Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Mechanisms of reaction in cytochrome P450: hydroxylation of camphor in P450cam†

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Supporting Information

1. Protein preparation

The protein model was based on the crystal structure of P450cam from *Pseudomonas putida* complex with camphor[S1] (PDB entry 1DZ9). Hydrogen atoms were added according to standard pK_a values, using the HBUILD[S2] module of CHARMM[S3] program version c27b2. The exception to this rule was Asp297 where both protonation states were used; the two different models are referred to in the text as the protonated and ionized models, respectively. In the protonated case, a hydrogen atom was added to the OD2 oxygen, forming a hydrogen bond between the OD2 and O2A atoms. Histidine tautomers were assigned on the basis of the local hydrogen bonding environment. The protein was truncated to a 25 Å sphere centred around haem, and the positions of the hydrogen atoms were minimized using 1000 steps of steepest descent minimization (SD) followed by 500 steps of conjugate gradient minimization (CONJ). In pure forcefield calculations, all polar residues (Asp, Lys, Glu, Arg) located 20 Å or more from the centre of the system (buffer region) were neutralized, unless they were forming salt bridges with charged residues in the inner region. This led to a system with a total charge of -1e for the ionized model, and with no charge for the protonated model. A non-bonded cutoff of 13 Å was used. All minimization and molecular dynamics simulations were performed with the CHARMM program version c27b2, using the CHARMM27 forcefield.

The system was then immersed in a 25 Å sphere of water (CHARMM TIP3P[S4] model), centred on the haem iron atom. All overlapping water molecules, *i.e.* whose oxygen atom was 2.6 Å or closer to existing heavy atoms, were deleted. The position of newly added water molecules was minimized (1000 SD, 500 CONJ), followed by minimization of all (added and crystal) water molecules atoms (600 SD, two series of 750 steps of Adopted Basis Newton-Raphson minimization (ABNR)). The position of water atoms (all other atoms were kept fixed) was then equilibrated in a stochastic boundary molecular dynamics (SBMD)[S5] simulation: 10 ps of heating from 0 K to 300 K, followed by 25 ps of equilibration; a timestep of 1 fs was used. SBMD simulations included full Newtonian dynamics for water molecules within a 20 Å radius from haem iron and Langevin dynamics for the remaining water molecules. A friction coefficient of 62 ps^{-1} was applied to water oxygen atoms. A deformable boundary potential was applied to water oxygens to keep them within the 25 Å radius sphere. The whole system was then minimized (500 SD and 1500 ABNR) and equilibrated (50 ps of heating to 300K followed by 50 ps of equilibration) with SBMD. As before, SBMD simulation was performed with a 20 Å reaction region and a 5 Å buffer. The haem unit was frozen in all calculations, all other atoms were mobile. Protein atoms in the buffer were harmonically restrained to their initial positions, with force constants increasing with the distance from the centre of the

system. Friction coefficients used in the Langevin dynamics were 62 ps^{-1} for water oxygens, and 250 ps^{-1} for protein heavy atoms. The system was subsequently minimized (500 SD, 1750 ABNR). This geometry was used as a starting point for three independent SBMD runs (following analogous protocol as before), which consisted of 50 ps of heating and 50 ps of equilibration. The final geometry from each run was minimized (500 SD, 1750 ABNR). As a result, three independent geometries were generated for both the ionized and the protonated models. These geometries were used as starting points for the QM/MM calculations.

Table S1 Shortest O-O distances between an A-propionate oxygen of haem group and a carboxylate oxygen of Asp297. Measurements were done for all P450cam structures present in PDB database with resolution of 2.00 Å or better. The shortest of four possible distances is given.

PDB code	Subunit A [Å]	Subunit B[a] [Å]
1AKD	2.77	
1CP4	2.56	
1DZ4	2.41	2.58
1DZ6	2.34	2.69
1DZ8	2.41	2.62
1DZ9	2.36	2.73
1GEK	2.59	
1GEM	2.55	
1IWI	2.57	
1IWJ	2.56	
1IWK	2.69	
1K2O	2.64	2.69
1O76	2.39	2.67
1PHA	2.63	
1PHB	2.60	
1PHC	2.44	
1PHD	2.78	
1PHE	2.79	
1PHF	2.67	
1PHG	2.82	
1QMQ	2.66	3.55[b]
1RE9	2.69	
1T85	2.49	
1T86	2.54	2.59
1T87	2.61	2.43
1T88	2.58	2.44
1UYU	2.35	2.62
1YRC	2.54	
1YRD	2.58	
2A1N	2.51	2.33
2A1O	2.58	2.41
2CPP	2.74	
3CPP	2.70	
5CP4	2.74	
6CP4	2.80	
6CPP	2.65	
7CPP	2.60	

