How \( \text{O}_2 \) Binds to Heme

REASONS FOR RAPID BINDING AND SPIN INVERSION*

Kasper P. Jensen and Ulf Ryde‡

From the Department of Theoretical Chemistry, Lund University, Chemical Center, P. O. Box 124, S-221 00 Lund, Sweden

Received for publication, December 22, 2003, and in revised form, January 28, 2004
Published, JBC Papers in Press, January 29, 2004, DOI 10.1074/jbc.M314007200

We have used density functional methods to calculate fully relaxed potential energy curves of the seven lowest electronic states during the binding of \( \text{O}_2 \) to a realistic model of ferrous deoxyheme. Beyond a \( \text{Fe}–\text{O} \) distance of \( \sim 2.5 \text{ Å} \), we find a broad crossing region with five electronic states within 15 kJ/mol. The almost parallel surfaces strongly facilitate spin inversion, which is necessary in the reaction of \( \text{O}_2 \) with heme (deoxyheme is a quintet and \( \text{O}_2 \) a triplet, whereas oxyheme is a singlet). Thus, despite a small spin-orbit coupling in heme, the transition probability approaches unity. Using reasonable parameters, we estimate a transition probability of 0.06–1, which is at least 15 times larger than for the ble parameters, we estimate a transition probability of 0.06–1, which is at least 15 times larger than for the

A chemical reaction can normally not change the spin state of an electron. Therefore, reactions between singlet and triplet states are formally spin-forbidden, which means that they are slow. This is the reason why organic matter may exist in an atmosphere containing much \( \text{O}_2 \). There is a strong thermodynamically unfavorable reaction of \( \text{O}_2 \) to oxidize organic matter to \( \text{H}_2\text{O} \) and \( \text{CO} \), but because these products (as well as the organic molecules) are singlets (whereas \( \text{O}_2 \) is a triplet), this reaction is spin-forbidden and therefore very slow at ambient temperatures. On the other hand, this is a problem when living organisms want to employ \( \text{O}_2 \) in their metabolism; the reactions are still spin-forbidden and slow.

Nature has handled this problem by using transition metals to carry, activate, and reduce \( \text{O}_2 \). There are many reasons for this choice. First, most transition metals also contain unpaired electrons, allowing reactions with triplet \( \text{O}_2 \). Second, transition metals are relatively heavy atoms, which increases spin-orbit coupling (SOC), and thereby provide a quantum mechanical mechanism to change the spin state of an electron, called spin inversion. However, the SOC of the first-row transition metals is too small alone to allow for spin transitions. Third, transition metals often have several excited states with unpaired electrons close in energy to the ground state. This can also be used to enhance the probability of spin inversion.

One of the most simple biological reactions involving molecular oxygen is the binding of \( \text{O}_2 \) to hemoglobin, i.e. the binding of \( \text{O}_2 \) to the \( \text{Fe}^{2+} \) ion in a heme group. This reaction is formally spin-forbidden, because the reactant deoxyheme contains four unpaired electrons in the \( 3d \) orbitals of iron (it is a quintet), and triplet \( \text{O}_2 \) has two unpaired electrons. Thus, depending on the relative direction of these two sets of unpaired electrons, the adduct would be expected to have two \((4 - 2)\) or six \((4 + 2)\) unpaired electrons (i.e. a triplet or a septet state). However, experimentally, the product complex is a singlet state with an equal number of \( \alpha \) and \( \beta \) electrons. As discussed already by Pauling and Coryell (2, 3), this problem makes the hemoglobin reactions troublesome to understand (4), and it is not clear how nature has coped with the spin-forbidden nature of this reaction. The importance of spin inversion is also reflected in the Perutz model of hemoglobin cooperativity (5–7). The movement of iron into the heme plane is assumed to trigger a transition from a tense state to a relaxed state after the binding of two oxygen molecules, and this trigger, in the form of the \( \text{Fe}–\text{N}_\text{ax} \) pull, depends on the spin state of heme.

Theoretical methods have been successfully applied to many problems in heme chemistry. Already the simple Hartree-Fock formalism correctly predicts the bent form of the \( \text{O}_2 \) adduct (8), whereas state-of-the-art density functional theory (DFT) pro-

* This investigation was supported by grants from the Swedish Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 46-46-2224502; Fax: 46-46-2224543; E-mail: Ulf.Ryde@teokem.lu.se.

