

Evidence for Two Ferryl Species in Chloroperoxidase Compound II

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Supporting Information

Reference:

32. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03; Gaussian, Inc.: Wallingford CT, 2004.

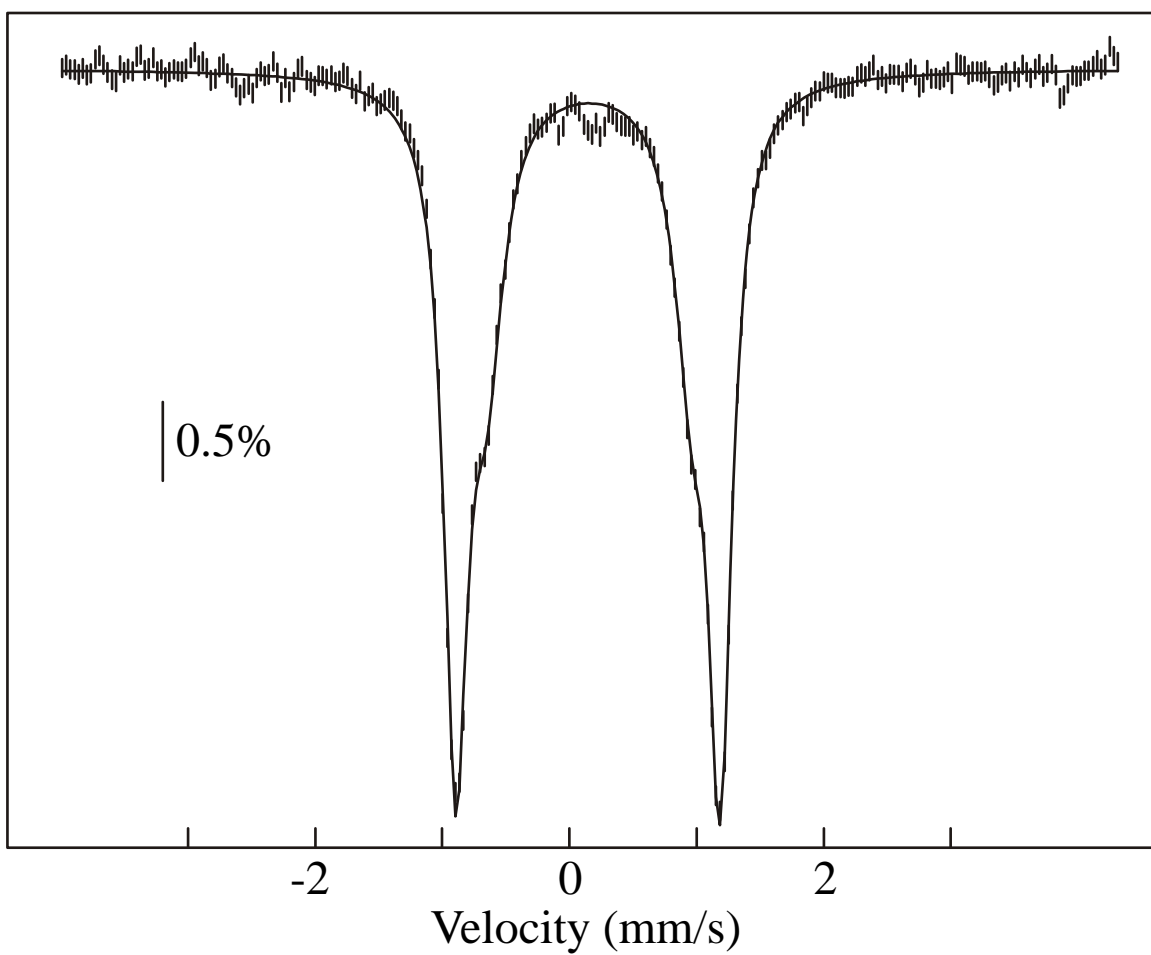
Legends for Supporting Figures and Table:

Figure S1: Mössbauer spectrum of CPO-II, pH 4.5, recorded at 4.2K in a 40mT magnetic field applied parallel to the γ beam. CPO-II was prepared in a rapid mixing experiment where ^{57}Fe -enriched CPO/ascorbate in 0.1M acetate, pH 4.5, (4mM and 37.5mM, respectively) was mixed with peracetic acid in 0.1M acetate, pH 4.5, (75mM) in a 2:1 ratio and quenched in a cold isopentane bath (-145°C) after 28ms. The spectrum shows raw data; solid line is best fit to two quadrupole doublets. The parameters for the protonated ferryl ($\Delta E_Q=2.08$ mm/s, $\delta=0.10$ mm/s, $\sim 66\%$) and the ferryl ($\Delta E_Q=1.58$ mm/s, $\delta=0.11$ mm/s, $\sim 34\%$) are similar to parameters measured for CPO-II at pH 6.5.

