The mechanism for the reversible oxygen addition to heme. A theoretical CASPT2 study†

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By performing CASPT2 calculations, the lowest energy pathway for oxygen addition to an isolated heme center of a heme-protein is evaluated and found to be reversible (the oxyheme compound is just 14.9 kcal mol^{-1} more stable than the deoxyheme + O_2 reactants, and the energy barriers to dissociation are even smaller).

The reversible addition of molecular oxygen to the heme group of myoglobin, hemoglobin or their synthetic functional analogues presenting the same active sites has been a topic of high interest due to its relevance in respiratory and metabolic processes.[1] Strong experimental evidence indicates that in such additions one O_2 molecule (triplet ground state) binds the Fe(II) atom of a deoxyheme group (quintet ground state) to form an oxyheme complex (singlet ground state). Therefore, this is a spin-crossing reaction. Although many experimental[1d] and theoretical studies[2] have been devoted to finding a mechanism that fits the available experimental information, none of the currently available ones is fully satisfactory, mostly due to the use of the density functional theory (DFT) methodology. However, as will be shown here, DFT fails to properly describe the electronic structure of oxyheme and deoxyheme, and thus cannot properly reproduce the shape of the oxyheme → deoxyheme + O_2 potential energy profile. In this work we present a mechanism for such a reaction based on multi-configurational second-order perturbation (CASPT2) calculations[3] performed using large active spaces capable of reproducing the experimental data for oxyheme and deoxyheme. These CASPT2 calculations give a new perspective of the changes produced when oxygen is added to a heme group. This addition is slightly exothermic (14.9 kcal mol^{-1}). It first gives rise to the formation of a weakly stable deoxyheme···O_2 van der Waals complex, which, by crossing a low energy barrier (whose maximum sits below the energy of the starting fragments), converts into the oxyheme complex with the formation of an Fe–O_2 coordination bond. This mechanism is reversible and, as we will show, fits all available experimental data.

There is wide agreement that the ground state of oxyheme is a singlet.[1b,4] Magnetic susceptibility data initially suggested[5] the presence of a thermally populated low-lying triplet excited state. However, later studies[6] attributed these results to small amounts of (high-spin) deoxyheme molecules. The exact nature of such a singlet ground state was also controversial.[4] However, recent CASPT2 calculations[7] demonstrated that the wave function is multiconfigurational, with a 50% weight of the Pauling[4a] and Weiss[4b] configurations, in good agreement with the data from Mössbauer studies.[8] On the same issue, DFT calculations[9] predicted a 75–80% weight of the Weiss configuration, with the Pauling configuration providing the remaining 20–25%.[2a,10] This is an almost monoconfigurational situation, which contradicts the experimental and CASPT2 results. Concerning deoxyheme, experimental Mössbauer studies indicated[11] that the quintet is the ground state for deoxyheme. There are no previous CASPT2 studies on this molecule. DFT calculations predicted a singlet, triplet, or quintet ground state, depending on the functional and geometry employed.[2,12,13]

Experimental studies also indicated that the O_2 addition to hemoglobin and myoglobin is exothermic by about −12 to −18 kcal mol^{-1}.[14] Such exothermicity cannot be directly assigned to the binding energy of the deoxyheme and O_2, as the experimental energy depends on many factors: the number of O_2 molecules already attached, pH and concentration of phosphates and chloride ions. Previous DFT computed potential energy curves show a very different exothermicity,[2] but they are questionable, due to the inability of the DFT functionals used so far on this complex to properly describe the oxyheme and deoxyheme electronic structures (described above). Therefore, we decided to perform CASPT2 calculations, using active spaces flexible enough to properly describe the ground states of oxyheme and deoxyheme, and thus compute the lowest energy pathway that connects these ground states.

