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Assessing the Heme Group Interactions of Hemoglobin and Myoglobin in Deoxy and Oxy Form

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Abstract:
Heme proteins such as hemoglobin and myoglobin's role are primarily associated to binding molecular oxygen for proper functioning. Heme moiety in both these proteins is found to be incorporated in a hydrophobic cleft and is responsible for oxygen binding. The interactions of amino acids surrounding heme group support in ligand binding and contribute to protein's functionality. Moreover, nano-ligands, drugs and dyes may bind near heme involving the nearby residues and accordingly those residues possibly take part in regulating the overall functionality of these proteins. Hence, studying the heme group interactions with neighbouring residues will unravel contribution of these residues in determining structural functionality of the proteins. Apparently, this work focuses on identifying the key residues and the nature of interactions surrounding heme of hemoglobin and myoglobin in deoxy and oxy form. These residues can be used to design and engineer novel peptide molecules of clinical significance. Our study also highlights contribution of specific Tyr/ Trp molecules in heme group interactions suggesting their roles not only in binding interactions but also in the development of fluorescence-based protein sensors and bio-imaging.

Keywords: Hemoglobin, Myoglobin, Heme-group interactions

INTRODUCTION:

Proteins are large macromolecules necessary for structure, function and regulation of the body's tissues and organs. The fundamental principle behind protein function is molecular recognition and distinct events involving macromolecule -ligand interactions. Two such proteins involved in ligand binding and expressing their functionality are hemoglobin and myoglobin. In vertebrates, they are basically the companions in transport and storage of oxygen.

Hemoglobin is an iron-containing metalloprotein whose key function is transporting oxygen to cells and CO_2. The functional hemoglobin has ferrous (Fe^{2+}) iron atom at the center of heme group located in crevices at the exterior of the subunit along with an organic component known as protoporphyrin IX. The heterocyclic ring system contains tetrapyrrole ring each connected by methene bridges with four methyl, two vinyl and two propionate substituents. The iron-iron distances in the tetramer of heme groups ranges

from 24-40 Å. In oxyhemoglobin, the iron atom is coordinated by six atoms: four nitrogen atoms of pyrrole groups, proximal histidine residue of the globin subunit occupies the fifth binding site and finally oxygen occupying the sixth ligandin site [1-10].

Myoglobin on the other hand, structurally and functionally like hemoglobin is a globular protein with oxygen storing capacity during resting period required for oxidative phosphorylation [3, 11-12]. The amphipathic pocket comprises of eight helices aides in binding iron protoporphyrin IX [9-12]. The iron is coordinated on four sides by the pyrrole nitrogen and one with His through the proximal side. The remaining side for both the proteins is attached to the ligand from the distal face of the heme moiety [13]. The interaction with ligands is greatly affected by protein structure and is often accompanied by conformational changes [14-17]. Moreover, the affinity with which heme binds its different ligands is altered when the heme is a component of myoglobin/ hemoglobin. Binding of such ligands is associated with differences in the way the orbital structures of the ligands interact with Fe^{2+} atom. These orbital structures display different binding geometries for ligands when they are bound to heme. The change in relative binding characteristics of ligands with heme when it is attached to globin is facilitated by the globin structure [11].

The binding of ligands to the heme in myoglobin and hemoglobin also depends on molecular motions, within the protein structure. The heme molecule is buried in the folded polypeptide, with limited direct pathway for ligands to reach the ligand-binding site from the surrounding solution. Fast molecular flexing of the side chains of the amino acid residues produces transient cavities in the protein structure creating paths for the ligands to reach its binding sites [11]. Thus, structural alterations during ligand binding to heme (for ligands like O_2 and CO) or to the globin part (for ligands like nanoparticles, nano-medicines, and dyes) of hemoglobin and myoglobin is apparent. The primary focus here is to study about the interactions of heme moiety with the nearby residues in the pocket which might influence the binding ability, structural, functional, and spectroscopic property of the protein.

Materials and Methods

For analysis of the three-dimensional structure of proteins, the RCSB protein data bank (RCSB PDB, https://www.rcsb.org) database is utilised to obtain the PDB ids of deoxy myoglobin (PDB id- 5MBN), oxy myoglobin (PDB id- 1MBO) [18], deoxy hemoglobin (PDB id- 2DN2), and oxy hemoglobin (PDB id- 2DN1) [19].

The interaction of amino acid residues surrounding 3.5 Å of heme are carried out using PyMOL (https://www.pymol.org/). Bond distances are also checked in PyMOL.

