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SPECTRA OF CHLOROPEROXIDASE COMPOUNDS II AND III

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SUMMARY : Chloroperoxidase was present as Compound II during the peroxidatic oxidation of ascorbic acid. Compound III (oxy-form) was formed when excess hydrogen peroxide was added to Compound II. By decreasing the temperature it was possible to measure the spectra of Compounds II and III in the Soret and visible regions. Each spectrum was found to resemble that of the corresponding form of lactoperoxidase. Under the experimental conditions, chloroperoxidase Compound III was apparently converted to Compound II in parallel with the decomposition of hydrogen peroxide and finally to the ferric enzyme.

Since cytochrome P-450 was found to catalyze both oxidation and oxygenation at the expense of hydroperoxides (1), comparison between cytochrome P-450 and peroxidase has been an interesting subject (2). Of various peroxidases, chloroperoxidase is unique, particularly when compared with cytochrome P-450. Despite the failure to detect a free sulfhydryl group in chloroperoxidase (3), the extensive data on optical absorption (4), MCD (5), resonance Raman (6), Mössbauer (7) and X-ray absorption (8) spectroscopy support sulfhydryl ligation for the structure of chloroperoxidase, as well as cytochrome P-450. The most striking similarity is, however, the location of the Soret band of ferrous CO complexes for chloroperoxidase at 443 nm (4) and for cytochrome P-450 at 450 nm, as opposed to about 420 nm for most other protoheme proteins.

Like other peroxidases, chloroperoxidase gives a typical spectrum of Compound I upon reaction with hydroperoxides and peracids (9). The spectrum indicates the formation of a porphyrin π-cation radical (10, 11). On the

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other hand, the product of the reaction of cytochrome P-450 with hydroperoxides differs from Compound I in the optical absorption spectrum and is regarded as a molecular complex of the two species (12). To clarify the relation between cytochrome P-450 and chloroperoxidase from the point of view of the structure and function it seems also necessary to compare the optical absorption spectra of Compound II (ferryl form) and Compound III (oxy-form). The spectrum of oxy-form has been measured for cytochrome P-450 (13-17), but not for chloroperoxidase. The spectrum of ferryl form is reported for cytochrome P-450 (18) and chloroperoxidase (19), but the data are not sufficient to compare the two spectra. In this paper, we show optical absorption spectra of Compounds II and III of chloroperoxidase and conclude that these spectra resemble those of peroxidases but not of cytochrome P-450.

MATERIALS AND METHODS

Chloroperoxidase was purchased from Sigma (c-2387) and the RZ value (Ratio of $A_{403\ nm}$ to $A_{280\ nm}$) of our enzyme used in this experiment was 1.05 in 50 mM sodium acetate (pH 4.0). Reactions were carried out in 50 mM sodium acetate and at 5°C. The spectrophotometer used was Shimadzu UV 300.

RESULTS AND DISCUSSION

The spectrum of chloroperoxidase Compound II was reported by Thomas et al (19). The spectrum was measured only in the Soret region during the steady state of the peroxidation of ascorbate at pH 4.6. The spectrum was reported to be stable for about 30 s and showed that chloroperoxidase existed as a mixture of Compound II with Compound I or the ferric enzyme. In a similar reaction system containing ascorbate and hydrogen peroxide, we could measure the spectrum of pure Compound II of chloroperoxidase, not only in the Soret region but also in the visible region (Fig. 1). To slow down the reaction we carried it out at pH 5.6 and at 5°C. The spectrum was stable during the reaction, indicating that under such a condition the reduction of Compound II by ascorbate was rate-limiting as reported in the horseradish peroxidase reaction (20). The spectrum of chloroperoxidase Compound II resembled the spectra of Compounds II of horseradish peroxidase, lactoperoxidase and intestine peroxidase (21, 22). Particularly, it should be noted that the

