Combination of Cytochrome c Peroxidase and Carbon Monoxide

ANN M. ENGLISH*, DEVENDRA SHARMA and MARK WALSH

Department of Chemistry, Concordia University, 1455 de Maisonneuve Boulevard West, Montreal, Quebec, Canada H3G 1M8

Received August 25, 1983

Introduction

The kinetics of CO binding by heme and heme-substituted proteins has been the focus of recent attention [1, 2]. The CO combination rate has been correlated with both the electron-withdrawing power [1] and the bulkiness of the porphyrin side chains [2]. A comparison of the protein rate data with free heme data shows that the CO combination rates follow the reverse order in the absence of the protein [3]. This suggests that the protein structure has a considerable effect on the ligand-binding properties of the heme. Indeed, such an effect is evident from the combination rates of myoglobin (Mb) and horseradish peroxidase (HRP) with CO; although both enzymes contain a protoheme group coordinated to the protein via a histidine residue, their CO binding rate constants differ by two orders of magnitude (Table I).

Cytochrome c peroxidase (CCP), whose biological function is believed to involve the catalysis of ferrocytochrome c oxidation by H_2O_2 [4], also contains a protoheme group bonded to the protein via a histidine residue. Thus, a comparison of its CO combination rate with those of Mb and HRP is of interest, since this would indicate whether the peroxidases exhibit similar ligand-binding properties. We present below our results for the combination of CO with CCP and Mb. Because of the extreme photosensitivity of carboxyheme complexes, flash photolysis is used to generate the reactants *in situ*, and a probe beam monitors their recombination after the photolyzing flash.

Experimental

CCP was isolated from baker's yeast by the procedure of Nelson *et al.* [5]. Horse heart Mb was obtained from Sigma (type III). The proteins were transferred to sodium phosphate buffer (0.1 *M*, pH 7.0), and reduced with sodium dithionite. CO was bubbled into the protein solutions in 10-mm sealed cuvettes at a pressure of 1 atm, giving a CO concentration of 10^{-3} *M* [6]. The visible absorption spectra of the proteins were recorded on a Perkin Elmer 552 spectrophotometer. A coaxial flashlamp pumped dye laser (Model DL-2100C, Phase-R) was used to photolyse the samples. With rhodamine 6G in the dye cell, a 30-nsec, 500-mJ pulse with a maximum at 590 nm was obtained. The photoflash was screened from the detector by means of a $CuSO_4$ solution. Changes in the Soret region of the proteins following the flash were monitored using a 250-W quartz tungsten-halogen lamp. A grating monochromator was placed between the sample and the photomultiplier, the output of which was displayed on a storage oscilloscope and recorded photographically. Transmittance changes were measured at the absorbance maxima of both the reduced and carboxy forms of the proteins to ascertain the wavelength dependence of the recombination rates.

TABLE I. Recombination Rate Constants for P(II) + CO after Flash Photolysis.[a,b]

P	$k\ (M^{-1}\ s^{-1})$	Ref.
Mb	4.3×10^5	This work
Mb	$5.1-3.0 \times 10^5$	[1]
CCP	4.6×10^3	This work
HRP	5.7×10^3	[6]

[a]P(II) represents the Fe(II) form of the protein; Mb = myoglobin, CCP = cytochrome c peroxidase, HRP = horseradish peroxidase. [b]Experimental conditions: phosphate buffer, 0.1 *M*, pH 7.0, 20 °C.

Results and Discussion

The absorption spectra of carboxy- and ferroCCP have Soret maxima at 423 and 438 nm, respectively (Fig. 1). Thus, following the photoflash, an increase in absorbance is expected at 423 nm corresponding to the growth of the carboxy peroxidase, while the 438-nm peak should decrease due to the decay of the reduced enzyme. Pseudo first order kinetics are expected because of the excess CO present, and Fig. 2 shows the first order plot of the oscilloscope trace at 438 nm. A similar plot was obtained for the growth of the 423-nm signal. Table I gives the bimolecular rate constant obtained from this data,

*Author to whom correspondence should be addressed.

