Supplemental materials

Discrimination between CO and O_2 in heme oxygenase: Comparison of static structures and dynamic conformation changes following CO photolysis

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Figure S1. Absorption spectrum of O_2-bound heme-rHO-1 crystal. The major peaks of the absorption spectrum at 540 nm and 573 nm are consistent to the spectrum of the O_2-bound heme-rHO-1 solution.(*1*)

Figure S2. Conformation changes depending on temperature change. Weighted difference Fourier map between before and after warm-up to 160 K, which is contoured by ±4.0 σ, is superimposed on the model of CO-bound form. Arrow shows the conformation change of Phe-167 by warm-up.

Figure S3. Fluctuated conformations in B-helix and the loop between B- and C-helices. O_2-bound heme-rHO-1 structure (yellow) is superimposed onto CO-bound form (cyan; PDB ID: 1IX4). The peptide bond between Gln-41 and Val-42 shows two conformations and the major conformation in each form is shown for clarity. The minor conformation of each form is identical to the major conformation of the other. The orientation of the benzene ring in Phe-37 of B-helix, which is close to the site-2, is slightly different each other.

Figure S4. Superimposition of the CO-bound form (orange) on the ferrous form (gray) to minimize rms differences of heme atoms except for the propionate groups.

References for the supplementary materials

1. Yoshida, T., Noguchi, M., and Kikuchi, G. (1980) Oxygenated form of heme・heme oxygenase complex and requirement for second electron to initiate heme degradation from the oxygenated complex, *J. Biol. Chem. 255*, 4418-4420.

Table S1. Data collection and refinement statistics for O_2 bound form

Crystallographic data	
Space group	$P3_221$
Unit cell dimensions (Å)	a = b = 65.14, c = 120.39
Diffraction statistics	
Resolution (Å)	30-1.8
No. of observations	115,375
No. of unique reflections	27,933
Completeness (%)*	99.2 (99.1)
Mean I_o/σ	14.6 (3.4)
R_{sym}[a,*](%)	4.2 (45.4)
Refinement statistics	
R_{cryst}/R_{free} (%) [d]	15.6/18.3
R.m.s. deviations	
Bond length (Å)	0.024
Bond angle (°)	2.596
No. of atoms per asymmetric unit,	2004
Average of B-factors	
Protein	16.4
Water	34.7
Heme/O_2/Formate	15.2/19.6/32.0
Deposited PDB ID	4G7L

[a] $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, $\langle I(hkl)\rangle$ is the mean intensity for multiple recorded reflections.

[d] $R_{cryst} = \Sigma|F_{obs}(hkl) - F_{calc}(hkl)| / \Sigma|F_{obs}(hkl)|$. R_{free} is the R_{cryst} calculated for the five percent of the dataset not included in the refinement.

* Values in parentheses are for the outermost shells (1.83-1.80 Å).

Table S2. Data collection and refinement statistics for CO photolysis datasets

Crystallographic data	Dark1	Light1	Dark2	Light2
Space group		$P3_221$		
Unit cell dimensions (Å)	a = b = 65.84, c = 120.27		a = b = 65.82, c = 119.88	
Diffraction statistics				
Laser illumination	dark	1 hr	dark	16 hr
Laser power density (mW/mm^2)	-	314	-	768
Resolution (Å)	50 - 1.9	50 - 1.9	50 - 1.9	50 - 1.9
No. of observations	121,926	122,839	144,581	145,576
No. of unique reflections	22,075	22,725	24,237	24,439
Completeness (%)[*]	92.7 (95.6)	92.7 (95.4)	99.3 (98.3)	99.2 (98.6)
Mean I_o/σ	16.1 (3.5)	15.8 (3.4)	10.4 (2.7)	11.8 (3.0)
R_{sym}[a,*](%)	4.2 (44.9)	4.2 (46.6)	6.4 (62.1)	5.3 (52.9)
Refinement statistics				
R_{cryst}/R_{free} (%) [d]	16.6/20.1	17.3/21.0	16.2/20.5	16.6/20.9
R.m.s. deviations				
Bond length (Å)	0.022	0.022	0.021	0.023
Bond angle (°)	2.938	2.926	2.976	2.462
No. of atoms per asymmetric unit,	1930	1912	2017	2006
Average of B-factors				
Protein	21.7	27.8	17.0	16.5
Water	34.8	34.0	37.0	36.3
Heme/CO/Formate	25.5/25.9/28.1	31.6/26.4/33.2	19.4/19.9/21.3	18.9/18.3/21.8
Deposited PDB ID	4G7P	4G7T	4G7U	4G8P

[a] $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, $\langle I(hkl)\rangle$ is the mean intensity for multiple recorded reflections.

[d] $R_{cryst} = \Sigma|F_{obs}(hkl) - F_{calc}(hkl)| / \Sigma|F_{obs}(hkl)|$. R_{free} is the R_{cryst} calculated for the five percent of the dataset not included in the refinement.

[*] Values in parentheses are for the outermost shells (1.93-1.90 Å).

