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SPECTRAL PROPERTIES OF MYELOPEROXIDASE AND ITS LIGAND COMPLEXES

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The effects of ligands with various field strengths on the optical absorption spectrum of myeloperoxidase have been investigated. As is the case with other hemoproteins, the Soret peak in the optical absorption spectra at 77 K moves to longer wavelengths when strong-field ligands are present, whereas binding of such ligands as chloride and fluoride, which stabilize the high-spin state, shows the opposite effect. With a ligand of intermediate field strength, such as azide, the optical spectrum is not affected at room temperature, but lowering of the temperature results in the formation of the low-spin form of the enzyme. Similarly, in native myeloperoxidase a spin state equilibrium is found in which the low-spin state is favoured at high ionic strength and displays corresponding changes in the optical spectra. From the ligand- and the temperature-induced changes in the optical spectra of the ferric enzyme it is concluded that the band at 620–630 nm is an α band of the low-spin heme iron species, whereas the bands at 500 and 690 nm are probably 'charge-transfer' bands * of the heme with the iron in the high-spin state.

Introduction

Myeloperoxidase from polymorphonuclear leukocytes is capable of catalysing the oxidation of Cl^- to hypochlorous acid [1–4]. This reactive product is responsible for the microbicidal activity of the enzyme when combined with H_2O_2 and Cl^- [5,6]. The optical absorption spectra of the enzyme, which contain high-spin heme groups [7–9], are rather peculiar. The Soret peak and the α band of the reduced enzyme, which are at 473 and 636 nm, respectively, are shifted far to the red as compared to other hemoproteins [10,11]. In addition, the band at 625 nm observed in the optical spectrum of the ferric enzyme [10,11] does not appear to correspond to the 'charge-transfer' band normally found at this wavelength in high-spin hemoproteins [12]. The band at 625 nm is intense in low-spin cyanomyeloperoxidase [13,14], whereas it should have been absent if this band were indeed of the charge-transfer type.

Pyridine hemochrome spectra [11,15] and spectra of the enzyme treated with sodium dodecyl sulphate (SDS) or acid [16–18] resemble those of cytochrome *c* oxidase. In addition carbonyl reagents react with myeloperoxidase [11,18,19] and it is likely that myeloperoxidase contains a formyl heme moiety similar to heme *a* from cytochrome *c* oxidase. However, the positions of the γ and α bands in the optical spectrum of reduced cytochrome *c* oxidase are 28–32 nm lower than those of reduced myeloperoxidase. It is conceivable that, as in cytochrome *P*-450, a mercaptide anion is one of the axial ligands of the heme iron and is responsible for the shift to the red in the bands of myeloperoxidase. However, the EPR spectra and ligand-field parameters of myeloperoxidase complexes are not very different from those of catalase

* 'Charge transfer' does not refer to the pure charge-transfer transitions, which occur in the near infrared, but to mixed transitions of ligand and charge transfer.

[20], suggesting that a mercaptide is unlikely to be an axial ligand. The unique spectral properties of myeloperoxidase must therefore be due to interaction of the porphyrin with the protein part of the molecule.

The present paper deals with a description of the optical properties of the enzyme-ligand complexes. Classification of the absorption bands is given according to Brill and Williams [12]. It is shown that in myeloperoxidase, as in other peroxidases [21,22], temperature-dependent spin state transitions can occur.

Materials and Methods

Myeloperoxidase was purified from human blood leukocytes as described elsewhere [23] and the concentration was determined spectrophotometrically using an absorption coefficient of 89 $mM^{-1} \cdot cm^{-1}$ at 428 nm [10]. The ratio A_{428nm}/A_{280nm} of the preparations used was 0.8. Absorbance spectra were recorded on an Aminco Chance DW-2 spectrophotometer. This instrument was equipped with a brass cell holder with two cells (apparent optical path length 3 mm) with perspex windows. The cells, one for the sample, the other for the reference, were placed in a transparent Dewar filled with liquid nitrogen. Spectra at temperatures higher than 77 K were obtained by allowing the brass holder to warm up. Since warming up of the sample from 77 K to room temperature required several hours, temperature changes during recording of the spectra were small (<2 K). The temperature of the sample was measured using thermocouples (gold 0.03% iron/chromel), one of which was frozen in the sample just beneath the light beam and the other was kept in liquid nitrogen. For the measurement of absorption spectra at room temperature a Cary-17R recording spectrophotometer was used.