[a] in the cases where two protein units were present in coordinate file, distances for each unit are given

[b] the number corresponds to alternative conformation of Asp297 side chain in subunit A, not to Asp297 in subunit B

2. Asp297-propionate O-O distances in P450cam structures

3. Additional Forcefield parameters

Two new residues for CHARMM forcefield were created: compound I (as described in earlier work by our group[S6]) and camphor residue, shown below.

```
RESI CAM 0.000
GROUP
ATOM C1 CT2 -0.078
ATOM C2 CC  0.602
ATOM O O -0.558
ATOM C3 CT1 -0.278
ATOM C4 CT1 -0.128
ATOM C5 CT2 -0.078
ATOM C6 CT2 -0.078
ATOM C7 CT1 -0.278
ATOM C8 CT3 -0.067
ATOM C9 CT3 -0.067
ATOM C10 CT3 -0.078
ATOM H1 HA  0.073
ATOM H2 HA  0.073
ATOM H3 HA -0.007
ATOM H4 HA  0.073
ATOM H5 HA  0.073
ATOM H6 HA  0.073
ATOM H7 HA  0.073
ATOM H8 HA  0.073
ATOM H9 HA  0.073
ATOM H10 HA  0.072
ATOM H11 HA  0.072
ATOM H12 HA  0.073
ATOM H13 HA  0.073
ATOM H14 HA  0.073
ATOM H15 HA  0.073
ATOM H16 HA  0.073
```

Additional parameters based on ref [S7]:

ANGLES	k_θ	θ_o
CT1 CC CT2	40.000 [kcal mol^{-1} rad^{-2}]	118.0000°
CT2 CT1 CT2	53.35	112

IMPROPERS	k_ψ	ψ_o
CC CT2 CT1 O	45 [kcal mol^{-1} rad^{-2}]	0.00°

4. Detailed QM/MM results for reaction pathways

Table S2 Energy barrier in model with protonated Asp297

Pathway	Barrier [kcal mol^{-1}]
1	18.46
2	18.86
3	17.68
Average	18.33
Standard deviation	0.60

Table S3 Energy barrier in model with ionized Asp297

Pathway	Barrier [kcal mol^{-1}]
1	15.63
2	15.45
3	14.95
Average	15.34
Standard deviation	0.35

Table S4 Key distances and spin density on sulfur for highest energy point for each reaction pathway in model with protonated Asp297, QM 1

Pathway	Fe-O [Å]	Fe-S [Å]	C-H [Å]	O-H [Å]	TS spin density
1	1.795	2.575	1.417	1.216	0.265
2	1.790	2.576	1.391	1.227	0.269
3	1.784	2.582	1.401	1.218	0.281
Average	1.790	2.578	1.403	1.220	0.272

Table S5 Key distances and spin density on sulfur for highest energy point for each reaction pathway in model with ionized Asp297, QM 1

Pathway	Fe-O [Å]	Fe-S [Å]	C-H [Å]	O-H [Å]	TS spin density
1	1.778	2.548	1.360	1.237	0.209
2	1.781	2.582	1.390	1.234	0.191
3	1.778	2.542	1.374	1.236	0.240
Average	1.779	2.557	1.375	1.236	0.213

Table S6 Comparison of key distances and spin density on sulfur for different QM regions for protonated model (results for pathway 1)

	Barrier [kcal mol^{-1}]	Spin density	Fe-O [Å]	Fe-S [Å]	C-H [Å]	O-H [Å]
QM 1	18.46	0.209	1.80	2.575	1.417	1.216
QM 2	21.06	0.230	1.795	2.570	1.386	1.238

Table S7 Comparison of key distances and spin density on sulfur for different QM regions for ionized model (results for pathway 1)

	Barrier [kcal mol^{-1}]	Spin density	Fe-O [Å]	Fe-S [Å]	C-H [Å]	O-H [Å]
QM 1	15.63	0.209	1.778	2.548	1.360	1.237
QM 2	17.84	0.151	1.780	2.570	1.350	1.220

Table S8 Comparison of key distances and spin density on sulfur for different QM regions for ionized model (results for pathway 2)

	Barrier [kcal mol^{-1}]	Spin density	Fe-O [Å]	Fe-S [Å]	C-H [Å]	O-H [Å]
QM 1	15.45	0.191	1.781	2.582	1.390	1.234
QM 2	17.14	0.155	1.777	2.550	1.354	1.218

References

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