

SAC/SAC–CI Study of the Ground, Excited, and Ionized States of Cytochromes P450CO

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The SAC (symmetry adapted cluster)/SAC–CI method was used to calculate the ground, excited, and ionized states of carboxy P450 model complexes. The excited and ionized states were calculated up to 5.9 and 3.4 eV, respectively. Our initial calculations were with the D_{4h} -skeleton model structure. The Q-band was well described, but the BI band (lower of the split Soret bands) was higher than the experimental value by 0.7 eV and the BII band (higher of the split Soret bands) did not appear. Next, we used the distorted X-ray crystallographic structure with side chains on porphyrin. The calculated results reproduced not only the Q-band but also both the BI and BII bands in reasonable agreement with experimental findings with regard to both absorption positions and intensities, thus demonstrating the importance of using the actual molecular geometry. In particular, the BII band is attributed to excitation from the π bond of the porphyrin side chain. We examined the effect of proteins on the spectrum by using the point charge model for environmental proteins. We then examined the effect of the double bond of the porphyrin side chain by saturating it with the thiohydrate bond and clarified that the experimental geometry is important for reproducing the BI band and that the double bond of the porphyrin side chain is important for reproducing the BII band. We also performed calculations for ionized states as a model of compound I (oxo-Fe(IV) porphyrin π -cation radical complex) of P450s. The lowest energy state was the porphyrin π cation radical with A_{2u} -like symmetry.

I. Introduction

Cytochromes P450s are a group of b-type heme enzymes that have similar biological functions and spectral properties.¹ They catalyze N- and C-hydroxylation,^{1–3} aromatic and aliphatic epoxidation,^{1–9} and the addition of atomic oxygen to a heteroatom.^{1,2}

The most characteristic feature of all P450s is the unusual 450 nm (2.78 eV) Soret band of the ferrous CO complex, which is considerably red-shifted relative to those of most other ferrous CO heme proteins:^{10,11} for example, the Soret bands of hemoglobin and horseradish peroxidase CO appear at 419 nm (2.96 eV)¹² and 423 nm (2.93 eV),¹³ respectively. The unusual 450 nm band of the CO complex has been attributed to the proximal cysteine heme ligand in P450, whereas globins and peroxidases contain a histidine ligand. In a theoretical semiempirical analysis of the CO complex, Loew et al.¹⁴ attributed the 450 nm band as being due to charge-transfer interaction between the anionic mercaptide cysteine ligand and the heme. The origin of the split Soret band was explained as a result of a mixing of the sulfur orbitals with the porphyrin π orbitals. Although their analysis offered many interesting suggestions, our understanding of the electronic structure of the ground, excited, and ionized states of P450CO is still incomplete.

Recently, Sundholm¹⁵ has reported density functional calculations of the electronic absorption spectra of porphyrins and chlorins. The excitation energies and oscillator strengths obtained at density-functional time-dependent perturbation theory level were in good agreement with experiment, though P450CO was not studied by this method.

We reported the ground and excited states of hemoglobin CO (HbCO)^{16,17} and horseradish peroxidase CO¹⁷ (HRPCO) using the SAC (symmetry adapted cluster) theory¹⁸ for the ground state and the SAC–CI theory¹⁹ for the excited states. The SAC/SAC–CI method has been established as a powerful method for studying ground, excited, ionized, and electron-attached states of molecules of various spin multiplicities.^{20,21} It has been found that an elaborate study, which includes a large amount of electron correlation, is necessary for a sound and reliable understanding of the electronic structure of the ground and excited states of HbCO and HRPCO.^{16,17} This is also supported by our studies on the ground and excited states of porphyrins: free-base porphin,²² Mg porphin,²³ tetrazaporphin,^{24a} phthalocyanine,^{24b} and oxyheme.²⁵ In addition, it has been clarified that the inclusion of polarization functions on all of the carbon and nitrogen atoms of the porphyrin ring is very important for describing the ionic π – π^* excited states of porphyrins.²⁶

We report here our studies on the ground and excited states of P450CO using the SAC/SAC–CI method. In these calculations, we used two different geometries: one with the C_s D_{4h} -skeleton model structure and the other with the X-ray structure including the side chains on the porphyrin ring. Next, we examined the protein effect with the point charge model. We also examined the effect of the double bond in the porphyrin side chain by capping it with a SH molecule. Furthermore, as a model of compound I (oxo-Fe(IV) porphyrin π -cation radical), we also performed calculations for the ionized states of P450CO using the SAC–CI method.

II. Methods

We performed five different calculations: (A) to (E). We hereafter call the cytochrome P450camCO model simply P450CO.

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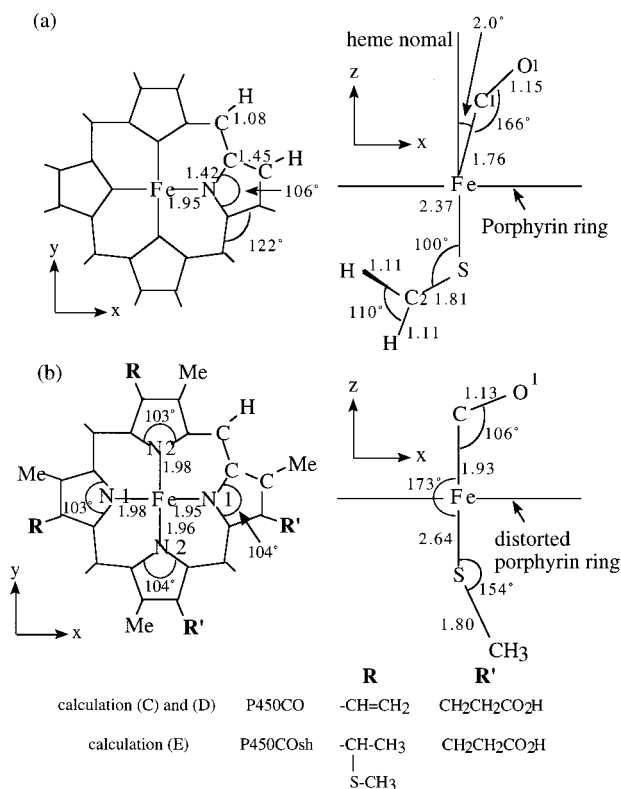


Figure 1. (a) Geometry of P450CO in calculations (A) and (B); porphyrin skeleton has D_{4h} symmetry. (b) Geometries of P450CO in calculations (C) and (D) and of P450COsh in calculation (E).

In calculations (A) and (B), P450CO has the formula $\text{FeC}_{22}\text{N}_4\text{OSH}_{15}$, as shown in Figure 1a, and consists of 44 atoms and 226 electrons. The geometry is based on X-ray crystallographic data and an IR analysis of cytochrome P450cam binding with CO and camphor²⁷ with some modification; the porphyrin skeleton is modified to have D_{4h} symmetry, and the Fe-S-C plane, one of the Npor-Fe-Npor axes, and the Fe-C-O plane are put on a mirror plane, as shown in Figure 1a. The symmetry of the entire molecule is C_s , and porphyrin is put on the xy plane. Other geometrical parameters are taken from those used previously for oxyheme.²⁵ Note that the Fe-C1 bond is offset from the normal line of the porphyrin ring by 2° .^{27b} Further, the CO bond is offset from the Fe-C1 bond: the Fe-C1-O1 angle is 166° .^{27a}

The basis sets^{28,29} for calculations are summarized in Table 1. The basis set for calculation (A) is of valence double- ζ quality, and we added single polarization d and p functions on sulfur and iron atoms, respectively, and single anion p functions on the oxygen and sulfur atoms. This calculation level is similar to those used previously for HbCO and HRPCO investigations.^{16,17} The Hartree-Fock (HF) SCF orbitals consist of 113 occupied and 168 unoccupied MOs. The SAC/SAC-CI calculations were carried out using SAC85³⁰ and its modified version.³¹ The HF molecular orbitals were used as reference orbitals. The 72 higher occupied MOs and 154 lower unoccupied MOs were included in the active space, for a total of 226 MOs. All valence orbitals were included in the active space, and only the core orbitals (1s orbitals of C, N, and O atoms; 1s, 2s, and 2p orbitals of S; and 1s, 2s, 2p, 3s, and 3p orbitals of Fe) were treated as frozen MOs. All of the single excitations and selected double excitations within this active space constituted linked operators, and their products were unlinked operators. The configuration selection^{22,32} was performed with an energy threshold of 1×10^{-5} au for excitations within the π space and

2×10^{-5} au for others for the ground state, and with the thresholds of 5×10^{-7} au for excitations within the π space and 2×10^{-5} au for others for the excited states. The number of HF SCF orbitals, the active space, and energy thresholds are summarized in Table 2.

In calculation (B), we added to calculation (A) a single polarization d function on all of the carbon, nitrogen, and oxygen atoms. Since the size of the HF SCF orbital is very large, as seen from Table 2, it was impossible to include all valence MOs in this case. Therefore, we included all valence 72 occupied MOs and 168 of the 358 unoccupied MOs in the active space. The other conditions were the same as in calculation (A) (Tables 1 and 2).

