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MYELOPEROXIDASE OF THE LEUKOCYTE OF NORMAL BLOOD

V. THE SPECTRAL CONVERSION OF MYELOPEROXIDASE TO A CYTOCHROME OXIDASE LIKE DERIVATIVE

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SUMMARY

Myeloperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) was converted by the addition of HCl to a new myeloperoxidase derivative (named acid myeloperoxidase) with light absorption peaks at 410, 500 (shoulder), 550 and 600 nm. Upon the gradual neutralization of the solution by dialysis the acid myeloperoxidase was then converted to an another characteristic form with light absorption peaks at 428 and 600 nm; the spectrum being similar to that of cytochrome oxidase.

The modified myeloperoxidase still showed a peroxidase activity comparable to the untreated enzyme and k_1 and k_4 (for guaiacol) were found to be $2.3 \cdot 10^6$ and $1.3 \cdot 10^4$ $M^{-1} \cdot s^{-1}$, respectively. The optimum pH of 8 was the same as that of the untreated enzyme.

The light absorption spectra of the reduced form and of the complexes with cyanide and carbon monoxide were measured. These spectra were also similar to those of the corresponding forms of cytochrome oxidase. Unusual binding of heme groups (analogous to *a*-type) in myeloperoxidase is suggested.

INTRODUCTION

Myeloperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) is known to have hematin groups quite different from protohematin. Nicholls[1] has pointed out the similarity in the spectra of reduced myeloperoxidase and (ferro-) sulfmyoglobin. Schultz and Shmukler[2] have reported that the pyridine hemochromogen spectrum of myeloperoxidase from human leukocytes is similar to that of formyl or diacetyl heme or of heme *a*. Newton *et al.*[3] have concluded that at least one of the conjugated electrophilic substituents of the myeloperoxidase heme is a carbonyl and the heme contains two electrophilic groups on opposite pyrroles as the heme of cytochrome oxidase does.

Because of difficulty in splitting the heme from myeloperoxidase and of an insufficient amount of the enzyme being available for chemical analysis, the chemical nature of the myeloperoxidase heme is not clearly identified as yet. In the present

paper we will describe spectral and functional properties of a modified myeloperoxidase which retains nearly normal peroxidase activity.

MATERIALS AND METHODS

Myeloperoxidase was prepared from leukocytes of normal pig blood by the method reported in the previous paper[4]. The concentration was calculated on the basis of a value of 95 $mM^{-1} \cdot cm^{-1}$ for the extinction coefficient at 430 nm[5]. The $A_{430\ nm}/A_{280\ nm}$ ratios for the enzyme preparations used in this experiment varied from 0.7 to 0.55.

All other materials were obtained from commercial sources at the highest available states of purity. The reaction and estimation were carried out at 25 °C except where stated otherwise.

The recording spectrophotometers used were Hitachi instruments, Type 124 and EPS-3T. Circular dichroism was measured with a model ORD/UV-5 (Jasco) spectrophotometer.

RESULTS

Fig. 1 demonstrates changes in the light absorption spectra of myeloperoxidase which occured when the HCl concentration was increased. Around pH 2 a new myeloperoxidase derivative could be observed. This species with absorption peaks at 410, 500 (shoulder), 550 and about 600 nm is here termed "acid myeloperoxidase". The acid myeloperoxidase was spectrophotometrically stable for several days at 5 °C and pH 2.

