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Short Communication

Docking of Smaller Ligands in the Heme Pocket of Hell's Gate Globin IV

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ABSTRACT

HGbIV is unique truncated hemoglobin (tHb) that differs from other bacterial tHbs due to its large size, polar heme pocket residues and large cavity for exogenous ligand in the heme pocket. The crystal structures of HGbIV showed that its heme pocket can accommodate and interact with bulky PO_4^{-3} ion. Here, we have docked different smaller molecules/ion like O_2, NO, CO, CO_2, NO_3^{-1}, CH_3COO^{-1} and CN^{-1} in the heme pocket of HGbIV. Analysis of the structure showed that the smaller ligands docked far away from most of the heme pocket residues namely His70(B9), His71(B10), Ser97(E11) and Trp137(G8), and only PO_4^{-3} docked at a position where it can interact with all these residues. It appears that HGbIV is not designed for transport of smaller gaseous molecule like the other heme containing proteins and it may be a phosphate sensor.

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Authors' Contribution
FJ designed the study. SR and HR performed the experiments, collected and analyzed the data. FJ gathered the data, supervised and prepared the manuscript.

Key words
Truncated hemoglobins, Phosphate sensor, Oxygen carrier, Enzymes.

Truncated hemoglobins (tHbs) are heme containing proteins (Hbs) that are ubiquitous to bacteria, protozoa, nemertea, cyanobacteria and plants (Ascenzi and Pesce, 2017; Wittenberg *et al*., 2002). These proteins are almost 20 to 40 amino acid short than novertebrate Hbs and constitute a separate lineage in the globin superfamily (Bustamante *et al*., 2016; Wittenberg *et al*., 2002; Vinogradov *et al*., 2013). Structurally, tHbs are based on 2/2 α-helical fold, showing an editing in classical 3/3 α-helical globin fold (Pesce *et al*., 2000). Although, real physiological roles of different bacterial tHbs have not yet been established, though different studies have proposed different roles for tHbs such as oxygen and nitrogen transport, as sensor, oxygen carrier, ligand storage and in nitric oxide detoxification (Dikshit *et al*., 1992; Couture *et al*., 1999; Pesce *et al*., 2000; Ouellet *et al*., 2002, 2003; Igarashi *et al*., 2011; Jamil *et al*., 2014; Minaeva *et al*., 2017; Ascenzi *et al*., 2017).

tHGbs are further classified into four phylogentic groups: group I (tHbN), group II (tHbO), group III (tHbP) and group IV (tHbQ) (Vinogradov *et al*., 2013; Bustamante *et al*., 2016). These groups separate from each other due to different heme pocket residues located at topological positions (B9, B10, E7, E11, E14 and G8). The most highly conserved residues in group II tHgbs are Phe, Tyr and Trp at B9, B10 and G8 positions, respectively (Wittenberg *et al*., 2002). These residues play a vital role in stabilization of heme-bound ligand as reported in crystal structures of different bacterial tHbs (Pesce *et al*., 2000; Bonamore *et al*., 2005; Ilari *et al*., 2007; Igarashi *et al*., 2011; Jamil *et al*., 2014; Tariq *et al*., 2016; Rana *et al*., 2017).

A truncated hemoglobin, designated Hell's Gate Globin IV (HGbIV), has been reported from a thermophilic, acidophilic obligate methotroph bacterium *Methylacidiphilim infernorum* (Hou *et al*., 2008). HGbIV differs from all the other structurally characterized group II tHbs due to large size (>197) compared to the typical size of other group II tHbs (120-140), and by their polar heme pocket residues: His(B9), His(B10), Ser(E11) and Trp(G8). The crystal structure of HGbIV (PDB: 4NK1) showed a bulky heme-bound phosphate ligand in the heme pocket (Jamil *et al*., 2014). It is observed that the two consecutive His residues at B9 and B10 positions provide more space for phosphate ion, suggesting that HGbIV heme pocket is designed for bulky ligand. This finding was reinforced by another crystal structure of HGbIV (4NK2) showing a very large electron density for an unknown heme bound ligand (Jamil *et al*., 2014).

This study is designed to determine the possible interaction of HGbIV with different already reported smaller ligands, such as O_2, NO, CO, CO_2, CH_3COO^{-1}, CN^{-1} for other bacterial tHbs (Dikshit *et al*., 1992; Couture *et al*., 1999; Pesce *et al*., 2000; Ouellet *et al*., 2002, 2003; Igarashi *et al*., 2011). It will be interesting to understand how smaller ligands will be stabilise in the bulky heme pocket of HGbIV.