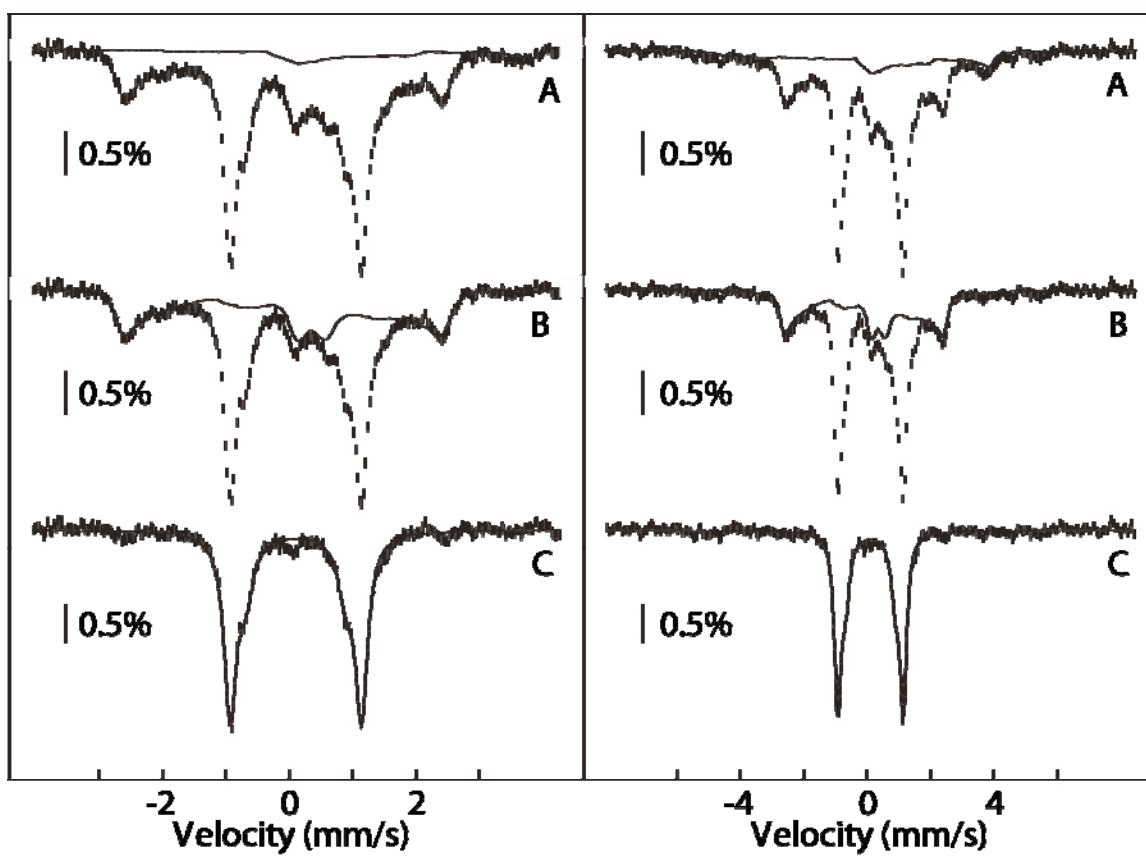
Figure S2: Mössbauer spectrum of CPO-II, pH 6.5, prepared with *p*-phenolsulfonic acid as the reductant. CPO-II was prepared in a rapid mixing experiment where ^{57}Fe -enriched CPO/*p*-phenolsulfonic acid (4mM and 37.5mM, respectively) in 0.1M KPhos, pH 6.5, was mixed in a 2:1 ratio with peracetic acid (75mM). The reaction was quenched in a cold isopentane bath (-145°C) after 5ms. Spectra were collected over a range of Doppler velocities of approximately ± 4 mm/s (left panel) or ± 8 mm/s (right panel). Removal of 20% of ferric CPO from the raw data (A) yields the spectra shown in (B). Removal of 35% of CPO-I yields the spectra shown in (C). These spectra have been simulated with two quadrupole doublets with the following parameters: $\delta(1) = 0.10$ mm/s, $\Delta E_Q(1) = 2.07$ mm/s, 32% of total intensity, and $\delta(2) = 0.10$ mm/s, $\Delta E_Q(2) = 1.58$ mm/s, 13% of total intensity. These parameters and the ratio of the relative intensities are similar to those observed for CPO-II with ascorbate as the reductant.

