Spin-Dependent O₂ Binding to Hemoglobin

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ABSTRACT: We report results of our study on the mechanism of spin-dependent O₂ binding to hemoglobin, which we represent as FePIm (Fe = iron, P = porphyrin, Im = imidazole). This involves the transition between two states, viz., the oxyhemoglobin state and the deoxyhemoglobin state. The deoxyhemoglobin state pertains to FePIm and a free O₂ molecule, while the oxyhemoglobin state pertains to an O₂ bound to FePIm. The deoxyhemoglobin and oxyhemoglobin systems have triplet and singlet total magnetizations, respectively. We found that a spin transition from triplet to quartet to singlet mediates the O₂ binding process, and this accelerates the reaction. We also found that the position of the Fe atom out of the porphyrin plane is an important indicator of O₂ affinity.

INTRODUCTION

Hemoglobin is an iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates. O₂ binds to the hemoglobin at high concentrations and desorbs at low concentrations, as it is transported throughout the body. This reversible O₂ binding process has been studied both theoretically and experimentally.1–3 Previous studies show that the electronic structure of the hemoglobin’s active site, that is, heme, plays an important role in determining oxygen affinity.4,5 A heme group consists of an iron (Fe) atom held in a heterocyclic ring, known as porphyrin. The porphyrin ring consists of four pyrrole (C₄H₄NH) molecules, cyclically linked together by methine (=CH=) bridges, with the Fe atom bound in the center. Fe atom coordinates with four N atoms in the center of the ring, which all lie in the plane. Fe covalently binds to the globular protein via the N atom of the imidazole ((CH)₂N(NH)CH) ring of (F₈) histidine residue below the porphyrin plane. In addition, it is well known that metals, such as Fe, determine the electronic, optical, and magnetic properties of metalloproteins. Thus, it is important to examine the metal complex composed of Fe atom (=heme) in order to clarify the physical properties of heme protein.

Oxygen affinity to hemoglobin has been studied experimentally using two model systems. One involves the substitution of the amino acid residue, and the other involves substitution of the heme itself by a similarly modified heme. The former is suitable for studying the regulation of delicate physiological reactions.6,7 The latter is used to improve or to introduce new functions.8 In this study, we focus on the former and represent the hemoglobin using FePIm (P = porphyrin, Im = imidazole), as shown in Figure 1.

The electronic structures of deoxyhemoglobin and oxyhemoglobin have been theoretically studied.9,10 In the deoxyhemoglobin state, we have FePIm and a free O₂ molecule (Figure 2). In this state, the O₂–FePIm distance R = 7.21 Å and the distance of the Fe atom from the porphyrin plane d = 0.18 Å. The total magnetization (spin multiplicity) of the deoxyhemoglobin state M = 1 μₛ (S = 3) [Fe (sz = 2) + O₂ (sz = −1)].11 On the other hand, in the oxyhemoglobin state, we have an O₂ attached to FePIm (Figure 3). The O₂–FePIm distance R = 1.84 Å and the distance of the Fe atom from the porphyrin plane d = 0.01 Å. The total magnetization in the oxyhemoglobin state is M = 0 μₛ (S = 1) [Fe (sz = −1/2) + O₂ (sz = 1/2)]. Thus, we see that reversible O₂ binding involves spin transitions. In this study, we analyzed the change in the spin multiplicity from the initial state to the final state, through various possible intermediate states. We also studied the structural change and energetics, to understand how these factors affect O₂ affinity.

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RESULTS AND DISCUSSION

Optimized Geometry. First, we study how the geometry of O₂ binding changes from the initial deoxyhemoglobin state to the final oxyhemoglobin state (Figures 2 and 3), using CINEB with 4 intermediate states. Table 1 shows the parameters defining the geometry of the deoxyhemoglobin and the oxyhemoglobin states, including the intermediate states. Θ gives the angle subtended by the imidazole plane with the −Z-axis. The optimized structure obtained from this study (Table 1) agrees with experimental results.18 We observed an elongated O1−O2, at a distance of R = 2.40 Å from Fe, in the intermediate state. This suggests the potential use of FePIm as a catalyst for O₂ dissociation, for example, as a cathode electrode in polymer electrolyte fuel cells (PEFCs),19−22 where the oxygen reduction reaction takes place. However, we still need to consider how we can overcome the high activation energy barrier associated with this reaction.