Results

The optical spectra of native myeloperoxidase at room temperature and at liquid nitrogen temperature are shown in Fig. 1. The spectrum at room temperature with its bands at 428, 500, 570, 620 and 690 nm is similar to those reported for myeloperoxidase isolated from other sources [10,18,24]. Upon cooling to 77 K, the spectrum changes markedly, the Soret peak

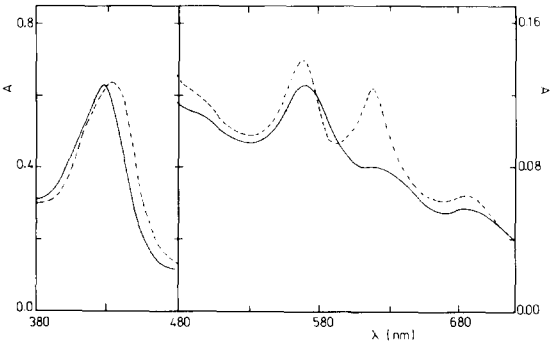

Fig. 1. Effect of temperature on the optical spectrum of myeloperoxidase. ———, room temperature; 7.1 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8). - - - - - -, 77 K; 6.6 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8).

at 428 nm moves to 433 nm and, compared with the other bands, the band at 620 nm intensifies considerably. In other peroxidases temperature-dependent changes in the optical spectrum have been observed which are due to spin state changes in these enzymes. EPR studies on myeloperoxidase have already demonstrated the presence of both high-spin signals as well as a low-spin one ($g_x = 2.56, g_y = 2.31, g_z = 1.81$) the intensity of which depends on the preparation studied [20]. At that time it was not known which factors determined the equilibrium between the two species.

From EPR studies on cytochrome *P*-450 it is known that the equilibrium between the high-spin state and the low-spin state is affected by the potassium concentration or by the ionic strength of the medium used [25]. Therefore, optical spectra of myeloperoxidase were recorded at several concentrations of potassium phosphate (pH 7.0). It is obvious from Fig. 2 that a high ionic strength favours the formation of a state of the enzyme with a high intensity of the band at 620 nm and a Soret peak that is shifted to a higher wavelength. Under these conditions EPR spectra at 15 K demonstrate the presence of significant amounts of the low-spin species (data not shown). Thus, as in other hemoproteins [12], the position of the γ band is a measure for the spin state of the enzyme.

EPR studies [20] at 15 K on myeloperoxidase have already shown that in the presence of weak-field ligands such as Cl^- or F^-, only rhombic high-spin

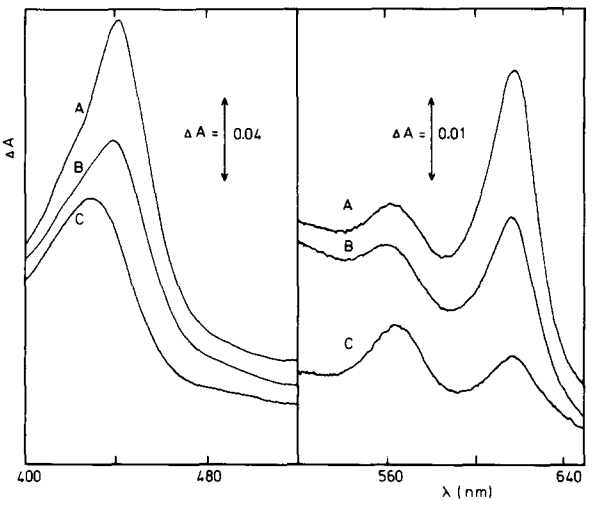

Fig. 2. Effect of potassium phosphate on the optical absorption spectrum of myeloperoxidase. 1.5 μM myeloperoxidase in potassium phosphate (pH 7.0); temperature 77 K. A, 1.0 M potassium phosphate; B, 0.1 M potassium phosphate; C, 0.01 M potassium phosphate.

