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DFT Computation of the Intrinsic Barrier to CO Geminate Recombination with Heme Compounds

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Dissociation and recombination of CO, O$_2$, and NO with heme proteins such as myoglobin or hemoglobin are of tremendous biological importance. Despite extensive experimental work,[1] the reaction mechanisms are still not fully understood, in part due to the inapplicability of standard computational methods even to modest sized models of the active sites. In this study, a newly developed approach is used to characterize the reaction barrier of the prototypical CO recombination reaction.

There are two steps in the addition of CO to free (or deoxy) heme proteins: diffusion of the ligand into the heme pocket and geminate recombination with the iron atom, which is rate determining. The kinetics are complex: there is a distribution of barriers, centered at 2.5 kcal/mol in the case of the low-temperature reaction of photolyzed myoglobin-CO.[1b] The distribution shifts upon warming, so that above 170 K, the mean activation energy *increases* to 5 kcal/mol.[1b] This has been construed to mean that dissociation leads to an ensemble of conformations, with different activation barriers, which interconvert very slowly at low temperature. At the higher temperatures, the metastable distribution of high-energy, low-barrier conformations of deoxymyoglobin, referred to as Mb*, relaxes to low-energy, high-barrier conformations, Mb.

Modeling the recombination using ad hoc potential energy surfaces, with one or more "intrinsic" degrees of freedom, such as the Fe−C distance, and unspecified "protein" coordinates, successfully reproduces the kinetics.[2] However, this does not help to assign the nature of the protein relaxation, or to explain *how* it affects the recombination barrier. One suggestion is that the geometry of the heme group, especially the position of the iron atom, is different in Mb* and Mb.[1b] Other explanations relate the relaxation to rearrangement of the protein's tertiary structure. Such complex mechanistic questions remain hard to solve in the absence of a sound understanding of the intrinsic barrier, in the absence of protein matrix effects.

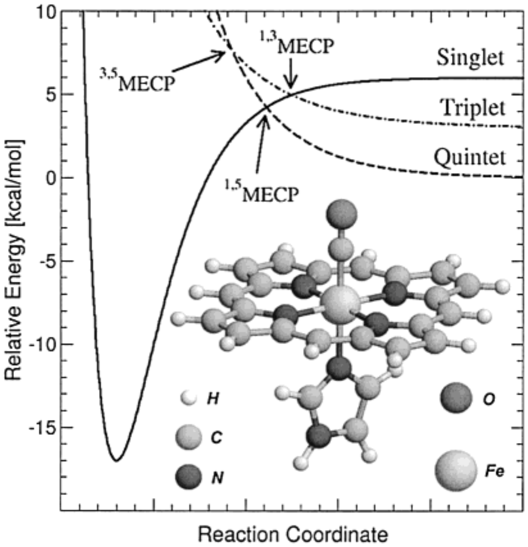

Figure 1. Qualitative potential energy curves for heme-CO, showing the computational model and the surface crossings.

Ab initio quantum mechanical computations are increasingly reliable tools for understanding biochemical and bioinorganic reaction mechanisms, and many aspects of heme chemistry have already been explored.[3] While the static properties of heme compounds, such as geometries or bond energies, are well reproduced by theory, the kinetic features have not been addressed. This is because, like many reactions of transition metal compounds,[4] recombination involves two different spin states, quintet deoxyheme and singlet heme-CO. Although such "spin-forbidden" processes[5] can occur close to the adiabatic limit, due to strong spin−orbit coupling, the avoided crossing of the different spin-state surfaces often leads to an activation barrier. The latter has been hard to study using ab initio methods, because transition state-locating routines are limited to single spin states. The spin-forbidden reaction barrier to recombination of heme with CO is located here by characterizing the spin-state surface crossing behavior, i.e., by finding the minimum energy crossing point (MECP) between the surfaces,[5] using a recently developed algorithm.[5c] MECPs are analogous to transition states for spin-forbidden reactions, and the feasibility and benefits of locating them have recently been demonstrated in a study of spin-forbidden phosphine dissociation from CpMoCl$_2$(PR$_3$)$_2$.[6]

The heme potential energy surfaces, as a function of the reaction coordinate (roughly equivalent to the Fe−C distance, see discussion below), are shown qualitatively in Figure 1. Deoxyheme is a quintet, with low-lying triplet and singlet excited states.

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Table 1. Properties of Iron Porphyrin/Imidazole/CO Stationary Points

species	E_{rel}[a]	r(Fe−C)[b]	d(Fe oop)[c]	jawsize[d]	r(Fe−N$_{\mathrm{his}}$)[e]
^{5}A′ deoxy	0.00		0.289[f]	2.077	2.209
^{3}A″ deoxy	1.27		0.142	2.019	2.282
^{1}A′ deoxy	5.78		0.151	2.013	1.981
^{1}A′-CO	−17.74[f]	1.802[f]	−0.025	2.029	2.090
^{1,5}MECP	2.38	2.327	0.065	2.079	2.236
^{3,5}MECP	1.98	3.005	0.135	2.044	2.270
^{1,3}MECP	2.63	2.422	0.045	2.030	2.292

[a] In kcal/mol. [b] All distances in Å. [c] distance from Fe to the center of mass of the 4 porphyrin nitrogens. [d] Half the average porphyrin cross-ring N−N distance. [e] Distance from Fe to the coordinating imidazole N atom. [f] Experimental values for myoglobin: d(Fe oop), 0.36 (ref 1k); BDE(Mb-CO), 19 ± 2 kcal/mol (ref 1c); r(Fe−C), 1.82 Å (ref 1k).

