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THE HALIDE COMPLEXES OF MYELOPEROXIDASE AND THE MECHANISM OF THE HALOGENATION REACTIONS

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Summary

The spectral changes caused by the addition of halides to myeloperoxidase (donor:hydrogen-peroxide oxidoreductase, EC 1.11.1.7) have been investigated and the dissociation constants of the enzyme-halide complexes have been determined. The pH dependence of the dissociation constants suggests that halide binding is associated with a protonation step in myeloperoxidase.

Myeloperoxidase catalyzes the peroxidative chlorination and bromination of monochlorodimedone. It is shown that at low pH, chloride acts as a competitive inhibitor with respect to H_2O_2, whereas at higher pH, H_2O_2 inhibits the chlorination reaction. The dissociation constant (K_d) of the spectroscopically detectable complex and the K_m for chloride are considerably smaller than the inhibition constant (K_i) for chloride.

These halogenation reactions are strongly pH dependent, the logarithm of the K_m for chloride varies linearly with pH. The position of the pH optimum of the chlorination and bromination reaction is a linear function of the logarithm of the [halide]/[H_2O_2] ratio. A mechanism of the chlorination and bromination reaction is suggested with substrate inhibition for both hydrogen peroxide and the halide.

Introduction

Myeloperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) the peroxidase present in human granulocytes, is considered to function in the killing of bacteria and fungi [1]. As proposed by Agner [2] in 1958 and later confirmed by him [3] and others [4,5], the bactericidal activity of the peroxidase is due to its capacity to oxidize chloride, bromide and iodide in the presence of hydrogen peroxide. The products formed in these reactions are able to halogenate bacteria, proteins, DNA, NADH and ATP [6]. These halogenation reactions may lead to irreversible damage of the organism and subsequent cell-death, presumably by decarboxylation of amino acids and by oxidative peptide cleavage [7—9]. The first product of the oxidation of Cl^- by H_2O_2 and myeloperoxidase is probably HOCl which can react with another H_2O_2 molecule to form the bactericidal singlet oxygen [10]. However, in vivo HOCl will be so rapidly scavenged by biological materials that $^1O_2$ formation will not play a major role in the killing process. Since probably HOCl is the pertinent bactericidal product, we have studied in detail the interaction of halides with myeloperoxidase and the halogenation reactions of monochlorodimedone catalyzed by the enzyme.

Materials and Methods

Myeloperoxidase was obtained from human granulocytes as previously described [11]. The absorbance ratio ($A_{430\text{nm}}/A_{280\text{nm}}$) of the enzyme used was 0.7. The halogenating activity of myeloperoxidase was measured in the assay system for chloroperoxidase as described by Hager et al. [12]. Monochlorodimedone was synthesized by chlorination of dimedon [12]. H_2O_2 solutions were freshly prepared by dilution of a 30% stock solution (Merck). The stock solution was periodically standardized by the MnO_4^- method [13]. All other chemicals were of analytical grade.

The stopped-flow experiments were carried out on a Durrum stopped-flow spectrophotometer. The changes in light transmittance with respect to time were recorded on a Datalab transient-recorder. Difference spectra were measured on a Aminco-Chance DW-2 spectrophotometer; all other spectrophotometric measurements were carried out on a Cary-17 recording spectrophotometer.

Results

Reaction of myeloperoxidase with halides

The halides fluoride, chloride, bromide and iodide all react with myeloperoxidase to spectrally distinguishable complexes. Fluoride shifts the Soret maximum from 428 nm 7 nm towards the red, whereas chloride, bromide and iodide shift the position of the Soret peak to 434 nm, 432 nm and 432 nm, respectively. Fig. 1 illustrates the difference spectra of myeloperoxidase plus halides minus free myeloperoxidase at pH 5.0 with the four halides present in great excess. A peak at 446—448 nm and a trough at 421—427 nm was induced by all halides and in the case of chloride and bromide a shoulder at 408—410 nm is present. The extent of the halide-induced absorbance change in the enzyme-fluoride and enzyme-chloride complexes is nearly pH independent in the range 3.8—7.0 whereas both the bromide and iodide complexes show an optimum at pH 5.5—6.0.