We also performed three additional calculations: (C), (D), and (E). In calculation (C), we used the X-ray crystallographic structure without modification, including the side chains on the porphyrin ring, as shown in Figure 1b. This model ($\text{FeC}_{36}\text{N}_4\text{O}_5\text{SH}_{35}$) consists of 82 atoms and 362 electrons, and the symmetry of the entire molecule is C_1 . The porphyrin skeleton is not planar, but rather distorted C_1 . The N1-Fe-S-C dihedral angle is 7.8° , and the N1-Fe-C-O dihedral angle is 5.5° . The Fe-S-C plane, the N1-Fe-N1 axis, and the Fe-C-O plane are not on the plane. The S-Fe-C angle is 173° , the Fe-S-C angle is 154° , and the Fe-C-O angle is 106° . These angles are different from those in calculations (A) and (B), and this difference is due to the fact that the geometries in calculations (A) and (B) were taken from that used previously for oxyheme. The basis set is shown in Table 1. The quality of the basis sets is the same as that in calculation (B), except that we excluded a single polarization d function from the carbon, nitrogen, and oxygen atoms that are not coordinated to Fe, since this effect is small and the calculation level is too large. Again, it was impossible to include all of the valence MOs, and therefore we included the MOs with orbital energies of -0.75 to $+0.97$ au in the active space; i.e., 87 higher occupied MOs and 180 lower unoccupied MOs, for a total of 267. The configuration selection^{22,32} was performed with an energy threshold of 1×10^{-5} au for the ground state and with a threshold of 3×10^{-5} au for the excited state. The other conditions are the same as in calculation (B).

In calculation (D), we further included the effect of the protein environment by approximating it by the point charge model. The geometries of the heavy atoms in P450CO are taken from the X-ray structure³³ (2ROM in Brookhaven DATA Bank³⁴). The positions of the hydrogens are estimated by using AMBER molecular-modeling software.³⁵ The published charges^{36,37} are put on all of the atoms of proteins and water molecules. The ionized form is adopted for the ionizable residues ASP, LYS, ARG, and GLU. The other conditions are the same as in calculation (C) (Tables 1 and 2).

In calculation (E), the double bonds of the porphyrin side chain in calculations (C) and (D) are modified by thioether bonds, modeling cytochrome C, as shown in Figure 1b. Other geometrical parameters are the same as in calculation (C). This model is referred to as P450COsh ($\text{FeC}_{38}\text{N}_4\text{O}_5\text{S}_3\text{H}_{43}$) and consists of 94 atoms and 414 electrons. We do not consider the effect of proteins in this calculation. The basis set of this model is the same as that in calculation (C), except for the sulfur. The basis sets are shown in Table 1. The other conditions of this calculation are shown in Table 2.

III. HF MOs and Ground State by Calculations (A) and (B)

The energy and character of the HF MOs of P450CO in calculations (A) and (B) are shown in Table 3, and the shapes

TABLE 1: Basis Set of Calculations (A)–(E)

atom	basis set ^a	polarization function	anion basis
calculation (A)			
Fe	(5333/53/5)/[53321/53/41]	p-type ($\alpha=0.082$)	
C	(63/5)/[63/41]		
N	(63/5)/[63/41]		
O	(63/5)/[63/41]		p-type ($\alpha=0.059$)
S	(533/53)/[533/521]	d-type ($\alpha=0.421$)	p-type ($\alpha=0.041$)
H	(4)/[4]		
calculation (B)			
Fe	(5333/53/5)/[53321/53/41]	p-type ($\alpha=0.082$)	
C	(63/5)/[621/41]	d-type ($\alpha=0.600$)	
N	(63/5)/[621/41]	d-type ($\alpha=0.864$)	
O	(63/5)/[621/41]	d-type ($\alpha=1.154$)	p-type ($\alpha=0.059$)
S	(533/53)/[5321/521]	d-type ($\alpha=0.421$)	p-type ($\alpha=0.041$)
H	(4)/[4]		
calculations (C), (D)			
Fe	(5333/53/5)/[533111/521/41]	p-type ($\alpha=0.082$)	
C ^b	(63/5)/[621/41]	d-type ($\alpha=0.600$)	
C	(63/5)/[621/41]		
N	(63/5)/[621/41]	d-type ($\alpha=0.864$)	
O ^c	(63/5)/[621/41]	d-type ($\alpha=1.154$)	p-type ($\alpha=0.059$)
O	(63/5)/[621/41]		p-type ($\alpha=0.059$)
S	(533/53)/[53111/521]	d-type ($\alpha=0.421$)	p-type ($\alpha=0.041$)
H	(4)/[4]		
calculation(E)			
Fe	(5333/53/5)/[533111/521/41]	p-type ($\alpha=0.082$)	
C ^b	(63/5)/[621/41]	d-type ($\alpha=0.600$)	
C	(63/5)/[621/41]		
N	(63/5)/[621/41]	d-type ($\alpha=0.864$)	
O ^c	(63/5)/[621/41]	d-type ($\alpha=1.154$)	p-type ($\alpha=0.059$)
O	(63/5)/[621/41]		p-type ($\alpha=0.059$)
S ^b	(533/53)/[53111/521]	d-type ($\alpha=0.421$)	p-type ($\alpha=0.041$)
S	(533/53)/[53111/521]		
H	(4)/[4]		

^a References 28 and 29. ^b Atom coordinated to Fe. ^c Oxygen of CO binding to Fe.

TABLE 2: Number of Active MOs and Energy Thresholds in the SAC/SAC–CI Calculations (A)–(E)

calc	number of HF–SCF MOs			number of active MOs			threshold (au)	
	occupied	unoccupied	total	occupied	unoccupied	total	ground state	excited state
(A)	113	168	281	72	154	226	1×10^{-5} (π) ^a 2×10^{-5} (other) ^b	5×10^{-7} (π) ^a 1×10^{-6} (other) ^b
(B)	113	358	471	72	168	240	8×10^{-6}	8×10^{-7}
(C)	181	363	544	87	180	267	1×10^{-5}	3×10^{-5}
(D)	181	363	544	87	180	267	1×10^{-5}	3×10^{-5}
(E)	207	391	598	97	193	290	1×10^{-5}	3×10^{-5}

^a For excitations within π space. ^b For other excitations.

of MOs 67–74 in calculation (B) are shown in Figure 2. The numbering of the MOs excludes core orbitals and starts from the lowest valence MO. $\text{SCH}_3(\pi_b)$ and $\text{CO}(\pi_a)$ denote π -type bonding and antibonding orbitals localized at mercaptide and carbon monoxide, respectively. The superscripts “o” and “p”, as in $\text{SCH}_3(\pi_b^o)$ and $\text{CO}(\pi_a^p)$, denote that the orbitals are on and perpendicular to the mirror plane, respectively. The higher occupied MOs 69, 70, 71, and 72 have a mercaptide σ or π nature. In particular, the HOMO (MO 72) does not have a porphyrin π nature but is localized on the Fe–S bond. This is different from other Fe–porphyrin–CO complexes such as HbCO and HRP CO,^{16,17} in which the MOs in the HOMO–LUMO region have a porphyrin π nature. MOs 69, 70, and 71 have a porphyrin π nature, though these MOs mix with proximal mercaptide σ or π orbitals. On the other hand, the natures of MOs 73 (LUMO) and 74 (next-LUMO) are the same as those for HbCO and HRP CO.^{16,17} Based on these results, it has been thought that the unique VUV spectrum of P450CO may be attributed to mixing of the proximal mercaptide σ or π orbitals with the higher occupied porphyrin π orbitals, and that some

main peaks may have a nature of charge-transfer (CT) from the proximal mercaptide ligand to the heme.¹⁴

Comparing the MOs of calculations (A) and (B), as shown in Table 3, the porphyrin π -type orbitals with a mercaptide π_b^p nature, MOs 61, 71, and 79 in calculation (B), are destabilized by the addition of polarization functions on the porphyrin ring, while there is little change in the energies of other MOs.

Next, we analyzed the Mulliken population and the bond order of the ground state of P450CO at the SAC level. The results are shown in Table 4. In calculation (B), which is of better quality than calculation (A), the gross charges on Fe, the porphyrin ring, and other ligands are neutralized more than in calculation (A). This is probably due to the fact that the description of the Fe–porphyrin bond in calculation (B) is better than that in calculation (A), and that direct sulfur–porphyrin π -interaction in calculation (B) is greater than that in calculation (A).