In Fig. 2 it is shown that, by dialysis against 0.0125 M acetate buffer (pH 5.5)

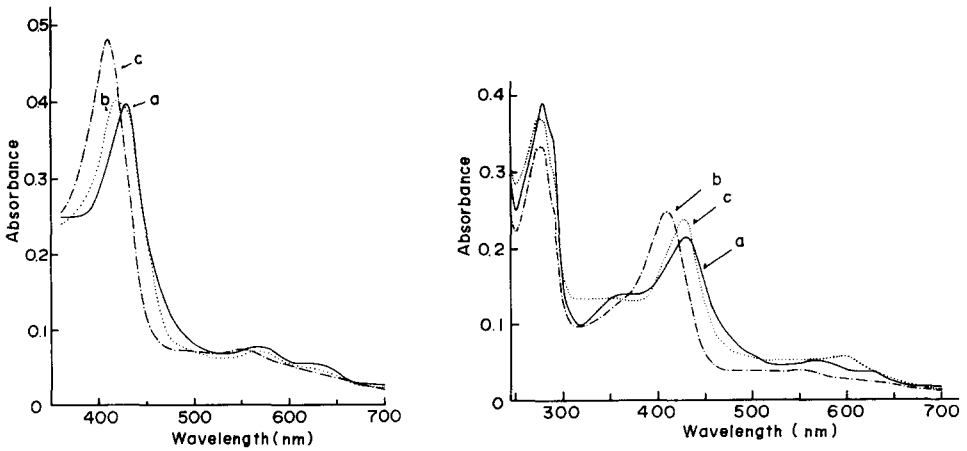


Fig. 1. Effect of HCl on the absorption spectrum of myeloperoxidase. Curve a, 4.1 μM myeloperoxidase in water; Curve b, in a 10 mM HCl solution (pH was about 2.6); Curve c, in a 30 mM HCl solution (pH was about 2.0).

Fig. 2. Formation of a cytochrome oxidase like derivative of myeloperoxidase. Curve a, 2.2 μM myeloperoxidase in water; Curve b, 2.2 μM myeloperoxidase in a 20 mM HCl solution (pH was about 2.0); Curve c, the solution of b was dialyzed against 0.0125 M acetate buffer (pH 5.5) at 5 °C for 24 h. The correction for dilution was made in the measurement of absorbance.

for 24 h, acid myeloperoxidase was further converted into a characteristic compound having absorption peaks at 428 and 600 nm. The spectrum of the compound was very similar to that of cytochrome oxidase (cytochrome $a + a_3$). This modified enzyme was also spectrophotometrically stable for several days at 5 °C. Dialysis of the acid myeloperoxidase solution against a neutral or slightly alkaline buffer solution caused partial nonspecific denaturation of the protein and the solution became turbid.

Fig. 2 also shows that by acid treatment the spectra of myeloperoxidase in the ultraviolet region were affected. Fig. 3 shows circular dichroism spectra in the far ultraviolet region of 3 forms of the enzyme. It might be concluded from the results that modified myeloperoxidase has a lower α-helix content than the untreated and acid enzymes.

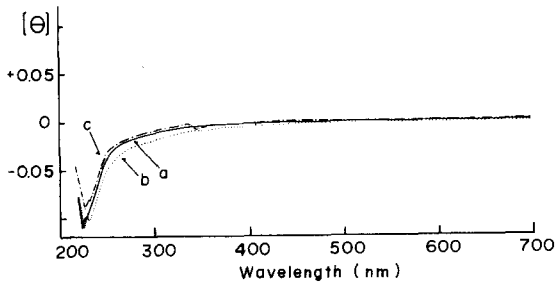
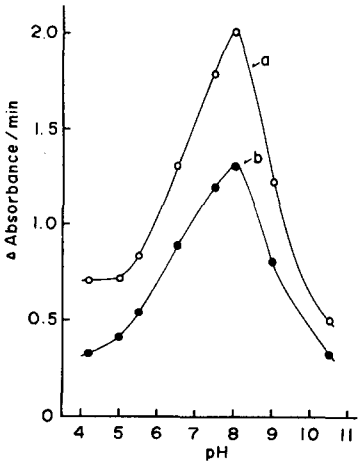


Fig. 3. Effect of the acid treatment on the circular dichroism spectrum of myeloperoxidase. Samples of Curves a, b and c are described in Fig. 2.