Results and Discussion

Interaction of heme group with surrounding amino acids of deoxy and oxy myoglobin

The interactions between heme and nearby amino acid of deoxy myoglobin (PDB id-5MBN) are depicted in **Figure1**. As observed, the C=O group of Lys42 is 3.5 Å away from the methyl group of heme. Whereas the benzene ring of Phe43 is 3.3 Å from methene bridges and 3.4 Å from vinyl group of the tetrapyrrole ring suggesting probable formation of hydrophobic interactions. The oxygen of propionate group of heme is at 2.9Å from the guanidino group of Arg45 indicating possible formation of hydrogen bond. The hydroxyl group of Ser92 is at 3.0 Å and NH-of imidazole group of His97 is 2.6 Å away from oxygen of another propionate group of heme, may be indicating formation of hydrogen bonding. Similarly, NH-of imidazole group of His93 is 2.2 Å from the Fe^{2+} of heme group. From the above observations we can

deduce that a combination of both hydrophobic interactions and hydrogen bonding is feasible.
Apart from these interactions within 3.5 Å surrounding heme, we also try to identify the nearby Tyr/ Trp residues of myoglobin. The myoglobin molecule has two Trp and three Tyr residues. The phenol ring of Tyr103 is 3.6 Å distant from the vinyl group of heme thereby specifying possible formation of hydrophobic interactions. An influence of two aromatic amino acids Tyr146 and Tyr151 are also observed, as the C=O group of His93 is 3.9 Å distant from hydroxyl group of Tyr146. Additionally, the C=O group of Tyr146 is 2.9 Å from NH_2 group of Tyr151, suggesting hydrogen bonding interactions among them.
As such, alterations in spectroscopic properties (UV-vis absorption, fluorescence) of deoxy myoglobin is expected during binding of ligands by involvement of heme and its direct interactions with Tyr103 and indirect interactions with Tyr146 and Tyr151.

Figure 1: Interaction of Heme with nearby Amino Acids in Deoxy Myoglobin (PDB Id- 5MBN) is shown. Heme Group in Cyan, Amino Acid Residues in Green and Tyr Residues in Pink colour are shown.