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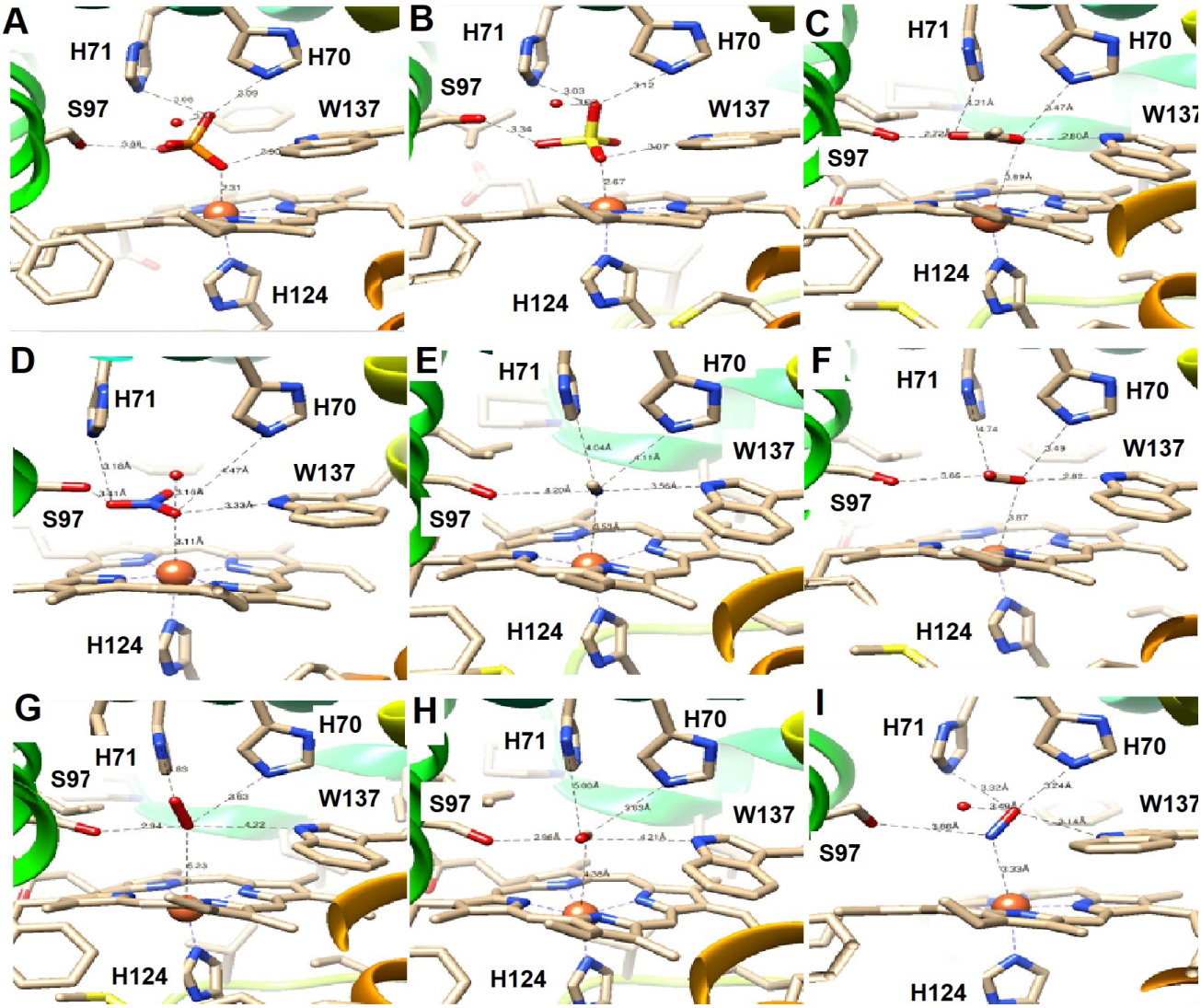

Fig. 1. Heme pockets of HGbIV. A to I, the docked PO_4^{-3}, SO_4^{-2}, CH_3COO^{-1}, NO_3^{-1}, CN^{-1}, CO_2, O_2, CO and NO in the heme pocket.

Methods

The PDB files of HGbIV (4NK1) and different ligands (SO_4^{-2}, NO_3^{-1}, CH_3COO^{-1} and CN^{-1}, O_2, NO, CO and CO_2) were retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) (Berman *et al*., 2000). Chimera was used for the energy minimization of HGbIV and ligands (Petterson *et al*., 2004). PDBQT files were generated by using the autodock 4 and autodock vina (Trott and Olson, 2010), and Gasteiger charges were added to convert protein and all ligands into PDBQT files. By using Autodock Vina, targeted docking was performed at the heme pocket of HGbIV with the above mentioned ligands and structure was visualized by using Chimera (Petterson *et al*., 2004).

Results

A targeted docking strategy was used for docking a PO_4^{-3} ion in the heme pocket of HGbIV (4NK1) where it docked with the binding affinity of -17.16 KJ/mol (Fig. 1A; Table I). Superimposition of the docked PO_4^{-3} ion in HGbIV to the real 3D structure of HGbIV showed that the ion docked at same orientation as observed in crystal structure of HGbIV. Moreover, it shows similar interactions with heme pocket residues namely His70(B9), His71(B10), Ser97(E11) and Trp137(G8) as observed in its real 3D crystal structure of HGbIV (Jamil *et al*., 2014). SO_4^{-2} ion is a structural analogue of PO_4^{-3} ion, it docked in the heme pocket of HGbIV relatively far (2.67 Å) from heme-iron compared to the position of PO_4^{-3} ion with binding affinity of -17.16 KJ/mol (Fig. 1B; Table I).