The modified myeloperoxidase had a peroxidase activity about 60% of the untreated enzyme. Fig. 4 shows pH–activity curves of the treated and untreated enzyme. Rate constants, k_1 for the reaction between myeloperoxidase and H_2O_2 and k_4 for the reaction between myeloperoxidase peroxide compound and guaiacol could be measured by the use of Chance's equation[6] for overall kinetics. From the results of Fig. 5, k_1 and k_4 were calculated to be $5 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ and $1.8 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ for the native enzyme and $2.3 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ and $1.3 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ for the modified enzyme, respectively.

It was reported in the preceding paper[5] that cyanide gives two complexes with myeloperoxidase. It was suggested that one of the two heme groups is complexed with cyanide by the addition of a stoichiometric amount of cyanide and a considerably high concentration of cyanide is needed to complete the formation of the second cyanide complex. Similar experiments with modified myeloperoxidase are shown in Figs 6 and 7. Fig. 6 shows that contrary to the case of native myeloperoxidase no characteristic change in the light absorption was found in the visible region even in the presence of a large amount of cyanide. Fig. 7 shows a spectrophotometric titration experiment which was carried out in the Soret region. The formation of the first cyanide complex was completed at lower cyanide concentrations and the complex was found to be formed by the reaction of one molecule of cyanide with the modified enzyme. The complex could be reduced by hydrosulfite, giving a complex between the reduced form and cyanide with an absorption spectrum slightly different from that of reduced form of modified myeloperoxidase.



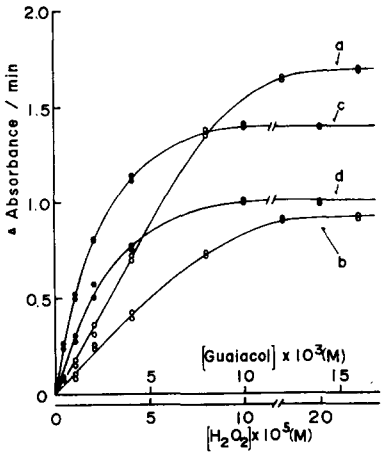
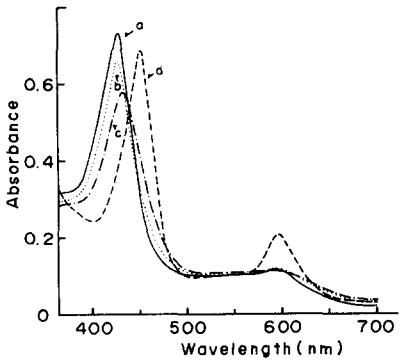


Fig. 4. Effect of pH on the peroxidase activities of myeloperoxidase(a) and its cytochrome oxidase like derivative (b). $2.2 \cdot 10^{-8}$ M myeloperoxidase, 40 μM H_2O_2 and 8 mM guaiacol, at 25 °C. The buffers used were acetate for pH of 4.2, 5.0 and 5.5, phosphate for pH of 6.5, 7.5 and 8.0 and borate for pH of 9.0 and 10.5 at concentrations of 0.1 M. Absorbance was measured at 475 nm.

Fig. 5. Effect of concentrations of guaiacol and H_2O_2 on the peroxidase activities of myeloperoxidase and its cytochrome oxidase like derivative. $2.2 \cdot 10^{-8}$ M untreated (a and c) and treated (b and d) myeloperoxidase and 0.1 M acetate, pH 5.5. Curves a and b, the activity was assayed at a concentration of 40 μM H_2O_2 and at variable concentrations of guaiacol; Curves c and d, the activity was assayed at a concentration of 8 mM guaiacol and at variable concentrations of H_2O_2.