0020-1693/84/$3.00

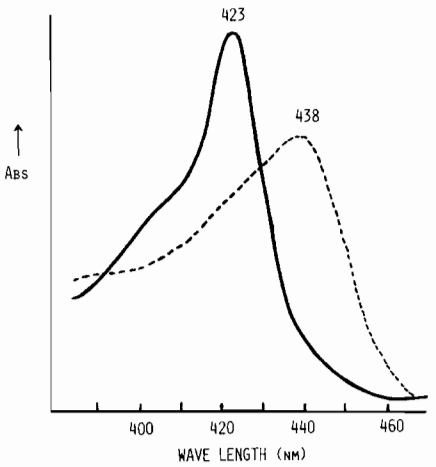

Fig. 1. Soret region of the absorption spectrum of dithionite-reduced cytochrome c peroxidase in the presence (solid line) and absence of CO (dotted line).

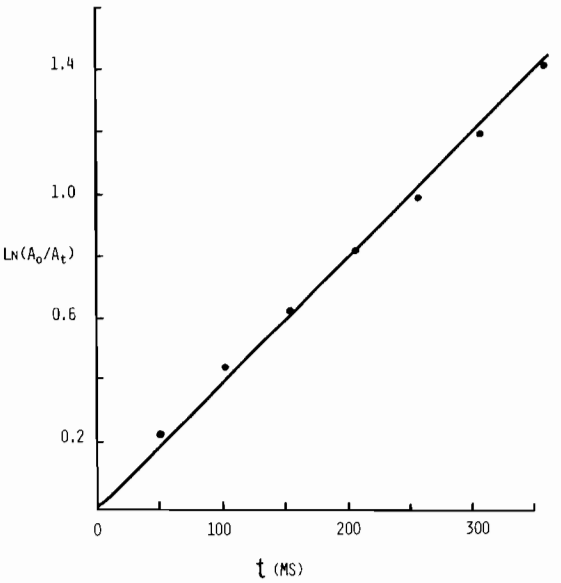

Fig. 2. Kinetic plot of the recombination of dithionite-reduced cytochrome c peroxidase (10^{-4} *M*) and CO (10^{-3} *M*) in phosphate buffer (0.1 *M*, pH 7.0, 20 °C). A_0 and A_t are the absorbance changes at 438 nm at times 0 and t ms, respectively (in the above plot, t = 0 corresponds to 50 ms after the photoflash).

and also our value for Mb which is in good agreement with the values obtained previously [1].

The literature value for the combination of HRP with CO [7] is also given in Table I. The rate constants for the combination of the two peroxidases with CO are almost identical. This suggests that the ligand-binding sites of the two proteins are somewhat similar, which is not surprising since peroxidase catalysis is believed to involve a number of invariant residues at the heme site [4]. Thus, the heme pockets should be similar in the two peroxidases, giving rise to similar electronic and steric effects on the CO recombination rates.

The rate constants extracted from the changes in the 423- and 438-nm signals agree within 10% (the expected experimental error). This suggests that only one process is occurring, *viz.*, the recombination of the peroxidase with CO following the photoflash. Hence, the photodissociated form of CCP is presumed to remain five-coordinate before recombination (as was presumed previously for Mb [3]), since no absorbance changes corresponding to the formation of an aquo ligand appear after photolysis.

Acknowledgements

This work was supported by NSERC Grant # A1530 and Concordia University.

References

1 (a) M. Sono, P. D. Smith, J. A. McCray and T. Asakura, *J. Biol. Chem., 251,* 1418 (1976);
(b) J. A. McCray, M. Sono and T. Asakura, in 'Porphyrin Chemistry Advances', P. R. Longo Ed., Ann Arbor Science, Ann Arbor, 1979, p. 205.
2 T. Iizuka, K. Watanabe, R. Makino, K. Sakaguchi, F. Mitani, Y. Ishimura, K. Kawabe, Z. Yoshida and H. Ogoshi, in 'Oxygenases and Oxygen Metabolism', M. Nozaki, S. Yamamoto and Y. Ishimura, Eds., Academic Press, New York, 1982, p. 445.
3 M. Sono, J. A. McCray and T. Asakura, *J. Biol. Chem., 252,* 7475 (1977).
4 T. L. Poulos and J. Kraut, *ibid., 255,* 8199 (1980), and references therein.
5 C. E. Nelson, E. V. Sitzman, C. H. Kang and E. Margoliash, *Anal. Biochem., 83,* 622 (1977).
6 A. J. Gordon and R. A. Ford, in 'The Chemist's Companion', Wiley–Interscience, New York, 1972, p. 37.
7 M. Brunori, E. Antonini, C. Phelps and G. Amiconi, *J. Mol. Biol., 44,* 563 (1969).