The deoxyheme form of the heme active center was modeled as shown in Fig. 1. The model is neutral and includes neither the substituents attached to the porphyrin rings, nor the imidazole group of the proximal histidine. The oxyheme form is obtained by attaching an O_2 molecule to the Fe atom. Geometry optimizations on these two compounds were performed using the BP86 functional[15] present in GAUSSIAN 03[16] and the SVP all-electron basis set of Ahlrichs *et al.*[17] The C_s symmetry was preserved. On the optimum BP86/SVP geometry (in the potential energy curves, the optimization was done at fixed Fe–O distances) CASPT2(16,14) calculations were done using MOLCAS 6.2.[18] A (16,14) active space was chosen because it reproduces the experimental data for oxyheme and deoxyheme, and is stable against inactive–active and active–secondary orbital rotations during the curve calculations. The G2 variant of the Fock matrix[3] and a level shift[3] of 1.6 hartrees were used. The 1s orbitals of C, N, O and Fe, plus the 2s and 2p orbitals of Fe were not correlated. The basis set was the Roos augmented double zeta ANO in Fe and

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† Electronic supplementary information (ESI) available: Figures and tables describing the optimized geometry employed, the active space used, the electronic structure of the species involved, and comparing the CASPT2 and DFT results. See DOI: 10.1039/b704871h

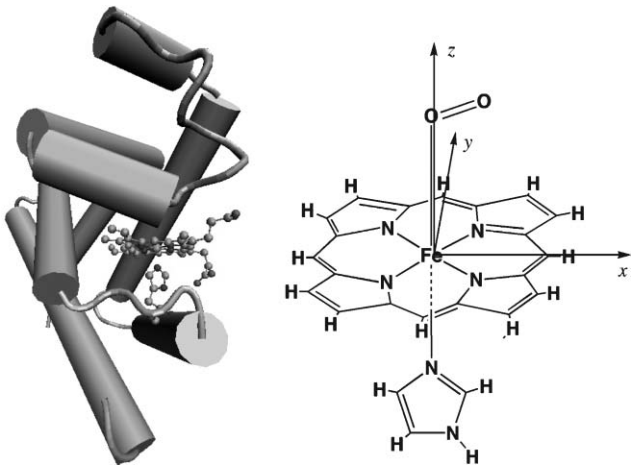

Fig. 1 *Left*: Structure of the deoxymyoglobin, showing the position of the heme group. *Right*: Model of the oxyheme molecule employed in this work, indicating the coordinates employed (origin at the Fe atom).

O ([6s5p4d2f] contraction on Fe,[19a] and the [4s3p2d] on O[19b]). The ANO-S basis set[19c] with [3s2p] contraction was used for C and N. The contraction[19c] [2s] was used for H.

The ground state of oxyheme was first systematically searched by doing CASPT2 calculations (with a (14,14) active space; see Fig. S1 and S2 of ESI† for a detailed description of this active space and how it is related to the (16,14) active spaces employed for the calculation of the potential energy curves) for the lowest ^{1}A′, ^{1}A″, ^{3}A′, ^{3}A″, ^{5}A′, ^{5}A″, ^{7}A′ and ^{7}A″ states, at the optimum BP86 geometry of the ^{1}A′ state (η^1-coordination, optimum parameters: Fe–O = 1.82 Å, O1–O2 = 1.27 Å, ∠ FeOO = 120.6°, average Fe–N_{porph} = 2.02, Fe–N_{imid} = 2.09 Å), which is in very good agreement with the best crystallographic data.[20] At the CASPT2 level, the lowest energy state is the ^{1}A′ (Table 1), separated by no more than 3.2 kcal mol^{-1} from ^{5}A″ and ^{3}A″ states. The next states are 7.5 kcal mol^{-1} above the ground state, and thus will not be considered hereafter. Such state ordering agrees with the experimental results,[11] but differs from that obtained with DFT calculations (See ESI†).