1 The abbreviations used are: SOC, spin-orbit coupling; DFT, density functional theory.
O$_3$ Binding to Heme

Model System—All calculations were performed on the Fe$^{II}$PorImO$_2$ model, where Por is porphine (heme without side chains) and Im is imidazole, a model of the proximal histidine. We calculated the structure and energetics of Fe–O bond breaking for the seven lowest states by systematically increasing the Fe–O bond distance and optimizing the structure with a fixed Fe–O distance. From the fully optimized potential energy surfaces, we obtain the crossing points of the various spin states involved in the binding mechanism. Strictly speaking, proper crossing points would require identical structures of the states (true transition states). Methods to obtain such structures have been developed, but the geometric effect is usually small and does not significantly affect the location of the crossing point along the reaction coordinate (17, 35).

We found that the most stable state of the Fe$^{II}$PorImO$_2$ model had C$_x$ symmetry. This is consistent with the most accurate crystal structure of oxymyoglobin (1.0 Å resolution) (36), in which the imidazole ligand has a staggered conformation, with a C–N–Fe–N$_2$ torsion angle of 45°. O$_2$ adopts a 4-fold occupancy in the thermally disordered structure at staggered conformations with respect to the equatorial Fe–N$_2$ bonds: two coplanar with imidazole and two orthogonal to it. Hence, this structure indicates that there are two binding modes of O$_2$, one with C$_x$ symmetry and the other unsymmetrical C$_2$. We have optimized the energies and the geometries of both states, and the symmetric one is more stable than the unsymmetric one. The geometries, charges, and spin densities of the two states were identical to within the accuracy of the present work, i.e. ±0.001 Å and 0.001 e. In addition, the calculation shows that π-bonding and trans electronic effects, including back-bonding to iron d-orbitals, are absent. This implies that imidazole is an innocent ligand. In fact, it has been shown that the rotation of the O$_2$ group in the model has a barrier similar to that obtained in the calculations (77).

Likewise, another unsymmetrical conformation arising from a 45° rotation of imidazole (staggered oxygen and eclipsed imidazole with respect to the Fe–N$_2$ bonds) was 2 kJ/mol less stable than the C$_x$ conformation. The spin densities and charges were similar to within 0.02e of the C$_x$ state, but the geometry showed differences of up to 0.07 Å for the Fe–O bond. Thus, we conclude that the C$_x$ structure is the most stable geometry of this system and we have therefore employed C$_x$ symmetry in all the calculations. This strongly facilitates the optimization and characterization of the various excited states. In C$_x$ symmetry, the electronic states are labeled as symmetric A' or antisymmetric A'', respectively, depending on whether their wave function preserves or changes sign upon reflection in the symmetry plane (xz, yz, or xy). The states of the same symmetry and multiplicity are numbered (in brackets) after their optimum energy. For example, the ground state is a symmetric singlet, 1$^A$(1).

Throughout this work, we used a coordinate system with Fe in the origin, the z-axis along the N$_{ax}$–Fe–O bonds, the x-axis through the methine bridge atoms (not the nitrogens), and the y-axis through the two other methine bridges (Fig. 1). Thus, the imidazole and O$_2$ molecules lie in the xz plane. The two unpaired π-electrons on oxygen are situated in two degenerate antibonding π orbitals, which transform as a' and a' in the reduced C$_x$ symmetry when binding to deoxymyoglobin. In our coordinate system, three of the Fe d-orbitals transform as a', viz. xx, yz, and z$^2$−x$^2$ and would therefore couple to the a' unpaired π-electron of O$_2$, whereas the other two orbitals, xy and yz, would interact with the a'' electrons instead.

Selection of States—We have searched for low-energy states that could contribute to the process of oxygen binding. The states were obtained from a systematic permutation of the occupations of 6 electrons in an active space consisting of molecular orbitals 73–75 and 45–47 a'. There are four classes of orbitals: symmetric and antisymmetric a' and b orbitals. Some restrictions were introduced to minimize the search, based on the ground state. We have only examined those configurations that distinguish themselves by one occupied orbital per class from the ground-state configuration (74 45 74 45, i.e. 74 electrons in a a' orbitals, 45 electrons in a a'' orbitals, 45 electrons in b' a' orbitals, and 45 electrons in b' a'' orbitals). Some orbitals were found to be very high in energy and were subsequently avoided. For example, the state (74 45 45 45) had 212 kJ/mol higher energy than the ground state. Hence, we avoided the (45–75) excitation. Such selections reduced our number of states to 20, and the seven lowest are presented in this work. The electronic configurations and optimized energies of these states are shown in Table 1. The states are unrestricted Kohn-Sham wave functions with a large degree of spin polarization in most cases.
O₂ Binding to Heme

