Hydrogen Bonding and O_2 Affinity of Hemoglobins

Damián A. Scherlis and Darío A. Estrin*

Departamento de Química Inorgánica Analítica y Química Física/INQUIMAE Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires Ciudad Universitaria, Pabellón II, piso 3 C1428EHA Buenos Aires, Argentina

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Hemoglobin (Hb) is well-known for its ability to transport oxygen and carbon dioxide in the vascular system of animals. However, through the design of the heme pocket, it can be adapted to many other biological functions as well. By substituting only a few amino acid residues the O_2 affinity can be increased several orders of magnitude, and thus the reversible oxygen binding, essential to physiological transport, is replaced by catalytic activities involving O_2 metabolism.[1] An example of such an extraordinary affinity for dioxygen is found in the hemoglobin of the parasitic nematode *Ascaris lumbricoides*.[2] This is an anaerobic organism, and recent studies have shown that its hemoglobin is actually a deoxygenase that enzimatically consumes O_2 in a reaction driven by nitric oxide, thus keeping hypoxic conditions in the perienteric fluid of the worm.[3] It has been proposed that the unusually high equilibrium constant observed in this Hb is due to the formation of hydrogen bonds between oxygen and two amino acids of the heme pocket: tyrosine B10 and glutamine E7 (Figure 1a).[2,4,5]

Human Hb is a tetrameric ($\alpha_2\beta_2$) protein: each of its four subunits contains an iron(II)-heme group capable of binding molecular oxygen. On the basis of X-ray analysis, Shaanan[6,7] proposed that a hydrogen bond to E7 histidine, called the distal residue, stabilized the heme-linked O_2 in the active site of the α-subunit (Figure 1b), while no hydrogen bonding was present in the β-chain. These conclusions were also supported by Olson et al.,[8] who demonstrated by means of site-directed mutagenesis that substitution of the distal histidine by glycine in R-state Hb produced a marked decrease in the oxygen affinity of the α-subunit and little or no effect on the ligand binding properties of the β-subunit. Despite this structural difference, oxygen affinities are of the same order in both subunits,[8,9] while in the ascaris hemoglobin it is much higher. Regarding human Hb, the accepted explanation is that proximal effects and a more accessible distal pocket compensate for the lack of H-bonding in the β-subunit to yield almost equal equilibrium constants in the α- and β-chains.[7,8] Taken together, these results demonstrate that the contribution of hydrogen bonding to the oxygen affinity is still one of the most puzzling aspects of hemoglobin chemistry.

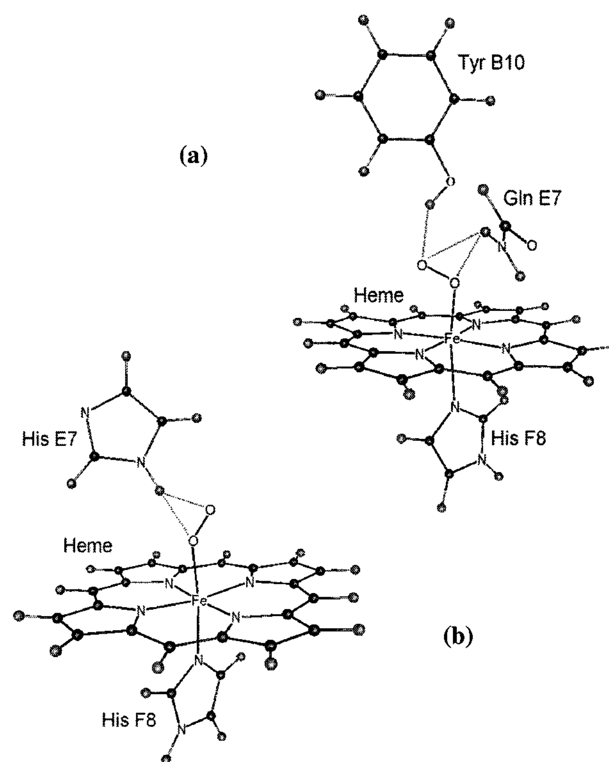

Figure 1. Model of the active sites for the (a) ascaris and (b) human hemoglobins. Dashed lines indicate hydrogen bonds.

A large number of quantum chemistry calculations of porphyrin models have been performed over the past decade to investigate structural, thermodynamic, and spectroscopic aspects of heme proteins.[10] The role of the protein electrostatic potential on the reactivity of heme systems has been assessed using a Poisson−Boltzmann scheme based on the protein atomic partial charges[11a,b] and a hybrid QM-MM approach.[11c] Hydrogen bonding effects of the distal amino acid have also been studied in myoglobin using density functional theory (DFT) calculations for a model system.[12] In the present study DFT[13] in combination with the SAM1 semiempirical electronic structure method[14] were used to estimate the interaction energies associated with H-bonds in the active site of ascaris Hb, as well as in the α- and β-subunits of human Hb in the R-state. The model employed to represent the active site

* To whom correspondence should be addressed. Fax: +5411 4576 3341. E-mail: dario@q1.fcen.uba.ar.

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Table 1. Interaction Energies (E_{Int}) and Mulliken Charges on Relevant Atoms

	E_{int} (kcal/mol)	Mulliken Charges		
		Fe	O1[a]	O2
ascaris (B10 + E7)	−12.8	1.13	−0.15	−0.22
ascaris (B10)	−7.2	1.11	−0.12	−0.21
ascaris (E7)	−4.3	1.09	−0.11	−0.20
α-subunit	−4.1	1.08	−0.10	−0.21
β-subunit	−6.8	1.10	−0.19	−0.16

[a] O1 refers to the iron-linked oxygen.

