Interaction thermodynamics of human hemoglobin with environmental and toxic gases: A density functional theory study

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Abstract. The interaction of human hemoglobin with environmental and other gases and molecules is investigated using density functional theory. The investigation includes gases and molecules such as O₂, N₂, Ar, CO₂, H₂O, CO, and Cl₂. Thermodynamic quantities usually include Gibbs free energy, enthalpy, and entropy. These thermodynamic quantities can be used to distinguish how much strong these molecules are bonded to hemoglobin. The interaction with the two different heme molecules, singlet and triplet states, is shown. Results show that the bonding strength differs greatly between these gases. Most of the investigated molecules remain at their monoatomic, diatomic, or triatomic structure except for O₂ and Cl₂ that may dissociate into two atoms attached to hemoglobin. The Gibbs free energy of interaction of these atoms and molecules reveals the toxicity of some of these gases, such as CO and Cl₂.

Keywords: Hemoglobin; Density functional theory; Gibbs free energy.

1. Introduction

Hemoglobin is known for being the carrier of many materials in human blood. The most important among these is oxygen (oxyhemoglobin) and carbon dioxide (carbaminohemoglobin) in breathing operations [1–3]. In addition to carrying vitally important gases and materials, it is exposed to gases in the environment that may have different effects on its work effectiveness. Some gases such as carbon monoxide [4] turns hemoglobin (Carboxyhemoglobin) into a stable compound that is unable to carry oxygen any more [5–7]. Other gases in the environment may interact with hemoglobin in less destructive ways. The most abundant gas in the atmosphere is nitrogen (N₂) that forms 78.1% of Earth's air by volume. The second abundant gas is oxygen (O₂) that forms 21% of air volume. Ar noble gas is the third abundant gas in Earth’s atmosphere, with less than 1% by volume. The interaction of these gases with hemoglobin is investigated in the present work. In addition to these gases, other gases with variable content in the atmosphere, less abundant or toxic gases are investigated. This includes CO₂, H₂O, CO, and Cl₂.

Density functional theory (DFT) is a cutting-edge theoretical method that can be used in chemistry [8], physics [9], engineering [10], and biology [11]. One of the current applications of DFT in modern...
physics and chemistry is gas sensing [12,13]. In order to use DFT in gas sensors effectively, other related methods such as transition state theory can be combined with DFT calculations [12,14].

The analysis of hemoglobin structure and reactions using DFT is approached several times in literature [15–18]. Hemoglobin structure and reactions can be analyzed through the use of thermodynamic quantities such as Gibbs free energy, enthalpy, and entropy [19–22]. The importance of Gibbs free energy relies on that it can predict the relative degree of stability of the chemical structure and spontaneous reactions. Enthalpy that is one of the components of Gibbs free energy, can explain the quantity of heat absorbed or emitted in reactions. On the other hand, the other constituent of Gibbs free energy, namely entropy, represents the randomness or disorder of the system.

In the present work, the structure of the heme part of hemoglobin is analyzed. The electronic charges and electrostatic potential are also analyzed to inspect the best interaction parts of heme. The heme structure is used to calculate the change in thermodynamic quantities, namely Gibbs free energy, enthalpy, and entropy due to reaction with different kinds of gases. These gases include O$_2$, N$_2$, Ar, CO$_2$, H$_2$O, CO, and Cl$_2$. The results manifest the degree of stability of the different reaction products and the anticipated final structure.

2. Theory

The B3LYP version of the DFT method is used in the present calculations. B3LYP is one of the successful methods that is frequently used in DFT calculations. The B3LYP method is also used reliably with good agreement with the experiment [23,24]. The basis states used in present calculations are 6-311G** [25]. These basis states are currently used in gas interaction, sensing, and biology, providing reliable and good results [13,26–29]. Gaussian 09 program that is used to perform present calculations can be considered as a standard program in DFT and other related molecular and nanoscale calculations [30].

Due to the existence of the Fe ion with its d electrons in the heme molecule, the lowest energy occurs at total spin states greater than zero spin. In fact, the lowest spin state is at S=1 triplet state (M=3), as we shall show in our results in the next section. This explains the magnetic behavior of the heme molecules [31–33]. The second-lowest spin state of heme is S=0. The other spin states have energies that are higher than S=0 and 1 spin states. As a result, we shall confine our discussion to the upper mentioned lowest two states.