There are 238 electrons in the model, and they are partitioned into the α or β orbitals of symmetry a' or a", as is specified in this table. A surplus of zero, two, four, or six α electrons gives a singlet, triplet, quintet, or a septet, respectively. The total wave function is antisymmetric (A') if the total number of α electrons is odd; otherwise it is symmetric (A").

<table>
<thead>
<tr>
<th>State</th>
<th>a'α</th>
<th>a&quot;α</th>
<th>a'β</th>
<th>a&quot;β</th>
<th>E_{rel}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'(1)</td>
<td>74</td>
<td>45</td>
<td>74</td>
<td>45</td>
<td>0.0</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>74</td>
<td>45</td>
<td>73</td>
<td>44</td>
<td>22.0</td>
</tr>
<tr>
<td>A'(2)</td>
<td>74</td>
<td>46</td>
<td>73</td>
<td>45</td>
<td>24.1</td>
</tr>
<tr>
<td>A&quot;(2)</td>
<td>74</td>
<td>46</td>
<td>73</td>
<td>45</td>
<td>19.7</td>
</tr>
<tr>
<td>A'(1)</td>
<td>75</td>
<td>47</td>
<td>72</td>
<td>44</td>
<td>28.5</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>73</td>
<td>46</td>
<td>74</td>
<td>45</td>
<td>28.9</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>74</td>
<td>46</td>
<td>74</td>
<td>44</td>
<td>24.7</td>
</tr>
</tbody>
</table>

Antiferromagnetic state obtained from the septet dissociation product.

TABLE I
Relative energies (kJ/mol) and occupation numbers of the seven lowest states of oxyheme in C₂v symmetry

<table>
<thead>
<tr>
<th>State</th>
<th>Fe–O</th>
<th>Fe–Nax</th>
<th>Fe–Nex</th>
<th>Fe–Neq</th>
<th>Fe–Nexl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'(1)</td>
<td>1.807</td>
<td>2.096</td>
<td>2.024</td>
<td>2.001</td>
<td>0.034</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>2.679</td>
<td>2.557</td>
<td>2.012</td>
<td>1.998</td>
<td>0.081</td>
</tr>
<tr>
<td>A'(2)</td>
<td>1.952</td>
<td>2.100</td>
<td>2.011</td>
<td>2.005</td>
<td>0.008</td>
</tr>
<tr>
<td>A&quot;(2)</td>
<td>1.892</td>
<td>2.133</td>
<td>2.079</td>
<td>2.065</td>
<td>0.018</td>
</tr>
<tr>
<td>A'(1)</td>
<td>2.519</td>
<td>2.200</td>
<td>2.086</td>
<td>2.069</td>
<td>0.144</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>1.878</td>
<td>2.071</td>
<td>2.012</td>
<td>2.008</td>
<td>0.012</td>
</tr>
<tr>
<td>A&quot;(1)</td>
<td>2.080</td>
<td>2.135</td>
<td>2.075</td>
<td>2.075</td>
<td>0.044</td>
</tr>
</tbody>
</table>

O₂ Binding to Heme

RESULTS AND DISCUSSION

The Ground State of the Adduct—The lowest energy was obtained for the (74 45 74 45) open-shell singlet A'(1) state in Table I (the lowest closed-shell singlet with the same occupation numbers is 5 kJ/mol higher in energy). Its geometry is displayed in Table II and Fig. 1. It can be seen that it closely resembles the x-ray structure of oxymyoglobin (36). The Fe–O bond lengths differ by only 0.001 Å. For the more soft Fe–Nax bond, the error is what can be expected with state-of-the-art DFT methods, 0.03 Å, whereas for the average equatorial Fe–Neq bonds, the difference is only 0.006 Å. This gives us confidence that this is the correct ground state and that the description of the Fe–O bond is essentially correct.