Table S3. Data collection and refinement statistics for O_2 photolysis datasets

Crystallographic data	Dark	Light
Space group		
Unit cell dimensions (Å)	a = b = 65.17, c = 120.64	
Diffraction statistics		
Laser illumination	dark	13 hr
Laser power density (mW/mm^2)	-	611
Resolution (Å)	30 – 2.1	30 – 2.4
No. of observations	61,412	41,076
No. of unique reflections	17,780	12,112
Completeness (%)[*]	99.0 (99.1)	98.7 (99.2)
Mean I_o/σ	10.7 (2.7)	9.4 (2.0)
R_{sym}[a,*](%)	6.0 (52.9)	8.0 (64.8)
Refinement statistics		
R_{cryst}/R_{free} (%) [d]	15.5/18.8	16.6/22.8
R.m.s. deviations		
Bond length (Å)	0.018	0.016
Bond angle (°)	2.397	2.223
No. of atoms per asymmetric unit,	1997	1896
Average of B-factors		
Protein	32.6	40.0
Water	49.2	58.2
Heme/O_2/Formate	31.2/35.1/43.4	41.3/28.0/50.0
Deposited PDB ID	4G8U	4G8W

[a] $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, $\langle I(hkl)\rangle$ is the mean intensity for multiple recorded reflections.

[d] $R_{cryst} = \Sigma|F_{obs}(hkl) - F_{calc}(hkl)| / \Sigma|F_{obs}(hkl)|$. R_{free} is the R_{cryst} calculated for the five percent of the dataset not included in the refinement.

[*] Values in parentheses are for the outermost shells (2.14-2.10 Å for dark data and 2.44-2.40 Å for light data, respectively).

Table S4. Data collection and refinement statistics of CO-bound form for temperature change

Crystallographic data		
Space group	$P3_221$	
Unit cell dimensions (Å)	a = b = 65.97, c = 120.02	
Diffraction statistics		
Warm-up temperature (K)	none	160
Resolution (Å)	50 - 2.3	50 - 2.3
No. of observations	86,086	85,845
No. of unique reflections	14,009	13,968
Completeness (%)*	99.8 (100)	99.8 (100)
Mean I_o/σ	13.2 (6.2)	11.2 (4.9)
R_{sym}[a,*](%)	6.8(38.2)	7.2 (44.3)
Refinement statistics		
R_{cryst}/R_{free} (%) [d]	15.3/21.8	16.5/22.4
R.m.s. deviations		
Bond length (Å)	0.017	0.023
Bond angle (°)	2.862	1.833
No. of atoms per asymmetric unit,	1944	1944
Average of B-factors		
Protein	26.7	34.1
Water	41.8	43.2
Heme/O_2/Formate	27.2/24.0/44.1	34.3/28.8/47.8
Deposited PDB ID	4G98	4G99

[a] $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, $\langle I(hkl)\rangle$ is the mean intensity for multiple recorded reflections.

[d] $R_{cryst} = \Sigma|F_{obs}(hkl) - F_{calc}(hkl)| / \Sigma|F_{obs}(hkl)|$. R_{free} is the R_{cryst} calculated for the five percent of the dataset not included in the refinement.

* Values in parentheses are for the outermost shells (2.34-2.30 Å).

Figure S1

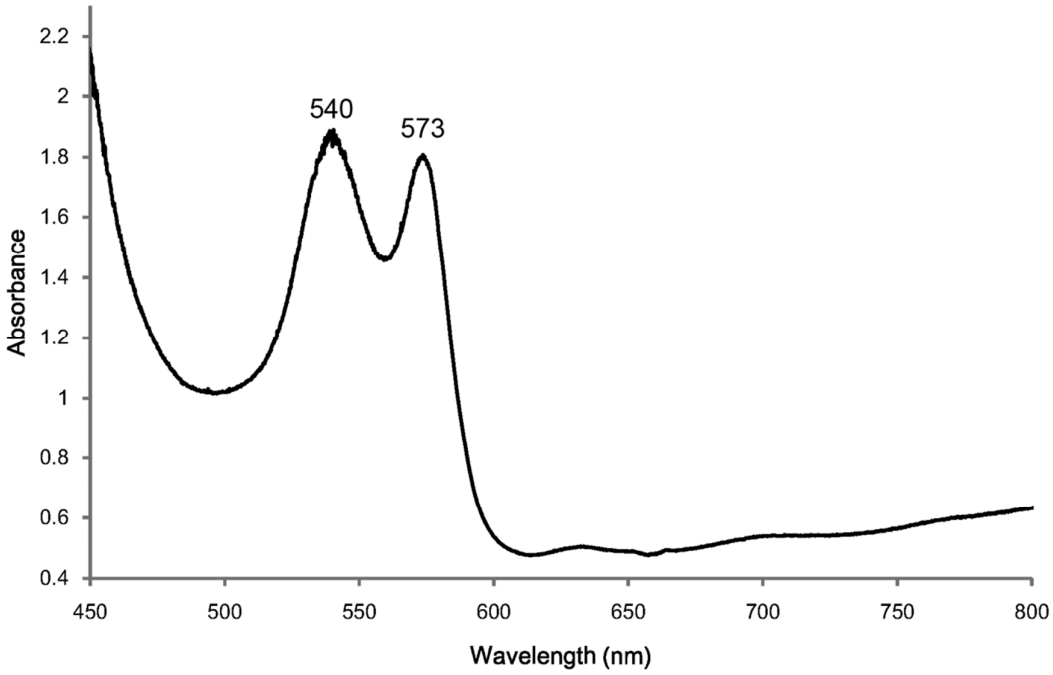
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
Absorbance
540
573
450
500
550
600
650
700
750
800
Wavelength (nm)