From the absorbance difference between myeloperoxidase and the enzyme-halide complexes in the Soret region, the degree of saturation α can be determined at each halide concentration. The dissociation constant (K_d) of the

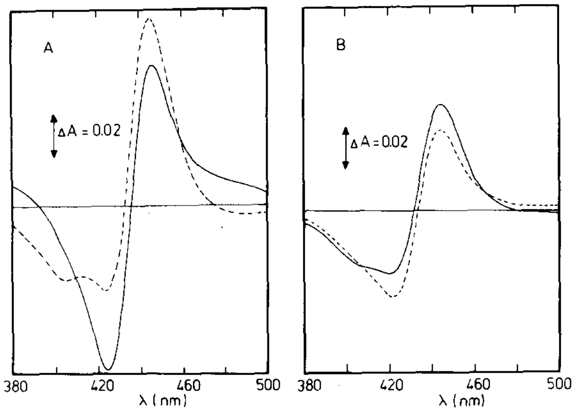


Fig. 1. Difference spectra of myeloperoxidase plus halides minus myeloperoxidase in the Soret region. 4.3 μM myeloperoxidase in 100 mM sodium acetate (pH 5.0). (A) ———, 100 mM NaF; - - - - - -, 100 mM NaCl. (B) ———, 100 mM NaBr; - - - - - -, 100 mM NaI.

enzyme-halide complex can be obtained from a Hill plot in which $\log \alpha/1 - \alpha$ is plotted against the logarithm of the ligand concentration. Fig. 2 shows that for each halide a straight line ($n = 1$) is observed from which the dissociation constant can be calculated. Fig. 2 illustrates also that at pH 5.0 the affinity of the halides for myeloperoxidase decreases in the order Cl^-, F^-, I^-, Br^-. Fig. 3 shows the pH dependence of the dissociation constants (K_d) of the various enzyme-halide complexes. It is evident that for fluoride and chloride a linear relationship exists between the logarithm of the K_d and the pH with a slope of 0.9—1.0. However, for iodide and bromide the slope of the line is less, being 0.6 and 0.8, respectively.

The rate of formation of the enzyme-halide complexes was studied for chloride with a stopped-flow spectrophotometer. However, under the conditions used, the reaction was too fast to be followed by our instrument. Therefore, the rate of dissociation was studied by means of a pH jump technique.

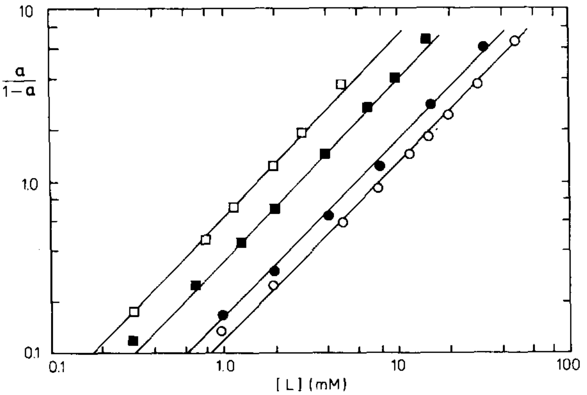


Fig. 2. Hill plot of the myeloperoxidase-halide complexes. The saturation degree α was calculated from the absorbance difference between the halide-induced peak at 446—448 nm and trough at 421—427 nm. 3.2 μM myeloperoxidase in 100 mM sodium acetate (pH 5.0). □———□, KCl; ■———■, KF; ○———○, KBr; ●———●, KI.

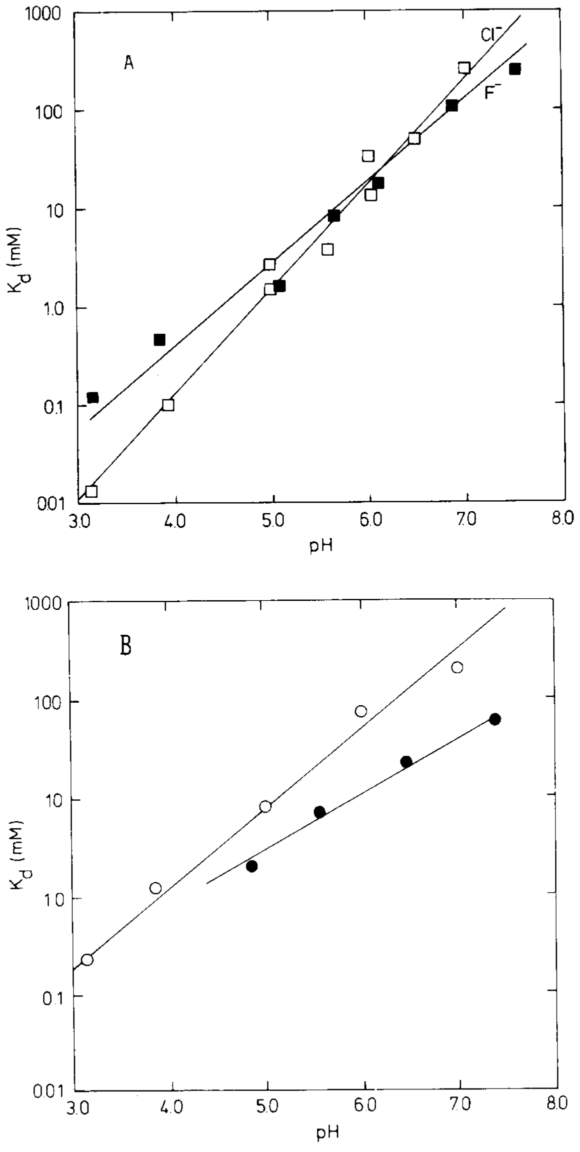
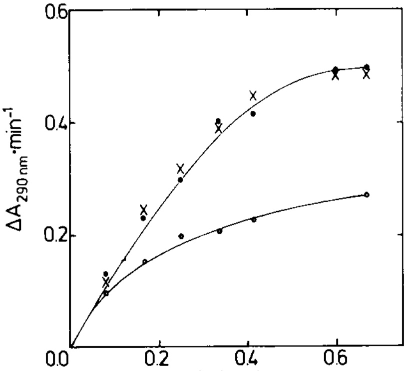