Since the level of calculation (A) is almost the same as that in our previous HbCO and HRP CO studies,^{16,17} the results of calculation (A) can be compared directly with the previous

TABLE 3: Energies and Characters of Hartree–Fock MOs of P450CO in Calculations (A) and (B)

MO no. ^a	symmetry	orbital energy (eV)		character ^b
		calc (B)	calc (A)	
occupied orbitals				
55	a''	-9.40	-9.34	CO(O1 ^c)p _y + Fe d _{yz} + Por(σ)
56	a'	-9.39	-9.35	CO(O1 ^c)p _x + Fe d _{xz} + Por(σ)
57	a'	-9.28	-9.28	Fe d _{x²-y²} , d _{xz} + Por(σ)
58	a'	-9.18	-9.17	Fe d _{xz} , p _x + Por(σ)
59	a''	-9.13	-9.16	Fe d _{yz} , p _y + Por(σ)
60 (61) ^d	a''	-8.89	-8.79	Fe d _{xy}
61 (60)	a''	-8.84	-9.05	SCH ₃ (C2 ^c)p _y + Por(π)
62	a'	-7.13	-7.33	Por(π)
63	a''	-7.07	-7.28	Por(π)
64	a'	-6.94	-6.95	SCH ₃ (σ) + Fe d _{xz} + Por(π)
65	a'	-6.30	-6.37	SCH ₃ (π _b ^o) + Fe d _{xz} + Por(π)
66	a'	-6.12	-6.24	Por(π)
67	a''	-5.79	-5.86	Fe d _{yz} + Por(π)
68	a'	-5.77	-5.84	Fe d _{xz} + Por(π)
69	a'	-4.16	-4.09	S s, p _x , p _z + Fe s + Por(π)
70 (71) ^d	a'	-3.09	-2.94	S p _z + Fe s, p _x + CO(C1 ^c)s, p _z + Por(π)
71 (70) ^d	a''	-2.93	-3.24	S p _y + Fe p _y + Por(π)
72	a''	-2.61	-2.45	S p _y + Fe p _y
unoccupied orbitals				
73	a'	3.32	3.19	Fe d _{xz} - Por(π)
74	a''	3.33	3.20	Fe d _{yz} - Por(π)
75	a''	6.02	5.82	Por(π)
76	a'	6.04	6.43	SCH ₃ (σ) - Fe s, p _z - CO(σ) - Por(σ)
77	a'	7.19	7.05	SCH ₃ (σ) - Fe s, p _z - CO(σ) - Por(σ)
78 (79) ^d	a'	7.36	7.50	SCH ₃ (σ) - Fe s, p _z , d _{xz} - CO(π _a ^o) - Por(σ)
79 (78) ^d	a''	7.40	7.18	S p _y - Fe d _{yz} - CO(π _a ^p) - Por(σ)
80	a'	7.40	7.82	SCH ₃ (σ) - Fe s, p _x , p _z , d _{x²-y²} - CO(π _a ^o) - Por(σ)
106 (96) ^d	a'	12.23	12.52	SCH ₃ (σ) - Fe s, p _x , p _z , - CO(σ)
111 (101) ^d	a'	13.44	13.14	SCH ₃ (σ) - Fe s, p _x , p _z , - CO(σ)
112 (102) ^d	a'	14.12	13.50	SCH ₃ (π) - Fe s, d _{x²-y²} - CO(C1 ^c)p _x , p _z - Por(σ)

^a The numbering starts from the lowest valence orbital. ^b The plus (+) and minus (-) signs denote bonding and antibonding interactions, respectively. The " π _b" and " π _a" denote the π -type bonding and antibonding orbitals localized on carbon monoxide and thiolate. The superscripts "o" and "p" denote that the orbitals are on and perpendicular to the mirror plane, respectively. ^c O1, C1, and C2 atoms are illustrated Figure 1. ^d The brackets are the number of calculation (A).

results. In P450CO, the positive charge on Fe is smaller than that in HRPCO and HbCO. The C–O polarization and the Fe–CO bond order of P450CO are, respectively, a little smaller and larger than those of HRPCO.¹⁷ In addition, the Fe–S bond of P450CO is much stronger than the Fe–imidazolate bond of HRPCO.¹⁷ These tendencies are enhanced in calculation (B). On the other hand, the C–O bond order is almost the same as that of HRPCO.¹⁷

IV. Excited States by Calculations (A) and (B)

We calculated the excited states of P450CO from visible to UV up to 3.4 eV in calculation (A) and up to 5.9 eV in calculation (B). Figure 3 shows the experimental electronic absorption spectrum of P450camCO³⁸ compared with the SAC–CI excitation spectrum (calculation (B)) in the VUV energy region, and Table 5 gives more detailed information on the excited states. Since calculation (B) is more accurate than calculation (A), we mainly use the results of calculation (B).

In the lower energy region, 0.8–1.6 eV, small peaks due to the 1¹A', 1¹A'', 2¹A', and 2¹A'' states appear in the theoretical spectrum. The 1¹A' and 1¹A'' states have main configurations corresponding to the excitations from HOMO 72 with a SFe π nature to LUMO 73 or the next LUMO 74 with a porphyrin π nature. The main configurations of the 2¹A' and 2¹A'' states correspond to excitations from the MO with a porphyrin π plus SFe σ nature to MOs with a porphyrin π nature. As expected, these states have a large CT nature from the proximal methylmercaptide ligand to heme, as shown in Table 5.

We note that although these excitations from the proximal mercaptide ligand (HOMO) to the heme (LUMO and next LUMO) might play an important role¹⁴ as the origin of the unusual 450 nm peak described in the Introduction, this is not supported by the present result. The intensities calculated for the 2¹A' and 2¹A'' states are too small to be attributed to the BI band.

The 3¹A' and 3¹A'' states with polarizations x and y , respectively, appear in the Q-band energy region around 2.3 eV. Recognizing that MOs 69, 71, 73, and 74 are pseudo Gouterman's 4 orbitals,³⁹ these states are explained by Gouterman's 4 orbital model.³⁹ In addition, since there are no other states with significant oscillator strength, the Q-band of P450CO with an energy of 2.25 eV is assigned to the 3¹A' and 3¹A'' states with energies of 2.295 and 2.304 eV, respectively, which are closely degenerate.

The BI band is assigned to the 7¹A' and 8¹A'' states with polarizations x and y , respectively, since the natures of the 7¹A' and 8¹A'' states are the same as those for the BI band obtained by calculation (C) described later, though the calculated excitation energies are certainly larger than the experimental values. These are the first two states that have strong intensities comparable to the BI band. The excitation natures can also be explained by the pseudo Gouterman's 4 orbital model.³⁹ However, states with a strong oscillator strength do not appear in the higher energy region in calculations (A) and (B). The reason for this will become clear by calculation (C) described below. Note however that the 8¹A' state, which is mainly the

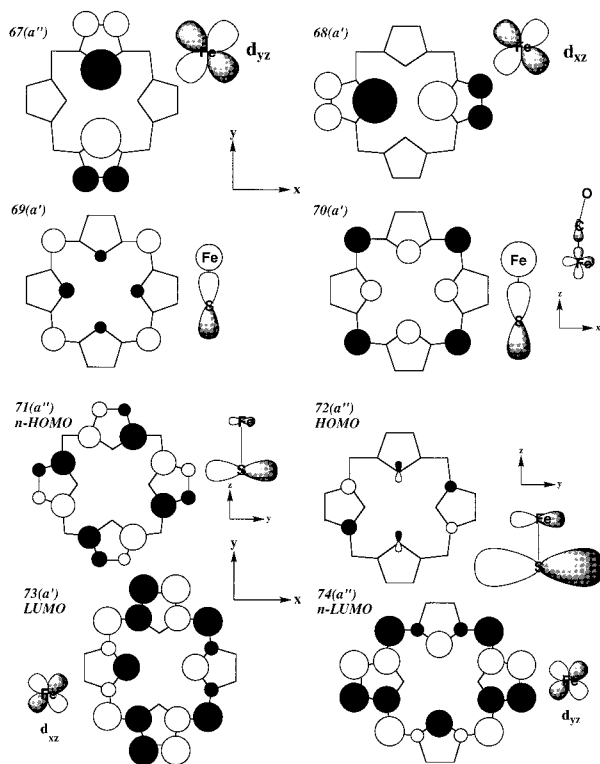


Figure 2. The occupied MOs (67, 68, 69, 70, 71, and 72) and the unoccupied MOs (73 and 74) of P450CO in calculation (B).

TABLE 4: Mulliken Population and Bond Order in the Ground State of P450CO by Calculation (A) and (B) Compared with Those for HbCO and HRPCCO Calculated at the SAC Level

	P450CO		HbCO ^a	HRPCO ^a
	calc (A)	calc (B)		
Mulliken population analysis				
Fe (net charge)	+1.45	+0.76	+1.87	+1.79
d _{x²}	0.22	0.26	0.22	0.21
d _{y²}	0.22	0.26	0.22	0.22
d _{z²}	0.30	0.28	0.23	0.26
d _{xy}	1.98	1.98	1.98	1.98
d _{xz}	1.90	1.91	1.92	1.91
d _{yz}	1.91	1.91	1.93	1.91
C1 ^b (net charge)	+0.21	+0.19	+0.23	+0.23
p _x	0.59	0.54	0.59	0.58
p _y	0.57	0.52	0.56	0.55
p _z	0.97	1.04	0.98	0.97
C1 ^b (net charge)	-0.24	-0.14	-0.22	-0.25
p _x	1.51	1.49	1.51	1.52
p _y	1.52	1.50	1.51	1.52
p _z	1.38	1.37	1.37	1.37
Por ^c (net charge)	-1.77	-1.21	-1.71	-1.78
Im ^d or SMe ^e (net charge)	-0.64	-0.60	-0.17	-0.99
total	-1.00	-1.00	0.0000	-1.00
Mulliken bond order analysis				
Fe-CO	-0.14	-0.14	-0.25	-0.23
C-O	0.69	0.65	0.70	0.69
Fe-Im ^d or Fe-S	0.40	0.45	-0.05	0.09

^a Reference 17. ^b The numberings of atoms are given in Figure 1. ^c Porphyrin ring. ^d Imidazole. ^e Methanemercaptide.

excitation from HOMO 72 (Fe-S π -bond) to MO 75 (porphyrin π), has a relatively large oscillator strength. Since the results of calculations (C) and (D) are more reliable than those of calculations (A) and (B) for the reasons described below, we do not give further details here.