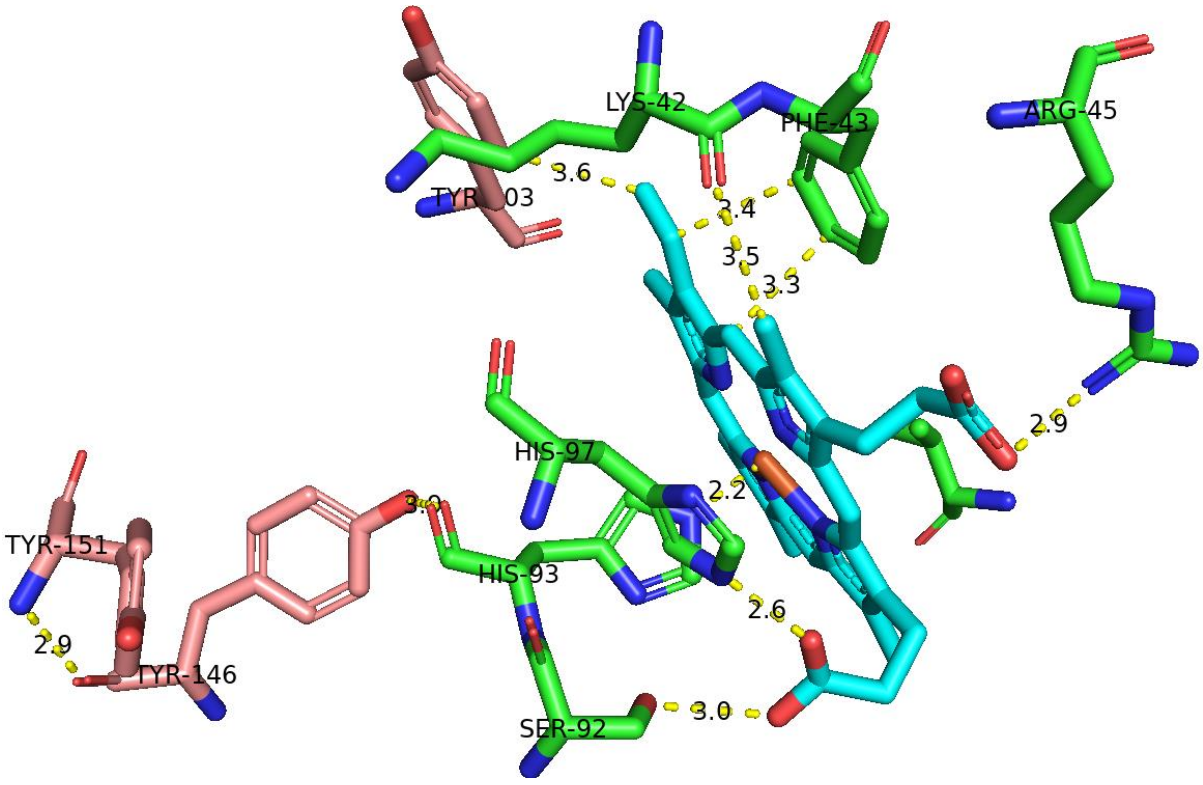


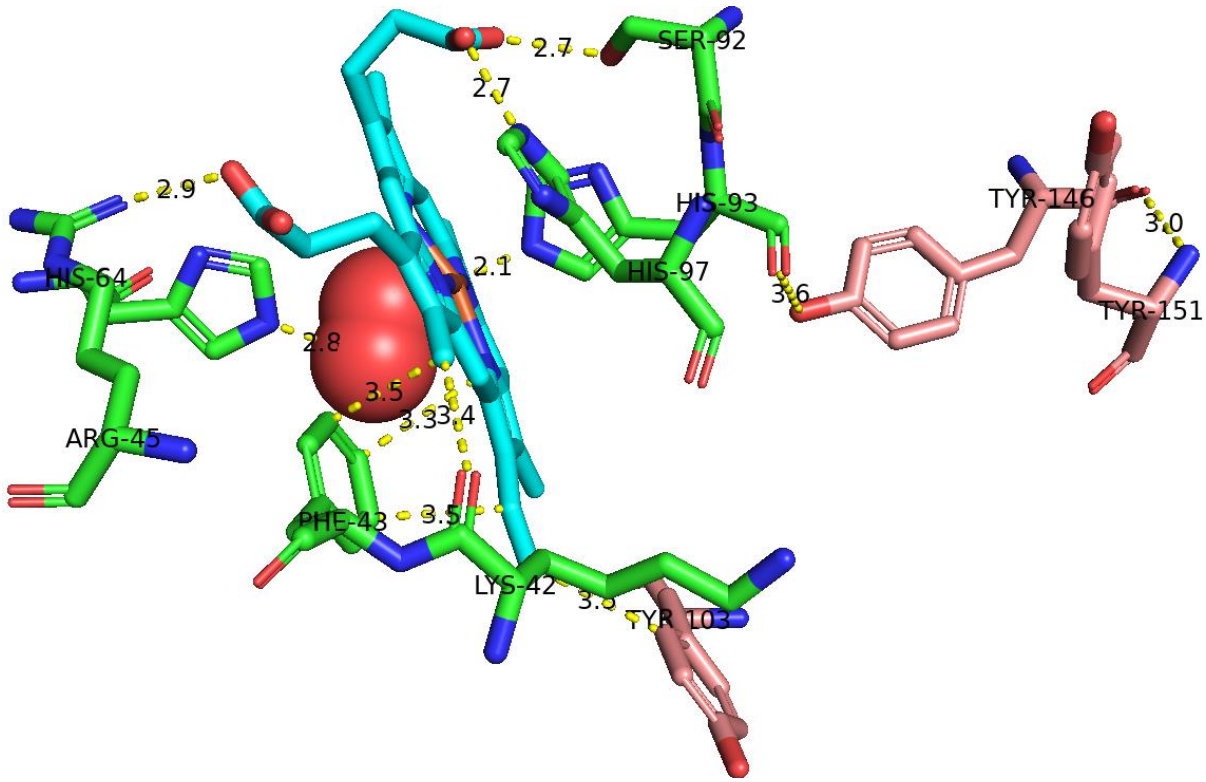
Figure 2 shows the interactions between heme and nearby amino acids of oxymyoglobin (PDB id-1MBO). As noted, the C=O group of Lys42 is 3.4 away from the methyl group of heme. As observed, the C=O group of Lys42 is 3.5 Å away from the methyl group of heme. The benzene ring of Phe43 is at 3.3 Å from methylene bridge, 3.5 Å from methyl and adjacent vinyl group of heme suggesting formation of hydrophobic interactions. A bond distance of 2.9 Å is seen between NH of guanidino group of Arg45 and propionate group of heme indicating strong hydrogen bonding. The propionate group of heme is at 2.7 Å from both the imidazole group of His97 and hydroxyl group of Ser92 once more demonstrating possible formation of hydrogen bond. The His64 is 2.8 Å from O_2 ligand. The Fe^{2+} of heme group is 2.1 Å from NH of imidazole group of His93, the iron atom in heme on binding oxygen becomes planar with the rest of the heme group pulling the histidine and causing a structural change in the protein.