Fig. 3. pH dependence of the dissociation constants of the myeloperoxidase-halide complex. The dissociation constants were calculated from Hill plots of the halide-induced spectral changes. 3—4 μM myeloperoxidase, 50 mM sodium citrate buffer (pH 3.2), 50 mM sodium acetate (pH 3.8—6.0) and 50 mM sodium phosphate (pH 6.0—7.5). (A) □——□, KCl; ■——■, KF. (B) ○——○, KBr; ●——●, KI.

Because the K_d is strongly pH dependent and the halides bind reversibly to myeloperoxidase, it is possible to study the kinetics of the dissociation reaction by mixing suddenly in a stopped-flow spectrophotometer a solution containing the enzyme-halide complex at low pH with an equal volume of Tris-SO_4 buffer at high pH. The dissociation reaction of the myeloperoxidase-chloride complex induced by raising the pH from 4.0 to 7.3 is first-order and the measured dissociation rate constant (k_{-1}) of 300 s^{-1} is independent of the Cl^- concentration (1—10 mM). Since the dissociation constant at pH 7.3 is 0.5 M (cf. Fig. 3) and equals k_{-1}/k_1, it is possible to calculate a value of $6 \cdot 10^2$ $M^{-1} \cdot s^{-1}$ for the on constant k_1. A similar experiment in which the pH was raised from 3.2 to 5.5 yields an off constant (k_{-1}) of 231 s^{-1} and a calculated on constant (k_1) of $3.4 \cdot 10^4$ $M^{-1} \cdot s^{-1}$. This demonstrates that the increase of the value of the dissociation constant upon increasing the pH is mainly due to a pH effect on the on constant rather than on the off constant. It is interesting to note that the value of the on constant at pH 5.5 is of the same magnitude as that reported for chloroperoxidase [14].

Enzymatic halogenation of monochlorodimedone

Incubation of myeloperoxidase with H_2O_2 and chloride together with monochlorodimedone results in a rapid decrease in the absorbance of monochlorodimedone at 290 nm, due to the formation of dichlorodimedone [12]. The sequence of adding substrates and enzyme into the cuvette affects the observed reaction rate (Fig. 4). When the reaction was started with either myeloperoxidase or hydrogen peroxide the chlorination rate did not differ, but when chloride was added to the cuvette in which enzyme and hydrogen peroxide were already present for 10 s a two times lower rate was obtained (Fig. 4). This confirms the observations of Harrison [15] that H_2O_2 in the absence of chloride inactivates myeloperoxidase. Therefore, all halogenation reactions described were initiated by addition of myeloperoxidase. The rate of the chlorination reaction is proportional to the amount of enzyme present and independent of monochlorodimedone concentration from 10 to 100 μM (not



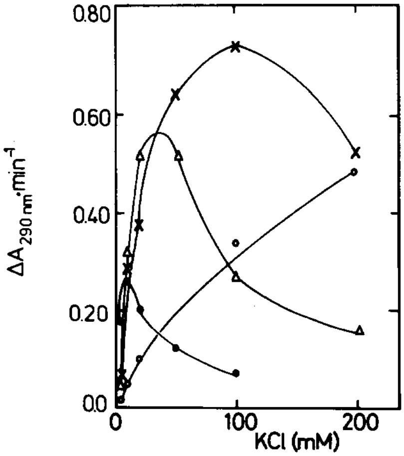


Fig. 4. Chlorination of monochlorodimedon catalyzed by myeloperoxidase. the reaction mixture contained KCl, 0.1 M $NaCH_3COO$ (pH 4.6), 0.2 M Na_2SO_4, 16.7 μM monochlorodimedone, 10 nM myeloperoxidase. H_2O_2 as indicated in the figure. The reaction was started by either myeloperoxidase (•——•), of H_2O_2 (X——X), or chloride (○——○). Chloride was added 10 s after addition of H_2O_2 to the cuvette. Temperature, 20°C.