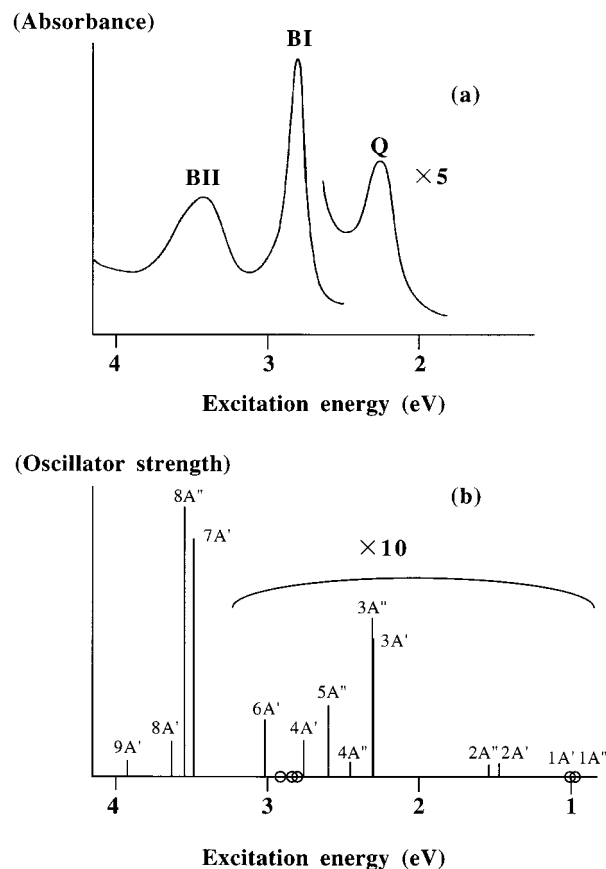


Figure 3. Electronic spectrum of P450CO. (a) Single-crystal spectrum from Hanson et al.³⁸ (b) SAC-CI theoretical spectrum by calculation (B). Open circle (O) indicates singlet state with small oscillator strength.

V. HF MOs and Excited States by Calculation (C)

The HF MO energy and character of P450CO obtained by calculation (C) are shown in Table 6, and the shapes of MOs 117–125 are shown in Figure 4. These MOs, except for HOMO 122, have a porphyrin π nature. The higher occupied MOs 119, 121, and 122 have a mercaptide π or σ nature. In particular, MO 122 without a porphyrin π nature appears in HOMO. This is the same as in calculations (A) and (B).

Comparing calculation (C) with (B), MOs 117 and 118 correspond to MOs 67 and 68, respectively, but MOs 117 and 118 have some double bond nature in the porphyrin side chain. MOs 119 and 121 correspond to MOs 69 and 70, respectively, and the MO natures are almost the same. MOs 120 and 122 correspond to MOs 71 and 72, respectively. MOs 71 and 72 have a mercaptide π nature, while MO 120 has a double bond nature of the side chain but does not have a mercaptide π nature. HOMO 122 does not have a porphyrin π nature, while HOMO 72 has a mercaptide π nature mixed with a porphyrin π nature. MOs 123 (LUMO) and 124 (next-LUMO) correspond to MOs 74 (next-LUMO) and 73 (LUMO), respectively, but MO 123 has a double bond nature of the side chain. MOs 117, 118, 120, 123, and 125 in calculation (C) have some double bond nature of the porphyrin side chains, thus demonstrating the importance of the double bonds of the porphyrin side chains through conjugation with the porphyrin skeleton.

We calculated the excited states of P450CO by calculation (C) from visible to UV up to 4.5 eV. Figure 5a, b shows the experimental electronic absorption spectrum of P450CO³⁸ compared with the theoretical spectrum calculated by the SAC-CI method. Table 7 gives more detailed information on the excited states.

TABLE 5: Excited States of P450CO Calculated by the SAC-CI Method (Calculations (A) and (B))

state	SAC-CI						SECI		exptl. ^a	
	(B)	(A)	(B)							
main config ^b ($ C\rangle > 0.25$)	nature ^c	excitation energy (eV)	polarization	oscillator strength	charge transfer ^d	polarization	oscillator strength	excitation energy (eV)	excitation energy (eV)	
1 ¹ A''	0.88(72-73)+0.36(71-73)	SFe(π), π - π^*	0.99	y	1.02×10^{-3}	0.73	y	7.46×10^{-4}	1.36	2.34
1 ¹ A'	0.89(72-74)+0.36(71-74)	SFe(π), π - π^*	1.04	x,z	2.06×10^{-3}	0.73	x,z	2.77×10^{-3}	1.17	2.55
2 ¹ A'	-0.90(70-73)+0.31(71-74)	SFeCO(σ), π - π^*	1.49	x	4.21×10^{-3}	0.36	x	6.93×10^{-3}	0.91	2.34
2 ¹ A''	0.89(70-74)+0.32(71-73)	SFeCO(σ), π - π^*	1.55	y	3.48×10^{-3}	0.38	y	7.25×10^{-3}	0.87	2.54
3 ¹ A'	-0.68(69-73)-0.60(71-74)	SFe(σ , π), π - π^*	2.30	x	4.85×10^{-2}	0.24	x	7.79×10^{-3}	2.05	3.32
3 ¹ A''	-0.62(69-74)+0.59(71-73) -0.29(68-74)	SFe(σ , π), π - π^*	2.30	y	5.58×10^{-2}	0.19	y	3.63×10^{-3}	2.06	3.32
4 ¹ A''	0.80(67-73)-0.27(55-73) +0.25(59-73)	d, σ , π - π^*	2.46	y	4.58×10^{-3}	0.15				4.71
5 ¹ A''	0.75(68-74)+0.25(56-74)	d, σ , π - π^*	2.60	y	2.51×10^{-2}	0.08	y	1.01×10^{-2}	2.27	4.68
4 ¹ A'	0.56(67-74)+0.55(68-73)	π - π^*	2.78	x	1.33×10^{-2}	0.07	x	6.79×10^{-3}	2.42	5.11
6 ¹ A''	-0.56(60-112)-0.26(55-111)	d, σ - σ^*	2.81	y	3.77×10^{-4}	-0.02				2.92
7 ¹ A''	0.64(60-112)-0.24(55-111)	d, σ - σ^*	2.84	y	8.16×10^{-6}	-0.02				3.06
5 ¹ A'	0.38(56-111)-0.31(58-111) +0.27(68-111)+0.25(56-106)	d, σ , π - σ^*	2.92	x,z	8.04×10^{-5}	-0.04				3.14
6 ¹ A'	0.60(67-74)-0.52(68-73)	π - π^*	3.05	x,z	1.96×10^{-2}	0.07	x,z	7.96×10^{-3}	2.81	5.53
7 ¹ A'	0.59(69-73)-0.51(71-74)	SFe(σ , π), π - π^*	3.49	x	0.849	0.14	x	0.941	3.33	4.67
8 ¹ A''	0.65(69-74)+0.50(71-73) +0.27(65-74)	SFe(σ , π), π - π^*	3.56	y	0.971	0.10	y	0.872	3.33	4.62
8 ¹ A'	0.85(72-75)+0.33(71-75)	SFe(π), π - π^*	3.64	x	0.116	0.69				5.59
9 ¹ A'	0.90(66-73)	π - π^*	3.93	x	5.10×10^{-2}	0.01				5.22
9 ¹ A''	0.78(72-77)+0.36(71-77)	SFe(π), π -SFe(σ^*), σ^*	4.39	y	7.18×10^{-2}	0.24				4.97
10 ¹ A''	0.60(60-111)+0.41(60-106) +0.26(60-78)	d- σ^*	4.46	y	5.31×10^{-5}	-0.05				4.89
11 ¹ A''	0.44(55-112)-0.39(67-112) -0.39(59-112)	d, σ , π - σ^*	4.60	y	6.44×10^{-4}	0.02				2.34
12 ¹ A''	-0.78(66-74)+0.43(65-74)	d, π - π^*	4.67	y	0.176	0.00				5.28
10 ¹ A'	-0.49(56-112)+0.41(58-112) -0.40(68-112)	d, σ , π - σ^*	4.71	x,z	6.39×10^{-5}	-0.01				4.85
11 ¹ A'	0.71(65-73)-0.43(71-75) -0.25(68-73)	SFe(π),d, π - π^*	4.85	x	0.273	0.14				6.14
13 ¹ A''	0.48(65-74)+0.39(56-74) -0.38(68-74)-0.36(58-74) -0.33(67-74)	SFe(π),d, σ , π - π^*	4.87	y	0.115	0.22				5.42
12 ¹ A'	0.75(71-75)+0.44(65-73) -0.29(72-75)	SFe(π),d, π - π^*	4.92	x	0.139	0.03				5.82
14 ¹ A''	0.60(70-75)-0.43(55-73) +0.43(59-73)-0.29(67-73)	SFeCO(σ),d, σ , π - π^*	5.20	y	4.48×10^{-3}	0.09				5.79
13 ¹ A'	0.55(55-74)-0.55(59-74) +0.47(67-74)	d, σ , π - π^*	5.68	x,z	4.69×10^{-3}	0.08				6.10
14 ¹ A'	0.65(70-77)+0.43(70-76) +0.37(69-77)-0.25(70-80)	SFeCO(σ), π - SFe(σ^*), σ^*	5.88	x,z	4.71×10^{-2}	0.24				5.74