The ground state is an open-shell singlet, in accordance with the experimental observation that the O₂ adduct is silent in electron paramagnetic resonance experiments (38). However, the spin is unevenly distributed in the complex with a surplus of α spin on O₂ (0.75 electrons) and a surplus of β spin on iron (0.79e), as is quantified in Table III and illustrated in Fig. 1, bottom. The literature is rich in discussions about the nature of the Fe–O bond (38, 39). In particular, it has been argued whether the electronic structure of oxyheme is better described as singlet oxygen bound to low-spin FeIII (2) or as a superoxide radical antiferromagnetically coupled to low-spin FeIII (40). Some consensus has arisen on the point that the FeIII–O₂ form agrees better with experiments, e.g. the O–O frequency of 1100 cm⁻¹, which is close to what is expected for O₂ (5), some aspects of the chemical reactivity (41), and changes in the electric field gradient studied with Mössbauer spectroscopy (42).

Our results are closest to the FeIII–O₂ description in accordance with earlier DFT calculations (14) (the FeII–O₂ form would be a closed-shell singlet). However, the spin densities are far from ±1, which clearly shows that the electronic structure cannot be fully described by a single configuration (such as FeIII–O₂ or FeII–O₂), but rather as a mixture of both these and possibly also other configurations. Thus, our spin densities could be interpreted as a mixture of 75–80% FeIII–O₂ and 20–25% FeII–O₂. This is in accordance with the experimental observation that a quantum mixture of approximately two-thirds ferric and one-third ferrous states gives the best agreement with Mössbauer spectra (43). Thus, oxyheme is inherently multiconfigurational, with an electronic structure that is somewhat analogous to that found in ozone (44). Early complete active space self-consistent field studies (on a simplified heme model with ammonia instead of imidazole) gave a mainly closed-shell A' ground state (45), as did a symmetry-adapted cluster configuration interaction (SAC-CI) study (46), with the lowest open-shell singlet 150 kJ/mol higher in energy. However, the present results give a better description of the ground state in terms of geometry.

The Dissociated States—Isolated deoxyheme is experimentally a high-spin quintet (38). The optimized structure of this complex (Table II) agrees well (within 0.02 Å) with the crystal structure of deoxyhemoglobin at 1.15 Å resolution (36). It is notable that both structures show a strongly distorted porphyrin with the iron ion ~0.3 Å out of the ring plane, illustrating that high-spin iron is too large to fit into the ring cavity.

When this complex is associated with triplet O₂, there are six unpaired electrons in the total system. The unpaired spin on deoxyheme and O₂ may be either parallel, giving rise to a septet, A'(1), or antiparallel, which gives rise to a triplet state, which turns out to be A'(2). At long (noninteracting) Fe–O distances, these two states are degenerate, as expected. Ideally, both states should give rise to rapid O₂ binding (i.e. all active sites of hemoglobin should be able to bind all O₂ molecules, independent of their spin states). However, as the Fe–O distance is decreased, the degeneracy is lifted. In the optimal structure, the A'(1) state has a Fe–O bond length of 2.52 Å, whereas it is 1.89 Å for state A'(2) (cf. Table II). The potential energy surface of the A'(1) state is flat around the minimum, and the energy is close to the dissociation limit, which is at 27 kJ/mol when calculated from separated species. The two states have very similar energies in their optimum geometries. Interestingly, the B3LYP method gives a quite different behavior of the A'(2) state. The energy of this state increases steadily as the Fe–O bond length is decreased, with an energy of ~50 kJ/mol at the BP86 minimum. The B3LYP curve shows a very shallow minimum at Fe–O = 2.39 Å, with an energy close to the dissociation limit. The lowest triplet (intermediate-spin) state of deoxyheme is close in energy to the lowest quintet state. In fact, in the present calculations (as well as in most previous DFT calculations (11, 34)), it is actually 3 kJ/mol more stable (4 kJ/mol when optimized at the B3LYP level; hence the dissociation limit of the lowest triplet state is 24 kJ/mol). Thus the states are degenerate to within the uncertainty of the method. If this triplet state is associated with triplet O₂, we once again obtain two states, depending on the relative orientation of the two sets of unpaired spin, a quintet state A'(1) and a singlet state, which actually turns out to be the dissociation product of the singlet ground state A'(1).

