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COMPOUND I OF MYELOPEROXIDASE

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SUMMARY

The optical spectrum of the primary peroxide compound of myeloperoxidase (compound I) is reported. The spectrum, obtained in 1 msec after mixing native ferric myeloperoxidase with excess hydrogen peroxide, exhibits a Soret maximum at 425 nm (ε = 52 mM^{-1} cm^{-1}) and an increase in extinction of the ferric peroxidase at wavelengths higher than 607 nm. The spectrum suggests a structure for compound I of myeloperoxidase similar to those of horseradish peroxidase and catalase. Compound I spontaneously decays to the secondary compound (compound II) in a half time of $\simeq$ 100 msec. The role of compound I in chloride peroxidation is discussed.

INTRODUCTION

Myeloperoxidase is the brown-green heme protein of mammalian polymorphonuclear leukocytes which functions in the phagocytic process to contribute to the killing of invasive microorganisms (1). The peroxidase, molecular weight 140,000 contains two hemes which are distinct from protoheme (2,3,4) and optically similar to heme a (5,6). Previous studies have shown that myeloperoxidase is uniquely capable of catalysing the oxidation of chloride ion to HOCl at the expense of H_2O_2 (7,8), a capability which can account both for the antimicrobial activity of the enzyme (7,9,10) and the various chlorination reactions reported by a number of groups (7,11,12).

Previous studies on the mechanism of chloride peroxidation by myeloperoxidase have led to the conclusion that it is an unstable primary peroxide compound which alone represents the oxidizing side of the chloride peroxidation cycle (13) as opposed to the more stable secondary compound studied and implicated earlier (14). The primary compound, which was first reported by Chance (15),

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was subsequently found to form (at pH 8.6) at the expense of either hydrogen peroxide or hypochlorous acid and to decay rapidly to the secondary peroxide compound (13). However, while these optical data were compatible with the initial formation of a compound I-like species, the full optical properties of the unstable primary compound have not been obtained in detail.

We have now obtained optical data on myeloperoxidase compound I using a stopped flow/rapid scan instrument. The optical properties of the compound are broadly parallel to those obtained with other peroxidases, suggesting that the structure of the compound is similar to those of catalase and horseradish peroxidase.

MATERIALS AND METHODS

Canine myeloperoxidase was isolated as previously described (3). The peroxidase utilized in this study exhibited an RZ (A_{430nm}/A_{280nm}) of 0.82. The preparation was diluted to the appropriate concentration with phosphate buffer pH 7.0 for stopped-flow/rapid scan experiments. Hydrogen peroxide was standardized by the oxidation of iodide ion to I_3^- using horseradish peroxidase as catalyst (16).

Stopped-flow/rapid scan observations were made using a Union Giken Rapid Reaction Analyser (Model RA601). For observations in the Soret region ferric myeloperoxidase (9.9 uM in heme) was mixed with 384 uM H_2O_2 (concentrations after mixing: 4.95 uM heme, 192 uM H_2O_2) at 20° and the data obtained in two 100 nm scan ranges overlapping near 390 nm. Each region was scanned in 1 msec immediately after the flow stopped. The observations were made similarly in the visible region in three overlapping scans. For this region the initial peroxidase (heme) concentration was adjusted to 50.2 uM (25.1 final) and the peroxide concentration raised to 576 uM (289 final). The different dilutions of stock peroxidase used for the Soret and visible regions resulted in slightly different pH values after mixing, 7.14 for the Soret region and 7.55 for the visible region.

To confirm the stability of the primary compound with respect to the time frame employed, each 100 nm scan was repeated in the next 1 msec interval after the initial scan (i.e. from 1 to 2 msec after flow stopped) and the spectra compared. For recording the spectrum of the native enzyme across the region reported, the peroxide solution was replaced with water and the spectra acquired in the same time interval for each 100 nm scan.