^a Reference 38. ^b The numbering starts from the lowest valence orbital. ^c " σ " and " π " denote the porphyrin σ and π MOs, respectively. " d " denotes the Fe d orbitals. ^d The net charge differences between the excited and ground states on the proximal methylmercaptide. The positive values indicate the magnitude of the charge transfer from proximal methylmercaptide ligand to heme.

TABLE 6: Energies and Characters of the Hartree–Fock MOs of P450CO by Calculation (C)

MO no. ^a	orbital energy (eV)	nature ^b
occupied orbitals		
105	-9.53	Por(σ), Fe d_{yz}
106	-9.41	Fe d_{yz}
107	-9.35	Fe d_{xz}
108	-9.01	Por(π), Fe d_{xy}
109	-8.95	Fe d_{xy}
110	-7.41	Por(π)
111	-7.22	Por(π)
112	-7.06	SCH ₃ (σ), Por(π)
113	-6.62	SCH ₃ (σ), Por(π)
114	-6.51	C=C, Por(π)
115	-6.13	Por(π), C=C, Fe d_{xz}
116	-6.01	Por(π), Fe d_{yz}
117	-5.81	Por(π), C=C, Fe d_{xz}
118	-4.68	C=C, Por(π)
119	-3.80	S p_z , Fe s , Por(π)
120	-3.01	Por(π), C=C
121	-2.86	S p_z , Fe s , Por(π)
122	-2.66	Sp _{1s} , p _z , Fe p _z , p _z
unoccupied orbitals		
123	2.92	C=C, Por(π)
124	3.57	Por(π)
125	4.05	C=C, Por(π)
126	4.57	COOH
127	4.69	COOH
128	5.01	C=C
129	5.45	COOH
130	5.61	COOH
131	5.67	COOH(π)
132	5.73	Fe s , CO
136	6.55	CO(π), Fe s
140	7.45	CO(π)
144	8.30	COOH
177	13.04	Fe s , d_z^2 , COOH, Por(σ^*)
179	13.24	Por(σ), Fe s
181	13.47	Fe s , d_z^2 , xy , Por(σ)
182	13.52	Fe s , p _z , C p _z , COOH
185	13.84	Por(σ), Fe s , x^2-y^2
186	13.99	Fe s , x^2-y^2 , Por(σ)

^a The numbering starts from the lowest valence orbital. ^b C=C and COOH denote double bond and propionate of porphyrin side chain, respectively.

In the lower energy region, small peaks due to the 1^1A and 2^1A states appear at 1.8 and 2.19 eV, respectively, in the theoretical spectrum, while we cannot find such peaks in the experimental spectrum. The 1^1A state has main configurations corresponding to HOMO–LUMO excitation from HOMO 122 with a SFe π nature to LUMO 123 with a porphyrin π nature and a double bond nature of the porphyrin side chain. The 2^1A state represents HOMO 122 to next-LUMO 124 excitation. The 1^1A and 2^1A states correspond to the $1^1A''$ and $1^1A'$ states of calculations (A) and (B).

The 3^1A and 4^1A states appear in the Q-band region around 2.3 eV. Assuming that MOs 120, 121, 123, and 124 are pseudo Gouterman's 4 orbitals,³⁹ these states can be explained by Gouterman's 4 orbital model.³⁹ In addition, since no other states have significant oscillator strengths, the Q-band of P450CO observed at 2.25 eV is assigned to the 3^1A and 4^1A states with calculated energies of 2.20 and 2.51 eV, respectively. These states correspond to the $3^1A'$ and $3^1A''$ states in calculation (B), since they have the same nature as in calculation (C).

The BI band is assigned to the 7^1A state. The BI band is at 2.78 eV, while the 7^1A state is calculated at 2.98 eV with an oscillator strength of 0.422, as shown in Figure 5b. The main

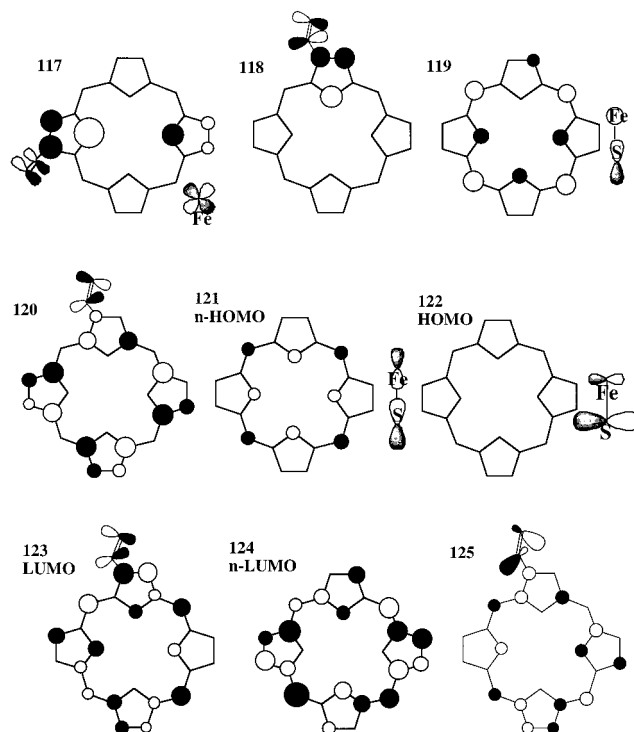


Figure 4. The occupied MOs (117, 118, 119, 120, 121, and 122) and the unoccupied MOs (123, 124, and 125) of P450CO in calculation (C).

configuration of this state has excitations from MO 120 to MO 123 (LUMO) and from MO 119 to MO 124 (next-LUMO), as shown in Table 7. MOs 120 and 123 (LUMO) have some double bond nature of the porphyrin side chain, and MO 119 has a mercaptide σ nature mixed with the porphyrin π nature. The 7^1A and 8^1A states of calculation (C) correspond to the $7^1A'$ and $8^1A''$ states of calculation (B). However, the oscillator strength of the 8^1A state of calculation (C) is very small, in contrast to that of the $8^1A''$ state. In calculation (B), the main configurations of the $7^1A'$ and $8^1A''$ states are the excitations from MOs 69 or 71 to MOs 73 or 74. The corresponding MOs are similar to each other, except that MOs 120 and 123 are different from MOs 71 and 74, respectively, since MOs 120 and 123 have some double bond nature of the porphyrin side chain.

The $7^1A'$ (3.49 eV) and $8^1A''$ (3.56 eV) states in calculation (B) shift to the 7^1A (2.98 eV) and 8^1A (3.05 eV) states in calculation (C). The reasons for this are that in calculation (C) the geometry of P450CO is based on X-ray crystallographic data and the double bonds of the porphyrin side chain play an important role in calculation (C).

In calculation (C), the 10^1A state (3.51 eV) with strong oscillator strength (0.304) appears in the BII band energy region of around 3.4 eV, as seen in Figure 5b. We assign this 10^1A state to the BII band. The main configuration of this state is the excitations from MO 118 to MOs 123 (LUMO) and 125, as shown in Table 7. The natures of MOs 118, 123, and 125 are the double bond orbitals of the porphyrin side chain interacting with the porphyrin π orbitals. Thus, the BII band strongly originates from the double bonds of the porphyrin side chain, which were neglected in calculation (B). Therefore, we can clearly understand why the state corresponding to the BII band did not appear in calculation (B).

