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# Energy Decomposition Analysis of the Protein Environmental Effect: The Case of Cytochrome P450cam Compound I

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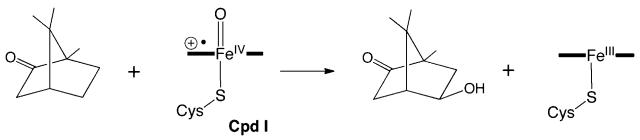
The effect of the protein environment on the short-lived compound I state of cytochrome P450cam was analyzed in detail by decomposing the ONIOM(B3LYP:AMBER) interaction energy between the active site and the surrounding protein environment into electrostatic, van der Waals (vdW), and polarization terms. The electrostatic effect was the largest in magnitude, followed by the vdW effect, and then by the polarization effect. The contributions of individual residues to the environmental effect were also evaluated.

Cytochromes P450 (P450s) are ubiquitous heme-containing monooxygenases that play vital roles in many physiologically important processes such as metabolism of xenobiotics and steroid hormone biosynthesis.[1,2] Although it is believed that an oxoiron(IV) porphyrin $\pi$-cation radical intermediate, so-called compound I (Cpd I), is responsible for the monooxygenase activity of P450s,[3,4] the difficulty in trapping and characterizing Cpd I experimentally has hampered the full mechanistic understanding of these enzymes. Over the past decade, many computational studies have been performed using density functional theory and hybrid quantum mechanical and molecular mechanical methods, which have significantly enhanced our understanding of P450s.[5–7] Cytochrome P450cam (P450cam or CYP101), a bacterial P450 that performs camphor hydroxylation in a selective manner (Scheme 1), has been particularly well studied by computational chemists, and these studies have produced invaluable insights into the reactivity of Cpd I and the roles of multiple spin states.




**Scheme 1.** Key step of camphor hydroxylation by P450cam.

A full mechanistic understanding of P450s requires knowledge of the relationship between protein structure and reactivity.[2a] In our opinion, past computational studies have focused primarily on the properties of Cpd I, and the effect of the protein environment has not necessarily been investigated in depth, despite a few attempts to evaluate the environmental effect.[6] Because of the diverse types of environmental effects and a number of amino-acid residues comprising the whole protein environment, we believe that some kind of energy decomposition is necessary to understand better this intricate aspect of enzymes. We applied our recently proposed energy decomposition scheme to P450cam Cpd I (Figure 1), with the aim of evaluating these effects quantitatively.[8]

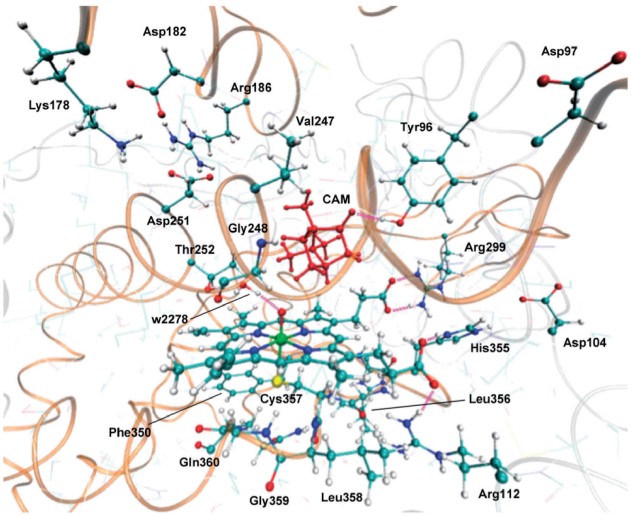



**Figure 1.** Key residues around the active site.

A model for our computational study was constructed from an X-ray structure of P450cam (PDB code 1DZ9, chain B).[4a] The protonation states of amino-acid residues were the same as those of "Prot1" in ref 9. ONIOM(B3LYP/[SDD(Fe),6-31G*(others)]:AMBER) calculations were performed using Gaussian 09.[10–14] The ONIOM calculation requires definition of the real system (entire enzyme) and the model system (active site). In this work, protoporphyrin IX, oxoiron(IV), deprotonated Cys357, and camphor (CAM) were defined as the model system, for which a doublet spin state was assumed.[15] Geometry optimization was performed with the electronic embedding (EE) scheme of ONIOM (ONIOM-EE). The electrostatic ($E_{es}$), van der Waals (vdW, $E_{vdW}$), and polarization ($E_{pol}$) interaction energies between the model system and the outer region were evaluated as described recently by the author (see also the Supporting Information for details).[8,17] In brief, these terms were evaluated as