Importantly, direct hydrophobic interactions with a bond distance of 3.5 Å between vinyl group of heme and phenyl group of Tyr103 is observed. Similar patterns of interactions with differences in bond distances are noticed for Tyr146 and Tyr151 in deoxy and oxy myoglobin. As noted, His93 is at 3.6 Å from hydroxyl group of Tyr146, the C=O group of the later in turn is 3.0 Å from the backbone NH group of Tyr151. Here also, adjustments in spectroscopic properties (UV-vis absorption, fluorescence) of oxy myoglobin is expected during binding of ligands via involvement of heme and its direct interactions with Tyr103 and indirect interactions with Tyr146 and Tyr151.**Table 1** shows the interacting moiety of amino acid residues of globin and corresponding moiety of heme. Increase/ Decrease in bond lengths between them are observed upon ligand binding (O_2) of heme (**Table 1**).

Table 1: A comparative study of deoxy and oxy myoglobin

Amino acid (interacting group)	**Interacting moiety of heme**	**Distance (Å) in 5MBN**	**Distance (Å) in 1MBO**	**Increase/Decrease in bond length in presence of O_2**
Phe43 (Benzene ring)	CH_2 of vinyl group methene bridge	3.4 3.3	3.5 3.3	↑ by 0.1 No change
Arg45 (NH of guanidino group)	O of propionate group	2.9	2.9	No change
His64 (NH of imidazole group)	Fe^{2+} O_2	4.6	 2.8	Not compared
Ser92 (hydroxyl group)	O of propionate group	3.0	2.7	↓ by 0.3
His93 (NH of imidazole group)	Fe^{2+}	2.2	2.1	↓ by 0.1
His97 (NH of imidazole group)	O of propionate group	2.6	2.7	↑ by 0.1
Tyr103 (Phenyl group)	CH_2 of vinyl group	3.6	3.5	↓ by 0.1

Figure 2: Interaction of Heme with nearby Amino Acids in Deoxy Myoglobin (PDB Id- 1MBO) is shown. Heme Group in Cyan, Amino Acid Residues in Green and Tyr Residues in Pink colour are shown.



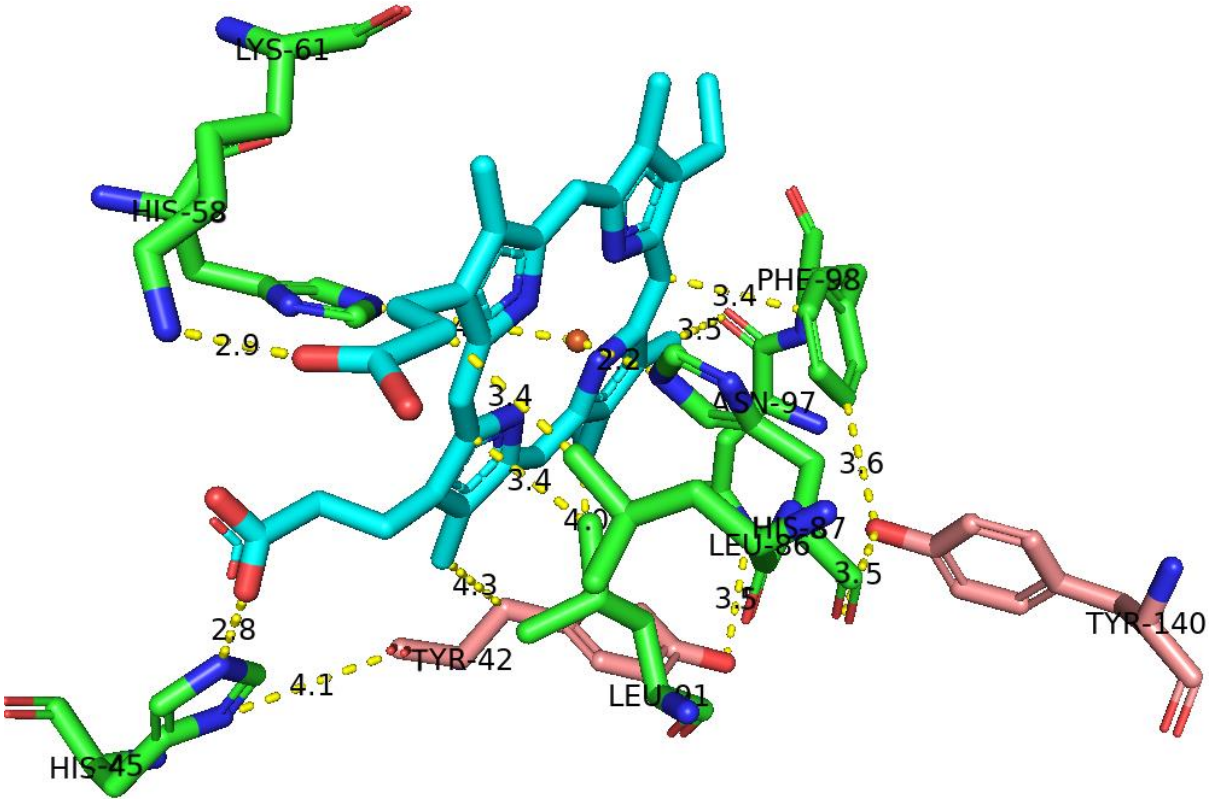
Interaction of heme group with surrounding amino acids of subunit A of deoxy and oxy hemoglobin