These results show that the use of the X-ray crystallographic structure and inclusion of the double bonds of the porphyrin skeleton are very important for studying the excitation spectra of P450CO. In section VII we will study the effects of the

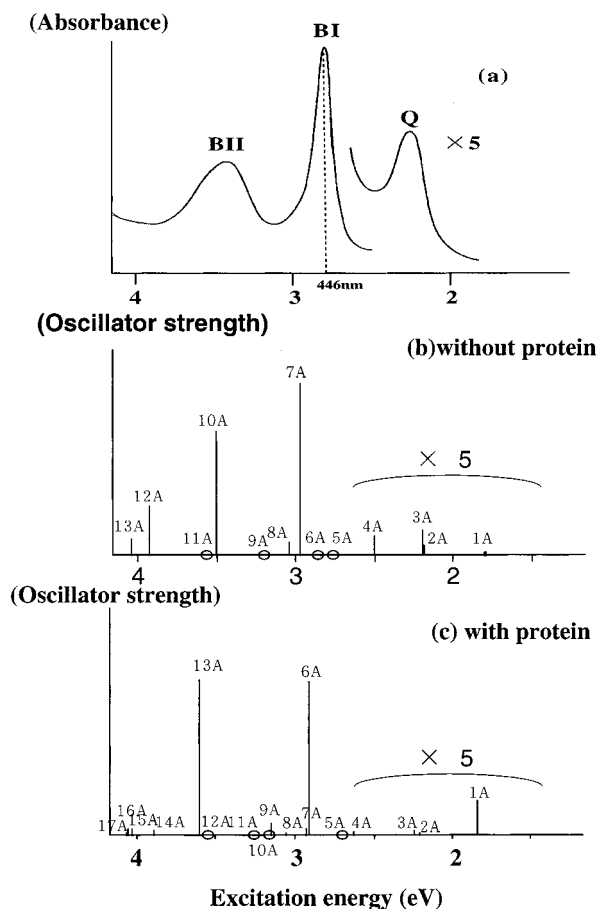


Figure 5. Electronic spectrum of P450CO. (a) Single-crystal spectrum from Hanson et al.³⁸ (b) SAC-CI theoretical spectrum by calculation (C). (c) SAC-CI theoretical spectrum by calculation (D). Open circle (○) indicates singlet state with small oscillator strength.

structure and double bonds of the porphyrin side chain in greater detail.

Table 7 and Figure 5b show that several states starting from the 12¹A state at 3.94 eV have oscillator strengths larger than 0.1. These states involve the electrons in the SFe bond and the double bonds in the porphyrin side chains as well as the porphyrin π orbitals. Since there are no experimental data available regarding this energy region, such a study is greatly needed.

VI. Protein Effect by Calculation (D)

We examined the protein effect by performing SAC/SAC-CI calculations for the ground and excited states of P450CO within a protein environment represented by the point charge model (calculation (D)). Figure 5 compares the SAC-CI excitation spectra calculated with and without the point charge model to the experimental spectrum, and Table 8 gives more detailed information on the excited states.

In the protein model, the BI band is assigned to the 6¹A state, instead of the 7¹A state. The peak position is calculated at 2.91 eV instead of 2.98 eV obtained without a protein effect, which is closer to the experimental BI peak position (2.78 eV): the peak is red-shifted by 10 nm by the effect of protein. The main configuration of this state has excitations from MO 121 (next-HOMO, MO 120 in calculation (C)) to MO 124 (next-LUMO, MO 125 in calculation (C)) and from MO 119 (MO 119 in calculation (C)) to MO 126 (MO 124 in calculation (C)), as shown in Table 8. The nature of this state is essentially the same as that of the 7¹A state in calculation (C).

The BII band is assigned to the 13¹A state, instead of 10¹A. The main configuration of this state has excitations from MO 118 (MO 118 in calculation (C)) to MO 124 and MO 130 with porphyrin π and Fe-CO σ nature, as shown in Table 8. This excitation originates from the double bond of the side chains of the porphyrin skeleton as in calculation (C) without the protein effect. Though the peak position of the calculated BII band of calculation (D) is further from the experimental value, the oscillator strength of BII band is almost the same as that of BI band, which is closer to the finding in the experimental spectrum.

VII. Effects of Side-Chain Double Bonds and Distorted Porphyrin

Figure 6 shows the experimental electronic absorption spectrum of P450CO (upper panel (a))³⁸ compared with that of cytochrome C (lower panel (b))⁴⁰ in the VUV energy region. The BII band of P450CO does not seem to exist in the spectrum of cytochrome C. We thought that this may reflect the structural difference between P450CO and cytochrome C: P450CO has double bonds in the porphyrin side chain, while cytochrome C does not, since it is saturated by the thioether linkage, as shown by the circles in Figure 7.

To examine the effects of double bonds in the side chain, we calculated the excited states of a model compound of P450CO in which the double bonds in the side chain are saturated by adding SH to form thioether linkages (calculation (E)). This model compound is called P450COsh, and its geometry is shown in Figure 1.

In Figure 6b, we compare the SAC-CI theoretical excitation spectrum of P450COsh by calculation (E) with the experimental spectrum of cytochrome C in the VUV energy region, and Table 9 gives more detailed information on the excited states obtained by calculation (E). The MOs around the HOMO-LUMO region by calculation (E) are similar to those by calculation (C), except that the double bond nature of the porphyrin side chain is not present in calculation (E).

The BI band of P450COsh is assigned to the 7¹A and 8¹A states obtained by calculation (E). These states are calculated to be about 0.1 eV higher than the corresponding 7¹A and 8¹A states of P450CO. They are assigned to the Soret band of cytochrome C, which is also higher than BI band of P450CO by about 0.2 eV. The main configurations of the 7¹A and 8¹A states are the excitations from MO 133 (119 in calculation (C)) or 134 (120 in calculation (C)) to MO 137 (123 in calculation (C)) or 138 (124 in calculation (C)). The nature of these states is the same as in calculation (C).

It is remarkable that the peak corresponding to BII band does not appear in calculation (E) for P450COsh. This is direct proof of the previous result that the BII band of P450CO originates from the double bonds of the side chains of the porphyrin skeleton. Since cytochrome C does not have such double bonds on its side chains, it does not show absorption at the position of the BII band of P450CO.

Comparison of the two calculations (B) and (E) shows that the 7¹A' (3.49 eV) and 8¹A'' (3.56 eV) states corresponding to the BI band in calculation (B) shift to the corresponding states at 3.07 and 3.09 eV in calculation (E), since the porphyrin geometry is distorted, as implied by the X-ray crystallographic data. Calculation (B) assumes C_s symmetry where the porphyrin skeleton is modified to have D_{4h} symmetry, while calculation (E) assumes C_1 symmetry where the porphyrin skeleton is not strictly planar, but rather distorted, as shown by X-ray crystallographic data (see Figure 1). The models in calculations (B)

TABLE 7: Excited States of P450CO Calculated by the SAC-CI Method (Calculation (C))

SAC-CI					exptl. ^a
state	main configurations ^b ($ C > 0.25$)	nature ^c	excitation energy (eV)	oscillator strength	excitation energy (eV)
1 ¹ A	0.954(122-123)	SFe(π)- π^* , C=C	1.80	1.31×10^{-3}	
2 ¹ A	0.964(122-124)	SFe(π)- π^*	2.19	5.04×10^{-3}	
3 ¹ A	-0.921(121-123)	SFe(σ), π - π^* , C=C	2.20	1.26×10^{-2}	2.25 Q
4 ¹ A	0.862(121-124)-0.422(120-123)	SFe(σ), C=C, π - π^*	2.51	9.89×10^{-3}	2.25 Q
5 ¹ A	0.344(106-177)+0.291(106-136)-0.281(106-182) 0.267(106-186)	d-d, σ^* , CO(π^*), COOH	2.76	4.27×10^{-4}	
6 ¹ A	0.310(107-177)-0.305(107-179)+0.276(107-136)	d-d, σ^* , CO(π^*), COOH	2.85	1.01×10^{-3}	
7 ¹ A	-0.723(120-123)-0.478(119-124)-0.361(121-124)	SFe(σ), C=C, π - π^* , C=C	2.98	0.422	2.78 BI
8 ¹ A	0.673(119-123)-0.670(120-124)	SFe(σ), C=C, π - π^* , C=C	3.05	3.35×10^{-2}	
9 ¹ A	-0.461(109-185)-0.437(109-186)-0.254(109-181)	d-d, σ^*	3.21	2.22×10^{-5}	
10 ¹ A	-0.619(118-123)+0.537(118-125)+0.315(119-124) 0.287(120-125)	SFe(σ), C=C, π - π^* , C=C	3.51	0.304	3.40 BII
11 ¹ A	0.912(122-125)	SFe(π)- π^* , C=C	3.56	1.97×10^{-4}	
12 ¹ A	-0.823(121-125)	SFe(σ), π - π^* , C=C	3.94	0.121	
13 ¹ A	-0.422(122-132)+0.399(122-140)-0.298(119-124) -0.292(122-144)	SFe(σ , π), π - SFe(σ^*), π^* , CO, COOH	4.05	4.02×10^{-2}	
14 ¹ A	-0.377(116-123)+0.364(119-123)+0.348(120-124) +0.339(120-125)+0.274(119-124)	SFe(σ), d, C=C, π , - π^* , C=C	4.17	0.659	
15 ¹ A	-0.844(122-128)	SFe(π)-C=C	4.20	0.126	
16 ¹ A	-0.370(122-128)+0.317(119-123)+0.312(120-124) +0.253(116-123)	SFe(σ , π), d, C=C, π - π^* , C=C	4.21	0.527	
17 ¹ A	-0.314(120-125)-0.297(118-123)+0.284(120-124) +0.266(109-136)+0.261(119-123)	SFe(σ), d, C=C, π - π^* , C=C, CO(π^*)	4.26	0.521	
18 ¹ A	0.573(116-123)+0.311(119-124)	SFe(σ), d, π - π^* , C=C	4.33	0.288	
19 ¹ A	-0.486(120-125)+0.418(119-124)+0.254(117-123)	SFe(σ), C=C, π - π^* , C=C	4.47	0.565	

^a Reference 38. ^b The numbering starts from the lowest valence orbital. ^c " σ " and " π " denote the porphyrin σ and π MOs, respectively. "d" denotes the Fe d orbital. C=C and COOH denote double bond and propionate of porphyrin side chain, respectively.