Fig. 5. Effect of chloride concentration on the chlorination reaction at various pH values. The reaction was started by addition of myeloperoxidase (final concentration 10 nM) to the sample which contained 0.2 M Na_2SO_4, 0.1 M $NaCH_3COO$, 16.7 μM monochlorodimedon, 166 μM H_2O_2 and the indicated concentration of chloride. •——•, pH 4.0; △——△, pH 4.5; X——X, pH 5.0; ○——○, pH 5.5.

shown). This latter observation indicates that the affinity of the oxidation product of chloride for monochlorodimedone is high.

Fig. 5 illustrates the dependence of the chlorination rate on the chloride concentration at various pH values and a fixed amount of hydrogen peroxide (166 μM). It is evident that particularly at low pH, excess of chloride inhibits the reaction. This is more clearly illustrated in Fig. 6. The Lineweaver-Burk plot shows that at pH 4.5 the chloride ion is not only a substrate for myeloperoxidase but also acts as a competitive inhibitor with respect to H_2O_2 in the chlorination reaction. The data from Fig. 6 replotted in a Dixon plot (not shown) show a common intercept from which an inhibition constant K_i of 14 mM can be obtained. Fig. 6 illustrates that the slopes of the lines increase linearly with the chloride concentration, in line with the competitive type of inhibition, and from a replot (not shown) of these slopes against the chloride

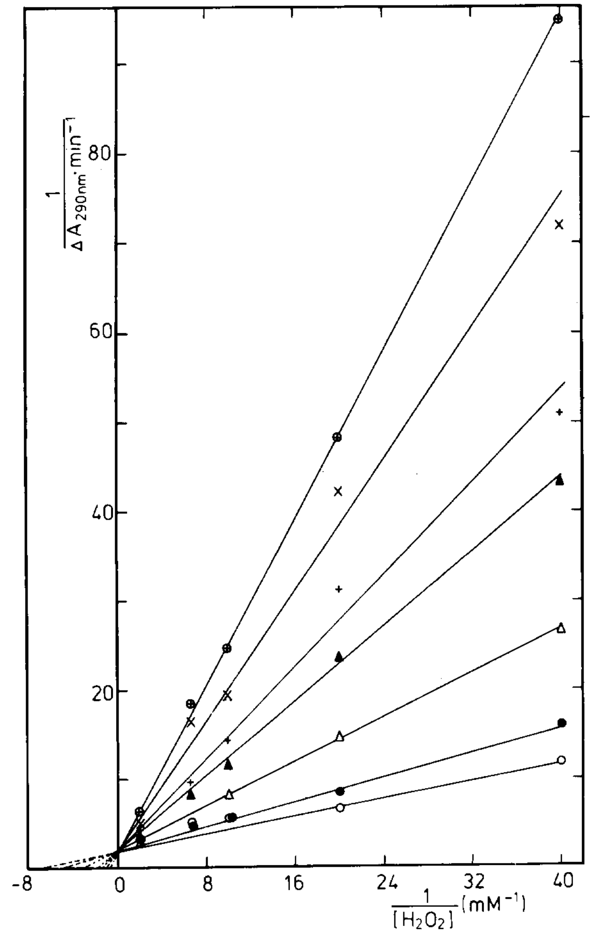


Fig. 6. Lineweaver-Burk plot of the chlorination of monochlorodimedon at various chloride concentrations. The enzymic reaction was started by the addition of myeloperoxidase to a final concentration of 10 nM. 100 mM $NaCH_3COO$ (pH 4.5), 0.2 M Na_2SO_4 and 16.7 μM monochlorodimedone; temperature, 20° C. ○———○, 10 mM NaCl; ●———●, 20 mM NaCl; △———△, 40 mM NaCl, ▲———▲, 80 mM NaCl; +———+, 100 mM NaCl; X———X, 150 mM NaCl; ⊕———⊕, 200 mM NaCl.

concentration it is possible to obtain the slope at zero concentration of chloride. When a line with this slope is replotted in Fig. 6 a Michaelis constant for H_2O_2 of 0.11 mM is obtained. The inhibition by chloride is not due to an effect of ionic strength on the chlorination reaction, since the experiments were carried out in 0.2 M Na_2SO_4 and an increase of the salt concentration had no effect on the chlorinating activity of the enyzme.

At pH 4.5 precise determination of the apparent Michaelis constant (K_m) for chloride at various H_2O_2 concentrations seems not to be possible. At low non-inhibitory chloride concentrations (0.4—3 mM) the rate of the chlorination reaction decreases rapidly and initial velocities are difficult to determine. Under these conditions the Lineweaver-Burk plots of the initial velocity versus the chloride concentration are hyperbolic concave up suggesting inhibition by H_2O_2 at low chloride concentrations. The apparent K_m for chloride can only be estimated from such a Lineweaver-Burk plot and is smaller than 2.5 mM at 20 μM H_2O_2 and increases approximately linearly with the H_2O_2 concentration to 11 mM at 166 μM H_2O_2. This K_m for chloride not only depends on the H_2O_2 concentration but varies strongly with pH, from 4 mM at pH 4.0 to 200 mM at pH 6.0, while the maximal velocity is much less affected. Fig. 7 shows that at a fixed H_2O_2 concentration the logarithm of this apparent K_m increases linearly ($n = 1.0$) with the pH.