Hemoglobin is a tetrameric protein with two identical subunits A/C and B/ D. For simplicity, we have considered two non-identical subunits A and B. The interplay between the nearby amino acids of heme group of subunit A of deoxy hemoglobin (PDB id- 2DN2) is represented in **Figure 3**. The NH of imidazole group of His45 is at 2.8 Å from the propionate group of heme. Another NH of imidazole group of His45 is at 4.1 Å from C=O group Tyr42, representing weak hydrogen bonding interactions. Moreover, CH_2 group of Tyr42 is spaced 4.3 Å from methyl group of heme and the phenyl group of Tyr42 is 4 Å from vinyl group of heme thus indicating formation of hydrophobic interactions between Tyr42 and the heme moiety. Moreover, the C=O group of Asn97 is 3.5 Å from the methyl group of heme whereas the side chainNH_2 group of Asn97 is 3.5 Å from the OH side chain of Tyr42. Furthermore, the phenyl group of Phe98 is3.4 Å from methylene bridges of tetrapyrrole ring system of heme thereby signifying formation of hydrophobic interactions. The aromatic ring of this Phe98 also makes a bond distance of 3.6 Å with phenolic OH of Tyr140.Theε-amino group of Lys61 is 2.9 Å away from propionate group of heme again implying possible formation of hydrogen bonding interactions. The methyl group of Leu86 is 3.4 Å away from propionate (CH_2) group of heme. The pyrrole C of heme is 3.4 Å away from the methyl side chain of Leu91. Finally, NH of imidazole group of His87 is at 2.2 Å from the Fe^{2+} of heme. Interestingly, the C=O group of His87 is 3.5 Å from the phenolic OH of Tyr145 indicating formation of hydrogen bonding interactions. Overall, apart from few strong and weak hydrogen bonding interactions, hydrophobic interactions are mostly observed.

Apparently, changes in UV-vis absorption, fluorescence spectroscopic properties of deoxy hemoglobin are expected during interactions of ligands with heme by obvious involvement through multiple

interactions of Tyr42 and Tyr140.

Figure 3: Interaction of Heme with nearby Amino Acids of Subunit A Of Deoxy Hemoglobin (PDB Id-2DN2) is Displayed. Heme Group in Cyan, Amino Acid Residues in Green And Tyr Residue in Pink colour are shown.



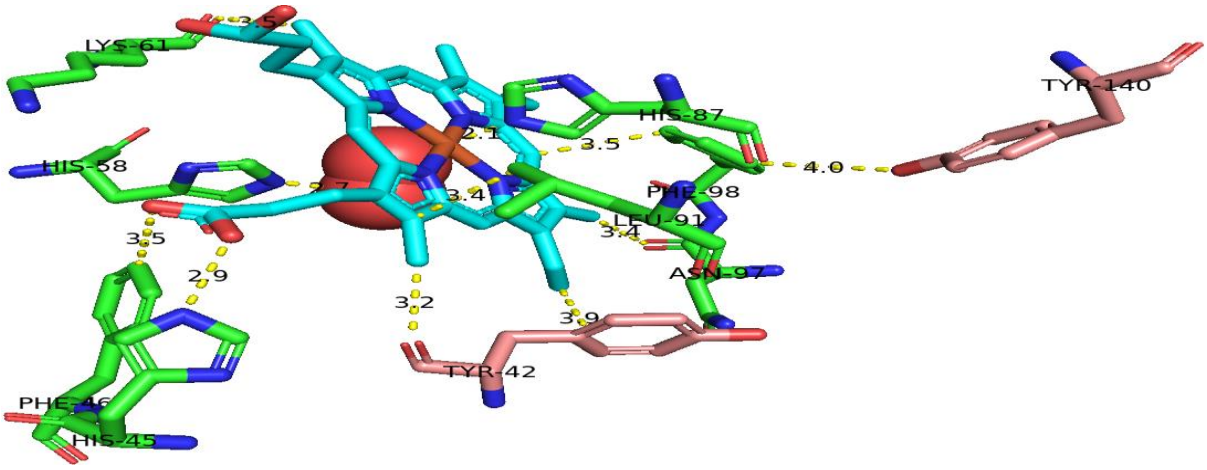
For a comparative study, subunit A of oxy hemoglobin (PDB id- 2DN1) is also considered (**Figure 4**). As observed, the methyl group of heme is at 3.2 Å from C=O group of Tyr42. The phenol ring is also 3.9 Å away from the adjacent vinyl group of heme indicating hydrophobic interactions between them. Moreover, the NH of imidazole group of His45 is 2.9 Å from propionate group of heme through possible formation of hydrogen bond. Also, propionate group of heme interacts with aromatic ring of Phe46 with a bond distance of 3.5 Å. One of the methyl groups of heme is 3.5 Å from C=O group of Lys61 and another methyl group is 3.4 Å from backbone C=O group of Asn97. Also, the methyl side chain of Leu91 is 3.4 Å distant from the pyrrole C of the tetra pyrrole protoporphyrin ring system. Furthermore, the ligand O_2 of heme is at 2.7 Å from the imidazole NH of His58, highlighting its role to act as agate in binding the ligand O_2. Also, the imidazole N of His87 is 2.1 Å away from central Fe^{2+} atom of heme. Moreover, the benzene ring of Phe98 is 3.5 Å away from the methylene bridge, whereas this aromatic ring also makes a bond distance of 4 Å with phenolic OH of Tyr140.