TABLE 8: Excited States of P450CO in the Protein Environment Calculated by the SAC-CI Method (Calculation (D))

SAC-CI					exptl. ^a
state	main configurations ^b ($ C > 0.25$)	nature ^c	excitation energy (eV)	oscillator strength	excitation energy (eV)
1 ¹ A	0.942(122-124)	SFe(σ), π - π^* , C=C	1.85	1.44×10^{-2}	2.25 Q
2 ¹ A	-0.878(122-126)+0.259(122-125)	SFe(σ), π - π^* , COOH	2.21	1.33×10^{-3}	
3 ¹ A	-0.893(120-124)+0.299(121-124)	SFe(π), C=C, π - π^* , C=C	2.25	2.30×10^{-3}	
4 ¹ A	0.851(120-126)+0.305(121-126)-0.254(120-125)	SFe(π), C=C, π - π^* , COOH	2.63	7.98×10^{-3}	
5 ¹ A	-0.372(106-181)	d-FeC(σ^*)	2.78	4.20×10^{-4}	
6 ¹ A	0.722(121-124)-0.438(119-126)-0.278(120-124)	SFe(π , σ), C=C, π - π^* , C=C	2.91	0.311	2.78 BI
7 ¹ A	0.926(122-123)	SFe(σ), π -COOH	2.93	1.54×10^{-2}	
8 ¹ A	-0.563(121-126)-0.554(119-124)	SFe(π , σ), C=C, π - π^* , C=C	3.05	2.55×10^{-2}	
9 ¹ A	0.311(119-124)+0.300(107-181)+0.284(121-126)	SFe(π , σ), C=C, d, π - π^* , C=C, FeC(σ)	3.06	5.58×10^{-3}	
10 ¹ A	0.490(109-186)-0.477(109-185)-0.313(109-183)	d-d, FeC(σ), S, COOH	3.09	4.30×10^{-6}	
11 ¹ A	-0.917(120-123)-0.291(121-123)	SFe(π), C=C, π -COOH	3.27	6.37×10^{-5}	
12 ¹ A	0.897(122-125)	SFe(σ), π -COOH	3.59	1.22×10^{-4}	
13 ¹ A	-0.680(118-124)+0.415(118-130)	C=C, π - π^* , C=C, COOH	3.61	0.317	3.40 BII
14 ¹ A	-0.638(122-131)-0.376(122-132)+0.261(122-130) -0.255(122-128))	SFe(σ), π - C=C, CO, COOH	3.89	9.97×10^{-3}	
15 ¹ A	0.863(120-125)+0.270(121-125)	SFe(π), C=C, π -COOH	3.94	2.76×10^{-4}	
16 ¹ A	0.835(122-127)+0.358(122-128)	SFe(σ), π -COOH	4.00	1.20×10^{-3}	
17 ¹ A	-0.403(109-181)-0.309(109-136)	d-CO(π^*), FeC(σ^*)	4.03	1.42×10^{-2}	
18 ¹ A	0.523(122-130)-0.441(122-128)+0.432(122-132)	SFe(σ), π -C=C, CO, COOH	4.06	1.56×10^{-2}	
19 ¹ A	-0.915(121-123)+0.292(120-123)	SFe(π), C=C, π -COOH	4.07	5.49×10^{-3}	
20 ¹ A	0.650(122-128)-0.436(122-127)+0.371(122-130)	SFe(σ), π -C=C, COOH	4.15	3.27×10^{-2}	
21 ¹ A	-0.414(119-124)+0.413(121-126)-0.395(118-124) 0.286(115-124)	SFe(π , σ), C=C, d, π - π^* , C=C	4.21	1.194	

^a Reference 38. ^b The numbering starts from the lowest valence orbital. ^c " σ " and " π " denote the porphyrin σ and π MOs, respectively. "d" denotes the Fe d orbital. C=C and COOH denote double bond and propionate of porphyrin side chain, respectively.

and (E) do not have double bonds in the porphyrin side chain. These results show that the geometry of the porphyrin ring is an important factor in reproducing the position of the BI band in the absorption spectra.

Comparison of the calculations (C) and (E) clearly shows that the existence of double bonds in the porphyrin side chain is the origin of the BII band. The model for calculation (C) has double bonds in the porphyrin side chain, while those for

calculations (B) and (E) do not. Therefore, the double bonds of the porphyrin side chain are necessary for reproducing the BII band in the experimental spectrum of P450CO.

VIII. Ionized States

Finally, we studied the ionized states of P450CO. In the Koopmans model, the ionization energy is the negative of the

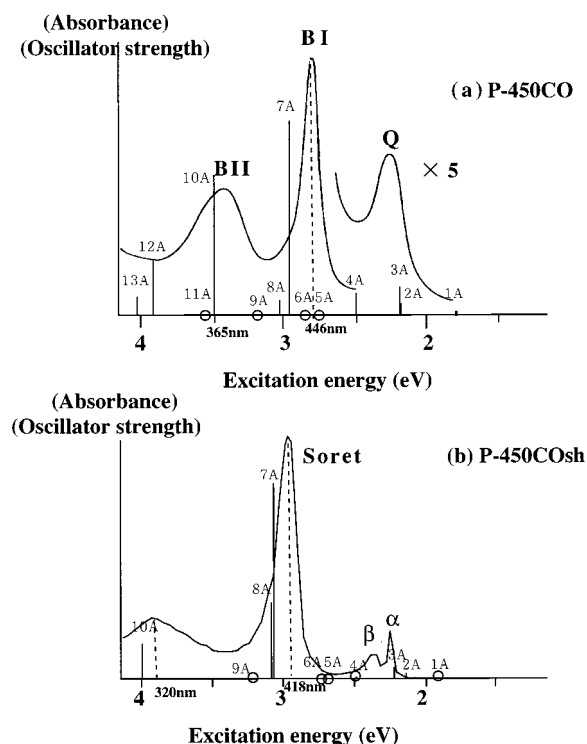


Figure 6. (a) SAC–CI theoretical spectrum by calculation (C) and electronic spectrum of P450CO from Hanson et al.³⁸ (b) SAC–CI theoretical spectrum by calculation (E) and electronic spectrum of cytochrome C from Frohvirt et al.⁴⁰ Open circle (○) indicates singlet state with small oscillator strength.

occupied orbital energy given in Table 3 (calculations (A) and (B)) and Table 6 (calculation (C)). The lowest ionization potential is estimated to be about 2.6 eV, and ionization occurs mainly from the Fe–S bond. Ionization from the porphyrin π region occurs at about 2.9 eV. However, since the ionized state is greatly affected by electron correlations, a higher-level calculation that includes electron correlation is necessary to obtain a reliable result. In this study, we calculated the ionized states of P450CO using the SAC–CI method.

Since it has been shown that the ionization energies of free base porphyrin are considerably improved by including the polarization d-functions on the carbon and nitrogen atoms,²⁶ we report here the results of calculation (B) for only some lower ionized states. Table 10 shows detailed information regarding the ionized states. The $2^2A'$ and $2^2A''$ states with a porphyrin π nature correspond to the 2^2A_{2u} and 2^2A_{1u} states in D_{4h} symmetry, respectively. The lowest ionization potential is calculated to be 1.06 eV, which is much smaller than the Koopmans' value of 2.6 eV. This state is the $1^2A''$ state derived from ionization from MO 72 (HOMO) localized at the SFe π orbital, suggesting that the porphyrin π -cation radical of P450CO cannot be formed by a simple oxidation reaction. Actually, it is known that model complexes of P450s are very unstable when they do not have a protecting group around the proximal ligand.⁴¹ However, since the oxygenation of substrates occurs at the distal site *in vivo*, it is difficult for the ionized state localized at the proximal SFe π orbital to take part in the enzymatic reaction. Thus, this state may not be important for a biological system.