The formation of compound II from compound I was observed in later time intervals. A holmium oxide filter was used for wavelength calibration. A Cary 219 recording spectrophotometer was employed to estimate the concentration of myeloperoxidase used in the stopped flow work. Extinction coefficients are based on iron analysis (4) and are expressed on a heme basis. Absorbances reported are for the cell of the Union Giken instrument.

RESULTS

Full formation of the primary peroxide compound of 5 uM myeloperoxidase (compound I) required the 40 fold excess of H_2O_2 used to obtain the spectrum

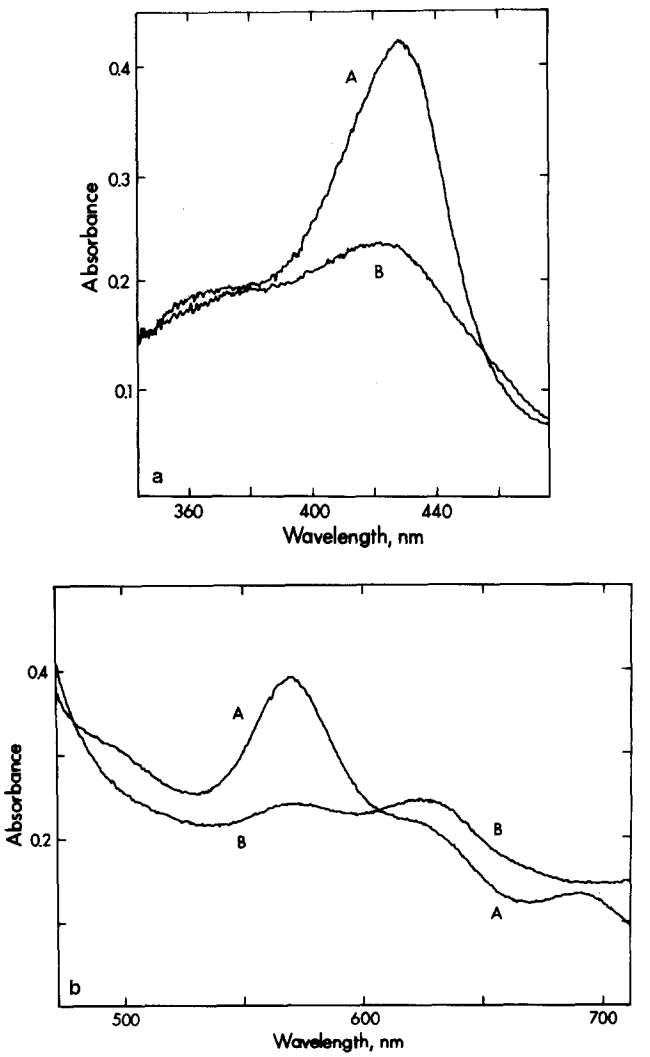

Figure 1.

a) Soret region spectra of native ferric myeloperoxidase (A) and myeloperoxidase compound I (B) formed at the expense of hydrogen peroxide. The final concentration of myeloperoxidase (heme) and hydrogen peroxide for the compound I spectrum were 4.95 uM and 192 uM. For further details see Materials and Methods.

b) Visible region spectra of native ferric myeloperoxidase (A) and compound I (B). The final concentration of myeloperoxidase (heme) and hydrogen peroxide for the compound I spectrum were 25.1 uM and 289 uM respectively.

in the Soret region. Lower excess led to incomplete compound I formation in an appropriate time, possibly due to the catalatic activity of the peroxidase.

Fig. 1 shows the optical spectrum obtained for the primary peroxide compound after mixing the peroxidase with a 40-fold excess of H_2O_2 and scanning each

100 nm spectral region in the first millisecond after flow had stopped (Materials and Methods). No significant change in spectrum was observed, in the second millisecond after flow stopped, at 455 nm, the wavelength most diagnostic of compound II. Formation of compound I from ferric peroxidase was isosbestic at 456 nm, and involved a decrease in Soret extinction of some 45%, from 95 mM^{-1} cm^{-1} to 52 mM^{-1} cm^{-1} at 430 nm.