Majority of the interactions mentioned above involves hydrophobic interactions along with few hydrogen bonding and other non-covalent interactions, between amino acid residues and heme moiety. As expected, changes in UV-vis absorption, fluorescence spectroscopic properties of oxy hemoglobin will probably occur during interactions of ligands with heme by direct involvement of Tyr42 and indirect involvement of Tyr140.The interacting moiety of amino acid residues of globin and corresponding moiety of heme are mentioned in **Table 2**. From **Table 2** alterations in bond lengths between them are evident in absence and presence of ligand (O_2).

Table 2: A comparative study of interactions in subunit A of deoxy and oxy hemoglobin

Amino acid	Interacting moiety of heme	Distance (Å) in 2DN2	Distance (Å) in 2DN1	Increase/ Decrease in bond length in presence of O_2
Tyr42 (Phenyl group in 2DN2) C=O group in 2DN1	Vinyl group Methyl group, phenol ring	4	3.2, 3.9	Not compared
His45 (Imidazole group)	O of propionate group	2.8	2.9	↑ by 0.1
His58 (NH of imidazole group)	Fe^{2+} O_2	4.5	2.7	Not compared
Lys61 (ε-amino group in 2DN2) (C=O group in 2DN1)	O of propionate group Methyl group	2.9	3.5	Not compared
His87 (Imidazole group)	Fe^{2+}	2.2	2.1	↓ by 0.1
Leu91 (methyl group)	Pyrrole C	3.4	3.4	No change
Asn97 (backbone C=O group)	Methyl group	3.5	3.4	↓ by 0.1
Phe98 (Phenyl group)	Methylene bridge	3.4	3.5	↑ by 0.1

Figure 4: Interaction of heme with nearby amino acids of subunit A of oxy hemoglobin (PDB id-2DN1) is shown. Heme group in cyan, amino acid residues in green and Tyr residues in pink colour are shown.