The next ionized state is $1^2A'$, which has a porphyrin π nature, calculated at 1.54 eV. It is predominantly ionization from MO 70, the third MO from the HOMO: the Koopmans value is 3.09 eV, which again is much lower than the SAC–CI value, and demonstrates the importance of electron correlation for the ionized state. Generally, it is expected that the activity of

compound I with A_{2u} porphyrin π -cation radical nature is greater than that with A_{1u} nature. This is because the A_{2u} porphyrin π -cation radical has frontier orbital densities on the α -nitrogen and iron atoms, so that the activity of the distal oxo ligand may be higher than that with A_{1u} symmetry:⁴² in the 2^2A_{1u} state, the porphyrin π -cation radical orbital has a node at the α -nitrogen and iron atoms. Therefore, we are interested in the symmetry of the π -cation radical appearing in a lower energy region, though in this study the distal ligand is not an oxo-oxygen, but rather a carbon monoxide.

The HF orbital energies of the higher porphyrin π MOs 70 (a') and 71 (a'') are very close, -3.09 and -2.93 eV, respectively. Therefore, at the HF level, we cannot say which of the A' and A'' states is lower in porphyrin π -cation radicals. SAC–CI calculations are very important for determining which is lower in energy. At the SAC–CI level, the $1^2A'$ state (1.54 eV) is calculated to be about 0.7 eV higher than the $2^2A''$ state (2.21 eV): the ordering is different from that given by Koopmans. In both states, the porphyrin π nature mixes with the SFe π and σ nature. Thus, the SAC–CI calculation shows that the nature of the porphyrin π -cation radical of P450CO is definitely A_{2u} -like in symmetry. Furthermore, the $1^2A'$ state can have a frontier density on the distal CO ligand, as shown in Figure 2, though the magnitude is small: we attribute the higher reactivity of P450s to the A_{2u} porphyrin π -cation radical nature in its cation radical (compound I). In addition, the $1^2A'$ state is not derived from the pseudo Gouterman's 4 orbitals,³⁹ as shown in section IV. This is also a specific nature of P450s, since compound I of peroxidases usually has a porphyrin π -cation radical nature derived from the Gouterman's 4 orbitals.³⁹

IX. Conclusions

The ground, excited, and ionized states of P450CO were studied by performing five different calculations (A)–(E) with the SAC/SAC–CI method. The results are summarized as follows.

(1) In the ground state, the positive charge on Fe in P450CO is smaller than those in HRPCO and HbCO studied previously.^{16,17} The Fe–CO bond in P450CO is stronger than those in HbCO and HRPCO.

(2) The Q-band was assigned to the 3^1A and 4^1A states. These states were explained by excitations within the pseudo Gouterman's 4 orbitals: MOs 120, 121 (next-HOMO), 123 (LUMO), and 124 (next-LUMO), shown in Figure 4 for calculation (C).

(3) The BI band was assigned to the 7^1A state. This state was explained by excitations from the orbitals with porphyrin π nature and double bond nature of the porphyrin side chain (MO 120) and a mercaptide σ nature (MO 119) to the orbital with porphyrin π nature (MOs 123 and 124) and double bond nature of the porphyrin side chain (MO 123)(see Figure 5b). The position of the BI band is sensitive to the geometry of the porphyrin ring: the use of a distorted geometry based on X-ray data was important for reproducing the position of the BI band in the theoretical spectrum.

(4) The BII band was assigned to the 10^1A state. This state was explained by excitations from the double bond of the porphyrin side chain (MO 118) to the LUMO with porphyrin π nature and double bond of the porphyrin side chain (MO 123) and to MO 184, which is delocalized between porphyrin and the side chain.

(5) We confirmed theoretically by calculation (E) that when the double bond of the porphyrin side chain was saturated by the addition of SH, the BII band disappeared. The resultant spectrum was very similar to that of cytochrome C. Thus, the

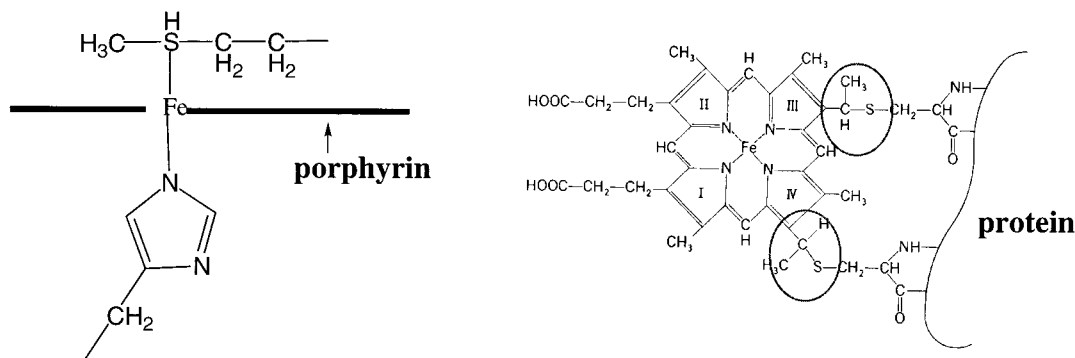


Figure 7. Structure of cytochrome C.

TABLE 9: Excited States of P450COsh Calculated by the SAC-CI Method (Calculation (E))

state	main configurations ^b ($ C > 0.25$)	nature ^c	excitation energy (eV)	oscillator strength	exptl. ^a excitation energy (eV)	
					P450CO	cytochrome C
1 ¹ A	0.964(136-137)	SFe(π)- π^*	1.91	3.18×10^{-4}		
2 ¹ A	-0.962(136-138)	SFe(π)- π^*	2.14	4.38×10^{-3}	2.25 Q	
3 ¹ A	-0.918(135-137)	SFe(σ), π - π^*	2.32	9.66×10^{-3}	2.25 Q	2.25 α
4 ¹ A	-0.894(135-138)+0.333(134-137)	SFe(σ), π - π^*	2.49	1.45×10^{-3}	2.25 Q	2.38 β
5 ¹ A	-0.299(118-194)+0.290(118-148) -0.277(118-198)-0.263(118-202)	d-	2.72	8.06×10^{-5}		
6 ¹ A	-0.335(119-196)-0.318(119-194) 0.279(119-148)	d, σ^* ,CO(π^*),COOH,FeC(σ^*)	2.76	5.32×10^{-4}		
7 ¹ A	0.720(134-137)+0.560(133-138) -0.250(135-138)	SFe(σ), π - π^*	3.07	0.168	2.78 BI	2.98 Soret
8 ¹ A	-0.684(134-138)+0.644(133-137)	SFe(σ), π - π^*	3.09	6.62×10^{-2}	2.78 BI	2.98 Soret
9 ¹ A	0.487(122-202)-0.373(122-199) -0.278(122-201)	d-d, σ^* ,Sside	3.23	1.10×10^{-5}		
10 ¹ A	0.458(136-152)+0.410(136-144) -0.320(136-142)-0.298(136-153) 0.297(133-138)-0.281(136-156)	SFe(σ , π), π - π^* ,CO(π^*),COOH	4.00	3.11×10^{-2}		
11 ¹ A	0.812(132-137)	Sside,d, π - π^*	4.20	1.84×10^{-2}		
12 ¹ A	0.529(133-137)+0.464(134-138) -0.407(136-146)	SFe(σ , π), π - π^* ,COOH	4.25	0.959		
13 ¹ A	0.638(136-146)+0.366(136-145) 0.358(133-137)+0.309(134-138)	SFe(σ , π), π - π^* ,COOH	4.31	0.543		
14 ¹ A	0.633(133-138)-0.426(134-137)	SFe(σ), π - π^*	4.35	1.364		

^a Reference 40. ^b The numbering starts from the lowest valence orbital. ^c " σ " and " π " denote the porphyrin σ and π MOs, respectively. "d" denotes the Fe d orbital. Sside and COOH denote together bond and propionate of porphyrin side chain, respectively.

TABLE 10: Ionized States of P450CO by the SAC-CI Method (Calculation (B))

state	main configurations ^a ($ C > 0.25$)	nature ^b	ionization energy (eV)	polarization
1 ² A''	0.91(72)+0.35(71)	SFe(π)	1.06	y
1 ² A'	0.96(70)	SFeCO(σ), π	1.54	x
2 ² A''	-0.91(71)+0.35(72)	SFe(π), π	2.21	y
2 ² A'	-0.96(69)	SFe(σ), π	2.77	x
3 ² A''	-0.95(67)	d, π	3.29	y
3 ² A'	-0.95(68)	d, π	3.33	x

^a Numbering starts from the lowest valence orbital. ^b " σ " and " π " denote the porphyrin σ and π MOs, respectively. "d" denotes the Fe d orbital.

existence of BII band is considered to be an indicator of the existence of double bonds on the side chain of the porphyrin ring: these double bonds conjugate with the porphyrin π bonds.

(6) The effect of the protein environment on the excitation spectrum was small, but red-shifted the BI peak by 10 nm, which was more consistent with experimental findings. On the other hand, the BII peak was blue-shifted.

(7) The first ionized state for P450CO was the 1²A'' state (A_{1u} -like in D_{4h}), which mainly has Fe-S π bond nature (see MO 72 in Figure 2). On the other hand, the second ionized

state was the 1²A' state, which has a porphyrin π nature and A_{2u} -like symmetry in D_{4h} .

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