In the visible region (Fig. 6) the characteristic 3 band spectrum of the ferric peroxidase (571 nm, ε =15.9 mM^{-1} cm^{-1}; 620 nm, ε =8.9 $mM^{-1}cm^{-1}$; 689 nm, ε = 5.4 mM^{-1} cm^{-1}) is replaced with a two band spectrum (572 nm and 625 nm, ε = 9.7 mM^{-1} cm^{-1} and 9.9 mM^{-1} cm^{-1} respectively) with extinction increasing at all wavelengths higher than 607 nm. In addition the weak band at 496 nm in the spectrum of the ferric peroxidase is abolished. Experiments in the visible region which utilized half the peroxide concentration reported gave identical results.

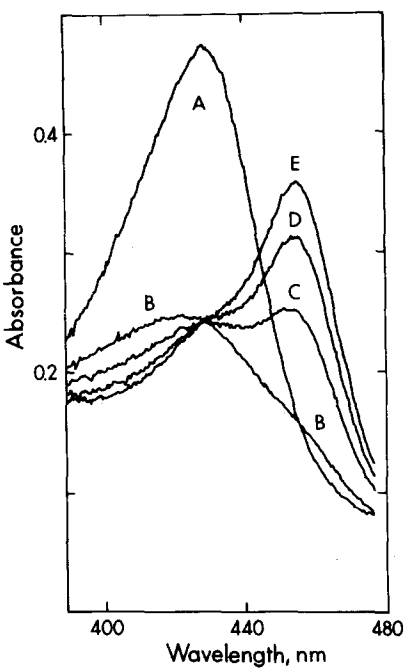

Figure 2.
Spectral changes in the Soret region accompanying the formation of compound I (scan B) from ferric peroxidase (scan A) and the decomposition of compound I to compound II (scans C,D, and E). Scans a and b were obtained as described in Materials and Methods, scans C,D, and E in time intervals 30-31 msec, 70-71 msec and 130-131 msec respectively. The concentrations of the peroxidase (heme) and H_2O_2 after mixing were 5.05 uM and 288 uM respectively.

The primary peroxide compound gave rise to the stable secondary compound (compound II) exhibiting a Soret maximum at 455 nm with a half time of the order of 0.1 sec (Fig. 2). This compound is easily prepared and observed in a conventional time scale (12,14,17). The compound I to compound II transition was isosbestic at 429 nm.

DISCUSSION

The optical spectrum of the primary peroxide compound of myeloperoxidase reported here confirms and extends two previous reports (13,15) but is somewhat at variance with the only other full spectrum reported (17). The data show that the hemes of myeloperoxidase react with hydrogen peroxide to form a compound exhibiting optical properties broadly similar to those of other compounds I (18,19,20,21). That is, despite the fact that the prosthetic groups of myeloperoxidase are not heme IX, formation of the primary peroxide compound still produces the broad diminution in Soret intensity characteristic of the primary peroxide compounds of horseradish peroxidase (18,19) and catalase (20,21).

It has been suggested that the optical properties of compounds I may result from the one equivalent oxidation of the porphyrin moiety to a cation radical (22), while the second oxidizing equivalent of these compounds resides in the ferryl iron centre. It will be of interest to determine whether a radical can be detected in myeloperoxidase compound I as has proved to be the case in horseradish peroxidase compound I (23,24).

Previous studies have shown that the primary peroxide compound of myeloperoxidase forms rapidly at the expense of the physiological product of chloride peroxidation (HOCl) at pH 8.6 (13). If, as is the case with the chloroperoxidase-peracid system (25), the oxygen of HOCl is alone retained in compound I of myeloperoxidase, then we can assume that transfer of oxygen from compound I to HCl (or $H^+ + Cl^-$), the reverse process, is the mechanism whereby the HOCl generating cycle is completed (8). This implies that the ferric peroxidase/compound I couple functions as an oxygen transferring system (8).

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