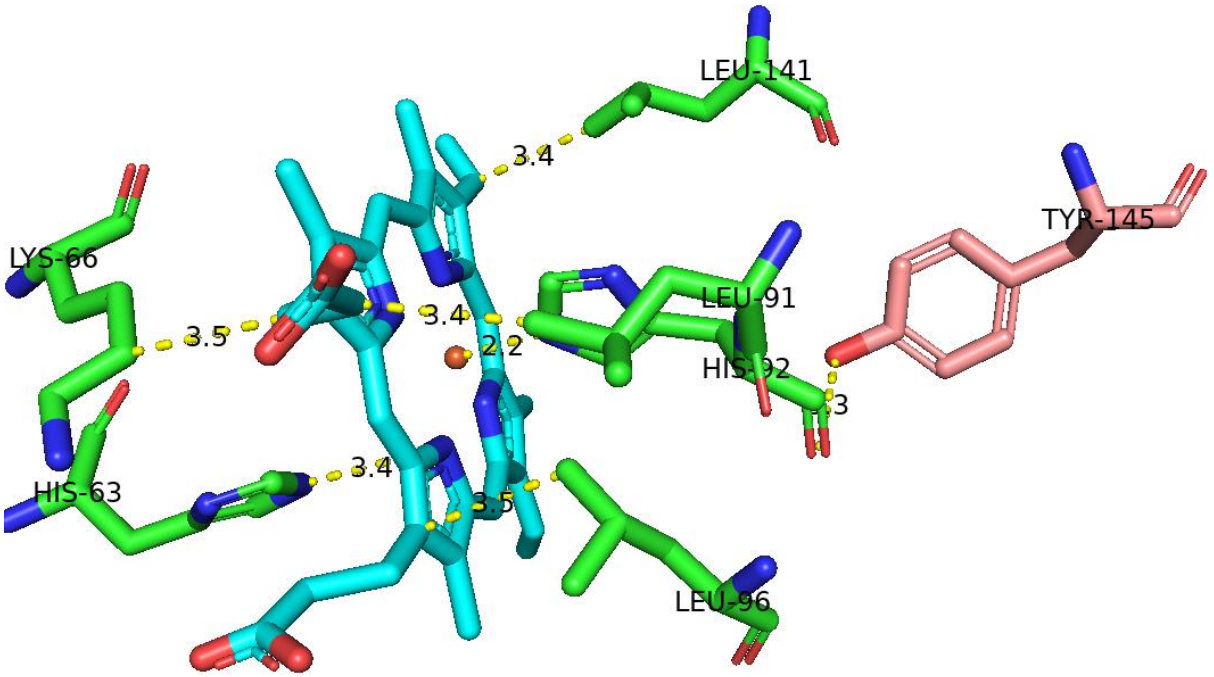


Interaction of heme group with surrounding amino acids of subunit B of deoxy and oxy hemoglobin

The interplay between the nearby amino acids and heme group of subunit B of deoxy hemoglobin (PDB id-2DN2) is shown in **Figure5**.The NH of imidazole group of His63 is at a distance 3.4 Å from the tetrapyrrole ring N of heme. The CH_2 group of Lys66 is at 3.5 Å from propionate (CH_2) group of heme indicating hydrophobic interactions. Again, methyl side chain of Leu91 is 3.4 Å from the propionate (CH_2) group of heme thereby indicating hydrophobic interactions. The NH of imidazole group of His92 is 2.2Å distant from Fe^{2+} of heme. Interestingly, the C=O group of His92 is 3.3 Å from phenolic OH group of Tyr145 showing hydrogen bonding interactions. Also, the methyl group of Leu96 is 3.5 Å from the pyrrole C atom of tetrapyrrole system of heme. Moreover, the methyl group of aliphatic side chain of Leu141 is 3.4 Å distant the vinyl group of heme. The observations clearly illustrates that the predominant force involved is hydrophobic interactions around the heme moiety with its surrounding amino acids.

For subunit B of deoxy hemoglobin also, changes in UV-vis absorption and steady-state and time-resolved fluorescence characteristics are anticipated during interactions of ligands involving heme and its nearby Tyr145.

Figure 5: Interaction Of Heme With Nearby Amino Acids Of Subunit B Of Deoxy Hemoglobin (PDB Id-2DN2) Is Shown. Heme Group In Cyan, Amino Acid Residues In Green And Tyr Residue In Pink Colour Are Shown.



The interplay between the nearby amino acids and heme group of subunit B of deoxy hemoglobin (PDB id-2DN1) is shown in **Figure 6**. The carbonyl group of Phe41 is 3.3 Å from the methyl group of heme moiety of subunit B of 2DN1.The phenyl ring of Phe42 is 3.5 Å from the methylene bridge of heme. The methyl group of Leu96 is 3.5 Å from the pyrrole C of tetrapyrrole ring system of metalloporphyrin heme. The methyl group of Leu96 is 3.5 Å from the pyrrole C atom of heme. The NH of the imidazole group of His63 is 3 Å from the O_2 ligand. The NH of imidazole group of His92 is 2.1 Å distant from Fe^{2+} atom of heme. Importantly, the backbone C=O group of this His92 is 3.7 Å distant from the phenolic OH of Tyr145. The backbone C=O group of Asn102 is 3.5 Å from methyl group of heme. Notably, the amide carbonyl group of side chain of Asn102 is 2.9 Å from the indole N of Trp37 indicating hydrogen bonding