The singlet surface leads to the heme-CO minimum, whereas the other spin states are repulsive. The computations reported here explore the reaction pathway by locating the stationary points and the MECPs between singlet, triplet, and quintet states: ^{1,5}MECP, ^{1,3}MECP, and ^{3,5}MECP. Heme-CO is modeled by the realistic yet manageable $\mathrm{FeC_{24}N_6OH_{16}}$ model shown in Figure 1. B3LYP density functional computations, known to be accurate for transition metal compounds in general[7] and heme derivatives in particular,[3h] were performed using the Jaguar software,[8] with flexible polarized basis sets.[9] Full geometry optimization was performed for all minima and MECPs,[5c] with the assumption of C_s symmetry.

Selected computed energies and geometrical parameters are reported in Table 1. The ground state of deoxyheme is found to be a quintet (^{5}A′ in C_s symmetry), in agreement with experiment.[1h] Excited ^{5}A″, ^{3}A′, and ^{3}A″ states lie 1−2 kcal/mol higher, with the latter being the lowest triplet. The closed-shell ^{1}A′ state is slightly higher. The computed geometries and energies are in good agreement with experimental data.

The three MECPs are of comparable energy. The direct and indirect recombination pathways, respectively via ^{1,5}MECP, and via ^{3,5}MECP then ^{1,3}MECP, therefore have comparable barriers, but because the former is the lowest-energy route leading to heme-CO, it will be the focus of discussion here.

^{1,5}MECP, the barrier or transition state for recombination of CO with deoxyheme, is found to have a very "product-like" structure, with the iron atom moved almost completely into the porphyrin plane. This motion is the cause of the repulsive barrier on the quintet surface: at the geometry of ^{1,5}MECP, quintet deoxyheme lies 2.63 kcal/mol above its optimized energy, almost the same as the relative energy of the MECP itself, 2.38 kcal/mol.[10] Despite the fairly short Fe−C distance of 2.33 Å, there is thus no repulsive Fe−CO interaction at the MECP.[11] The reaction coordinate, which could be assumed to be mostly the Fe−C distance, in fact is also made up of the Fe out-of-plane distance. This shows the importance of full MECP geometry optimization: like transition states on a single spin-state surface, MECPs are inherently multidimensional.

The intrinsic barrier height found here of ca. 2.5 kcal/mol[10] matches the most probable barrier to recombination in Mb*,[1b] suggesting that the protein matrix does not much alter the mechanism in that case. The energy required to reach the barrier mostly serves to bring the iron atom close to the porphyrin plane in the quintet state. The most likely means by which the protein matrix can affect the barrier height is by modifying the energy requirement of that motion, through a change in the iron atom's deoxy equilibrium position, or in the corresponding force constant. Movement of the coordinating F-helix proximal histidine or changes in the hydrogen-bonding network to which it belongs could have such an effect.

The gradients on both surfaces at an MECP are parallel (or antiparallel); their common direction provides information on the mechanism, and their magnitude can be helpful in constructing models of the surfaces and of the nonadiabatic behavior.[5] At ^{1,5}MECP, the singlet gradient points toward contraction of all the Fe−ligand bond distances, whereas the quintet gradient is toward their increase. The reduced mass along the common direction is 12.75 amu. The singlet state is strongly attractive, with a gradient of 16.5 kcal/(mol•Å), while the quintet is repulsive by 4.8 kcal/(mol•Å).

The ^{3}A″ state is close in energy to the singlet and quintet states throughout the crossing region; at ^{1,5}MECP, it lies just 3.5 kcal/mol higher. The spin−orbit coupling between singlet and quintet states is, roughly speaking, inversely proportional to this energy gap. Estimates of the spin−orbit coupling have assumed the gap to be large, and have therefore led to low values, of the order of 1−10 cm^{-1}.[12] Using similar approximations, and the present energy gap, the coupling can be estimated to be closer to 100 cm^{-1}. Given the frictional nature of the CO motion within the protein pocket, this means that the reaction is probably close to the adiabatic limit.[13]

Such hypotheses can be examined by molecular dynamics studies of the whole protein.[14] Previous work in this area has had to rely on arbitrary potential energy functions fitted to the assumed properties of the different states.[15] By explicitly locating the minimum energy crossing point between the singlet and quintet states, which forms the barrier to reaction, the present study can serve as a guide for the design of more realistic potential energy surfaces for use in these studies.

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Supporting Information Available: Computational details; total energies and Cartesian coordinates for all species in Table 1, and some less important ones; gradients at ^{1,5}MECP; single-point total energies; results of counterpoise computations (ASCII). This material is available free of charge via the Internet at http://pubs.acs.org.

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[9] Fe: LACV3P ECP basis (ref 8). CO ligand and Fe-bound N atoms: 6-311G*. All other atoms: 6-31G* basis.

[10] How reliable is this number? The B3LYP method can be assumed to be of high accuracy in the MECP region, given the good agreement with experiment on geometries and relative energies, so that the main source of error should be the limited basis set size. Counterpoise calculations indicate a nonnegligible basis set superposition error (BSSE). Single-point calculations using a much larger basis (see Supporting Information) at ^{1,5}MECP find the singlet and quintet states to lie respectively 2.7 and 3.5 kcal/mol above quintet deoxyheme. This suggests that the barrier value in Table 1 may be slightly too low.

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[15] Since this article was submitted, another B3LYP study of the CO-heme reaction was published (McMahon, B. H.; Stojković, B. P.; Hay, P. J.; Martin, R. L.; García, A. E. *J. Chem. Phys.* **2000**, *113*, 6831−6850) in which a small subset of the geometrical parameters were varied to generate ~1000 points on the singlet and quintet surfaces, which were then fitted to an analytical potential. That study uses a substantially smaller basis set than in the present calculations and finds *no* intrinsic barrier.