Fig. 8 demonstrates that when the H_2O_2 concentration and the pH are varied at a fixed chloride concentration of 100 mM also H_2O_2 can act as an inhibitor of the chlorination reaction. In this case, however, at 100 mM chloride inhibition by H_2O_2 is seen only at higher pH. From the non-inhibited part of the curves the apparent K_m for H_2O_2 can be obtained and the value of this K_m increases when the concentration of chloride increases. Furthermore, the logarithm of

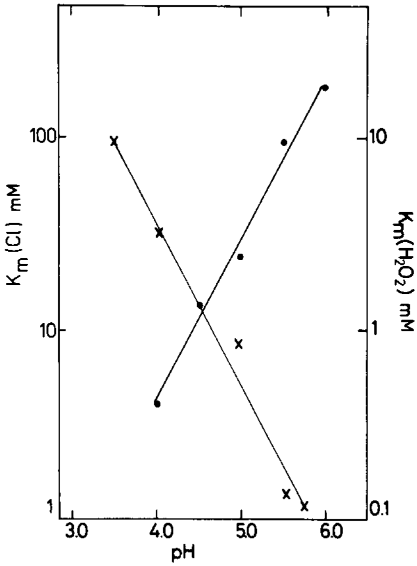


Fig. 7. pH dependence of the K_m values for chloride and H_2O_2 in the chlorination reaction of monochlorodimedone. The K_m values were calculated from Lineweaver-Burk plots by extrapolation of the non-inhibited part. ○———○, apparent K_m for chloride; X———X, apparent K_m for H_2O_2.

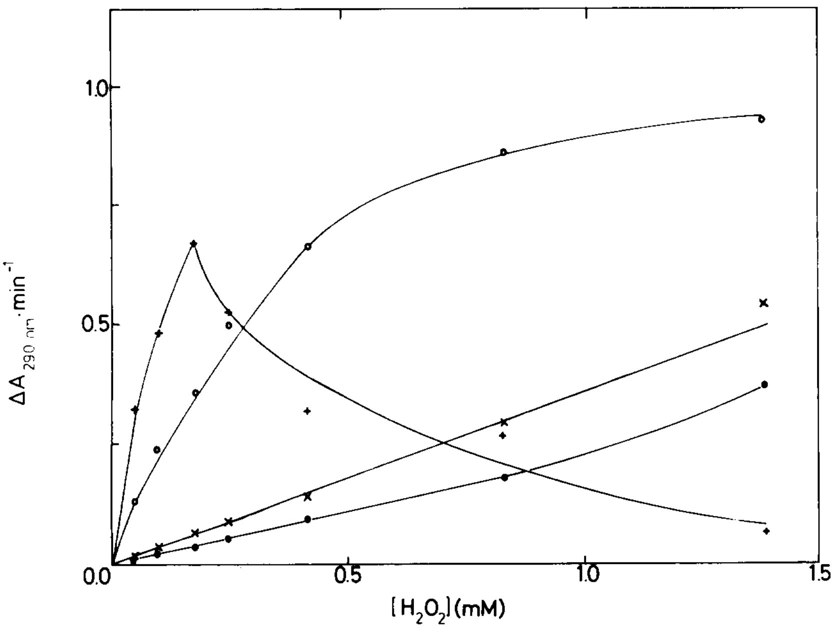


Fig. 8. Effect of hydrogen peroxide on the chlorination of monochlorodimedone at various pH values. Conditions as described in Fig. 5, except that 100 mM KCl was present and H_2O_2 concentration varied. ●——●, pH 3.5; X——X, pH 4.0; ○——○, pH 4.5; +——+, pH 5.5.

the apparent K_m for H_2O_2 at a fixed chloride concentration decreases linearly with increasing pH (Fig. 7) from 10 mM at pH 3.7 to 0.12 mM at pH 5.6.

