STRUCTURE OF FERROUS FORMS OF THE YELLOW LUPIN LEGHEMOGLOBIN

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Legume hemoglobin – leghemoglobin (Lb) – was discovered by Kubo [1] in root nodules of leguminous plants. These nodules are a natural nitrogen-fixing system which is a result of the symbiosis of high plants with the bacteria *Rhysobium*. Lb does not directly take part in nitrogen fixation. Possessing very high affinity for oxygen (P_{50} = 0.05 torr), Lb has a high combination rate constant k = 118 $\mu M^{-1} \cdot sec^{-1}$ and a comparatively fast dissociation rate constant k = 4.4 sec^{-1} [2]. These properties mean that Lb may, on the one hand, maintain proper tension of oxygen in nodules and, on the other hand, provide transport of oxygen.

In many respects (magnetic susceptibility data, spectroscopic data, etc.) Lb resembles animal hemoglobins and myoglobins. Its molecular weight is about 17000. Lb has the same type of polypeptide chain folding as other hemoglobins. Lb consists of 7 α-helices and a porphyrine group – the heme group (figure 1).

We have studied deoxy-Lb and three ferrous complexes of leghemolgobin with CO, NO and O_2. In nodules Lb was detected only in oxy and deoxy ferrous states.

All experimental data sets were collected on a CARD-diffractometer with a multiwire detector at the Institute of Crystallography in Moscow [3]. Experimental data for LbO_2 were obtained in the atmosphere of argon at –20° C. Data sets for other complexes were collected at room temperature.

All complexes have been refined by the Hendrickson-Konnert restrained least-square method [4]. Deoxy-Lb was refined by Jack-Levitt procedure [5]. The best results were obtained for LbO_2. Models include 139, 150 and 254 water molecules for LbCO, LbNO and LbO_2, respectively. Summary of the refinement is given in Table 1.

Table 1. Summary of the refinement.

Complex	deoxy-Lb	LbCO	LbNO	LbO_2
Program	J – L*	H – K	H – K	H – K
Resolution (Å)	2.0	1.8	1.8	1.7
N_o of reflections, I σ (I)	10172	10925	11512	13063
N_o of solvent atoms	68	139	150	254
$R = \Sigma\|F_o\| - \|F_c\|/\Sigma\|F_o\|$(%)	20	18	18	15

*: J–L – Jack & Levitt [5], H–K – Hendrickson & Konnert [4].

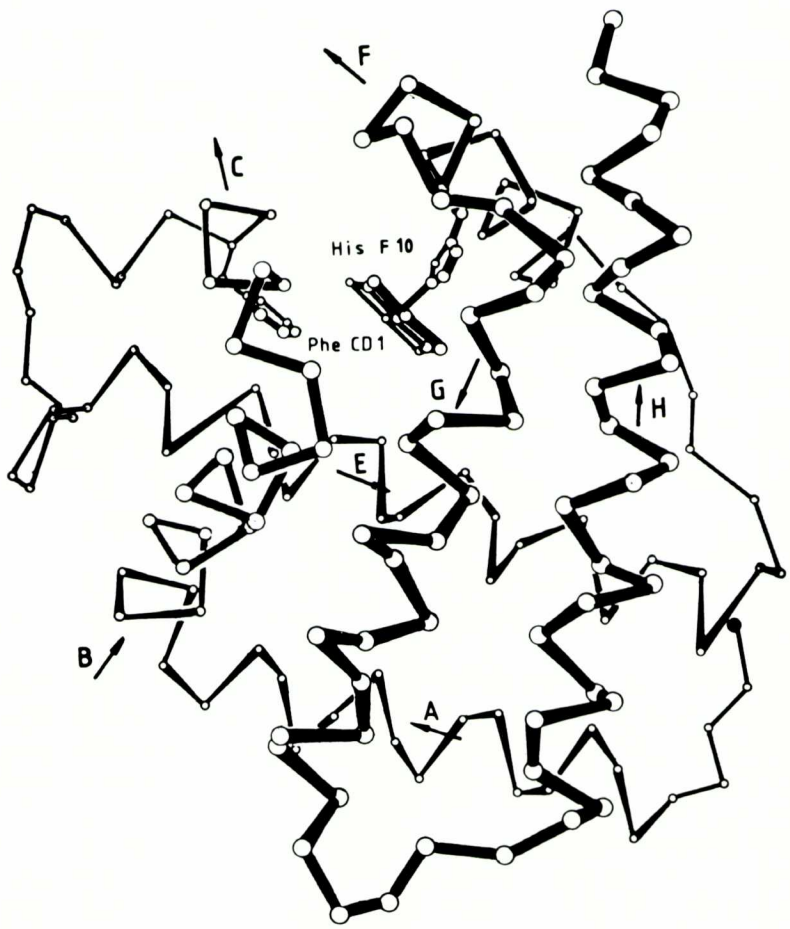

Fig. 1. Polypeptide chain of leghemoglobin. Arrows indicate directions of helices.