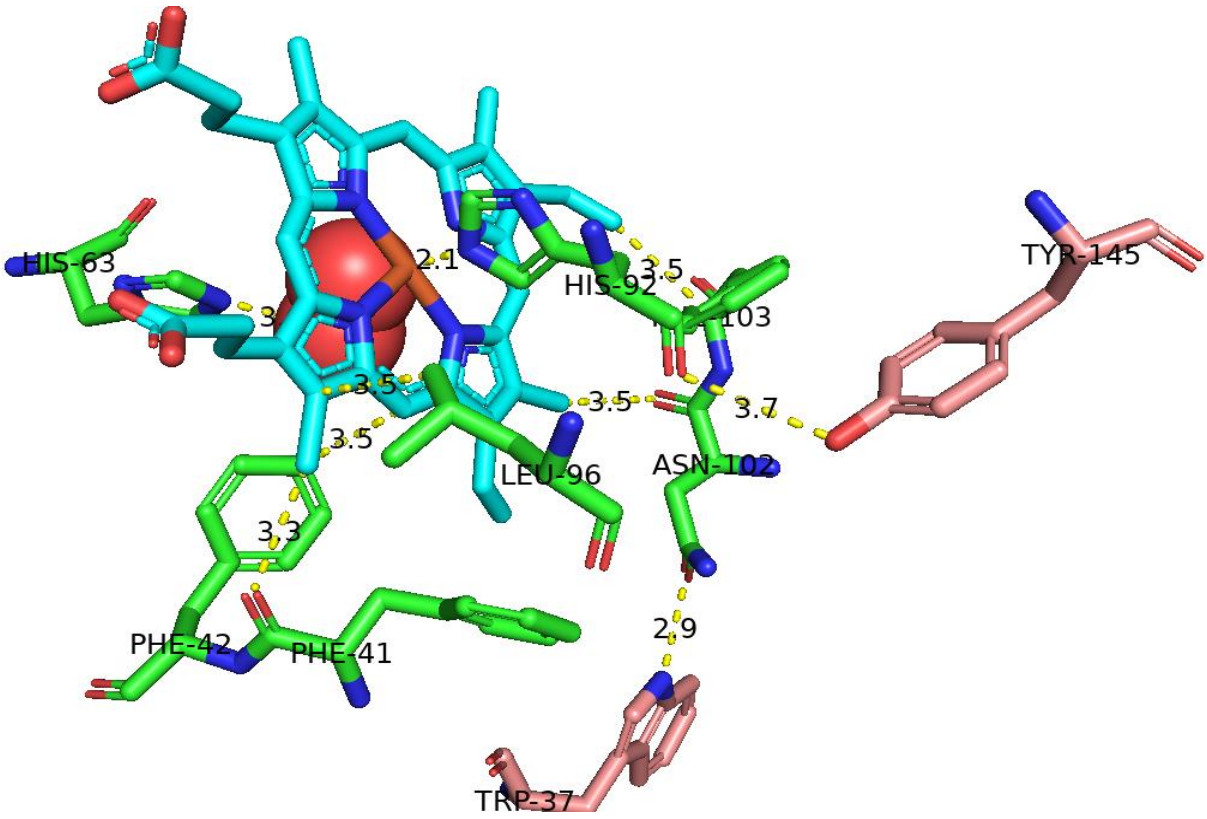
interactions. The CH_2 group of Phe103 is 3.5 Å from vinyl group of heme suggesting hydrophobic interactions. Thus, it appears that the heme is interacting predominantly through hydrophobic interactions and few other non-covalent interactions with its surrounding amino acids. Considering subunit B of oxy hemoglobin too, changes in UV-vis absorption and fluorescence properties are probable during interactions of ligands involving heme and its nearby Tyr145 and Trp37.

Table 3: A comparative study of interactions in subunit B of deoxy and oxy hemoglobin

Amino acid	Interacting group of heme	Distance (Å) of 2DN2	Distance (Å) of 2DN1	Increase/Decrease in bond length
His63 (Imidazole N)	Pyrrole N in 2DN2 O_2 in 2DN1	3.4	3	↓ by 0.4
His92 (Imidazole N)	Fe^{2+}	2.2	2.1	↓ by 0.1
Leu96 (Methyl group)	Pyrrole C	3.5	3.5	No change

The interacting moiety of amino acid residues of globin and corresponding moiety of heme are mentioned in **Table 3**. From **Table 3**alterations in bond lengths between them are evident in absence and presence of ligand (O_2).

Figure 6: The Interacting Nearby Residues Of Heme Moiety Of Subunit B Of Oxy Hemoglobin (PDB-ID-2DN1) Is Shown. Heme Group In Cyan, Amino Acid Residues In Green And Tyr/ Trp Residues In Pink Colour Are Shown.



Conclusions

Hydrophobic interactions are the main connections between the nearby residues and heme moiety apart from few hydrogen bonding interactions and other forms of non-covalent interactions. The interplay of hydrophobic interactions stabilizes the heme-protein conjugate strongly. We cannot possibly overlook the influence of aromatic amino acids in the hydrophobic crevice which might impact ligand binding of heme group in general. As such direct involvement of Tyr103 and indirect involvement of Tyr146 and Tyr151 during heme group interactions of myoglobin are noted. Our study also demonstrates direct interactions of Tyr42 and indirect interaction of Tyr140 of subunit A with heme in oxy as well as deoxy hemoglobin. Also, for subunit B, the heme group interaction involve Tyr145 of both oxy and deoxy hemoglobin. However, the involvement of Trp37 is seen only for oxy hemoglobin of subunit B. Overall, we may conclude that there is a possibility of an influence of the aromatic amino acids on the hydrophobic pocket which probably might influence ligand binding to the heme moiety. The contribution of these specific Tyr/ Trp residues in heme group interactions indicate their promising roles not only in binding interactions but also in biosensing and bioimaging.

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