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Controlled intersystem crossing in iron porphycene substituted myoglobin for cyclopropanation reaction: a theoretical study

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【Abstract】

In the experiment, it was found that comparing with wild-type myoglobin, iron porphycene base myoglobin has a much higher catalytic efficiency in the cyclopropanation reaction where styrene and ethyl diazoacetate (EDA) were used. Especially, the step that EDA adduction to myoglobin to form carbene was found important in this reaction. To figure out the difference of carbene formation process with two different myoglobins we focused on the iron spin state changes along the reaction pathway. Minimum energy intersystem crossing (ISC) points were located with the program package developed by our group. One step further, QM/MM method combined with density functional theory was used to study the protein environment effect.

【Introduction】

In nature, cytochrome P450 is a huge family of enzymes that related to various important oxidative reaction. Because of their vital roles in metabolism reactions, they soon become hot spot in research field since they were first recognized around 60 years ago. [1] Not only wild-type cytochrome P450 has attracted researchers' interests, the artificially modified cytochrome also shows high catalytic performance in specific reactions. Hayashi's group has been focusing on developing mutagenesis of heme inside the cytochrome P450 protein for years. Recently, they found the reconstituted myoglobin can accelerate the cyclopropanation reaction than the wild-type myoglobin due to the rapid formation of carbene. [2] We employed theoretical method to study the inner mechanism and explain the reason why the formation of carbene species plays an important role in such a reaction.

【Computational details】

All the calculations are based on the natural-type myoglobin (nMb) crystal structure 1YMB and reconstituted myoglobin (rMb) crystal structure 2V1K. In our computational model, to have a better comparison, the metal atom Mn in 1YMB was replaced by Fe since they have similar electronic structure. The sulfate was removed for its far distance to the active region. The hydrogen atoms were added by using Amber tool. Histidine residues were carefully checked with visual software VMD. The Amber tool MCPB.py was used to apply force field to the model system, where RESP charges were calculated at the B3LYP/6-31G* level and TIP3P water model was used. The MM optimizations were carried out with Amber16 program. To reduce the computational cost, after relaxing and heating the system, all the counterious and the solvent water that are further than 10 Å to the QM atoms were removed.

All the QM and QM/MM calculations were carried out by using Gaussian16 program. For the QM calculations, DFT method with uB97D functional were used. ECPs and valence electrons of Fe were represented with SDD. The 6-31g* basis set was adopted for C, H, O, and N. For ONIOM calculations, a two-layer scheme was performed where the QM method was same as QM calculations and amber method was used for MM region. Since EDA and styrene were

used in the experiment as the reactants of cyclopropanation reaction, we also adopted EDA in this work as the source of carbene formation.

【Results and discussions】

At first, only the active sites, iron-based porphyrin and iron-based porphycene where the histidine connected to Fe was replaced by imidazole group, were used as model. We investigated the process that EDA adduction to the porphyrin or porphycene ring to form the carbene. Figure 1 shows the potential energy profiles of this process. Comparing the relative energy of different spin states in each step, several important conclusions can be concluded.

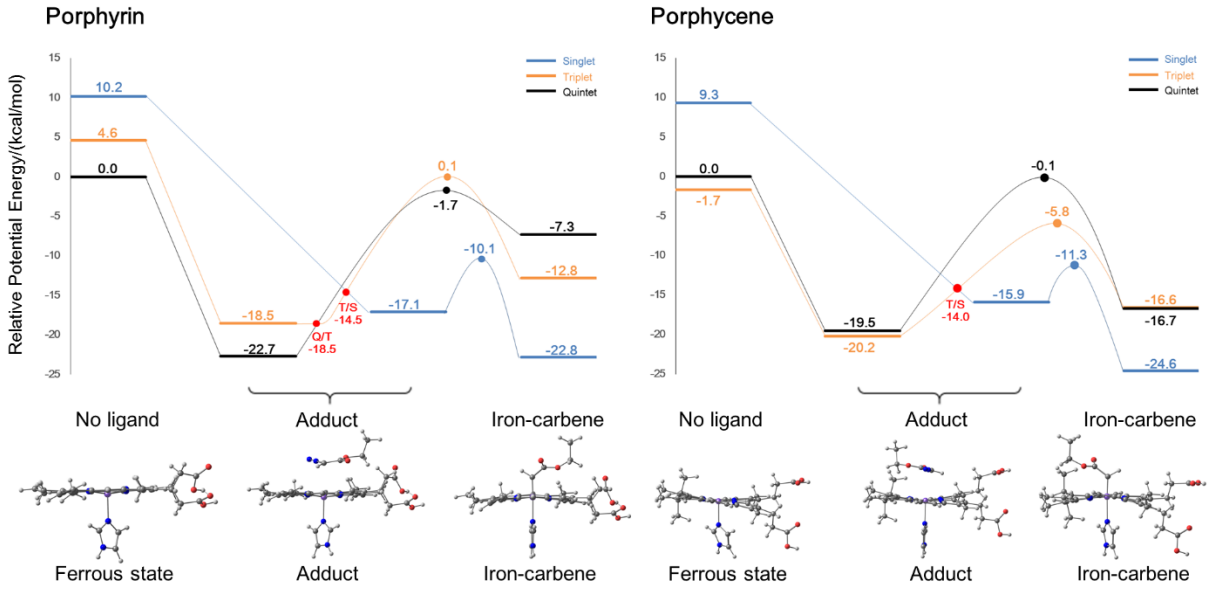

Fig. 1 Potential energy diagrams for the reactions of (left) the native heme-imidazole complex with EDA and (right) the FePc-imidazole complex withEDA. Optimized structures of stable intermediates in different spin states at each step in the reaction are shown below the diagrams.

First, in the case of nMb, the most stable ferrous state is the quintet while the most stable iron-carbene state is the singlet. Two ISC points were found along the reaction pathway. The triplet and quintet crossing point is near the minimum of triplet adduct complex, indicating that the triplet species is efficiently quenched to the stable quintet state of the EDA adduct via a negligible kinetic barrier. In the case of rMb, the most stable state of ferrous state and carbene are triplet and singlet, respectively. Thus, only one ISC point is necessary. After yielding singlet adduct complex, the case of rMb needs a smaller energy barrier to form carbene. These affects suggest that rMb is easier to react with EDA to form carbene.

Next, the protein environment effect was considered. We put the active sites back into the protein environment and optimized the structures in an iterative way. The potential energy profile of nMb shows a similar reaction path to the case that without protein. Two ISC points were found close to triplet adduct minimum and singlet adduct minimum, respectively. For the case of rMb, a little bit different potential energy surface was found where the ISC point of singlet and triplet was found near the triplet carbene minimum. The main conclusion remains same as the case without protein. Then we will figure out how did the protein environment influence the energy surface. Also, we are trying to calculate the free energy surface.

【References】

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