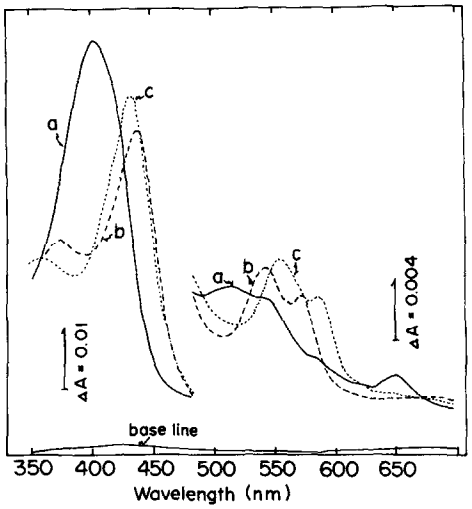

Figure 1. Absorption spectra of chloroperoxidase Compounds II and III. a, 0.71 μM chloroperoxidase, pH 5.6. b(Compound II), 500 μM ascorbate and 170 μM H_2O_2 were added to a. c(Compound III), 1.4 mM H_2O_2 was added to b. The experimental conditions are as described in MATERIALS AND METHODS.

wavelengths of the three main bands of chloroperoxidase, lactoperoxidase and intestine peroxidase resembled each other (Table 1).

It is well known that peroxidase Compound II is converted to Compound III in the presence of excess hydrogen peroxide. Figure 1 shows also that the same conversion occurred in the reaction of chloroperoxidase Compound II with hydrogen peroxide. The product was identified as Compound III from the characteristic visible spectrum of this species. The similarity of the

Table 1

Absorption bands (nm) of peroxidase Compounds II and III

	Compound II	Compound III (oxy-form)	Reference
Chloroperoxidase	438, 542, 571	432, 555, 586	This paper
Lactoperoxidase	433, 537, 568	428, 551, 590	(21)
Intestine peroxidase	436, 538, 565	430, 553, 590	(21)
Horseradish peroxidase	420, 527, 555	418, 546, 583	(22)
Cytochrome $P\text{-}450_{cam}$		418, 555	(14)
Cytochrome $P\text{-}450_{LM}$		418, 555	(17)

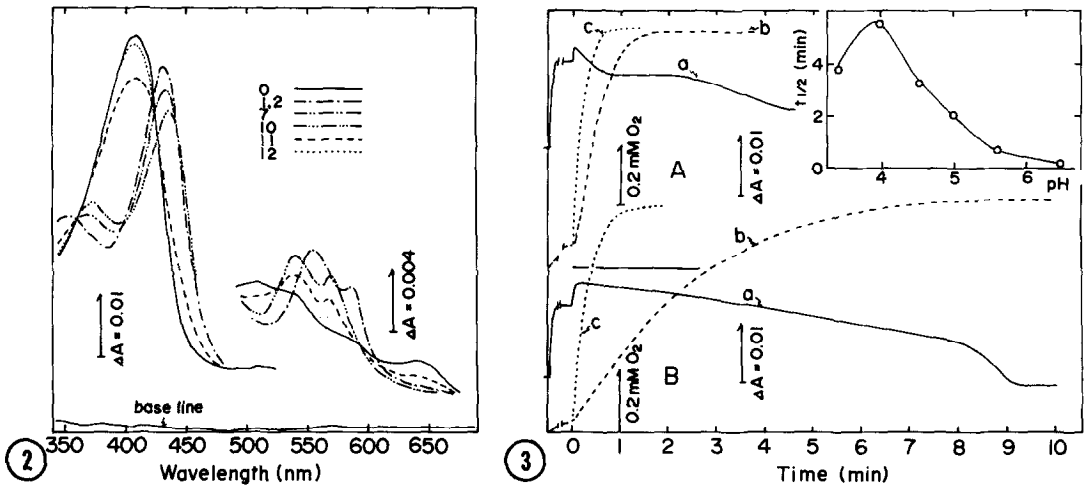

Figure 2. Decomposition of chloroperoxidase Compound III. The spectra were scanned every 55 s from 700 nm at a speed of 600 nm/min, after 1.4 mM H_2O_2 was added to Compound II. Numerals in the figure denote the scanning number. No. 0, 0.75 μM chloroperoxidase at pH 4.0. The change from No. 1 to 10 corresponds to the conversion from Compound III to Compound II and No. 11 denotes an intermediate spectrum during the conversion from Compound II to the ferric enzyme.