Figure S2

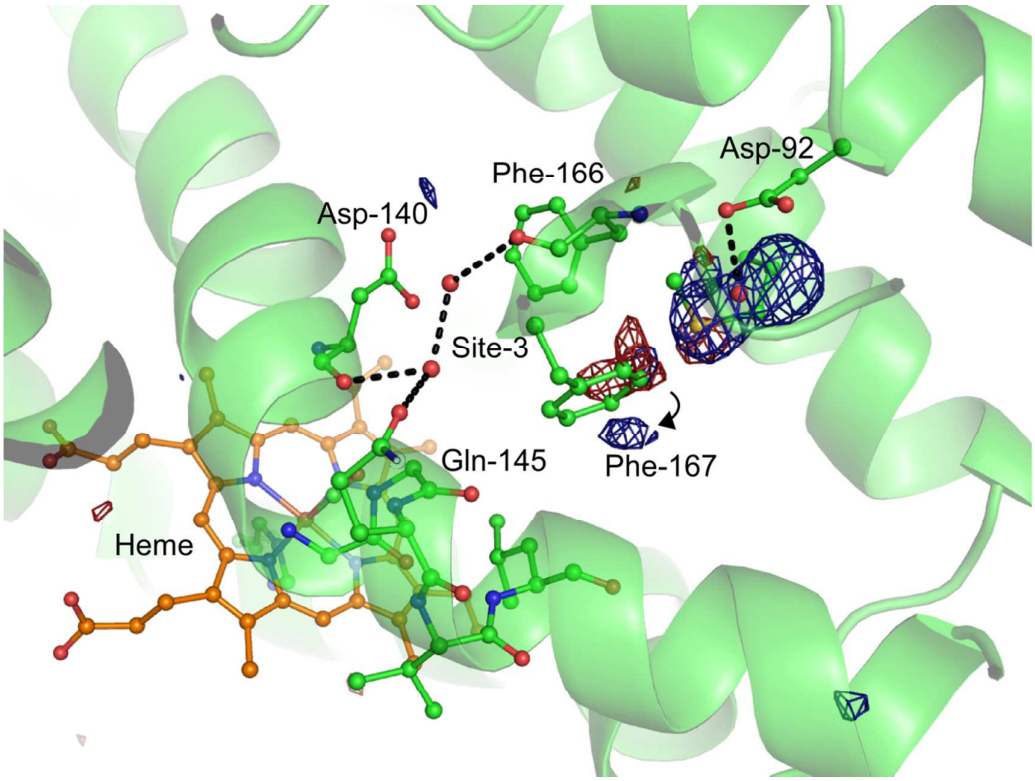

Figure S3

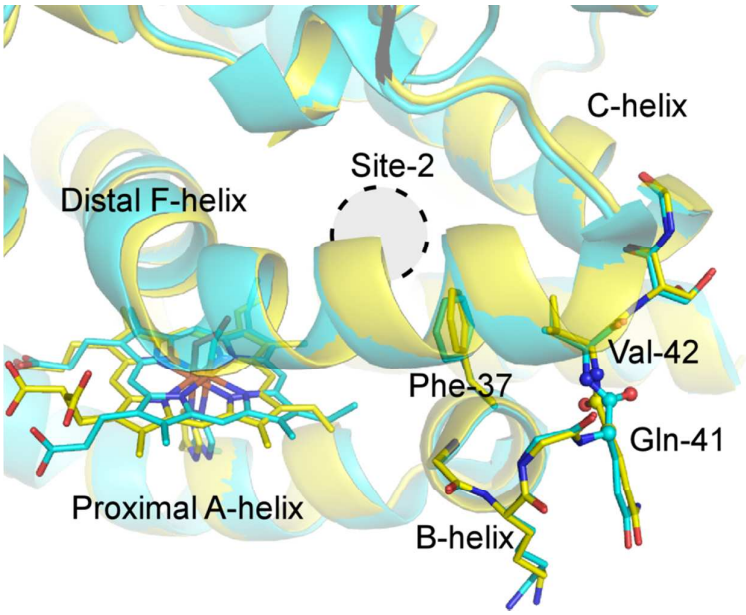
C-helix
Site-2
Distal F-helix
Val-42
Phe-37
Gln-41
Proximal A-helix
B-helix

Figure S4