$$E_{es} = \sum_i \sum_j s_{ij}^{es}(q_i q_j / r_{ij}) \quad (1)$$

$$E_{vdW} = \sum_i \sum_j s_{ij}^{vdW}(A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^{6}) \quad (2)$$

$$E_{pol} = E^{ONIOM}(EE) - E^{ONIOM}(ME) \quad (3)$$

where $i$ and $j$ are atoms in the model system (excluding H-link atoms) and in the rest of the system, respectively; $s_{ij}^{es}$ and $s_{ij}^{vdW}$ are scale factors for 1–2, 1–3, and 1–4 interactions; $q_i$ and $q_j$ are atomic charges; $r_{ij}$ is the internuclear distance; $A_{ij}$ and $B_{ij}$ are AMBER vdW parameters, $E^{ONIOM}(EE)$ is ONIOM-EE energy, and $E^{ONIOM}(ME)$ is ONIOM energy evaluated with the mechan-

**Table 1.** Decomposed energy components (in kcal mol$^{-1}$)

| Type | Energy |
|---|---|
| $E_{es}$ | −289.9 |
| $E_{vdW}$ | −105.8 |
| $E_{pol}$ | −101.6 |

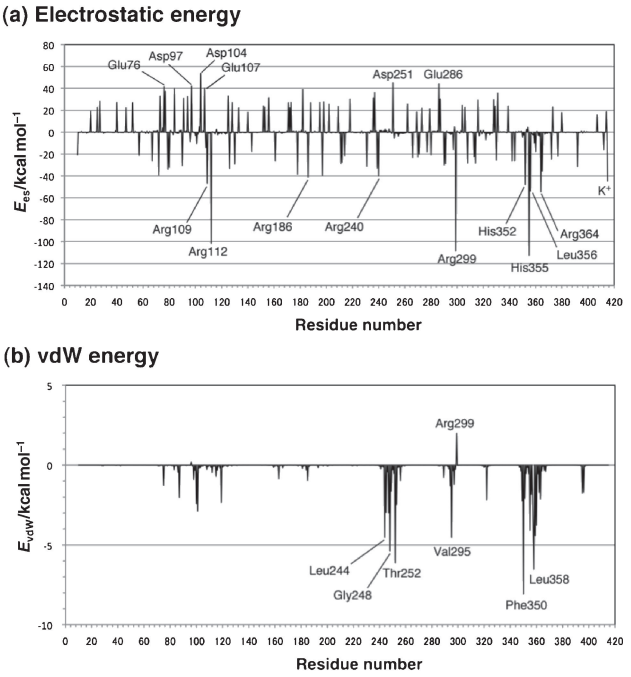



**Figure 2.** Contributions of residues to (a) $E_{es}$ and (b) $E_{vdW}$. The last residue (#415) refers to a $K^+$ ion (#515 in the PDB file).

ical embedding (ME) scheme. $E_{es}$ and $E^{ONIOM}$(ME), and $E^{ONIOM}$(EE) were calculated with gas-phase point charges assigned to the model system.

Table 1 summarizes the three different energy terms. All terms are seen to be negative, meaning that these interactions stabilize the active site. The electrostatic effect appears to stabilize the active site substantially, because $E_{es}$ (−289.9 kcal mol$^{-1}$) is the largest in magnitude. $E_{vdW}$ (−105.8 kcal mol$^{-1}$) also has a negative value, which reflects the fact that the model system fits well in the active-site cavity of the enzyme. The polarization effect ($E_{pol}$) is associated with the polarization of the electronic wave function of the model system in the presence of other atoms in the protein. This was found to stabilize the system by 101.6 kcal mol$^{-1}$.