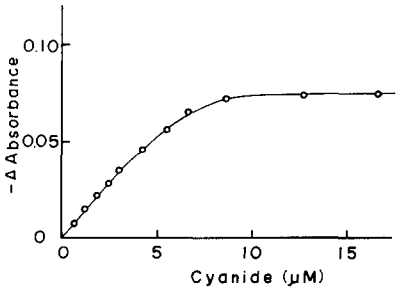


Fig. 6. Cyanide complexes of the cytochrome oxidase like derivative of myeloperoxidase. 0.01 M acetate, pH 5.5. Curve a, 6.3 μM enzyme; Curve b, 6.6 μM cyanide was added to a; Curve c, 2 mM cyanide was added to b; Curve d, c was reduced by the addition of solid sodium hydrosulfite.

Fig. 7. Stoichiometry of the reaction between cyanide and the cytochrome oxidase like derivative of myeloperoxidase. 6.3 μM enzyme, 0.01 M acetate, pH 5.5. Absorbance was measured at 428 nm.

Fig. 8 shows that the reduced form of modified myeloperoxidase could easily react with CO to form a complex having absorption maxima at 438 and 598 nm. This seems to be a characteristic property of the modified enzyme since it is known[4] that CO–myeloperoxidase complex can be observed only under restricted conditions probably dependent on temperature and CO concentration.

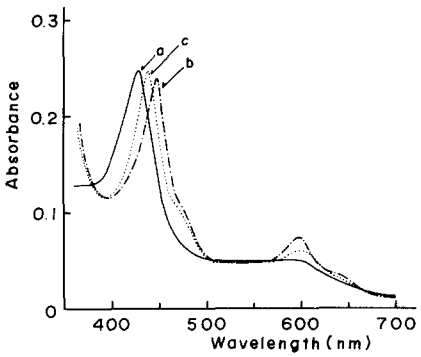


Fig. 8. Formation of a CO complex of the reduced form of the cytochrome oxidase like derivative of myeloperoxidase. Curve a, 2.2 μM enzyme; Curve b, excess hydrosulfite was added to a; Curve c, CO was bubbled into the solution of b.

DISCUSSION

The prosthetic group of myeloperoxidase has not so far been identified. The heme is firmly attached to the protein and its chemical structure has been assumed only from spectral analogy with known hemoproteins. Some similarity to the prosthetic group of choleglobin was described by Lemberg and Legge[7]. A close analogy with sulfhemoprotein has been suggested by Nicholls[1] and Newton *et al.*[3]. Of special interest might be a comparison with heme *a*. Schultz and Shmukler[2] have shown that the alkaline pyridine hemochromogen of myeloperoxidase has an α-band at 586 nm, which is not essentially different from the 587 nm band found for cytochrome oxidase. Judging from the fact that pyridine reacts with the reduced enzyme to form a ferrohemochrome with an α-band at 590 nm and no β-band, Newton *et al.*[3] have concluded that two strongly electrophilic substituents exist on opposite pyrrole rings. They have suggested that at least one of the conjugated electrophilic substituents of the heme is a carbonyl.

Table I shows a comparison of spectral properties between native myeloperoxidase, modified myeloperoxidase and cytochrome oxidase. It can be seen in this table that, from the spectrophotometric point of view, modified myeloperoxidase is much more similar to cytochrome oxidase than is the original myeloperoxidase. These observations strongly support the possibility that the prosthetic group of myeloperoxidase is analogous to heme *a*. The question may be raised as to why the initial spectral properties between myeloperoxidase and cytochrome oxidase are so different. One of the probable explanations might be that the electronic structure of the myeloperoxidase heme is "distorted" in the enzyme molecule by an interaction with the protein moiety, which is removed by the acid treatment. This phenomenon appears to be similar to the conversion of microsomal P450 to P420. P450 contains protoheme as the prosthetic group but its spectral properties are very different from those of other protoheme proteins. By certain chemical modifications it can be converted into P420, which has normal spectral properties. By a modification, like that of microsomal P450, the Soret peak of the CO–ferrous myeloperoxidase complex is moved to shorter wavelengths by about 30 nm.