Excited States—In Table I, the relaxed electronic spectrum is presented for the oxyheme model. It shows that there are six states within 30 kJ/mol (25 kJ/mol if optimized B3LYP structures are used) of the open-shell singlet ground state of oxy-
Scalar-relativistic corrections have only a minor effect on these energies (less than 5 kJ/mol, usually in favor of the low-spin states). Besides the three dissociation states discussed previously, \(^7\)A'(1), \(^5\)A'(1), and \(^7\)A'(2), there is another low-lying antiasymmetric triplet state (\(^3\)A'(1)), a symmetric triplet (\(^3\)A'(2)), and an antiasymmetric singlet (\(^1\)A'(1)).

Thus, these states are nearly degenerate within the uncertainty of current methods (\(-10\) kJ/mol), which makes it hard to assign the spectrum in detail. However, the result is in qualitative agreement with the fact that all three spin states have been found from Mössbauer spectroscopy within 10 kJ/mol ferrous myoglobin and hemoglobin (corresponding to an excitation at 12,000 nm) (47–49) and the observation of a low-lying triplet state in thermal equilibrium with the singlet ground state of oxyheme at temperatures between 25 and 250 K (50).

The vertical electronic excitation spectrum was recently calculated (46) with a model identical to ours using the symmetry-adapted cluster configuration interaction (SAC-CI) method on the experimental geometry. This approach resulted in a similar ground state and low-lying \(^3\)A' and \(^1\)A' states (at 0.47 and 1.54 eV), but in general, the spectrum had much larger energy separations than we had. The reason for this is probably that they used one geometry (from experiments) to compute all states, whereas we have optimized the geometry of all states. If the excited-state geometries are optimized, the states will come substantially closer in energy.

The spin densities of the seven low-lying states are shown in Table III. From these, it can be seen that the two dissociative states, \(^7\)A'(1) and \(^5\)A'(1), are quite close to triplet \(\mathrm{O}_2\) and high- or intermediate-spin Fe\(^{II}\), also in their optimum structures. The \(^3\)A'(2) state is quite well described as intermediate-spin Fe\(^{III}\) (2.98 unpaired electrons) antiferromagnetically coupled to \(\mathrm{O}_2\) (1.13e) and the \(^3\)A'(1) state is low-spin Fe\(^{III}\) (1.05e) ferromagnetically coupled to \(\mathrm{O}_2\) (0.96e). The \(^1\)A'(1) state has spin densities similar to the singlet ground state (i.e. low-spin Fe\(^{III}\) antiferromagnetically coupled to \(\mathrm{O}_2\)), whereas the \(^5\)A'(1) state

<table>
<thead>
<tr>
<th>State</th>
<th>Fe</th>
<th>O1</th>
<th>O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1)A'(1)</td>
<td>-0.79</td>
<td>0.26</td>
<td>0.49</td>
</tr>
<tr>
<td>(^5)A'(1)</td>
<td>2.25</td>
<td>0.90</td>
<td>0.92</td>
</tr>
<tr>
<td>(^7)A'(1)</td>
<td>3.35</td>
<td>-0.73</td>
<td>-0.84</td>
</tr>
<tr>
<td>(^3)A'(2)</td>
<td>2.98</td>
<td>-0.68</td>
<td>-0.45</td>
</tr>
<tr>
<td>(^3)A'(1)</td>
<td>3.89</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td>(^3)A'(1)</td>
<td>-0.77</td>
<td>0.35</td>
<td>0.41</td>
</tr>
<tr>
<td>(^1)A'(1)</td>
<td>1.05</td>
<td>0.38</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Fig. 1. Optimized structure (top) and spin density (bottom) of the oxyheme model in the \(^1\)A'(1) ground state. All wave functions that change sign when reflected in the \(xz\) plane are antisymmetric, \(A'\), whereas those that keep their sign are symmetric, \(A'\).
is intermediate between $O_2$ and $O_2^-$ (1.57 unpaired electrons).

Mulliken charges for the various excited states are compiled in Table IV. Interestingly, whereas the spin densities on iron and $O_2$ vary appreciably for the various states, the charges are much more similar. For example, the variation in the charge of the $N_{ax}$ and $N_{eq}$ atoms is 0.05e and 0.09e, respectively. A somewhat larger variation is seen for the charge on the iron ion, varying between 0.58 and 0.73e. However, this variation is fully consistent with the spin densities, giving a lower charge for low-spin states, which are better shielded from the nuclei, as rationalized by Slater’s rule (51). For the same reason, the high-spin states tend to have more charge in the porphyrin ring as measured at the $N_{eq}$ atoms in Table IV.