Ligands were localized on the difference electron density maps. In the course of the refinement we did not use restraints for the bond between Fe and the first ligand atom and for the angle Fe–L_1–L_2 (L_1 – first ligand atom, L_2 – second ligand atom). Standard lengths for the bond between two ligand atoms were 1.15, 1.13 and 1.22 Å for LbCO, LbNo and LbO_2, respectively. Occupancies for ligand atoms were refined. Occupancies for atoms of CO and NO ligands were 1.0. Occupancies for ligand atoms in LbO_2 were 0.4 and 0.5. Such low values of occupancies in the last case are probably due to the disorder of the oxygen molecule.

The geometrical parametes for ligand binding are given in Table 2. Fe-C bond in CO-complex is markedly shorter than the same bonds obtained by X-ray analysis in CO-complexes of myoglobin [6] (2.27 Å) and erythrocruorin [7] (2.40 A). Still, these lengths determined by EXAFS-spectroscopy [8] and Raman spectroscopy [9] in myoglobin (Mb) and erythrocruorin (Ery) complexes are in good agreement (1.97 and 1.8 Å) with our results.

Stereochemistry of molecular oxygen binding has some specific features. Two oxygen atoms of the ligand are located at approximaltely equal distances from the Fe atom (Fe–L_1 is 2.00 Å, Fe–L_2 is 2.17 Å). The angle Fe–L_1–L_2 is 81° but not 120° as predicted by Pauling [10]. The values of this angle in model systems are 129° and 131° [11, 12], in MbO_2 and $EryO_2$ – 115° and 170°, respectively.

Table 2. Geometrical parameters of ligand binding.

Complexes	deoxy-Lb	LbCO	LbNO	LbO_2
Distances (Å)				
Fe–L_1	–	1.95	1.79	2.00
Fe–$N^{\varepsilon 2}$ (His)	2.25	2.27	2.20	2.21
ΔFe*)	0.08	0.02	0.03	0.30
ΔC_M 2*)	0.13	0.27	0.20	0.25
Angles (degrees)				
Fe–L_1–L_2 3*)	–	151	154	81
between Fe–L_1 and the normal to the heme plane	–	0.6	8.6	20.1
torsion angle $C^{\varepsilon 1}$ – $N^{\varepsilon 2}$ – Fe – N1 4*)	– 55	– 60	– 60	16

*) ΔFe – distance between Fe atom and the heme plane.
2*) ΔC_m – average deviation of methine C atoms from the heme plane.
3*) L_1, L_2 – atoms of the ligand.
4*) $C^{\varepsilon 1}$, $N^{\varepsilon 2}$, – atoms of the proximal His, N1 – atom of the heme.

Angles Fe–L_1–L_2 are 151, 154 and 81° for CO, NO and O_2 complexes. Angles between Fe–L_1 bond and the normal to the heme plane are 0.6, 8.6 and 20° for CO, NO and O_2 complexes, respectively. Probably the ligand's bent binding geometry is largely determined by its direct interaction with the heme than with the protein environement.

The binding of the ligands in CO and NO complexes causes a shift of the Fe atom towards the ligand into the heme plane and the rotation of the heme relative to the globin. On the contrary, transition of Lb from deoxy to oxy-form causes the shift of Fe in the direction of the proximal His (figure 2). The distance between Fe and the heme plane is 0.30 Å. Thus, the most important difference of LbO_2 from other ferrous Lb derivatives lies in the relationship between the Fe atom and the heme.

Oxygenation leads to the 70° rotation of the His F11 imidazole ring thus breaking the hydrogen bond between His F11 and Leu F7. In this conformation the angle between the imidazole ring and the heme plane is 71°. Due to the rotation of the proximal His ring the plane of imidazole ring passes through the line connecting two nitrogen atoms of the heme. The angle between planes of proximal and distal His changes from 74 to 124°.

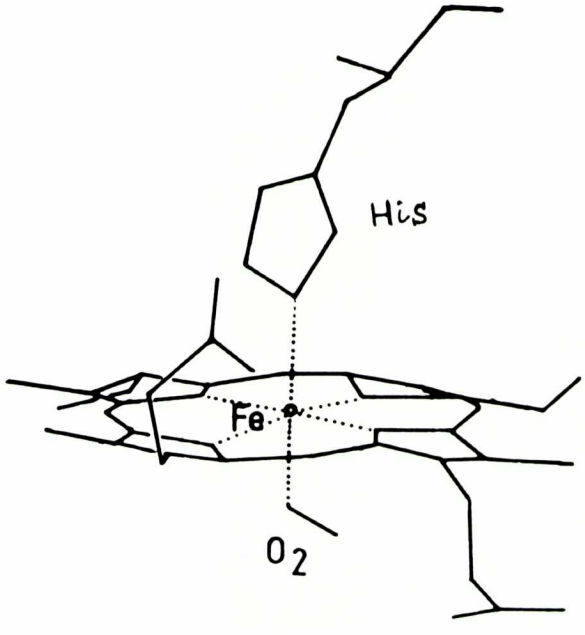

Fig. 2. The heme, proximal His and the ligand in LbO_2.

Table 3. Mean-square deviations of C_α–coordinates (1) and all atom coordinates (2) in CO, NO and O_2 complexes after superimposition with deoxy-Lb.