A plot of the chlorination rate against the pH reveals that the enzymic activity shows optima in pH, the position of which shift to higher pH values upon increasing the concentration of chloride. However, when the chloride

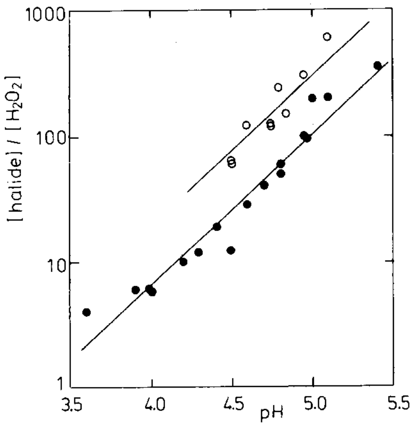


Fig. 9. Position of the pH optimum in the chlorination reaction (○——○) and bromination reaction (●——●) as a function of the $[halide]/[H_2O_2]$ ratio. Conditions of the chlorination reaction as in Figs. 5 and 8. The bromination reaction was measured in 0.2 M Na_2SO_4, 0.1 M $NaCH_3COO$, 16.7 μM monochlorodimedone, and 10 nM myeloperoxidase. The concentration of H_2O_2 varied from 0.05 to 1.66 mM and the concentration of KBr from 0.33 to 60 mM.

concentration is fixed and the H_2O_2 concentration varied, the pH optima shift to lower pH values with increasing concentrations of H_2O_2. Fig. 9 shows that in fact the logarithm of the $[Cl^-]/[H_2O_2]$ ratio determines the position of the pH optimum in the chlorination reaction.

The aspects of the chlorination of monochlorodimedone described are similar for bromination. However, the concentration of bromide needed to reach the same reaction rate is about ten times lower than the concentration of chloride under otherwise identical conditions, probably because bromide is oxidized more easily than chloride. As is observed in the chlorination reaction, the bromination also shows pH optima, which are strongly dependent on the concentration of hydrogen peroxide and bromide. Fig. 9 illustrates that like in the chlorination reaction the position of the optimum is a linear function of the logarithm of the $[Br^-]/[H_2O_2]$ ratio.

Fluoride is not utilized by myeloperoxidase as a halogen donor in the reaction with monochloridimedone but it is in fact an inhibitor of the halogenating activity of the enzyme. Lineweaver-Burk plots (not shown) demonstrate that fluoride is a competitive inhibitor with respect to H_2O_2 in the bromination reaction. The inhibition constant increases with increasing pH from 3 mM at pH 4 to 20 mM at pH 5.

Discussion

Based on iron analysis [16], myeloperoxidase contains two heme iron groups, which appear to be bound to the protein in an inequivalent way [17]. This may suggest that the hemes show differences in their ligand binding properties. However, the Hill constants of 1.0 observed in the Hill plots of the spectral changes induced by halides (Fig. 2) show that if both heme groups bind a halide associated with absorbance changes, the binding sites are identical and non-interacting. The value of the K_d for the enzyme-chloride complex at pH 4.5 (0.45 mM) is in good agreement with the value reported by Harrison [15] but in disagreement with the K_d of 4 mM found by Stelmaszynska and Zgliczynski [4]. These authors also report spectral differences between myeloperoxidase at neutral and slightly acidic pH. These differences are, however, not observed in our preparation of myeloperoxidase.

The increase of the value of the dissociation constant of the enzyme-halide complex with increasing pH suggests that an ionizing group in the enzyme has to take up a proton simultaneously with binding of the halide to the enzyme. Since the relation between the logarithm of the K_d and pH is linear between pH 7 and pH 3, the pK of this ionizing group is lower than 3.0. Another possibility, suggested by Chance [18] for horseradish peroxidase and by Litchfield et al. [19] for catalase, is that the protonated ligand is bound to the enzyme. However, this is less likely for myeloperoxidase since the log K_d versus pH plot should show a break at a pH value corresponding to the pK_a of the HF (3.45) [20] and this is not observed.

The steady-state kinetics of the chlorination reaction catalyzed by myeloperoxidase are complex. It is obvious that substrate inhibition may occur by either substrate, depending on the pH and the concentration of the substrates used. At low pH, chloride acts as a competitive inhibitor with respect to H_2O_2

in the chlorination reaction confirming the observations of Zgliczynski et al. [8] and Harrison [15]. The K_i observed for chloride is 14 mM at pH 4.5 and this is nearly two orders of magnitude larger than the K_d (0.45 mM) of the spectroscopically detectable enzyme-chloride complex and at least five times larger than the K_m (2.5 mM) for the chlorination reaction at this pH. Thus, myeloperoxidase can form two types of complexes with chloride, a complex with a high affinity and a second inhibitory complex in which chloride is weakly bound to the same enzyme locus where normally hydrogen peroxide reacts with the heme iron, since it is a competitive inhibitor with respect to H_2O_2. This suggestion is supported by the observation that heme-iron ligands like azide and fluoride are expelled from the sixth heme ligand position when chloride is added [21]. For intestinal peroxidase, lactoperoxidase and catalase it has been reported [19,22] that the catalytic activity is inhibited by chloride. In the peroxidases it appears that chloride also reacts at the same site as cyanide, which probably binds at the heme iron.