Electrostatic and vdW interaction energies were decomposed futher into the contributions from individual residues. Figure 2a shows the $E_{es}$ values for all residues. Arg112, Arg299, and His355 (Figure 1), which form salt-bridge interactions with propionates of heme, have large attractive electrostatic interactions with the active site (<−100 kcal mol$^{-1}$). It follows, therefore, that these residues play important roles in anchoring the protoheme cofactor firmly in the active site. Negatively charged Asp and Glu residues are not very close to heme and do not form a direct H-bond or salt-bridge interaction with the active site. However, they have relatively large destabilizing

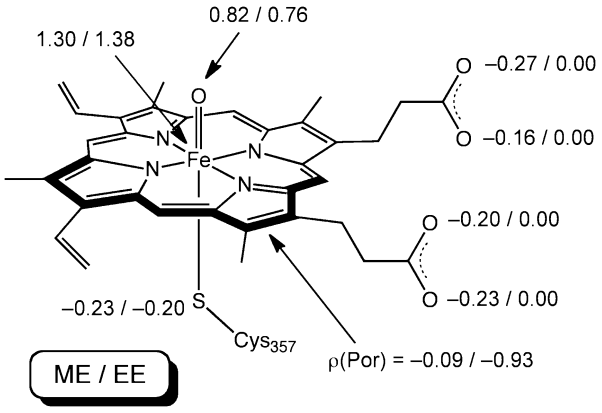



**Figure 3.** Key atomic spin population values at the ONIOM-ME and ONIOM-EE levels. $\rho$(Por) is the value for the porphyrin ring (excluding the non-hydrogen substituents).

effects (>40 kcal mol$^{-1}$), reflecting the long-range nature of the electrostatic effects. Overall, stabilizing interactions prevail; therefore, the total $E_{es}$ is negative (Table 1). It is well known that the H bond of Tyr96 with the carbonyl group of CAM confers high regioselectivity of the hydroxylation reaction (Scheme 1).[16] Our analysis shows that Tyr96 has an $E_{es}$ value of −8.9 kcal mol$^{-1}$. Mutation of this residue to Phe, which provides flexibility of the substrate, diminishes the regioselectivity. A water molecule (w2278) is H-bonded to the oxo moiety of Cpd I (Figure 1), and its $E_{es}$ value was calculated as −13.1 kcal mol$^{-1}$. A previous study demonstrated that this coordinating water lowers the activation barrier for the H-abstraction step.[6d]

Figure 2b shows the vdW energies of individual residues. The values are mostly negative (i.e., stabilizing), even though their magnitudes are small (<10 kcal mol$^{-1}$). The largest stabilizing contributions are from Phe350 and Leu358, which lie below the heme, and from Gly248 and Thr252, which lie above the heme (Figure 1). The destabilizing vdW interaction of Arg299 occurs because of the very large electrostatic stabilization (Figure 2a), which strongly pulls the residue toward the propionate group.

Figure 3 shows the key atomic spin population values obtained from ONIOM-ME//EE and ONIOM-EE calculations. At the ONIOM-EE level, the porphyrin ring has a spin population value of around −1, consistent with the nature of a porphyrin $\pi$-cation radical. However, it can be clearly seen that at the ONIOM-ME level, the spin population on the porphyrin is significantly delocalized toward the propionate oxygen atoms, which is also consistent with the results obtained by Schöneboom et al.[6b] The main role of the polarization effect is, therefore, to shift the spin populations on propionate groups back to the porphyrin ring.

In conclusion, an ONIOM(B3LYP:AMBER)-based energy decomposition analysis has been performed and has provided profound insights into the protein environmental effect on P450cam Cpd I. This analysis can be applied to any states on reaction paths (e.g., transition states), thus helping identify the factors that determine the reactivity of P450 Cpd I. Applications of this method to other enzymes should also improve our understanding of enzyme functions.

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17 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

  