring [12, 18].

In this study, the AnOxPePred tool was utilized to predict the FRS score and chelating properties of peptides. The predicted antioxidant activity is solely based on the amino acid sequence of the peptide, which provides information about its intrinsic properties such as size, local structure, and charge. The FRS score indicates the likelihood of a peptide donating hydrogen to a free radical, which can sometimes be predicted from the reduction potential of a standard electron (E0). In our research, regions with the highest FRS score on the outer surface of alpha- and beta-globin chains in hemoglobin were found at the sites of loops and junctions between helices that interact with the cytoplasmic environment. This positioning suggests that hemoglobin can effectively neutralize free radicals in its surroundings without interfering with its primary function of oxygen transport [12, 18-24].

Prediction of transmembrane alpha helices of globin chains sequences

Prediction of transmembrane helices in integral membrane proteins is one of the most important aspects of bioinformatics. The most successful methods to date not only predict the transmembrane helices but also attempt to predict the complete protein topology, including the total number of transmembrane helices and their orientation relative to the membrane. The TMHMM software, based on the hidden Markov model, correctly predicts 97%-98% of transmembrane helices. It can distinguish between soluble and membrane proteins with a specificity and sensitivity of more than 99%, although the accuracy is reduced in the presence of signal peptides in the sequence [13, 14, 21-25].

In our study, based on the results of the TMHMM software, beta-globin chains show a high tendency to be located in the outer layer of the cell membrane, while the likelihood of them being in the inner layer is very low. This suggests that beta chains have less deposition in the cell membrane compared to alpha chains. On the other hand, the alpha chain is less likely to be positioned in the outer membrane but has a slight tendency to be in the inner membrane, which explains the higher deposition of alpha globin chains in the cell membrane in beta-thalassemia patients' red blood cells (RBCs).

Conclusion

This study investigates the physicochemical and biochemical features of the amino acid sequences of alpha- and beta-globin chains in the hemoglobin tetramer. We found similarities in the physicochemical and biochemical characteristics of the two sequences. Hydrophobicity is directly related to membrane penetration tendency. Additionally, domains in contact with the cytoplasmic environment possess antioxidant and free radical neutralizing properties, with some showing chelating properties. Both alpha- and beta-globin chains have a high tendency to span the outer layer of the membrane, but the alpha chain has a greater tendency to be in the inner layer compared to the beta chain, explaining the deposition of alpha globin in the cell membrane in beta-thalassemia patients' RBC.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Faculty of Medicine, Tarbiat Modares University, Tehran, Iran (Code:IR.MODARES.REC.1401.149).

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Authors contribution's

Conceptualization: Roohallah Yousefi, Seyed Hossein Khaleghinejad, and Sadegh Hasannia; Methodology and formal analysis: Roohallah Yousefi and Seyed Hossein Khaleghinejad; Software and visualization: Roohallah Yousefi and Dariush Gholami; Validation: Seyed Hossein Khaleghinejad and Sadegh Hasannia; Investigation, data curation, and writing the original draft: Roohallah Yousefi; Resources: Sadegh Hasannia, Dariush Gholami; Review and editing: Seyed Hossein Khaleghinejad, Dariush Gholami, Sadegh Hasannia; Supervision:and project administration: Seyed Hossein Khaleghinejad and Sadegh Hasannia; Funding acquisition: Sadegh Hasannia and Seyed Hossein Khaleghinejad.

Conflict of interest

The authors declared no conflicts of interest.

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