Thus, the total electron density (charge) is quite rigid in the states. This applies for the polarity of the Fe–$O$ bond as well. The spin density, however, differs significantly within the various states. Therefore, the notion of FeIII–$O_2$ and FeII–$O_2$ is only justified in terms of spin density and not in terms of the charge.

**Binding of $O_2$**—We now turn to the actual association mechanism. How does $O_2$ bind to heme in hemoglobin or myoglobin, facing the restrictions of spatial and spin symmetry? To answer this question, we calculated the fully relaxed geometry and energy at various fixed Fe–$O$ bond distances to obtain the binding curves of the seven lowest states shown in Fig. 2. The most prominent feature is that there are five potential curves, viz. those for the $1^A(1)$, $5^A(2)$, $5^A(1)$, $7^A(1)$, and $1^A(1)$ states, which, from a Fe–$O$ distance of 2.5 Å and outwards, are near degenerate and virtually parallel within the accuracy of the present method. Solvent effects, calculated by the continuum conductor-like screening model (71) in a water-like solvent, ready before $O_2$ approaches heme (53). Of course, such an association mechanism would have essentially the same energetics as the triplet association (because of the near degeneracy at large Fe–$O$ distances) but different dynamics, because the crossing probabilities may differ.

Alternatively, the binding of $O_2$ also could take place directly to the triplet state of deoxyheme, which would lead directly to the right low-spin state upon binding to triplet O$_2$ (52). In this case, the primary electronic reorganization takes place in iron at an equilibrium between the quintet and triplet states already before $O_2$ approaches heme (53). Of course, such a mechanism would also be facilitated by the near degeneracy of the spin states of deoxyheme.

This topology with five nearly degenerate states at long and intermediate Fe–$O$ distances explains the rapid and reversible binding of $O_2$. To see this, we will consider the Eyring expression of the reaction rate constant $k$ of $O_2$ binding,

$$k = \frac{h}{k_BT} \exp(-\Delta G^\ddagger/2k_BT)$$  \hspace{1cm} (Eq. 1)

where $h$ and $k_B$ are Planck and Boltzmann constants, and $\Delta G^\ddagger$ is the activation energy. For a simple one-step reaction involving two states (A and B), the transmission coefficient $\kappa$ can be approximated by the probability $P_{AB}$ in the Landau-Zener equation (54, 55).

$$P_{AB} = 1 - \exp[-(2\pi\Delta E_{AB})^2/(hv|S_A - S_B|)]$$ \hspace{1cm} (Eq. 2)

Here, $v$ is the crossing velocity (the classical velocity of the particle moving along the potential energy surface) and $|S_A - S_B|$ is the absolute value of the difference in slope of the potential energy as a function of the reaction coordinate at the crossing point. In addition, $2\Delta E_{AB}$ is the difference in energy of
the states when the perturbation that lifts their degeneracy has been applied, in our case the SOC.

To get a feeling for the various terms in this expression, we will insert reasonable values and compare the results for oxyheme with a similar but nonbiological process, the dissociation of the diatomic Fe–O system, studied by Danovich and Shaik (56). The term $h\nu$ describes the normal mode of Fe–O dissociation, which can be obtained from temperature and the reduced mass. It will be similar in the biological and nonbiological system, $5\text{kJ/mol}$. The SOC constant for iron is intermediate between what you find for first-row elements and for heavy metals, because it grows approximately as $Z^4$. It is $1\text{kJ/mol}$ between various spin states in free iron (57), and it is smaller in metal complexes than in the free ions (58). For ferrous deoxyheme in hemoglobin and myoglobin, it has been estimated from Mössbauer spectroscopy to be $0.8\text{kJ/mol}$ (47).

Finally, the gradient differences $|S_A - S_B|$ will strongly depend on the system under study and the location of the crossing point. For the FeO system, the crossing point was between two surfaces with very different slopes, one negative and the other positive. The calculated $|S_A - S_B|$ was $300\text{kJ/mol/Å}$ for sextet and quartet states (56). With such a large difference in the slopes, Danovich and Shaik (56) obtain a transmission probability of only 0.004 for the crossing between the high- and intermediate-spin states, with a SOC of 0.61 kJ/mol.