Transition state theory provides a mature understanding of chemical interaction [12,34–36]. The theory describes a barrier that must be overcome so that the reaction can proceed between constituents. The value of the energy of this barrier is called the activation energy, which will be described below. The reaction rate of the heme group in hemoglobin with oxygen can be given by the following equation [37]:

\[
\frac{d[heme]}{dt} = -C[heme][O_2]e k(T).
\] (1)

In the above equation, [heme] is the concentration of the heme group in blood. [O$_2$]$_e$ is the effective concentration of oxygen that diffuses through the blood vessels' wall. C is the reaction proportionality constant, and k(T) is given by:

\[
k(T) = T \exp \left( \frac{-\Delta G_a}{k_B T} \right).
\] (2)

In the above equation, T is the temperature, $k_B$ is Boltzmann constant, and $\Delta G_a$ is the activation energy or Gibbs free energy of activation for this reaction. Since the temperature is nearly constant inside the human body, the reaction rate depends only on the concentration of [heme], [O$_2$]$_e$, and the activation energy $\Delta G_a$. $\Delta G_a$ is different for each interacting gas with hemoglobin. The activation energy is smaller for gases with dipole moment since a dipole-dipole interaction will take place between the heme group dipole moment and the gas dipole moment that will reduce the value of activation energy.
3. Results and discussion

Figure 1a shows the heme group in its unoxidized state. The heme group is an Fe complex in the center of the porphyrin molecule group (C\textsubscript{20}H\textsubscript{12}N\textsubscript{4}). The Fe is also connected to an axial 4-Imidazoleacetate (C\textsubscript{5}H\textsubscript{5}N\textsubscript{2}O\textsubscript{2}) molecule. Figure 1b shows the charges on each atom in the heme molecule using natural bond order analysis (NBO). This figure shows that the highest positive charge in the heme molecule is located at the Fe ion. The highest negative charges are located at oxygen and nitrogen ions.

Figure 2 shows the electrostatic potential energy surface of the heme molecule. We can see from this figure that the positive energy surface covers most of the molecule due to the existence of hydrogen ions near the surface. An exception occurs near the negative nitrogen ions around the Fe ion and O ions near the end of the axial structure of the heme molecule. This agrees with figure 1b that represents the charges on each atom in heme.

Figure 3 shows the oxidized heme molecule in the singlet state (\(M=1\)). Both two atoms are connected to one heme molecule. This state has lower energy than that of one oxygen atom connected to one heme molecule in the singlet state (\(M=1\)). The difference in energy is in the order of 1 eV, which is high enough to render the formation of a single oxygen atom connected to the heme molecule. The reverse is true for the triplet state, as we shall see in Table 2 below. Since oxygen is divalent, the structure in figure 3 has a dangling bond at the far oxygen molecule (from Fe ion). This dangling bond eases the operation of breaking the bonding between the oxygen molecule and the heme molecule so that the oxygen molecule can be transformed to other parts of the human body that has lower oxygen concentration which is the opposite operation in Eq. 1. This reduction appears in the value of \(\Delta G_a\) in Eq. 2. This dangling bond disappears when discussing poisonous gases such as CO or Cl\textsubscript{2} that bond to the heme molecule, as we shall discuss in Table 2.
Figure 1. (a) The heme molecule, (b) charges on each atom in the heme molecule using natural bond order analysis.

Figure 2. The electrostatic potential of the heme molecule. The pink color represents the positive surface, while the brown color represents the negative surface.
Figure 3. This figure shows the oxidized heme molecule in the singlet state (M=1).

Table 1 shows the Gibbs free energy comparison of the two most stable multiplicities of the heme group molecule. The M=3 basis state is more stable than the M=1 state. The difference between the Gibbs free energies of the two states is 0.016 atomic units (0.426 eV). Higher multiplicities such as 5, 7, etc., are less stable than the M=3 and M=1 state. The final state is a combination of these two multiplicities with the M=3 as the dominant multiplicity.

Table 1: Gibbs energy and its components (enthalpy and entropy) at standard conditions in atomic units (a. u.).

| Molecule | Multiplicity | G (a. u.)       | H (a. u.)       | ST (a. u.)     |
|----------|--------------|-----------------|-----------------|---------------|
| 1 heme   | M=1          | -2706.295625    | -2706.211765    | 0.08385475    |
| 2 heme   | M=3          | -2706.311269    | -2706.223113    | 0.08815472    |

Table 2 shows the thermodynamic quantities of Gibbs free energy (ΔG_r), enthalpy (ΔH_r), and entropy energy (ΔS_rT) of the interaction of several gases with heme molecule in standard conditions with different spin states. These thermodynamic quantities are connected by the equation:

\[ \Delta G_r = \Delta H_r - \Delta S_r T \]  

We can see from Table 2 that all the entropy energies (ΔS_rT) are negative. This is due to the fact that the number of product molecules is less than the reactants [38]. However, the enthalpy of the reaction depends on the heat produced or absorbed by the reaction. Finally, the sign of Gibbs free energy can indicate whether the reaction is spontaneous or non-spontaneous (negative or positive, respectively). From Table 2, we can see that the heme connection with the oxygen molecule (O_2) (n=1,2 in Table 2) is a weak connection (positive Gibbs free energy) both in M=3 and M=1 multiplicity. However, the
interaction of an oxygen atom (O) (n=3,4 in Table 2) has a negative Gibbs energy in the case of the triplet state. The oxidized heme molecule transforms oxygen to the body tissue with the help of catalysts. Both oxygen molecules and atoms that are connected to the heme molecule have dangling bonds that ease their transformation to human cells.