TABLE I

COMPARISON OF ABSORPTION PEAKS OF VARIOUS FORMS OF MYELOPEROXIDASE AND CYTOCHROME OXIDASE (CYTOCHROME $a+a_3$)

A number in parentheses shows a shoulder of the absorption spectrum.

Enzyme	*Derivative*	*Wavelength (nm)*					*Reference*
Myeloperoxidase							
Untreated	Ferric	430	(500)	570	634	690	4 and 5
	Ferrous	475	(500)	(585)	637		
	CN^- complex I	433			634		
	CN^- complex II	454			634		
	Reduced CO- complex	468			634		
Acid form	Ferric	410	(500)	550	600		This paper
Modified	Ferric	428			600		This paper
	Ferrous	447			595		
	CN^- complex I	428			600		
	CN^- complex II	433			600		
	Reduced-CN^- complex	452			596		
	Reduced-CO complex	438			598		
Cytochrome oxidase	Ferric	421–425*			597–600*		8–14
(cytochrome $a+a_3$)	Ferrous	443–444*			603–605*		
	CN^- complex	424–428*			600		
	Reduced-CN^- complex	444			605		
	Reduced-CO complex	432		(590)	603		

* Many results have been reported but the values lie within this range.

Newton *et al.*[3] have indicated that myeloperoxidase differs structurally from other peroxidases by (1) the covalent binding of the heme to the enzyme protein, (2) the unknown but unusual conjugated electrophilic substituents of the heme and (3) the structural entity responsible for the sulfmyoglobin-like spectrum of the reduced enzyme. The last two can be now explained as follows; for (2), that the heme is very similar to heme *a* and for (3), that some kind of structural strain is induced in the periphery of the heme by interaction with the protein, which results in the formation of a dihydro-pyrrole-type (chlorin) structure.

Heterogeneity of myeloperoxidase has been discussed by many workers[2,15–19]. Agner[15] has found that myeloperoxidase splits into two parts in 50% pyridine solution and each of them contains a heme. Their absorption spectra are not identical with each other but both of them are rather similar to that of our modified myeloperoxidase. Schultz *et al.*[16] have shown that myeloperoxidase is separated into at least ten components in 6 M urea solution and these components can be divided into two groups on the basis of light absorption in the Soret region; a group characterized by maxima close to that of the native enzyme and an other with maxima about 20–25 nm toward the shorter wavelengths. Unlike modified myeloperoxidase, members of the latter group have no peroxidase activity and bleach on treatment with hydrosulfite. Felberg *et al.*[17] have indicated that the heterogeneity observed is not due to partial digestion during the trypsin solubilization procedure.

The presence of a myeloperoxidase isozyme in guinea pig leukocytes has been also reported by Himmelhoch *et al.*[18]. It was found that the new myeloperoxidase was spectrally different from known myeloperoxidases. However, because of a lack of available information on the properties of the isozyme it is premature to discuss its

relationship with the present modified enzyme. Archer *et al.*[19] have isolated a peroxidase from eosinophils which has spectra of the same type as milk peroxidase.

In spite of marked changes in spectral properties compared with untreated myeloperoxidase, the difference in the activity between the two heme groups is retained in the modified enzyme. It might be worthwhile to emphasize here the striking similarities between cytochrome oxidase and modified myeloperoxidase not only with regard to spectral properties but also in the existence of "two heme groups" in each hemoprotein. Since a large amount of experimental data on cytochrome oxidase has been accumulated the study of modified myeloperoxidase will be promoted by comparison with cytochrome oxidase. Unlike cytochrome oxidase, the α-bands of complexes of the reduced form of the modified myeloperoxidase with CO and cyanide are symmetrical and there is no difference in the spectrum of the cyanide complex whether cyanide is added before or after the enzyme is reduced. Thus, it can be said that there are small but distinct differences in spectral properties between these hemoproteins. At the present moment the data on myeloperoxidase are quite insufficient to draw any effective comparison between them.

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