The ground state of deoxyheme was computed at the CASPT2(8,11) level. The quintet (hereafter indicated as $^5Fe^{2+}$) is the ground state (Table 1), in agreement with the available experimental data.[11]

The previous CASPT2 results on the oxyheme and deoxyheme ground states allows us to conclude that the lowest energy pathway for the deoxyheme + O_2 → oxyheme reaction is a spin-crossover reaction, as found experimentally but not in many DFT studies. The lowest energy configuration for deoxyheme and O_2 at dissociation is the $^5Fe^{2+}$–$^3O_2$ one (obtained by combining a quintet Fe^{2+} with a triplet O_2). Angular momentum coupling rules indicate that a $^5Fe^{2+}$–$^3O_2$ configuration can only generate a ^{7}A″, ^{5}A″ and ^{3}A″ state. On the other hand, the ^{1}A′ state of oxyheme dissociates into the $^3Fe^{2+}$–$^3O_2$ configuration of deoxyheme + O_2. Thus, the lowest energy pathway necessarily involves a change in the spin along the reaction.

Table 1 Energy ordering of the lowest electronic states of oxyheme (relative to the ^{1}A′ state, taken as zero) and deoxyheme (relative to the ^{5}A′). All the values are given in kcal mol^{-1}

Oxyheme	CASSCF[a]	CASPT2	Deoxyheme	CASSCF	CASPT2
^{3}A″	12.1	3.2	^{5}A′	**0.0**	**0.0**
^{5}A″	2.5	2.0	^{3}A″	22.8	13.6
^{1}A′	**0.0**	**0.0**	^{1}A′	41.1	21.9

[a] CASSCF refers to a Complete-Active Space Self Consistent Calculation.

Fig. 2 shows the potential energy curves computed at the CASPT2(16,14) level for the ^{1}A′ state of oxyheme, and the ^{3}A″ and ^{7}A″ curves that originate from the $^5Fe^{2+}$–$^3O_2$ configuration (the ^{5}A″ state at long distance was computed only at a few long distances, one of them is shown in Fig. 2, and was found to be always placed in between the ^{3}A″ and ^{7}A″ curves; at short distances the quintet converts into a charge-transfer quintet, placed above the ^{1}A′ ground state, that is, no short distance minimum seems to exist).

The following main points emerge from the analysis of Fig. 2: (a) the ^{1}A′ curve has only one minimum (at 1.82 Å), whose energy is 14.9 kcal mol^{-1} below that for deoxyheme plus O_2 at dissociation; (b) the ^{3}A″ and ^{7}A″ curves that originate from the $^5Fe^{2+}$–$^3O_2$ configuration of the deoxyheme–O_2 complex have only long-distance minima (placed at 2.6 Å for the triplet and 3.1 Å for the septet, corresponding to van der Waals minima where the O_2 molecule does not form an Fe–O_2 coordination bond) located 17.2 and 15.2 kcal mol^{-1} below the deoxyheme plus O_2 energy at dissociation; (c) the ^{3}A″ crosses the ^{1}A′ curve and becomes the most stable one at an Fe–O distance of around 1.9 Å, the energy of that crossing point being 12.5 kcal mol^{-1} *below* the energy of deoxyheme plus O_2 at dissociation; (d) the ^{7}A″ curve crosses the ^{1}A′ curve at an Fe–O distance of around 2.1 Å, the crossing point energy being around 5.4 kcal mol^{-1} (the ^{5}A″–^{1}A′ crossing was not computed, but is expected to be placed between the previous two crossing points). Notice that the current DFT functionals cannot find such van der Waals minima.[2]

The existence of the long-distance van der Waals minima of the ^{3}A″ and ^{7}A″ states was confirmed on doing MP2 calculations[21] on the ^{7}A″ state (a monoreference state). Although the van der Waals