Reactions 5 and 6 in Table 2 shows that nitrogen molecules react weakly with heme molecule. However, the Gibbs free energy of the singlet state nearly vanishes, indicating a possible moderate interaction in this state. As we increase the pressure over the nitrogen gas, the interaction turns to have a negative value. This leads to the transport of nitrogen by the heme molecule inside the human body. Serious effects can result from such transformation since nitrogen interacts with the lipids inside the human body that results in Nitrogen narcosis [39,40]. The same is true for Ar gas as in reactions 7 and 8. Since Argon is a heavy element, its interaction with heme leads to more than twice narcosis effects than nitrogen molecule [41]. On the other hand, the interaction of CO2 gas is weaker than N2 and Ar that results in less CO2 carried gas connected to the heme molecule (as in reactions 9 and 10 in Table 2). In fact, most CO2 molecules are carried out in the human body by the formation of a dissolved solution of carbonic acid.

The interaction of the heme molecule with water is a moderate interaction, as can be seen from the values of Gibbs free energy in reactions 11 and 12 in Table 2. Since H2O already exists in the human body, the heme molecules are already carrying H2O molecules with them in most cases.

Reactions 13 to 18 in Table 2 show the interaction of strongly interacting gases CO and Cl2 with the heme molecule. The interaction of CO with the heme molecule in a singlet state results in a strongly bonded CO molecule with heme without a dangling bond. The resultant molecule cannot be easily broken. As a result, the heme molecule will not be able to carry oxygen again to the human body. The same is true for the Cl2 gas. As a heme molecule that is connected to Cl2 gas interacts with another free heme molecule, two heme molecules that are each connected to a single Cl atom are formed. The two formed heme-Cl molecules will not be able to carry oxygen again, as we can see from the values of the Gibbs free energy of interaction. Since the Cl atom has an odd number of electrons, the final multiplicity of the heme-Cl molecule is (M=2 or M=4).

Table 2: Thermodynamic quantities of the spin states of heme molecule and its reaction with several gases. This includes Gibbs free energies, enthalpy, and entropy energy in eV units at standard temperature (298.15 Kelvin) and pressure (1 atm).

| n  | Reaction       | Multiplicity | ΔG(r) (eV) | ΔH(r) (eV) | ΔS,T (eV) |
|----|----------------|--------------|------------|------------|-----------|
| 1  | heme+O2→heme-O2 | M=1          | 0.722      | 0.147      | -0.575    |
| 2  | heme+O2→heme-O2 | M=3          | 0.510      | -0.026     | -0.536    |
| 3  | 2heme+O2→2heme-O| M=1          | 1.782      | 1.389      | -0.393    |
| 4  | 2heme+O2→2heme-O| M=3          | -0.040     | -0.669     | -0.620    |
| 5  | heme+N2→heme-N2 | M=1          | 0.0001     | -0.4537    | -0.4538   |
| 6  | heme+N2→heme-N2 | M=3          | 0.203      | 0.004      | -0.199    |
| 7  | heme+Ar→heme-Ar| M=1          | 0.299      | -0.040     | -0.340    |
| 8  | heme+Ar→heme-Ar| M=3          | 0.190      | -0.014     | -0.204    |
| 9  | heme+CO2→heme-CO2| M=1         | 0.320      | -0.116     | -0.436    |
| 10 | heme+CO2→heme-CO2| M=3         | 0.288      | -0.044     | -0.331    |
| 11 | heme+H2O→heme-H2O| M=1        | -0.180     | -0.610     | -0.180    |
| 12 | heme+H2O→heme-H2O| M=3        | 0.151      | -0.219     | -0.370    |
| 13 | heme+CO→heme-CO | M=1          | -0.549     | -1.015     | -0.466    |
| 14 | heme+CO→heme-CO | M=3          | 0.776      | 0.270      | -0.506    |
| 15 | heme+Cl2→heme-Cl2| M=1         | 0.129      | -0.262     | -0.391    |
| 16 | heme+Cl2→heme-Cl2| M=3         | -0.175     | -0.643     | -0.468    |
| 17 | 2heme+Cl2→2heme-Cl | M=2       | -2.352     | -2.789     | -0.437    |
| 18 | 2heme+Cl2→2heme-Cl | M=4       | -1.506     | -2.120     | -0.614    |
4. Conclusions
The interaction of several gases (O$_2$, N$_2$, Ar, CO$_2$, H$_2$O, CO, and Cl$_2$) with heme blood molecule is discussed. The two spin multiplicities of the heme molecules, i.e., $M=1$ and $M=3$, are included in the discussion. Results show that the reaction of the heme molecule with O$_2$ can have different values of Gibbs free energy that can determine the rate of reaction. The oxygen atom at the triplet state has the highest reaction rate with negative Gibbs free energy. The interaction of N$_2$ is weak, with the Gibbs free energy of the singlet state having a 0.0001 (eV) value. The interaction of Ar and CO$_2$ is very weak, while the interaction of H$_2$O is relatively strong with the singlet heme state. Finally, the interaction of CO and Cl$_2$ is very strong with the heme molecule that leave the heme molecule unsensitive to any future reactions. The values of Gibbs free energy illustrate the ability of the heme molecule to transfer the interacting species inside the human body or the destruction of the human blood molecule by permanent adhesion of the interacting species with the heme molecule.

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