Analyses of the structure showed that most of the heme pocket residues can stabilize SO_4^{-2} ion except Ser97(E11) which is relatively far (3.34 Å) away (Fig. 1B).

Table I.- Ligands and their binding affinity.

S No.	Ligands	Binding affinity (kJ/mol)	Hydrogen bonds
1	PO_4^{3-}	-17.16	His70(B9), His71(B10), Ser97(E11), Trp137(G8)
2	SO_4^{2-}	-17.16	His70(B9), His71(B10), W137(G8)
3	CH_3COO^-	-12.13	Ser97(E11), Trp137(G8)
4	NO_3^{-1}	-11.30	His70(B9)
5	CN^-	-5.44	No residue
6	CO_2	-10.04	Ser97(E11), Trp137(G8)
7	O_2	-8.37	Ser97(E11)
8	CO	-6.69	Ser97(E11)
9	NO	-5.85	Trp137(G8)

CH_3COO^{-1}, NO_3^{-1} and CN^{-1} ions docked in the heme pocket of HGbIV at a distance of 3.89, 3.11 and 3.53 Å away from the heme iron with binding affinities of -12.13,-11.30 and -5.44 KJ/mol, respectively (Fig. 1C-E). Analyses of the structures showed that the docked CH_3COO^{-1} ion is stabilized by Ser97(E11) and Trp137(G8), whereas the His70(B9) and His71(B10) are far away (> 3.2 Å) from the ion. Previously, CH_3COO^{-1} ion has been reported in a crystal structure of a tHb from *Thermobifida fusca* (tf-tHgb). A notable difference between the two structures is long distance of His(B10) from the docked CH3COO^{-1} ion in HGbIV compared to that of Tyr(B10) in tf-tHgb. On the other hand, the Trp(G8) plays a vital role in interacting and stabilizing the acetate ion in tf-tHgb as well as in HGbIV. Analysis of the docked NO_3^{-1} ion in HGbIV showed that only His70(B9) can interact with NO_3^{-1}while the other heme pocket residues are far away (> 3.2 Å) (Fig. 1D). The CN^{-1} ion docked very far from all the heme pocket residues so no residue can form hydrogen bonding with it (Fig. 1E).

CO_2, O_2, CO and NO were docked in the heme pocket of HGbIV at distance 3.87, 5.23, 4.38, 3.33, 3.51 Å away from the heme iron with binding affinity -10.04, -8.37, -6.69 and -5.85 KJ/mol, respectively (Table I; Fig. 1F-I). Analyses of these structures showed that the CO_2 is stabilized by interaction of Ser97(E11) and Trp137(G8), whereas CO and O_2 are stabilised only by Ser97(E11). On the other hand, NO may only interact with Trp137(G8).

Discussion

This work is an extension of our previous work in which we have determined three dimensional structure of HGbIV under two different conditions (Jamil *et al*., 2014). Structurally, HGbIV belongs to group II tHbs with several unique features. For instance, HGbIV is a large protein (194 amino acids) compared to other bacterial group II tHbs (120-140). Second, the heme pocket of HGbIV is more polar and host two His residues at B9 and B10 positions compared to highly conserved Phe(B9) and Tyr(B10) residues in other bacterial tHbs . Third, the crystal structure of HGbIV (4NK1) showed that the heme pocket can accommodate a bulky heme-bound phosphate ion. On the other hand, smaller ligands like CH_3COO^{-1}, NO_3^{-1}, CO_2, O_2, CO, NO, CN^{-1} and H_2O have been reported in other bacterial tHbs (Pesce *et al*., 2000; Bonamore *et al*., 2005; Ilari *et al*., 2007; Igarashi *et al*., 2011; Jamil *et al*., 2014). In this study we docked smaller ligands in the heme pocket of HGbIV.

The PO_4^{-3}, SO_4^{-2}, CH_3COO^{-1}, NO_3^{-1}, CO_2, O_2, CO, NO and CN^{-1} were docked in the heme pocket of HGbIV with the binding affinities of -17.16,- 17.16, -12.13, -11.30, -10.04, -8.37, -6.69, -5.85 and -5.44 Kj/mol, respectively (Table I). Analysis of these values clearly showed that the heme pocket of HGbIV is designed for bulky ligands like PO_4^{-3} as it is only ligand that can interact with all the active site residues namely His70(B9), His71(B10), Ser97(E11) and Trp137(G8) (Fig. 1). On the other hand, smaller heme-iron bound molecules are not so stabilized in this pocket due to far location of the active site residues. In other group II bacterial tHbs smaller heme-bound ligands are mostly stabilized by highly conserved Tyr(B10) which is replaced by His71(B10) in HGbIV. In conclusion, it appears that heme pocket of HGbIV is specific for bulky ligands and we forsee remarkable displacement in HGbIV for smaller ligands stabilization.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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