heme signals are detectable. The optical spectra at 77 K (Fig. 3) confirm this observation: in chloridomyeloperoxidase the band at 620 nm has diminished and the Soret peak has shifted to a slightly lower wavelength than that of native myeloperoxidase at 77 K. The position and intensity of the bands at 500, 570 and 690 nm are only slightly or not at all affected. The effect of F^- on the spectrum is similar to that of Cl^-, the bands in the visible region, however, move about 20 nm to the blue and a shoulder appears at 412 nm. At room temperature weak bands can also be detected in the near infrared at 800 and 910 nm (data not shown).

When cyanide, a strong-field ligand, is added to myeloperoxidase, this enzyme is converted into a low-spin derivative [20]. In this species, the position of the Soret peak is at 450 nm and an intense band is present at 630 nm (Fig. 3, trace D), whereas the bands at 500 and 690 nm have disappeared completely from the spectrum. The spectrum is not affected when the temperature is raised to 294 K, in line with previous reported data [13,14]. In addition, the bands in the near infrared have disappeared completely.

At room temperature a very similar spectrum (λ_{max} = 454 and 625 nm) is observed when myeloperoxidase is brought to pH 12.0. The pK of the spectral change is about 11.6 (data not shown). Formation of this low-spin species is reversible as upon lowering the pH to 7 the Soret peak shifts back to 428 nm. This demonstrates that, as in horseradish peroxidase [26], an alkaline form of the enzyme is present at high pH in line with previous reported data [20].

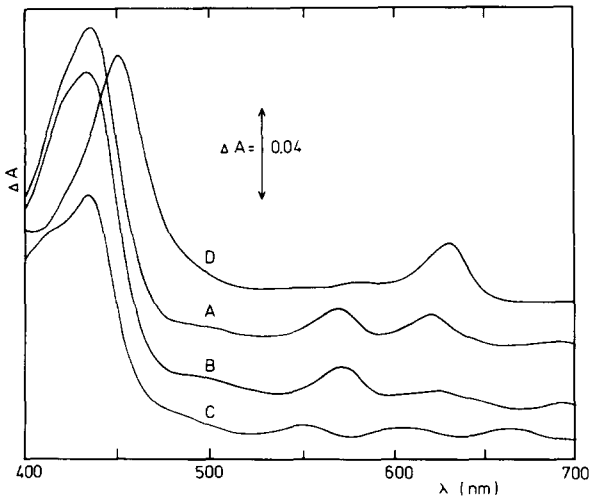

Fig. 3. Effect of ligands on the optical absorption spectrum of myeloperoxidase. 1.5 μM myeloperoxidase in 100 mM potassium phosphate (pH 6.8); temperature 77 K. A, native enzyme; B, 0.4 M KCl; C, 0.4 M KF; D, 0.5 mM KCN.

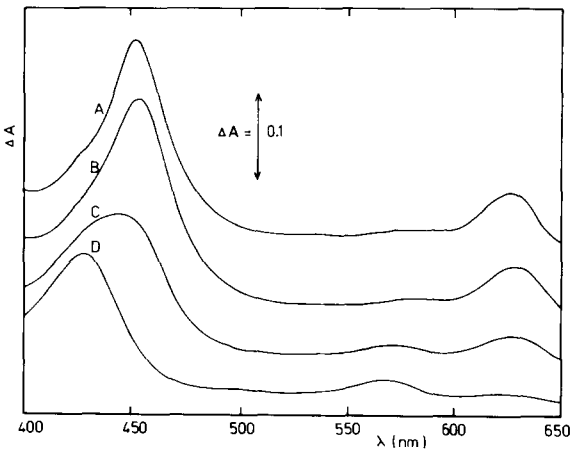

Fig. 4. The temperature dependence of the absorption spectra of the myeloperoxidase-azide complex. 1.5 μM myeloperoxidase in 100 mM potassium phosphate (pH 7.0); 0.5 mM sodium azide. A, 77 K; B, 219 K; C, 249 K; D, 270 K.