However, our results indicate that the behavior of oxyheme
is quite different. At Fe–O distances longer than 3 Å, the potential energy curves of the relevant $^1\text{A'}(1)$, $^3\text{A'}(2)$, $^5\text{A'}(1)$, and $^7\text{A''}(1)$ states are nearly degenerate, making up a crossing region of flat and almost parallel potential energy surfaces. We do not know the exact locations of the crossing points between the various spin states, because the energy differences are so small, and the triplet and quintet states of deoxyheme are almost degenerate. However, it is known from experiments that the triplet-quintet splitting in deoxyheme is 10 kJ/mol, with the quintet lower in energy. This gives us an experimental bound to the crossing points in Fig. 2. Translating the septet and triplet curves (corresponding to quintet deoxyheme) down to such a dissociation energy shows that all crossing points must be at Fe–O distances above 2.5 Å. This means that all curves have a slope of less than $\pm 25$ kJ/mol/Å. Moreover, the maximum value of $|S_A - S_B|$ for the curve crossings of interest is less than 20 kJ/mol/Å (all curves have a negative slope except that of $^1\text{A'}(1)$, for which the slope is 4 kJ/mol/Å or less).

However, even more important for the binding of O$_2$ to heme is the activation energy of the reaction, $\Delta G^\ddagger$ in Equation 1. From Fig. 2, it can be seen that for the possible crossing points discussed above (i.e. for Fe–O distances longer than 2.5 Å), all curves for the relevant four spin states are less than 15 kJ/mol above the energy of the dissociated states (allowing for a downshift of the heptet and triplet curves to the experimentally observed quintet-triplet splitting (47–49)). This means that the activation enthalpy should be lower than this. For the FeO$^+$ system, the analogous reaction from the high-spin state has an activation enthalpy of binding of 75 kJ/mol (56). Thus, the design of deoxyheme gives a barrier decrease of $\sim 60$ kJ/mol compared with the simplest Fe–O binding complex imaginable.

Provided that the entropy of binding is similar in both reactions (it is most likely dominated by the removal of six degrees of freedom from free O$_2$), this corresponds to a rate enhancement of $\sim 10^{10}$. Thus, we can conclude that the facile binding of O$_2$ to hemo- and myoglobin arises primarily as an effect of the topology of the binding curves for the four relevant spin states. This topology, with nearly degenerate and parallel curves, is caused by the near degeneracy (within 10 kJ/mol) of the triplet and quintet states of deoxyheme. Therefore, the design by nature of iron porphines having close-lying spin states of a particular symmetry and energy is a means to tune binding of small ligands and overcome the activation barriers of these spin-forbidden reactions, despite the moderate SOC of first-row transition metals. The resulting barrier height makes up most of the rate enhancement due to the exponential dependence on the rate, whereas one or two orders of magnitude may come from the increase in the transmission coefficient.

The different relative rates for the rebinding of NO, CO, and O$_2$ to heme have recently been studied by DFT (16). That study also used a Landau-Zener formalism to explain the importance of spin states for the rates of ligand binding. Unfortunately, it was based on curves obtained with a fixed geometry, except for
the Fe–O bond. Moreover, it resulted in a closed-shell ground state for oxyheme, which gives a Fe–O geometry different from the experiment. Relaxation effects are very large for these systems, in particular for the Fe-imidazole and iron out-of-plane distances. This was also observed when three different values of the distance of the iron ion out of the porphyrin plane were tested, giving rise to changes by up to 100 kJ/mol in the energies and a reordering of the spin states. Therefore, none of the curves has any clear significance for the binding of O₂. An accurate description of spin surfaces and the topology at crossing points can only be obtained with fully relaxed potential energy surfaces, such as those presented in Fig. 2.

Comparison with Peroxidases—We have seen that the facile binding of O₂ to heme in the globins is essentially an effect of the near degeneracy of quintet and triplet states of deoxyheme. It is then natural to ask whether other heme proteins have solved the problem in a similar way and whether proteins are designed to facilitate the binding of ligands. We will show that this is probably the case by a comparison with the peroxidases.

Peroxidases are heme proteins that oxidize various substrates in one-electron reactions, using H₂O₂ as the oxidant. The resting state of these proteins is high-spin ferric heme (a sextet), in contrast to the ferrous high-spin state in the globins. It is this state that binds H₂O₂, and this reaction is spin-forbidden like the globin binding of O₂, because H₂O₂ is a singlet, whereas peroxymethemoglobin is a doublet (57).