Complex	LbCO		LbNO		LbO_2	
	1	2	1	2	1	2
Helices						
A	0.17	0.31	0.19	0.34	0.22	0.41
B	0.16	0.28	0.18	0.30	0.15	0.36
C	0.17	0.32	0.17	0.38	0.15	0.35
E	0.19	0.35	0.18	0.39	0.32	0.40
F	0.22	0.51	0.23	0.44	0.35	0.70
G	0.18	0.38	0.16	0.44	0.20	0.36
H	0.21	0.47	0.17	0.42	0.31	0.48
Non-helical regions						
NA	0.63	0.87	0.61	0.70	1.00	1.07
CD	0.26	0.50	0.26	0.59	0.28	0.59
EF	0.19	0.33	0.12	0.30	0.24	0.46
FG	0.14	0.26	0.17	0.25	0.29	0.32
GH	0.10	0.26	0.21	0.26	0.23	0.36
The heme	–	0.30	–	0.19	–	1.08
R.m.s. deviation	0.21	0.40	0.21	0.41	0.30	0.51

Average deviations of heme methine bridges are 0.27, 0.20 and 0.25 Å for CO, NO and O_2 complexes, respectively. These deviations are equal to rotation of pyrrole rings relative to the least-square plane of the heme through 6°. In deoxy-Lb this average deviation is 0.13 Å. So we can suggest that maximum distortion of the heme electron system occurs due to the binding of CO and O_2 ligands. The degree of the heme ruffle is dependent only on the valent and spin state of the Fe atom, but not on the size of the ligand. This suggestion is confirmed by the geometry of ferrous Lb complex with nitrozobenzole, where the average deviation of methine bridges is only 0.18 Å.

Oxygenation changes the degree but not the character of the heme distortion. Therefore the type of the heme distortion should be connected with the affinity of hemoglobins to molecular oxygen. In myoglobin and hemoglobins the domeshaped distortion of the heme is observed and their affinity to oxygen is significantly lower than in Lb.

In deoxy-Lb both propionic acids of the heme are oriented towards the distal side, thus covering the heme pocket. In CO, NO and O_2 complexes one of these residues turns away towards the proximal side.

In the course of ligand binding some conformational changes in the side chains, in the secondary structure and in the heme occur. The most mobile is the N-terminus of the molecule. Results of the superimposition of CO, NO and O_2 complexes with deoxy-Lb based on C_α–atoms are given in Table 3. Ligand binding causes rotation of the heme relative to the protein part. This rotaton for CO and NO complexes can be described as a rotation of the protein about the line that passes through the Fe atom parallel to B-helix. The angle of rotation in the CO complex is larger than in the NO complex. The direction of rotation in LbO_2 is opposite to that observed in CO and NO complexes.

The main peculiarity of oxygen binding is the marked disorder of O_2 molecule. It may be due either to the different geometry of binding in different Lb molecules or to significant thermal motion of oxygen molecule. It may be possible that it is this ability of Lb to bind oxygen in arbitrary orientation that provides the high affinity of Lb to oxygen.

Systems of hydrogen bonds and ionic interactions in these complexes were compared. It was shown that the principal differences are observed on the surface of the molecule. Still, there are also some changes in the bonds, in which heme pocket residues take part.

Abstract

Crystal structures of deoxy-leghemoglobin and three ferrous complexes of leghemoglobin (Lb) with CO, NO and O_2 were studied by X-ray analysis at 2.0, 1.8, 1.8 and 1.7 Å resolution for deoxy-Lb, LbCO, LbNO and LbO_2, respectively. All experimental data arrays were collected on a CARD-diffractometer with a multiwire detector. Experimental data for LbO_2 were obtained at –20° C. Data arrays for other complexes were collected at room temperature. All the complexes were refined by the Hendrickson-Konnert method. Deoxy-Lb was refined by Jack-Levitt procedure. The final R-values are 0.20, 0.18, 0.18 and 0.15 for deoxy-Lb, LbCO, LbNO and LbO_2 respectively. Angles Fe–L_1–L_2 are 151, 154 and 81° for CO, NO and O_2 complexes. The binding of the ligands in CO and NO complexes causes a shift of the Fe atom towards the ligand into the heme plane. On the contrary, transition of Lb from deoxy to oxy-form causes the shift of Fe in the direction of the proximal His. The distance between Fe and the heme plane in LbO_2 is 0.30 Å. Oxygenation leads to the 70° rotation of the His F11 imidazole ring. Geometrical parameters of the heme show that the degree of the heme ruffle is dependent only on the valent and spin state of the Fe atom, but not on the size of the ligand. Oxygenation changes the degree but not the character of the heme distortion. The main peculiarity of oxygen binding is the marked disorder of O_2 molecule (occupancies for ligand atoms 0.4 and 0.5). It may be due either to the different geometry of binding in different Lb molecules or to significant thermal motion of O_2 molecule. It may be possible that it is this ability of Lb to bind O_2 in arbitrary orientation that provides the high affinity of Lb to oxygen.

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