It has been reported [11] that azide does not affect the optical spectrum of myeloperoxidase at room temperature. However, as is illustrated in Fig. 4 in the presence of azide, an intermediate-field ligand, the absorption spectrum of myeloperoxidase at 77 K has changed drastically. The spectrum is characteristic of a low-spin species with a Soret peak position at 454 nm and an intense band at 628 nm. When the sample was allowed to warm up, the Soret peak moved to shorter wavelengths and the band at 628 nm bleached, showing stabilization of the high-spin state of the enzyme. Concomitantly, bands reappear at 500, 570, and also 690 nm (data not shown). Thus, in azido-myeloperoxidase, as in the azide complex of horseradish peroxidase [21], a temperature-dependent transition between a low-spin state and a high-spin state is present. This transition from a low-spin form to a high-spin form is seen at temperatures above 200 K; the temperature at which the fraction of both spin states is about equal is 250 K.

Discussion

The optical data presented in this paper show that myeloperoxidase at room temperature is mainly in the high-spin state. A thermal equilibrium exists between this state and a low-spin state, the position of which is clearly affected by the concentration of the buffer used. The addition of CN^-, OH^- or N_3^- results in a marked shift of the high/low-spin equilibria, so that at low temperature all of the enzyme is in a low-spin state. In other peroxidases similar temperature-dependent spin state equilibria are present [21,22] in which the low-spin state is populated at low temperature.

High-spin peroxidase complexes typically show a Soret peak at 403–407 nm, low intensity α and β bands at about 575 and 535 nm, and 'charge-transfer' bands at about 490 and 610–640 nm. When cyanide is present these enzymes are converted into a low-spin state with a concomitant shift in the Soret peak of about 20 nm to the red and the appearance of two visible bands, a β band at about 540 nm, and a weaker α band at 575 nm. In these low-spin complexes 'charge-transfer' bands are absent or have a very low intensity [12,27].

Very little information is available regarding the interpretation of the optical spectrum of myeloperoxidase, although this is of particular interest. The visible spectrum of high-spin myeloperoxidase is complex. In addition to the Soret peak, weak bands are observed at 500, 620 and 690, 800 and 910 nm, and a more intense band is found at 570 nm. The bands at 500, 690, 800 and 910 nm disappear completely when the enzyme is converted into a low-spin state and it is probable that these transitions are of the 'charge-transfer' type.

Upon addition of cyanide the Soret peak at 428 nm of ferric myeloperoxidase shifts to 450 nm. Two bands appear in the spectrum, an intense band at 630 nm and a much weaker band at about 580 nm. Other ligands such as N_3 or OH^-, which also convert high-spin hemoproteins to the low-spin state, give rise to the same typical spectra. Thus, these bands at 620–630 and 580 nm are the α and β bands of myeloperoxidase. Surprisingly, the fluoride complex of myeloperoxidase still has bands at 550 and 600 nm which may correspond to α and β bands of a low-spin state of the enzyme, suggesting that the myeloperoxidase-fluoride complex is not completely high-spin at 77 K. This is, however, in contrast to the observations on myoglobin fluoride [28] and the EPR spectra at 15 K of myeloperoxidase-fluoride which only show a high-spin signal [20]. It is conceivable that α and β bands in myeloperoxidase are always present to some extent, irrespective of the spin state.