In the deoxyhemoglobin state, the Fe atom lies at a distance d = 0.18 Å out of the porphyrin plane, which is larger than in the oxyhemoglobin state (d = 0.01 Å). Table 1 shows that d decreases as the system changes from the deoxyhemoglobin state to the oxyhemoglobin state. d = 0.15 Å and remains constant when 2.40 Å < R < 4.24 Å. d suddenly decreases to d = 0.01 Å at the oxyhemoglobin state, when R < 2.40 Å. We found a similar trend for the distance Fe−ImN. For Fe−porphyrin without imidazole, the Fe atom lies in the porphyrin plane, that is, d = 0 Å. Because of the interaction between the imidazole and the porphyrin, the Fe atom moves out of the porphyrin plane. The interaction between the Fe (dᵧ) orbital and the N atom in the porphyrin plane shows antibonding character (Figure 2). With O₂ interacting with the Fe atom of the FePIm through the σ-bond between the Fe (dᵧ) orbital and O₂ (π) orbital, the electron in the Fe (dᵧ) orbital flips its spin and moves to the Fe (dᵧ) orbital. Because the Fe (dᵧ) orbital is now empty, the antibonding interaction between the Fe (dᵧ) orbital and the N (π) orbital vanishes. Therefore, the Fe−ImN distance and d becomes shorter, 2.04 and 0.01 Å, respectively. As O₂ approaches the Fe atom, the subtended angle Θ decreases, and we find O₁, Fe, and N forming a linear arrangement.

Spin Multiplicity of O₂ Binding. To understand the O₂ binding process, we investigated the adsorption energy as a function of the corresponding O₁−Fe distance R, starting with the deoxyhemoglobin state through to the oxyhemoglobin state (Figure 4). As reference, we took the corresponding energy of the deoxyhemoglobin state at R = 7.21 Å. We found an activation barrier 0.38 eV for O₂ binding and 0.92 eV for O₂

MATERIALS AND METHODS

We performed spin-polarized density functional theory (DFT)-based calculations as implemented by the Vienna ab initio simulation package (VASP),12−15 using the Perdew–Burke–Ernzerhof exchange–correlation functional,16 energy cutoff of 400 eV, and a (1 × 1 × 1) gamma centered grid. The projector augmented wave method was used to describe the ionic cores. All atoms were relaxed in ionic calculations with an optimization criterion of 0.01 meV/Å inside a 20 × 20 × 27 Å³ supercell. We adopted the climbing image nudged elastic band (CINEB) method for finding the saddle points and minimum energy paths between the initial state (deoxyhemoglobin state) and the final state (oxyhemoglobin state).17

Table 1. Optimized Parameters Defining the Geometry of the Deoxyhemoglobin State, the Oxyhemoglobin State, and the Intermediate States

| State                       | theoretical results | experimental results |
|-----------------------------|----------------------|----------------------|
|                             | deoxyhemoglobin      | intermediate states  | oxyhemoglobin      | deoxyhemoglobin      | oxyhemoglobin      |
|                             | state                | state                | state              | state                | state              |
| Fe−O₁ (=R)                  | 7.21                 | 5.33                 | 4.24               | 3.43                 | 2.40               | 1.84               | 1.81               |
| O₁−O₂                       | 1.26                 | 1.26                 | 1.25               | 1.25                 | 1.37               | 1.29               |
| Fe−ImN                      | 2.20                 | 2.21                 | 2.22               | 2.22                 | 2.22               | 2.04               |
| Fe−PN                       | 2.00                 | 2.00                 | 2.00               | 2.00                 | 2.00               | 2.01               |
| Fe−porphyrin plane (=d)     | 0.18                 | 0.17                 | 0.15               | 0.15                 | 0.15               | 0.01               |
| ImN−Fe−(−Z)-axis (=Θ)       | 9.85                 | 9.26                 | 7.98               | 7.45                 | 6.28               | 3.39               |

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desorption. These values are high for a reversible O2 binding process, as suggested by other studies.23

In Figure 4, we see that at R = 7.21 Å, Fe and O2 have corresponding magnetizations M = 2.128 μB and M = −1.446 μB, respectively. We round each magnetization (spin multiplicity) to the nearest integer and then sum them up to get the total magnetization. This means that the total magnetization in the deoxyhemoglobin state M ≈ 1 μB (S = 3) [Fe (sz = 2), O2 (sz = −1)]. At R = 4.24 Å, the unpaired electrons in the O2 (π*) orbital flip its spin state and the corresponding magnetizations of Fe and O2 become M = 2.145 μB and M = 1.443 μB. At R = 4.24 Å, the total magnetization M ≈ 3 μB (S = 7) [Fe (sz = 2), O2 (sz = 1)]. This change in the total magnetization can be attributed to the magnetic coupling between Fe and O2, which has been estimated in a non-heme Fe–O2 interaction in the superoxo form.24 We found a small difference in the magnetization of Fe, as R decreases from 7.21 to 3.43 Å. This indicates weak interaction between Fe and O2 when the R > 3.43 Å. Furthermore, the small energy difference going from R = 7.21 Å to R = 3.43 Å (Figure 4) indicates degenerate triplet (R = 7.21, 5.33 Å) and septet (R = 3.43, 4.24 Å) states. At an O2 distance of R = 2.4 Å, the Fe and O2 magnetizations become M = 2.149 μB and M = −0.454 μB, respectively (Figure 5). This corresponds to a total magnetization M ≈ 2 μB (S = 5) [Fe (sz = 2), O2 (sz = 0)]. We can also confirm that O2 changes to the singlet state, because the distance O1–O2 is larger than that of the triplet state (Table 1). As O2 approaches FePIm, the electron in the Fe (dxz) orbital flips its spin state and moves to the Fe (dz2) orbital, and the electron in the Fe (dxz) orbital flips its spin state, when the spin multiplicity converts from quintet to singlet in Figure 6. Finally, in the oxyhemoglobin state (R = 1.84 Å), the O2 molecule binds with the FePIm forming σ-bond between Fe (dxz) orbital and O2 (π*) orbital. The magnetizations of Fe atom and O2 molecule are M = −0.679 μB and M = 0.53 μB which means the total magnetization at the final (oxyhemoglobin) state M ≈ 0 μB (S = 1) [Fe (sz = −1/2), O2 (sz = 1/2)]. In summary, we found that the O2 adsorption on the FePIm process involves a change in total spin multiplicity from triplet (septet) to quartet to singlet.