Figure S3: Mössbauer spectrum of CPO-II prepared with hydrogen peroxide as the oxidant. CPO-II was prepared in a rapid mixing experiment where ^{57}Fe -enriched CPO/ascorbate (4mM and 37.5mM, respectively) in 0.1M KPhos, pH 6.5 was reacted in a 2:1 ratio with hydrogen peroxide (75mM) and quenched in a cold isopentane bath (-140°C) after 28ms. The spectrum shows raw data; solid line is best fit to two quadrupole doublets. A) The sample contained 65% ferric CPO and was removed from the raw data. B) The remaining iron showed two doublets: 69% ($\Delta E_Q=2.08$ mm/s, $\delta=0.10$ mm/s) assigned as the protonated ferryl and 31% ($\Delta E_Q=1.58$ mm/s, $\delta=0.11$ mm/s) assigned as the ferryl. These parameters are similar to parameters measured for CPO-II with peracetic acid as the oxidant.

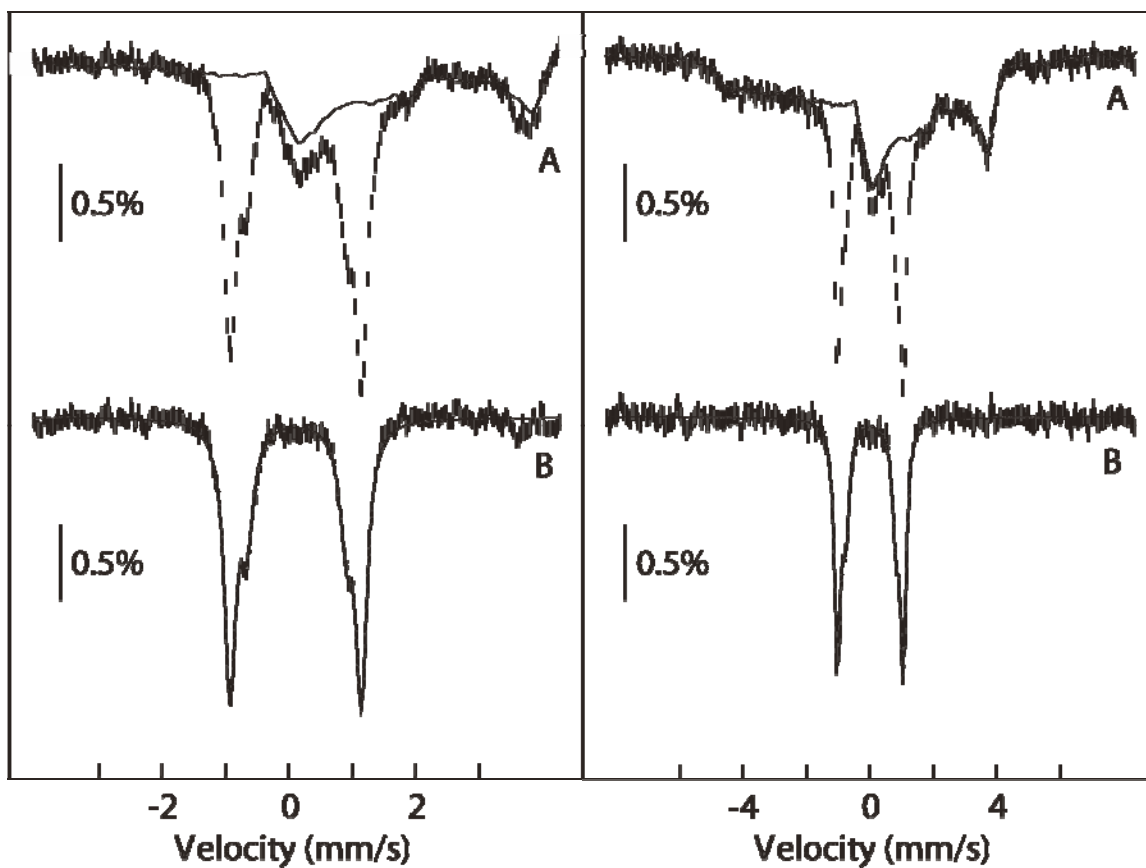
Table S1: Mössbauer parameters under various conditions for CPO-II formation.



Stone, et al. Figure S1



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Table S1: Mössbauer parameters for CPO-II

	Protonated Ferryl		Ferryl	
	$\delta(\text{mm/s})$	$\Delta E_Q(\text{mm/s})$	$\delta(\text{mm/s})$	$\Delta E_Q(\text{mm/s})$
PA/Ascorbate pH 4.5	0.10	2.08	0.10	1.61
PA/Ascorbate pH 6.5	0.10	2.06	0.11	1.59
Annealed CR-CPO-I	0.10	2.08	0.11	1.6
<i>p</i> -PSA/PA pH 6.5	0.10	2.07	0.10	1.58
H ₂ O ₂ /Ascorbate pH 6.5	0.10	2.08	0.11	1.58

Abbreviations: CPO-II: Chloroperoxidase compound II; PA: peracetic acid; CR-CPO-I: cryoreduced Chloroperoxidase compound I; *p*-PSA: *p*-phenolsulfonic acid

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