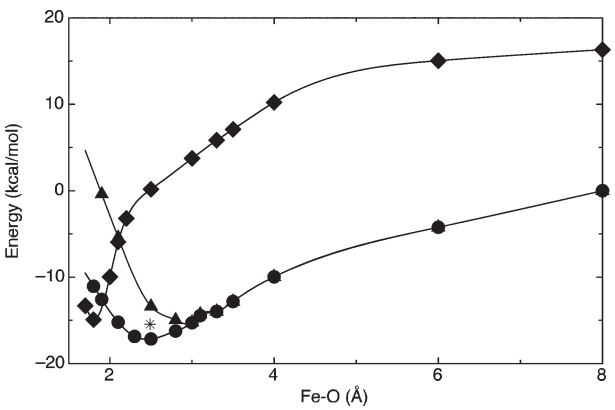

Fig. 2 CASPT2 potential energy curves for the ^{1}A′ (◆), ^{3}A″ (●) and ^{7}A″ (▲) electronic states of oxyheme. The energy of the ^{5}A″ state (*) at 2.5 Å is also given for comparison. The energy of all points (*E*) is referred to the energy of the dissociation products of oxyheme into $^5Fe^{2+}$ + $^3O_2$, here approximated as the energy of the complex at Fe–O = 10 Å (to avoid possible size-consistency errors and problems of stability of the active space). The BSSE-error was not corrected for any of these points.

minimum at the MP2 level is 5 kcal mol^{-1} weaker than at the CASPT2 level, it is 10.4 kcal mol^{-1} more stable than the deoxyheme–O_2 complex at dissociation. When the basis set superposition error (BSSE)[22] of the MP2 interaction energy was corrected (using the counterpoise method), the van der Waals complex was still 2 kcal mol^{-1} more stable than two deoxyheme and O_2 fragments in their ground states. This is remarkable, given that the trend that the counterpoise method presents is towards over-correcting the BSSE with moderately-sized basis sets (as that used in this computation).

The overall physical picture that emerges from the previous results for the lowest energy pathway of the O_2 addition to deoxyheme is the following: (a) the deoxyheme + O_2 → oxyheme process is slightly exothermic (14.9 kcal mol^{-1}) and its most stable pathway goes initially along the $^3A''$ curve (where a van der Waals minimum energy complex is formed at 2.6 Å with an energy of 17.2 kcal mol^{-1}), while at about 1.9 Å (and at 12.5 kcal mol^{-1} *below* the reactants energy) crosses into the $^1A'$ curve, where an Fe–O_2 coordination bond is formed, and a minimum is found at 1.82 Å; (b) the oxyheme → deoxyheme + O_2 back-transformation is slightly endothermic (14.9 kcal mol^{-1}) and its most stable pathway goes initially along the $^1A'$ curve, but crosses into the $^3A''$ state around 1.9 Å (and 2.3 kcal mol^{-1} above the $^1A'$ minimum), where a van der Waals minimum is formed (at 2.6 Å, 2.3 kcal mol^{-1} below the $^1A'$ minimum), which then dissociates into deoxyheme plus O_2; (c) there is also an alternative deoxyheme + O_2 → oxyheme $^1A'$–$^7A''$ pathway, with slightly higher crossing points and van der Waals minimum than those for the $^1A'$–$^3A''$ pathway, but with an interaction energy always below the energy of reactants at the CASPT2 level (a $^1A'$–$^5A''$ pathway is also possible; it is demonstrated to be placed in between the $^1A'$–$^3A''$ and $^1A'$–$^7A''$ pathways, although was not fully computed). The use of larger basis sets and active spaces in the CASPT2 calculations, or correcting the CASPT2 BSSE error in the potential energy curves at large distance is not expected to change the overall picture for the mechanism, although could affect the reported energy differences.

In summary, CASPT2 calculations using the large active spaces employed here provide a proper theoretical description of the mechanism for O_2 addition to an isolated active center of a heme protein that reproduces all available experimental data for the first time. The process is found to be thermodynamically and kinetically reversible at room temperature. The key points behind such reversibility are: (1) the small energy difference between oxyheme and the deoxyheme–O_2 complex (14.9 kcal mol^{-1}), and (2), the existence of a long-distance van der Waals deoxyheme···O_2 complex, whose energy is similar to that for the oxyheme $^1A'$ coordination compound. These results permit an understanding of the main features of the central step of the O_2 addition to heme proteins. Further studies should address how the potential energy curves are affected by the protein groups adjacent to the heme center and not included in our current heme model, and the diffusion of the O_2 towards and from the heme center (the O_2 will not dissociate in the heme protein into deoxyheme and free O_2, but it will be transferred from the heme group to one of the nearby groups).

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