Hemoproteins which contain a heme with unsaturated substituents have bands in the spectrum which have shifted to the red [27]. In addition the α band in such compounds has a much higher intensity relative to the β band. This is also observed in these myeloperoxidase complexes and is in agreement with the observation that myeloperoxidase, like cytochrome *c* oxidase, contains heme *a* with a formyl substituent. Yet, the positions of the bands in native myeloperoxidase are at higher wavelengths than those in cytochrome *c* oxidase. It has already been shown [16,17,29] that disruption of the interaction of the heme group with the protein leads to spectra of the more usual heme *a* type. Therefore, the protein part of the molecule greatly affects the electronic structure of the heme group in myeloperoxidase.

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References

1 Agner, K. (1972) in Structure and Function of Oxidation-Reduction Enzymes (Åkeson, Å. and Ehrenberg, A., eds.), pp. 329–335, Pergamon Press, Oxford
2 Stelmaszyńska, T. and Zgliczyński, J.M. (1974) Eur. J. Biochem. 45, 305–312
3 Harrison, J.E. and Schultz, J. (1976) J. Biol. Chem. 251, 1 371–1 374
4 Bakkenist, A.R.J., De Boer, J.E.G., Plat, H. and Wever, R. (1980) Biochim. Biophys. Acta 613, 337–348
5 Klebanoff, S.J. (1968) J. Bacteriol. 95, 2 131–2 138
6 Lehrer, R.I. and Cline, M.J. (1969) J. Clin. Invest. 48, 1 478–1 488
7 Ehrenberg, A. (1962) Ark. Kemi 19, 119–128
8 Wever, R., Roos, D., Weening, R.S., Vulsma, T. and Van Gelder, B.F. (1976) Biochim. Biophys. Acta 421, 328–333
9 Rotilio, G., Brunori, M., Concetti, A., Dri, P. and Patriarca, P. (1977) FEBS Lett. 73, 181–184
10 Agner, K. (1958) Acta Chem. Scand. 12, 89–94
11 Newton, N., Morell, D.B., Clarke, L. and Clezy, P.S. (1965) Biochim. Biophys. Acta 96, 476–486
12 Brill, A.S. and Williams, R.J.P. (1961) Biochem. J. 78, 246–253
13 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 360–367
14 Zgliczyński, J.M. and Stelmaszyńska, T. (1979) Biochim. Biophys. Acta 567, 309–314
15 Schultz, J. and Shmukler, H.W. (1964) Biochemistry 3, 1 234–1 238
16 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 368–374
17 Harrison, J.E. (1979) J. Biol. Chem. 254, 4 536–4 539
18 Odajima, T. (1980) J. Biochem. (Japan) 87, 379–391
19 Harrison, J.E. and Schultz, J. (1978) Biochim. Biophys. Acta 536, 341–349
20 Wever, R. and Bakkenist, A.R.J. (1980) Biochim. Biophys. Acta 612, 178–184
21 Tamura, M. (1971) Biochim. Biophys. Acta 243, 239–248
22 Hollenberg, P.F., Hager, L.P., Blumberg, W.E. and Peisach, J. (1980) J. Biol. Chem. 255, 4 801–4 807
23 Bakkenist, A.R.J., Wever, R., Vulsma, T., Plat, H. and Van Gelder, B.F. (1978) Biochim. Biophys. Acta 524, 45–54
24 Harrison, J.E., Araiso, T., Palcic, M.M. and Dunford, H.B. (1980) Biochem. Biophys. Res. Commun. 94, 34–40
25 Lange, R. and Debey, P. (1979) Eur. J. Biochem. 94, 485–489
26 Tamura, M. and Hori, H. (1972) Biochim. Biophys. Acta 284, 20–29
27 Smith, D.W. and Williams, R.J.P. (1970) in: Structure and Bonding (Hemmerich, P., Jørgensen, C.K., Neilands, J.B., Nyholm, R.S., Reinen, D. and Williams, R.J.P., eds.), Vol. 7, pp. 1–45, Springer Verlag, Berlin
28 Smith, D.W. and Williams, R.J.P. (1968) Biochem. J. 110, 297–301
29 Bakkenist, A.R.J. (1981) Ph.D. Thesis, University of Amsterdam, Rodopi, Amsterdam