Relation between Energy and Spin Crossing. To study how the spin state affects the activation energy barrier, we calculated the corresponding energies of the system at fixed O2 spin states, that is, adsorption where O2 is in a singlet and triplet state, as shown in Figures 6 and 7, respectively. We can observe that the O2 binding on the FePIm in the septet state is stable when the d and R values are large. On the other hand, the O2 binding on the FePIm in the singlet state is stable when the d and R values are small. With that, we found that the distances d (between the imidazole and Fe) and R (between Fe and O2) are two important reaction coordinates that can be used to control O2 affinity. We also observe that the activation energy barrier of the O2 binding process in the septet (S = 7)
the activation energy barrier of the O₂ binding becomes 0.02 eV and that of the O₂ desorption becomes 1.23 eV. Therefore, diCINEB is conducted with optimization. This accounts for the reference in activation energy barriers found in Figures 4 and 6. Blue line represents the minimum energy path from R = 3.43 Å to R = 1.84 Å. The darker color shows more stability than the brighter color in 3D graphics. Contour spacing is 0.01 eV.

Figure 7. Potential energy of O₂ adsorption in the singlet (S = 1) state referred to the energy at R = 1.84 Å, d = 0.01 Å as a function of two reaction coordinates, d (Å) and R (Å). The distance d and R are same in Figure 6. Blue line represents the minimum energy path from R = 3.43 Å to R = 1.84 Å. The darker color shows more stability than the brighter color in 3D graphics. Contour spacing is 0.01 eV.

Figure 8. Spin crossing between the septet (S = 7) and singlet (S = 1) state as the O₂ molecule adsorbs on the FePIm. R represents the distance between the Fe atom and the O₂ molecule.

We studied spin-dependent O₂ binding on FePIm using DFT-based calculations. We found that as O₂ approaches the Fe atom, binding between O1 and O2 weakens, particularly at distances R < 3.43 Å. This hints at the potential application of FePIm as PEFC catalysts. In the deoxyhemoglobin state, the Fe atom protrudes a distance of 0.18 Å out of the porphyrin plane. On the other hand, in the oxyhemoglobin state, the Fe atom protrudes a distance of 0.01 Å from the porphyrin plane. These results are in good agreement with experimental results. As O₂ approaches the Fe atom, the electron in the Fe (dₓᵧ) orbital flips its spin state and moves to the Fe (dₓᵧ) orbital. Because of the absence of electrons in the Fe (dₓᵧ), the antibonding interaction between the Fe (dₓᵧ) orbital and the N (π) orbital vanishes. This explains the movement of the Fe atom towards the porphyrin plane, when the O₂ approaches. This also indicates that the position of Fe atom from the porphyrin plane is an important reaction coordinate that can be used to control O₂ affinity.

We also investigated the minimum energy pathway from the deoxyhemoglobin state to the oxyhemoglobin state. We found that the activation barrier for O₂ binding is 0.38 eV, while that for O₂ desorption is 0.92 eV. We also found that the total spin multiplicity upon O₂ adsorption changes. At the initial state (deoxyhemoglobin state), the total magnetization is M = 1 μₛ (S = 3) [Fe (sₓ = 2) + O₂ (sₓ = −1)]. At the distance of R = 2.4 Å, the total magnetization changes to M = 2 μₛ (S = 5) [Fe (sₓ = 2) + O₂ (sₓ = 0)]. Finally, at the oxyhemoglobin state, the total magnetization is M = 0 μₛ (S = 1) [Fe (sₓ = −1/2) + O₂ (sₓ = 1/2)]. These spin crossings are important for obtaining the minimum energy pathway of the O₂ binding process as we confirmed that spin crossing affects the activation energy barrier.

■ CONCLUSIONS

The authors declare no competing financial interest.

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