Interestingly, many experiments indicate that the sextet and quartet states are very close in energy for the ferric resting state of peroxidases. In fact, the ground state of some peroxidases seems actually to be a quantum chemical (by SOC) mixture of these two states (59–68). This indicates that the same mechanism as we have suggested for O₂ binding to globins also applies for the binding of H₂O₂ to the peroxidases, i.e. that the spin surfaces are nearly degenerate and parallel, caused by a near degeneracy of the dissociated states.

This suggestion is further strengthened by the fact that peroxidases and globins have a slightly different axial bonding of the heme group. In the globins, the axial histidine ligand makes only weak hydrogen bonds to the backbone of the surrounding protein. However, in all known heme peroxidases, the axial histidine ligand instead forms a strong hydrogen bond to the carboxylate side chain of a conserved aspartate residue (69). It has been suggested that such a hydrogen bond may change the properties of the axial ligand (impose some imidazole character onto it) (70).

Interestingly, we have recently shown that such a hydrogen bond to a carboxylate group changes the relative energies of the spin states of ferrous and ferric deoxyheme (33). Without the hydrogen bond, the high- and intermediate-spin states are nearly degenerate in the ferrous state, but not in the ferric state. With the hydrogen bond, the opposite is true; the two states are degenerate to within 3 kJ/mol in the ferric state. Thus, it seems that evolution has selected an axial ligand that favors spin degeneracy and thereby a facile binding of a proper ligand in the opposite axial site in both peroxidases and the globins. This would provide a new explanation for the selection of the axial ligand in heme proteins, a subject of much debate (33, 70).

Conclusions—We have provided evidence that the spin-forbidden reversible binding of oxygen to globins is strongly facilitated by the shape of the potential energy curves of the various spin states during O₂ binding. We have found that the four relevant low-lying spin states, with zero, two, four, or six unpaired electrons, form nearly parallel surfaces with almost the same energy along the O₂-binding coordinate beyond a Fe–O distance of 2.5 Å. Such a topology has three important biological consequences for O₂ binding. First, it ensures that the protein may bind all O₂ molecules, independent of their spin state (two unpaired α or β electrons). Second, the relative slope of the crossing spin surfaces is small in the crossing region. This leads to a large probability for the necessary spin crossing, despite a modest spin-orbit coupling for iron. In fact, the transition probability, and therefore also the rate constant of O₂ binding, is increased by at least a factor of 15 compared with similar nonbiological iron complexes. Third, the detailed shape of curves ensures that the energy barrier (activation enthalpy) for the curve crossing is small (<15 kJ/mol). This has a large effect on the rate acceleration of O₂ binding. Altogether, these parallel and nearly degenerate energy surfaces may accelerate oxygen binding by 11 orders of magnitude.

The unusual topology of the binding surfaces is caused by the near degeneracy of the two lowest spin states of ferrous deoxyheme. Such near degeneracy is a basic feature of many porphyrins (7) and is supported by Mössbauer spectroscopy, which finds all three spin states within 10 kJ/mol in ferrous hemo- and myoglobin (47-49). In fact, we have earlier shown that it is an intrinsic property of porphyrin to produce a small splitting between the various spins states of iron, because the central cavity of the ring is too large for low-spin iron (11). However, we have also seen that the axial ligand has an important influence of the spin-splitting energies. Imidazole with only weak hydrogen bonds (as found in the globins) results in near degeneracy for the Fe(II) state, whereas imidazolate or imidazole, hydrogen-bonded to a carboxylic group (as is found in the peroxi- dases), instead produces degeneracy for the Fe(III) state (33). Thus, we have obtained an important explanation for the selection of the axial ligand and the structural design of heme proteins, with the aim of enhancing the binding of substrates to the proteins.

Acknowledgment—We are grateful for computer resources support from Lunar at Lund University.

REFERENCES

How $O_2$ Binds to Heme: REASONS FOR RAPID BINDING AND SPIN INVERSION
Kasper P. Jensen and Ulf Ryde

doi: 10.1074/jbc.M314007200 originally published online January 29, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M314007200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 64 references, 3 of which can be accessed free at http://www.jbc.org/content/279/15/14561.full.html#ref-list-1