Figure 3. Time courses of absorbance at 341 nm and O_2 evolution. At time 0, 1.4 mM H_2O_2 was added to 0.71 μM chloroperoxidase Compound II at pH 6.5 (A) and 4.0 (B). Compound II was formed as described in Fig. 1. a, traces of absorbance at 314 nm and b, traces of O_2 concentration. Curves c denote the O_2 evolution when 1.4 mM H_2O_2 was added to 0.71 μM chloroperoxidase (ferric). The inset shows the pH dependence of half time of the conversion from Compound III to Compound II.

spectrum of chloroperoxidase Compound III with those of Compounds III of lactoperoxidase and intestine peroxidase (21) is remarkable (Table I). It was unstable and converted into the ferric form via Compound II under the experimental conditions (Fig. 2). The half time of the conversion from Compound III to Compound II depended on pH and Compound III was most stable at about pH 4 (Fig. 3). However, it was found that the decomposition rate measured in Fig. 3 did not directly reflect the stability of Compound III itself. The conversion of Compound III to Compound II occurred in parallel with the decomposition of hydrogen peroxide (Fig. 3). This decomposition of hydrogen peroxide was much slower than the catalatic activity of chloroperoxidase reported by Thomas et al. (23), as demonstrated in Fig. 3. As expected, the disappearance of Compound III was accelerated by catalase. The conversion of Compound II to the ferric enzyme was seen after hydrogen

peroxide had almost disappeared (Fig. 3), and was attributed to the reduction of Compound II by ascorbate. Figure 3 shows that the reduction was faster at pH 4.0 than at pH 6.5. Compound III was not formed when m-chloroperbenzoic acid was added to chloroperoxidase Compound II.

The restoration of the spectrum was more than 96% after the decomposition of Compound III (Fig. 2), which shows that Compound III was not a derivative of denatured chloroperoxidase. This conclusion was also supported by the experiment of Fig. 4, which shows that the characteristic spectrum of CO complex of reduced chloroperoxidase could be observed upon the addition of dithionite to Compound III in a CO-saturated solution.

From the present result we conclude that chloroperoxidase and cytochrome P-450 give similar spectra for their ferrous CO complexes but different spectra for their oxy-forms. In this respect it is interesting to note a recent report (24) on spectroscopic similarities between thiol-binding forms of the two hemoproteins. As regards Compound II, the

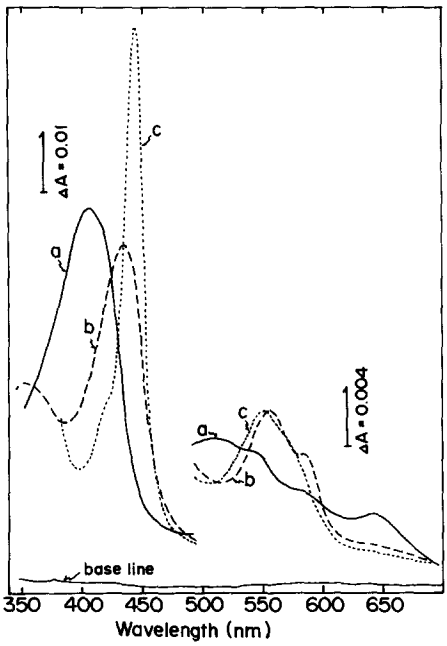

Figure 4. Conversion of Compound III to CO complex of reduced chloroperoxidase by the addition of dithionite. Compound III (b) was formed as described in Fig. 1 after an 0.7 μM chloroperoxidase (a) solution was saturated with CO at pH 4.0. c, a few crystals of sodium dithionite were added to b. The absorption peaks of spectrum c were at 445 and 550 nm.

similarity of the two hemoproteins cannot be discussed because the information on the ferryl form of cytochrome P-450 is obscure.

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