Table 2. Selected Distances (Å) in the Three Hemoglobin Active Sites[a]

	ascaris	α-subunit	β-subunit
Fe−N(F8)	2.201	1.934	2.065
Fe−O1	1.837	1.857	1.817
H(E7)-O1	2.691	2.073	2.312
H(E7)-O2	2.722	1.717	2.831
H(B10)-O1	2.646	-	-
H(B10)-O2	1.900	-	-

[a] Atoms belong to the residues indicated in parenthesis (see Figure 1).

of the ascaris protein, depicted in Figure 1a, is a 67-atom structure consisting of the heme group plus the (truncated) distal and proximal residues (glutamine E7, tyrosine B10, and histidine F8) with a geometry taken from crystallographic data.[5] In addition we also have considered two model systems in which only one of the distal amino acids was included to analyze cooperative effects. On the other hand, to model the active sites of the α- and β-chains we used the system of 57 atoms shown in Figure 1b, which includes the heme unit plus the (truncated) proximal and distal histidines. In this case the geometry corresponds to the X-ray experimental structure of ref 7. Since the motion of the porphyrin ring and the nearby residues is constrained by the protein environment, relaxation from the crystal geometry was not allowed; only the coordinates of hydrogen atoms and of the bound O_2 were optimized with the SAM1 method.

The hydrogen-bond energies computed using DFT at the generalized gradient approximation (GGA) level are displayed in Table 1. This methodology has proved to be reliable to describe H-bonding in a variety of systems.[15] E_{int} values in Table 1 correspond to the difference between the energy of the oxygenated active site and the energy of a model where the distal residue or residues lie infinitely apart from the rest of the system. Therefore, energies computed in this way account specifically for the interaction of the oxygenated active site with the distal amino acids.

We found that the strength of the H-bonds is significant in all considered Hb. This result was predictable since a large extent of negative density is transferred from the iron toward the oxygen molecule, which can be explained in terms of a significant metal-to-ligand π^* back-donation (see the Mulliken atomic charges in Table 1). In the case of the ascaris molecule, data in Tables 1 and 2 show that the hydrogen of tyrosine B10 [H(B10)] forms a strong H-bond with the second oxygen (O2), while the contribution of the glutamine hydrogen [H(E7)], located more than 2.5 Å away from any of the oxygens, is lower. We found also that the interaction of glutamine with tyrosine polarizes the hydroxyl tyrosine moiety, leading to enhanced H-bonds with O_2 when both amino acids are included in the calculation. Results in Table 2 show also that H(E7)−O distances are shorter in the distal pocket of the α-chain, but larger in the β-chain. However, the Fe−O bond length is longer in the former: this is suggesting that there is a competition between the distal histidine and the iron for the O_2 molecule, which results in a larger net interaction energy with the distal amino acid in the β subunit. Our H-bond stabilization for human Hb are of the same order of magnitude of the experimental estimate of 4 kcal/mol obtained in myoglobin using site directed mutagenesis data.[16]

The present results confirm the existence of strong hydrogen bonds in the ascaris Hb, in the α-subunit of the human Hb, and reveal a strong hydrogen interaction also in the β-subunit, in contradiction with previous observations.[7,8]

At this point it is worth noting that the global equilibrium constant for ligand binding of an heme protein corresponds to a complex phenomena, which may be modeled in three stages, for example, ligand partitioning between the solvent phase and the protein matrix, transport across the polipeptidic matrix from the outside part to the coordination position, and finally the binding process itself, which involves the reactivity of the metal center and distal effects.[17] H-bonding is just a single contribution among other factors which play important roles in determining the affinity of the Hb. Proximal effects, mediated by the Fe−N distance and the orientation of the F8 histidine, are likely to modulate the reactivity toward O_2.[7,18] In the ascaris Hb case it seems possible, as discussed by Peterson et al.,[19] that the structure and electrostatics of the distal cavity conform an effective cage where the O_2 molecule remains trapped. There is evidence[2,5] of an H-bond between H(E7) and the hydroxyl of the tyrosine residue that brings the distal chains together, favoring the probability of ligand rebinding.

Of much interest is the work of Miele et al.,[20] who replaced the native B10 and E7 residues in human Hb by tyrosine and glutamine respectively, to mimic the behavior of the ascaris protein. Conversely to what we expected, the mutants displayed decreased affinity in the binding of O_2. In this context the comparison between the ascaris and Lucina hemoglobins described in ref 17 is also interesting. Both proteins contain tyrosine B10 and glutamine E7 in the heme pocket; however, their oxygen affinities are astonishingly different.

Mutagenesis experiments using different Hbs show that the increase of the dissociation rate resulting from the suppression of an H-bond in the distal pocket is almost always accompanied by a rise in the association kinetic constant.[8] This means that the residues involved in H-bonding are also involved in the kinetic barrier, and in this way a strong H-bond does not necessarily imply a high affinity constant.

Our results confirm that H-bonding plays an important role in determining oxygen affinity in hemoglobins. However, the role of factors other than proximal effects and H-bonding should be also investigated to obtain a more complete picture of O_2 affinity of hemoglobins.

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Supporting Information Available: Details on the computational techniques and the optimized coordinates of the model systems (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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