At low pH and sufficiently large chloride concentration the kinetics of the chlorination reaction can be described by the rate equation:

$$\frac{V}{v} = 1 + \frac{K_m^{H_2O_2}}{[H_2O_2]}\left\{1 + \frac{[Cl^-]}{K_i^{Cl^-}}\right\} \tag{1}$$

where $K_i^{Cl^-}$ is the inhibition constant of the chloride complex. It is obvious from this equation that the apparent K_m for H_2O_2 is a linear function of the chloride concentration and will increase upon increasing the chloride concentration as has been observed (cf. Fig. 6). The measured K_m for H_2O_2 at a fixed chloride concentration is strongly pH-dependent (Fig. 7). It is conceivable that the K_m for H_2O_2 decreases when the pH increases, but it is more likely that the K_i^{Cl} for chloride is strongly pH dependent, since this is also observed for the dissociation constant (K_d) of the spectroscopically detectable enzyme-chloride complex and the Michaelis constant (K_m) for chloride.

At higher pH values (5.5—6.5) or alternatively at low chloride and high H_2O_2 concentrations the kinetic pattern of the chlorination reaction changes. Under these conditions H_2O_2 inhibits the reaction and since the K_m for chloride ($K_m^{Cl^-}$) increases with the H_2O_2 concentration, the rate equation which includes formation of the inhibitory complex may be written:

$$\frac{V}{v} = 1 + \frac{K_m^{H_2O_2}}{H_2O_2}\left\{1 + \frac{[Cl^-]}{K_i^{Cl^-}}\right\} + \frac{K_m^{Cl^-}}{[Cl^-]}\left\{1 + \frac{[H_2O_2]}{K_i^{H_2O_2}}\right\} \tag{2}$$

At low pH and concentrations of chloride which are high compared to the $K_m^{Cl^-}$ the first term in Eqn. 2 dominates. However, at higher pH values and at concentrations of H_2O_2 which are high as compared with $K_m^{H_2O_2}$, the second term determines the kinetic pattern. This term will increase further in magnitude when the pH is raised, since the K_m for chloride increases with the pH (Fig. 7). Whether the inhibition by H_2O_2 is indeed of the competitive type as suggested here or of another type is not possible to decide for reasons discussed in the Results.

In the absence of chloride, myeloperoxidase is inactivated by H_2O_2 (Fig. 4). In fact Compound II will be rapidly formed and this Compound II is a form of the enzyme which, according to Harrison [15], does not function in the HOCl formation. When the pH increases, the affinity of chloride for the enzyme will decrease and as a consequence the probability of formation of the inhibitory Compound II, will increase which may account for the observed inhibition by H_2O_2. Naskalski [23] also reported that above pH 5.6 the rate of enzyme inactivation increases. However, it cannot be excluded that the enzyme is inactivated by its own product (HOCl).

Rate Eqn. 2 is in fact that of a 'ping-pong' mechanism with a single substrate site and double competitive substrate inhibition [24]. In peroxidases mechanisms are observed in which H_2O_2 first has to modify a site on the enzyme before the electron donor can react with that modified site [25]. However, there is an important observation which makes this kind of mechanism less likely for myeloperoxidase. Myeloperoxidase reacts already with chloride before H_2O_2 has reacted with the enzyme. This spectroscopically detectable enzyme-chloride complex is, however, not responsible for the inhibition by chloride, since the inhibition constant K_i^{Cl} is nearly two orders of magnitude larger than the dissociation constant of this enzyme-chloride complex. Thus the rate equation probably has the form of a sequential bi-bi mechanism with competitive substrate inhibition [24]:

$$\frac{V}{v} = 1 + \frac{K_m^{H_2O_2}}{[H_2O_2]}\left\{1 + \frac{[Cl^-]}{K_i^{Cl^-}}\right\} + \frac{K_m^{Cl}}{[Cl^-]}\left\{1 + \frac{[H_2O_2]}{K_i^{H_2O_2}}\right\} + \frac{K_d^{Cl^-} \cdot K_m^{H_2O_2}}{[Cl^-][H_2O_2]} \qquad (3)$$

In this mechanism the last term is probably small compared with the other terms. It is also clear from Eqn. 3 why the chlorination reaction shows sharp pH optima. The first term which describes the inhibition by chloride will decrease when the pH increases whereas the second term due to the increase of K_m^{Cl} with pH will increase in magnitude. Differentiation of Eqn. 3 with respect to pH yields a formula which will give the pH optimum of the reaction as a function of both substrate concentrations. When the assumption is made that the inhibition constant for H_2O_2 ($K_i^{H_2O_2}$) is smaller than the concentration of H_2O_2 the position of the pH optimum should depend linearly on the logarithm of the [chloride]/[H_2O_2] ratio with a slope of 1.0. This is in good agreement with the observations in the literature [8] and those found by us for the chlorination and bromination reaction (Fig. 9).

These observations show that in vitro myeloperoxidase is able to carry out halogenation reactions over a broad pH range. The position of the optimum in the enzymic activity is governed by both the chloride and H_2O_2 concentration. The concentration of chloride in plasma of man is 103 mM and the intracellular concentration in human leucocytes is 110 mM [26]. At least part of the plasma will enter the phagocytic vacuole during ingestion of a particle by a granulocyte and thus the concentration of chloride in the vacuole will be similar to the plasma concentration. The pH in the vacuole immediately after uptake of the particle will be near neutral. This requires that for full halogenating activity of myeloperoxidase (cf. Fig. 9) the initial concentration of H_2O_2 formed by the phagocytic granulocyte is very low. This condition seems to be fullfilled since a lagtime is observed in the formation of H_2O_2 by phagocytosing granulocytes [27]. As shown by Jensen and Bainton [28] the pH in the phagocytic vacuole after uptake of a particle drops to slightly acidic pH values and this requires that for example at pH 4.5 for optimal activity of myeloperoxidase in vivo a concentration of H_2O_2 should be present in the vacuole of about 1 mM. Whether this concentration is indeed reached is not known since only rates of H_2O_2 formation by granulocytes have been reported [29].

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References

1 Klebanoff, S.J. and Clark, R.A. (1978) The Neutrophil: Function and Clinical Disorders, pp. 409—488, North-Holland Publishing Comp., Amsterdam

2 Agner, K. (1964) Abstr. IVth Int. Congr. Biochem. Vienna, p. 64, Pergamon Press, New York

3 Agner, K. (1970) in Structure and Function of Oxidation-Reduction Enzymes (Åkesson, Å. and Ehrenberg, A., eds.), pp. 329—335, Pergamon Press, Oxford

4 Stelmaszyńska, T. and Zgliczyński, J.M. (1974) Eur. J. Biochem. 45, 305—312

5 Harrison, J.E. and Schultz, J. (1976) J. Biol. Chem. 251, 1371—1374

6 Odajima, T. (1978) Abstr. VIth Int. Biophys. Congr., Kyoto, Japan, p. 193

7 Sgliczyński, J.M., Stelmaszyńska, T., Domański, J. and Ostrowski, W. (1971) Biochim. Biophys. Acta 235, 419—424

8 Zgliczyński, J.M., Selvaraj, R.J., Paul, B.B., Selmaszyńska, T., Poskitt, P.K.F. and Sbarra, A.J. (1977) Proc. Soc. Exp. Biol. Med. 154, 418—422

9 Thomas, E.L. (1979) Infect. Immun. 23, 522—531

10 Allen, R.C. (1975) Biochem. Biophys. Res. Commun. 63, 675—683

11 Bakkenist, A.R.J., Wever, R., Vulsma, T., Plat, H. and Van Gelder, B.F. (1978) Biochim. Biophys. Acta 524, 45—54

12 Hager, L.P., Morris, D.R., Brown, F.S. and Eberwein, H. (1966) J. Biol. Chem. 241, 1769—1777

13 Vogel, A.I. (1961) Quantitative Inorganic Analysis, pp. 295—296, Longmans, London

14 Thomas, J.A., Morris, D.R. and Hager, L.P. (1970) J. Biol. Chem. 245, 3135—3142

15 Harrison, J.E. (1976) in Cancer Enzymology (Schultz, J. and Ahmed, F., eds.), Miami Winter Symp. 12, pp. 305—316, Academic Press, New York

16 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 360—367

17 Harrison, J.E. and Schultz, J. (1978) Biochim. Biophys. Acta 536, 341—349

18 Chance, B. (1952) Arch. Biochem. Biophys. 40, 153—164

19 Litchfield, W.J. (1977) FEBS Lett., 83, 281—284

20 Handbook of Chemistry and Physics (Weast, R.L., ed.), 57th ed., D-151, CRC Press, Cleveland, OH

21 Wever, R. and Bakkenist, A.R.J. (1980) Biochim. Biophys. Acta 612, 178—184

22 Kimura, S. and Yamazaki, I. (1978) Arch. Biochem. Biophys. 189, 14—19

23 Naskalski, J.W. (1977) Biochim. Biophys. Acta 485, 291—300

24 Fromm, H.J. (1975) Mol. Biol. Biochem. Biophys. 22, 121—160

25 Dunford, H.B. and Stillman, J.S. (1976) Coord. Chem. Rev. 19, 187—251

26 Baron, D.N. and Ahmed, S.A. (1969) Clin. Sci. 37, 205—220

27 Root, R.K., Metcalfe, J., Oshino, N. and Chance, B. (1975) J. Clin. Invest. 55, 945—955

28 Jensen, M.S. and Bainton, D.F. (1973) J. Cell Biol. 56, 379—388

29 Homan-Müller, J.W.T., Weening, R.S. and Roos, D. (1975) J. Lab. Clin. Med. 85, 198—207